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Myeloid neoplasms with concurrent *BCR-ABL1* and *CBFB* rearrangements: A series of 10 cases of a clinically aggressive neoplasm

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Abstract

Chronic myeloid leukemia (CML) is defined by the presence of t(9;22)(q34;q11.2)/*BCR-ABL1*. Additional chromosomal abnormalities confer an adverse prognosis and are particularly common in the blast phase of CML (CML-BP). *CBFB* rearrangement, particularly *CBFB-MYH11* fusion resulting from inv(16)(p13.1q22) or t(16;16)(p13.1;q22), is an acute myeloid leukemia (AML)-defining alteration that is associated with a favorable outcome. The co-occurrence of *BCR-ABL1* and *CBFB* rearrangement is extremely rare, and the significance of this finding remains unclear. We identified 10 patients with myeloid neoplasms harboring *BCR-ABL1* and *CBFB* rearrangement. The study group included six men and four women with a median age of 51 years (range, 20–71 years). The sequence of molecular alterations could be determined in nine cases: *BCR-ABL1* preceded *CBFB* rearrangement in seven, *CBFB* rearrangement preceded *BCR-ABL1* in one, and both alterations were discovered simultaneously in one patient. BCR-ABL1 encoded for p210 kD in all cases in which *BCR-ABL1* preceded *CBFB* rearrangement; a p190 kD was identified in the other three cases. Two patients were treated with the FLAG-IDA regimen (fludarabine, cytarabine, idarubicin, and G-CSF) and tyrosine kinase inhibitors (TKI); seven with other cytarabine-based regimens and TKIs, and one with ponatinib alone. At last follow up (median, 16 months; range 2–85), 7 of 10 patients had died. The co-existence of *BCR-ABL1* and *CBFB* rearrangement is associated with poor outcome and a clinical course similar to that of CML-BP, and unlike *de novo* AML with *CBFB* rearrangement, suggesting that high-intensity chemotherapy with TKI should be considered in these patients.

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CONFLICT OF INTEREST DISCLOSURE

The authors have no relevant conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

Design and conception: JDK; Data gathering and analysis: AS, LJM, GT, SL, JDK; Manuscript preparation: AS, LJM, GT, SL, WW, JDK. All authors have read and approved the final version of this manuscript.

1 | INTRODUCTION

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm that arises from a clonal pluripotent bone marrow (BM) stem cell. CML is defined by the presence of *BCR-ABL1* fusion resulting from a reciprocal translocation between chromosomes 9 and 22, t(9;22)(q34;q11.2) that creates a minute derivative chromosome 22, also known as the Philadelphia (Ph) chromosome.[1] The translocation is also detected in a subset of B-cell lymphoblastic leukemia (B-ALL) and less commonly in *de novo* AML.[2,3] The most common form of *BCR-ABL1* fusion (b2a2 or b3a2) in CML results in a 210 kDa product, whereas in B-ALL the main fusion form (e1a2) results in the 190 kDa product.[4] The BCR-ABL1 fusion protein is a constitutively activated receptor tyrosine kinase that results in dysregulated growth and cell replication through the activation of downstream effectors such as RAS, RAF, MYC, and JAK/STAT.[4]

CML is further divided into three phases: chronic phase (CP), accelerated phase (AP), and blast phase (BP) based on the presence of persistent or increasing WBC ($>10\times10^9$/L), splenomegaly, thrombocytosis or thrombocytopenia; clonal cytogenetic evolution; 20% or more basophils in the peripheral blood; the number of myeloblasts in the BM or extramedullary tissues; and response to tyrosine receptor kinase inhibitors (TKI).[1,4] The 10-year survival of patients with CML has increased dramatically in the era of targeted therapy, approaching 80%–90%.[5]

The occurrence of additional cytogenetic alterations other than t (9;22) is observed in up to 80% of cases of CML-BP.[6–12] The most common additional cytogenetic abnormalities include trisomy 8, an extra copy of the Ph chromosome, 3q26 rearrangements, monosomy 7/del(7q), i(17)(q10), trisomy 21, minus Y, and trisomy 19.[6,7]

CBFB rearrangement, particularly *CBFB-MYH11* fusion, resulting from inv(16)(p13.1q22) or less commonly t(16;16)(p13.1;q22), is an acute myeloid leukemia (AML)-defining alteration that is associated with a favorable outcome.[13–15] CBFB is a member of the core binding factor (CBF) family of transcription factors and stabilizes the interaction of the α subunits RUNX1, RUNX2, and RUNX3 with DNA. RUNX1 regulates hematopoietic stem cell self-renewal, survival, and differentiation of B-cells, T-cells, and megakaryocytes. The fusion product encodes the protein CBFB-SMMHC which is thought to be necessary but insufficient for the development of AML. The fusion protein induces defective hematopoietic differentiation; however, usually additional genetic alterations, mostly mutations, are needed for fully developed leukemogenesis.[16] CBFB-SMMHC induces a dominant negative effect on wild-type CBFB via its more potent binding ability to RUNX, thereby repressing RUNX1. More recently, it has been suggested that the CBFB-SMMHC fusion protein cooperates with RUNX1 to act as a transcription activator and induce differential gene expression.[16] Because of the variability of the genomic breakpoints in *CBFB* and *MYH11* over 10 fusion products of different sizes have been described. The most common form is type A, occurring in more than 85% of cases; type D and E are seen in up to 5%–10% of cases and other fusion forms have been reported in isolated cases.[17]

The co-occurrence of *BCR-ABL1* fusion and *CBFB* rearrangement is extremely rare and its clinical significance remains largely unknown.[18–21] Since therapeutic approaches to neoplasms harboring these potent oncogenic fusion products are different, the co-occurrence of *BCR-ABL1* fusion and *CBFB* rearrangement might pose a clinical management challenge. Herein, we describe a series of patients with myeloid neoplasms harboring *BCR-ABL1* fusion and *CBFB* rearrangement and provide detailed clinicopathologic details, genotype-phenotype correlation, and outcome data.

2 | METHODS

2.1 | Patients and study design

We identified retrospectively 10 patients with AML carrying both *BCR-ABL1* and *CBFB* rearrangement seen and treated at The University of Texas MD Anderson Cancer Center (UTMDACC). These patients included a subset with a well-documented antecedent CML in chronic phase and another group that harbored both alterations at the time of initial diagnosis. Clinical and laboratory data were obtained by electronic chart review. This study was approved by the Institutional Review Board of UTMDACC and was conducted in accordance with the declaration of Helsinki.

2.2 | Morphologic evaluation

All diagnostic BM samples were reviewed. BM cellularity was assessed relative to age according to the EUMNET criteria.[22] BM blast, eosinophil, and monocyte percentages were enumerated by a 500-cell count using Wright-Giemsa-stained aspirate smears and/or touch imprints.

2.3 | Flow cytometry immunophenotyping

Multiparameter flow cytometry was performed on BM samples using a standard stain-lyse-wash procedure with ammonium chloride lysis and the FACSCanto II cytometer and FACSDiva software (BD Biosciences). Data were analyzed using FCS Express (De Novo Software, Glendale, CA). The following antibodies were used in various combinations: CD2, sCD3, cytoCD3, CD4, CD5, CD7, CD13, CD14, CD15, CD19, CD22, CD25, CD33, CD34, CD36, CD38, CD41, CD45, CD56, CD64, CD117, CD123, HLA-DR, MPO, and TDT.

2.4 | Cytogenetic analysis

Conventional cytogenetic analysis was performed on unstimulated cultured BM aspirate specimens using standard GTG-banding as described previously.[23] At least 20 metaphases were analyzed. Results were reported using the 2013 International System for Human Cytogenetic Nomenclature (ISCN).[24]

2.5 | Fluorescence in situ hybridization for BCR-ABL1 and CBFB rearrangement

Fluorescence *in situ* hybridization (FISH) analysis for *BCR-ABL1* and *CBFB* rearrangement was performed on cultured BM cells or G-banded slides with LSI BCR-ABL1 ES fusion probes or LSI CBFB dual-color breakapart probes (Abbott Molecular/Vysis, Des Plaines,

IL) using previously described methods.[18] A total of 200 interphases were analyzed. The positive cutoff value of 2.0% for *BCR/ABL1* rearrangement and 4.2% for *CBFB* rearrangement has been established in our laboratory.

2.6 | Quantitative reverse transcription PCR for BCR-ABL1 and CBFB-MYH11

Reverse transcription quantitative polymerase chain reaction (Q-PCR) for detection of *BCR-ABL1* and *CBFB-MYH11* was performed using RNA extracted from BM or PB samples according to methods described previously.[25,26] Briefly, the *BCR-ABL1* Q-PCR is a multiplex assay designed to detect the e1a2, e13a2 (b2a2), and e14a2 (b3a2) transcripts in a single tube. *BCR-ABL1* and *ABL1* transcript levels were detected simultaneously and quantitative results were expressed as the percent ratio of *BCR-ABL1* to *ABL1*. The specific fusion transcripts were distinguished using capillary electrophoretic separation of the fluorochrome-labeled products. The *CBFB-MYH11* assay is designed to detect type A *CBFB-MYH11* fusion transcript.[26] The *CBFB-MYH11* was also normalized to *ABL1* transcript levels for quantification. The sensitivity of detection of *BCR-ABL1* and *CBFB-MYH11* transcripts was between 1 in 10,000 and 1 in 100,000.

2.7 | Mutation analysis

Mutation analysis was performed using DNA extracted from BM aspirate samples in a subset of patients using the following techniques: Next-generation sequencing-based mutation analysis of exonic regions of *ABL1, EGFR, GATA2, IKZF2, MDM2, NOTCH1, RUNX1, ASXL1, EZH2, HRAS, JAK2, MLL, NPM1, TET2, BRAF, IDH1, KIT, NRAS, TP53, DNMT3A, GATA1, IDH2, KRAS, MYD88, PTPN11,* and *WT1* was performed using the Illumina MiSeq (Illumina, San Diego, CA) sequencer as described previously.[27] *FLT3* (internal tandem duplication and D835) and *NPM1* (exon12, codons 956–971) mutations were assessed by polymerase chain reaction (PCR) followed by capillary electrophoresis on a Genetic Analyzer (Applied Biosystems, Foster City, CA), as described previously.[28] In some cases, mutations in *NRAS* and *KRAS* (codons 12, 13, and 61), and *KIT* exon 17 (codons 778 to 838) were analyzed using pyrosequencing (Biotage, Uppsala, Sweden). PCR-based cDNA sequencing of BCR-ABL1 was performed to detect mutations in codons 221 to 500 of the *ABL1* kinase domain, including codon 315.

3 | RESULTS

3.1 | Clinical and laboratory findings

The salient clinical features for the 10 patients are summarized in Table 1. There were six (60%) men and four (40%) women with a median age of 51 years (range, 20–71 years) at diagnosis. Patients were classified into three groups based on the sequence of genetic alterations: those who presented with *BCR-ABL1* first and later acquired *CBFB* rearrangement (n =7); those who presented with simultaneous *BCR-ABL1* and *CBFB* rearrangement (n =1); and those who initially presented with *CBFB* rearrangement and later acquired *BCR-ABL1* (n =1). For one patient, the sequence of events is unknown. Accordingly, the first group represented patients who had CML with progression to blast phase at the time of acquiring *CBFB* rearrangement, whereas the other two groups represented patients who, by definition, had AML. The median interval from CML diagnosis

to acquiring *CBFB* rearrangement was 11 months (range, 5–43) in patients who originally presented with CML in chronic phase.

At presentation, all patients had anemia (range, 7–13.8 g/dL; normal range, 12–16 g/dL for women and 14–18 g/dL for men), five patients had leukocytosis (range, 21.3–362.7×10^3/ul; reference range, 4.0–11.0 ×10^3/ul), and four patients had leukopenia (range, 1.9–3.1 ×10^3/ul). Eight patients had thrombocytopenia (range, 12–73 ×10^3/ul; normal range, 140–440×10^3/ul) at presentation.

3.2 | Morphologic features

The median BM cellularity was 90% (range, 30–100%). The median BM blast and eosinophil percentages were 26% (range, 20–87%) and 8% (range, 2–30%), respectively, at the time of *BCR-ABL1* and *CBFB* rearrangement co-occurrence. The median BM monocyte percentage was 3% (range, 0–26%). Morphologically abnormal eosinophils and eosinophilic precursors with immature eosinophilic granules were identifiable in 6 of 10 cases. (Figures 1 and 2)

3.3 | Flow cytometry immunophenotyping results

Aberrant myeloid blasts were identified in all cases (n =9) with available data. The most common alterations included increased expression of CD13 and decreased expression of CD33, CD38, and HLA-DR. Flow cytometric immunophenotyping details for each case are provided in Table 2.

3.4 | Cytogenetics results

Detailed cytogenetic results at the time of initial presentation and at the time of co-detection of *BCR-ABL1* and *CBFB* rearrangements are provided in Table 3. Case #1 had two clones at initial diagnosis: a clone with inv(16) only as stemline and a clone with both inv(16) and t(9;22) as a sideline (Figure 3A); Case #2 had inv(16) at initial diagnosis, 14 months later the patient developed clonal evolution with acquisition of t(9;22); Case #3 and #4 had only one clone with t(9;22) and inv(16) detected simultaneously; The remaining six cases (cases #5–10) had t(9;22) at initial diagnosis of CML and acquired inv(16) during blast crisis.

FISH analysis using LSI *BCR/ABL1* ES probe and *CBFB* breakapart probe showed equal or similar percentages of nuclei positive for *BCR-ABL1* and *CBFB* rearrangement (Figure 3B, C) in all cases except case #2 who showed *CBFB* rearrangement in 92% of nuclei and *BCR-ABL1* rearrangement in 4.5% of the nuclei. This patient presented with AML with *CBFB* rearrangement and later acquired *BCR-ABL1* as a secondary genetic alteration.

3.5 | Molecular results

A p210 kD BCR-ABL1 product was identified in all cases in which *BCR-ABL1* fusion preceded *CBFB* rearrangement, whereas a p190 kD product was identified in the other three cases. Among six cases analyzed for *ABL1* kinase domain mutations, one case showed two distinct mutations (p.G254E and p.E329G). All evaluated cases were negative for *FLT3* (n =5), *NRAS* and *KRAS* (n =4), and *KIT* (n =3) mutations.

3.6 | Treatment and outcome

Treatment details are provided in Table 1. Briefly, two patients were treated with the FLAG-IDA regimen (fludarabine, cytarabine, idarubicin, and G-CSF) and TKIs; one of which ultimately received an allogeneic stem cell transplant. Seven patients were treated with cytarabine-based regimens and TKIs; in two of these patients treatment was followed by allogeneic stem cell transplant. One patient was treated with ponatinib alone.

Seven of 10 patients were dead at time of last follow-up [median, 16 months; range 2–85 from the time of t(9;22) and inv(16)]. Of the three patients alive, two received FLAG-IDA and TKI; one had CML-BP and another had AML with both alterations discovered simultaneously; the sequence of events is unknown in the third patient. The latter two patients harbored the e1a2 fusion transcript and the former had a b3a2/b2a2 fusion.

4 | DISCUSSION

We describe 10 patients with simultaneous occurrence of *BCR-ABL1* and *CBFB* rearrangement. The co-occurrence of *BCR-ABL1* and *CBFB* rearrangement is an extremely rare event with less than a total of 20 cases reported in the literature.[18,20,29–38] There is a male predominance, and the disease can present in any age, although patients tend to be older than those with AML with inv(16).[13]

These patients can generally be classified into two major groups: those who present with chronic phase CML and progress to CML-BP by means of acquiring inv(16)(p13q22) and those who present with *de novo* AML in which both genetic alterations are discovered simultaneously or, in very rare cases, inv(16) precedes *BCR-ABL1*. Among the patients included in this study, those with an antecedent history of CML carried the p210 kD fusion protein whereas all patients with *de novo* AML with inv(16) and *BCR-ABL1* carried the p190 fusion protein. These results are similar to what has those reported by previous authors[19,21] and suggests that the biology of the two processes is distinct. Although rare patients with CML-BP with inv(16) have been reported to carry the p190 fusion protein, there were no cases in this study group.[21]

Although flow cytometry is not routinely used for follow-up of patients with CML, aberrant myeloid blasts were identified in all cases included in this study suggesting that flow cytometry panels designed for the detection of minimal residual acute myeloid leukemia may be useful in monitoring disease in patients with concurrent inv(16) and *BCR-ABL1*. This approach may be useful in patient where RNA may not be readily available for minimal residual disease detection by PCR.

Among six patients evaluated for the *ABL1* kinase domain mutation, one showed two distinct mutations whereas the other five had wild-type *ABL1*. Of note, the patient with mutated *ABL1* presented initially with AML with inv(16) and had not been treated with tyrosine kinase inhibitors prior to the discovery of the *ABL1* kinase domain mutations. Mutations in *NRAS, KIT* and *FLT3* have been reported to occur in 35% of cases of AML with inv(16).[39,40] All of the cases evaluated in this series were wild type for these three genes, suggesting that the potent leukemogenic effect executed by the simultaneous presence

of both chromosomal alterations precludes the need for additional somatic mutations for survival of the leukemic clone.

Based on their review of the literature, Ninomiya and colleagues suggested that *de novo* AML with *BCR-ABL1* has a favorable prognosis when compared with other acute myeloid leukemias, similar to that of AML with inv(16) alone.[21] Two such patients were included in this cohort; one patient was treated with the FLAG-Ida regimen plus dasatinib and had a favorable clinical course and was in remission 21 months following presentation. The second patient was treated with the 3 +7 regimen and tyrosine kinase inhibitors (details included in Table 1), relapsed 8 months after presentation and succumbed to disease two years following initial diagnosis. The prognosis of patients with CML and inv(16) as a secondary genetic alteration on the other hand seems to be very poor.[21] In fact, six of seven patients included in our series died despite intensive chemotherapy and targeted therapy with tyrosine kinase inhibitors (median OS from the time of inv(16): 14 months, range 4–85 months). One patient was alive and free of disease at the time of last follow-up; this patient was treated with the FLAG-IA regimen plus ponatinib and later received an allogeneic stem cell transplant. Although the number of patients in this series, and in the literature in general is too small to draw a definite conclusion, it appears that patients with concurrent inv(16) and *BCR-ABL1* may benefit from intensive chemotherapy regimens such as the FLAG-Ida plus tyrosine kinase inhibitors.

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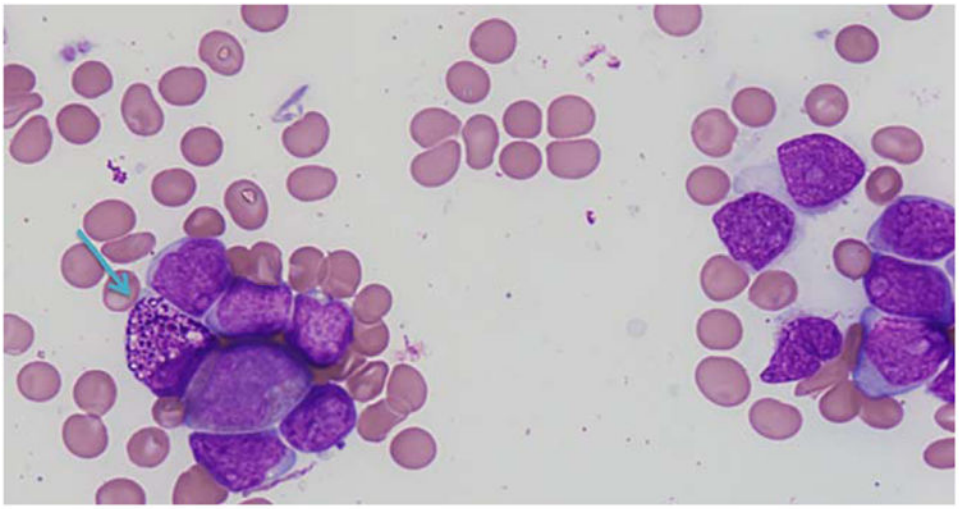

FIGURE 1.

Bone marrow aspirate smear from patient #2 showing increased blasts and an abnormal eosinophilic precursor (blue arrow). (Giemsa stain, 600×) This patient presented with *de novo* AML harboring concurrent *BCR-ABL1* (e1a2) and *CBFB* rearrangement [Color figure can be viewed at wileyonlinelibrary.com]

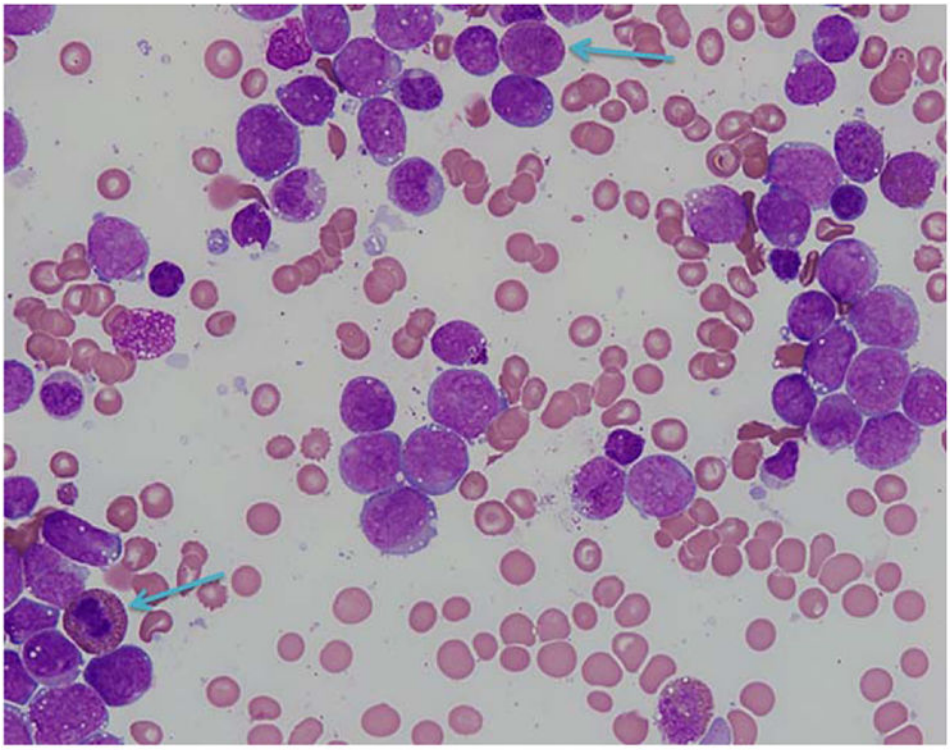

FIGURE 2.

Bone marrow aspirate smear from patient #4 showing increased blasts with monocytic features and occasional eosinophilic precursors (blue arrows). (Giemsa stain, 600×) This patient presented with CML blast phase with *BCR-ABL1* (b3a2 and b2a2) and later acquired *CBFB* rearrangement. The photograph depicts the sample with concurrent *BCR-ABL1* and *CBFB* rearrangement [Color figure can be viewed at wileyonlinelibrary.com]

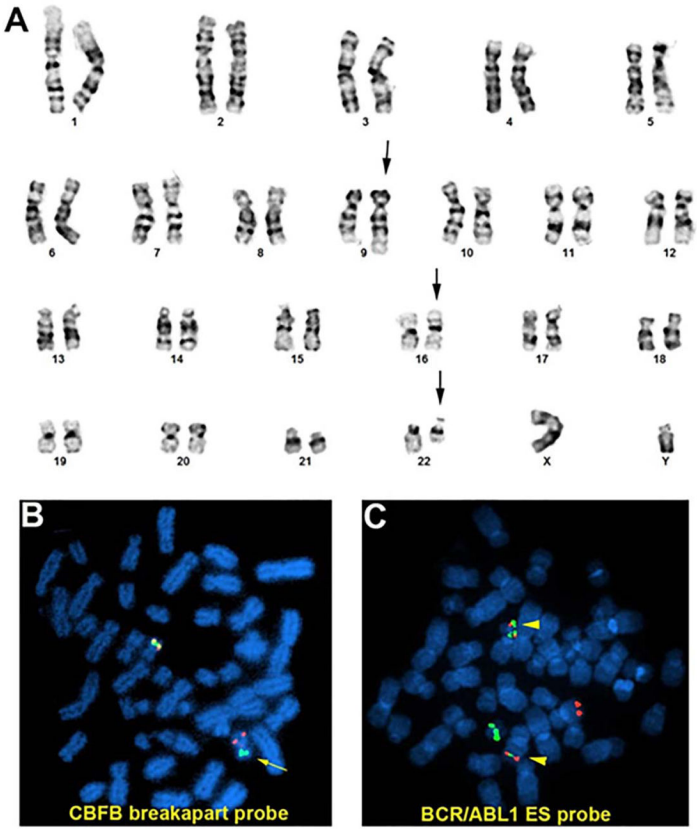

FIGURE 3.

Karyotype and FISH analysis of case #1. **A.** Karyotype of 46,XY,t(9;22)(q34;q11.2),inv(16)(p13q22). **B.** FISH analysis using LSI *CBFB* breakapart probe on a metaphase. Chromosome with break signal (red & green, marked by arrow) indicates *CBFB* rearrangement; **C.** FISH analysis using LSI *BCR/ABL1* ES probe on a metaphase. Two fusion signals (yellow, marked by arrow head) indicate *BCR/ABL1* rearrangement. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1

Summary of clinical features

Patient	Age at initial dx	Sex	Initial dx	Sequence of CG alterations[a]	Time from initial dx to *CBFB* rearrangement (months)	Therapy	Response to therapy	Status at last FU	Interval from *CBFB* rearrangement to relapse	Length of FU from initial diagnosis (months)	Length of FU from t(9:22) and *CBFB* rearrangement (months)
1	71	M	AML	A	0	FLAG-Ida+ dasatinib	Remission	Alive	NA	21	21
2	66	M	AML	C	0	3 +7 and consolidation with high-dose cytarabine; MEK inhibitor, GSK1120212; single agent decitabine	Relapse	Dead	8	24	24
3	55	F	AML	U	0	High dose cytarabine and idarubicin; imatinib+clofarabine and cytarabine; dasatinib	Relapse/persistent	Alive	7[c]	11	2
4	20	F	CML-CP	B	8	Interferon alpha and leukophoresis[b]; imatinib and Hydrea; fludarabine and cytarabine and IT idarubicin and cytarabine; Allo-SCT	Relapse/persistent	Dead	8	27	18
5 (3)	61	F	CML-BP	B	5	Hydrea; imatinib; idarubicin, and cytarabine; cytarabine+ imatinib	Relapse/persistent	Dead	4	11	6
6 (1)	43	F	CML-CP	B	11	Hydrea +; imatinib; cytarabine +fludarabine	Relapse	Dead	3	20	9
7 (2)	48	M	CML-AP	B	30	Hydrea +leukophoresis; imatinib; cytarabine+ idarubicin+etoposide	Persistent	Dead	0	115	85
8 (4)	47	M	CML-CP	B	6	Interferon alpha+ cytarabine +ATRA; allo-SCT	Remission	Dead[d]	NA	20	14
9	54	M	CML-CP	B	26	FLAG-IA+ponatinib; allo-SCT	Remission	Alive	NA	41	27
10	40	M	CML-CP	B	43	Ponatinib	Persistent	Dead	0	47	4

Abbreviations: Allo-SCT, allogeneic stem cell transplant; AML, acute myeloid leukemia; CG, cytogenetic; CML-BP, chronic myelogenous leukemia-blast phase; CML-CP, chronic myelogenous leukemia-chronic phase; dx, diagnosis; F, female; FU, follow up; IT, intrathecal.

[a]Sequence of CG alterations: A: simultaneous *BCR-ABL1* and *CBFB* rearrangement at initial presentation; B: *BCR-ABL1* first *CBFB* rearrangement later; C: *CBFB* rearrangement first *BCR-ABL1* later; U: unknown.

[b]The patient was pregnant at this time.

[c]Time from initial diagnosis.

[d]Because of graft versus host disease.

TABLE 2

Summary of clinical features and immunophenotypic features at the time of *BCR-ABL1* and *CBFB* rearrangement co-occurrence

Case	BM blasts%	Blast immunophenotype	BM eosinophil %	BM monocyte %	Abnormal eosinophils in BM
1	20	CD2dim+, sCD3−, CytoCD3−, CD4partial+, CD5−, CD7−, CD10−, CD13+/increased, CD14−, CD15partial +, CD19−, CD22−, CD33+/decreased, CD34+, CD36−, CD38+/decreased, CD41−, CD45dim+, CD56−, CD64 partial+, CD117+, CD123+/increased, HLA-DR+/decreased, MPO+, TDT dim+.	10	26	Yes
2	62	CD2−, sCD3−, CytoCD3−, CD5−, CD7−, CD10−, CD13+, CD14−, CD15partial +, CD19−, CD33+/decreased, CD34+, CD38+/decreased, CD41−, CD45dim+, CD56−, CD64 −, CD117+, HLA-DR+/decreased, MPO+, TDT −.	10	1	Yes
3[a]	54	CD2−, sCD3−, CytoCD3−, CD5−, CD7−, CD10−, CD13+, CD14−, CD15partial +, CD19−, CD33+, CD34+, CD38+/decreased, CD41−, CD45dim+, CD56−, CD64 −, CD117+, HLA-DR+/decreased, MPO+, TDT −.	4	2	No
4	87	CD7−, CD10−, CD13+, CD14−, CD19−, CD33+, CD34+, CD38+, CD45dim+, CD56−, CD64 partial+, CD117+, HLA-DR+, MPO+, TDT −.	2	0	No
5 (3)	20	CD7−, CD10−, CD13+, CD14−, CD19−, CD33+, CD34 partial+, CD38+, CD45dim+, CD56−, CD64 −, CD117+, HLA-DR+, MPO partial+, TDT −.	5	7	Yes
6 (1)	30	CD7−, CD10−, CD13+, CD14−, CD19−, CD33+, CD34 partial+, CD38+/decreased, CD45dim+, CD56−, CD64 partial+, CD117+, HLA-DR+/decreased, MPO partial+, TDT −.	22	19	Yes
7 (2)	20	N/A	30	9	Yes
8 (4)	40	CD2−, CD7−, CD10−, CD13+, CD19−, CD33+/increased, CD34 small subset+, CD64+, HLA-DR+	11	2	Yes
9[a]	20	CD2−, sCD3−, CytoCD3−, CD4−, CD5−, CD7−, CD10−, CD13+, CD14−, CD15partial +, CD19−, CD22−, CD33+/decreased, CD34+, CD36−, CD38+/decreased, CD41−, CD45dim+, CD56−, CD64−, CD117+, CD123+, HLA-DR+/decreased, MPO+, TDT dim+.	5	16	No
10	21	CD2−, CD5−, CD7−, CD10−, CD13+, CD14−, CD15 partial+, CD19−, CD33+/decreased, CD34 partial+, CD38+, CD45dim+, CD56−, CD64 −, CD117+, HLA-DR+/decreased, MPO partial+, TDT −.	2	0	No

[a] These patients were previously treated with chemotherapy.

[b] Intensities are reported in comparison with normal myeloid blasts.

[c] Patients 5–8 are those previously reported by Merzianu, M et al. (Am J Clin Pathol. 2005 Nov;124(5):807–14.). Designations in that article are provided parenthetically.

TABLE 3

Summary of cytogenetic and molecular features

Case	Karyotype at initial diagnosis	Karyotype at *BCR-ABL1* and *CBFB* rearrangementco-occurrence	% nuclei positive for *BCR-ABL1*:and *CBFB* rearrangement by FISH	BCR-ABL1 fusion transcript	*ABL1* kinase mutation	Other mutations
1	46,XY,inv(16)(p13.1q22)[3] 46,idem,t(9;22)(q34;q11.2)[17]	Same	NA:NA	e1a2	ND	None (next generation sequencing, multiple genes.)
2	46,XY,inv(16)(p13.1q22)	46,XY,inv(16)(p13q22)[2] 48,idem,t(9;22)(q34;q11.2),+13,+22[16] 47~48,idem,+13[cp2]	4.5%:92%	e1a2	p.G254E and p.E329G	None (tested for *FLT3, KRAS, NRAS, NPM1, KIT*)
3	NA	46,XX,t(9;22)(q34;q11.2),inv(16)(p13.1q22)[19] 46,XX[1]	91.5%:81%	e1a2	ND	None (tested for *FLT3, KRAS, NRAS, KIT*)
4	NA	46,XX,t(9;22)(q34;q11.2),inv(16)(p13.1q22)[20]	99.5%:83%	b3a2 and b2a2	None	None (tested for *FLT3, KRAS, NRAS*)
5 (3)	46,XX,t(9;22)(q34;q11.2)[20]	46,XX,t(9;22)(q34;q11.2)[3] 46,XX,idem,inv(16)(p13q22)[7]/47,idem,+8[2] 47,idem,+8,inv(16)(p13q22)[4] 46~47,XX,t(9;22)(q34;q11.2),inv(16)(p13q22)[cp2] /46,XX[2]	59%:57.5%	b3a2 and b2a2	None	None (tested for *FLT3*)
6 (1)	46,XX,t(9;22)(q34;q11.2)	46,XX,t(9;22)(q34;q11.2),inv(16)(p13q22)[20]	91%:NA	b2a2	ND	ND
7 (2)	46,XY,t(9;22)(q34;q11.2)[20]	46,XY,t(9;22)(q34;q11.2),inv(16)(p13q22)[6]/46,XY[14]	NA:NA	b2a2	None	ND
8 (4)	46,XY,t(9;22)(q34;q11.2)[20]	46,XY,t(9;22)(q34;q11),inv(16)(p13q22)[25]	NA:NA	b2a2	ND	ND
9	46,XY,t(9;22)(q34;q11.2)[10]	46,XY,t(9;22)(q34;q11.2),inv(16)(p13.1q22)[8] 47,idem,+der(22)t(9;22)[4] 46,XY[8]	52%:45%	b3a2 and b2a2	None	ND
10	46,XX,t(9;22)(q34;q11.2)	46,X,-Y,+8,t(9;22)(q34;q11.2)[5] 46,idem,inv(16)(p13q22)[7] 47,XY,+Y[2] 46,idem,t(3;18)(p21;q23)[1] 46,XY[5]	41%: 59.5%	b3a2	None	ND

Abbreviations: NA: not available; ND: not done.

+++ Patients 5–8 are those previously reported by Merzianu, M et al. (Am J Clin Pathol. 2005 Nov;124(5):807–14.). Designations in that article are provided parenthetically.