Brief report

Clinical and genetic features of therapy-related myeloid neoplasms after chemotherapy for acute promyelocytic leukemia

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Acute promyelocytic leukemia (APL) is a highly curable disease with excellent complete remission and long-term survival rates. However, the development of therapy-related myeloid neoplasms (t-MN) is being reported with increasing frequency in patients successfully treated for APL. We attempted to clarify the different clinical features and hematologic findings between t-MN and relapse cases, and to identify gene alterations involved in t-MN. We compared 10 relapse and 11 t-MN cases that developed in 108 patients during their first complete remission from APL. At APL diagnosis, t-MN patients had lower white blood cell counts than did relapse patients ($P = .048$). Overall survival starting from chemotherapy was significantly worse in t-MN patients than in relapse patients ($P = .022$). The t-MN cases were characterized as CD34$^+$/HLA-DR$^+$ and *PML-RARA*$^-$, and 4 *RUNX1/AML1* mutations were detected. T-MN is easily distinguished from APL relapse by evaluating these hematologic features, and it may originate from primitive myeloid cells by chemotherapy-induced *RUNX1* mutations. (*Blood*. 2010;116(26):6018-6022)

Introduction

Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia (AML) characterized by a t(15;17) translocation leading to a *PML-RARA* fusion gene. APL is a highly curable disease with excellent complete remission (CR) and long-term survival rates. All-*trans* retinoic acid (ATRA) combined with anthracycline-based chemotherapy yields a CR rate of approximately 90% for newly diagnosed APLs. The relapse rate is approximately 20%, and with the development of new molecular target therapies such as arsenic trioxide, a cure can now be expected even for relapsed patients.

However, the development of therapy-related myeloid neoplasms (t-MN) is being reported with an increasing frequency of 0.97% to 6.5% in patients successfully treated for APL.[1-3] Patients are typically in hematologic and cytogenetic remission for APL, and at follow-up after treatment, hematologic abnormalities may be detected without evidence of the original *PML-RARA* fusion gene seen at APL diagnosis. The t-MN secondary to APL is usually difficult to treat, and it is one of the prognosis-limiting factors for the curable APL disease. Therefore, we attempted to clarify the different clinical features and hematologic findings between t-MN and relapsed APL cases, and to identify gene alterations associated with t-MN.

Methods

Patients

Patients with APL were diagnosed at Hiroshima University Hospital and its affiliated hospitals between 1996 and 2008. Diagnoses of APL were initially made morphologically according to the French-American-British classification and were confirmed by the presence of t(15;17) and/or presence of the *PML/RARA*. Patients were treated with ATRA combined with chemotherapy and with intensified maintenance chemotherapy.[4,5] Patients who were resistant to ATRA therapy were treated with arsenic trioxide. Drugs used during APL treatment were idarubicin, cytarabine, mitoxantrone, daunorubicin, etoposide (VP-16), behenoyl cytarabine, mercaptopurine, vindesine, and aclarubicin. All patients received an intrathecal administration of methotrexate, cytarabine, and prednisolone during consolidation therapy. Patients with APL were examined as approved by the Institutional Review Board at Hiroshima University. Patients gave written informed consent in accordance with the Declaration of Helsinki.

Identification of *RUNX1*, *CEBPA*, *FLT3*, and *NRAS* mutations

Mononuclear cells were isolated from bone marrow samples, and genomic DNA and total RNA were prepared as described previously.[6] Identification of *RUNX1*, *CEBPA*, *FLT3*, and *NRAS* mutations was performed as described previously.[6-8]

Statistical analysis

Probabilities of survival were estimated using the Kaplan-Meier method and compared by the log-rank test.

Results and discussion

A total of 124 patients with APL were newly diagnosed and were consecutively enrolled in the study. The median age at diagnosis was 52 years (range, 18-86 years). An 86-year-old patient died of

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Table 1. Treatments for primary APL and for t-MN

	At APL				At t-MN	
Disease type/patient no.	Protocol*	VP-16 in consolidation therapy, mg/m²†	VP-16 in maintenance therapy, mg/m²‡	Presence of myelodysplastic phase	Therapy	Outcome
t-MN with progression to AML						
10	APL92	500	480	+	Intensive chemotherapy	Died of disease progression
18	APL97	500	480	+	Intensive chemotherapy	Died of disease progression
19	APL97	500	480	+	Intensive chemotherapy	Died of disease progression
22	APL97	500	480	–	Intensive chemotherapy	Died of disease progression
48	APL97	500	480	+	Intensive chemotherapy	Alive in CR
79	APL97	500	480	+	Intensive chemotherapy	Died of disease progression
83	APL97	500	480	+	Intensive chemotherapy	Died of disease progression
108	APL97	500	0	+	Intensive chemotherapy then allogeneic stem cell transplantation	Died of disease progression
109	APL97	500	0	+	Intensive chemotherapy	Died of disease progression
t-MN without progression to AML (t-MDS-RCMD)						
7	APL92	500	480	+	Supportive care	Alive without progression
49	APL97	500	480	+	Supportive care	Alive without progression

*Two consecutive protocols (APL92[4] or APL97[5] of the Japan Adult Leukemia Study Group) were used.
†A total of 100 mg/m² for 5 days.
‡A total of 80 mg/m² on days 1, 3, and 5 in the second and the sixth course.

lung hemorrhage before starting induction therapy. Other patients received induction therapy, including ATRA with chemotherapy. Two patients were resistant to ATRA therapy and were treated with arsenic trioxide. Six patients who were 70 years of age or older died during induction therapy, whereas 117 patients (94.4%) achieved CR. After achieving CR, 2 patients dropped out of the study because they moved to a distant place, and 2 patients could not continue the therapy because of neuropsychologic diseases and died within 1 year. Others received intensive consolidation chemotherapy, but 5 patients died of therapy-related complications. All of the remaining 108 patients completed consolidation therapy. The predicted 10-year overall survival was 74.6% for the whole population, and 81.4% and 36.9% in patients younger than 70 years and 70 years or older, respectively. Thus, the efficacy of the treatment for APL patients in this study is comparable with that reported in clinical trials.[4,5]

After a median follow-up of 8.6 years (range, 1.7-16.3 years), 5 patients died of nonhematologic causes, 10 patients (9.3%) relapsed, and 11 patients (10.2%) developed t-MN among the 108 patients during their first CR from APL. We also analyzed 61 patients with *RUNX1-ETO* or *CBFB-MYH11* (core-binding factor leukemia) during the same research period and found 13 relapses (21.3%) but no t-MN. Thus, there is a possibility that t-MN after successful treatment of APL may be more popular than AML other than APL. We noted more t-MN patients instead of fewer relapse patients compared with other reports. The fact that all patients in this study had received VP-16 for consolidation therapy whereas no VP-16 was administered in other trials could explain, in part, the higher incidence of t-MN.[3] Furthermore, the accumulation of chemotherapeutic agents in the maintenance phase may increase the risk of t-MN.[5] Meanwhile, there is a possibility that t-MN might have been diagnosed as relapsing APL because of positive *PML-RARA* from coexisting APL cells. Therefore, we attempted to clarify the differences in clinical features and hematologic findings between t-MN and relapsed APL patients after successful treatment of APL.

The patients who developed t-MN with progression to AML, therapy-related myelodysplastic syndrome, refractory cytopenia with multilineage dysplasia (t-MDS-RCMD), or relapse are shown in Tables 1 and 2. The median white blood cell (WBC) count at APL diagnosis was 6940/μL (range, 1.1-42.6/μL) in the t-MN patients and 33 630/μL (range, 1.1-148.6/μL) in the relapse patients, whereas it was 13 130/μL (range, 0.3-152.8/μL) in the disease-free patients who remained continuously in their first CR for 1.5 to 16.1 years (median, 8.3 years). The WBC count was significantly lower in the t-MN patients than in the relapse patients ($P = .048$). Relapse risk based on pretreatment WBC count and platelet count indicated that most of the t-MN patients were distributed in low- and intermediate-risk groups (Figure 1A). In particular, the WBC count at APL diagnosis was less than or equal to 4500/μL in most of the t-MN patients, whereas it was more than 4500/μL in the relapse patients. Previous reports also showed that most of the t-MN patients had a low WBC count.[3,9] After the first APL diagnosis, the median intervals were 2.6 years (range, 0.6-10.1 years) to relapse, 2.3 years (range, 1.6-3.0 years) to t-MDS-RCMD, and 3.3 years (range, 1.0-9.7 years) to t-MN with progression to AML ($P = .83$). However, overall survival from the start of chemotherapy was significantly worse for the t-MN with progression to AML patients than for the relapse patients ($P = .022$, Figure 1B). VP-16 or intensive maintenance chemotherapy may reduce the risk of relapse; however, the frequency of t-MN may increase. Recent therapeutic approaches in the treatment of refractory APL may improve the second CR rate. Our results suggest that preventing t-MN may be more important than preventing relapse, especially in patients with low WBCs.

We examined surface markers of the leukemic cells (Figure 1C). At the primary APL diagnosis, most of the leukemic cells from the APL patients showed $CD34^-HLA\text{-}DR^-CD33^+$ phenotypes. At relapse, the phenotypes showed the same pattern, whereas at the t-MN CD34 and HLA-DR phenotypes changed to positive and the positivity of CD33 became lower. The relapse patients and the t-MN patients were easily distinguished by their CD34/HLA-DR distribution pattern; APL cells were $CD34^-HLA\text{-}DR^-$, whereas t-MN cells were $CD34^+$ and/or $HLA\text{-}DR^+$ (Figure 1D). These results indicate that the leukemic cells changed to primitive myeloid cells when t-MN developed after successful treatment of APL, and they were considered to have an independent clonal origin.[10]

Table 2. Clinical features and genetic findings for the patients with t-MN and relapsed APL

Disease type/ patient no.	At APL diagnosis (A)							Interval from A to B, y	At relapse or t-MN (B)					Survival from B, y
	Age, y/sex	WBCs, $\times 10^9$/L	Platelets, $\times 10^9$/L	FAB classification	Karyotype*	*PML-RARA*	Class I mutations†		FAB classification	Karyotype*	*PML-RARA*	Other class II abnormalities†	Class I mutations†	
t-MN with progression to AML														
10‡	38/F	1.10	17	M3	46,XX[20]	+	−	5.1	RAEBt	45,XX,-7[19]/46,idem,+21[1]	−	*RUNX1* D171N	*NRAS* G12V	0.8
18	38/M	1.10	43	M3	46,XY,t(15;17)(q22;q21)[17]/ 46,XY[3]	+	−	4.0	RAEB	45,XY,-7[3]/46,XY[17]	−	*RUNX1* D171G	−	2.0
19	35/M	42.60	10	M3	46,XY,t(15;17)(q22;q21)[20]	+	−	2.4	RAEBt	46,XY,t(7;15)(q11;q11),der(12)t(12;17)(p11;q21), t(16;21)(q24;q22), add(17)(q11),add(19)(p13), del(21)(q21)[3]/46,idem, der(18)t(15;18)(q11;p11) [6]/ 46,XY[11]	−	*RUNX1-MTG16*	−	1.9
22	48/M	1.80	110	M3	46,XY,t(15;17)(q22;q21)[18]/ 46,XY[2]	+	−	9.7	M0	46,XY,add(13)(q32)[19]/46,XY[1]	−	−	−	0.8
48	57/F	4.10	16	M3	46,XX,t(15;17)(q22;q21)[19]/ 46,XX[1]	+	−	1.4	M1	46,XX,t(6;11)(q21;q23)[16]/46,XX[4]	−	*MLL-FOXO3*	−	> 8.7
79	62/M	2.20	54	M3	46,XY,t(15;17)(q22;q21)[19]/ 46,XY[1]	+	−	2.6	RAEB	46,XY,add(2)(p23),inv(5)(p11q13), add(11)(q23)[14]/46,idem, inv(2) (p23q11)[4]/47,idem,+13[2]	−	*RUNX1* S295fsX571	−	1.3
83	42/M	1.20	144	M3	46,XY,t(15;17)(q22;q21)[17]/ 46,XY[3]	+	−	2.5	RAEB	45,XY,-7[19]/46,XY[1]	−	*RUNX1* G172W	−	1.7
108	18/M	14.40	36	M3	46,XY,t(15;17)(q22;q21)[20]	+	−	1.0	M4	46,XY,t(11;16)(q23;p13.3)[6] / 46,XY[14]	−	*MLL-CBP*	*FLT3*ITD	2.6
109	65/M	1.68	41	M3	46,XY,t(15;17)(q22;q21)[20]	+	−	1.3	RAEB	46,XY[20]	−	*CEBPA* Q305P	−	1.7
t-MN without progression to AML (t-MDS-RCMD)														
7	54/F	4.50	6	M3	46,XX,t(15;17)(q22;q21)[20]	+	−	1.6	RA	46,XX,del(20)(q11)[15]/46,XX[5]	−	−	−	> 13.4
49	67/M	1.62	42	M3	45,X,-Y,t(15;17)(q22;q21), del(14)(q13q22)[18]/ 46,XY[2]	+	−	3.0	RA	46,XY,del(20)(q1?)[3]/46,XY[17]	−	−	−	> 6.9
Relapse														
8	54/M	1.30	258	M3	46,XY,t(15;17)(q22;q31)[20]	+	−	3.4	M3	46,XY,add(9)(q22),add(13)(p11), t(15;17)(q22;q21)[3]/46,XY[17]	+	−	−	> 11.4
12	32/M	12.67	7	M3	46,XY,t(9;X)(p24;q22), t(15;17)(q22;q21)[20]	+	−	0.6	M3	ND	+	−	*FLT3*ITD	2.4§
13	38/M	95.70	35	M3	47,XY,+mar1[17]/46,XY[3]	+	−	10.1	M3	48,XY,+mar1x2[13]/46,XY[7]	+	−	−	> 3.6
17	53/F	4.80	52	M3	46,XX[20]	+	−	2.0	M3	46,XX[20]	+	−	−	> 11.1
25	58/F	148.60	31	M3v	46,XX[20]	+	*FLT3*ITD	1.5	M3v	46,XX[20]	+	−	*FLT3*ITD	0.8
38	64/F	15.70	27	M3	46,XX,t(15;17)(q22;q21)[20]	+	*FLT3*ITD	1.0	M3	46,XX,t(15;17)(q22;q21)[20]	+	−	*FLT3*ITD	1.7
78	39/M	7.03	13	M3	48,XY,+8,+8,t(15;17)(q22;q21) [10]/49,idem,+21[9]/46,XY[1]	+	−	1.1	M3	ND	+	−	−	0.9‖
85	19/M	1.10	152	M3v	49,XY,t(15;17)(q22;q21),+mar1, +mar2, +mar3[16]/46,XY[4]	+	−	2.5	M3v	49,XY,t(15;17)(q22;q21),+mar1, +mar2, +mar3[15]/46,XY[5]	+	−	−	> 4.2
88	46/M	4.60	47	M3	46,XY[20]	+	*FLT3*ITD	1.8	M3	46,XY[20]	+	−	*FLT3*ITD	> 4.8
95	55/M	44.80	21	M3v	46,XY,t(15;17)(q22;q31)[20]	+	−	1.8	M3v	46,XY,t(15;17)(q22;q21)[17] / 46,XY[3]	+	−	−	> 4.0

M3v indicates M3 variant; ND, not done; +, positive; and −, negative.

*Numbers in square brackets are cell numbers.

†Class I mutations include the *FLT3* and *NRAS* mutations, and other class II abnormalities include the chimeric proteins and the *RUNX1* and *CEBPA* mutations.

‡This patient was reported previously[6] (case 8).

§This patient died from an accident during the third CR.

‖This patient refused reinduction therapy and died of cerebral bleeding.

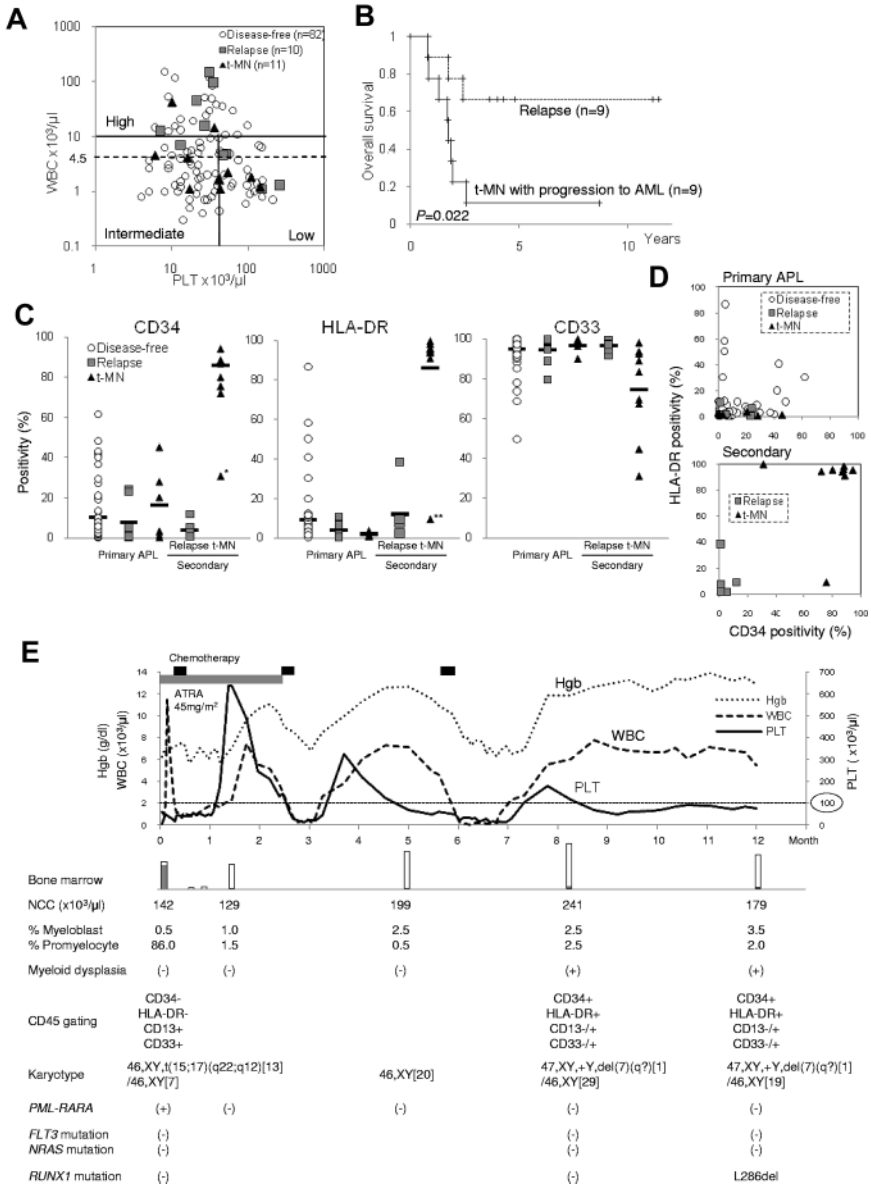

Figure 1. T-MN is easily distinguished from APL relapse by evaluating hematologic features. (A) Relapse risk was predicted by pretreatment WBC and platelet counts of each patient at APL diagnosis. Patients with APL could be stratified into low-risk (WBC count $\leq 10 \times 10^3/\mu L$, platelet count $> 40 \times 10^3/\mu L$), intermediate-risk (WBC count $\leq 10 \times 10^3/\mu L$, platelet count $\leq 40 \times 10^3/\mu L$), and high-risk (WBC count $> 10 \times 10^3/\mu L$) groups. The number of patients in high-, intermediate-, and low-risk groups were 21 (25.6%), 40 (48.8%), and 21 (25.6%), respectively, in the disease-free population, whereas they were 5 (50%), 1 (10%), and 4 (40%), respectively, in the relapse patients and 2 (18.2%), 3 (27.3%), and 6 (54.5%), respectively, in the t-MN patients. (B) Overall survival from date of starting chemotherapy for the relapsed APL or t-MN patients after successful APL treatment. Patients with t-MDS-RCMD and patient 78 who were treated without chemotherapy were excluded. (C) Surface-marker analysis of the leukemic cells by the CD45RO gating method. Because of their low blast percentages, t-MDS-RCMD patients were excluded. *Patient 48 and **patient 109 were mixed lineage leukemia with *MLL* chimera. (D) APL relapse and t-MN were distinguished by CD34/HLA-DR expression patterns. (E) Clinical course of a 69-year-old man. The patient developed APL in 2009, although he had a normal blood count until 2008. Eight months after starting the treatment for APL with ATRA and chemotherapy, the patient showed persistent thrombocytopenia and trilineage dysplasia (megaloblastic changes of erythroblasts, decreased granules of granulocytes, and micromegakaryocytes), and he was diagnosed as t-MN.

To confirm their clonality, gene abnormalities that may play an important role in leukemogenesis were analyzed.[11] All of the relapse patients had the *PML-RARA* gene, whereas none of the patients with t-MN had *PML-RARA*. Instead, we found translocations involving 21q22 of *RUNX1* (*RUNX1-MTG16*) or 11q23 of *MLL* (*MLL-FOXO3* and *MLL-CBP*; Table 2), as previously reported.[9,12,13] Both of the t-MDS-RCMD patients had an isolated del(20q), which may be associated with a good prognosis. Furthermore, 4 *RUNX1* mutations and one *CEBPA* mutation were detected. These abnormalities were not detected at the primary APL diagnosis or in the relapsed patients with APL or core-binding factor leukemia. *RUNX1* mutations are known to be frequently involved in t-MN; however, no such reports included t-MN from APL. One patient with a *RUNX1* mutation also acquired an *NRAS* mutation at t-MN. Three of 4 *RUNX1* mutations were associated with monosomy 7, which is frequently reported in t-MN after successful treatment of APL.[3,9] It is possible that previously reported monosomy 7 cases were also associated with *RUNX1* mutations, but this was not investigated in the earlier studies.

In addition, a recent case of t-MN at 8 months after APL diagnosis showed a phenotype of increasing $CD34^+HLA\text{-}DR^+$ cells, gating on the CD45RO (Figure 1E). Karyotypic analysis showed a del(7q) abnormality, although the percentage of blasts was less than 5% in the bone marrow, and the *RUNX1* mutation was detected at 12 months. Monitoring of surface markers, karyotype, and the *RUNX1* mutation, as

well as *PML-RARA* monitoring, may be useful for the early diagnosis of t-MN in patients successfully treated for APL.

A previous report showed that *RUNX1* mutations may have a leukemogenic potential in $CD34^+$ cells from patients in the chronic phase of myeloproliferative neoplasms.[7] In addition, in APL, it is assumed that *RUNX1* or other abnormalities may be induced in $CD34^+$ cells during chemotherapy resulting in t-MN after successful treatment of APL. Some patients with *JAK2*$V617F^+$ myeloproliferative neoplasms were reported to transform to *JAK2*$V617F^-$ AML, suggesting that leukemia developed from a pre-*JAK2* hematopoietic stem cell[14] or from a normal hematopoietic stem cell.[15] *PML-RARA*$^-$ t-MN may also develop from a "receptive" hematopoietic stem cell or from a normal hematopoietic stem cell, which is a myeloid committed progenitor, by the accumulation of chemotherapy-induced gene abnormalities, including *RUNX1* mutations.

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Authorship

Contribution: J.I. collected data and wrote the paper; Y.H. assembled and analyzed the data and revised the manuscript; T.S., H.T., Y.O., H. Hyodo, and A.K. provided patient samples and clinical information; and H. Harada designed the research and revised the manuscript.

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