Brief Communication
Diagnostic Hematology

Ann Lab Med 2022;42:678-682
https://doi.org/10.3343/alm.2022.42.6.678
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Immunohistochemical Staining to Identify Concomitant Systemic Mastocytosis in Acute Myeloid Leukemia with *RUNX1::RUNX1T1*

Sang Mee Hwang, M.D., Ph.D.[1,2], Beom Joon Kim, M.D.[1], Jee-Soo Lee, M.D.[2,3], Moon-Woo Seong, M.D., Ph.D.[2,3], Soo Hyun Seo, M.D., Ph.D.[1,2], Jin Ho Paik, M.D., Ph.D.[4], Sang-A Kim, M.D.[5], Ji Yun Lee, M.D.[5], Jeong-Ok Lee, M.D., Ph.D.[5], Yoon Hwan Chang, M.D., Ph.D.[2,3,*], and Soo Mee Bang, M.D., Ph.D.[5,*]

[1]Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seongnam, Korea; [2]Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul, Korea; [3]Department of Laboratory Medicine, Seoul National University Hospital, Seoul, Korea; [4]Department of Pathology, Seoul National University Bundang Hospital, Seongnam, Korea; [5]Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, Korea

Systemic mastocytosis with associated hematological neoplasm (SM-AHN) poses diagnostic challenges because of the coexistence of atypical mast cell proliferation and hematological neoplasms. We assessed the presence of SM-AHN in patients with acute myeloid leukemia (AML) with *RUNX1::RUNX1T1* from 2014 to 2020. Bone marrow (BM) samples were evaluated for mast cell aggregates using CD117 and CD25 immunohistochemical (IHC) staining. The *KIT* D816V variant burden at diagnosis and post induction was assessed using droplet digital PCR. Among 23 patients diagnosed as having AML with *RUNX1::RUNX1T1*, four (17.4%) were also diagnosed as having SM-AHN. No significant differences in clinical characteristics or overall survival (P=0.565) were observed between patients with or without SM-AHN, except for the presence of *KIT* variants (P=0.040). After induction therapy, IHC staining revealed the presence of mast cell aggregates in the BM, and the *KIT* D816V variant burden decreased with decreasing blast count and was similar in BM aspirates, smear slides, and sections. Concomitant SM-AHN was not infrequent in AML patients with *RUNX1::RUNX1T1*. This study showed the importance of CD117 and CD25 IHC staining after induction chemotherapy for SM-AHN screening, especially in patients with *KIT* variants.

Key Words: Systemic Mastocytosis, Acute myeloid leukemia with *RUNX1::RUNX1T1*, *KIT* variant, Immunohistochemistry, Droplet digital PCR

Received: February 4, 2022
Revision received: April 4, 2022
Accepted: June 3, 2022

Corresponding author:
Yoon Hwan Chang, M.D., Ph.D.
Department of Laboratory Medicine, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 03080, Korea
Tel: +82-2-2072-3519
Fax: +82-2-747-0359
E-mail: cyh1969@snu.ac.kr

Co-corresponding author:
Soo Mee Bang, M.D., Ph.D.
Department of Internal Medicine, Seoul National University Bundang Hospital, 82 Gumi-ro 173beon-gil, Bundang-gu, Seongnam 13620, Korea
Tel: +82-31-787-7038
Fax: +82-31-787-4098
E-mail: smbang7@snu.ac.kr

Systemic mastocytosis (SM) is characterized by the clonal neoplastic proliferation of mast cells accumulating in one or more organ systems [1]. SM with associated hematological neoplasm (SM-AHN) poses diagnostic challenges because of the coexistence of non-mast cell lineage hematological malignancies, including myelodysplastic syndrome (MDS), myeloproliferative neoplasm (MPN), and acute myeloid leukemia (AML), which mask the SM components [1-4]. We evaluated SM-AHN in patients with AML with *RUNX1::RUNX1T1* as they are known to have a high prevalence of *KIT* variants, which are one of the di-

agnostic criteria for SM-AHN.

SM diagnosis depends on histopathological findings and the identification of mast cell aggregates, especially via immunohistochemical (IHC) staining, where mast cell aggregates are identified by CD117 staining and aberrant CD25 with or without CD2 expression indicate SM [3, 5]. *KIT* variants detected in SM are often simultaneously found in AHN, especially in core-binding factor (CBF)-AML: 35% in AML with inv(16)(p13.1q22); *CBFB::MYH11* and 25% in AML with *RUNX1::RUNX1T1* have *KIT* variants [6, 7]. As the *KIT* D816V variant is a minor criterion for SM-AHN diagnosis, patients diagnosed as having AML with inv(16) or AML with *RUNX1::RUNX1T1* may have concurrent SM-AHN [2, 8, 9]. *KIT* variants are present in both mast cells and myeloid blasts [10]. The variant burden has prognostic significance in advanced SM, including SM-AHN [11].

Although cases of masked SM-AHN with AML have been previously reported, IHC staining of CD117 and CD25 for differential diagnosis are not routinely included in the work-up of SM-AHN in AML with *RUNX1::RUNX1T1*. We assessed the clinical significance of SM-AHN in patients with AML with *RUNX1::RUNX1T1*, focusing on IHC staining results and *KIT* variant detection after induction chemotherapy. As mast cells are enriched in tissues, we investigated whether the variant burden differs based on the sample type (bone marrow (BM) aspirate, a smear slide, or trephine biopsy tissue) and evaluated the changes in these differences post induction therapy.

The presence of SM-AHN was assessed in patients diagnosed as having AML with *RUNX1::RUNX1T1* in Seoul National University Bundang Hospital, Seongnam, Korea, from December 2014 to April 2020, and SM-AHN was diagnosed according to WHO criteria [1]. This study was approved by the Institutional Review Board of Seoul National University Bundang Hospital (B-1711/435-004, B-2005/612-106) and Seoul National University Hospital (J-2108-080-1245). Informed consent was obtained from those patients in whom additional studies were performed. CD117 and/or CD25 IHC staining results at diagnosis, post induction and/or follow-up, were compared in patients with SM-AHN.

KIT variants were detected in BM aspirates by Sanger sequencing and/or next-generation sequencing (NGS). NGS was performed on BM aspirates of patients when requested by the clinician, using the Oncomine Myeloid Research Assay (Thermo Fisher, Waltham, MA, USA), IonS5 XL (Thermo Fisher), or a customized panel with the NextSeq 550 platform (Illumina, San Diego, CA, USA).

The *KIT* D816V variant burden in different samples, including BM aspirates, smear slides, and trephine biopsy sections, was evaluated at diagnosis and follow-up using droplet digital PCR (ddPCR). DNA was extracted using a QIAamp DNA Blood Mini *kit* (Qiagen, Hilden, Germany) and QIAcube (Qiagen). ddPCRs were run in a QX200 Droplet Digital PCR System (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. The thermal cycles were as follows: enzyme activation at 95°C (10 minutes); 40 cycles of denaturation at 94°C (30 seconds), annealing and extension at 55°C (1 minute), and enzyme inactivation at 98°C (10 minutes); and holding at 4°C. Gating was performed based on positive and negative controls and analyzed using the QuantaSoft software v1.7.4 (Bio-Rad). Data were considered valid when at least 15,000 droplets were available.

Survival analysis was performed using the Kaplan-Meier method to compare overall survival (OS) in AML with *RUNX1::RUNX1T1* with or without SM-AHN. OS was defined as the time from the date of diagnosis to the date of death or last follow-up. Categorical values were compared using Fisher's exact test, and continuous variables were compared using the Mann-Whitney test. Statistical analyses were performed using SPSS version 25 (IBM Corporation, Armonk, NY, USA) and Prism 9.3.1 (GraphPad, San Diego, CA, USA).

Twenty-three patients were diagnosed as having AML with *RUNX1::RUNX1T1* during the study period, four of whom (17.4%) were also diagnosed as having SM-AHN. The clinical characteristics of the patients with or without SM-AHN are summarized in Table 1. No significant differences in clinical characteristics were observed between the two groups, except for the presence of *KIT* variants ($P=0.040$). *KIT* variants were present in six (26.1%) AML patients with *RUNX1::RUNX1T1*, in three out of four patients with SM-AHN (75%; two D816V and one N822K) and in three patients without SM-AHN (15.8%; D816V, A820G, Y418delinsSVYIYIH). Two (50%) patients with D816V *KIT* variants were diagnosed as having SM-AHN. Patients with or without SM-AHN did not show a significant difference in OS ($P=0.565$).

We evaluated whether SM-AHN and AML diagnoses were concurrent. All patients were diagnosed as having SM-AHN based on follow-up BM tests. Patients with SM-AHN at the time of AML diagnosis did not show evident mast cell aggregation on hematoxylin-eosin slides. Thus, IHC staining for CD117 and CD25 was performed for diagnostic and follow-up BM samples (Fig. 1). In retrospective analysis, CD117 expression was observed in mast cell aggregates at diagnosis in two patients, whereas it was not evident in the other two patients, with diffuse CD117-positive staining observed on the blasts at diagnosis of

Table 1. Characteristics of patients with AML with *RUNX1::RUNX1T1*

Characteristics	Without SM-AHN (N=19)	With SM-AHN (N=4)	*P*
Age (yr)			
Median (range)	43 (4–74)	38.5 (8–56)	0.683
Sex, N (%)			
Male	12 (63.2)	2 (50)	1.000
Female	7 (36.8)	2 (50)	
Hb (g/L)			
Median (range)	84 (52–116)	86 (69–101)	0.892
Leukocytes ($\times 10^9$/L)			
Median (range)	5,810 (750–26,610)	10,540 (4,690–29,930)	0.366
Absolute neutrophil count ($\times 10^9$/L)			
Median (range)	1,243 (66–13,017)	730 (125–1,373)	0.457
Platelets ($\times 10^9$/L)			
Median (range)	57 (22–110)	61 (16–81)	0.953
KIT variant, N (%)	3 (15.8)	3 (75)	0.040
D816V	2 (10.5)	2 (50)	
Other	1 (5.3)	1 (25)	
Outcome			
Follow-up, months, median (range)	22 (2–68)	33 (11–70)	0.494
Hematopoietic stem cell transplantation, N (%)	11 (57.9)	4 (100)	0.257
Death, N (%)	2 (11.1)	1 (25.0)	0.470

Abbreviations: AML, acute myeloid leukemia; SM-AHN, systemic mastocytosis with associated hematological neoplasm.

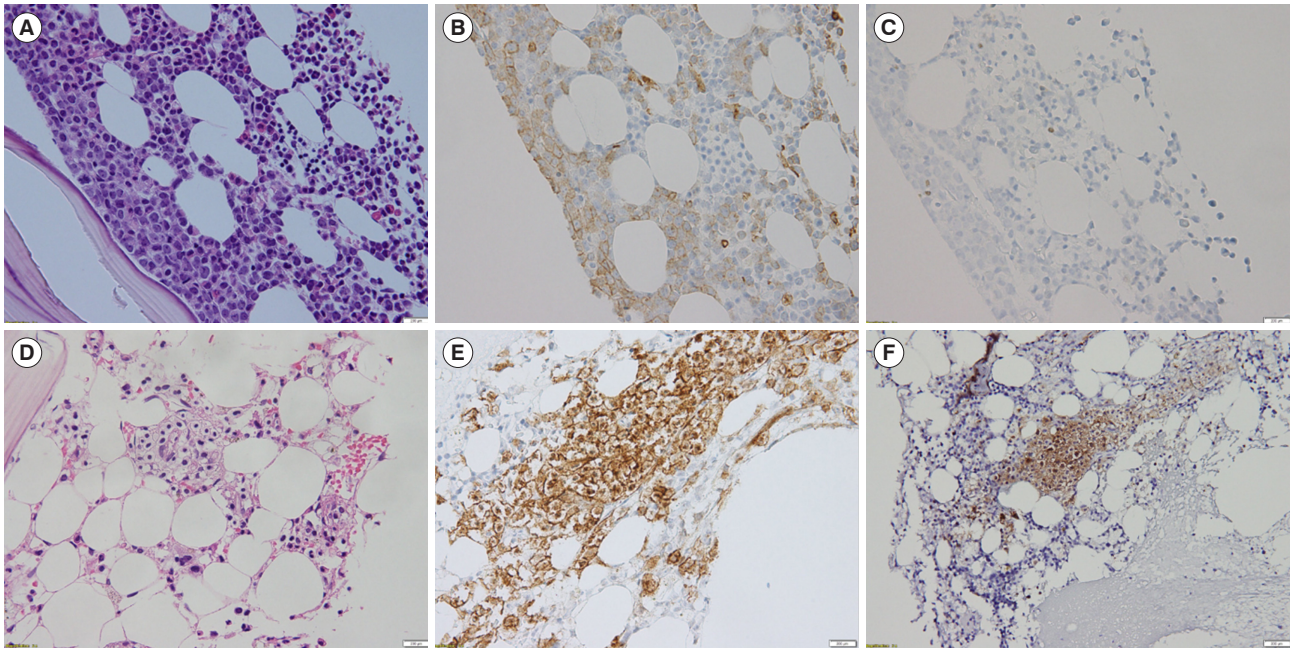

Fig. 1. BM biopsy section and IHC staining results at diagnosis showing an increase in blasts and scattered mast cells (A, B, C) and evident mast cell aggregates post induction (D, E, F). (A, D) Hematoxylin-eosin stain (×400). (B, E) CD117 positivity in mast cells (×400). (C, F) Aberrant CD25 expression in atypical mast cells (×400).
Abbreviations: BM, bone marrow; IHC, immunohistochemical.

https://doi.org/10.3343/alm.2022.42.6.678

Table 2. ddPCR results for *KIT* D816V in BM aspirates, smear slides, and tissue sections

Sample number	Sampling time	Sample type	Blast count (%)	Mutant allele burden (%)
S1	Diagnosis	BM smear slide	52.2	46.5
		BM aspirate		46.9
		BM tissue		47.3
	Post-induction	BM smear slide	0.5	1.3
		BM aspirate		0.6
		BM tissue		1.2
S2	Diagnosis	BM smear slide	80.7	32.1
		BM aspirate		31.8
		BM tissue		50.0
	Post-induction	BM smear slide	2.6	3.2
		BM aspirate		0.1
		BM tissue		3.2

Abbreviations: ddPCR, droplet digital PCR; BM, bone marrow

AML. Aberrant CD25 expression on mast cells at diagnosis was evident in only one patient. However, IHC staining of BM samples after induction revealed the presence of mast cell aggregates with CD117 expression and aberrant CD25 expression in all patients.

The *KIT* D816V variant burden in two patients with SM-AHN who had *KIT* D816V was assessed using diagnostic and follow-up samples (Table 2). The variant allele burden decreased with decreasing blast count and was similar in all three sample types. The variant burden was low in post-induction samples and lower in BM aspirates than in smear slides and tissue sections for both patients. However, the *KIT* D816V variant persisted after induction. Targeted sequencing results were obtained for two patients with SM-AHN and revealed additional variants in *RUNX1* (N=2), *FLT3* (N=1), *TP53* (N=1), and *NRAS* (N=1) at diagnosis.

Concomitant SM-AHN was diagnosed in 17.4% of the AML patients with *RUNX1::RUNX1T1* via CD117 and CD25 IHC staining in post-induction BM sections. Many studies have reported SM-AHN diagnosis in patients with AML [4, 8, 12, 13]; however, the clinical characteristics of patients with and without SM-AHN have not been compared. The OS and clinical characteristics did not differ significantly, but *KIT* variants were more prevalent in patients with SM-AHN. SM-AHN is difficult to diagnose in AML with *RUNX1::RUNX1T1* because of a marked expansion of blasts and CD117 expression in blasts [4]. As NGS is routinely performed as a diagnostic test for myeloid malignancies, the presence of *KIT* D816V has been reported to predict concurrent SM-AHN in MDS, MPN, and AML [9]. A higher *KIT* variant burden in tissue samples than in liquid samples has been reported in mastocytosis, including advanced SM [11]. In our study, although only two patients were tested, the variant allele frequency in the BM smear slides was similar to that in the BM tissues, but greater than that in the BM aspirates, because BM components are enriched in tissues and smear slides. Thus, when fresh BM aspirate is not available, archived BM smear slides can serve as a reliable source of DNA for variant analysis [14]. The genomic analysis of AML with *RUNX1::RUNX1T1* showed that the *KIT* D816V variant was present in 17.4% of patients (N=4). As these patients tested positive for one minor criterion, it is advisable to screen for mast cell collections. However, according to the European Competence Network on Mastocytosis (ECNM), non-D816V *KIT* variants are present in <10% of patients with mastocytosis, and therefore, the ECNM recommends whole *KIT* gene sequencing when *KIT* variant is not detected by codon 816-targeted assays, and non-D816V *KIT* variants may show a different response to treatment [15-17]. The persistence of *KIT* variants after induction therapy may be attributed to the SM or AHN component. Further, neoplastic mast cells of SM associated with AML with t(8;21) also carried *RUNX1::RUNX1T1* as well as *KIT* variant as indicated by targeted FISH and single-cell analysis [10, 18]. Thus, it is difficult to predict the SM burden or use *KIT* variant burden as a minimal residual disease marker for SM-AHN [9]. Since therapies targeting the SM component of SM-AHN are available, confirmation of the diagnosis is clinically significant, especially for advanced SM [17, 19]. SM-AHN diagnosis requires additional testing with IHC staining after induction, and testing for *KIT* variants may be performed using various BM samples with high mutation burdens, such as tissues or smear slides. Although a previous report has suggested that SM-AHN is uncommon in patients with CBF-AML, in this study, SM-AHN diagnosis was confirmed in 50% of the patients with *KIT*-mutated AML with *RUNX1::RUNX1T1* [20]. Therefore, it is necessary to screen mast cell aggregates for CD117 expression and subsequently for CD25 expression through IHC staining of samples collected from AML patients with *RUNX1::RUNX1T1* after induction.

ACKNOWLEDGEMENTS

None.

AUTHOR CONTRIBUTIONS

Hwang SM, Lee JS, Seong MW, Seo SH, Paik JH, Kim SA, Lee JY, Lee JO were involved in study organization, data collection and revision of the draft. Hwang SM, Kim BJ, Lee JS, Seong MW, Seo SH, Paik JH, Chang YH interpreted the data. Hwang SM, Kim BJ wrote the first draft of the manuscript. Hwang SM, Chang YH, Bang SM designed the study and composed the final draft of the manuscript. All the authors have read the final manuscript and approved the submission.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

RESEARCH FUNDING

This study was supported by the SNUBH Research Fund (16-2017-006).

ORCID

Sang Mee Hwang	https://orcid.org/0000-0003-3390-1932
Beom Joon Kim	https://orcid.org/0000-0002-2801-5453
Jee-Soo Lee	https://orcid.org/0000-0002-7005-5686
Moon-Woo Seong	https://orcid.org/0000-0003-2954-3677
Soo Hyun Seo	https://orcid.org/0000-0002-6899-4967
Jin Ho Paik	https://orcid.org/0000-0002-2792-0419
Sang-A Kim	https://orcid.org/0000-0002-8529-0232
Ji Yun Lee	https://orcid.org/0000-0002-5835-7219
Jeong-Ok Lee	https://orcid.org/0000-0001-9402-6372
Yoon Hwan Chang	https://orcid.org/0000-0002-9010-5281
Soo Mee Bang	https://orcid.org/0000-0002-0938-3007

REFERENCES

1. Swerdlow SH, Campo E, et al. eds. WHO classification of tumours of haematopoietic and lymphoid tissues. Revised 4th ed. Lyon: IARC Press, 2017.
2. Pullarkat ST, Pullarkat V, Kroft SH, Wilson CS, Ahsanuddin AN, Mann KP, et al. Systemic mastocytosis associated with t(8;21)(q22;q22) acute myeloid leukemia. J Hematop 2009;2:27-33.
3. Horny HP, Sotlar K, Sperr WR, Valent P. Systemic mastocytosis with associated clonal haematological non-mast cell lineage diseases: a histopathological challenge. J Clin Pathol 2004;57:604-8.
4. Johnson RC, Savage NM, Chiang T, Gotlib JR, Cherry AM, Arber DA, et al. Hidden mastocytosis in acute myeloid leukemia with t(8;21)(q22;q22). Am J Clin Pathol 2013;140:525-35.
5. Sotlar K, Horny HP, Simonitsch I, Krokowski M, Aichberger KJ, Mayerhofer M, et al. CD25 indicates the neoplastic phenotype of mast cells: a novel immunohistochemical marker for the diagnosis of systemic mastocytosis (SM) in routinely processed bone marrow biopsy specimens. Am J Surg Pathol 2004;28:1319-25.
6. Reiter A, George TI, Gotlib J. New developments in diagnosis, prognostication, and treatment of advanced systemic mastocytosis. Blood 2020;135:1365-76.
7. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017;129:424-47.
8. Yabe M, Masukawa A, Kato S, Yabe H, Nakamura N, Matsushita H. Systemic mastocytosis associated with t(8;21) acute myeloid leukemia in a child: detection of the D816A variant of KIT. Pediatr Blood Cancer 2012;59:1313-6.
9. Craig JW, Hasserjian RP, Kim AS, Aster JC, Pinkus GS, Hornick JL, et al. Detection of the KIT^{D816V} variant in myelodysplastic and/or myeloproliferative neoplasms and acute myeloid leukemia with myelodysplasia-related changes predicts concurrent systemic mastocytosis. Mod Pathol 2020;33:1135-45.
10. Pullarkat V, Bedell V, Kim Y, Bhatia R, Nakamura R, Forman S, et al. Neoplastic mast cells in systemic mastocytosis associated with t(8;21) acute myeloid leukemia are derived from the leukemic clone. Leuk Res 2007;31:261-5.
11. Greiner G, Gurbisz M, Ratzinger F, Witzeneder N, Class SV, Eisenwort G, et al. Molecular quantification of tissue disease burden is a new biomarker and independent predictor of survival in mastocytosis. Haematologica 2020;105:366-74.
12. Nagai S, Ichikawa M, Takahashi T, Sato H, Yokota H, Oshima K, et al. The origin of neoplastic mast cells in systemic mastocytosis with AML1/ETO-positive acute myeloid leukemia. Exp Hematol 2007;35:1747-52.
13. Escribano L, García-Montero A, Núñez-López R, López-Jiménez J, Almeida J, Prados A, et al. Systemic mastocytosis associated with acute myeloid leukemia: case report and implications for disease pathogenesis. J Allergy Clin Immunol 2004;114:28-33.
14. Al Hinai ASA, Grob T, Kavelaars FG, Rijken M, Zeilemaker A, Erpelinck-Verschueren CAJ, et al. Archived bone marrow smears are an excellent source for NGS-based mutation detection in acute myeloid leukemia. Leukemia 2020;34:2220-4.
15. Arock M, Sotlar K, Akin C, Broesby-Olsen S, Hoermann G, Escribano L, et al. KIT mutation analysis in mast cell neoplasms: recommendations of the European Competence Network on Mastocytosis. Leukemia 2015;29:1223-32.
16. Laine E, Chauvot de Beauchêne I, Perahia D, Auclair C, Tchertanov L. Mutation D816V alters the internal structure and dynamics of c-KIT receptor cytoplasmic region: implications for dimerization and activation mechanisms. PLoS Comput Biol 2011;7:e1002068.
17. Gotlib J, Kluin-Nelemans HC, George TI, Akin C, Sotlar K, Hermine O, et al. Efficacy and safety of midostaurin in advanced systemic mastocytosis. N Engl J Med 2016;374:2530-41.
18. Grootens J, Ungerstedt JS, Ekoff M, Rönnberg E, Klimkowska M, Amini RM, et al. Single-cell analysis reveals the KIT D816V mutation in haematopoietic stem and progenitor cells in systemic mastocytosis. EBioMedicine 2019;43:150-8.
19. Gotlib J, Reiter A, Radia DH, Deininger MW, George TI, Panse J, et al. Efficacy and safety of avapritinib in advanced systemic mastocytosis: interim analysis of the phase 2 PATHFINDER trial. Nat Med 2021;27:2192-9.
20. Kristensen T, Preiss B, Broesby-Olsen S, Vestergaard H, Friis L, Møller MB. Systemic mastocytosis is uncommon in KIT D816V mutation positive core-binding factor acute myeloid leukemia. Leuk Lymphoma 2012;53:1338-44.