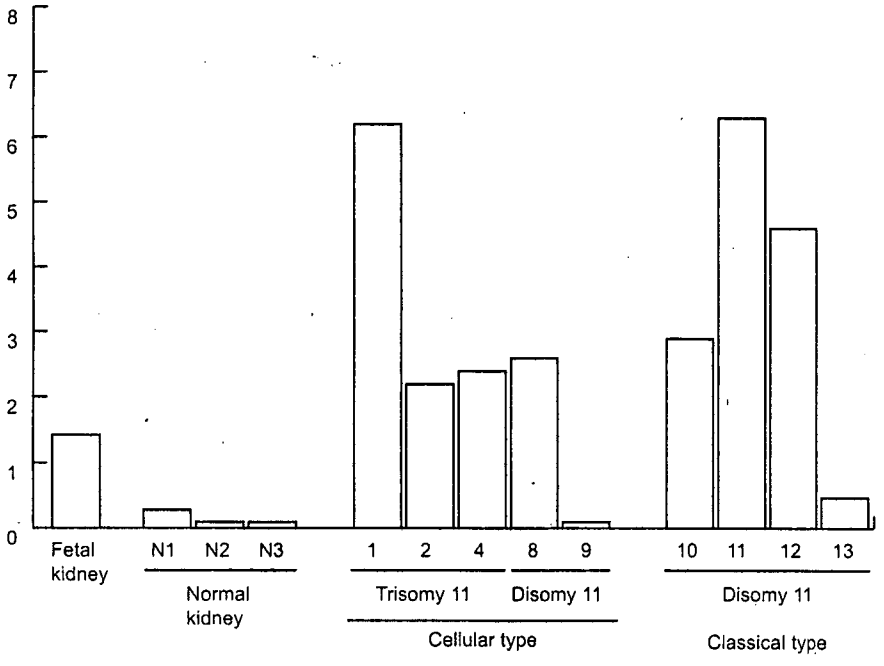

Figure 4. Relative mRNA expression (Y axis) of *IGF2* is plotted in three cellular type tumors with trisomy 11, in two cellular type tumors with disomy 11, in four classical type tumors with disomy 11, in fetal kidney total RNA, and in three normal kidney tissues.

genetic studies have disclosed that the *ETV6-NTRK3* fusion gene is always present in cellular or mixed type CMN and CFS, but not in classical type CMN and IFS (Knezevich et al., 1998a; Rubin et al., 1998; Watanabe et al., 2002). The most common chromosome change in cellular or mixed type CMN is trisomy 11, followed by trisomy 8, 17, or 20. These findings were detected by the classical cytogenetic method or FISH analysis using centromere probes, and there seems to have been no mCGH study on various CMN tumors to disclose the whole cytogenetic pattern. The present mCGH and RT-PCR study showed that cellular or mixed type tumors characterized by the *ETV6-NTRK3* fusion gene are classified into tumors with trisomy 11, and tumors with disomy 11 and other chromosomal changes, and that classical type tumors are characterized by the absence of *ETV6-NTRK3* and no chromosomal changes.

Nonrandom extra chromosomes 7 and 10 are found in hereditary papillary renal carcinoma and MEN2-associated pheochromocytoma, respectively, and mutated *MET* or *RET* is shown to be present on duplicated chromosomes (Zhuang et al., 1998; Huang et al., 2000). Both groups of investigators suggested that cells containing two mutant *MET* or *RET* alleles may gain a growth advantage and eventually develop into tumors. These findings made us speculate that active growth-promoting genes or mutated oncogenes may be present in duplicated chromosome 11 in cellular or mixed type CMN. The prime candidate is the *IGF2* gene, because the overexpression of *IGF2* mRNA has been reported in CMN (Sharifah et al., 1995; Morrison et al., 2002). LOH with loss of the maternal allele or LOI, resulting in the overexpression of *IGF2* mRNA, has been reported in other embryonal tumors, including Wilms tumor, rhabdomyosarcoma, and hepatoblastoma (Zhan et al., 1994; Rainier et al., 1995; Ravenel et al., 2001; Yuan et al., 2005; Watanabe et al., 2006). In addition, in an experimental system using the *ETV6-NTRK3* fusion vector and R-murine fibroblasts derived from mice with targeted disruption of the IGF1 receptor gene (*IGFRI*), Morrison et al. (2002) showed that an intact IGF signaling axis is essential for in vitro *ETV6-NTRK3* mediated transformation. These findings suggest that the *ETV6-NTRK3* fusion gene and *IGF2* may collaborate in the tumorigenic process of CMN.

IGF2 is a fetal polypeptide growth factor, which is expressed at high levels in embryos, and continues to be expressed only in the liver after birth (Foulstone et al., 2005). Thus, normal kidney tissues of young children express almost no or small amounts of *IGF2* mRNA, and fetal kidney tissues express moderate amounts of *IGF2* mRNA, as shown in the present study. Elevated expression

levels of *IGF2* mRNA detected in three of four classical type tumors indicate that IGF2 is needed for development of the majority of classical type tumors. Elevated expression levels were also found in all three cellular type tumors with trisomy 11 and one of two cellular type tumors with disomy 11. The present study showed duplication of the paternal *IGF2* allele in trisomy 11, analyzing the methylation status of CTCF6 in *H19*-DMR. The expression levels of cellular type tumors with trisomy 11 were high; however, the levels were not necessarily twice as high as those of cellular or classical type tumors with disomy 11 (Table 1 and Fig. 4). The findings may be explained by the speculation that tumors with trisomy 11 expressed *IGF2* mRNA twice as much as those with disomy 11 at the critical time of tumorigeneis, but not at the time of surgical resection. Thus, CMN tumors with trisomy 11 overexpress *IGF2* mRNA by the mechanism of duplication of the paternal *IGF2* allele. Similar to the role of mutated oncogenes in the tumorigenesis of hereditary tumors, duplicated paternal *IGF2* may provide CMN or its precursor cells with a proliferative advantage. Non-random chromosome gains in cancer may be a mechanism to increase the copy number of genes that are genetically or epigenetically altered for the acquisition of a growth advantage.

Histological studies have suggested that cellular type CMN might arise from classical type CMN (Knezevich et al., 1998a). The present study showed elevated expression levels of *IGF2* mRNA in the majority of both cellular and classical type tumors, and the *ETV6-NTRK3* fusion transcript in only cellular or mixed type tumors, but not in classical type tumors. Some investigators reported that the overexpression of *IGF2* mRNA promotes tumor susceptibility (Feinberg and Tycko, 2004; Prawitt et al., 2005). It is tempting to speculate that the overexpression of *IGF2* mRNA helps some classical type tumors acquire the *ETV6-NTR3* fusion gene. In contrast, some investigators previously reported that trisomy 11 is secondary to other genetic events in some CFS and cellular type CMN tumors (Schofield et al., 1993, 1994). In addition, one experimental study reported that IGF2 plays a role as the second signal in oncogene-induced tumorigenesis (Christofori et al., 1994). Thus, *IGF2* clearly plays an important role in the tumorigenic process of CMN as the primary genetic event shown in classical type tumors, or as the secondary genetic event shown in cellular type tumors with trisomy 11, although it seems difficult to assess the exact role at present.

Previous studies reported LOI or LOH of *IGF2* in CMN with disomy 11 (Becroft et al., 1995; Speleman et al., 1998). Furthermore, Huang et al. (2000) showed that there was either duplication of the mutant *RET* allele in trisomy 10 or loss of the wild-type *RET* allele in disomy 10 in *MEN2*-associated pheochromocytoma. Thus, we speculated that CMN tumors with disomy 11 might have LOI or LOH of *IGF2*; however, we found almost equal percentages of CTCF6 methylation indicating ROI of *IGF2*, but not LOI or LOH of *IGF2* in two cellular type tumors with disomy 11. As the two CMN tumors with LOI or LOH of *IGF2* were reported before identification of the *ETV6-NTRK3* gene (Becroft et al., 1995; Speleman et al., 1998), and histological diagnosis is sometimes difficult in differentiating CMN from clear cell sarcoma of the kidney, LOI or LOH of *IGF2* is unlikely to occur even in CMN tumors with disomy 11 and *ETV6-NTRK3*.

ACKNOWLEDGMENTS

The authors thank the physicians who participated in the Japan Wilms tumor study group and supplied samples for this study.

REFERENCES

Becroft DMO, Mauger DC, Skeen JE, Ogawa O, Reeve AE. 1995. Good prognosis of cellular mesoblastic nephroma with hyperdiploidy and relaxation of imprinting of the maternal *IGF2* gene. Pediatr Pathol Lab Med 15:679–688.

Christofori G, Naik P, Hanahan D. 1994. A second signal supplied by insulin-like growth factor II in oncogene-induced tumorigenesis. Nature 369:414–418.

D'Angio GJ, Breslow N, Beckwith JB, Evans A, Baum H, deLorimier A, Fernbach D, Hrabovsky E, Jones B, Kelalis P, Othersen HB, Tefft M, Thomas PRM. 1989. Treatment of Wilms' tumor: Results of the third national Wilms' tumor study. Cancer 64:346–360.

Feinberg AP, Tycko B. 2004. The history of cancer epigenetics. Nat Rev Cancer 4:143–153.

Foulstone E, Prince S, Zaccheo O, Burns JL, Harper J, Jacobs C, Chrurch D, Hassan AB. 2005. Insulin-like growth factor ligands, receptors, and binding proteins in cancer. J Pathol 205:145–153.

Huang SC, Koch CA, Vortmeyer AO, Pack SD, Lichtenauer UD, Mannan P, Lubensky IA, Chrousos GP, Gagel RF, Pacak K, Zhuang Z. 2000. Duplication of the mutant *RET* allele in trisomy 10 or loss of the wild-type allele in multiple endocrine neoplasia type 2-associated pheochromocytomas. Cancer Res 60:6223–6226.

Japanese Pathological Society. 1988. Committee on histological classification of childhood tumors: Tumors of the urinary system. Tokyo: Kanahara Shuppan.

Knezevich SR, Garnett MJ, Pysher TJ, Beckwith JB, Grundy PE, Sorensen PHB. 1998a. *ETV6-NTRK3* gene fusions and trisomy 11 establish a histogenetic link between mesoblastic nephroma and congenital fibrosarcoma. Cancer Res 58:5046–5048.

Knezevich SR, McFadden DE, Tao W, Lim JF, Sorensen PHB. 1998b. A novel *ETV6-NTRK3* gene fusion in congenital fibrosarcoma. Nat Genet 18:184–187.

Kumon K, Kobayashi H, Namiki T, Tsunematsu Y, Miyauchi J, Kikuta A Horikoshi Y, Komada Y, Hatae Y, Eguchi H, Kaneko Y. 2001. Frequent increase of DNA copy number in the 2q24 chromosomal region and its association with a poor clinical outcome in hepatoblastoma: Cytogenetic and comparative genomic hybridization analysis. Jpn J Cancer Res 92:854–862.

Morrison KB, Tognon CE, Garnett MJ, Deal C, Sorensen PHB. 2002. ETV6–NTRK3 transformation requires insulin-like growth factor 1 receptor signaling and is associated with constitutive IRS-1 tyrosine phosphorylation. Oncogene 21:5684–5695.

Prawitt D, Enklaar T, Gartner-Rupprecht B, Spangenberg C, Oswald M, Lausch E, Schmidtke P, Reutzel D, Fees S, Lucito R, Korzon M, Brozek I, Limon J, Housman DE, Pelletier J, Zabel B. 2005. Microdeletion of target sites for insulator protein CTCF in a chromosome 11p15 imprinting center in Beckwith-Wiedemann syndrome and Wilms' tumor. Proc Natl Acad Sci USA 102:4085–4090.

Rainier S, Dobry CJ, Feinberg AP. 1995. Loss of imprinting in hepatoblastoma. Cancer Res 55:1836–1638.

Ravenel JD, Broman KW, Perlman EJ, Niemitz EL, Jayawardena TM, Bell DW, Haber DA, Uejima H, Feinberg AP. 2001. Loss of imprinting of insulin-like growth factor-II (IGF2) gene in distinguishing specific biologic subtypes of Wilms tumor. J Natl Cancer Inst 93:1698–1703.

Rubin BP, Chen CJ, Morgan TW, Xiao S, Grier HE, Kozakewich HP, Perez-Atayde AR, Fletcher JP. 1998. Congenital mesoblastic nephroma t(12;15) is associated with ETV6-NTRK3 gene fusion: Cytogenetic and molecular relationship to congenital (infantile) fibrosarcoma. Am J Pathol 153:1451–1458.

Satoh Y, Nakadate H, Nakagawachi T, Higashimoto K, Joh K, Masaki Z Uozumi J, Kaneko Y, Mukai T, Soejima H. 2006. Genetic and epigenetic alterations in the short arm of chromosome 11 are involved in a majority of sporadic Wilms' tumor. Br J Cancer 95:541–547.

Schofield DE, Yunis EJ, Fletcher JA. 1993. Chromosome aberrations in mesoblastic nephroma. Am J Pathol 143:714–724.

Schofield DE, Fletcher JA, Grier HE, Yunis EJ. 1994. Fibrosarcoma in infants and children. Application of new techniques. Am J Surg Pathol 18:14–24.

Sharifah NA, Yun K, McLay J. 1995. Insulin-like growth factor II gene expression by congenital mesoblastic nephroma. Diagn Mol Pathol 4:279–285.

Speleman F, van den Berg E, Dhooge C, Oosterhuis W, Redeker B, De Potter CR. Tamminga RY, Van Roy N, Mannens M. 1998. Cytogenetic and molecular analysis of cellular atypical mesoblastic nephroma. Genes Chromosomes Cancer 21:265–269.

Takai D, Gonzales FA, Tsai YC, Thayer MJ, Jones PA. 2001. Large scale mapping of methylcytosines in CTCF-binding sites in the human *H19* promoter and aberrant hypomethylation in human bladder cancer. Hum Mol Genet 10:2619–2626.

Uejima H, Lee MP, Cui H, Feinberg AP. 2000. Hot-stop PCR: A simple and general assay for linear quantitation of allele ratios. Nat Genet 25:375–376.

Watanabe N, Kobayashi H, Hirama T, Kikuta A, Koizumi S, Tsuru T, Kaneko Y. 2002. Cryptic translocation t(12;15)(p13;q26) producing *ETV6-NTRK3* fusion gene and no loss of *IGF2* imprinting in congenital mesoblastic nephroma with trisomy 11: FISH and *IGF2* allelic expression analysis. Cancer Genet Cytogenet 136:10–16.

Watanabe N, Nakadate H, Haruta M, Sugawara W, Sasaki F, Tsunematsu Y. Kikuta A, Fukuzawa M, Okita H, Hata J, Soejima H, Kaneko Y. 2006. Association of 11q loss, trisomy 12, and possible 16q loss with loss of imprinting of insulin-like growth factor-II in Wilms tumor. Genes Chromosomes Cancer 45:592–601.

Xiong Z, Laird PW. 1997. COBRA: A sensitive and quantitative DNA methylation assay. Nucleic Acids Res 25:2532–2534.

Yuan E, Li CM, Yamashiro DJ, Kandel J, Thacker H, Murty VV, Tycko B. 2005. Genomic profiling maps loss of heterozygosity and defines the timing and stage dependence of epigenetic and genetic events in Wilms' tumors. Mol Cancer Res 3:493–502.

Zhan S, Shapiro DN, Helman LJ. 1994. Activation of an imprinted allele of the insulin-like growth factor II gene implicated in rhabdomyosarcoma. J Clin Invest 94:445–448.

Zhuang Z, Park WS, Pack S, Schmidt L, Vortmeyer AO, Pak E Pham T, Weil RJ, Candidus S, Lubensky IA, Linehan WM, Zbar B, Weirich G. 1998. Trisomy 7-harbouring non-random duplication of the mutant *MET* allele in hereditary papillary renal carcinomas. Nat Genet 20:66–69.

Bone Marrow Transplantation (2007), 1–6

www.nature.com/bmt

ORIGINAL ARTICLE

High incidence of secondary failure of platelet recovery after autologous and syngeneic peripheral blood stem cell transplantation in acute promyelocytic leukemia

H Narimatsu[1,2], N Emi[2], A Kohno[1], M Iwai[3], M Yanada[2], T Yokozawa[2], S Saito[1], K Shimada[1], H Kiyoi[3], T Naoe[2], K Yamamoto[2,4,5] and Y Morishita[1]

[1]*Department of Hematology and Oncology, JA Aichi Showa Hospital, Konan, Japan;* [2]*Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan;* [3]*Department of Infectious Diseases, Nagoya University School of Medicine, Nagoya, Japan;* [4]*Department of Hematology and Cell Therapy, Aichi Cancer Center Hospital, Nagoya, Japan and* [5]*Department of Preventive Medicine/Biostatistics and Medical Decision Making, Nagoya University Graduate School of Medicine, Nagoya, Japan*

Secondary failure of platelet recovery (SFPR), which is a delayed decline in platelet count after primary recovery following myeloablative hematopoietic SCT, is a significant problem in allogeneic SCT. However, its clinical characteristics have not been well described in autologous SCT for acute myeloid leukemia. We reviewed 11 consecutive patients who had received autologous or syngeneic SCT for acute promyelocytic leukemia. Seven of 11 patients (64%) had SFPR, which is defined as a decline in the platelet count to less than 30 000/μl for more than 7 days. The median onset of SFPR was day 36 (range, 25–51 days) and the median duration of thrombocytopenia was 13 days (range, 4–25 days). Of nine patients who received busulfan-containing preparative regimens, seven (78%) had SFPR and one had delayed primary platelet count recovery. Neither patient who received cyclophosphamide and total body irradiation as preparative regimens had SFPR. The clinical courses of SFPR were transient and self-limited. SFPR was not associated with relapse of underlying diseases, graft failure or other fatal morbidities. The unexpectedly high prevalence and the characteristics of SFPR may provide additional information on management following autologous SCT for acute myeloid leukemia.

Bone Marrow Transplantation advance online publication, 13 August 2007; doi:10.1038/sj.bmt.1705820

Keywords: autologous hematopoietic stem cell transplantation; platelet decline; acute myeloid leukemia; cytomegalovirus

Introduction

Thrombocytopenia is a critical problem after myeloablative hematopoietic SCT.[1–5] Recovery of platelets is affected by several factors, including the stem cell source, the infused cell dose, disease status, graft-versus-host disease, infections, and, especially, CMV. Veno-occlusive disease of the liver (VOD), thrombotic thrombocytopenic purpura/hemolytic uremic syndrome (TTP/HUS) and allo-immunization to random donor platelets also contribute to thrombocytopenia.[1,3,6]

A delayed, persistent decline in platelets count after primary platelet recovery is termed secondary failure of platelet recovery (SFPR). Isolated thrombocytopenia may occur without the decline of any other cell lineage. In a large study by Bruno *et al.*,[6] SFPR was observed in 20% of patients undergoing allogeneic transplantation and in 8% of patients undergoing autologous transplantation. Although it is not related to disease recurrence or graft rejection, SFPR is associated with poor outcomes after transplant. Various factors that either affected platelet production in the marrow or caused decreased platelet survival in the peripheral circulation have been implicated in the pathophysiology of SFPR.[1,3,6]

Autologous HSCT offers favorable outcomes for patients with acute promyelocytic leukemia in the second or later remission with minimal residual disease.[7,8] We conducted a series of autologous or syngeneic HSCTs for patients with acute promyelocytic leukemia using mobilized PBSCs with granulocyte CSF. An unexpectedly high incidence of SFPR was observed. In this paper, we describe clinical and pathological features that will lead to a new method of SFPR management after HSCT.

Patients and methods

Study patients

We reviewed medical records of 11 consecutive patients who had received autologous or syngeneic HSCT for AML

Correspondence: Dr H Narimatsu, Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan.
E-mail: narimt54@med.nagoya-u.ac.jp
Received 23 April 2007; revised 30 May 2007; accepted 6 July 2007

at Nagoya University Hospital, Aichi, Japan, or JA Aichi Showa Hospital, Konan, Japan from 1 April 2000, to 31 December 2004. All patients were evaluated from the day of transplant, defined as day 0, until death or the last routine follow-up.

Transplant procedures

Schedules and doses of preparative regimens are shown in Table 1. Granulocyte CSF (filgrastim (patients 1–3 and 9–11) or nartograstim (patients 4 and 7–8) was administered intravenously from day 1 until neutrophil engraftment. Sodium valproate 400 mg/day (patients 1–3) or phenytoin 300 mg/day (patients 4–6 and 9–11) were administered for prophylaxis against BU-induced seizure. Prophylaxis against herpes virus infection was administered with acyclovir 1000 mg/day in patients 4–8. Fluoroquinolone and fluconazole or itraconazole were also administered for prophylaxis against bacterial and fungal infections, respectively. Patients 1 and 2 received trimethoprim/sulfamethoxazole for prophylaxis against *Pneumocystis jiroveci* from day −7 to −1.

Definitions

Primary platelet recovery after myeloablative conditioning regimens was defined as an increase in platelet count to $\geqslant 50 \times 10^3/\mu l$ without transfusion support. SFPR was defined as a decline in the platelet count to less than $30 \times 10^3/\mu l$ for more than 7 days or on two consecutive laboratory examinations, or as the requirement of platelet transfusions after primary platelet recovery. The first day of thrombocytopenia with a platelet count less than $30 \times 10^3/\mu l$ was designated as the onset of SFPR. Neutrophil engraftment was defined as an absolute neutrophil count $>0.5 \times 10^9/l$ for three consecutive days for neutrophil recovery.

Laboratory examinations

Complete blood cell count and blood chemistry were analyzed 2–3 times a week during hospitalization. Bone marrow aspiration was performed from the sternum to confirm engraftment in all eight patients, and additional examinations were carried out at the onset of SFPR in some patients. CMV pp65 antigenemia[9,10] was analyzed as a method of rapid CMV antigen detection in all patients. The results are reported as the number of antigen-positive cells per slide containing 150 000 polymorphonuclear leukocytes. Platelet-associated immunoglobulin G (PA-IgG) was assayed in two patients (patients 4 and 5) around the time of SFPR onset. CMV pp65 antigenemia was monitored weekly after engraftment. CMV antigenemia was managed according to the method reported by Kanda *et al*.[11] with some modifications. If CMV pp65-positive cells were detected, patients preemptively received ganciclovir (5 mg/kg) two times daily. Ganciclovir could also be started with less than 10 positive cells in the patients who had received more than 0.5 mg/kg of prednisolone.

Statistical analysis

Differences in the incidence of SFPR between patients conditioned with BU-containing regimens and the other

Table 1 Patient characteristics

Patient no.	*Age*	*Sex*	*Donor*	*Preparative regimens*	*Status at transplantation*	*CD34+cell dose (× 10⁶/kg)*	*Previous treatment*
1	**59**	**F**	**Autologous**	**BU 8 mg/kg/day (day −6 to −3), MEL 70/m²/day (day −2 and −1)**	**Second CR**	**3.0**	**High-dose cytarabine**
2	**34**	**M**	**Syngeneic**	**BU 8 mg/kg/day (day −6 to −3), MEL 70/m²/day (day −2 and −1)**	**Second CR**	**2.5**	**Arsenic trioxide**
3	**30**	**M**	**Autologous**	**BU 8 mg/kg/day (day −6 to −3), MEL 70/m²/day (day −2 and −1)**	**Third CR**	**5.0**	**High-dose cytarabine, arsenic trioxide**
4	**63**	**F**	**Autologous**	**BU 8 mg/kg/day (day −8 to −5), CY 60 mg/kg/day (day −3 and −2)**	**Second CR**	**9.2**	
5	**55**	**M**	**Autologous**	**BU 8 mg/kg/day (day −8 to −5), CY 60 mg/kg/day (day −3 and −2)**	**Second CR**	**8.9**	
6	65	M	Autologous	BU 8 mg/kg/day (day −8 to −5), CY 60 mg/kg/day (day −3 and −2)	Third CR	3.7	Arsenic trioxide
7	36	M	Autologous	CY 60 mg/kg/day (day −4 and −3), TBI 6 Gy/day (day −6 and −5)	Second CR	4.0	
8	51	F	Autologous	CY 60 mg/kg/day (day −4 and −3), TBI 5 Gy/day (day −7 and −6)	Second CR	1.9	
9	41	M	Autologous	BU 8 mg/kg/day (day −6 to −3), MEL 70/m²/day (day −2 and −1)	Second CR	2.8	High-dose cytarabine, arsenic trioxide
10	**46**	**F**	**Autologous**	**BU 8 mg/kg/day (day −6 to −3), MEL 70/m²/day (day −2 and −1)**	**Second CR**	**3.4**	**High-dose cytarabine, arsenic trioxide**
11	**54**	**F**	**Autologous**	**BU 8 mg/kg/day (day −6 to −3), MEL 70/m²/day (day −2 and −1)**	**Third CR**	**3.1**	**High-dose cytarabine, arsenic trioxide**

Abbreviation: MEL = melphalan.
Bold type indicates patients who expressed SFPR.

patients (conditioned with CY and TBI) were analyzed with Fisher's exact probability test for independent groups. The Mann–Whitney *U*-test was used to analyze the difference in the dose of $CD34^+$ cells between patients with SFPR and others. Statistical analyses were performed with the STATA version 8.2 Software (STATA Corp., College Station, TX, USA).

Results

Patient's characteristics

Patient's characteristics are shown in Table 1. The median age of the patients was 49 (range, 30–65) years, with five male and six female patients. All patients had acute promyelocytic leukemia. Eight patients were in second CR, and the other three patients were in third CR. The median dose of infused $CD34^+$ cells was 3.4 (range, 1.9–9.2) $\times 10^6$/kg. Six patients received BU and melphalan, three patients received BU and CY, and two patients received CY and TBI as preparative regimens. The stem cell source in 11 patients was PBSCs.

Primary platelet recovery after transplantation

All 11 patients achieved primary neutrophil engraftment. In all patients, except patient 6, the platelet count was greater than $50 \times 10^3/\mu l$ within 20 days after transplant (range, 10–18 days; median, 14 days). In patient 6, platelet recovery to greater than $50 \times 10^3/\mu l$ did not occur until day 85.

Clinical course of SFPR

Seven of 11 patients (64%) had SFPR (patients 1–5, 10 and 11). The median onset of SFPR was day 36 (range, 25–51 days), and the median duration of thrombocytopenia was 13 days (range, 4–25). Platelet counts recovered in all patients developing SFPR (Table 2). None of the seven patients with SFPR showed the decline in WBC count or RBC count during SFPR. Patients 1 and 2 received platelet transfusions. In patient 1, the platelet count declined to $14 \times 10^3/\mu l$ on day 63 and recovered to $47 \times 10^3/\mu l$ on day 70. In patient 6, the primary platelet count recovery did not occur until after day 50, but SFPR was not observed after platelet recovery. Patients 4 and 5 had preceding febrile episode. In patient 10, platelet count declined to $29 \times 10^3/\mu l$ on day 51, and then to $22 \times 10^3/\mu l$ on day 53. Platelet count recovered to $30 \times 10^3/\mu l$ on day 55, and then to $52 \times 10^3/\mu l$ at day 85. In patient 11, platelet count declined to $42 \times 10^3/\mu l$ on day 33, and then to $20 \times 10^3/\mu l$ on day 36. Platelet count recovered to $33 \times 10^3/\mu l$ on day 44, and then to $74 \times 10^3/\mu l$ on day 58. Four of the seven patients with SFPR developed hepatic dysfunction during SFPR (Table 2). No patients developed renal dysfunctions (Table 2). No other remarkable clinical manifestations developed in the seven patients during SFPR.

Correlation between SFPR and conditioning regimens or infused cell doses

Of the nine patients who received BU-containing conditioning regimens, that is non-TBI regimens, seven (78%) had SFPR and one (patient 6) had delayed primary recovery of platelet count. Neither patient who received conditioning regimens without BU, that is, CY and TBI, had SFPR ($P = 0.067$: patient 6 was excluded from analysis because of prolonged thrombocytopenia following transplantation). The median number of infused $CD34^+$ cells was 3.4 (range, 2.5–9.2) $\times 10^6$/kg for patients with SFPR and 3.3 (1.9–4.0) $\times 10^6$/kg for patients without SFPR ($P = 0.35$).

Bone marrow findings

Bone marrow aspiration at the onset of SFPR was performed on day 51 for patient 1, on day 23 for patient 2, on day 20 for patient 3, on day 31 for patient 4 and on day 38 for patient 5. As shown in Figure 1, all specimens showed normal cellularity, without relapse of leukemia or decrease in the number of megakaryocytes.

CMV antigenemia

In Patient 1, CMV antigenemia (9 positive cells out of 150 000) was detected on day 14, almost at the same time that the stomatitis and fever appeared, and was successfully treated with ganciclovir. In patient 2, the antigenemia was negative on day 23, before onset of SFPR. At day 37, the antigenemia (2 positive cells out of 150 000) was detected at day 37 and it became negative after the administration of ganciclovir. In patients 3–5, the antigenemia was negative at the time of the onset of SFPR. None of the patients had documented CMV diseases.

Outcome of SFPR

Table 2 shows the outcomes of seven patients with SFPR. Of the seven patients, six are alive without recurrence of leukemia. One patient (patient 5) died of leukemia relapse on day 278.

Discussion

Here, we have demonstrated a high incidence of SFPR following autologous PBSC transplantation (PBSCT) for AML. Bruno *et al.*[6] have reported a low incidence (3.8%) of SFPR after autologous SCT.[12] Unlike their study, in which the 1-year mortality of patients with SFPR was high (44%), in our study SFPR was self-limiting and did not affect the relapse of underlying diseases. The different feature from their report was that SFPR in our patients were self limiting and did not affect the relapse of underlying disease; in contrast the 1-year mortality of patients developing SFPR was relatively high (44 %) in their study. This contrast might be due to differences in patient profiles.

A high prevalence (64%) of SFPR was consistently observed in our patients with AML who received autologous PBSCT. One report has shown that 5 (56%) of 9 patients with AML had SFPR, whereas 16 (11%) of 146 patients with diseases other than AML who received autologous PBSCT had secondary thrombocytopenia.[1] Underlying disease of AML was a statistically significant predictor of poor platelet engraftment after both autologous BMT and PBSCT, compared with transplants for

Table 2 Clinical and laboratory findings of the patients with SFPR

Patient no.	*Onset of SFPR*	*Lowest count of platelet during SFPR ($\times 10^3/\mu l$)*	*PA-IgG at SFPR onset*[a]	*Duration of SFPR (days)*	*Treatment*	*Outcome*	*Onset (peak level) of CMV antigenemia*	*Use of ganciclovir*	*Laboratory findings during SFPR*				*Concomitant medication at onset of SFPR*	*Status at last follow up*
									Maximum creatinine level (mg dl)	*Maximum total-bilirubin level (mg dl)*	*Maximum aspartate aminotransferase level (IU)*	*Maximum alanine aminotransferase level (IU)*		
1	Day 44	20	NA	18	Prednisolone	Resolved	Day 14 (9)	Days 15–34	0.5	0.7	25[b]	26[d]	Trimethoprim sulfamethoxazole. rebamipide. amphotericin B	Alive 60 months after transplant
2	Day 25	16	NA	25	Fresh-frozen plasma	Resolved	Day 37 (2)	Days 39–53	0.8	0.8	171[b]	207[d]	Rebamipide. glycyrrhizic acid	Alive 42 months after transplant
3	Day 35	17	NA	22	None	Resolved	NA	NA	0.7	0.9	47[b]	68[d]	None	Alive 12 months after transplant
4	Day 34	17	118	13	None	Resolved	NA	NA	0.5	0.4	76[c]	55[e]	Teprenone. glibenclamide. lactomin	Alive 61 months after transplant
5	Day 43	25	43.6	10	None	Resolved	NA	NA	0.7	0.7	31[c]	95[e]	Teprenone	Dead at day 278 due to relapse
10	Day 51	22	NA	5	None	Resolved	Day 12	Not used	0.4	0.6	28[b]	38[d]	Trimethoprim sulfamethoxazole. teprenone	Alive 41 months after transplant
11	Day 36	20	NA	8	None	Resolved	NA	NA	0.5	NA	32[b]	19[d]	Trimethoprim sulfamethoxazole. pantethine. magnesium oxide. teprenone	Alive 32 months after transplant

Abbreviations: NA = not applicable; PA-IgG indicates = platelet associated IgG.
[a]Calculated in $ng/10^7$ cells (normal: 9.0–25.0).
[b]Upper normal limit is 41 IU.
[c]Upper normal limit is 31 IU.
[d]Upper normal limit is 45 IU.
[e]Upper normal limit is 37 IU.

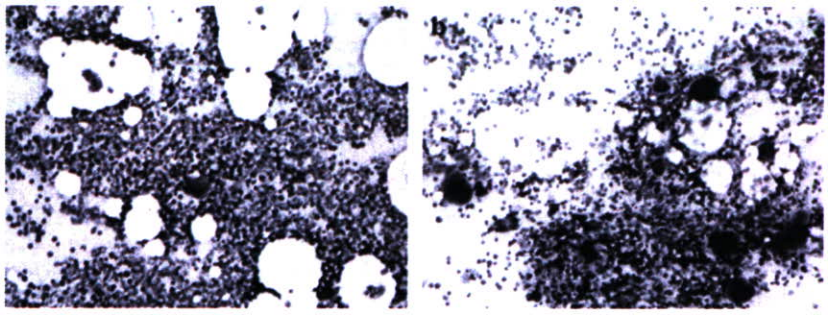

Figure 1 Bone marrow findings at SFPR onset (May-Giemsa stain, × 10) (**a**) patient 4 (**b**) patient 5.

other diseases.[6] Previous intensive chemotherapy for AML before PBSC harvest might deplete the primitive precursor cells that maintain long-term hematopoiesis.

Interestingly, seven of nine patients receiving BU-containing conditioning regimens had SFPR, whereas neither patient receiving TBI-based regimens had SFPR, although the difference was not statistically significant. The BU-containing regimen has particularly been used in transplants in patients with myeloid malignancies, and has been suggested to be associated with the development of VOD. Although VOD was not clinically evident in our patients, BU may have injured endothelial cells to induce platelet activation and sequestration resulting in subclinical VOD or TTP/HUS.

The elevation of PA-IgG in patients 4 and 5, and the preceding febrile episodes of possibly infectious origins in patients 1 and 3 suggest immunomediated thrombocytopenia. Bone marrow megakaryocytes did not decrease in any patients during SFPR. Auto-immune thrombocytopenia after high-dose chemotherapy and autologous BMT/PBSCT has been reported, but occurs infrequently in patients with AML, lymphoblastic lymphoma or breast cancer.[13–18] Although the exact mechanism for the development of autoimmune thrombocytopenia is unknown, several possible mechanism have been proposed including transient immune system perturbation, such as impaired suppressor T-cell function, immune deregulation related to thymic damage caused by irradiation and chemotherapy, and altered expression of self antigens as a result of stem cell damage during harvest and storage and viral infections after transplant.[13,18] Serum cytokine profiles observed in immunological imbalances might contribute to the activation of monocytes and macrophages, which are critical components in the elimination pathway of platelets.[1] Thus, immunological perturbations following transplants possibly caused decreased platelet survival by activating the elimination system in the peripheral circulation.

CMV infection after primary platelet recovery was most significantly associated with the incidence of SFPR in a previous report.[6] In the present study, CMV disease was not documented in any patient. Moreover, preemptive ganciclovir use was not related to the onset of SFPR or to clinical outcome. Thus, although CMV infection is a potential cause of SFRR, it was not involved in our series.

In summary, SFPR was observed frequently after autologous and syngeneic transplantation in patients with acute promyelocytic leukemia. The high prevalence of SFPR may be related to the use of PBSC as a stem cell source and the use of BU-containing preparative regimens. While multiple mechanisms are involved in the development of SFPR, the identification of etiology in each patient with precise descriptions of clinical characteristics should improve patient care.

References

1 Nash RA, Gooley T, Davis C, Appelbaum FR. The problem of thrombocytopenia after hematopoietic stem cell transplantation. *Oncologist* 1996; **1**: 371–380.
2 Bernstein SH, Nademanee AP, Vose JM, Tricot G, Fay JW, Negrin RS *et al*. A multicenter study of platelet recovery and utilization in patients after myeloablative therapy and hematopoietic stem cell transplantation. *Blood* 1998; **91**: 3509–3517.
3 First LR, Smith BR, Lipton J, Nathan DG, Parkman R, Rappeport JM. Isolated thrombocytopenia after allogeneic bone marrow transplantation: existence of transient and chronic thrombocytopenic syndromes. *Blood* 1985; **65**: 368–374.
4 Behringer D, Bertz H, Schmoor C, Berger C, Dwenger A, Finke J. Quantitative lymphocyte subset reconstitution after allogeneic hematopoietic transplantation from matched related donors with CD34$^+$ selected PBPC grafts unselected PBPC grafts or BM grafts. *Bone Marrow Transplant* 1999; **24**: 295–302.
5 Sierra J, Perez WS, Rozman C, Carreras E, Klein JP, Rizzo JD *et al*. Bone marrow transplantation from HLA-identical siblings as treatment for myelodysplasia. *Blood* 2002; **100**: 1997–2004.
6 Bruno B, Gooley T, Sullivan KM, Davis C, Bensinger WI, Storb R *et al*. Secondary failure of platelet recovery after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2001; **7**: 154–162.
7 Lo Coco F, Diverio D, Avvisati G, Petti MC, Meloni G, Pogliani EM *et al*. Therapy of molecular relapse in acute promyelocytic leukemia. *Blood* 1999; **94**: 2225–2229.
8 Meloni G, Diverio D, Vignetti M, Avvisati G, Capria S, Petti MC *et al*. Autologous bone marrow transplantation for acute promyelocytic leukemia in second remission: prognostic relevance of pretransplant minimal residual disease assessment by reverse-transcription polymerase chain reaction of the PML/RAR alpha fusion gene. *Blood* 1997; **90**: 1321–1325.
9 van der Bij W, Torensma R, van Son WJ, Anema J, Schirm J, Tegzess AM *et al*. Rapid immunodiagnosis of active cytome-

galovirus infection by monoclonal antibody staining of blood leucocytes. *J Med Virol* 1988; **25**: 179–188.

10 The TH, van der Bij W, van den Berg AP, van der Giessen M, Weits J, Sprenger HG *et al*. Cytomegalovirus antigenemia. *Rev Infect Dis* 1990; **12** (Suppl 7): S734–S744.

11 Kanda Y, Mineishi S, Saito T, Seo S, Saito A, Suenaga K *et al*. Pre-emptive therapy against cytomegalovirus (CMV) disease guided by CMV antigenemia assay after allogeneic hematopoietic stem cell transplantation: a single-center experience in Japan. *Bone Marrow Transplant* 2001; **27**: 437–444.

12 Bacigalupo A, Lamparelli T, Bruzzi P, Guidi S, Alessandrino PE, di Bartolomeo P *et al*. Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). *Blood* 2001; **98**: 2942–2947.

13 Jillella AP, Kallab AM, Kutlar A. Autoimmune thrombocytopenia following autologous hematopoietic cell transplantation: review of literature and treatment options. *Bone Marrow Transplant* 2000; **26**: 925–927.

14 Ashihara E, Shimazaki C, Hirata T, Okawa K, Oku N, Goto H *et al*. Autoimmune thrombocytopenia following peripheral blood stem cell autografting. *Bone Marrow Transplant* 1993; **12**: 297–299.

15 Klumpp TR, Herman JH, Macdonald JS, Schnell MK, Mullaney M, Mangan KF. Autoimmune neutropenia following peripheral blood stem cell transplantation. *Am J Hematol* 1992; **41**: 215–217.

16 Sivakumaran M, Hutchinson RM, Pringle H, Graham S, Primrose L, Wood JK *et al*. Thrombocytopenia following autologous bone marrow transplantation: evidence for autoimmune aetiology and B cell clonal involvement. *Bone Marrow Transplant* 1995; **15**: 531–536.

17 Matsuishi E, Anzai K, Dohmen K, Taniguchi S, Gondo H, Kudo J *et al*. Sonographic diagnosis of veno-occlusive disease of the liver and danazol therapy for autoimmune thrombocytopenia in an autologous marrow transplant patient. *Jpn J Clin Oncol* 1990; **20**: 188–192.

18 Garcia Vela JA, Ona F, Monteserin MC, Lastra AM, Garcia Larana J, Lopez J *et al*. Autoimmune thrombocytopenia after autologous bone marrow transplantation. *Am J Hematol* 1994; **46**: 375.

Leukemia (2007), 1–4

www.nature.com/leu

LETTER TO THE EDITOR

Clinical characteristics and outcomes in patients with t(8;21) acute myeloid leukemia in Japan

Leukemia advance online publication, 16 August 2007; doi:10.1038/sj.leu.2404905

In acute myeloid leukemia (AML), t(8;21)(q22;q22) translocation is one of the most common karyotype abnormalities, occurring in 7–8% of adult patients.[1] This change is closely associated with AML-M2 subtype in the French-American-British (FAB) classification, a type of AML with high complete remission (CR) rate (85–90%) and favorable survival rate.[1–3] Studies conducted in western countries have demonstrated that survival is prolonged when consolidation chemotherapies comprising high-dose cytarabine are administered.[2]

A research group from the Cancer and Leukemia Group B reported that clinical characteristics of t(8;21) AML could alter depending on ethnicity, suggesting that direct adoption of treatment strategies based on clinical studies conducted in western countries to Japanese patients may not be advisable.[4] A recent study from Japan focused mainly on the clinical impact of cytogenetics.[5] Information on clinical characteristics and optimal treatments, such as the clinical impact of high-dose cytarabine, thus remains unavailable for Japanese patients.

To establish optimal therapeutic strategies applicable to Japanese patients, clarification of clinical characteristics and outcomes in Japanese patients with t(8;21) AML is crucial. The present retrospective multicenter study was conducted to investigate the clinical characteristics of Japanese patients with t(8;21) AML.

From January 2000 to December 2005, a total of 147 Japanese adult patients (≥15-years-old), who were newly diagnosed with *de novo* AML (FAB: M2) according to FAB classifications, were consecutively enrolled in nine collaborating hospitals. We retrospectively reviewed the medical records of these patients. These 147 patients included 46 patients with t(8;21) AML and 101 AML(M2) patients without t(8;21).

Diagnosis of t(8;21) AML was established based on chromosomal analysis (G-banding, $n=45$) and/or detection of RUNX1(AML1)/MTG8(ETO) fusion gene by real-time reverse transcription-polymerase chain reaction ($n=16$). Overall survival was calculated from diagnosis to death from any causes and event-free survival was defined as the time from diagnosis to the following events: first relapse of AML; treatment-failure; or death from any cause except leukemia. High-, standard- and low-dose cytarabine were defined as ≥2 g/m^2/day, 100–200 mg/m^2/day and ≤40 mg/m^2/day, respectively. No patients received any other doses of cytarabine. Of the 46 patients with t(8;21), 4 were enrolled in the AML 202 study of the Japan Adult Leukemia Study Group.

Overall survival rate was calculated using the Kaplan-Meier product limit method. A log-rank test was applied to assess impact by the factor of interest when appropriate. Estimated survival was calculated as of January 31, 2007. Uni- and multivariate Cox proportional hazard models were applied to estimate the impact of potential prognostic factors. Factors associated with at least borderline significance ($P<0.10$) in univariate analyses were subjected to multivariate analysis using backward stepwise proportional-hazard modeling. Values of $P<0.05$ were considered statistically significant. Multivariate Cox proportional hazard models were used to determine the influence of age, sex and karyotype (with or without t(8;21)) on survival of all 147 patients. All analyses were conducted using STATA version 9.2 software (STATA, College Station, TX, USA).

Characteristics of AML patients with t(8;21) are shown in Table 1. Patients with t(8;21) (median age, 49.5 years; range, 18–86 years) were significantly younger than AML(M2) patients without t(8;21) (median age, 60 years; range 17–90 years; $P<0.001$). AML(M2) patients without t(8;21) included 57 men and 44 women. The median follow-up of surviving patients was 27.0 months (range, 0.2–82.6 months) after diagnosis.

Twelve patients with t(8;21) AML died during follow-up at a median of 10.6 months (range, 3.1–80.1 months) after diagnosis due to primary disease ($n=10$), pneumonia ($n=1$) or sudden cardiac death ($n=1$). Overall survival rates at 3 years after diagnosis in patients with t(8;21) was 70% (95% confidence interval (CI), 51–83%). This rate was significantly better than that in AML (M2) patients without t(8;21) (overall survival at 3 years, 0.43 (95%CI, 0.32–0.54); log-rank test, $P=0.005$; Figure 1a). Among patients <60-years-old, overall survival rates of patients with t(8;21) AML and patients with non-t(8;21) AML(M2) were 71% (95%CI, 47–86%) and 58% (95%CI, 41–72%), respectively (log-rank test, $P=0.28$; Figure 1b). Event-free survival rate at 3 years in patients with t(8;21) was 54% (95%CI, 37–69%). Overall survival rates in patients with t(8;21) according to karyotype are shown in Figure 1c. No significant difference in overall survival (Figure 1c) or event-free survivals were noted between karyotypic groups (log-rank test, $P=0.27$ and $P=0.51$, respectively). There was not any significant association between presence of extramedullary involvement and additional karyotype abnormality ($P=0.49$).

Of the 45 patients who received induction therapy, 36 and 5 patients achieved CR after first and second courses of chemotherapy, respectively (Table 1). CR rate was 91%.

Of the 40 patients who received induction therapy containing standard-dose cytarabine, 38 achieved CR. Among those, 21 patients received high-dose cytarabine-containing consolidation therapy. One of the 21 patients died in CR during consolidation therapy, due to infection. Event-free survival rates in patients with and without high-dose cytarabine were shown in Figure 1d.

In multivariate analysis, age and white blood cell count at diagnosis represented significant unfavorable predictors of overall survival. White blood cell count and lactate dehydrogenase level at diagnosis represented significant unfavorable predictors of event-free survival. (Table 2) Among 147 patients with AML (M2), presence of t(8;21) was not a significant predictor of survival (hazard ratio, 0.65; 95%CI 0.34–1.24; $P=0.19$) in multivariate analysis.

The present study demonstrated a more favorable survival rate for patients with t(8;21) AML in Japan than seen in recent studies conducted in western countries,[4,6–8] even though median age in the present study (49.5 years) was higher than those in recent studies (28–43 years).[4,6–8] Median white blood cell count and platelet count in the present study, which have been reported as predictors of survival in previous studies,[4,6–8] were consistent

Table 1 Characteristics and treatment of patients with t(8;21) AML

Variables		*Number*
Age (years)	Median, range	49.5 (18–86)
Sex	Male/female	32/14
Karyotypic abnormality[a]		
(A) t(8;21)(q22; q22) without additional karyotypic abnormality		12
(B) t(8;21)(q22; q22) with loss of sex (Y) chromosome		13
(C) t(8;21)(q22; q22) with abnormal chromosome 9		4
(D) t(8;21)(q22; q22) with ⩾3 additional abnormalities		9
(E) t(8;21)(q22; q22) with loss of X chromosome		3
(F) Other karyotypic abnormalities[b]		3
White blood cell count (/μl)	Median, range	9350 (900–54 970)
Red blood cell count (10E6/μl)	Median, range	2.4 (0.6–4.0)
Hemoglobin at diagnosis (g/dl)	Median, range	8.5 (2.3–12.8)
Platelet count (10E3/μl)	Median, range	34 (6–99)
Lactate dehydrogenase level (IU/l)	Median, range	453 (162–3831)
Extramedullary involvement	Present[c]/absent	8/37
Surface antigens on leukemia cells		
CD 7	Present/absent	2/42
CD 13	Present/absent	41/3
CD 19	Present/absent	29/17
CD 33	Present/absent	35/11
CD 34	Present/absent	42/2
CD 56[d]	Present/absent	32/7
HLA-DR	Present/absent	44/2
First induction therapy[e]		
Idarubicine 12 mg/m^2 d1–3+cytarabine 100 mg/m^2 d1–7		37
Daunorubicine 50 mg/m^2 d1–5+cytarabine 100 mg/m^2 d1–7		3
Low-dose cytarabine-based chemotherapy		4
Other regimen		1
Outcomes of first induction therapy		
Complete remission		36
Complete remission not achieved		9[f]
Consolidation therapy[g]		
High-dose cytarabine-based chemotherapy		21
Courses of high-dose cytarabine (1/2/3/4)		1[h]/4/12/4
Standard cytarabine-based chemotherapy		16
Low-dose cytarabine-based chemotherapy		2
Hematopoietic stem cell transplantation		
In first complete remission (autologous/allogeneic)		3/4[i]
In other stage (autologous/allogeneic)		0/8

Abbreviation: AML, acute myeloid leukemia.
[a]Two patients were diagnosed by detection of RUNX1/MTG8 fusion gene using reverse transcription-polymerase chain reaction.
[b]Those included 46,XY, t(8;21)(q22;q22), del(11)(p11p13) ($n = 1$); 46,XY, t(7;21;8)(q22;q22;q22) ($n = 1$); 45,XY, t(8;12;21)(q22;p11;q22), del(9)(q?) ($n = 1$) and 46,XX,t(2;19)(q37;p13),t(8;21)(q22;q22) ($n = 1$).
[c]Including skin ($n = 2$), submandibular lymph nodes ($n = 1$), mediastinum ($n = 2$), cervical lymph nodes ($n = 2$), spleen ($n = 2$), submandibular lymph nodes ($n = 1$), liver ($n = 1$), lung ($n = 1$) and subcutaneous ($n = 1$).
[d]Mean fluorescence intensity of CD56 among karyotype group A-F was 68, 42, 48,83, 85 and 18%, respectively.
[e]One patient rejected chemotherapy.
[f]Five patients achieved complete remission after second course of induction therapy. Three of the remaining 4 patients died with disease progression at a median of 195 days (range, 92–243 days) after diagnosis. The final remaining patient underwent allogeneic peripheral blood stem cell transplantation from an HLA-matched sibling at day 114 after diagnosis.
[g]One patient did not receive consolidation therapy and data were unavailable for one patient.
[h]This patient received autologous hematopoietic stem cell transplantation in first complete remission.
[i]Those patients had received standard-dose cytarabine ($n = 2$) or high-dose cytarabine ($n = 2$) containing consolidation therapies before transplantation.

with those in previous studies. Differences in patient backgrounds between recent studies and ours are thus unlikely to have affected survival rates. These results indicate that t(8;21) AML in Japanese patients is associated with more favorable outcomes than seen in patients from western countries. Prognosis of t(8;21) AML may differ according to ethnicity, although statically analysis was not conducted. Further large-scale studies to investigate differences in clinical outcome among patients of various ethnicities thus appear warranted.

Interestingly, differences in overall survival between t(8;21) AML patients and AML(M2) without t(8;21) patients were unclear after adjusting for age (Figure 1b). One possible explanation is that AML (M2) in Japanese patients is associated with favorable outcomes. Another explanation is that favorable outcomes for t(8;21) AML are greatly related to low patient age. To date, information on clinical differences after adjusting for age between t(8;21) AML and AML(M2) without t(8;21) limited, and is worth investigating in future studies.

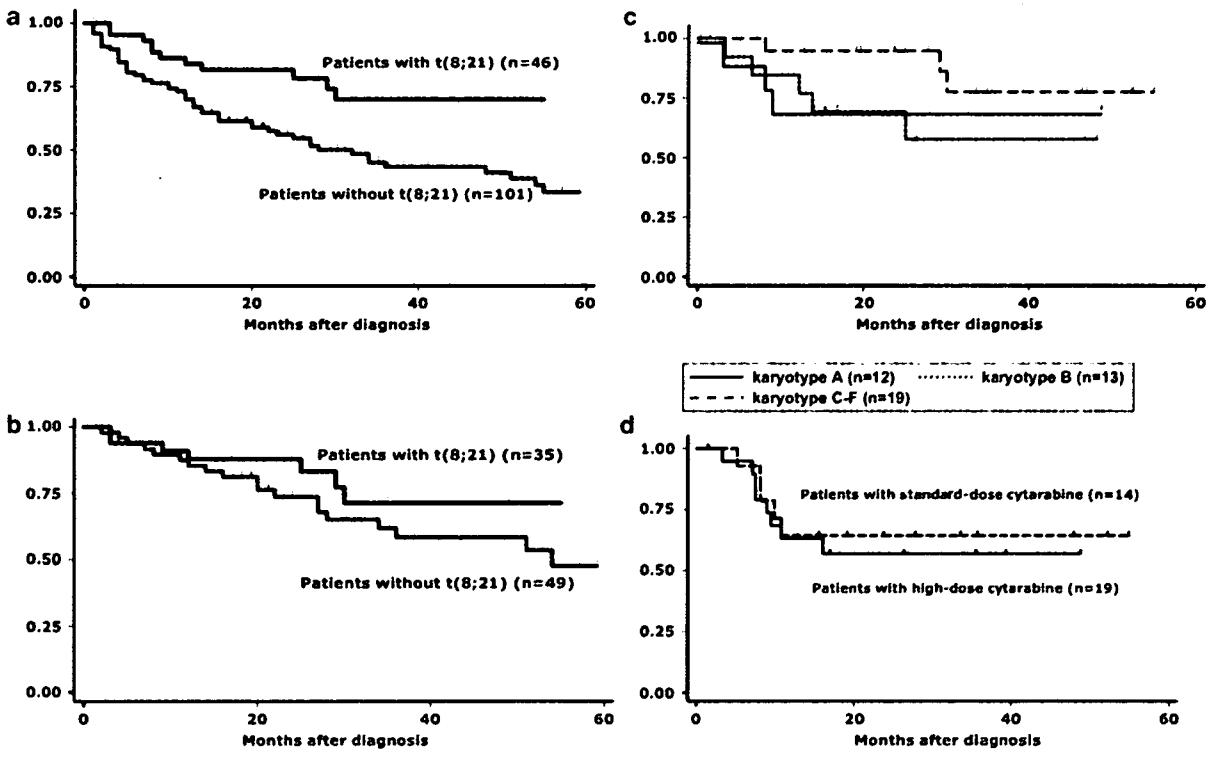

Figure 1 Overall survival rates. Overall survival rates of the AML(M2) patient with or without t(8;21) were shown in (**a**). Overall survival rate at 3 years after diagnosis in patients with t(8;21) was 0.70 (95%CI, 0.51–0.83). Overall survival rate at 3 years after diagnosis in patients with non-t(8;21) AML(M2) was 0.43 (95%CI, 0.32–0.54) (**a**). A significant difference was identified between groups (log-rank test, $P=0.005$). Overall survival rates for AML(M2) patients <60-years-old were shown in (**b**). Overall survival rate at 3 years after diagnosis in patients with or without t(8;21) was 0.71 (95%CI, 0.47–0.86) and 0.58 (95%CI, 0.41–0.72), respectively. No significant difference was seen between groups (log-rank test, $P=0.28$) (**b**). Overall survival rates according to karyotype at diagnosis were shown in Panel c. Survival rates of the following karyotype groups are shown: (A) t(8;21)(q22;q22) without other karyotype abnormality; (B) t(8;21)(q22;q22) with loss of sex (Y) chromosome; (C) t(8;21)(q22;q22) with abnormal chromosome 9; D) t(8;21)(q22;q22) with ⩾3 additional abnormalities; E) t(8;21)(q22;q22) with loss of X chromosome and F) other karyotype abnormalities. No significant difference was noted between groups (log-rank test, $P=0.27$) (**c**). Event-free survival rates with high- and standard-dose cytarabine were shown in (**d**). Event-free survival rates at 3 years after diagnosis in patients with consolidation therapy containing high- and standard-dose cytarabine were 57% (95%CI, 32–76%) and 64% (95%CI, 34-83%), respectively (log-rank test, $P=0.69$) The four patients who received the allogeneic stem cell transplantation in the first complete remission were excluded from the analysis (**d**). AML, acute myeloid leukemia; CI, confidence interval.

Table 2 Risk factors for overall and event-free survival in patients with t(8;21) AML

Univariate factors	*Overall survival*			*Event-free survival*		
	HR	*95% CI*	*P*	*HR*	*95% CI*	*P*
Age (years)	1.04	1.00–1.08	0.08	1.01	0.98–1.05	0.37
Sex (male vs female)	2.25	0.48–10.4	0.30	1.75	0.58–5.32	0.32
Induction therapies (low-dose vs standard-dose cytarabine-containing regimens)	2.57	0.55–12.0	0.23	2.65	0.76–9.23	0.13
White blood cell count at diagnosis (⩾10E4/μl vs <10E4/μl)	6.78	1.48–31.0	0.01	4.41	1.57–12.41	0.005
Hemoglobin at diagnosis (linear by 1 g/dl increase)	1.01	0.81–1.25	0.94	1.08	0.90–1.30	0.40
Platelet count at diagnosis (linear by 10E4/μl increase)	1.06	0.86–1.32	0.58	1.01	0.84–1.22	0.91
Lactate dehydrogenase level at diagnosis (linear by 1 IU increase)	1.00	1.000–1.001	0.06	1.001	1.0002–1.0011	0.004
CD56 expression of leukemia cell (positive vs negative)[a]	0.59	0.15–2.39	0.46	0.78	0.22–2.78	0.71
Karyotype (t(8;21) with other additional abnormality vs t(8;21) without additional abnormality or t(8;21) with loss of sex (Y) chromosome)[a]	1.01	0.31–3.31	0.99	1.05	0.42–2.66	0.91
Extramedullary involvement (present vs absent)	0.92	0.20–4.25	0.91	0.87	0.25–3.02	0.83
Stepwise multivariate factors						
Age (years)	1.04	1.00–1.09	0.04	NA	NA	NA
White blood cell count at diagnosis (⩾10E4/μl vs <10E4/μl)	7.70	1.66–35.7	0.009	3.68	1.29–10.50	0.02
Lactate dehydrogenase level at diagnosis (linear by 1 IU increase)	NA	NA	NA	1.001	1.000–1.001	0.02

Abbreviations: AML, acute myeloid leukemia; 95% CI, 95% confidence interval; HR, hazard ratio; NA, not applicable.
[a]Patients with an unknown variable were included in the analysis using a dummy variable indicating missing data.

Additional karyotype abnormalities have been reported as an unfavorable prognostic factor for t(8;21) AML.[1] The significance of these abnormalities may vary with ethnicity.[4] In the present study, the prognostic impact of additional karyotype abnormalities (including loss of the sex chromosome and abnormal chromosome 9) was uncertain, consistent with a previous study from Japan.[5] Additional karyotype abnormalities may not represent an important prognostic factor in Japanese patients. However, trisomy 4 still requires special consideration. All 3 Japanese patients with trisomy 4 in an earlier study died within 3 years.[5] This additional karyotype warrants further investigation, since the present study did not include these patients.

No clinical impact of high-dose cytarabine consolidation therapy in Japanese t(8;21) AML patients was demonstrated in the present study, inconsistent with previous studies from western countries.[1,2] Efficacy of high-dose cytarabine may differ between patients from Japan and western countries. Since intensive chemotherapy such as high-dose cytarabine carries a risk of treatment-related morbidity and mortality, clinicians must carefully select eligible patients who would benefit from this regimen. Overall survival rate in patients with high-dose cytarabine was not inferior to that in patients who received standard or low-dose regimens (data not shown). Our results indicate that high-dose cytarabine consolidation chemotherapy is feasible in Japanese patients with t(8;21) AML and that investigation of efficacy by conducting a randomized trial in Japan is warranted.

Despite providing novel and useful information on t(8;21) AML in Japan, some issues remain to be discussed. First, the patients known to have a less good prognosis, such as those with additional trisomy 4 was not included in the present study. Second, the information on tyrosine kinase mutations in the patients with t(8;21) was not presented in the present study. The tyrosine kinase mutations among various ethnicities are require to investigate in future studies, since those could influence the prognosis. Furthermore, specific mutations often associated with t(8;21), such as N-Ras and Flt3 besides c-kit are also worth investigating. The last detailed information of AML(M2) patients without t(8;21), including white blood cell count and karyotype at diagnosis, and induction and consolidation treatment were not available in the present study. Those require to be investigated in future studies.

In summary, the clinical characteristics of t(8;21) AML might differ between patients from Japan and western countries. Clinicians should be alert to potential clinical differences among ethnicities. Further large-scale studies on differences in clinical characteristics among various ethnicities including Japanese patients are required.

Acknowledgements

We thank all the staff and resident members of the participating institutions. A complete list of participating institutions appears in the Appendix.

H Narimatsu[1,2], T Yokozawa[3], H Iida[4], M Tsuzuki[5], M Hayakawa[6], T Takeo[7], M Iino[8], T Ichihashi[9], C Kato[3], A Sawamoto[1], H Sao[4], M Yanada[2], N Emi[5], H kiyoi[10], T Yamaguchi[11], T Naoe[2], R Suzuki[11] and I Sugiura[1]

[1]*Department of Hematology and Oncology, Toyohashi Municipal Hospital, Toyohashi, Japan;*
[2]*Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan;*
[3]*Department of Hematology/Oncology, Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya, Japan;*
[4]*Department of Hematology, Meitetsu Hospital, Nagoya, Japan;*
[5]*Department of Medicine, Fujita Health University School of Medicine, Toyoake, Japan;*
[6]*Department of Hematology, Komaki City Hospital, Komaki, Japan;*
[7]*Department of Hematology, Yokkaichi Municipal Hospital, Yokkaichi, Japan;*
[8]*Department of Hematology, Yamanashi Prefectual Central Hospital, Kofu, Japan;*
[9]*Department of Hematology, Okazaki City Hospital, Okazaki, Japan;*
[10]*Department of Infectious Diseases, Nagoya University School of Medicine, Nagoya, Japan and*
[11]*Department of HSCT Data Management, Nagoya University School of Medicine, Nagoya, Japan*
E-mail: narimt54@med.nagoya-u.ac.jp

References

1 Ferrara F, Del Vecchio L. Acute myeloid leukemia with t(8;21)/AML1/ETO: a distinct biological and clinical entity. *Haematologica* 2002; **87**: 306–319.
2 Byrd JC, Dodge RK, Carroll A, Baer MR, Edwards C, Stamberg J *et al.* Patients with t(8;21)(q22;q22) and acute myeloid leukemia have superior failure-free and overall survival when repetitive cycles of high-dose cytarabine are administered. *J Clin Oncol* 1999; **17**: 3767–3775.
3 Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G *et al.* The importance of diagnostic cytogenetics on outcome in AML: analysis of 1612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. *Blood* 1998; **92**: 2322–2333.
4 Marcucci G, Mrozek K, Ruppert AS, Maharry K, Kolitz JE, Moore JO *et al.* Prognostic factors and outcome of core binding factor acute myeloid leukemia patients with t(8;21) differ from those of patients with inv(16): a Cancer and Leukemia Group B study. *J Clin Oncol* 2005; **23**: 5705–5717.
5 Nishii K, Usui E, Katayama N, F Lorenzo V, Nakase K, Kobayashi T *et al.* Characteristics of t(8;21) acute myeloid leukemia (AML) with additional chromosomal abnormality: concomitant trisomy 4 may constitute a distinctive subtype of t(8;21) AML. *Leukemia* 2003; **17**: 731–737.
6 Nguyen S, Leblanc T, Fenaux P, Witz F, Blaise D, Pigneux A *et al.* A white blood cell index as the main prognostic factor in t(8;21) acute myeloid leukemia (AML): a survey of 161 cases from the French AML Intergroup. *Blood* 2002; **99**: 3517–3523.
7 Schlenk RF, Benner A, Krauter J, Buchner T, Sauerland C, Ehninger G *et al.* Individual patient data-based meta-analysis of patients aged 16 to 60 years with core binding factor acute myeloid leukemia: a survey of the German Acute Myeloid Leukemia Intergroup. *J Clin Oncol* 2004; **22**: 3741–3750.
8 Appelbaum FR, Kopecky KJ, Tallman MS, Slovak ML, Gundacker HM, Kim HT *et al.* The clinical spectrum of adult acute myeloid leukaemia associated with core binding factor translocations. *Br J Haematol* 2006; **135**: 165–173.

Appendix

This study was conducted at the following institutions: National Hospital Organization Nagoya Medical Center, Nagoya; Toyohashi Municipal Hospital, Toyohashi; Meitetsu Hospital, Nagoya; Fujita Health University Hospital, Toyoake; Komaki City Hospital, Komaki; Yokkaichi Municipal Hospital, Yokkaichi; Yamanashi Prefectural Central Hospital, Yamanashi; Okazaki City Hospital, Okazaki, Japan.