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Detection of a novel *CBFB-MYH11* fusion transcript in acute myeloid leukemia M1 with inv(16)(p13q22)

Keiji Kurata[a,*], Katsuya Yamamoto[a], Yoko Okazaki[b], Yoriko Noguchi[b], Keiji Matsui[b], Hisayuki Matsumoto[b], Yumiko Inui[a], Kimikazu Yakushijin[a], Mitsuhiro Ito[a], Yuji Nakamachi[b], Hiroshi Matsuoka[a], Jun Saegusa[b], Hironobu Minami[a]

[a]Department of Medical Oncology and Hematology, Kobe University Hospital, Japan

[b]Department of Clinical Laboratory, Kobe University Hospital, Kobe, Japan

***Corresponding Author**:

Keiji Kurata, MD, PhD

Department of Medical Oncology and Hematology, Kobe University Hospital

7-5-2 Kusunoki-cho, Chuo-ku, Kobe City, 650-0017, Japan

e-mail: kkurata@med.kobe-u.ac.jp

TEL: +81-78-382-5820

FAX: +81-78-382-5821

Abstract

Acute myeloid leukemia (AML) with an inv(16)(p13q22) or t(16;16)(p13;q22) chromosomal abnormality represents one of the most common subtypes of *de novo* cases. These chromosomal rearrangements result in multiple *CBFB-MYH11* fusion transcripts, with type-A being the most frequent. We here describe a unique case of *de novo* AML-M1, with inv(16)(p13q22), leading to an unusual *CBFB-MYH11* fusion transcript, and der(7)t(7;11)(q31;q21). The fusion transcript involves a *CBFB* exon 5 with a breakpoint at nucleotide 756, an insertion of a 13-bp sequence of *CBFB* intron 5 at the fusion point, and the *MYH11* exon 27 with a breakpoint at nucleotide 3466. To our knowledge, this *CBFB-MYH11* fusion transcript has never been reported previously. The clinical characteristics of the present case are in line with previous reports suggesting that rare *CBFB-MYH11* fusion transcripts lead to aberrant characteristics such as an atypical cytomorphology and additional cytogenetic abnormalities.

Key Words: AML, inv(16)(p13q22), CBFB, MYH11, fusion transcript

Introduction

Acute myeloid leukemia (AML) with inv(16)(p13q22) or t(16;16)(p13;q22) accounts for approximately 5–7% of all *de novo* AML cases [1-3]. These chromosomal rearrangements are generally associated with the subtype AML-M4Eo according to the French–American–British (FAB) classification, which is characterized by abnormal myelomonocytic differentiation and an increase of atypical bone marrow eosinophils [4]. The inv(16)/t(16;16) results in fusion of the core-binding factor subunit beta (*CBFB*) gene at 16p22 and the myosin heavy chain 11 (*MYH11*) gene at 16p13, creating a new chimeric gene, *CBFB-MYH11* [5]. To date, at least 12 differently sized *CBFB-MYH11* fusion transcripts have been reported, arising from multiple breakpoints in the *MYH11* gene [6-9]. The majority (85%) of such cases have type A fusion transcripts, followed by type D and E (5% each) [10, 11]. Descriptions of the rare fusion types B, C, and F–K, and an additional type identified by Albano and colleagues, are mostly based on single case reports [6-9]. The resultant CBFB-MYH11 fusion protein interferes with formation of the CBF complex and blocks the differentiation of hematopoietic cells. However, the biological and clinical significance of rare fusion transcripts has not yet been elucidated since they involve scantily reported cases [7, 8].

We here describe a case of *de novo* AML with inv(16)(p13q22) showing a new *CBFB-MYH11* fusion transcript associated with aberrant characteristics.

Case report

A 76-year-old woman was admitted to our hospital owing to general fatigue. She had no previous history of chemotherapy or radiotherapy. The peripheral blood count showed 7.0 g/dL

hemoglobin, 38 × 10^9/L platelets, and 77 × 10^9/L white blood cells with 3% monocytes, 7% lymphocytes, and 90% blasts. The bone marrow aspirate was hypercellular and revealed the presence of 94% myeloperoxidase-positive myeloblasts (Fig. 1). No findings of dysplasia and eosinophilia were observed. Immunophenotypic analysis of the bone marrow showed that the myeloblasts were positive for CD7 (52%), CD13 (60%), CD33 (60%), CD34 (67%), CD11c (25%), and HLA-DR (66%), and were negative for CD2 (5%). Immunohistochemical analysis of the bone marrow revealed a positive pattern for myeloperoxidase, CD34, CD117, and TdT in the blast cells. Accordingly, the diagnosis was determined to be AML without maturation according to the FAB classification (FAB-M1) and AML with inv(16)(p13q22);*CBFB/MYH11* according to the World Health Organization 2016 classification after cytogenetic analysis.

The patient underwent chemotherapy with cytarabine (20 mg/m^2, days 1–14), aclarubicin (14 mg/m^2, days 1–4), and granulocyte colony-stimulating factor (lenograstim; 5 μg/m^2, days 1–14), since high-intensity induction chemotherapy was not feasible due to her age-related frailty. Although she achieved complete hematological remission on day 43 after two cycles of chemotherapy, bone marrow aspiration revealed relapsed AML with 22% blasts on day 63 after starting the second cycle of chemotherapy and she was then transferred to palliative care.

Materials and methods

Chromosome and multicolor spectral karyotype (SKY) analyses

Chromosomal analysis was performed by using the G-banding technique on short-term cultured cells obtained from the bone marrow at diagnosis. SKY analysis was performed on

metaphase spreads of the cells obtained from the bone marrow at diagnosis, using SKY Paint Probes (Applied Spectral Imaging, Inc., Carlsbad, CA [12].

Reverse transcription polymerase chain reaction (RT-PCR), Real-time quantitative PCR (RT-qPCR), and sequencing

Total RNA was extracted from mononuclear cells in the bone marrow at diagnosis using an RNeasy Mini Kit (QIAGEN, Hilden, Germany) and transcribed to cDNA using a SuperScript VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, CA). The primer set was designed based on *CBFB* forward primer [5'- CTCCAAAGACTGGATGGTATGGGC-3', cDNA positions 607–630 in exon 4 (NM_001755.2)] and *MYH11* reverse primer [5'-CCATGAGCTGGAGAAGTCCAAG-3', cDNA positions 4709–4730 in exon 34 (NM_001040113.1)]. PCR was carried out using AmpliTaq Gold 360 Master MIX (Thermo Fisher Scientific, Waltham, MA). The PCR products were analyzed by electrophoresis on a 2.0% agarose gel using the 100 bp DNA ladder (TaKaRa, Shiga, Japan) as a size marker.

Sequencing reactions were carried out using a BigDye XTerminator Purification Kit (Applied Biosystems, Foster City, CA) and the primers mentioned above. The sequences were analyzed on a 3500 Dx Genetic Analyzer (Applied Biosystems, Foster City, CA).

RT-qPCR was performed in the QuantStudio 12K Flex Real-Time PCR System (Applied Biosystems, Foster City, CA) using TaqMan Fast Advanced Master Mix (Applied Biosystems, Foster City, CA). A standard curve was generated with serial dilutions of the PCR product extracted from a plasmid containing the *CBFB-MYH11* fusion transcript. PCR mixtures were denatured for 2 min at 50°C and for 20 sec at 95°C, followed by 40 cycles of denaturation at

95°C for 1 sec, annealing at 60°C for 20 sec, and extension at 95°C for 30 sec. The primer set was designed based on *CBFB* forward primer [cDNA positions 346–369 in exon 4 (NM_001755.2)] and *MYH11* reverse primer [5'-AAAGCAGAAGCGAGACCTCG-3', cDNA positions 3542–3561 in exon 27 (NM_001040113.1)], and the FAM-labeled TaqMan probe was based on *CBFB* [5'-TCTGGAGTTTGATGAGGAGCGAGCCC-3', cDNA positions 633–658 in exon 4 (NM_001755.2)].

Results

G-banding and SKY analysis of bone marrow cells at diagnosis of AML showed the following karyotype: 46,XX,der(7)t(7;11)(q31;q21),inv(16)(p13.1q22)[18]/46,XX[2] (Fig. 1B, 1C).

For further characterization of inv(16), we examined the expression of the *CBFB-MYH11* fusion transcript by RT-PCR. Unexpectedly, a large band of 1428 bp was detected using conventional primers (Fig. 2A). Sequencing reactions of the purified PCR product revealed the fusion *CBFB* exon 5 with a breakpoint at nucleotide 756 (NM_001755.2), insertion of a 13-bp sequence by *CBFB* intron 5 (NM_001755) at the fusion point, and the *MYH11* exon 27 with a breakpoint at nucleotide 3466 (NM_001040113.1) (Fig. 2B). As a consequence of this rearrangement, a fusion gene consisting of about 58 kb from the 5' end of *CBFB* and 34 kb from the 3' end of *MYH11* was generated. Although the fusion gene included 13 inserted nucleotides at the fusion point, it retained intact reading frames. Thus, the fusion gene is presumed to encode a 4264-bp *CBFB-MYH11* fusion mRNA and a CBFB-MYH11 fusion peptide composed of 995 amino acids, which is the largest subtype among previously reported rare *CBFB-MYH11* fusion transcripts.

We also established an RT-qPCR method for monitoring minimal residual disease (MRD) after chemotherapy. The expression level of *CBFB-MYH11* (relative to *GAPDH* × 10^6) was 66,100 at diagnosis, and then decreased to 2,420 and 652 after the first and second cycles of chemotherapy, respectively. The expression level of the fusion gene then increased again to 88,000 on day 63 after the second cycle of chemotherapy coinciding with the hematological relapse. Thus, the expression of *CBFB-MYH11* varied almost in parallel with the disease condition and expression level of Wilms tumor 1 (*WT1*) (Fig. 2C).

Discussion

We identified a novel *CBFB-MYH11* fusion transcript in a patient with *de novo* AML with inv(16)(p13q22). The fusion transcript consists of breakpoints within exon 5 of the *CBFB* gene and exon 27 of the *MYH11* gene, resulting in a novel M-type of the *CBFB-MYH11* fusion transcript. Moreover, a partial insertion of 13 bp derived from intron 5 of the *CBFB* gene was detected. Similarly, insertions of short nucleotides at genomic fusion points have previously been reported in *CBFB-MYH11* variant fusion transcripts with various sequences, including *CBFB* intron 4 or random sequences [9, 13, 14]. One explanation for these observations is incomplete splicing of the *CBFB* intron. Intron retention is a representative process of alternative splicing, by which specific introns remain unspliced in polyadenylated transcripts [15-17]. Intron retention is an important mechanism of gene expression regulation, and intron-retaining transcripts result in functionally distinct protein isoforms [18]. Furthermore, dysregulation of alternative splicing has been associated with cancers, and abnormal expression or mutations in splicing factors are known to contribute to tumorigenesis [19, 20].

Another possible explanation is that these insertions are due to V(D)J recombinase-mediated rearrangement. *CBFB* intron 5 contains various repetitive sequences, leading to genetic instability; the genomic breakpoints are clustered in this region, and several hepta- and nonamer homologous sequences have been found near the breakpoints in *CBFB* intron 5 [15]. Consistently, two heptamers were found within 100 bp up- and downstream of the 13 inserted nucleotides in the present case. Furthermore, two candidates of cryptic recombination signal sequences were identified within 1,000 bp upstream of the insertion [21].

Additional cytogenetic abnormalities are observed in almost half of the AML cases with inv(16)/t(16;16) and are relatively frequent among cases of non-type A inv(16)/t(16;16), including trisomies of chromosomes 8, 21 and 22, and [10, 11, 22]. “Atypical” additional cytogenetic abnormalities, including deletion of chromosome 7q, are particularly more frequent in non-type A cases than in type A cases (29% and 8.7%, respectively), while typical additional cytogenetic abnormalities (+8, +21, and +22) are mainly observed in type A cases (with frequencies of 25.3% and 16.1% in non-type A cases) [10, 11]. In the present case, der(7)t(7;11)(q31;q21) was also detected along with the inv(16)(p13q22), leading to deletion of 7q. Loss of chromosome 7 material, either as complete loss of one chromosome (i.e., -7) or as deletion of its long arm [del(7q)], is one of the most commonly observed chromosomal abnormalities in myeloid malignancies, and 5% of AML cases with inv(16)(p13q22) have del(7q) [23]. Deletions of 7q are variable in length and commonly occur in the region between 7q22 and 7q36 [24]. Although such chromosome 7 abnormalities are considered to be markers for poor prognosis in AML and myelodysplastic syndrome, previous reports suggest that del(7q) has no clinical impact on AML with inv(16)(p13q22) [22, 25-27].

Regarding morphological features, a diagnosis of AML-M1 was made in the present case according to the FAB classification, and there was no eosinophilia in the bone marrow. Although inv(16)/t(16;16) is typically associated with AML-M4Eo, 20–30% of cases are not accompanied by eosinophilia and are ultimately diagnosed as other FAB subtypes of AML [11]. Specifically, *de novo* AML with a non-type A *CBFB-MYH11* fusion tends to not be associated with eosinophilia, and AML-M1 accounts for 1% and 14% of all type A and non-type A cases, respectively [11]. The morphological features observed in the present case may also be associated with the rare type of *CBFB-MYH11* fusion identified. In contrast, the white blood cell count at diagnosis tends to be low in non-type A *CBFB-MYH11* fusion cases, and thus the leukocytosis observed in the present case appears to be an atypical feature associated with this fusion type [11].

At present, the fusion type of the *CBFB-MYH11* transcript is not considered to influence the clinical outcome [11]. In contrast, *KIT* and *FLT3* secondary genetic mutations in AML are considered to be important prognostic factors in cases with inv(16)/t(16;16) [11, 22]. To date, *KIT* gene mutations have only been identified in type-A cases and *FLT3* gene mutations are identified in 17% of all cases with inv(16)/t(16;16) [11, 22]. In the present case, neither *KIT* nor *FLT3* mutations were detected. Further, as more than 100 genes were identified to be differentially expressed among various *CBFB-MYH11* fusion types, the genomic lesions retained or missed through various fusions might affect the expression status of the associated genes and subsequent clinical outcomes [11].

In conclusion, we described a novel *CBFB-MYH11* fusion transcript resulting from a *MYH11* exon 27 breakpoint and 13-bp insertion at the fusion point with atypical cytomorphology

and other aberrant characteristics.

Conflicts of interest:

Dr. Minami reports grants, personal fees and other from Novartis, during the conduct of the study; grants from Asahi-Kasei Pharma, grants from Astellas, other from AstraZeneca, grants, personal fees and other from Bayer, grants and personal fees from Behringer, grants, personal fees and other from Bristol-Myers Squibb, personal fees from Celgene, grants, personal fees and other from Chugai, grants, personal fees and other from DaiichiSankyo, grants and personal fees from DaiNihonSumitomo, grants and personal fees from Eizai, personal fees from Janssen, personal fees from Kowa, grants and personal fees from Kyowa-Kirin, grants and personal fees from Lilly, grants and personal fees from Merck Serono, grants, personal fees and other from MSD, grants from Nihon Shinyaku, grants and personal fees from Nippon Chemiphar, grants and personal fees from Eizai, grants, personal fees and other from Ono Yakuhin, personal fees from Ohtsuka, grants, personal fees and other from Pfizer, grants and personal fees from Sanofi, personal fees from Shire Japan, grants, personal fees and other from Taiho, grants from Taisho-Toyama, grants and personal fees from Takeda, grants from Teijin Pharma, grants from Yakult, personal fees from Genomic Health, grants from CSL Behring, grants from Nihon Kayaku, grants from Shionogi, outside the submitted work;

Other Authors: none

Figure Legends

Figure 1. (A) Bone marrow smear with May-Giemsa stain (left and middle) and myeloperoxidase stain (right). The blast percentage exceeds 90%, and there is no significant maturation of leukemia cells. Leukemia cells are positive for myeloperoxidase staining. Scale bar = 10 μm. (B and C) Chromosome and multicolor spectral karyotype (SKY) analyses. G-banded karyotype and SKY analysis of the bone marrow cells at diagnosis: 46,XX,der(7)t(7;11)(q31;q21),inv(16)(p13.1q22). The arrowhead indicates the rearranged chromosome.

Figure 2. (A) Detection of the *CBFB-MYH11* fusion transcript by RT-PCR analysis. Lane M, 100-bp ladder size marker; lanes 1 and 2, bone marrow cells at diagnosis; lane 3, negative control sample; lane 4, negative control (H_2O); lane 5, positive control (type A *CBFB-MYH11* fusion transcript). PCR products of 1428 bp are amplified in lanes 1 and 2. (B) Schematic representation of the *CBFB* and *MYH11* genes and sequencing analysis of the PCR product. The scheme shows the localization of breakpoints, primer positions, and the fusion gene and cDNA generated in the present case. Sequencing analysis of the PCR product showed a novel *CBFB-MYH11* fusion transcript with distinct breakpoints. (C) Serial RT-qPCR analyses for *CBFB-MYH11* fusion transcripts.

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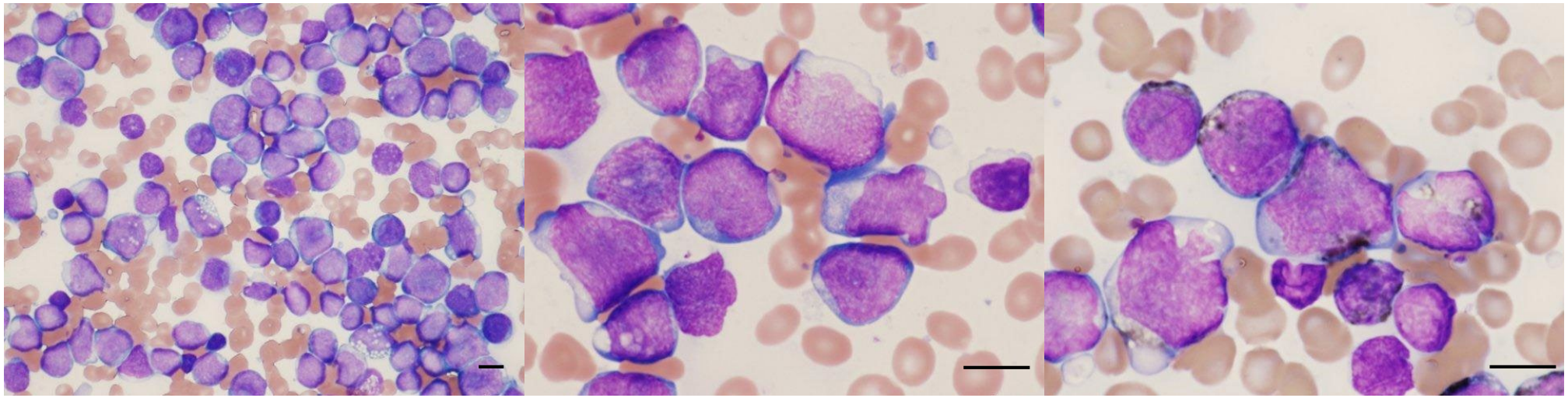

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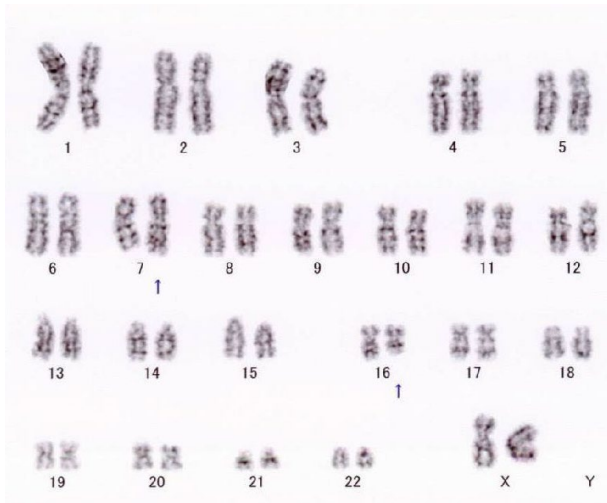
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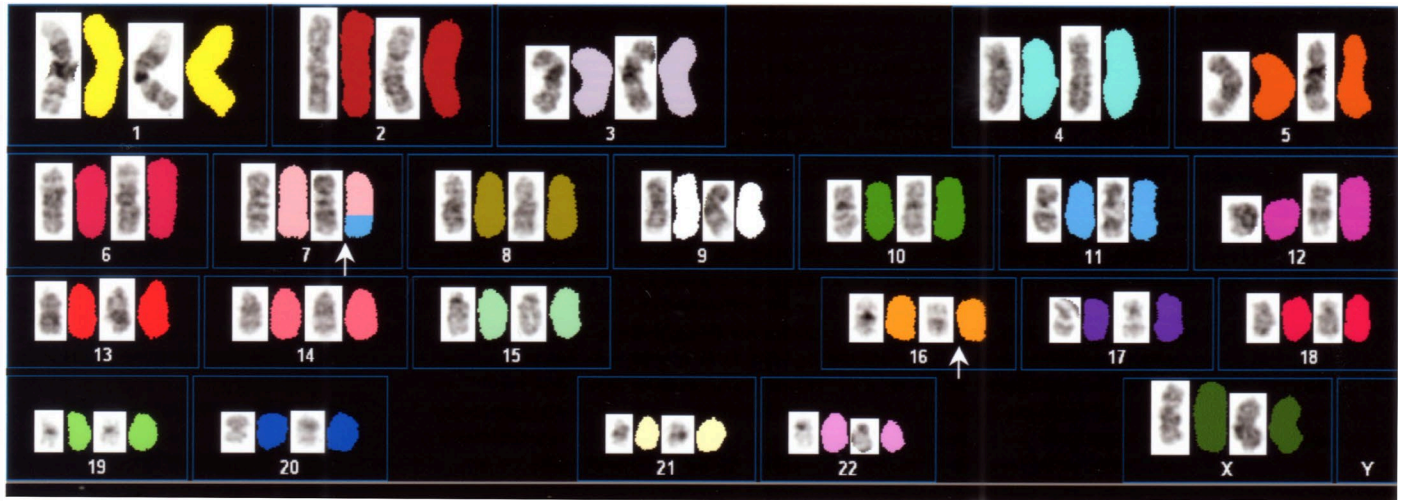
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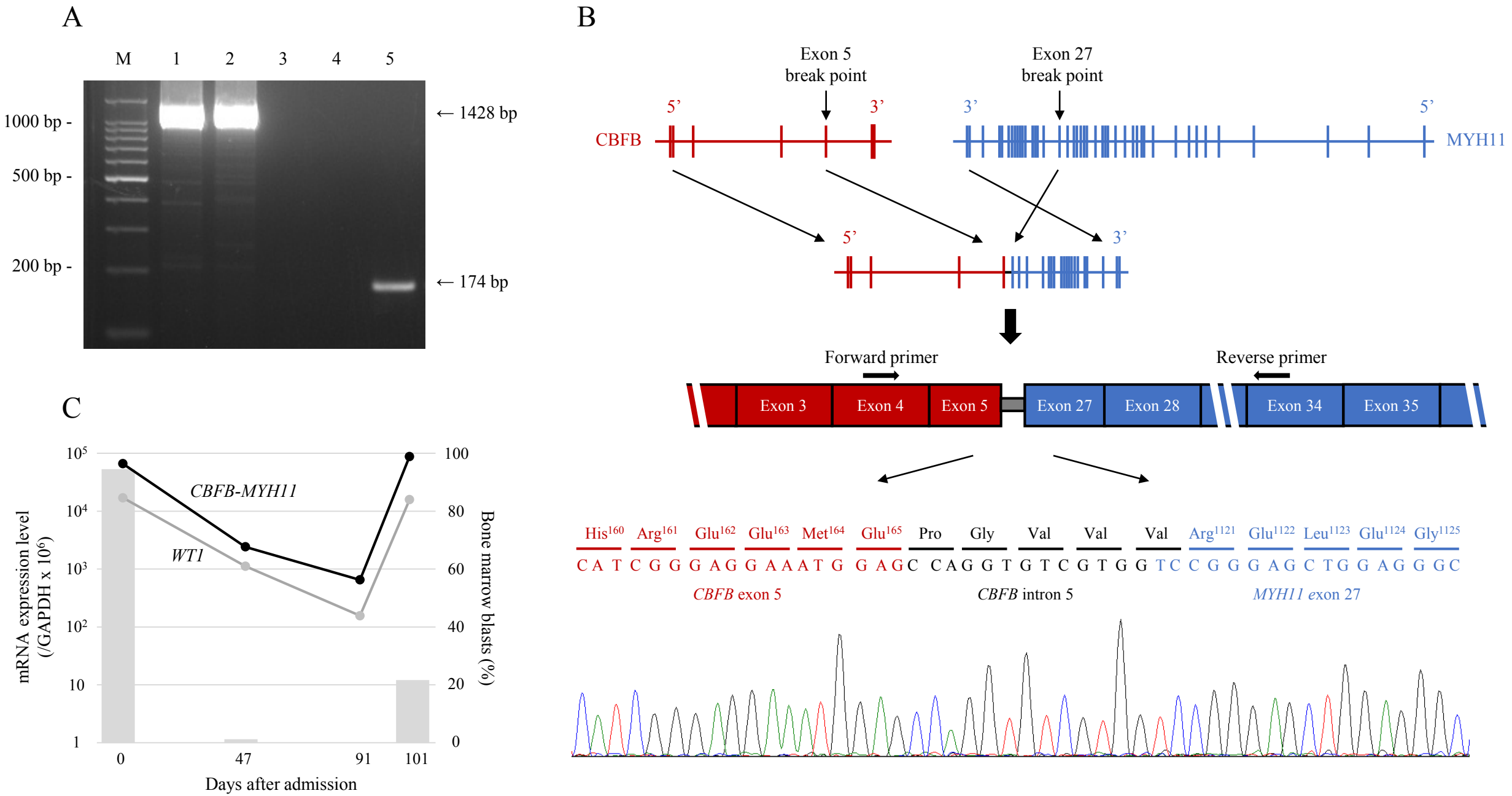
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(/GAPDH x 10^6)
Bone marrow blasts (%)
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60
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0
47
91
101
Days after admission

Clinical studies

Age, median (range)	Patient number	Sex (M:F)	Fusion transcript types, no. (%)									Morphology (FAB), %					Diagnosis		Karyotypes, %			WBC, no. (range)	BM blasts, %	KIT		Ref
			Avar	Bvar	D	E	F	G	H	I	J	I	M1	M2	M4	M4Eo	M5	de novo	tAML	sole	typical additional	atypical additional			Mutated	Wild	
41 (22-62)	208	54:46	-	-	6 (3)	18 (9)	-	-	-	2 (1)	-	2 (1)	14	0	19	67	0	100	0	42	58	0	21.9 (1.4-87.2)	53	0	100	Blood 2013
51 (18-83)	158	46:54	1 (1)	1 (1)	16 (10)	8 (5)	1 (1)	2 (1)	1 (1)	2 (1)	2 (1)	2 (1)	-	-	-	68	-	71	29	55	16	29	7.8 (0.8-148)	NA	NA	NA	Leukemia 2007

Case reports

Age	Sex	Fusion transcript types	Morphology (FAB)	Diagnosis	Karyotypes	WBC	BM blasts (%)	Treatment response/duration (month)	Ref
24	M	F	M2Eo	NA	47,XY,inv(16)(p13q22),del(8p21),+21	6.1	NA	NA	Shurtleff (1995)
14	M	G	M1Eo	NA	46,XY,inv(16)(p13q22)	5.8	NA	NA	Shurtleff (1995)
34	F	H	M1	NA	47,XX,inv(16)(p13q22),+8	2.7	NA	NA	Shurtleff (1995)
53	F	I	M4Eo	tAML	46,XY,inv(16)(p13q22)[13]/46,XX[23]	NA	NA	CR/16+	Dissing
58	M	I	M4Eo	de novo	NA	NA	NA	CR/18+	van der Rajiden
53	F	I	M2	tAML	46,XX,inv(16)(p13q22)[15]/46,XY[5]	5.5	34	CR/6+	Grardel
36	M	I	M2Eo	de novo	46,XY,inv(16)(p13q22)[9]/47,XY,+8,inv(16)(p13q22)[6]/46,XY[5]	7.5	31	CR/12+	Grardel
48	F	I	t-MDS (RAEB-t)	tAML	46,XX,inv(16)(p13q22)[5]/46,XX[15]	NA	NA	CR/28+	Yamamoto
68	M	J	M1	de novo	46,XY,inv(16)(p13q22) [6]/46,idem,i(17)(q10)[9]/46,XY[5]	12.6	83	CR/Relapse at 9	Springall (1998)
20	M	K	M2	de novo	46,XY [21]	6.9	50-70	CR/3+	Park (2009)
49	F	L	M4Eo	de novo	46,XX,inv(16)(p13q22)	50	47	CR	Rowe (2007)
52	F	M	M2	de novo	45,XX,t(16;16)(p13; q22),–13 [20]	2.5	53	CR	Albano (2014)
76	F	N	M1	de novo	46,XX,der(7)t(7;11)(q31;q21),inv(16)(p13.1q22)[18]/46,XX[2]	77	94	CR//Relapse at 3	Current case