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A Case of Therapy-related ALL with *MLL* Gene Rearrangement Following Treatment of Breast Cancer

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ALL with *MLL* gene rearrangement secondary to chemotherapy has been rarely reported. We report a case of therapy-related ALL (t-ALL) with *MLL* gene rearrangement in a patient who had undergone treatment for breast cancer. A 60-yr-old woman with breast cancer underwent breast-conserving surgery followed by 6 cycles of adjuvant chemotherapy (cyclophosphamide, epirubicin, and fluorouracil) and radiation therapy (dose, 5,040 cGy to the left breast and a 1,000 cGy boost to the tumor bed). A follow-up examination performed 14 months after the chemotherapy revealed no evidence of breast malignancy. However, the patient's complete blood cell count indicated acute leukemia: white blood cell count, 174.1×10^9/L with 88% blasts; Hb level, 12.5 g/dL; and platelet count, 103.0×10^9/L. Examination of the bone marrow aspirate smear revealed a high percentage of blasts (85.1% of all nucleated cells); the blasts showed a pro-B immunophenotype and were positive for CD19, CD79a, HLA-DR, CD34, and terminal deoxynucleotidyl transferase (TdT). Cytogenetic and FISH analyses revealed t(4;11)(q21;q23) and *MLL* gene rearrangement, respectively. The patient received induction chemotherapy with cyclophosphamide, vincristine, doxorubicin, and dexamethasone and achieved complete remission. Following consolidation chemotherapy, she underwent allogenic peripheral blood stem cell transplantation and has been clinically stable. To our knowledge, this is the first reported case of t-ALL with *MLL* gene rearrangement following treatment of breast cancer in Korea. *(Korean J Lab Med 2010;30:255-9)*

Key Words : *Therapy, ALL, Topoisomerase II inhibitor, MLL gene rearrangement*

INTRODUCTION

The widespread use of chemotherapy and the improvement in the survival rates of cancer patients has led to an increase in the cases of therapy-related leukemia. Therapy-related leukemias account for 10-20% of all cases of acute leukemia [1, 2], with therapy-related AML (t-AML) and therapy-related MDS (t-MDS) being the most common types of therapy-related leukemia. However, therapy-related ALL (t-ALL) is rare, and only few cases of t-ALL have been reported in the literature [3, 4]. In one study, t-ALL was reported to account for 10% of all therapy-related leukemias and for less than 2% of all lymphoid leukemias [5]. Thus far, 8 cases of therapy-related leukemia following systemic chemotherapy have been reported in Korea; among these, 7 cases were of t-AML and 1 was of t-ALL with t(9:22)(q34;q11.2) [6-10]. We report a case of t-ALL with *MLL* gene rearrangement in a patient who had undergone treatment for breast cancer and review the related literature.

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CASE REPORT

A 60-yr-old woman with breast cancer underwent breast-conserving surgery followed by 6 cycles of adjuvant chemotherapy (cyclophosphamide, epirubicin, and fluorouracil) and radiation therapy (dose, 5,040 cGy to the left breast and a 1,000 cGy boost to the tumor bed). A follow-up examination performed 14 months after the chemotherapy revealed no evidence of breast malignancy. However, the patient's complete blood cell count indicated acute leukemia: white blood cell count, 174.1×10^9/L with 88% blasts; Hb level, 12.5 g/dL; and platelet count, 103.0×10^9/L.

Examination of the bone marrow aspirate smear revealed a high percentage of blasts, which accounted for 85.1% of all nucleated cells (Fig. 1). The blasts showed block positivity for periodic acid-Schiff stain but were negative for myeloperoxidase (MPO) and esterase stains. The blast cells had a pro-B immunophenotype and were positive for CD19, CD79a, HLA-DR, CD34, and terminal deoxynucleotidyl transferase (TdT), and negative for CD10, CD13, CD33, and MPO. T-cell markers such as CD2, CD3, CD5, and CD7 were absent. Cytogenetic analysis of the bone marrow cells by using the Giemsa banding technique revealed the karyotype 46,XX,t(4;11)(q21;q23)[16]/46,XX[4]. FISH performed using LSI MLL dual-color, break-apart rearrangement probes (Vysis, Downers Grove, IL, USA) revealed *MLL* gene rearrangement in 91.5% of the analyzed cells (Fig. 2).

The patient was diagnosed with ALL and received induction chemotherapy with cyclophosphamide, vincristine, doxorubicin, and dexamethasone; she achieved complete remission. Subsequently, she received 3 courses of consolidation chemotherapy and 6 cycles of intrathecal chemotherapy according to the scheduled regimen. Then, she underwent allogenic peripheral blood stem cell transplantation from an HLA-matched male donor.

A follow-up bone marrow examination performed 1 month after transplantation revealed engraftment of trilineage cells. PCR analysis of short tandem repeats revealed complete donor chimerism, and FISH performed using a dual-color CEP X SpectrumOrange/CEP Y SpectrumGreen DNA probe kit (Vysis) showed XY signals in 100% of the analyzed cells. The patient was clinically stable at the follow-up examination and showed complete remission.

DISCUSSION

The clinical and cytogenetic pathologies of therapy-related leukemia can be attributed to 2 different classes of chemotherapeutic agents. Therapy-related leukemias induced by alkylating agents are preceded by myelodysplasia and are characterized by a prolonged latency period and unbalanced cytogenetic abnormalities of chromo-

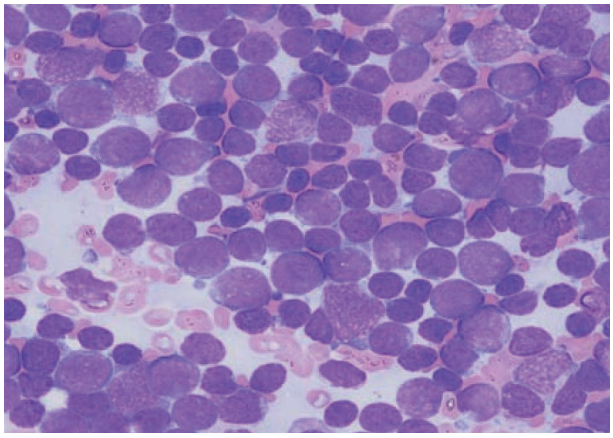

Fig. 1. Bone marrow aspirate smear showing lymphoblasts (Wright's stain, ×1,000).

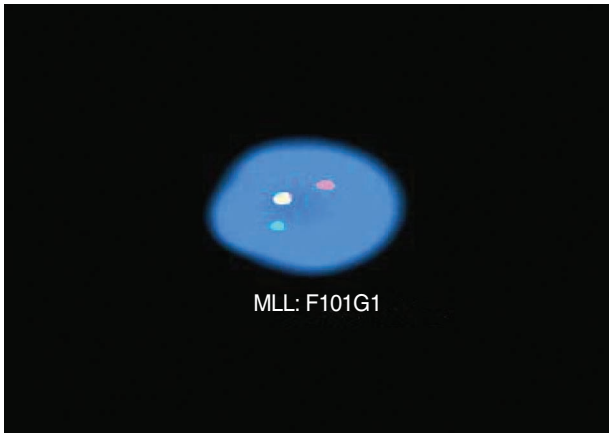

Fig. 2. FISH analysis showing *MLL* gene rearrangement with separate red and green signals (performed using LSI *MLL* dual-color, break-apart rearrangement probe, Vysis, USA; ×1,000).

some 5 and/or 7. In contrast, topoisomerase II inhibitor-related leukemias have a very short latency period without a preleukemic phase and are strongly associated with balanced translocations involving chromosome 11q23 (the site of the *MLL* gene) [11]. In this case, both DNA topoisomerase II inhibitor (epirubicin) and alkylating agent (cyclophosphamide) were used in the adjuvant chemotherapy for breast cancer. The incidence of DNA topoisomerase II inhibitor-related leukemia may be related to both the short latency period (14 months) after the initial chemotherapy and the balanced translocation of 11q23 with *MLL* gene rearrangement.

Most of the therapy-related leukemia cells have myeloid lineages, and the ratio of the cases of t-AML to those of t-ALL is 9/1; moreover only few studies have reported the clinical and laboratory characteristics of patients with t-ALL [3]. However, among therapy-related 11q23 abnormalities, the developments of t-AML and t-ALL show similar frequencies [12, 13]. Andersen et al. [4] reported that 22 cases of t-ALL with balanced translocations involving chromosome 11q23 have been described in the literature between 1992 and 2001. However, since 2001, only 9 such cases, including 2 cases reported by Andersen et al. [4], have been reported [13-15] (Table 1).

In all the 10 cases, including this case, patients were treated with regimens involving at least 1 topoisomerase II inhibitor. Although the latency period ranged from 10 months to 8 yr, the latency period in our case was short and less than 24 months. The leukemia cells in our case showed an immature pro-B immunophenotype and were negative for CD10. This finding is consistent with that reported by Pagano et al. [5], who compared 21 t-ALL cases with 695 *de novo* ALL cases [5]. They showed that the expression of B-cell and T-cell phenotypes in t-ALL

Table 1. Characteristics of the therapy-related ALL cases with 11q23 abnormalities reported since 2001

Cases	Sex/ Age	Primary malignancy	Treatment	RT	Secondary malignancy	Latency period (months)	Karyotype	Immunophenotype	Clinical status (months)*
Andersen et al. (2001) [4]	F/64	Breast cancer	Epirubicin	RT	Pro-B ALL	10	t(4;11)(q21;q23)	$CD10^-$, $CD19^+$, $CD22^+$, faint CD34, TdT^+	PR (NA)
	M/27	Testicular cancer	ETO, CIS, BLO	RT	Pro-B ALL	18	t(4;11)(q21;q23)	CD10, $CD19^+$, $CD22^+$, faint CD34	CR (NA)
Ishizawa et al. (2003) [13]	F/65	Follicular lymphoma	FLU, MIT, DEXA	ND	Pro-B ALL	13	t(4;11)(q21;q23)	$CD10^-$, $CD15^{+\dagger}$, $CD65^{+\dagger}$	Alive with disease (2.6)
	M/25	Hodgkin's lymphoma	MOPP/ABV, MIT, THIO, CYP with BMT	RT	Pro-B ALL	96	-X,t(2;15)(q?21;q15), der(11), t(11:?)(q23;?), del(13)(q14q34), etc	$CD10^-$, $CD15^{+\dagger}$, $CD65^{+\dagger}$	DFS (54)
	F/59	Breast cancer	CYP, DOX, 5-FU	ND	Pro-B ALL	24	t(4;11) (q21;q23), add (5), (q33)	$CD10^-$, $CD15^{+\dagger}$, $CD65^{+\dagger}$	DFS (21)
	F/60	Lung cancer	CAB, ETO	RT	Pro-B ALL	NA	t(4;11)(q21;q23)	$CD10^-$, $CD15^{+\dagger}$, $CD65^{+\dagger}$	Dead (0.4)
	M/69	Lung cancer	NA	NA	Pro-B ALL	NA	*MLL* rearrangement‡	$CD10^-$, $CD15^{+\dagger}$, $CD65^{+\dagger}$	NA
Tsujioka et al. (2003) [14]	M/57	APL with t(15;17)	ATRA, IDA, Ara-C, MIT, DNR, ETO	ND	Pro-B ALL	10	t(1;11)(p32;q23), t(15;17)(q22;q21)	$CD10^-$, $CD19^+$	Dead (10)
Millot et al. (2005) [15]	M/8	Burkitt's lymphoma	CYP, VIC, PD, MTX, DOX, Ara-C, ETO	ND	Pro-B ALL	13	t(4;11)(q21;q23), add(19)(q13)	$CD10^-$, $CD19^+$, $CD79a^+$, $HLA\text{-}DR^+$	CR (NA)
Present case (2010)	F/60	Breast cancer	CYP, EPR, 5-FU	RT	Pro-B ALL	14	t(4;11)(q21;q23)	$CD10^-$, $CD19^+$, $CD79a^+$, $HLA\text{-}DR^+$, $CD34^+$, TdT^+	CR with BMT (10)

*time of follow-up after the diagnosis of therapy-related leukemia; ‡aberrant expression; †confirmed by FISH.

Abbreviations: RT, radiation therapy; F, female; PR, partial remission; NA, not available; M, male; ETO, etoposide; CIS, cisplatin; BLO, bleomycin; CR, complete remission; FLU, fludarabine; MIT, mitoxantrone; DEXA, dexamethasone; ND, not done; MOPP, mustard/vincristine/prednisone/procarbazine; ABV, doxorubicin/bleomycin/vinblastine; THIO, thiotepa; CYP, cyclophosphamide; BMT, bone marrow transplantation; DFS, disease-free state; DOX, doxorubicin; 5-FU, 5-fluorouracil; CAB, carboplatin; APL, acute promyelocytic leukemia; ATRA, all-trans retinoic acid; IDA, idarubicin; Ara-C, cytosine arabinoside; DNR, daunorubicin; VIC, vincristine; PD, prednisone; MTX, methotrexate; EPR, epirubicin.

was not different from that in *de novo* ALL. However, the number of CD10-negative leukemia cells with immature pro-B immunophenotypes in t-ALL was greater than that in *de novo* ALL (8/20 vs. 128/609). Ishizawa et al. [13] reported 5 t-ALL cases with CD10-negative pro-B immunophenotype and aberrant expression of CD15 and CD65 (Table 1). Although the leukemia cells in this case showed the typical pro-B immunophenotype with CD10 negativity, these cells were not evaluated for aberrant expression of CD15 and CD65.

In cases of ALL with *MLL* rearrangement, especially t(4;11) translocation, the leukemia cells typically had a pro-B immunophenotype and were positive for CD19 and CD15 and negative for CD10 and CD24 [16]. Since all the 10 reported t-ALL cases had *MLL* rearrangements, the presence of CD10-negative cells with a pro-B immunophenotype and aberrant CD15 positivity may be a typical feature of ALL with *MLL* rearrangement, in the cases of both *de novo* ALL and t-ALL. However, in the series reported by Pagano et al. [5], there was no correlation between the pro-B immunophenotype and any specific cytogenetic abnormality. The predominance of the pro-B immunophenotype may be related to both *MLL* rearrangement and therapy-related leukemogenesis.

The prognosis of t-AML is generally known to be poorer than that of *de novo* AML, and the survival rates of patients with secondary leukemia are difficult to predict because of the recurrence of the primary cancer. However, recent studies have shown that the outcome of t-AML treatment does not differ from that of *de novo* AML treatment after adjustment for unfavorable cytogenetic findings [17]. The outcome of t-ALL treatment has been assessed only in small series, and the survival rates and rates of complete remission in cases of t-AML were not different from those in cases of t-ALL [3, 18].

Thus far, 8 cases of therapy-related leukemia, including 7 cases of t-AML and 1 case of t-ALL, have been reported in Korea [6-10] (Table 2). Similar to our case, the most

Table 2. Cases of therapy-related leukemia in Korea

Cases	Sex/ Age	Primary malignancy	Treatment	RT	Secondary malignancy	Latency period (months)	Karyotype	Immunophenotype	Clinical status (months)*
Song et al. (1992) [6]	M/15	Rhabdomyosarcoma	VIC, ACT-D, CYP, AD, CIS	RT	AML, M1	19	-8,+iso7q,del(7)(q32q36), 9p+,t(7;20)(p13;q13/1), t(9;11)(q13;q24), etc.	NA	Dead (0.4)
Hur et al. (1999) [7]	F/66	Lung cancer	ETO, IFS, CIS	RT	AML, M2	33	NA	NA	Alive with disease (4)
	M/7	ALL	I-ASP, MTX, DNR	RT	AML, M4	33	NA	NA	Dead (5)
	M/15	NHL	VIC, MTX, DNR, I-ASP, PD	RT	AML, M5b	24	NA	NA	Dead (2)
	F/18	ALL	I-ASP, VIC, MTX, DNR	RT	AML, M2	51	NA	NA	Dead (9)
Kim et al. (2006) [8]	F/43	Follicular lymphoma	FLU, MIT, DEXA	ND	AML, M4 with eosinophilia	10	inv(16)(p14.1q22), +8,t(9;22;14) (q34;q11.2;q22), etc.	$CD13^+$, $CD33^+$, $CD34^+$	CR with BMT (5)†
Lee et al. (2007) [9]	F/50	Ovarian cancer	CIS, PAT, TPT	ND	AML	43	inv(16)(p13.1q22),+22	$CD13^+$, $CD14^+$, $CD33^+$, $CD34^+$, $CD64^+$, $CD117^+$ $HLA\text{-}DR^+$, MPO^+	Dead (7)
Lee et al. (2009) [10]	F/60	Breast cancer	CMF, EPR, PAT	RT	Pre-B ALL	48	t(9;22)(q34;q11.2),-7	$CD10^+$, $CD19^+$, $CD79a^+$, TdT^+, $CD13^{+‡}$, $CD33^{+‡}$	Follow-up loss after diagnosis

*time of follow-up after the diagnosis of therapy-related leukemia; †time of follow-up after BMT; ‡aberrant expression.
Abbreviations: RT, radiation therapy; M, male; VIC, vincristine; ACT-D, actinomycin-D; CYP, cyclophosphamide; AD, adriamycin; CIS, cisplatin; NA, not available; F, female; ETO, etoposide; IFS, ifosfamide; L-ASP, L-asparaginase; MTX, methotrexate; DNR, daunorubicin; NHL, non-Hodgkin's lymphoma; PD, prednisone; FLU, fludarabine; MIT, mitoxantrone; DEXA, dexamethasone; ND, not done; CR, complete remission; BMT, bone marrow transplantation; PAT, paclitaxel; TPT, topotecan; CMF, cyclophosphamide/methotrexate/5-fluorouracil; EPR, epirubicin; 5-FU, 5-fluorouracil.

recent case of t-ALL was that of a patient with breast cancer and was related to the use of topoisomerase II inhibitor. However, the leukemia cells in the previous case showed a CD10-positive pre-B immunophenotype with aberrant expressions of CD13 and CD33 and the karyotype t(9;22)(q34;q11.2) [10].

In summary, this case is the second reported case of t-ALL and the first case of t-ALL with *MLL* rearrangement in Korea. This case showed the typical immunophenotypic and cytogenetic features of t-ALL with a CD10-negative pro-B immunophenotype and *MLL* rearrangement. Our findings suggest that t-ALL can be attributed to the use of topoisomerase II inhibitor. On the basis of the findings of our study and those of previous studies, the predominance of pro-B immunophenotype can be attributed to both *MLL* rearrangement and therapy-related leukemogenesis.

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