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Yamamoto, Katsuya ; Yakushijin, Kimikazu ; Ichikawa, Hiroya ; Kakiuchi, Seiji ; Kawamoto, Shinichiro ; Matsumoto, Hisayuki ; Nakamachi, Yuji ;…

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LETTER TO THE EDITOR

Expression of a novel *ZMYND11/MBTD1* fusion transcript in $CD7^{+}CD56^{+}$ acute myeloid leukemia with t(10;17)(p15;q21)

Katsuya Yamamoto[1], Kimikazu Yakushijin[1], Hiroya Ichikawa[1], Seiji Kakiuchi[1], Shinichiro Kawamoto[1], Hisayuki Matsumoto[2], Yuji Nakamachi[2], Jun Saegusa[2], Hiroshi Matsuoka[1] & Hironobu Minami[1]

[1]*Division of Medical Oncology/Hematology, Department of Medicine, Kobe University Graduate School of Medicine, Kobe, Japan*

[2]*Department of Clinical Laboratory, Kobe University Hospital, Kobe, Japan*

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Corresponding author: Katsuya Yamamoto

Address: Division of Medical Oncology/Hematology, Department of Medicine, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan

TEL: +81-78-382-5820

FAX: +81-78-382-5821

E-mail: kyamamo@med.kobe-u.ac.jp

The t(10;17)(p15;q21) translocation is a very rare but recurrent cytogenetic aberration in acute myeloid leukemia (AML), which would be classified as AML, not otherwise specified by World Health Organization (WHO), generally with classification as M0 or M1 by French–American–British (FAB) criteria. [1-8]. Recently, colocalization between the *ZMYND11* (zinc finger MYND-type containing 11, alias *BS69*) gene on 10p15.3 and the *MBTD1* (mbt domain containing 1) gene on 17q21.33 was shown by fluorescence *in situ* hybridization (FISH) in two cases of AML M1 with t(10;17)(p15;q21) [7]. Furthermore, molecular analyses identified a *ZMYND11/MBTD1* fusion transcript in a recent case of AML M0 with t(10;17) [8]. Nucleotide sequencing revealed an in-frame fusion of *ZMYND11* exon 12 to *MBTD1* exon 3 generated on der(17)t(10;17). The resultant ZMYND11/MBTD1 fusion protein was supposed to contribute to *HOXA* overexpression, which is a leukemogenic pathway to AML, whereas the reciprocal MBTD1/ZMYND11 fusion was predicted to lack a productive start codon [8]. Here, we describe an unusual case of AML with t(10;17)(p15;q21), demonstrating a novel *ZMYND11/MBTD1* fusion transcript and a $CD7^{+}CD56^{+}$ immunophenotype.

A 67-year-old man was admitted to our hospital because of the appearance of blasts in his peripheral blood. He did not show any extramedullary mass, including lymphadenopathy. The peripheral blood counts 6 months before were normal: hemoglobin 147 g/L, platelets 190×10^9/L and leukocytes 4.2×10^9/L with no blasts. Peripheral blood values on admission were hemoglobin 126 g/L, platelets 199×10^9/L and leukocytes 3.8×10^9/L with 31% segmented neutrophils, 4% monocytes, 45% lymphocytes, and 20% blasts. His bone marrow was normocellular with 18.2% blasts, 2.6% myelocytes, 3.8% metamyelocytes, 1.0% band forms, 20.8% segmented neutrophils, 3.2% monocytes, 22.4% lymphocytes, and 25.0% erythroblasts. Dysplastic changes of bone marrow cells were not apparent. Medium- to large-sized blasts showed fine nuclear chromatin, prominent nucleoli, a pale cytoplasm, and

a lack of azurophilic granules (Figure A). These blasts were negative for myeloperoxidase (MPO) and periodic acid–Schiff staining (Figure B).

Immunophenotyping by three-color flow cytometry using CD45/side scatter gating revealed that gated cells (21.0% of all bone marrow cells) were positive (>20%) for CD7 (94.2%), CD13 (39.0%), CD33 (97.7%), CD34 (91.3%), CD41 (60.7%), CD56 (94.4%), and HLA-DR (40.9%), but negative for other lymphoid markers, including CD3 (0.8%; Figure C). A diagnosis of *de novo* AML M0, or AML with minimal differentiation, was made. The patient received induction therapy with idarubicin and cytarabine according to a JALSG-AML201 protocol and achieved complete remission (CR). He then received a further four courses of conventional consolidation therapy, and has remained in hematological and cytogenetic CR for more than 14 months.

Chromosome analysis of bone marrow cells on admission showed 47,XY,+Y,t(10;17)(p15;q21)[8]/47,sl,del(12)(p?)[8]/46,XY[4] (Figure D). At hematological CR after induction therapy, the karyotype returned to 46,XY[20]. Spectral karyotyping (SKY) confirmed der(10)t(10;17)(p15;q21), whereas the small segment, 10p15→10pter, on der(17)t(10;17)(p15;q21) could not be visualized (Figure E). This segment may be smaller than the minimum genomic alteration detectable by SKY [9]. FISH with a *PML/RARA* probe revealed that the *RARA* signal at 17q21 remained on the der(17)t(10;17), indicating that the 17q21 breakpoint of t(10;17) was telomeric to *RARA*, as previously reported (data not shown) [6].

We next performed reverse transcription–polymerase chain reaction (RT–PCR) for the possible detection of *ZMYND11/MBTD1* fusion transcripts. We designed a forward primer, ZMYND11-F (from *ZMYND11* exon 11, 5’-GAGGACCGAGGTGAGGAAGA-3’, cDNA positions 1443–1462 according to NCBI reference sequence NM_006624.5), and a reverse primer, MBTD1-R (from *MBTD1* exon 3, 5’-TCACTCTCTTCGGAGCTGGA-3’, cDNA

positions 390–409 according to NM_017643.2). A PCR product of 170 bp was successfully amplified in the patient's bone marrow cells only (Figure F). Nucleotide sequencing of the PCR product revealed that the *ZMYND11* exon 11 was in-frame fused to the *MBTD1* exon 3 (Figure G). The predicted ZMYND11/MBTD1 fusion protein is composed of 1031 amino acids.

We next established a real-time quantitative PCR (RT–qPCR) strategy for the *ZMYND11/MBTD1* fusion transcript to monitor minimal residual disease (MRD) after chemotherapy. RT–qPCR was carried out with a forward primer covering the breakpoint (5'-CTCCACCAGTAATGAGCAGACT-3', from *ZMYND11* exon 11, cDNA positions 1481–1499, and from *MBTD1* exon 3, cDNA positions 297–299), reverse primer (5'-CTGCTGCTTGTGTCCTCACTG-3', from *MBTD1* exon 3, cDNA positions 368–388), PowerUp SYBR Green Master Mix, and QuantStudio 12K Flex (Applied Biosystems, Foster City, USA). Each sample was normalized against the corresponding expression of *GAPDH*. The expression level of *ZMYND11/MBTD1* (/*GAPDH* $\times 10^6$) decreased from 1200 at diagnosis to 2.09 after induction therapy, and to below the detection limit after four courses of consolidation therapy.

Thus, we have detected a rare translocation t(10;17)(p15;q21), confirmed the expression of a *ZMYND11/MBTD1* fusion transcript, and monitored MRD in a patient with AML M0. Considering the low percentage of blasts in the bone marrow (18.2%), it may be possible that the diagnosis was high-grade myelodysplastic syndrome (MDS). An older age and an additional chromosome abnormality, del(12)(p?), are generally associated with MDS. However, morphologically, there was no apparent dysplastic feature in the bone marrow cells, and the patient had no history of cytopenia. Therefore, according to the WHO criteria for blasts in the peripheral blood (20%), we made a diagnosis of AML in the early stage.

As shown in the Table, a total of nine cases of acute leukemias with t(10;17)(p15;q21)

have been reported: four presented with AML M0, four presented with AML M1, and one presented with B-cell acute lymphoblastic leukemia [1-8]. The *ZMYND11/MBTD1* fusion transcript has been previously detected in only one AML case (case 8); the present case is the second with a *ZMYND11/MBTD1* fusion transcript to be identified. In contrast to case 8, which demonstrated fusion with *ZMYND11* exon 12 and *MBTD1* exon 3, the present case exhibited fusion between *ZMYND11* exon 11 and *MBTD1* exon 3, indicating that this is a novel type of fusion transcript. The *ZMYND11* gene has 15 exons, of which 14 are coding, whereas the *MBTD1* gene contains 17 exons, of which 15 are coding [7,8]. The former showed seven alternative splice variants, but exons 11 and 12 were not involved in alternative splicing [9]. Thus, the genomic breakpoints of *ZMYND11* are supposed to be different between the two cases: introns 11 and 12. That is, the structures of *ZMYND11/MBTD1* fusion genes appear to be heterogeneous. At present, it is unknown whether this difference of breakpoints could influence hematologic and clinical findings of AML with t(10;17)(p15;q21).

ZMYND11 is a candidate tumor suppressor localized to the nucleus, and contains four functional domains: a PHD finger, a bromodomain, a PWWP domain, and a MYND domain [7,8]. ZMYND11 specifically recognizes H3.3K36me3 and suppresses phenotypes of cancer cells in a manner that depends on its H3.3K36me3-binding activity [10]. Wen et al. showed that deletion of the PHD, bromo, or PWWP domains greatly diminished full-length ZMYND11 binding to a H3K36me3 peptide and chromatin, while deletion of the MYND domain had no effect [10]. MBTD1 belongs to Polycomb Group of proteins that are critical epigenetic modifiers of chromatin, and functions as a transcriptional repressor of genes affecting tumor suppression [8,11,12]. MBTD1 specifically binds to mono- or di-methylated lysine on histone H4K20 through one of its four Malignant Brain Tumor (MBT) repeats.

Similar to case 8 in the Table, the *ZMYND11* exon 11/*MBTD1* exon 3 fusion results in the

replacement of the MYND domain of ZMYND11 by the full-length MBTD1. The fusion protein retains PHD, bromo, and PWWP domains of ZMYND11, and a FACS-type zinc finger and four MBT repeats of MBTD1, and may have a binding capacity for H3.3K36me3. Thus, the precise mechanism of leukemogenesis remains to be elucidated, but the fusion protein may play a role in gene repression by epigenetic mechanisms and eventually cause high *HOXA* expression [8]. Recently, in addition to *ZMYND11/MBTD1* fusion, *MBTD1* was shown to be involved in a low-grade endometrial stromal sarcoma with t(X;17)(p11.2;q21.33): *MBTD1* exon 16 was fused with *CXorf67* exon 1 [12]. Likewise, the resultant MBTD1/CXorf67 fusion protein retains almost all the MBTD1 coding region, suggesting a significant role of full-length MBTD1 in tumorigenesis.

We diagnosed the patient's disease as AML M0 because blasts were positive for myeloid markers (CD13, CD33), but negative for cytochemical MPO staining and other lymphoid markers. Furthermore, immunophenotypic analysis revealed that leukemic cells showed strong coexpression of CD7 and CD56. Interestingly, this immunophenotype corresponds to that of myeloid/natural killer cell precursor acute leukemia (MNKL), although this entity is not universally recognized and is not mentioned by the WHO classification [13]. As shown in the Table, another three AML cases with *ZMYND11/MBTD1* fusion (cases 6 to 8) presented similar immunophenotypes: blasts were commonly positive for CD7 and CD56 as well as CD33 and CD34. Clinically, extramedullary involvement was not described in all four cases with *ZMYND11/MBTD1*, and their prognoses were not always poor. Thus, *ZMYND11/MBTD1* fusion may be specifically associated with the $CD7^+CD33^+CD34^+CD56^+$ immunophenotype of MNKL, although several different clinical findings from typical MNKL exist. With regard to cytogenetic findings of MNKL, a specific aberration has not been detected to date [14,15]. Our results indicated that t(10;17)(p15;q21) could be one of the recurrent translocations observed in MNKL or $CD7^+CD56^+$ AML.

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Figure legends

Figure.

(A) Bone marrow smears showing blasts at diagnosis of AML. Medium- to large-sized blasts show fine nuclear chromatin, prominent nucleoli, a pale cytoplasm and no azurophilic granules (May–Grünwald–Giemsa staining, ×1000).

(B) Two blasts (left) are negative for myeloperoxidase staining, whereas one neutrophil (right) is positive (×1000).

(C) Flow cytometric analysis of bone marrow cells at diagnosis of AML by CD45/side scatter (SSC) gating. The corresponding cell percentage demarcated by the gate is 21.0%. The results of two-color analyses with CD7 and CD10, CD33 and CD2, CD19 and CD13, CD3 and CD20, CD34 and HLA-DR, CD56 and CD16, and CD41 and KOR-SA for the gated cells are shown. Corresponding cell percentages in each fraction are indicated. The gated cells are positive for CD7, CD13, CD33, CD34, CD41, CD56, and HLA-DR.

(D) G-banded karyotype of bone marrow cells at diagnosis of AML. The karyotype is 47,XY,+Y,t(10;17)(p15;q21). Arrows indicate rearranged chromosomes.

(E) Spectral karyotyping of metaphase spreads after spectrum-based classification (left side, reverse DAPI; right side, spectral karyotyping [SKY]). Only chromosomes 10 and 17 are shown. Arrows indicate rearranged chromosomes. SKY confirmed der(10)t(10;17)(p15;q21), whereas the small segment 10p15→10pter on der(17)t(10;17)(p15;q21) could not be visualized since this segment may be smaller than the minimum genomic alteration detectable by SKY.

(F) Detection of the *ZMYND11/MBTD1* fusion transcript by reverse transcription–polymerase chain reaction (RT–PCR) analysis. Lane M, the DNA of a 100 bp ladder as a size marker; lane 1, bone marrow cells of the patient at diagnosis; lane 2, normal

bone marrow cells (negative control). A PCR product of 170 bp is amplified only in the cells of the patient.

(G) Nucleotide and amino acid sequences surrounding the junction of the *ZMYND11/MBTD1* fusion transcript. *ZMYND11* exon 11 is in-frame fused to *MBTD1* exon 3. A vertical arrow indicates the breakpoint.

Figure

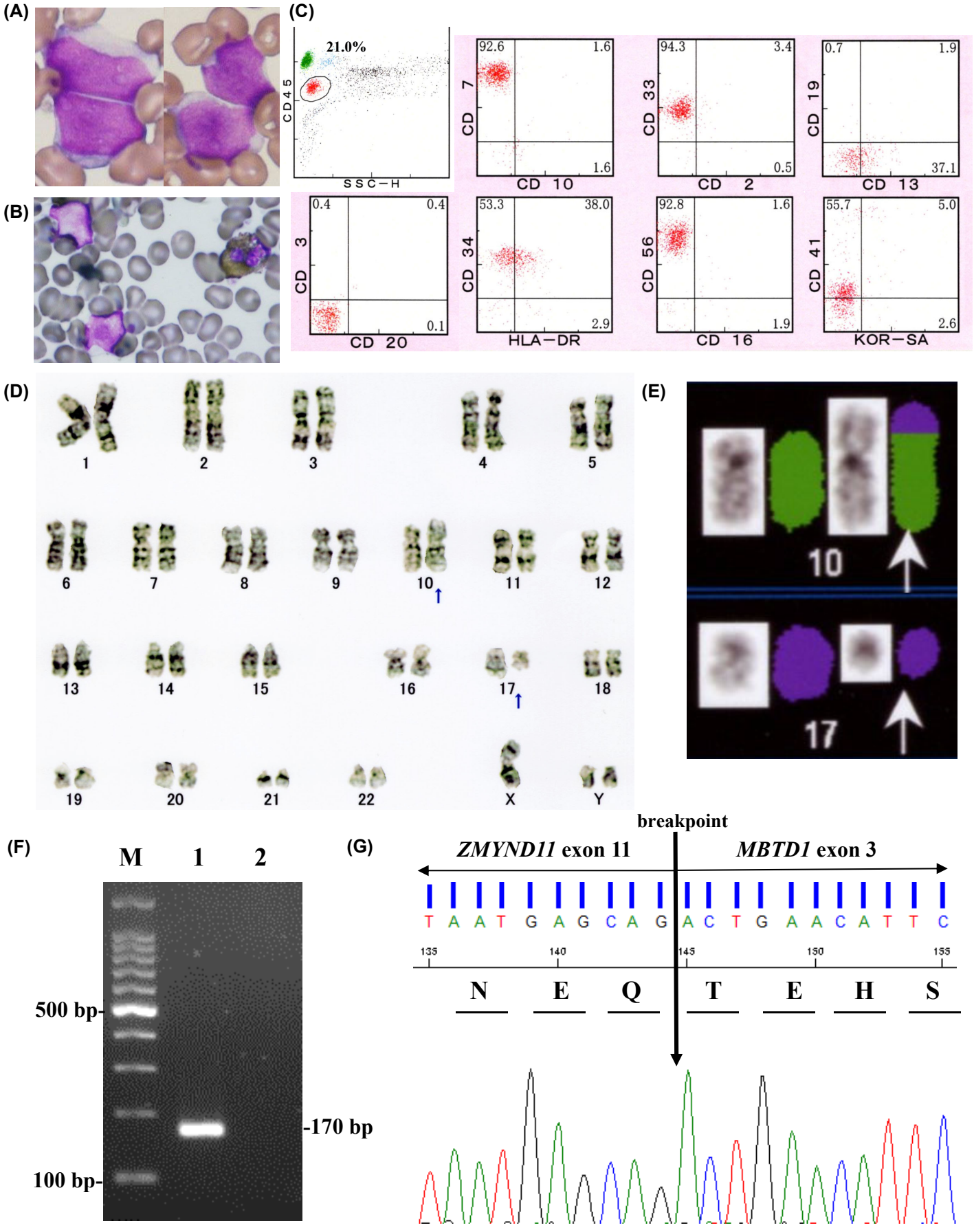
(A)
(B)
(C)
21.0%
CD45
SSC-H
92.6
1.6
1.6
CD 7
CD 10
94.3
3.4
0.5
CD 33
CD 2
0.7
1.9
37.1
CD 19
CD 13
0.4
0.4
0.1
CD 3
CD 20
53.3
38.0
2.9
CD 34
HLA-DR
92.8
1.6
1.9
CD 56
CD 16
55.7
5.0
2.6
CD 41
KOR-SA
(D)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
(E)
10
17
(F)
M
1
2
500 bp
100 bp
~170 bp
(G)
breakpoint
ZMYND11 exon 11
MBTD1 exon 3
T A A T G A G C A G A C T G A A C A T T C
135
140
145
150
155
N
E
Q
T
E
H
S

Table. Reported cases of hematological malignancies associated with t(10;17)(p15;q21)

Case No.	*Age/ Sex*	*Diagnosis*	*Karyotypes*	*ZMYND11/MBTD1 fusion gene*	*Positive surface markers*	*EI*	*OS (mo)*	*References*
1	16/M	AML M0	46,XY,**t(10;17)(p15;q21)**[30]/46,XY[31] 46,XY,del(6)(q15q23),del(9)(p21p22),**t(10;17)(p15;q21)**, del(14)(q21q24)[8]/46,XY[23]	NA	NA	no	4 died	[1]
2	11/M	AML M1	46,XY,**t(10;17)(p15;q21)**[5]/46,sl,i(7)(q10)[10]	NA	**CD7**, CD13, CD31, CD33, CD34, CD41, CD45, CD61	no	42+ CR	[2]
3	6/F	B-ALL L1	46,XX,**t(10;17)(p15;q21)**[24]/46,XX[3]	NA	NA	NA	NA	[3]
4	NA/M	AML M1	46,XY,del(6)(q14q16),**t(10;17)(p15;q21)**?der(11)[7]/ 46, XY[3]	NA	NA	NA	NA	[4]
5	NA/F	AML M0	46,XX,**t(10;17)(p15;q21)**[2]/47,sl,+7[7]/46,XX[16]	NA	NA	NA	NA	[5]
6	13/M	AML M1	46,XY,**t(10;17)(p15;q21)**[5]/47,sl,+13[9]/46,XY[9]	colocalization*	CD4, **CD7**, CD13, CD15, CD33, **CD56**, CD65, CD117	no	71+ CR	[6, 7]
7	40/F	AML M1	46,XX,**t(10;17)(p15;q21)**[3]/46,sl,der(11)(11pter→11q13:: 11q23→11q14::13q34→13qter),der(13)(13pter→13q34:: 11q23→11qter)[6]/46,XX[16]	colocalization*	**CD7**, CD13, CD33, CD34, **CD56**, CD117	no	37 died	[6, 7]
8	13/M	AML M0	46,XY,**t(10;17)(p15;q21)**[9]/46,XY[1] 47,XY,**t(10;17)(p15;q21)**,+13[1]/48,sl,+7[11]/46,XY[8]	exon 12-exon 3	**CD7**, CD33, CD34, CD45, **CD56**, CD117	no	30 died	[8]

9	67/M	AML M0	47,XY,+Y,**t(10;17)(p15;q21)**[8]/47,sl,del(12)(p?)[8]/ 46,XY[4]	exon 11-exon 3	**CD7**, CD13, CD33, CD34, CD41, **CD56**, HLA-DR	no	14+ CR	present case

Abbreviations: F, female; M, male; AML, acute myeloid leukemia; B-ALL, B-cell acute lymphoblastic leukemia; NA, not available; EI, extrameduallary involvement; OS, overall survival; CR, complete remission. + indicates alive. *Colocalization of both genes were shown by fluorescence *in situ* hybridization. The t(10;17)(p15;q21) translocation, CD7, and CD56 are described in bold letters.