BLOOD RESEARCH
VOLUME 49 · NUMBER 1
March 2014
ORIGINAL ARTICLE

Characteristics of hematologic malignancies with coexisting t(9;22) and inv(16) chromosomal abnormalities

Eunhee Han[1], Hyeyoung Lee[1], Myungshin Kim[1], Yonggoo Kim[1], Kyungja Han[1], Sung-Eun Lee[2], Hee-Je Kim[2], Dong-Wook Kim[2]

[1]Department of Laboratory Medicine, [2]Department of Hematology, Department of Internal Medicine, Catholic Blood and Marrow Transplantation Center, The Catholic University of Korea, Seoul, Korea

p-ISSN 2287-979X / e-ISSN 2288-0011
http://dx.doi.org/10.5045/br.2014.49.1.22
Blood Res 2014;49:22-8.

Received on November 13, 2013
Revised on February 17, 2014
Accepted on March 4, 2014

*This study was supported by a grant from the Korea Health Technology R&D Project, Ministry of Health and Welfare (A120175).

Correspondence to
Myungshin Kim, M.D., Ph.D.
Department of Laboratory Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 137-701, Korea
Tel: +82-2-2258-1645
Fax: +82-2-2258-1719
E-mail: microkim@catholic.ac.kr

Background
The coexistence of t(9;22)(q34;q11.2) and inv(16)(p13q22) chromosomal abnormalities is extremely uncommon, and only a small number of such cases have been reported. Here, we characterized 7 cases of hematologic malignancy exhibiting t(9;22) and inv(16) coexistence.

Methods
We reviewed the cytogenetic data for hematologic malignancies treated at the Catholic Blood and Marrow Transplantation Center between January 2004 and June 2013. We identified 7 cases exhibiting t(9;22) and inv(16) coexistence. In addition, we analyzed mutations in the *IKZF1*, *NPM1*, *FLT3*, *N-RAS*, *K-RAS*, *c-KIT*, and *TP53* genes.

Results
Four cases of chronic myelogenous leukemia (CML; 1 chronic phase, 2 accelerated phase, and 1 blast phase) and 3 cases of acute myeloid leukemia (AML; 1 de novo and 2 therapy-related) were identified. The percentages of circulating blasts and bone marrow eosinophils were higher in AML cases than in CML cases (53% vs. 5% and 30% vs. 5.5%, respectively). The proportions of each chromosomal abnormality were used along with follow-up karyotyping results to identify secondary changes. In *BCR/ABL*, a p210 fusion transcript was associated with CML, whereas a p190 fusion transcript was associated with AML. One patient with AML harbored 2 mutations: *c-KIT* D816V and *TP53* E11Q. All patients except 1 with CML blast phase sustained clinical remission after treatment, which included an imatinib mesylate regimen.

Conclusion
This study shows that observations of bone marrow morphology, initial and follow-up cytogenetic studies, and karyotyping of *BCR/ABL1* and *CBFB/MYH11* provide valuable information for characterizing hematologic malignancies exhibiting t(9;22) and inv(16) coexistence.

Key Words Chronic myelogenous leukemia; Acute myeloid leukemia; t(9;22); *BCR/ABL1*; inv(16); *CBFB/MYH11*

INTRODUCTION

The presence of chromosomal abnormalities, including inv(16)(p13q22) and its associated variant-t(16;16)(p13;q22), in acute myeloid leukemia (AML) is relatively common. Such abnormalities occur in 10%–12% of all AML cases [1]. AML with inv(16) has been defined as a distinctive morphologic subtype and is designated M4Eo by the French-American-British Cooperative Group. From a molecular standpoint, the inversion of chromosome 16 creates the pathologic fusion gene *CBFB/MYH11*, which reportedly alters transcriptional regulation [2]. The *BCR/ABL1* rearrangement, created by t(9;22)(q34;q11.2), is characteristic of chronic myelogenous leukemia (CML) but also occurs in precursor lymphoid neoplasm and AML [3]. Coexistence

of the t(9;22) and inv(16) chromosomal aberrations is a rare occurrence that has been described in CML (mainly the myeloid blast phase [CML-BP]), de novo AML, and a few cases of therapy-related AML (t-AML) [2-6]. Most of the de novo forms of AML with the t(9;22) and inv(16) chromosomal abnormalities have a favorable prognosis comparable to that of AML with inv(16) alone [6]. However, the majority of cases, in which these abnormalities coexist are CML-BP, which is typically characterized by an aggressive clinical course with rapid disease progression and resistance to chemotherapy [4]. Therefore, discriminating between these disease types is important in the formulation of appropriate treatment plans. However, few cases have been analyzed. Herein, we describe 7 cases of hematologic malignancy accompanied by the coexistence of t(9;22) and inv(16). In addition, we analyze the presence of mutations in several other genes associated with the hematologic malignancies in these cases.

MATERIALS AND METHODS

Patients

We reviewed all cytogenetic data for patients with hematologic malignancies who received treatment at the Catholic Blood and Marrow Transplantation Center between January 2004 and June 2013. We observed 716 cases of t(9;22), including 705 with CML, 151 cases of acute lymphoblastic leukemia/acute biphenotypic leukemia, and 56 cases of AML. AML with inv(16) occurred in 104 cases. Among these cases, we identified 7 patients exhibiting both t(9;22) and inv(16). This study was performed according to the Declaration of Helsinki guidelines, and full approval was obtained from the institutional review board of St. Mary's Hospital, which is affiliated with The Catholic University of Korea (IRB No: KC13ZISE0779).

Bone marrow examination and flow cytometry

We reviewed all available data, including peripheral blood (PB) smears, bone marrow (BM) aspirates, cytochemical staining, and core biopsy specimens. Clinical information was also reviewed when available; however, not all cases included the same types of data. Immunophenotyping of cells was performed with flow cytometry against CD3, CD41a, CD14, CD34, CD33, CD20, CD5, CD10, CD19, CD64, CD11c, CD13, CD117, CD56, CD2, CD7, HLA-DR, cytoplasmic CD22, cytoplasmic myeloperoxidase, cytoplasmic CD3, and cytoplasmic CD79a (BD Biosciences, San Jose, CA, USA). Leukemic blasts were analyzed with the FACSDiva program (BD Biosciences).

Conventional cytogenetics

Chromosomal analyses were performed by examining short-term cultures of BM specimens according to standard conventional cytogenetic protocols. At least 20 cells in metaphase were analyzed in each case. Clonal abnormalities were classified according to the 2009 International System for Human Cytogenetic Nomenclature guidelines [7].

Fluorescence in situ hybridization

In 6 of 7 cases, fluorescence in situ hybridization (FISH) analyses were performed to confirm Ph translocation and the presence of inv(16). These FISH analyses used pellets of cells remaining after conventional cytogenetic studies. Slides for FISH were prepared by using cells harvested for conventional cytogenetics and processing them for FISH according to the manufacturer's guidelines (Abbott Vysis, Des Plaines, IL, USA). Analyses were performed on cells in either interphase or metaphase. An LSI *BCR/ABL1* dual-color, dual-fusion probe (Abbott Vysis) was used to identify t(9;22) and its variants. A LSI *CBFB* dual-color break-apart rearrangement probe (Abbott Vysis) was used to identify rearrangements involving *CBFB*.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

RNA from 6 patients was extracted from BM cells, reverse transcribed into complementary DNA using a QIAamp RNA Blood Mini Kit (Qiagen, Chatsworth, CA, USA) according to the manufacturer's instructions, and analyzed using RT-PCR designed to detect the *BCR/ABL* fusion transcript of t(9;22) or the *CBFB/MYH11* fusion transcript of inv(16). Multiplex RT-PCR was performed to detect the presence of *BCR/ABL1* (N=6) and *CBFB/MYH11* (N=3) fusion products using a HemaVision kit (Bio-Rad Laboratories, Hercules, CA, USA).

Mutation analysis

Genomic DNA was extracted from BM specimens using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). We analyzed an internal tandem duplication in *FLT3*, a mutation in the tyrosine kinase domain of *FLT3* (Seegene, Seoul, Korea), mutations in *c-KIT* (BioSewoom Inc., Seoul, Korea), and mutations A and B in *NPM1* (Ipsogen Inc., Stamford, CT, USA) following the manufacturers' instructions for the appropriate kits. Mutations in *N-RAS*, *K-RAS*, and *TP53* were evaluated via direct sequencing using primers designed using primer sequences. Amplicons were bidirectionally sequenced with a BigDye Terminator v3.1. cycle sequence kit (Applied Biosystems, Foster City, CA, USA) by using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). The targeted copy number of *IKZF1* was analyzed using a SALSA MLPA probemix P202-B1 *IKZF1* kit (IKAROS, MRC-Holland, Amsterdam, The Netherlands) according to the manufacturer's instructions.

RESULTS

Clinical features

The clinical and laboratory characteristics of the patients analyzed in this study are summarized in Table 1. In total, 5 men and 2 women with a median age of 27 years (range, 10–61 years) were included. Four cases of CML and 3 cases of AML were identified. The incidence of inv(16) in the

Table 1. Patient characteristics.

Case No./ Gender/Age (y)	Diagnosis	Peripheral blood							Bone marrow	
		WBC 10^9/L	Nø %	Eø %	Bø %	Blasts %	Hb g/dL	Plt 10^9/L	Blast %	Eø %
1/M/18	CML-CP→BP[a)]	10	41	0	0	44	7.4	14	70	7
2/M/61	CML-CP[a)]	195.1	67	3	7	1	9	811	1	11
3/M/27	CML-BP[a)]	285.8	9	22	2	6	9.2	663	60	4
4/M/10	CML-CP→BP[a)]	73	6	0	0	90	9.9	56	80	4
5/M/30	AML, M4Eo	194.5	13	5	5	39	12.8	35	37	30
6/F/27	Hodgkin's lymphoma→t-AML	91.3	6	5	2	53	8.3	29	65	30
7/F/57	Follicular lymphoma→Eosinophilia→t-AML	10	10	0	0	80	8.2	24	60	30

[a)]Phase in which cytogenetic studies were performed.
Abbreviations: AML, acute myeloid leukemia; AP, accelerated phase; Bø, basophil; BP, blast phase; CML, chronic myelogenous leukemia; CP, chronic phase; Eø, eosinophil; Hb, hemoglobin; M4Eo, French-American-British Cooperative Group classification M4 with eosinophils; Nø, neutrophil; Plt, platelet; t-AML, therapy-related AML.

patients with CML was 0.6% (4/705), and the incidence of t(9;22) in patients with AML with inv(16) was 2.9% (3/104). Among the 4 CML patients, 1 patient (case 2) presented with CML-chronic phase (CML-CP). Two patients were previously diagnosed with and treated for CML-CP and later progressed to CML-BP (cases 1 and 4). One patient initially presented with CML-BP. Among the 3 patients diagnosed with AML, 1 had de novo AML M4Eo and the other 2 were diagnosed with t-AML. One t-AML patient presented with Hodgkin's lymphoma and was treated with doxorubicin, bleomycin, vinblastine, and dacarbazine chemotherapy (case 6), and the other presented with follicular lymphoma and was treated with fludarabine, mitoxantrone, and dexamethasone chemotherapy (case 7). Splenomegaly was documented in all cases except case 3. All patients received chemotherapy containing imatinib mesylate (Table 2). Two patients with CML and 3 patients with AML underwent either BM or PB stem cell transplantation. One patient died of sepsis 5 months after developing CML-BP (case 3). The other 6 patients were alive at the most recent follow-up examination.

Peripheral blood and BM findings

In the CML cases, clinical studies of PB revealed anemia (median Hb, 9.1 g/dL) and mild to severe leukocytosis (median WBC, 134.1×10^9/L). Thrombocytosis was observed in 2 patients with CML (cases 2 and 3). The other 2 patients with CML exhibited thrombocytopenia. In the AML cases, clinical studies of the PB revealed anemia (median Hb, 8.3 g/dL) and mild leukocytosis (median WBC, 91.3×10^9/L). All AML patients also exhibited thrombocytopenia (median platelet count, 29×10^9/L). The percentage of circulating blasts was variable and higher in AML patients than in CML patients (median, 53% vs. 5%, P=0.4). BM cellularity was nearly 100% and exhibited an increased number of blasts (median, 65% in CML; 60% in AML). The percentage of eosinophils in the BM of AML patients was higher than that in CML patients (median, 30% vs. 5.5%, P=0.057); furthermore, most of the eosinophils were abnormal with coarse basophilic granules. Flow cytometric analysis showed a large population of myeloid blasts with the following immunophenotype: CD13, CD33, CD34, CD11c, and myeloperoxidase.

Cytogenetic and molecular findings

The cytogenetic and molecular findings of this study are summarized in Table 2. All patients exhibited t(9;22) and inv(16) at some point during the course of their disease. Five patients showed complex karyotypes with additional numerical chromosomal aberrations, structural chromosomal aberrations, or both. Of the 4 patients with CML, 1 patient had both abnormalities detected in the CML-CP sample, whereas the other 3 had both abnormalities detected in CML-BP samples. Of the 3 CML-BP cases, 2 cases included karyotype results for the time at which the patients exhibited CML-CP, and both showed t(9;22) without inv(16). Of the 3 patients with AML, 1 of the patients with t-AML (case 7) had the karyotype result from the previous illness, which showed inv(16) without t(9;22). Follow-up karyotype results were available for all patients. Cells in metaphase were documented for all CML-BP cases, with the occurrence of t(9;22) observed only after treatment. One CML-CP and 3 AML cases showed normal karyotype results after treatment.

The *BCR/ABL1* fusion transcript resulting from the translocation of t(9;22) was analyzed with RT-PCR in 6 cases. A p210 fusion product was detected in all patients with CML and 1 patient with AML (case 5); moreover, a p190 fusion product was detected in the 2 patients with t-AML. The *BCR/ABL1* fusion transcript was also confirmed with FISH in 6 patients. The *CBFB/MYH11* fusion transcript was confirmed with RT-PCR or FISH. Interestingly, FISH analysis of 1 of the patients with CML (case 3) showed that the percentage of cells with *CBFB/MYH11* rearrangement was lower than that with *BCR/ABL1* rearrangement. On the contrary, the percentages of cells with both types of rearrangement were similar in patients with AML. In addition, each of the 3 cases in which *CBFB-MYH11* RT-PCR was performed displayed a different transcript type. The patient with CML (case 3) exhibited type B, a patient with de novo AML (case 5) exhibited type E, and the patient with t-AML

Table 2. Molecular and cytogenetic findings and clinical outcomes.

No.	Cytogenetic findings			Molecular findings			Treatment	Clinical outcome
	Initial BM	Presence of t(9;22) and inv(16) (months[a])	Follow up BM (months[b])	*BCR/ABL1* RT-PCR	*CBFB/MYH11* RT-PCR	*BCR/ABL1* FISH (%)	*CBFB/MYH11* FISH (%)		
1	46, XY, t(9;22)(q34;q11.2)[20]	46, XY, t(9;22)(q34;q11.2), inv(16)(p13.1q22)[5]/48, idem, +8, +19[15] (2 mon)	46, XY, t(9;22)(q34;q11.2)[17]/46, XY[3] (3 mon)	b2a2	ND	95%	80%	Imatinib, dasatinib, idarubicine, cytarabine, uBMT	Alive at 48 mon
2		46, XY, t(3;11)(p23;p15), t(6;16)(q15;p13.3), t(9;22)(q34;q11.2), inv(16)(p13.3q11.2)[20]	46, XY[20] (5 mon)	b2a2	ND	ND	ND	Hydroxyurea, imatinib	Alive at 41 mon
3		46, XY, t(9;22)(q34;q11.2), inv(16)(p13.1q22)[20]	46, XY, t(9;22)(q34;q11.2)[15]/46, XY[5] (2 mon)	b3a2	*CBFB/MYH11*(B)	95%	50%	Imatinib, dasatinib, uPBSCT	Alive at 6 mon
4	46, XY, t(9;22)(q34;q11.2)[25]	46, X, der(Y)t(y;1)(q12;q21), der(4)t(1;4)(q25;q31.3), t(9;22)(q34;q11.2), inv(16)(p13.1;q22)[17]/47, idem, +der(22)y(9;22)[1]/46, XY, der(5)t(1;5) (q12;q31), der(9)t(9;22)(q34;q11.2), inv(16)(p13.1;q22), t(1;22)(q21;q11.2)[2] (67 mon)	46, XY, t(9;22)(q34;q11.2)[1]/46, idem, der(4)t(1;4)(q25;q31.3), der(5)t(1;5)(q21;q31), inv(16)(p13.1q22)[6]/47, idem, der(5)t(1;5)(q21;q31), inv(16)(p13.1q22), der(19)t(1;19)(q21;q13.3), +der(22)t(9;22)[13] (3 mon)	ND	ND	93.50%	70.50%	Imatinib, hydroxyurea, cytarabine, etoposide	Died by sepsis
5		46, XY, t(9;22)(q34;q11.2), inv(16)(p13.1q22)[13]/47, idem, +17[15]/48, idem, +8, +17[2]	46, XY[20] (2 mon)	b3a2	*CBFB/MYH11*(E)	98.80%	98.50%	Idarubicine, cytosine arabinoside, imatinib, alloPBSCT	Alive at 15 mon
6		46, XX, inv(16)(p13.1q22)[20] Ish ins(9;22)(q34;q11.2q11.2)(ABL1+, BCR+; BCR+)[20]	46, XX[20] (2 mon)	e1a2	*CBFB/MYH11*(A)	98.80%	98.50%	Idarubicine, cytosine arabinoside, imatinib, alloPBSCT	Alive at 5 mon
7	46, XX, inv(16)(p13.1q22)[20]	46, XX, inv(16)(p13.1q22)[5]/47, idem, +8,t(9;22;14)(q34;q11.2;q22)[12]/46, idem, der(3)t(3;8)(p26;q11.2), t(9;22;14)(q34;q11.2;q22)[8] (I mon)	46, XX[20] (5 mon)		e1a2	67.50%	91%	Idarubicine, cytosine arabinoside, imatinib, alloPBSCT	Alive at 99 mon

[a]Months from previous diagnosis. [b]Months from presence of inv(16) and t(9;22).
Abbreviations: alloPBSCT, allogeneic peripheral blood stem cell transplant; BM, bone marrow; FISH, fluorescence in situ hybridization; ND, not done; RT-PCR, reverse transcriptase-polymerase chain reaction; uBMT, unrelated BM transplantation; uPBSCT, unrelated peripheral blood stem cell transplant.

(case 6) exhibited type A. Mutations in *IKZF1*, *NPM1*, *FLT3*, *N-RAS*, and *K-RAS* were not detected in any patient. The *c-KIT* D816V and *TP53* E11Q mutations were identified in case 6.

DISCUSSION

Coexistence of t(9;22) and inv(16) is rare in hematologic malignancies. The few cases in which both of these aberrations have been reported have had either an AML or a CML

Table 3. Previously reported cases of t(9;22) and inv(16) coexistence.

Authors	Gender/ age	Diagnosis	Molecular analysis	Cytogenetic study	Outcome from diagnosis at t(9;22) and inv(16)
Wu *et al*. [5]	M/33	CML-BP	M-bcr	46, XY, t(9;22)(q34.1;q11.2)[18]/46, XY, idem, inv(16)(p13.1q22)[2]	Died two failed allo-BMT
Wu *et al*. [5]	M/41	CML-BP	M-bcr	46, XY, t(9;22)(q34.1;q11.2), inv(16)(p13.1q22)[20]	Alive 4 years after allo-BMT
Wu *et al*. [5]	F/62	CML-BP	M-bcr	46, XX, t(9;22)(q34.1;q11.2, inv(16)(p13.1q22)[20]	Died 24 months
Ninomiya *et al*. [8]	F/63	CML-BP	m-bcr	46, XY, t(9;22)(q34;q11.2), inv(16)(p13q22)	Died 7 months
Merziau *et al*. [9]	F/43	CML-BP	ND	46, XX, t(9;22)(q34;q11.2), inv(16)(p13q22)[20]	Died 3 months
Merzianu *et al*. [9]	F/61	CML-BP	ND	46, XX, t(9;22)(q34;q11.2)[3]/46, XX, t(9;22)(q34;q11.2), inv(16)(p13q22)[7]/47, XX, +8, t(9;22)(q34;q11.2)[2]/47, XX, +8, t(9;22)(q34;q11.2), inv(16)(p13q22)[4]/46, X, add(X)(p22.3), t(9;22)(q34;q11.2), del(12)(p11.2), inv(16)(p13q22)[1]/47, XX, t(9;22)(q34;q11.2), inv(16)(p13q22), +der(22)t(9;22)[1]/46, XX[2]	Died 7 months
Merzianu *et al*. [9]	M/47	CML-BP	ND	46, XY, t(9;22)(q34;q11), inv(16)(p13q22)[25]	Died 1 month
Merzianu *et al*. [9]	F/36	CML-BP	ND	46, XX, t(9;22)(q34;q11.2), inv(16)(p13q22)[20]	Died 1 month
Asou *et al*. [10]	M/51	CML-BP	ND	46, XY, t(9;22)(q34;q11.2)[9], 46, XY, t(9;22)(q34;q11.2), inv(16)(p13q22)[12]	Died 3 months
Heim *et al*. [11]	M/21	CML-BP	M-bcr	45, X, -Y, t(9;22)(q34;q11), inv(16)(p13q22)	Alive 48 months
Myint *et al*. [12]	M/29	CML-BP	ND	46, XY, t(9;22)(q34;q11.2), inv(16)(p13q22)[30]	Died 0 month
Tirado *et al*. [3]	M/13	AML	ND	46, XY, inv(16)(p13.1q22)[2]/46, idem, del(7)(q22q32)[1]/46, idem, t(9;22;19)(q34;q11.2;p13.1)[2]	Alive 10 months
Secker-Walker *et al*. [13]	F/9	AML M4Eo	m-bcr	46, XX, inv(16)(p13q22)[21]/46, XX, t(9;22)(q34;q11.2), inv(16)[8]/46, XX[10]	Died 7 months
Secker-Walker *et al*. [13]	M/25	AML M4Eo	ND	46, XY, t(9;22)(q34;q11.2), inv(16)(p13q22)[16]	Died 15 months
Preudhomme *et al*. [14]	M/64	AML M4Eo	m-bcr	46, XY, inv(16)(p13q22), t(9;22)(q34;q11.2)[30]	Alive 13 months
Miura *et al*. [15]	M/40	AML M4Eo	m-bcr	46, XY, inv(16)(p13q22)[17]/46, idem, t(9;22)(q34;q11.2)[3]	Alive 27 months
Svaldi *et al*. [16]	F/40	AML M1	m-bcr	inv(16)[4]/Ph, inv(16)[18]	Unknown
Siddiqui *et al*. [17]	M/23	AML M4Eo	ND	46, XY, t(9;22)(q34;q11.2), inv(16)(p13q22)	Alive 36 months
Cividin *et al*. [18]	F/38	AML M4Eo	m-bcr	46, XX[22/71]/46, XX, inv(16)(p13q22)[1/71]/46, idem, t(9;22)(q34;q11)[25/71]/46, XX, t(2;9;22)(q32;q34;q11), inv(16)(p13q22)[23/71]	Alive 12 months

Abbreviations: AML, acute myeloid leukemia; AML M1, acute myeloblastic leukemia; allo-BMT, allogeneic bone marrow transplant; BP, blast phase; F, female; M, male; m-bcr, minor breakpoint region; M-bcr, major breakpoint region; M4Eo, French-American-British Cooperative Group classification M4 with eosinophils; ND, not done.

phenotype (Table 3). In this study, we identified 4 cases of CML and 3 cases of AML, in which both of these chromosomal abnormalities occurred. Although the prevalence of t(9;22) and inv(16) coexistence has not yet been fully evaluated, a previous study showed that the incidence of t(9;22) in AML with inv(16) is less than 1% (approximately 0.5%) [19]. Our data showed that the coexistence of these abnormalities was detected in 0.6% of CML cases and 2.9% of AML cases with inv(16).

The majority of documented AML patients with t(9;22) and inv(16) coexistence have exhibited M4Eo BM morphology, including an increased number of myelomonocytic cells and abnormal eosinophils with coarse basophilic granules [8]. The AML cases we studied had similar AML-M4Eo BM morphologies. However, because the CML-BP cases in this study also exhibited an increased number of myelomonocytic cells and abnormal eosinophils, we had difficulty in discriminating CML-BP from AML on a morphological basis alone. Patients with a history of CML-CP before the development of a "double positive" can be easily diagnosed with CML-BP. However, we found that patients with AML exhibited a higher number of circulating blasts and more severe BM

eosinophilia than did patients with CML-BP.

Cytogenetically, the proportion of each chromosomal abnormality was informative for identifying secondary changes [3]. This study documented a CML patient with secondary inv(16) who also exhibited a minor proportion of a *CBFB/MYH11* rearranged clone compared with that of a *BCR/ABL1* rearrangement. AML patients in this study also harbored minor proportions of the *BCR/ABL1* rearranged clone compared with that of the secondary *CBFB/MYH11* rearranged clone. Follow-up karyotyping results showed that only the t(9;22) aberration remained after CML treatment. However, this observation may not always apply in the era of tyrosine kinase inhibitors, because a complete cytogenetic response is achieved even in the early stages of CML-BP treatment [20].

In most double-positive cases, the major p210 chimeric BCR-ABL1 protein is observed in CML, whereas the minor p190 form correlates with AML presentation. However, some exceptions to this tendency have also been reported [9, 21, 22]. In the present study, a p210 fusion was detected in all patients with CML and 1 patient with de novo AML; moreover, a p190 fusion was detected in the 2 patients with t-AML. These findings suggest that the presence of a p190 BCR-ABL1 fusion protein is associated with AML. In addition, rare *CBFB/MYH11* fusion transcripts in AML with inv(16) have been shown to be associated with t-AML M4Eo [23]. However, 1 patient with t-AML (case 6) in the present study exhibited an A-type *CBFB/MYH11* fusion transcript. We detected variant E-type *CBFB/MYH11* fusion transcripts in the patient with de novo AML (case 5) and a variant B-type *CBFB/MYH11* fusion transcript in the patient with CML-BP (case 3).

We also analyzed the presence of commonly observed mutations associated with inv(16) and t(9;22). Nacheva *et al.* [24] have shown that AML with t(9;22) is accompanied by the loss of *IKZF1*, *CDKN2A*, or both, a hallmark of ALL with t(9;22), as well as cryptic deletions within immunoglobulin and T-cell receptor genes. Some studies have found that mutations in *RAS*, *c-KIT*, and *FLT3* are commonly observed in cases of AML with inv(16) [25]. In the study by Bacher *et al.* [19], cases of AML with t(9;22) carried mutations in *NPM1* at a frequency similar to that in cases of AML without the abnormality. In the present study, mutations in *IKZF1*, *NPM1*, *FLT3*, *N-RAS*, and *K-RAS* were not detected in any of the studied cases. Alterations in *TP53* are the most common molecular lesions in AML cases with complex karyotypes and have also been shown to be predictive for resistance to conventional chemotherapy and poor outcome [26]. In the present study, 1 patient with t-AML (case 6) exhibited *c-KIT* D816V and *TP53* E11Q mutations. However, the significance of this result was difficult to investigate owing to the limited number of cases in the study.

De novo AML with coexisting t(9;22) and inv(16) chromosomal abnormalities appears to have a prognosis as favorable as that in AML with inv(16) alone, whereas CML with both t(9;22) and inv(16) seems to have an unfavorable or uncertain prognosis [4, 5]. However, our data show that only 1 patient with CML-BP died of sepsis, and the other patients sustained clinical remission. Imatinib mesylate has been used effectively and safely to treat CML-BP [27]. With the benefits of better tolerance and fewer side effects, it has also been useful as a first-line interim therapy or maintenance strategy to bridge hematopoietic stem cell transplantation in patients with AML [28].

An interesting finding in the present study is that the 2 patients with t-AML exhibited t(9;22) and inv(16) coexistence. Patients with t(9;22) represent 2% of all therapy-related cases of myelodysplastic syndromes and t-AML. Moreover, t(9;22) is significantly associated with previous therapy with topoisomerase II inhibitors [5]. Although inv(16) is a well-known recurrent abnormality in t-AML, it is most frequently observed in patients with breast cancer or lymphoma who have been treated with alkylating agents, topoisomerase II inhibitors, radiation therapy, or a combination thereof. The response rates to intensive antileukemic chemotherapy in patients with t-AML with inv(16) are comparable to those of patients with de novo AML. Overall, 85% of all patients obtain complete remission after intensive chemotherapy [29]. In the present study, 1 patient had follicular lymphoma treated with an alkylating agent, and t-AML occurred 14 months after finishing lymphoma treatment. The other t-AML patient had received chemotherapy including topoisomerase II inhibitors for follicular lymphoma and developed t-AML 10 months after completing treatment. Complex karyotype, reportedly the strongest prognostic indicator, predicts a poor prognosis for t-AML patients. One study has reported that the t-AML M3 phenotype has a good prognosis, but overall survival is significantly shorter in t-AML with a complex karyotype [30].

The present study shows that evaluations of the percentage of circulating blasts and BM eosinophils, careful comparison of initial and follow-up cytogenetics studies including FISH results, and karyotyping of *BCR/ABL1* yield valuable information for characterizing hematologic malignancies with t(9;22) and inv(16) coexistence. The results also show that patients with CML-BP classified as double positive as well as patients with AML have favorable clinical courses in this era of targeted therapy. Future studies with larger numbers of patients, longer follow-up periods, and additional genetic evaluations are warranted to confirm and extend the results presented herein.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Swerdlow SH, Campo E, Harris NL, et al, eds. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon, France: IARC, 2008.

2. Patel BB, Mohamed AN, Schiffer CA. "Acute myelogenous leukemia like" translocations in CML blast crisis: two new cases of inv(16)/t(16;16) and a review of the literature. Leuk Res 2006;30:225-32.
3. Tirado CA, Valdez F, Klesse L, et al. Acute myeloid leukemia with inv(16) with CBFB-MYH11, 3'CBFB deletion, variant t(9;22) with BCR-ABL1, and del(7)(q22q32) in a pediatric patient: case report and literature review. Cancer Genet Cytogenet 2010;200:54-9.
4. Roth CG, Contis L, Gupta S, Agha M, Safyan E. De novo acute myeloid leukemia with Philadelphia chromosome (BCR-ABL) and inversion 16 (CBFB-MYH11): report of two cases and review of the literature. Leuk Lymphoma 2011;52:531-5.
5. Wu Y, Slovak ML, Snyder DS, Arber DA. Coexistence of inversion 16 and the Philadelphia chromosome in acute and chronic myeloid leukemias: report of six cases and review of literature. Am J Clin Pathol 2006;125:260-6.
6. Kim M, Lim J, Kim Y, et al. A case of therapy-related acute myeloid leukemia associated with inv(16), with subsequent development of t(9;22). Leukemia 2006;20:746-8.
7. Willatt L, Morgan S, Shaffer LG, Slovak ML, Campbell LJ (2009): ISCN 2009 an international system for human cytogenetic nomenclature. Hum Genet 2009;126:603-4.
8. Ninomiya S, Kanemura N, Tsurumi H, et al. Coexistence of inversion 16 and the Philadelphia chromosome comprising P190 BCR/ABL in chronic myeloid leukemia blast crisis. Int J Hematol 2011;93:806-10.
9. Merzianu M, Medeiros LJ, Cortes J, et al. inv(16)(p13q22) in chronic myelogenous leukemia in blast phase: a clinicopathologic, cytogenetic, and molecular study of five cases. Am J Clin Pathol 2005;124:807-14.
10. Asou N, Sanada I, Tanaka K, et al. Inversion of chromosome 16 and bone marrow eosinophilia in a myelomonocytic transformation of chronic myeloid leukemia. Cancer Genet Cytogenet 1992;61:197-200.
11. Heim S, Christensen BE, Fioretos T, Sorensen AG, Pedersen NT. Acute myelomonocytic leukemia with inv(16)(p13q22) complicating Philadelphia chromosome positive chronic myeloid leukemia. Cancer Genet Cytogenet 1992;59:35-8.
12. Myint H, Ross FM, Hall JL, Hamblin TJ. Early transformation to acute myeloblastic leukaemia with the acquisition of inv(16) in Ph positive chronic granulocytic leukaemia. Leuk Res 1997;21:473-4.
13. Secker-Walker LM, Morgan GJ, Min T, et al. Inversion of chromosome 16 with the Philadelphia chromosome in acute myelomonocytic leukemia with eosinophilia. Report of two cases. Cancer Genet Cytogenet 1992;58:29-34.
14. Preudhomme C, Lai JL, Plantier I, Demory JL, Zandecki M, Fenaux P. Cytogenetic and molecular remission in a case of acute myeloid leukaemia(AML) with inversion of chromosome 16 (Inv(16)) and Philadelphia chromosome (Ph). Br J Haematol 1992;82:623-6.
15. Miura I, Takatsu H, Yamaguchi A, et al. Standard Ph chromosome, t(9;22)(q34;q11), as an additional change in a patient with acute myelomonocytic leukemia (M4Eo) associated with inv(16)(p13q22). Am J Hematol 1994;45:94-6.
16. Svaldi M, Lanthaler A, Venturi R, Coser P, Mitterer M. Simultaneous occurrence of bcr-abl and inv16 in a case of M1 acute myeloid leukemia. Leukemia 2001;15:695.
17. Siddiqui AD, Sheikh ZS, Liu D, Seiter K. Coexistence of inversion 16 and the Philadelphia chromosome in patients with acute myelogenous leukemia. Leuk Lymphoma 2002;43:1137-40.
18. Cividin M, Brizard F, Sorel N, Renaud M, Guilhot F, Brizard A. p190(BCR-ABL) rearrangement as a secondary change in a case of acute myelo-monocytic leukemia with inv(16)(p13q22). Leuk Res 2004;28:97-9.
19. Bacher U, Haferlach T, Alpermann T, et al. Subclones with the t(9;22)/BCR-ABL1 rearrangement occur in AML and seem to cooperate with distinct genetic alterations. Br J Haematol 2011;152:713-20.
20. Malagola M, Breccia M, Skert C, et al. Long term outcome of Ph+ CML patients achieving complete cytogenetic remission with interferon based therapy moving from interferon to imatinib era. Am J Hematol 2014;89:119-24.
21. Dai HP, Xue YQ, Wu LL, et al. p210 BCR/ABL1 as a secondary change in a patient with acute myelomonocytic leukemia (M4Eo) with inv(16). Int J Hematol 2012;96:814-7.
22. Bustamante D, Chan KR, Czuchlewski DR, Saadi AA. Patterns of BCR breakpoints in patients with coexisting inv(16)(p13.1q22) and t(9;22)(q34;q11.2). Int J Hematol 2012;95:324-6.
23. Schnittger S, Bacher U, Haferlach C, Kern W, Haferlach T. Rare CBFB-MYH11 fusion transcripts in AML with inv(16)/t(16;16) are associated with therapy-related AML M4eo, atypical cytomorphology, atypical immunophenotype, atypical additional chromosomal rearrangements and low white blood cell count: a study on 162 patients. Leukemia 2007;21:725-31.
24. Nacheva EP, Grace CD, Brazma D, et al. Does BCR/ABL1 positive acute myeloid leukaemia exist? Br J Haematol 2013;161:541-50.
25. Goyama S, Mulloy JC. Molecular pathogenesis of core binding factor leukemia: current knowledge and future prospects. Int J Hematol 2011;94:126-33.
26. Rucker FG, Schlenk RF, Bullinger L, et al. TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome. Blood 2012;119:2114-21.
27. Palandri F, Castagnetti F, Testoni N, et al. Chronic myeloid leukemia in blast crisis treated with imatinib 600 mg: outcome of the patients alive after a 6-year follow-up. Haematologica 2008;93:1792-6.
28. Sun J, Wang Z, Luo Y, Tan Y, Allan DS, Huang H. Prolonged survival with imatinib mesylate combined with chemotherapy and allogeneic stem cell transplantation in de novo Ph+ acute myeloid leukemia. Acta Haematol 2012;127:143-8.
29. Andersen MK, Larson RA, Mauritzson N, Schnittger S, Jhanwar SC, Pedersen-Bjergaard J. Balanced chromosome abnormalities inv(16) and t(15;17) in therapy-related myelodysplastic syndromes and acute leukemia: report from an International Workshop. Genes Chromosomes Cancer 2002;33:395-400.
30. Park SH, Chi HS, Cho YU, Jang S, Park CJ. Evaluation of prognostic factors in patients with therapy-related acute myeloid leukemia. Blood Res 2013;48:185-92.