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Therapy-related, mixed phenotype acute leukemia with t(1;21)(p36;q22) and *RUNX1* rearrangement

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Abstract

We describe here a new case of therapy-related acute leukemia with t(1;21)(p36;q22). A 25-year-old man was admitted because of anemia and thrombocytopenia. Four years prior, he had received combination chemotherapy including etoposide for seminoma. Bone marrow was hypercellular with 49% myeloperoxidase (MPO) staining-negative blasts. Chromosome analysis showed 46,XY,t(1;21)(p36.3;q22)[11]/49,sl,+8,+16,+20[9]. Fluorescence *in situ* hybridization demonstrated that *RUNX1* signals at 21q22 were split onto the der(1)t(1;21) and der(21)t(1;21). Immunophenotypic analyses revealed that blasts were positive for CD19, CD79a, and cytCD22 as well as MPO, CD13, and CD33, fulfilling the diagnostic criteria of mixed phenotype acute leukemia, B/myeloid. The patient died of disease progression after 10 months. Thus, acute leukemia with t(1;21) and *RUNX1* rearrangement could be associated with B/myeloid mixed phenotype as well as prior topoisomerase II inhibitor therapy and poor prognoses.

1. Introduction

The *RUNX1* gene located at 21q22 is involved in more than 30 chromosomal translocations such as t(8;21)(q22;q22), t(3;21)(q26;q22), and t(12;21)(p13;q22) in acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and acute lymphoblastic leukemia (ALL) [1]. Among them, the t(1;21)(p36;q22) translocation is a very rare but recurrent cytogenetic aberration in AML although only a little data is available about immunophenotypes [2-7]. Recently, it has been shown that *RUNX1* is fused to *PRDM16* at 1p36, which is highly homologous to *EVI1* at 3q26 [6, 7]. On the other hand, translocations with *RUNX1* rearrangement, including t(1;21), are often associated with therapy-related myeloid neoplasms, especially those induced by prior topoisomerase II inhibitor therapy [3, 7, 8]. They usually present overt acute leukemia and lack a preceding myelodysplastic phase with a latency period of 1-5 years.

Acute leukemias that express surface markers with more than one lineage are classified as AML with lymphoid markers, ALL with myeloid markers, or biphenotypic acute leukemia (BAL). To distinguish BAL from AML or ALL with aberrant expression, it has been diagnosed according to the scoring system by European Group for the Immunological Characterization of Leukemia (EGIL) [9]. In the newly revised World Health Organization (WHO) classification, the term mixed phenotype acute leukemia (MPAL), including B/myeloid and T/myeloid, applies to BAL in general [10]. We describe here a new case of therapy-related acute leukemia with t(1;21) and *RUNX1* rearrangement, who first showed B/myeloid mixed phenotype.

2. Materials and methods

2.1. Case history

A 25-year-old man was admitted to our hospital because of anemia and thrombocytopenia in August 2007. Four years prior, he had been diagnosed as having testicular seminoma and embryonal carcinoma after orchiectomy. He had received four courses of systemic chemotherapy with PEB regimen comprising of cisplatin, etoposide and bleomycin.

Peripheral blood on admission showed hemoglobin 83 g/L, platelets 10 x 10^9/L and white blood cells (WBC) 17.6 x 10^9/L with 31% blasts, 2% promyelocytes, 6% myelocytes, 8% metamyelocytes, 22% segmented neutrophils, 16% monocytes, and 15% lymphocytes. Bone marrow was hypercellular with 49.2% blasts, 21.0% myeloid cells and 14.4% erythroblasts. Large blasts had slightly convoluted nuclei with fine nuclear chromatin and basophilic cytoplasm with some vacuoles (Fig. 1A). There was no morphological feature to suggest two mixed populations of blasts. These blasts were negative for myeloperoxidase and double esterase stainings. Trilineage dysplasia was not apparent in the bone marrow cells. An initial diagnosis of therapy-related myeloid neoplasms in the WHO classification was made [8].

An induction therapy with daunorubicin and cytosine arabinoside was started, and he achieved a hematological and cytogenetic complete remission (CR). Then, he underwent myeloablative cord blood transplantation (CBT) from an HLA two loci mismatched-unrelated female donor after conditioning regimen with total body irradiation and high-dose cyclophosphamide on October 2007. He obtained complete chimerism and maintained CR on day 30. However, peripheral blood and bone marrow examinations on day 53 revealed 8% and 60% blasts, respectively, indicating the first relapse after CBT. After discontinuation of immunosuppressive agents, tacrolimus and mycophenolate mofetil, reinduction treatment with high-dose cytosine arabinoside and mitoxantrone was performed without apparent effect. He died of disease progression on June 2008.

2.2. Cytogenetic and fluorescence in situ hybridization (FISH) analyses

Chromosome analyses were performed by the G-banding technique on unstimulated

short-term culture of the cells obtained from bone marrow during the clinical course. Karyotypes were described according to ISCN 2009 [11]. Spectral karyotyping (SKY) was carried out with SkyPaint kit (Applied Spectral Imaging, Migdal Ha'Emek, Israel) on five metaphase spreads.

To examine *RUNX1* rearrangement, we used LSI AML1/ETO Dual Color, Dual Fusion Translocation Probe (Abbott Molecular, Des Plaines, IL, USA) for FISH on 20 metaphase spreads and 100 interphase nuclei. To exclude cryptic rearrangements of *BCR-ABL1* and *MLL* genes, we also performed FISH with LSI BCR/ABL ES Dual Color Translocation Probe and LSI MLL Dual Color, Break Apart Rearrangement Probe (Abbott Molecular) on 100 interphase nuclei. Bone marrow cells obtained at the relapse after CBT (on day 53) were used for SKY and FISH analyses.

2.3. Immunophenotypic analyses

Immunophenotypes of the bone marrow cells were analyzed by three-color flow cytometry with CD45/side scatter (SSC) gating at the initial diagnosis and at the relapse after CBT. Expression of each antigen on the gated CD45 low/SSC low mononuclear cells was examined by monoclonal antibodies and defined as positive when at least 20% of cells showed fluorescence above the background staining. Expression of TdT and cytoplasmic antigens MPO, CD3 and CD79a was defined as positive when more than 10% of the cells exhibited nuclear or intracytoplasmic fluorescence compared with negative controls, due to their high degree of specificity, as described previously [9, 12]. The diagnosis of MPAL was based on the scoring system for the definition of BAL by EGIL and WHO classification [9, 10].

3. Results

Chromosome analysis of the bone marrow cells at the initial diagnosis showed 46,XY,t(1;21)(p36.3;q22)[11]/46,sl,+8,+16,+20[9] (Fig. 1B). The karyotype converted to

normal after hematological CR, whereas t(1;21)(p36.3;q22) reappeared under mixed chimerism at the relapse after CBT as follows: 46,XY,t(1;21)(p36.3;q22)[9]/46,sl,inv(3)(p21q27),add(17)(p11.2)[1]//46,XX[4]. In addition to t(1;21), several secondary abnormalities were detected although they were variable. SKY analysis confirmed the der(1)t(1;21), but it could not visualize the small segment 1p36.3→1pter on the der(21)t(1;21) as observed in other two cases with t(1;21) (Fig. 1C) [6, 13]. The size of this segment was supposed to be within a range of 1-2 Mb, which is minimum genomic alteration that SKY could detect.

For further characterization of t(1;21), we performed FISH analysis with probes for *RUNX1/RUNX1T1*. *RUNX1* signals were located on the der(21)t(1;21) and der(1)t(1;21) as well as a normal chromosome 21 in 13 of 20 metaphase spreads, indicating the rearrangement of *RUNX1* by t(1;21) (Fig. 2A). FISH on interphase nuclei confirmed that 82 of 100 cells had three *RUNX1* signals (Fig. 2B). We also performed additional FISH to exclude the cryptic *BCR-ABL1* or *MLL* rearrangements because blasts disclosed B/myeloid mixed phenotype as described below [10]. FISH on 100 interphase nuclei showed neither *BCR-ABL1* fusion signals nor *MLL* split signals (data not shown).

The results of immunophenotypic analyses were summarized in Table 1 and Fig. 3. Blasts were initially shown to be positive for CD19 as well as CD13, CD33, CD34 and HLA-DR. Two-color analysis with CD13 and CD19 demonstrated that CD13+CD19-, CD13+CD19+, and CD13-CD19+ cells were 34.2%, 25.6%, and 31.4%, respectively (Fig. 3B). Therefore, it was difficult to conclude clearly whether the leukemia was biphenotypic or bilineal. We next examined additional myeloid and B-lineage markers and found that blasts were also positive for CD24, cytCD22, CD79a, CD64, MPO, CD117, and TdT. These results indicated that myeloid and B-lineage scores by EGIL scoring system were five and six points, respectively [9]. That is, both scores were over two points, which defines BAL. Furthermore, the positiv-

ity for MPO, CD19, CD79a, and cytCD22 fulfilled requirements for assigning myeloid and B-lineages to a single blast cell population, indicating the diagnosis of MPAL, B/myeloid, not otherwise specified [10]. On the other hand, the expression of CD19 considerably decreased at the relapse after CBT although myeloid markers, CD13 and CD33, were consistently positive. Finally, according to the WHO classification, the disease was primarily classified as therapy-related myeloid neoplasms with a secondary notion that it had a mixed phenotype, B/myeloid [10].

4. Discussion

We have characterized the first case of therapy-related MPAL, B/myeloid, with t(1;21)(p36;q22) and *RUNX1* rearrangement. As summarized by Stevens-Kroef et al. [7], a total of five cases of AML with t(1;21) have been reported to date [2-7]. Among them, *RUNX1* rearrangements were shown in four cases, and two of them had previous treatment histories of topoisomerase II inhibitors (etoposide and epirubicin) [3, 7]. The present case had t(1;21) and *RUNX1* rearrangement as well, indicating the primary diagnosis of therapy-related myeloid neoplasms induced by etoposide for testicular tumor. In accordance with poor prognoses of reported AML cases (survival time, 6-7 months), overall survival in the patient was relatively short (10 months) although he underwent myeloablative allogeneic stem cell transplantation in the first CR.

In addition, another case of chronic myeloid leukemia (CML) was shown to have t(1;21) at the blast crisis (BC) as well as t(9;22)(q34;q11.2) by SKY and FISH analyses [13]. Cryptic *RUNX1-PRDM16* fusion transcripts were also identified in two cases of CML-BC and one case of Philadelphia chromosome-positive therapy-related ALL. Cytogenetically, all of them had secondary trisomy 21 but not t(1;21) [14]. It is suggested that these secondary-onset *RUNX1-PRDM16* may contribute to clonal evolution and imatinib resistance. As a

result, leukemias with t(1;21) or *RUNX1-PRDM16* appear to exhibit heterogeneous phenotypes such as AML, MPAL, CML and ALL. Unfortunately, we could not confirm the presence of *RUNX1-PRDM16* fusion transcripts in this patient.

In these leukemias with t(1;21) or *RUNX1-PRDM16*, results of surface marker analyses were hardly available except for one *de novo* AML M4 case: blasts were shown to be positive for CD13, CD14, CD33 and CD34 [6]. In the present case, blasts were positive for B-lymphoid markers including CD19, CD79a, and cytCD22 as well as myeloid markers including MPO. These results indicate that therapy-related acute leukemia with t(1;21) and *RUNX1* rearrangement could be associated with B/myeloid mixed phenotype whether this correlation is specific or not.

Therapy-related leukemias are often difficult to be characterized by lineage, and this is also seen in the CML-BC: the frequent lineage infidelity of the blasts makes immunophenotypic classification of CML-BC difficult [15]. According to WHO classification, there are no specific immunophenotypic findings in therapy-related myeloid neoplasms [8]. Blasts are generally positive for CD34 and pan-myeloid markers (CD13, CD33), and aberrantly positive for CD56 and/or CD7, as observed in the present case. Immunophenotypes reflect the heterogeneity of the underlying morphology and often show changes similar to their *de novo* counterparts. However, as described above, immunophenotypes differed between the present case and the *de novo* counterpart with t(1;21) [6]. Other reports also did not fully characterize immunophenotypic features of blasts in therapy-related AML and ALL [16, 17]. Furthermore, as far as we searched, there is only one case of therapy-related acute biphenotypic leukemia, indicating that it appears to be a very rare entity [17, 18].

RUNX1 can act as a key regulator of hematopoiesis through various target genes essential for definite hematopoiesis of all lineages [1]. By reciprocal translocations, *RUNX1* is involved in *de novo* and therapy-related hematological malignancies in almost all lineages in-

cluding AML, MDS, B-ALL and T-ALL [1]. With regard to acute leukemias of ambiguous lineage, *ETV6/RUNX1* was shown to be the most frequent cytogenetic abnormality [19]. Therefore, *RUNX1* could be implicated in the pathogenesis of therapy-related MPAL with t(1;21). The resultant RUNX1-PRDM16 fusion protein, highly homologous to RUNX1-MDS1/EVI1 in t(3;21), is thought to contain the runt DNA binding domain but lack the transactivation domain of RUNX1 [6, 7]. The RUNX1-PRDM16 or truncated RUNX1 may compete with wild-type RUNX1 and act as a dominant-negative inhibitor of the RUNX1. Furthermore, the almost entire PRDM16, which is a zinc finger protein normally absent in hematopoietic cells, will be expressed under the control of the active *RUNX1* promoter [7]. As a result, it is suggested that expression of *RUNX1-PRDM16* fusion gene and ectopic *PRDM16* expression induced malignant transformation to MPAL with t(1;21). Accumulation of more cases are necessary to clarify pathological roles of the fusion gene, most common immunological phenotypes and clinical findings in AML with t(1;21).

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Figure legends

Fig. 1.

(A) Bone marrow smear at the initial diagnosis of acute leukemia (x1000, May-Grünwald-Giemsa staining). Large blasts having slightly convoluted nuclei with fine nuclear chromatin and basophilic cytoplasm with some vacuoles are shown.

(B) G-banded karyotype of the bone marrow cells at the initial diagnosis. The karyotype is 46,XY,t(1;21)(p36.3;q22). Arrows indicate rearranged chromosomes.

(C) Spectral karyotyping of the metaphase spreads after spectrum-based classification (left side, reverse DAPI; right side, SKY). Chromosomes were assigned a pseudocolor according to the measured spectrum. Only partial karyotypes (chromosomes 1 and 21) are shown. Arrows indicate rearranged chromosomes. The der(1)t(1;21) is confirmed, but the small segment 1p36.3→1pter on the der(21)t(1;21) could not be visualized by SKY.

Fig. 2. FISH analyses with probes for *RUNX1/RUNX1T1* on (A) metaphase spreads and (B) interphase nuclei.

(A) Arrows indicate 1) *RUNX1TI* signals (red) on normal chromosomes 8, 2) *RUNX1* signal (green) on a normal chromosome 21, 3) *RUNX1* signal on the der(21)t(1;21), and 4) *RUNX1* signal on the der(1)t(1;21).

(B) Two *RUNX1T1* (red) and three *RUNX1* (green) signals are detected.

Fig. 3. Flow cytometric analyses of the bone marrow cells at the initial diagnosis.

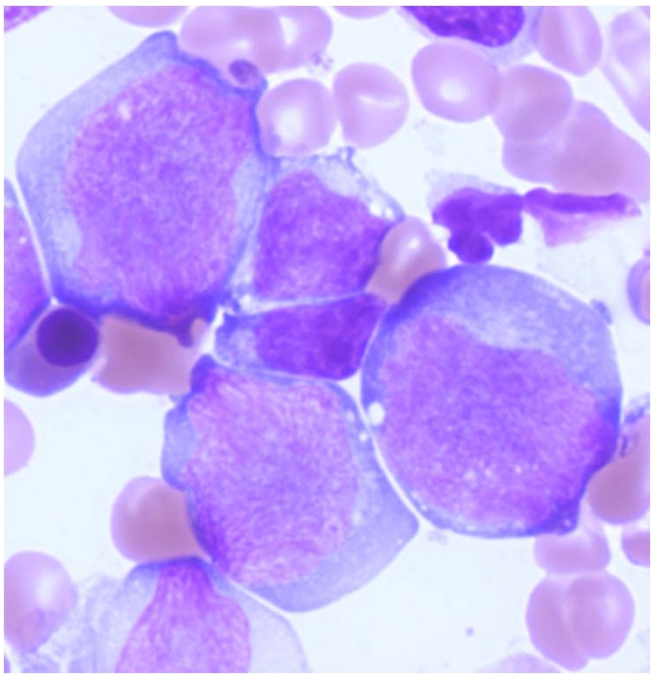
(A) By CD45 low/SSC low gating, the corresponding percentage of blasts is 74.4% of all bone marrow cells. A relatively homogeneous blast population is shown by a circle.

(B) Two-color analyses with CD13 and CD19, CD2 and CD33, MPO and CD117, TdT and

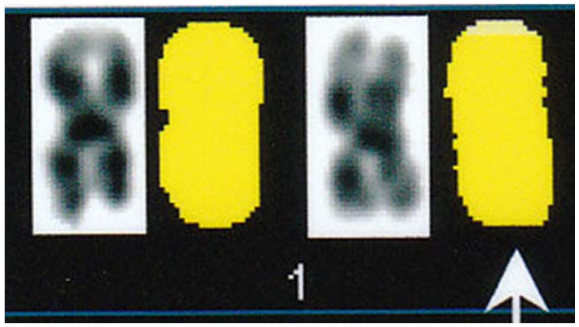
CD79a, and cytμ and cytCD22, for cells surrounded by CD45 low/SSC low gating. Corresponding cell percentages in each fraction are indicated.

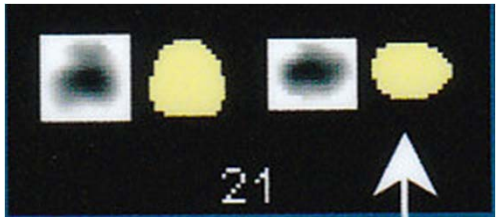
Fig. 1

A



C





B

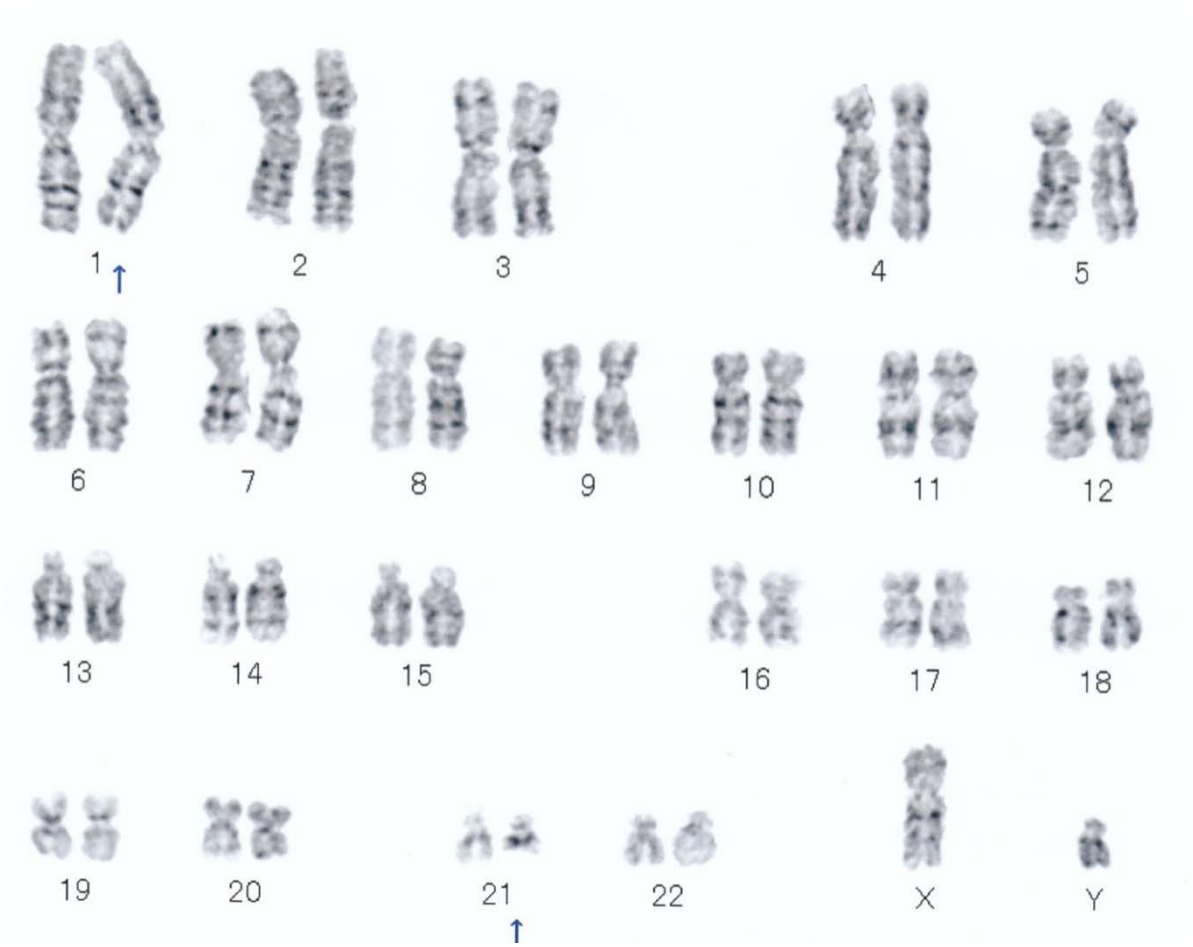


Fig. 2

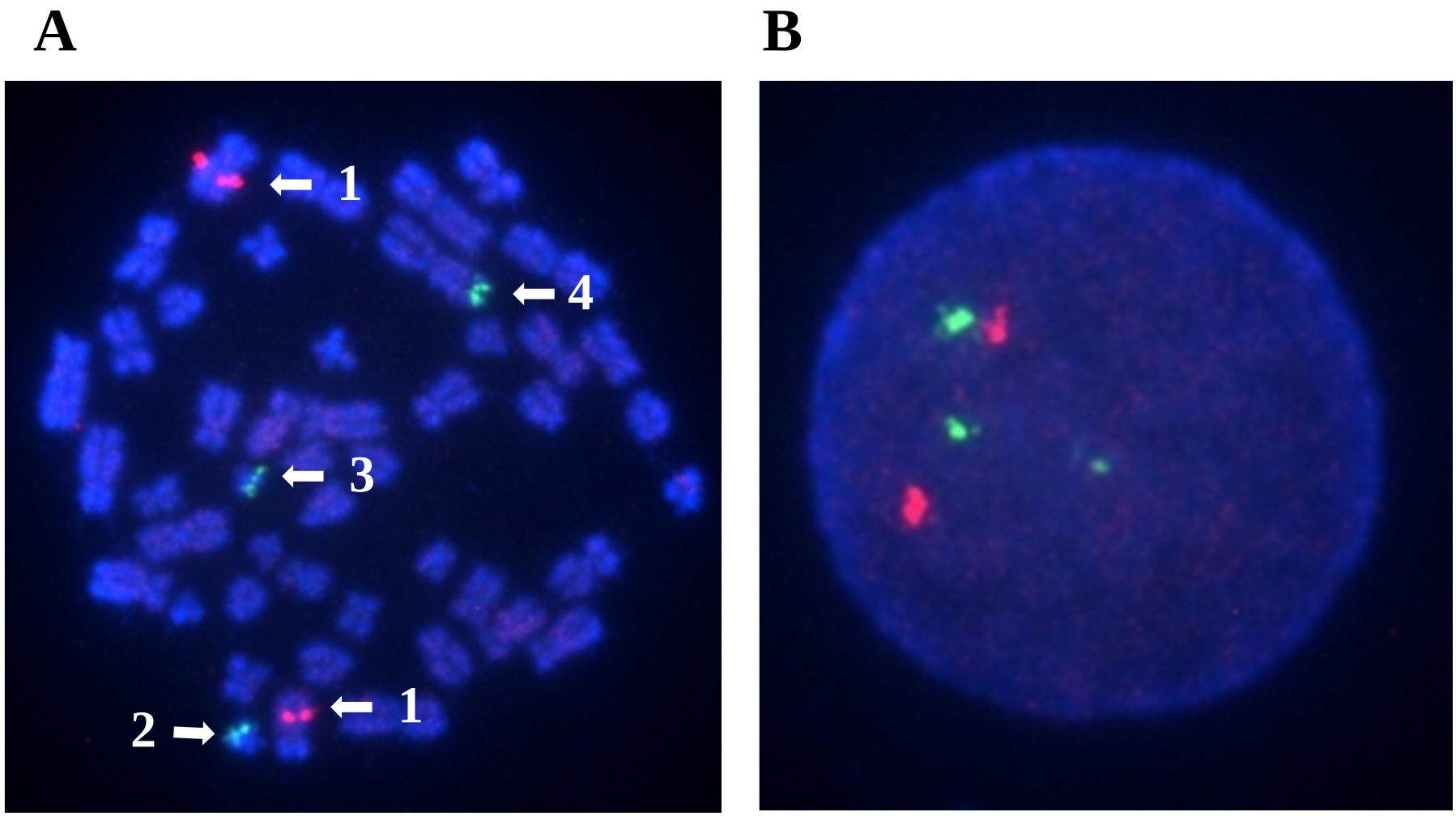


Fig. 3

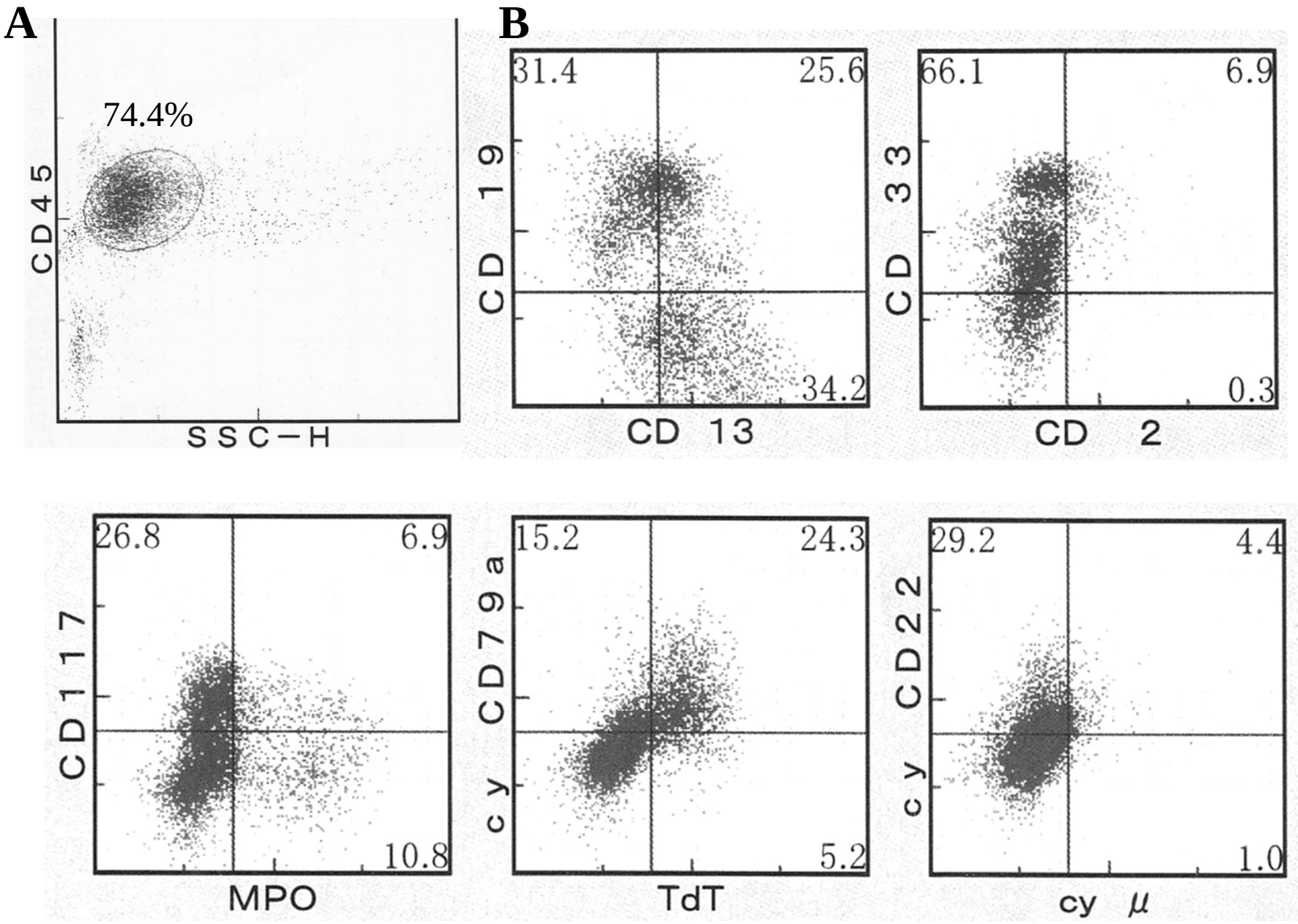

A
74.4%
CD45
SSC−H
B
31.4
25.6
34.2
CD 19
CD 13
66.1
6.9
0.3
CD 33
CD 2
26.8
6.9
10.8
CD117
MPO
15.2
24.3
5.2
cy CD79a
TdT
29.2
4.4
1.0
cy CD22
cy μ

Table 1. Results of surface marker analyses

	CD2	CD3	CD4	CD5	CD7	CD8	CD10	CD13	CD14	CD16	CD19	CD20	CD33	CD34	CD41
D	7.2	1.3	**26.8**	3.9	**33.6**	1.0	1.5	**59.8**	7.0	6.6	**57.0**	3.5	**73.0**	**56.4**	9.6
R	3.8	3.2	**39.9**	3.8	7.0	3.5	4.8	**46.7**	11.3	6.3	18.5	3.9	**75.1**	**57.5**	**29.1**

	CD56	HLA-DR	TCR-αβ	TCR-γδ	cyt-CD3	CD24	cyt-CD22	CD79a	cyt-μ	CD15	CD64	CD65	MPO	CD117	TdT
D	0.8	**89.9**	1.7	0.7	1.2	**22.2**	**33.6**	**39.5**	5.4	10.8	**28.1**	17.9	**17.7**	**33.7**	**29.5**
R	4.0	**96.7**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

The positive rates (%) of CD antigen expression in the bone marrow cells by CD45 low/SSC low gating are shown. The data at the initial diagnosis and at the relapse are shown in upper and lower lines, respectively. Positive data (more than 20%; MPO, more than 10%) are described in bold letters. Abbreviations: D, at the initial diagnosis; R, at the relapse; ND, not done.