# Therapy-related myelodysplastic syndrome with inv(16)(p13q22) and I type CBF$\beta$/MYH11 after autologous transplantation: Undetectable fusion transcript in pretransplant progenitor cells

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## Abstract

We describe here a unique case of therapy-related myelodysplastic syndrome (t-MDS) with inv(16)(p13q22) after autologous stem cell transplantation for lymphoma. The rare and smallest I type *CBFβ/MYH11* fusion transcript with a breakpoint at nucleotide 399 of *CBFβ* and at nucleotide 2134 of *MYH11* was detected in the bone marrow cells by reverse transcription polymerase chain reaction analysis. However, the fusion transcript was undetectable in the pretransplant peripheral blood stem cells. These results suggest that the stem cell damage leading to t-MDS may be induced mainly by the conditioning regimen for transplantation. Taken together with previous reports, the I type fusion transcript is preferentially induced with chemotherapy.

# 1. Introduction

High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) has been increasingly used for the treatment of hematological malignancies including non-Hodgkin's lymphoma (NHL) and other chemosensitive solid tumors. However, therapy-related myelodysplastic syndrome and acute myeloblastic leukemia (t-MDS/AML) after ASCT have become an important and serious problem [1, 2]. The cumulative risk of t-MDS/AML has varied widely from 1.1% at 20 months up to 24.3% at 43 months after ASCT, and is often very high if compared to the patients treated by conventional chemotherapy and radiotherapy [1]. There are 3 contributors to risk of t-MDS/AML, that is, pretransplantation therapy, method of stem cell mobilization, and conditioning regimens for transplantation [2]. It has been controversial whether t-MDS/AML results from exposure to previous chemotherapy, or it is a direct result of conditioning regimens for ASCT [1-5].

Pericentric inversion of chromosome 16, inv(16)(p13q22), has been specifically observed in the subtype AML M4Eo, which is characterized by abnormal myelomonocytic differentiation and increase of atypical bone marrow eosinophils [6]. In addition, it has been reported that inv(16)(p13q22) is detected in approximately 1.5% of t-MDS/AML [7, 8]. The breakpoints in the inv(16) involve the core binding factor β (*CBFβ*) gene at 16q22 and the smooth muscle myosin heavy chain gene (*MYH11*) at 16p13 [9, 10]. The breakpoints in the *MYH11* gene are heterogeneous and at least 10 types (type A to J) of the *CBFβ/MTH11* fusion transcripts have

been identified to date [11, 12]. The majority (85%) of cases have type A fusion transcripts. Many of the rest have types D and E, whereas other transcripts are rarely found [11, 12]. The resultant CBFβ/MYH11 fusion protein interferes with the formation of CBF complex and blocks differentiation of hematopoietic cells. However, the functional difference of each fusion transcript remains to be elucidated [12].

We describe here a unique case of t-MDS with inv(16) that developed following high-dose therapy with ASCT for NHL. We examined whether the *CBFβ/MYH11* fusion transcript was expressed in the pretransplant peripheral blood stem cells (PBSC) as well as bone marrow cells to speculate the origin of t-MDS after ASCT.

## 2. Materials and methods

### 2.1. Case History

A 48-year-old woman was referred to our hospital for the swelling of her right shoulder in August 2001. Magnetic resonance images demonstrated that the tumor originated from the right scapula and diffusely infiltrated into surrounding tissues. Biopsy of the tumor was consistent with NHL, diffuse large B-cell lymphoma. Serum levels of lactate dehydrogenase (LDH) and soluble interleukin-2 receptor (sIL-2R) were 292 IU/l (normal range, 117-205) and 625 U/ml (normal range, 135-483), respectively. Computed tomography, Ga-67 citrate scintigraphy and bone marrow biopsy showed no evidence of lymphoma cell infiltration except for the right scapula and sternum. She was treated with 3 cycles of CHOP regimen (cyclophosphamide 750 mg/m$^2$ day 1, doxorubicin 50 mg/m$^2$ day 1, vincristine 1.4 mg/m$^2$ day 1, prednisolone 100 mg/body days 1 to 5) and 2 cycles of ESHAP regimen (methylprednisolone 250 mg/body days 1 to 5, etoposide 40 mg/m$^2$ days 1 to 4, cisplatin 100 mg/m$^2$ days 1 to 4, cytosine arabinoside 2 g/m$^2$ day 5). The tumor was reduced in size, but still detected in the right scapula. Therefore, we decided to perform high-dose therapy with peripheral blood stem cell transplantation for the residual tumor. After high-dose etoposide (500 mg/m$^2$ days 1 to 4) and mobilization with granulocyte colony-stimulating factor (400 μg/m$^2$), 3.5 x 10$^6$/kg of CD34-positive cells were harvested from her peripheral blood. In February 2002, high-dose therapy with total body irradiation (TBI, 3 Gy/body, days -7 to -4) and cyclophosphamide (50 mg/kg, days -3 to -2) was carried out

and all stored stem cells were transfused into the patient on day 0. Engraftment was confirmed on day 13 and she achieved a complete remission (CR).

In August 2002, pancytopenia gradually appeared. Peripheral blood showed hemoglobin 8.9 g/dl, platelets 15 x $10^9$/l and white blood cells 2.6 x $10^9$/l with 63% segmented neutrophils, 2% eosinophils, 1% basophils, 2% monocytes and 32% lymphocytes. Bone marrow was normocellular with 16.2% myeloperoxidase-positive myeloblasts and 1.2% eosinophils. Auer rods were detected in some of the myeloblasts (Fig. 1A). Dysplastic changes, such as hypogranulation and pseudo-Pelger anomaly of neutrophils, were observed in the bone marrow cells (Fig. 1B). Surface marker analysis by three-color flow cytometry with CD45 gating revealed that myeloblasts were positive (more than 20%) for CD13 (97.4%), CD33 (56.0%), CD34 (92.3%) and HLA-DR (99.3%), but negative for B- and T-lymphoid markers. She was diagnosed as MDS, refractory anemia with excess of blasts in transformation (RAEB-t) in the French-American-British (FAB) classification, or MDS, therapy related, in the World Health Organization (WHO) classification. After 2 months, although eosinophils were still 1.8%, myeloblasts in the bone marrow increased to 22.3% and the disease evolved to AML, therapy related, in the WHO classification. An induction therapy with cytosine arabinoside, daunorubicin, 6-mercaptopurine and prednisolone was started and she achieved a CR. Then, she received unrelated cord blood transplantation following the conditioning regimen with fludarabine, busulfan and high-dose cyclophos-

phamide in December 2002. She remained in hematological and cytogenetic CR for more than 28 months.

## 2.2. Chromosome and fluorescence in situ hybridization (FISH) analyses

Chromosome analyses were performed by the G-banding technique on short-term culture of the cells obtained from bone marrow at the diagnosis of NHL (September, 2001), t-MDS (August, 2002), t-AML (October, 2002) and at a CR period after an induction therapy (November, 2002). Karyotypes were described according to the International System for Human Cytogenetic Nomenclature [13].

For FISH analyses, we used the LSI CBFB Dual Color, Break Apart Rearrangement Probe (Vysis, Downers Groove, IL, USA), which is a mixture of 5' (centromeric) CBFB probe labeled with SpectrumRed and 3' (telomeric) CBFB probe labeled with SpectrumGreen. FISH analyses were performed on interphase nuclei of cryopreserved PBSC specimen (January, 2002) as well as interphase nuclei and metaphase spreads of the bone marrow cells at the diagnosis of t-AML, according to the manufacturer's instructions. In a cell with an inv(16), separate red and green signals appear on opposite arms of the inverted 16 chromosome, whereas two red/green (yellow) fusion signals are detected in a normal cell.

## 2.3. Reverse transcription polymerase chain reaction (RT-PCR) and nucleotide sequence analyses

Total RNA was extracted from mononuclear cells in the bone marrow at

the diagnosis of t-MDS and at a CR period after an induction therapy and from cryopreserved PBSC specimen using QIAamp RNA Blood Mini (QIAGEN, Tokyo, Japan). Two micrograms of total RNA were transcribed to cDNA by using Ready-to-Go™ T-Primed First-Strand Kit (Pharmacia Biotech, Piscataway, NJ, USA) in a total volume of 33 µl according to the manufacturer's instructions. Designation of primers for the *CBFβ/MYH11* fusion transcript and nested PCR were performed as described previously with some modifications [14, 15]. The outer primer set of cd (*CBFβ* forward primer, cDNA position 274-296 according to GenBank accession number AF294326) and mm (*MYH11* reverse primer, cDNA position 2379-2401 according to GenBank accession number D10667) and the inner primer set of cmd1 (*CBFβ*, position 359-379) and mmd2 (*MYH11*, position 2345-2365) were used for the first and second PCR, respectively.

First PCR was carried out using 2 µl of synthesized cDNA, 2 µl of 10 x PCR buffer (10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100), 3.2 µl of 1.25 mM each dNTP, 1.2 µl of 25 mM $MgCl_2$, 1.4 µl of 10% DMSO, 8.2 µl of water, 20 pmol of each outer primer with 2.5 units of Taq DNA polymerase (Promega, Madison, WI, USA) in a total volume of 20 µl. PCR mixtures were denatured for 5 min at 94°C and 30 cycles of the first PCR (denaturation at 94°C for 1 min, annealing at 62°C for 1 min and extension at 72°C for 1 min) were performed in a thermal cycler followed by a final round of extension at 72°C for 10 min. Two microliters of the first PCR product were reamplified in the 20 µl reaction mixture containing each inner primer under the same condition as the first PCR. Four microliters of

the second PCR products were analyzed by electrophoresis on a 2.0% agarose gel. The 100 bp DNA ladder (New England Biolabs, Beverly, MA, USA) was used as a size marker.

Sequencing reactions were carried out by using BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and primers cmd1 and mmd2 according to the manufacturer's instructions. The sequences were analyzed on an ABI PRISM Model 310 Genetic Analyzer (Perkin Elmer, Foster City, CA, USA).

## 2.4. Sensitivity analysis of RT-PCR

To assess the sensitivity of RT-PCR for the *CBFβ/MYH11* fusion transcript, 1 µg of total RNA isolated from bone marrow cells of the patient at the diagnosis of t-MDS was serially diluted with 1 µg of total RNA extracted from normal bone marrow mononuclear cells. The resulting cDNA was then subjected to nested PCR under the same condition as described above.

# 3. Results

Chromosome analysis of the bone marrow cells at the initial diagnosis of NHL showed a normal karyotype. The karyotypes at the diagnosis of t-MDS and t-AML were 46,XX,inv(16)(p13q22)[5]/46,XX[15] and 46,XX,inv(16)(p13q22)[19]/46,XX[1], respectively (Fig. 2). After an induction therapy, the karyotype returned to normal. To confirm inv(16), we performed FISH analyses with the CBFB probe initially on bone marrow cells at the diagnosis of t-AML. The 5' portion of the CBFB probe moved to the 16p13 and the 3' CBFB remained on the 16q22 in all 10 metaphase spreads analyzed (Fig. 3A). FISH on interphase nuclei showed that 96.4% of 112 cells had split CBFB signals (negative control, 1.0%) (Fig. 3A). These results confirmed the rearrangement of the *CBFβ* gene. To investigate the origin of t-MDS with inv(16), we also performed FISH on cryo-preserved PBSC harvested for ASCT. However, 0.0% of 103 interphase nuclei had split CBFB signals (negative control, 0.0%) (Fig. 3B).

For further characterization of inv(16), we examined the expression of the *CBFβ/MYH11* fusion transcript by RT-PCR analysis. We designed the *MYH* primers more downstream region of the gene as recommended by van der Reijden et al. [15], because a preliminary result with primers generally used was negative. Similar PCR products of 273 bp were amplified in the bone marrow cells at the diagnosis of t-MDS and at the CR period after an induction chemotherapy (Fig.4A, lanes 3 and 4). Nucleotide sequencing analyses of both PCR products proved to be the *CBFβ/MYH11* fusion transcript with a breakpoint at nucleotide 399 of the *CBFβ* gene and at nucleo-

tide 2134 of the *MYH11* gene (Fig. 5A). That is, the *CBFβ* exon 4 was fused with the *MYH11* exon 34 (*MYH11* exon number is according to the GenBank accession number NM 002474.). This transcript is called as "I type" or "type S", and it is the smallest one identified to date [7, 15, 16].

On the other hand, two PCR fragments of 155 bp and 400 bp were amplified in the cryopreserved PBSC reproducibly (Fig. 4A, lane 2). The 155 bp fragment was composed of exon 4 and exon 5 of *CBFβ* and mmd2 18 bp primer. The primer sequences were corresponding to the nucleotide position from 2348 to 2365 of the *MYH11* gene. The nucleotide 2348 of *MYH11* was located within exon 35, and did not correspond to an exon-intron boundary. Although the *CBFβ* exon 5 usually terminates at nucleotide 495 (GenBank accession number NM_022845), another splicing variant of *CBFβ* exon 5 extends to nucleotide 526 (NM_001755). The nucleotide sequences from 493 to 501 of *CBFβ* were highly homologous to the mmd2 primer sequences (Fig. 5B). Therefore, the 155 bp PCR fragment was produced by non-specific annealing of the mmd2 primer to *CBFβ* transcript, but not a *CBFβ/MYH11* fusion transcript.

The nucleotide sequences of a 400 bp fragment were derived from intron 34 and exon 35 of the *MYH11* gene except the first 14 sequences of the cmd1 primer. Detection of intronic sequences may be due to unspliced primary RNA transcript or contamination with very small amounts of genomic DNA [17]. This fragment also seemed to be amplified by non-specific annealing of the cmd1 primer to intron 34, because they had highly homologous sequences as shown in Fig. 5C. As a result, we con-

cluded that any I type *CBFβ/MYH11* fusion transcript was undetectable in the cryopreserved PBSC.

We also performed dilution experiment to test the sensitivity of RT-PCR for the *CBFβ/MYH11* fusion transcript. RT-PCR could reliably detect 100 pg of rearranged RNA in 1 μg of normal RNA (Fig. 4B). That is, the sensitivity was found to be $1:10^4$. Our assay was as sensitive as that reported previously [15].

## 4. Discussion

In this study, we have detected the rare and smallest I type *CBFβ/MYH11* fusion transcript in the bone marrow cells of the patient with inv(16)-positive t-MDS after ASCT. The fusion transcript was undetectable in the pretransplant PBSC by RT-PCR with relatively high sensitivity, suggesting that the stem cell damage leading to t-MDS may be induced mainly by the conditioning regimen for ASCT rather than conventional chemotherapy. Naturally, this result does not necessarily prove the contention that the conditioning regimen was the cause of t-MDS, because there is a possibility that the fusion transcript might pre-exist under detectable level.

More than 60 cases of t-MDS/AML with inv(16) have been described in the literature [7, 8]. The majority of cases were AML M4Eo in the FAB classification, as observed in *de novo* AML. Only 6 and 5 cases were t-MDS with or without progression to t-AML, respectively [7, 8]. Furthermore, to our knowledge, there is no report of t-MDS/AML with inv(16) that developed following high-dose therapy with ASCT. Thus, t-MDS with inv(16) after ASCT seems to be a very uncommon phenotype. However, the present case had some clinical features usually found in t-MDS/AML with inv(16), such as a short latent period from the start of treatment for primary tumors to the development of t-MDS (12 months) and a favorable prognosis comparable to *de novo* AML M4Eo [8]. With regard to previous therapy, t-MDS/AML with inv(16) was predominantly related to topoisomerase II inhibitors (TIs), in particular anthracyclines, although it could be induced by radiotherapy and/or alkylating agents (AAs) alone [7, 8]. In the

present case, doxorubicin and etoposide were used for conventional chemotherapy and stem cell harvest, whereas cyclophosphamide was used for the conditioning regimen as well as previous therapy. These 3 drugs were the most frequently administered TIs and AAs in t-MDS/AML with inv(16) [8].

The cellular origin of t-MDS/AML following ASCT has been an important issue. Considering the very short latent period from ASCT to the development of t-MDS (6 months) and an association between TIs and inv(16), it is suspected that the stem cell damage occurred before ASCT in the present case. Moreover, Abruzzese et al. [3] demonstrated that cytogenetic abnormalities observed in t-MDS after ASCT were already present in the pretransplant stem cell harvest specimens from 9 of 12 patients. The percentages of abnormal cells were relatively high (11 to 46%), then these cytogenetic abnormalities, such as 5q-, -7, and +8, were easily detected by FISH. Lillington et al. [4] also showed that clonally abnormal cells were found before ASCT in all 20 t-MDS/AML patients screened using single locus-specific FISH probes. Nevertheless, we could not detect inv(16) or *CBFβ/MYH11* in the PBSC by RT-PCR as well as FISH. These findings indicate that the origin of t-MDS may be different from case to case. In contrast to our results, almost all karyotypes observed in the studies by Abruzzese et al. [3] and Lillington et al. [4] were unbalanced and complex abnormalities usually brought about by AAs, and there was no case having balanced translocation alone. Stem cells were harvested from bone marrow in all 20 patients [4] and in 8 of 9 patients with positive results [3], whereas

only one of two patients whose stem cells were harvested from peripheral blood was positive [3]. It is unknown whether abnormal clones could be detected more easily in bone marrow than PBSC by FISH, but these differences of stem cell sources and cytogenetic abnormalities might be associated with discrepant results.

On the other hand, several reports supported our results. Pedersen-Bjergaard et al. [1] suggested that some cases of t-MDS/AML could be directly initiated or triggered by transplantation procedure, because the use of TBI in the preparative regimen for ASCT has been reported to increase the risk of t-MDS/AML. Gilliland and Gribben [2] described that some patients who go on to develop t-MDS/AML have no detectable cytogenetic or interphase FISH abnormalities at the stem cell harvest. In addition, Weber et al. [5] screened whether chromosomal aberrations, including 5q-, -7, +8, and del(17)(p13), are already detectable in PBSC from 40 patients treated with ASCT. However, none of the stem cell preparations exhibited chromosomal abnormalities, indicating that chromosomal damage is a rare event in stem cell autografts. Consequently, the origin of t-MDS is varied, but at least a part of t-MDS/AML, including the present case, may be derived from transplantation procedure itself.

As shown in Table 1, a total of 5 cases with the I type *CBFβ/MYH11* fusion transcript has been reported [7, 15, 16]. The subtypes of the disease were not uniform, that is, only two AML cases were M4Eo. Morphologically, dysplastic changes of neutrophils were observed in all 4 cases examined and Auer rods of the blasts were detected in 3 cases. All patients

achieved and maintained CR, confirming that patients with t-MDS/AML and inv(16) have favorable prognoses, even if the fusion transcript was I type [8]. As expected, 3 of 5 cases developed to MDS/AML following treatment for primary malignancies after a relatively short latent period. Cyclophosphamide, TIs and radiotherapy were commonly used as previous therapy. This finding is not contradictory to our speculation that the conditioning regimen consisting of TBI and cyclophosphamide mainly affected the onset of t-MDS in the present case. Thus, the I type *CBFβ/MYH11* fusion transcript may be preferentially, but not exclusively, associated with atypical phenotypes other than M4Eo, prior chemotherapy, dysgranulopoiesis, Auer rods and favorable prognoses.

The high percentage of therapy-related diseases in patients with I type *CBFβ/MYH11* fusion transcript indicates that breakpoints in the *CBFβ* and *MYH11* genes may vary between *de novo* AML and t-MDS/AML. Particularly, breakpoints of the *MYH11* gene may cluster within the intron 33 in t-MDS/AML. It has been shown that genomic breakpoints, leading to the formation of A type fusion transcript, are clustered within 370 bp of *MYH11* intron 32 [18], but those in t-MDS/AML with I type fusion transcript have never been studied. Recently, Mistry et al. [19] analyzed t(15;17) translocation breakpoints in acute promyelocytic leukemia that developed after exposure to mitoxantrone. The *PML* breakpoints were tightly clustered in an 8-bp region within intron 6, which was a common site of mitoxantrone-induced cleavage by topoisomerase II. Similar mechanisms might exist in t-MDS/AML with I type fusion transcript. Analyses of

genomic breakpoints in the *CBFβ* and *MYH11* genes are needed to clarify the molecular mechanism of t-MDS/AML.

Amplification of *CBFβ/MYH11* artifacts due to non-specific annealing is a persistent problem with false-positive results. Hackwell et al. [17] demonstrated the amplification of the *MYH11* gene comprising of intron 30 and exon 31 using CBFβ3 and MYH11 5M primers, instead of the *CBFβ/MYH11* fusion transcript. Their results were due to contamination with genomic DNA or unspliced primary RNA transcript and 7 bp sequence homology of CBFβ3 primer to intron 30. Amplification of *MYH11* intronic sequences in the present study could be explained similarly. Other PCR artifacts due to aspecific annealing of MYH11 M1 primer to another position of *MYH11* cDNA were also reported [20]. We have shown here that the nested RT-PCR method described by van der Reijden et al. [15] using cd-mm and cmd1-mmd2 primer sets could also amplify PCR artifacts such as *CBFβ* cDNA and *MYH11* genomic DNA, resulting in false positive results. Therefore, even if these primers are used, it is essential to confirm the sequences of PCR products when the unexpected anomalous sized bands are detected.

# Acknowledgements

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## Figure legends

**Fig. 1.** Bone marrow smear at the diagnosis of t-MDS (x1000, May-Grunwald-Giemsa staining). (A) Myeloblasts with or without Auer rods and (B) hypogranulation and pseudo-Pelger anomaly of neutrophils are shown. An arrow indicates Auer rods.

**Fig. 2.** G-banded karyotype of the bone marrow cells at the diagnosis of t-MDS: 46,XX,inv(16)(p13q22). An arrowhead indicates the rearranged chromosome.

**Fig. 3.** Dual-color FISH analysis with 5' CBFB (red) and 3' CBFB (green) probes on (A) metaphase spreads and interphase nuclei of the bone marrow cells and (B) interphase nuclei of cryopreserved peripheral blood stem cells. (A) The inverted chromosome 16 (arrowhead, left) displays the 5' CBFB (red) at 16p13 and 3' CBFB (green) at 16q22. Normal colocalization of 5' and 3' CBFB signals (yellow) at 16q22 is observed on normal chromosome 16. Split CBFB signals (red and green) are also observed on an interphase cell (arrowhead, right).
(B) Only normal CBFB fusion signals are detected.

**Fig. 4.**
(A) Detection of the *CBFβ/MYH11* fusion transcript by RT-PCR analysis. Lane M, the DNA of 100 bp ladder as a size marker; lane 1, negative con-

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trol ($H_2O$); lane 2, cryopreserved peripheral blood stem cells; lane 3, bone marrow cells at the diagnosis of t-MDS; lane 4, bone marrow cells in hematological CR after an induction therapy. PCR products of 273 bp corresponding to the I type *CBFβ/MYH11* fusion transcripts are amplified in lanes 3 and 4. On the other hand, two PCR products of 155 bp and 400 bp, probably corresponding to the *CBFβ* cDNA and *MYH11* genomic DNA, respectively, due to non-specific annealing, are detected in the lane 2.

(B) Sensitivity of RT-PCR to detect the *CBFβ/MYH11* fusion transcript. One microgram of total RNA from bone marrow cells of the patient at the diagnosis of t-MDS was serially diluted in 1 μg of total RNA from normal bone marrow cells. Lane M, the DNA of 100 bp ladder as a size marker; lane 1, 1 μg of total RNA at the diagnosis of t-MDS; lane 2, 100 ng; lane 3, 10 ng; lane 4, 1 ng; lane 5, 100 pg; lane 6, 10 pg; lane 7, 1 pg; lane 8, negative control ($H_2O$). PCR products of 273 bp corresponding to the I type *CBFβ/MYH11* transcripts are amplified in lanes 1 to 5. That is, they are still detectable at a 1 in $10^4$ dilution.

**Fig. 5.** Schematic illustration and partial nucleotide sequences of the PCR products shown in Fig. 4A.

(A) The I type *CBFβ/MYH11* fusion transcript identified in lanes 3 and 4. The *CBFβ* exon 4 is fused to *MYH11* exon 34 and exon 35. The breakpoints are at nucleotide 399 of *CBFβ* and at nucleotide 2134 of *MYH11* genes and indicated by an arrow.

(B) The PCR product of 155 bp detected in lane 2. The *CBFβ* exon 5 at nu-

cleotide 495 is fused to 18 bp of the mmd2 primer. The sequences of the 3'
portion of the PCR product, the mmd2 primer, and another splicing variant
of *CBFβ* exon 5 which extends to nucleotide 526, are compared. The se-
quence of mmd2 primer is underlined. Homologous sequences are indi-
cated by colons.

(C) The PCR product of 400 bp detected in lane 2. It is composed of
*MYH11* intron 34 and exon 35. The sequences of the 5' portion of the PCR
product, cmd1 primer (underlined) and *MYH11* intron 34 are compared.
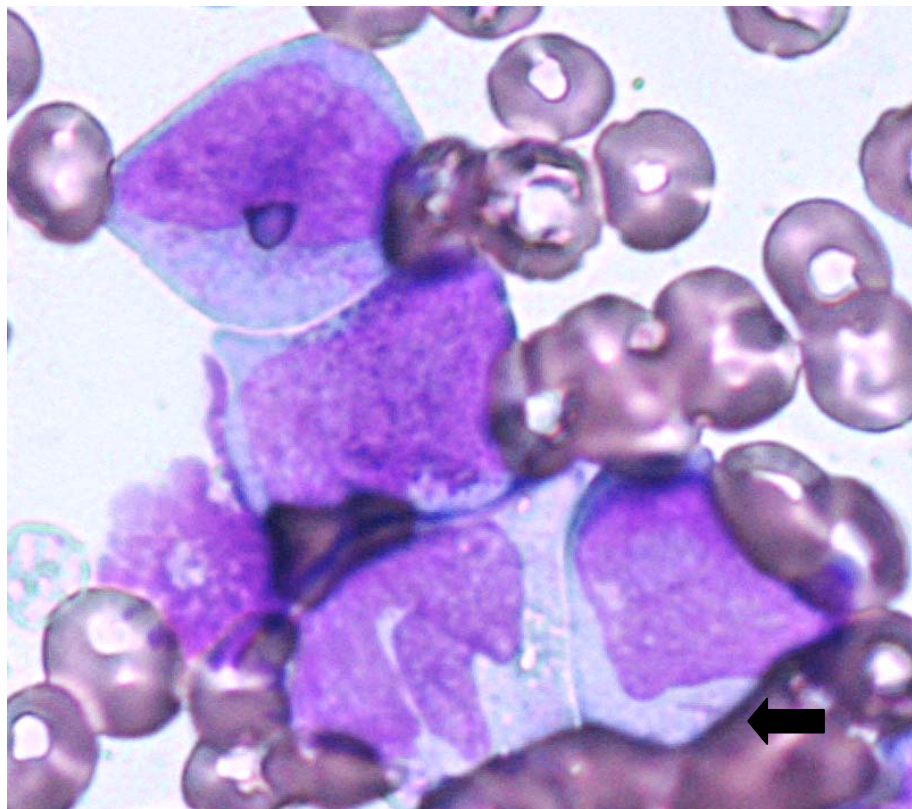Homologous sequences are indicated by colons.

## Table 1. Reported cases of myelodysplastic syndrome/acute myeloblastic leukemia with inv(16)(p13q22) and $CBF\beta^{399}/MYH11^{2134}$ (I type) fusion transcript

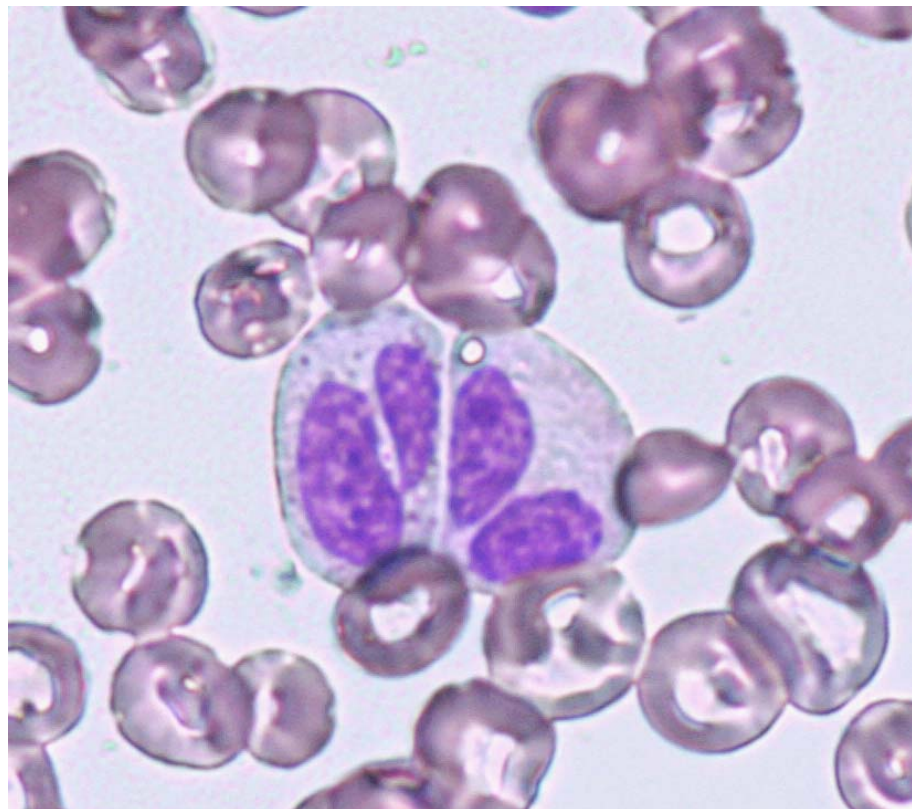| Age/ Sex | Diagnosis | Eosinophils in BM | Morphology of neutrophils and blasts | Karyotypes | Primary tumor | Previous therapy | Latent period (mo) | Treatment Response/ Duration (mo) | References |
|---|---|---|---|---|---|---|---|---|---|
| 53/F | t-AML M4Eo | NA | NA | 46,XY,inv(16)(p13q22)[13]/ 46,XX[23] | Breast cancer | CY, MIT, 5-FU, RT | 11 | CR/16+ | Dissing *et al*, 1998 [7] |
| 58/M | *de novo* AML M4Eo | 6%, abnormal | dysgranulopoiesis | poor quality of metaphase, inv(16) confirmed by FISH[10] | none | none | none | CR/18+ | van der Reijden *et al*, 2001[15] |
| 53/F | t-AML M2 | 5%, no abnormality | hypogranulation, hyposegmentation, Auer rods, MPO+ | 46,XX,inv(16)(p13q22)[15]/ 46,XY[5] | Breast cancer | CY, EPI, 5-FU, RT | 24 | CR/6+ | Grardel *et al*, 2002 [16] |
| 36/M | *de novo* AML M2Eo | 10%, dystrophic | hypogranulation, Auer rods, MPO+ | 46,XY,inv(16)(p13q22)[9]/ 46,XY,+8,inv(16)(p13q22)[6]/ 46,XY[5] | none | none | none | CR/12+ | Grardel *et al*, 2002 [16] |
| 48/F | t-MDS RAEB-t | 1.2%, no abnormality | hypogranulation, pseudo-Pelger anomaly, Auer rods, MPO+ | 46,XX,inv(16)(p13q22)[5]/ 46,XX[15] | NHL | CY, DXR, VCR, PSL, ETP, Ara-C CDDP, TBI | 12 | CR/28+ | Present case |

Abbreviations: M, male; F, female; t-AML, therapy-related acute myeloblastic leukemia; t-MDS, therapy-related myelodysplastic syndrome; NA, not available; MPO+, myeloperoxidase positive; NHL, non-Hodgkin's lymphoma; CY, cyclophosphamide; MIT, mitoxantrone; 5-FU, fluorouracil; RT, radiotherapy; EPI,

epirubicin; DXR, doxorubicin; VCR, vincristine; PSL, prednisolone; ETP, etoposide; CDDP, cisplatin; Ara-C, cytosine arabinoside; TBI, total body irradiation; CR, complete remission; CR/+ indicates alive.
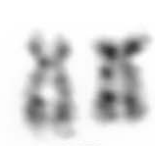
A
B

1  2  3  4  5

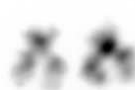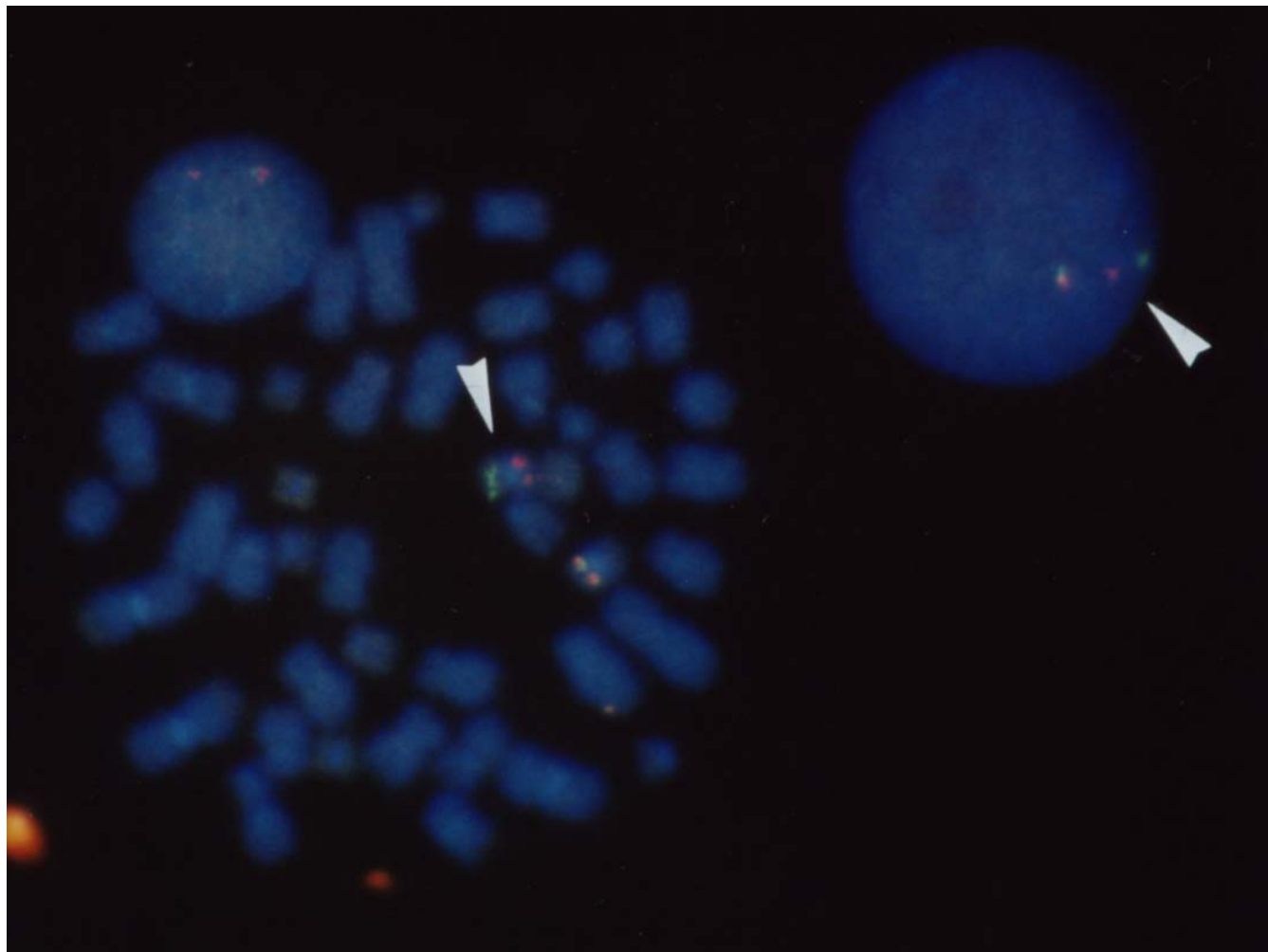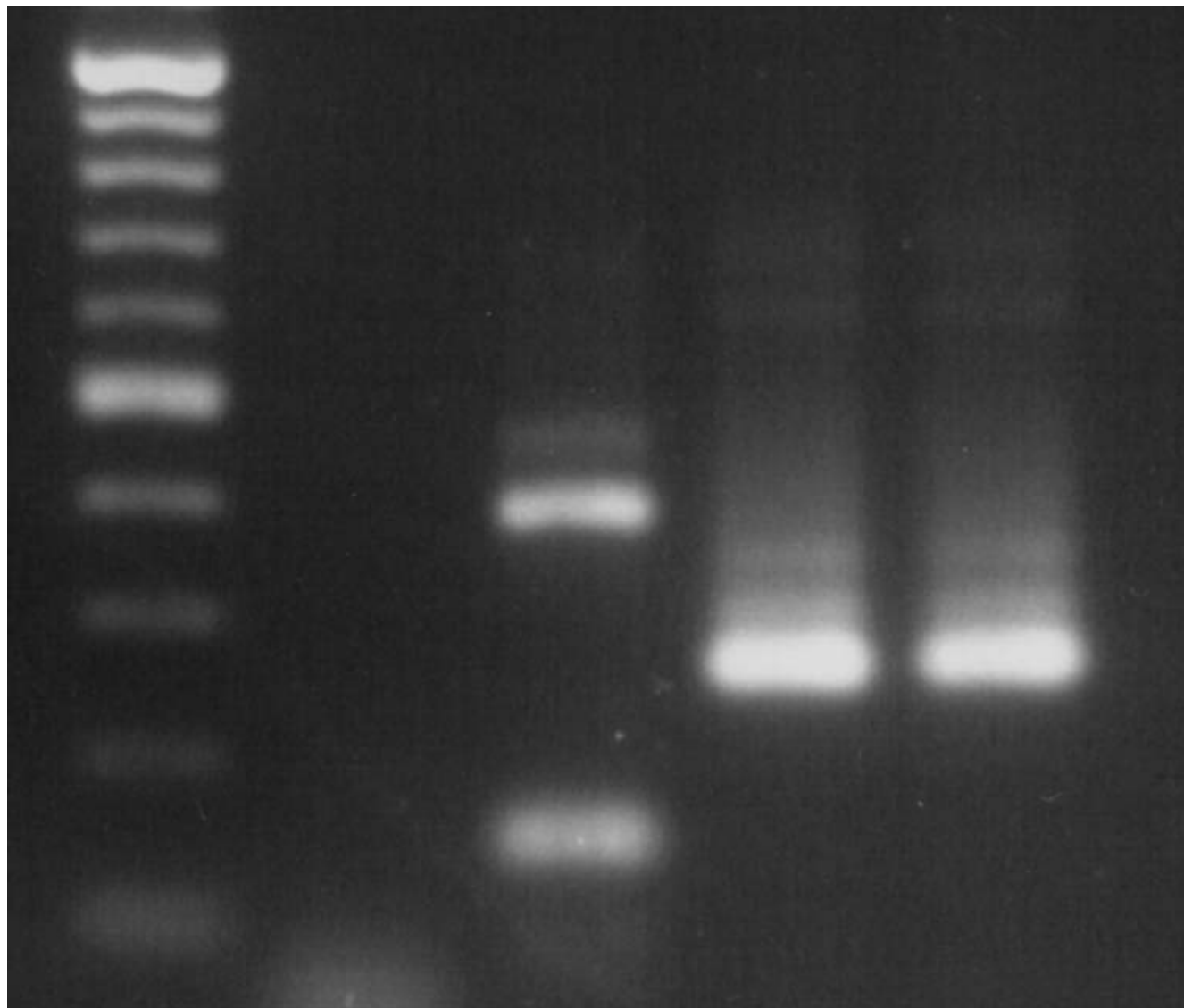6  7  8  9  10  11  12

13  14  15  16  17  18

19  20  21  22  X  Y

**A  273 bp**

cmd1 primer
→
mmd2 primer
←

359    399

| CBFβ Exon 4 | MYH11 Exon 34 | MYH11 Exon 35 |
|---|---|---|

2134              2296        2365

CBFβ 399   2134 MYH11

---GGAGCGAGCCCAG CTTCACGAGTATG---


**B  155 bp**

cmd1 primer
→
mmd2 primer
←

359       399                      495  513    526

| CBFβ Exon 4 | CBFβ Exon 5 | |
|---|---|---|

mmd2 primer     GAGATGAGATCTTTGCCACAG
                :: : :: :: : :: :: :: :: : ::
PCR product   ---GGAGGAAATGGAGATGAGATCTTTGCCACAG
                :: : : :: :: : ::   :: : : :   ::    :    : :
CBFβ Exon 5   ---GGAGGAAATGGAGGTGAGAGTTTCACAGCTG
(NM_001755)
                      CBFβ 495                    513


**C  400 bp**

cmd1 primer
→
mmd2 primer
←

| MYH11 Intron 34 | MYH11 Exon 35 |
|---|---|

2296        2365

cmd1 primer   ATGGTATGGGCTGT--CTGGAGT
                : : : :: : :: : :: :: : :: ::   ::
PCR product   ATGGTATGGGCTGT--CTGGGGTTTTT
                : :: :  :: : :  :        :: :: : :: :: ::
MYH11 Intron 34   TTGGTGTGGGTTCAGCTGGGGTTTTT