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Cryptic insertion of *CBFB* into *MYH11* leading to a type D fusion in acute myeloid leukemia with normal karyotype

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Dear Editors,

Acute myeloid leukemia (AML) with inv(16)(p13q22)/t(16;16)(p13;q22) has distinct clinicopathologic features, including myelomonocytic leukemic cells, abnormal bone marrow eosinophils, and a relatively favorable prognosis.[1,2] Most cases are classified as AML M4 with abnormal eosinophils (M4Eo) in the French–American–British (FAB) classification. The inv(16)/t(16;16) leads to a fusion gene between the core-binding factor beta subunit (*CBFB*) at 16q22 and myosin heavy chain 11 (*MYH11*) at 16p13. Over 10 types (types A–K) of *CBFB/MYH11* fusion transcripts have been characterized.[3] Furthermore, in addition to inversions and translocations, a few cases of insertions leading to the *CBFB*/*MYH11* fusion gene, namely, insertion of *CBFB* into *MYH11* or insertion of *MYH11* into *CBFB*, have been reported.[4-10] We describe here a rare case of AML with a normal karyotype and type D *CBFB/MYH11* fusion transcript. Fluorescence *in situ* hybridization (FISH) revealed an atypical signal pattern by cryptic insertion.

A 53-year-old man was admitted due to fever and leukocytosis. He had no history of chemotherapy or radiotherapy for malignancies. Peripheral blood showed hemoglobin 52 g/L, platelets 21×10^9/L, and white blood cells 41.0×10^9/L, with 5% neutrophils, 16% lymphocytes, 34% monocytes, and 45% blasts. Bone marrow was hypercellular, with 14.8% monocytes, 5.6% eosinophils, and 74.8% blasts staining positive for myeloperoxidase (Figure 1A, 1B). Flow cytometric analysis revealed blasts positive for CD13 (77.0%), CD33 (81.5%), CD34 (29.7%), and HLA-DR (85.8%).

G-banding analysis of bone marrow cells showed 46,XY[20] (Figure 2A). Reverse transcription polymerase chain reaction (RT–PCR) screening detected a *CBFB/MYH11* fusion transcript, whereas other fusions, including *BCR/ABL1*, *RUNX1/RUNX1T1*, *PML/RARA*, and *DEK/NUP214,* were negative. Furthermore, *NPM1*, *FLT3*-D835, and *KIT* (exons 7 to 17) mutations, and *FLT3*-internal tandem duplication were also negative. To perform RT–PCR

for *CBFB/MYH11*, we created a forward primer, CBFBF (*CBFB* exon 4, 5'-CTCCAAAGACTGGATGGTATGGGC-3', cDNA 605–628 in NCBI NM_001755.3), and reverse primer, MYHR (*MYH11* exon 34, 5'-CTTGGACTTCTCCAGCTCATGG-3', cDNA 4707–4728 in NM_001040113.2). One PCR band of 894 bp was generated in the bone marrow cells of the patient (Figure 1C) and was larger than the type A fusion transcript that is most commonly found. Nucleotide sequencing revealed *CBFB* exon 5 fused with *MYH11* exon 30 indicating a type D fusion transcript since sequences were identical to those of GenBank accession number AF249897.1 (Figure 1D). Namely, rearrangements of *CBFB* and *MYH11* occurred although G-banding analysis showed a normal karyotype but not inv(16)(p13q22)/t(16;16)(p13;q22).

The inv(16)/t(16;16) is a subtle chromosomal rearrangement that may be overlooked by G-banding analysis when metaphase preparations are not optimal.[2] Thus, to confirm the mechanism of the rearrangement, we carried out FISH using a Vysis LSI CBFB Break Apart Rearrangement Probe (Abbott Molecular, Abbott Park, IL) on interphase nuclei. This is the most commonly used probe for detecting inv(16)(p13q22).[8] The expected pattern in a nucleus containing inv(16)(p13q22) is separation into red (5' *CBFB*) and green (3' *CBFB*) signals besides one fused red/green (yellow, 5' and 3' *CBFB*) signal. However, FISH detected an atypical split signal pattern in 83 of 100 interphase cells: two fused yellow signals and one small red signal (Figure 2B). We next performed FISH using an LSI CBFB probe on metaphase spreads. A small red signal was located at 16p13 in addition to two fused red/green (yellow) signals at 16q22 in 18 of 20 metaphase spreads (Figure 2C) indicating only a part of the 5' *CBFB* probe moved to 16p13. That is, this suggests the 16q22 fragment, including *CBFB* exons 1 to 5, was inserted into the *MYH11* intron 29, resulting in the generation of a type D *CBFB/MYH11* fusion gene at 16p13 (Figure 2D). Accordingly, the karyotype was finally interpreted as ins(16)(p13q22q22).

With respect to these laboratory findings, we diagnosed the patient as having AML M4Eo and AML with *CBFB-MYH11* in FAB and World Health Organization classifications, respectively.[2] The patient received induction therapy, including daunorubicin and cytarabine, followed by three courses of high-dose cytarabine and remained in complete remission (CR). However, 16 months after the initial diagnosis, a bone marrow aspiration detected 23.4% blasts. G-banding showed 46,XY[20]. Subsequent RT–PCR showed an increased level of *CBFB/MYH11* fusion transcripts while FISH revealed similar signal patterns to those at initial diagnosis in 18 of 20 metaphase spreads. Consequently, the patient underwent re-induction therapy, three courses of consolidation therapy, and an unrelated allogeneic peripheral blood stem cell transplantation. The patient has been in a second CR for more than 12 months.

An insertion, cryptic or not, is an alternative mechanism of chromosomal rearrangement producing a *CBFB*/*MYH11* fusion gene.[5] To our knowledge, only two other cases of cryptic insertion of *CBFB* into *MYH11* have been reported to date (Table 1).[4,5] The diagnosis for both cases was AML M4Eo. The first case presented with a normal karyotype and type D fusion transcript.[4] FISH using an ON CBFB t(16;16);inv(16) break-apart probe (Kreatech Diagnostics, Amsterdam, The Netherlands) displayed a normal pattern, but FISH with a "homemade" CBFB/16q22 BAC clone on interphase cells yielded two fused red/green signals and an extra small red signal. In metaphase spreads, the extra red signal (3' *CBFB*) was evident at 16p13, indicating a part of *CBFB* was inserted into *MYH11*. An LSI CBFB probe was not used although the expectation was that a clear insertion into a 16p13 signal was produced by this probe. The second case presented with type A fusion and a normal karyotype.[5] FISH using a CBFB/MYH11 Translocation Dual Fusion Probe (Cytocell, Cambridge, UK) showed normal signals, whereas FISH with BAC clones showed co-localization of *CBFB* and *MYH11* signals at 16p13, indicating a possible insertion of *CBFB* into *MYH11*. An

LSI CBFB probe was not used, but it was suspected this insertion could be missed by this probe due to the level of resolution of FISH. In contrast to these two cases, we demonstrated that cryptic insertion of *CBFB* could be detected by FISH using a widely used, commercially available LSI CBFB probe. All three cases showed normal karyotypes, suggesting the rearrangement, ins(16)(p13q22q22), cannot be identified by G-banding analysis.[4]

In comparison, five reported cases exist of AML with a cryptic insertion of *MYH11* into *CBFB*.[5-10] In addition to two cases identified by conventional cytogenetics as ins(16)(q22p13p13), FISH detected the localization of *MYH11* on 16q in all cases. FISH with an LSI CBFB probe was performed in three cases.[8-10] In one case with ins(16)(q22p13p13), 5' and 3' *CBFB* signals were separated probably by an insertion.[9] As suspected from Figure 2D, 5' *CBFB* and 3' *CBFB* signals become separated on ins(16)(q22p13p13) if a large 16p13 fragment containing *MYH11*, which is visible by G-banding, is inserted between *CBFB* exons 5 and 6. However, FISH with an LSI CBFB probe yielded normal results in two cases with normal karyotypes.[8,10] Namely, the cryptic insertion of *MYH11* into *CBFB* might not be detected by an LSI CBFB probe. These findings indicate that knowing the exact location of FISH probes is very important for detecting and interpreting cryptic insertions of *CBFB* or *MYH11*.[4]

Interestingly, two of the three cases with an insertion of *CBFB* into *MYH11* displayed type D fusions, whereas all four cases examined with an insertion of *MYH11* into *CBFB* showed type A fusions. In AML with inv(16)/t(16;16), more than 85% of fusions are type A, and two fusions (types D and E) are approximately 5% each.[1,3] It has been shown that non-type A fusions were correlated with distinct clinical and genetic findings, such as an absence of *KIT* mutations and a distinctive gene expression profile.[3] The present case with a type D fusion also did not show a *KIT* mutation. Considering the low frequency of a type D fusion in all AML with inv(16)/t(16;16), the cryptic insertion of *CBFB* into *MYH11* might be associated

with a type D fusion.

Patients with AML harboring *CBFB/MYH11* achieve a longer CR once they are treated with high-dose cytarabine as consolidation therapy, but those with *KIT* mutations have a higher risk of recurrence and worse survival.[2] *KIT* mutations, not the type of fusion transcript, could affect clinical outcome.[3] That is, overall survival was similar between non-type A patients and type A patients without *KIT* mutation. Our patient was treated with high-dose cytarabine and showed a first CR of relatively short duration although the *KIT* mutation was negative. It might be that a cryptic insertion was associated with early relapse. In any case, it is critical not to miss a *CBFB/MYH11* fusion since this is a prognostic marker. This case highlighted the importance of FISH with an LSI CBFB probe and screening by RT–PCR to detect *CBFB/MYH11* in AML with a normal karyotype.

Key words

FISH, *CBFB*, *MYH11*, acute leukemias, cryptic insertion

Conflict of interest

The authors declare no conflicts of interest.

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None.

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Figure legends

Figure 1

(A) Bone marrow smear shows myeloblasts, monocytes, and eosinophils (May–Grünwald–Giemsa staining, ×1000). An immature eosinophil contains cyto-plasmic granules that are purple-violet in color (black arrow).

(B) Bone marrow smear shows myeloblasts and monocytes staining positive for myeloperoxidase (×1000).

(C) RT–PCR for a *CBFB/MYH11* fusion transcript. Lane M, DNA of a 100-bp ladder as markers; lane 1, positive control (type A fusion); lane 2, the patient's bone marrow cells; and lane 3, negative control (water). A PCR product of 894 bp, larger than type A (174 bp), was generated in the patient's cells.

(D) Nucleotide sequences that surround the junction of the PCR product. *CBFB* exon 5 is fused with *MYH11* exon 30, indicating a type D *CBFB/MYH11* fusion transcript. The vertical arrow points the breakpoint.

Figure 2

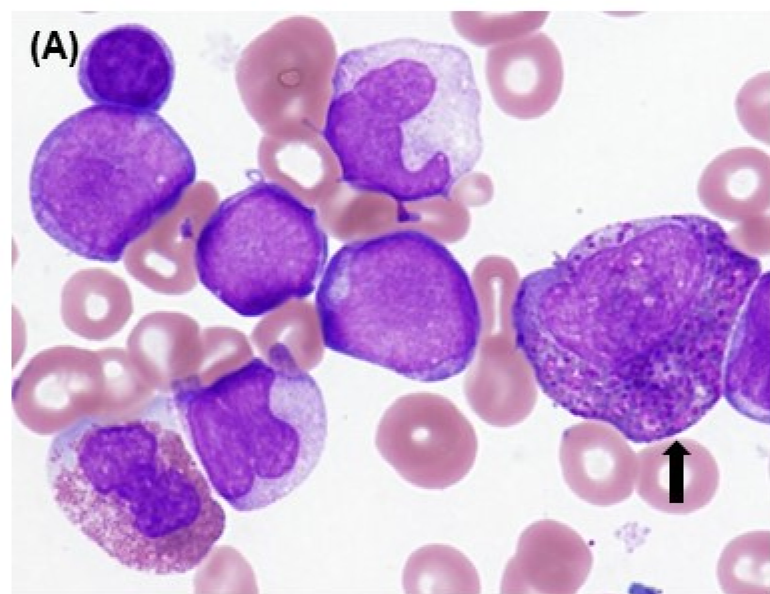
(A) G-banding of bone marrow cells shows a normal karyotype: 46,XY.

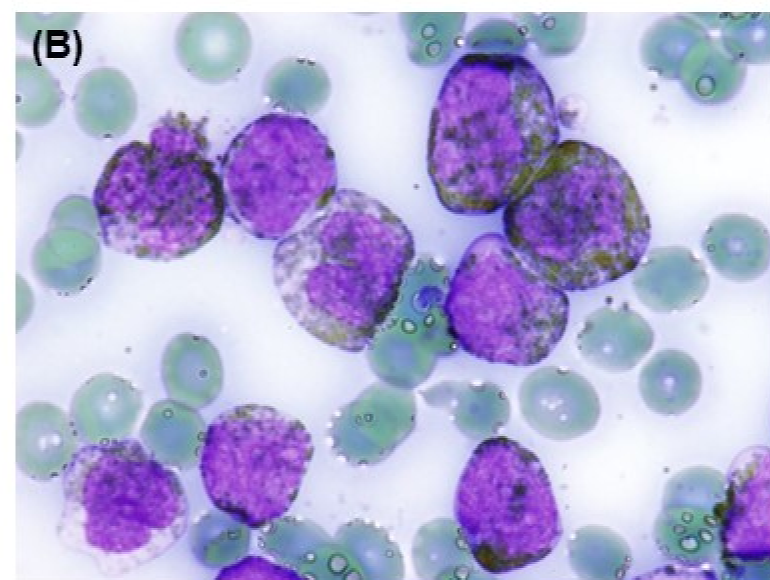
(B) Fluorescence *in situ* hybridization (FISH) using a Vysis LSI CBFB Break Apart Rearrangement Probe (Abbott Molecular, Abbott Park, IL, USA) on interphase cells. One small red (5' *CBFB*) signal and two yellow (red and green, 5' *CBFB* and 3' *CBFB*) fusion signals are observed.

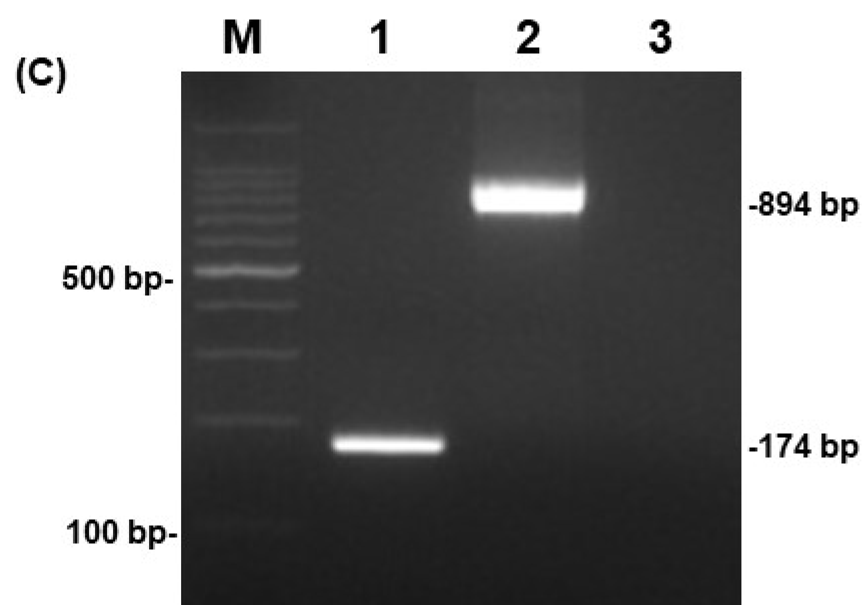
(C) FISH using a Vysis LSI CBFB Break Apart Rearrangement Probe on metaphase cells. Arrows show 1) 5' and 3' *CBFB* fusion signals (red/green, yellow) on a nor-mal chromosome 16; and 2) an inserted 5' *CBFB* signal (small red) at 16p13, and 5' *CBFB* and 3' *CBFB* fusion signals (yellow) at 16q22 on ins(16)(p13q22q22).

(D) Schematic presentation of insertion of *CBFB* into *MYH11*. The positions of two genes and Vysis LSI CBFB probes on normal chromosomes 16 are shown. Presumed breakpoints, namely, the 5' side of *CBFB* exon 1 and 3' side of *CBFB* exon 5, are demonstrated by vertical arrows. The resultant *CBFB/MYH11* fusion gene at 16p13 and corresponding 5' CBFB FISH probe are also shown. FISH results suggest that the 16q22 fragment, including *CBFB* exons 1 to 5, was inserted into the *MYH11* intron 29. Black and white squares show coding exons of *CBFB* and *MYH11* genes, respectively. The resultant FISH signals are shown on the right side.

FIGURE 1







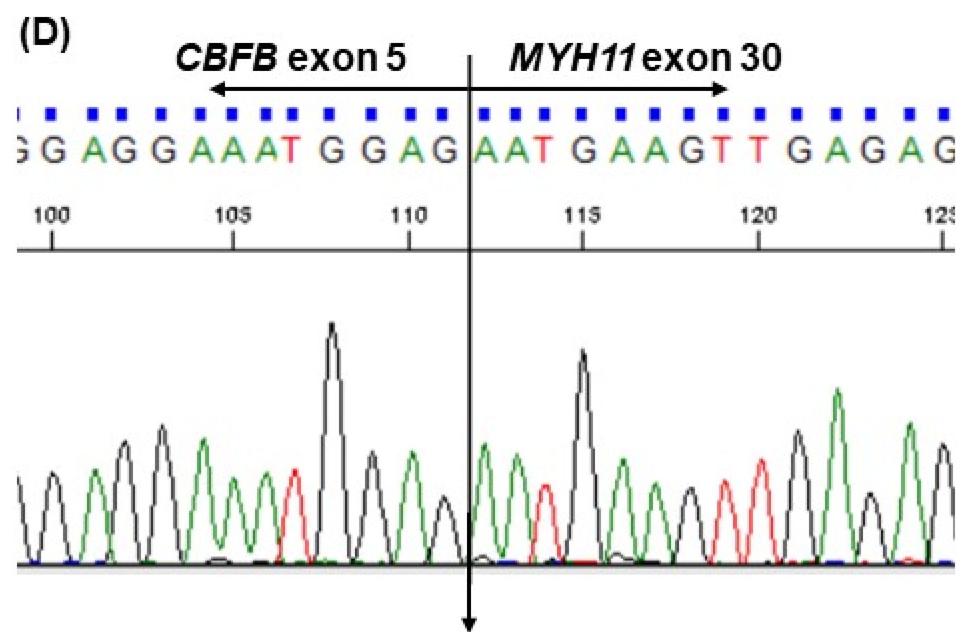
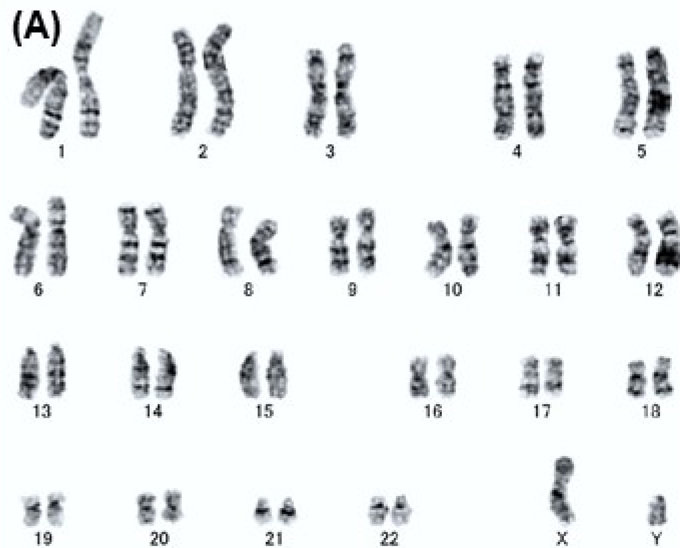
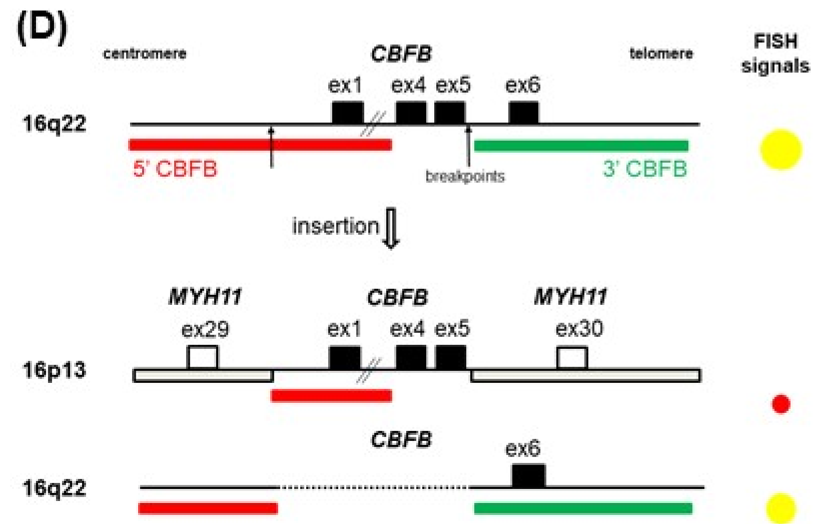
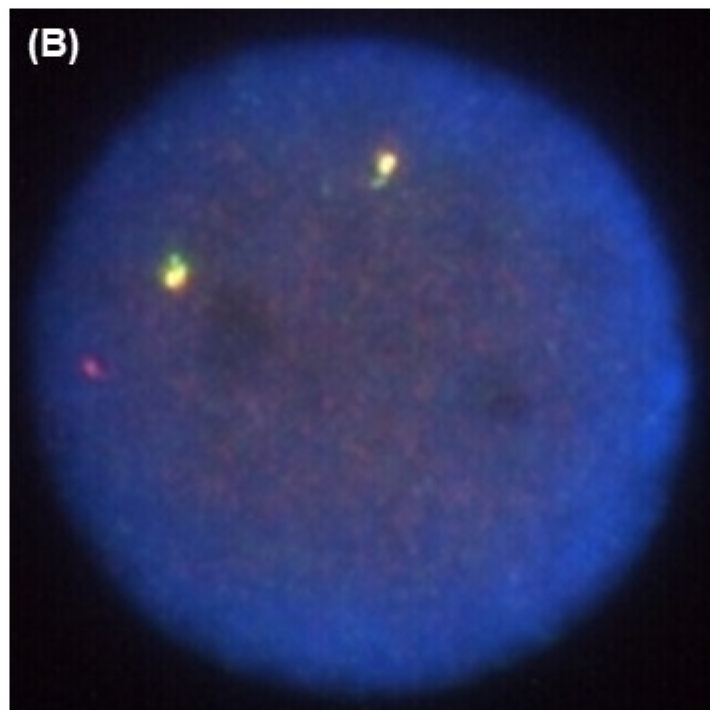


FIGURE 2







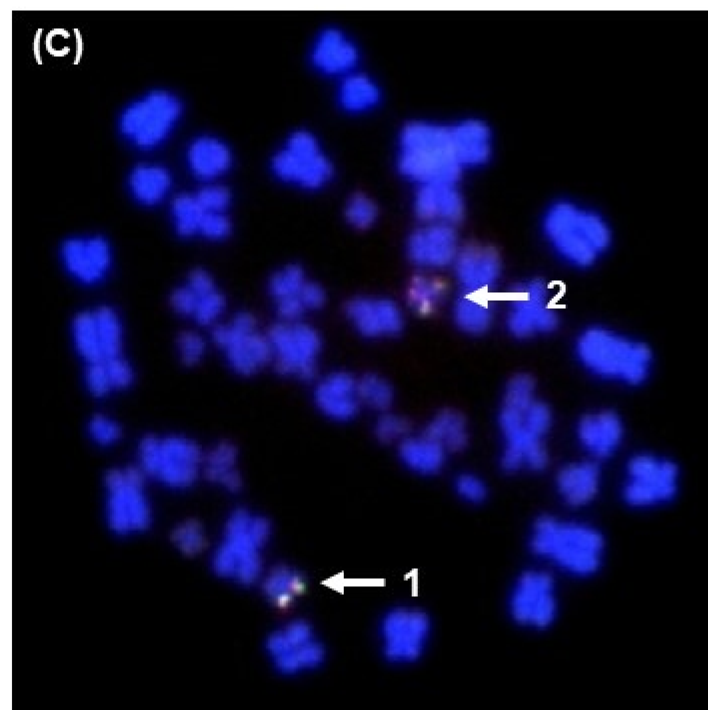


Table 1. Reported cases of hematological malignancies associated with cryptic insertion of *CBFB* into *MYH11*

Case No.	*Age/ Sex*	*Dx*	*Hb (g/L)*	*Plt ($x10^9/L$)*	*WBC ($x10^9/L$)*	*Blasts in PB (%)*	*Blasts in BM (%)*	*Eos in BM (%)*	*Karyotypes*	*CBFB/MYH11 fusion transcript*	*FISH with LSI CBFB probe*	*OS*	*References*
1	56/M	AML M4Eo	100	26	29	74	62	dysplastic	46,XY	type D	ND	NA	4
2	32/F	AML M4Eo	81	16	180	75	70.5	4.5	46,XX[25]	type A	ND	8 mo in 1st CR	5
3	53/M	AML M4Eo	52	21	41	45	74.8	5.6	46,XY[20]	type D	2Y1R	28 mo in 2nd CR	present case

Abbreviations: AML M4Eo, acute myeloid leukemia M4 with eosinophilia; BM, bone marrow; CR, complete remission; Dx, diagnosis; Eos, eosinophils; F, female; FISH, fluorescence *in situ* hybridization; Hb, hemoglobin; M, male; mo, months; NA, not available; ND, not done; OS, overall survival; PB, peripheral blood; Plt, platelets; WBC, white blood cells; 2Y1R, two yellow and one red signal.