radiotherapy; however, in December 1998 she had a BM relapse, involving the thoracic vertebrae and the spine. Salvage chemotherapy reduced the total number of leukemic blasts in BM to below 5%. In March 1999, she underwent BMT from her HLA-2-antigen-mismatched/haploidentical sister at Osaka University Hospital. A total of 3.0×10^8 per kg unmanipulated nucleated cells were infused. The transplant protocol consisted of a high dose of cytarabine and cyclophosphamide and total body irradiation (12 Gy), followed by a short course of methotrexate, tacrolimus and methylprednisolone (2 mg/kg) for graft-versus-host disease (GVHD) prophylaxis. The patient achieved an absolute neutrophil count above 0.5×10^9 per liter on day 20. The last platelet transfusion was performed on day 125. CR and complete donor chimerism was confirmed by a BM examination on day 24. No acute GVHD developed.

We sequentially measured *AML1-MTG8* expression levels using RQ-PCR methods during the clinical course as previously described.[4] *AML1-MTG8* levels in BM stayed below 1.0×10^{-5} after BMT, but increased to 3.8×10^{-5} on day 96. Thereafter, *AML1-MTG8* levels in BM and PB were monitored biweekly and weekly, respectively. Whereas *AML1-MTG8* levels in BM and PB showed parallel movement, those in PB were constantly over 2.0×10^{-4} and were higher than those in BM. Because a BM examination still revealed CR, we suspected a regrowth of leukemia cells in the patient and performed a systemic examination. Computed tomography scans of the pelvis revealed little ascites in the patient on day 109, but an enlargement of the left ovary with a diameter of 5 cm with moderate ascites on day 158 (Figure 1). The ovarian tumor was diagnosed as EMR due to the contamination of t(8;21)-positive leukemia cells in ascites by culdocentesis.

As tacrolimus and prednisolone were tapered rather rapidly for induction of a graft-versus-leukemia effect, skin GVHD developed on day 170. Following increase in tacrolimus and prednisolone, the skin rash disappeared in about a week, followed by shrinkage of the tumor to a diameter of 2.5 cm and

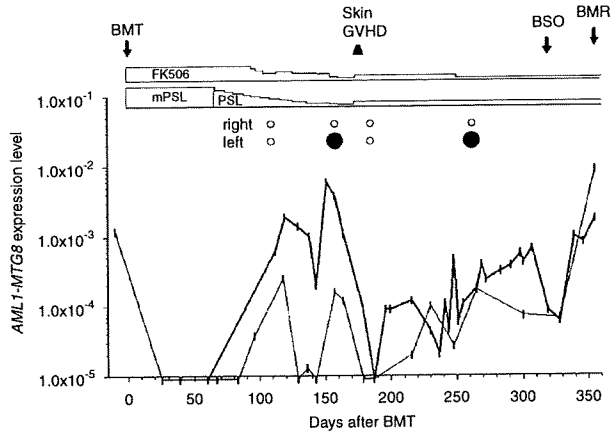

Figure 1 Clinical course and kinetics of the *AML1-MTG8* gene-transcript expression levels in bone marrow (BM) and peripheral blood (PB). Thin and thick lines indicate changes in *AML1-MTG8* expression levels in BM and PB, respectively. In the results of imaging studies of the ovary, open circles indicate the normal size ovary, whereas closed circles indicate the enlarged ovary. Real-time quantitative-PCR was performed with ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). *AML1-MTG8* expression levels in Kasumi-1 cell lines were defined as 1.0. BMR, bone marrow relapse; BMT, bone marrow transplantation; BSO, bilateral salpingo-oophorectomy; FK506, tacrolimus; GVHD, graft-versus-host disease; mPSL, methylprednisolone; PSL, prednisolone.

disappearance of the ascites on day 185. *AML1-MTG8* levels in both BM and PB decreased to below 1.0×10^{-5} on day 187; however, the levels increased again with some fluctuations. Magnetic resonance imaging of the pelvis on day 262 showed enlargement of the left ovarian tumor again. Because BM was still present in CR, bilateral salpingo-oophorectomy was performed on day 319. The bilateral ovaries appeared to be involved, making complete resection impossible due to tight adhesion with surrounding tissues. *AML1-MTG8* levels were highest in the BM greater than those in PB, when morphologic BM relapse occurred on day 355 (Figure 1). Despite chemotherapy and donor lymphocyte infusion, the patient died of renal failure due to obstruction of the bilateral ureters by abdominal mass on day 474.

EMR of leukemia after transplant occurs in diverse sites such as the central nervous system and testis, which makes an early diagnosis difficult. One feasible approach to overcome this problem would be monitoring MRD that is involved in BM.[5,6] In the present case, continuous detection of *AML1-MTG8* chimeric transcripts not only in BM, but also in PB was quite helpful in detecting the presence of EMR. So far nested PCR detection of *AML1-MTG8* chimeric transcripts in BM and PB have not been the indicators of subsequent relapse in t(8;21) AML after BMT.[7] Meanwhile, recent studies using quantitative PCR methods reported that there is a threshold of *AML1-MTG8* expression levels at which subsequent relapse occurs.[1,8,9] Therefore, frequent monitoring of *AML1-MTG8* expression levels or systemic screening for extramedullary disease should be considered, especially as *AML1-MTG8* chimeric transcripts continued to be detected despite CR.

Interestingly, the *AML1-MTG8* levels in BM and PB showed reversal between day 112 and 179, suggesting that t(8;21)-positive leukemia cells originated from extramedullary disease were constantly present in PB, rather than in BM. Given that this unusual relationship returned to the original state at the time of BM relapse, higher *AML1-MTG8* expression levels in PB compared to BM suggest a sign of isolated EMR. However, further studies are required to determine whether screening of PB is superior to BM for early detection of isolated EMR by PCR-based monitoring *AML1-MTG8* expression levels.

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The B-cell calcium sensor predicts progression of chronic lymphocytic leukemia

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Identifying mechanisms responsible for the clinical heterogeneity of chronic lymphocytic leukemia (CLL) is important to develop better treatments for this disease. Variations in responsiveness to immunoreceptor signaling may be responsible for differences in proliferation of CLL cells *in vivo*.[1] Accordingly, we examined the status of the B-cell calcium sensor (Ca_oS) in primary CLL cells, as it responds to extracellular calcium (Ca_o^{2+}) fluctuations by modulating subsequent signal transduction through immunoreceptors.[2]

In contrast to normal B cells, nearly half (23/51) of the CLL samples examined (with approval from the Sunnybrook Health Sciences Center Research Ethics Board) did not release intracellular calcium (Ca_i^{2+}) in response to $CaCl_2$ (labeled Ca_o^{2+} non-responders) (Table 1; Figure 1a). This impaired Ca_oS activity was not due to decreased stores of Ca_i^{2+} in the endoplasmic reticulum, as the Ca^{2+} ATPase inhibitor, thapsigargin, was able to mobilize Ca_i^{2+} in these cells (not shown). While normal B-cells mobilized Ca_i^{2+} in response to as little as 250 μM $CaCl_2$, Ca_o^{2+} non-responder CLL cells remained insensitive to doses as high as 1.5 mM (above which, calcium was toxic) (not shown). These results suggested that the defective responses to Ca_o^{2+} were not due to reduced expression or

Table 1 Summary of clinical properties of CLL patients classified on the basis of release of Ca_i^{2+} stores by their tumor cells in response to Ca_o^{2+}

Variable	*All patients*	*Ca_o^{2+} responders*	*Ca_o^{2+} non-responders*	P-*value*
No. of patients	51	28	23	
Median age, years	61	60.5	63	NS
Sex, no. (%)				
Female	27 (52.9)	14 (50.0)	13 (56.5)	NS
Male	24 (47.1)	14 (50.0)	10 (43.5)	NS
Years after diagnosis, mean ± s.e.	6.1 ± 0.6	6.1 ± 0.9	6.1 ± 0.7	NS
WBC count, × 1000 cells/μl, mean ± s.e.	65.9 ± 11.2	62.7 ± 14.2	69.8 ± 18.3	NS
Rai stage III–IV, no. (%)	30 (58.8)	19 (67.9)	11 (47.8)	0.08
CD38%, mean ± s.e.	16.8 ± 3.4 (*n* = 46)	25.3 ± 5.4 (*n* = 26)	5.9 ± 0.9 (*n* = 20)	<0.02
β2-Microglobulin, mg/l, mean ± s.e.	2.3 ± 0.3 (*n* = 16)	2.6 ± 0.4 (*n* = 11)	1.8 ± 0.3 (*n* = 5)	0.05
Genomic aberrations, no. (%)				
Deletion 11	3 (8.1)	2 (9.1)	1 (6.6)	
Deletion 17	5 (13.5)	4 (18.8)	1 (6.6)	
Trisomy 12	3 (8.1)	2 (9.1)	1 (6.6)	
Deletion 13	21 (56.8)	15 (68.2)	6 (40.0)	
Normal	11 (29.7)	6 (27.3)	5 (33.3)	
Not available	14	6	8	
High-risk cytogenetics[a], no. (%)	10 (27.0)	7 (31.8)	3 (20.0)	NS
LDTs, months, mean ± s.e.	28.3 ± 5.6 (*n* = 44)	10.9 ± 3.3 (*n* = 22)	49.5 ± 9.4 (*n* = 22)	<0.001
Received treatment, no. (%)	25 (49.0)	17 (60.7)	8 (34.8)	0.03
No. of treatments/patient, mean ± s.e.	1.3 ± 0.3	1.9 ± 0.4	0.7 ± 0.3	0.02

Abbreviations: CLL, chronic lymphocytic leukemia; LDTs, lymphocyte doubling times; NS, not significant; WBC, white blood cell.
Assume $n = 51$, unless otherwise indicated.
[a]High-risk cytogenetics include patients with $17p^-$ deletions, $11q^-$ deletions, trisomy 12 or complex multiple abnormalities.

Hematopoietic stem cell transplantation for core binding factor acute myeloid leukemia: t(8;21) and inv(16) represent different clinical outcomes

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We analyzed 338 adult patients with acute myeloid leukemia (AML) with t(8;21) and inv(16) undergoing stem cell transplantation (SCT) who were registered in the Japan Society for Hematopoietic Cell Transplantation database. At 3 years, overall survival (OS) of patients with t(8;21) and inv(16) was 50% and 72%, respectively ($P = .002$). Although no difference was observed when restricted to allogeneic SCT in first complete remission (CR; 84% and 74%), OS of patients with t(8;21) and inv(16) undergoing allogeneic SCT in second or third CR (45% and 86% at 3 years; $P = .008$) was different. OS was not different between patients in first CR who received allogeneic SCT and those who received autologous SCT for both t(8;21) AML (84% vs 77%; $P = .49$) and inv(16) AML (74% vs 59%; $P = .86$). Patients with inv(16) not in CR did better after allogeneic SCT than those with t(8;21) (70% and 18%; $P = .03$). Patients with t(8;21) and inv(16) should be managed differently as to the application of SCT. SCT in first CR is not necessarily recommended for inv(16). For t(8;21) patients in first CR, a prospective trial is needed to clarify the significance of autologous SCT and allogeneic SCT over chemotherapy. (Blood. 2009;113:2096-2103)

Introduction

Core binding factor (CBF) acute myeloid leukemia (AML) including t(8;21)(q22;q22) and inv(16)(p13q22)/t(16;16)(p13;q22) [t(8;21) and inv(16)] is considered to be a favorable cytogenetic subgroup in clinical studies.[1-4] Patients with t(8;21) and inv(16) have shown a markedly improved outcome with repetitive use of high-dose cytarabine.[5-13] However, the major treatment failure is disease recurrence.[14-16] These patients frequently become stem cell transplantation (SCT) candidates.

Both t(8;21) and inv(16) AMLs are associated with disruption of genes encoding subunits of the CBF, a heterodimeric transcriptional factor involved in the regulation of hematopoiesis.[17,18] Although these 2 different cytogenetics also share common clinical characteristics, they are associated with different clinical features such as morphologic presentation and immunophenotypic marker expression.[19]

Several reports demonstrated inferior outcome of t(8;21) compared with inv(16), but the number of patients who underwent transplantation was limited.[14,15,20] A recent study from the Dana-Farber Cancer Institute reported that both patients with t(8;21) and inv(16) de novo AML who underwent allogeneic transplantation performed favorably compared with other karyotypes.[21] To identify the survival data and prognostic factors among the CBF leukemia population who received SCT, we conducted a retrospective analysis using a Japanese multi-institution database with a large number of patients.

Methods

Study population

A total of 2802 adult patients who underwent autologous or allogeneic SCT from 1996 and 2004 for AML were registered in the Japan Society for Hematopoietic Cell Transplantation (JSHCT) database. Patients who underwent SCT from unrelated donors were registered in the different registry in the study period, but not all of the patients undergoing unrelated SCT were registered in the JSHCT database. Demographic, diagnostic, clinical, cytogenetics, induction, and outcome information were collected for each patient, and were sent to a central registration center. Cytogenetic studies were performed in each center, but a central review of cytogenetic analysis was not performed.

Patients with de novo AML aged 16 to 70 years who received hematopoietic SCT as the first transplant were included in the study. No patients with prior history of autologous or allogeneic SCT were included in the study. Of the remaining 2164 patients, 178 patients with t(15;17) or PML/RARα were excluded from the analysis below (Table 1). Finally, of the 1986 patients included in the analysis, 255 were reported to have t(8;21) abnormality, and 83 to have inv(16). A total of 194 patients had no available cytogenetic data. The remaining 1454 patients with normal karyotype and other cytogenetic abnormalities were further coded and analyzed according to published Southwest Oncology Group (SWOG) criteria.[3] The intermediate risk category included patients characterized by +8, −Y, +6, del(12p), or normal karyotype. The unfavorable risk category was defined by the presence of one or more of −5/del(5q), −7/del(7q), abn 3q, 11q, 20q, or

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Table 1. Cytogenetic risk groups of patients with AML who received autologous SCT and allogeneic SCT

Cytogenetic risk groups	No. patients		
	Auto-SCT	Allo-SCT	Total
t(8;21)	61	194	255
inv(16)	17	66	83
t(15;17)*	65	113	178
Intermediate	140	749	889
Unfavorable	35	325	360
Unknown			
Unknown cytogenetic risk	27	178	205
No available cytogenetic data	44	150	194
Total	389	1775	2164

Auto-SCT indicates autologous stem cell transplantation; Allo-SCT, allogeneic stem cell transplantation.

*Patients with t(15;17) were excluded from the analysis.

21q, del(9q), t(6;9), t(9;22), abn 17p, and complex karyotypes defined as 3 or more abnormalities. Patients with other cytogenetic aberrations were considered an unknown risk group, and were analyzed together with 194 patients with no cytogenetic data.

This study was approved by the Committee for Nationwide Survey Data Management of the JSHCT. Informed consent was obtained in accordance with the Declaration of Helsinki.

Transplantation

A total of 1662 patients underwent allogeneic SCT, and 324 underwent autologous SCT. Patients were treated with various conditioning regimens, but most of those who underwent autologous transplantation received non–total body irradiation (TBI) regimens (97%), including busulfan (BU), cytarabine (CA), and etoposide. The most frequently used conditioning regimens before allogeneic SCT were cyclophosphamide (Cy) plus TBI (n = 327 patients), and BU plus Cy (n = 267). Conditioning regimens before allogeneic SCT also included more intensified regimens such as CA plus Cy plus TBI (n = 262) and BU plus Cy plus TBI (n = 146), or reduced-intensity conditioning regimens with fludarabine (n = 241) or cladribine (n = 19).

Stem cell sources for allogeneic SCT were bone marrow in 871 patients, peripheral blood stem cell in 570 patients, bone marrow plus peripheral blood stem cell in 23 patients, and cord blood in 190 patients. A total of 1242 patients underwent allogeneic SCT from a related donor, and 404 patients underwent SCT from an unrelated donor.

Of the 1637 patients who had available data, 74% received transplants from human leukocyte antigen (HLA)–matched donors. Among patients who received unrelated bone marrow transplants, 156 patients were HLA genotypically matched and 51 were HLA mismatched. HLA data for 39 mismatched unrelated bone marrow transplantation patients were available. A total of 32 patients were one locus mismatched, and 7 patients were 2 loci mismatched. Among patients receiving unrelated cord blood transplants, 19 patients were serologically HLA matched and 170 patients were mismatched. HLA incompatibility was 5 of 6 HLA matched in 57 patients, 4 of 6 HLA matched in 99 patients, 3 of 6 HLA matched in 7 patients, and 1 of 6 HLA matched in 1 patient.

Graft-versus-host disease (GVHD) prophylaxis mostly consisted of methotrexate and a calcineurin inhibitor, either cyclosporin A or tacrolimus. Several other prophylaxes include mycophenolate mofetil, antithymocyte globulin, and $CD34^+$ selection. The incidence of acute GVHD was evaluated in 1488 patients who survived more than 28 days, and chronic GVHD was evaluated in 1302 patients who survived more than 100 days after allogeneic SCT. GVHD was evaluated in each center.

Statistical analysis

Correlation between the 2 groups was examined with the chi-square test, Fisher exact test, and the Mann-Whitney *U* test. Disease-free survival (DFS) was calculated from the date of transplantation until the date of relapse or the date of death in CR. Patient survival data were analyzed with the method of Kaplan and Meier and compared by the log-rank test.

Univariate and multivariate analyses for OS were performed with the aid of the Cox proportional hazard regression model, and variables were selected with the stepwise method. The following variables were evaluated: age, sex, and disease status at transplantation; CR versus not in CR; the number of induction courses to achieve CR; one course versus more than one course and failure; type of transplantation (allogeneic SCT vs autologous SCT); conditioning regimen (reduced intensity vs myeloablative); TBI regimen or not; and the existence of additional karyotype abnormalities or not. For those who received allogeneic SCT, in addition to these variables, the following were also evaluated: type of GVHD prophylaxis; short-course methotrexate plus cyclosporin A or short methotrexate plus FK506; acute GVHD, grade II to IV or grade III to IV; chronic GVHD; HLA mismatch; donor; and donor source. The doses of methotrexate were not surveyed. Each factor was considered to be prognostic if the *P* value was less than .05. Data were analyzed with the Stata 9.2 statistical software (College Station, TX).

Results

Initial characteristics of patients

The median age of all patients with AML in total was 41 years old (range, 16-70 years old). Median follow-up period of living patients was 37.3 months (range, 0.4-108 months). Patients were categorized into 5 cytogenetic subgroups: with t(8;21), with inv(16), intermediate risk cytogenetics, unfavorable cytogenetics, and an unknown risk group. Table 1 shows the number of patients in each cytogenetic subgroup and patients with t(15;17), who were excluded from the analysis.

Characteristics of the patients with CBF who underwent allogeneic SCT or autologous SCT are shown in Table 2. No significant difference was observed between characteristic of 2 groups of patients with CBF who received autologous SCT, except for the initial white blood cell count.

Of the 259 patients with CBF who received allogeneic SCT, significantly more patients with t(8;21) had failed to achieve CR with a single course of induction chemotherapy at diagnosis ($P = .002$), and were not in CR at the time of transplantation ($P < .001$). Among patients in CR at transplantation, the ratio of those in first, second, or third CR was not different between t(8;21) and inv(16) subgroups. Significantly more patients with inv(16) received transplants from an unrelated donor ($P = .004$). Table 3 and Table S1 (available on the *Blood* website; see the Supplemental Materials link at the top of the online article) summarize the transplantation data of those undergoing allogeneic SCT. More patients with inv(16) received unrelated transplants compared with t(8;21) patients ($P = .004$).

Overall survival

The OS of 1986 patients with AML at 3 years was 48%, and those with t(8;21), inv(16), intermediate, unfavorable, and unknown cytogenetic risks showed OS of 50%, 72%, 52%, 35%, and 45%, respectively ($P < .001$). Figure 1 shows survival curves of patients with AML patients who underwent allogeneic SCT in first CR (Figure 1A), in second or third CR (Figure 1B), or not in CR (Figure 1C), categorized by the cytogenetic abnormalities. Survival data are listed in Table 4. The OS of patients with t(8;21), inv(16), and intermediate, unfavorable, and unknown risk undergoing allogeneic SCT in first CR was 84%, 74%, 69%, 53%, and 52%, respectively ($P < .001$), and that of patients undergoing allogeneic-SCT

Table 2. Characteristics of patients with CBF AML

	Auto-SCT			Allo-SCT		
	t(8;21) (n = 61), no.	inv(16) (n = 17), no.	P	t(8;21) (n = 194), no.	inv(16) (n = 66), no.	P
Median age, y (range)	44 (17-68)	37 (19-61)	.59	39 (16-70)	34 (16-64)	.054
Median WBC, g/L (range)	8.8 (0.2-94)	33 (2.1-199)	.02	11 (.6-366)	53 (1.8-284)	< .001
Sex						
Male	41	12	.79	117	40	.93
Female	20	5		74	26	
No. of induction chemotherapy at diagnosis of AML						
1 course	48	15	.72	125	55	.002
> 1 or failure*	11	2		56	7	
Additional cytogenetic abnormalities						
None	53	15	> .999	153	54	.61
Positive	8	2		41	12	
Disease status at SCT						
CR	55	16	> .999	108	52	< .001
Not in CR	6	1		85	11	
CR1	43	13	.98	49	21	.29
CR2	7	1		45	26	
CR3	0	1		5	4	
Conditioning regimen						
TBI	0	1	.22	118	47	.078
Not TBI	61	16		71	16	

Correlation between the two groups was examined.
WBC indicates white blood cell count; g/L, 10^9/L; CR1, first complete remission; and CR2 or 3, second or third CR.
*More than 1 or failure includes patients who did not achieve complete remission after first course of induction chemotherapy, and those who were resistant to induction chemotherapy.

in second or third CR was 45%, 86%, 57%, 44%, and 64%, respectively ($P = .09$). OS of patients undergoing allogeneic SCT not in CR was 18%, 70%, 25%, 15%, and 18%, respectively ($P = .003$).

Table 3. Summary of allogeneic SCT

	t(8;21) (n = 194), no.	inv(16), (n = 66), no.	P
Conditioning regimen			
RIST	31	9	.66
Myeloablative	161	56	
GVHD prophylaxis*			
sMTX+CyA	136	48	.78
sMTX+FK	20	8	
HLA			
Match	146	47	.5
Mismatch	45	18	
Donor			
Related	161	44	.004
Unrelated	32	22	
Stem cell source			
BM	101	40	.27
PB	72	17	
CB	18	7	
aGVHD grade			
0-I	117	37	.54
II-IV	60	22	
cGVHD type			
None	64	28	.28
Lmt/Ext	67	20	

Correlation between the two groups was examined. Some of the missing data was not available, and total numbers do not add up to the number of the patients in each group.

RIST indicates reduced intensity stem cell transplantation; sMTX, short-course methotrexate; CyA, cyclosporin A; FK, tacrolimus; BM, bone marrow; PB, peripheral blood; CB, cord blood; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; Lmt, limited; and Ext, extensive.

*Dose of methotrexate was not surveyed in the study. Detail of other GVHD prophylaxis regimens are in Table S1.

When patients undergoing allogeneic SCT in first CR were analyzed, 3-year OS was not significantly different between patients with t(8;21) and inv(16) (84% and 74%, respectively; $P = .28$), between inv(16) and intermediate risk groups (74% and 69%, respectively; $P = .84$), or between t(8;21) and intermediate risk groups (84% and 69%, respectively; $P = .06$). However, when patients undergoing allogeneic SCT in second or third CR were analyzed, the 3-year OS of patients with inv(16) was significantly better than patients with t(8;21) (86% and 45%, respectively; $P = .008$), and better than intermediate risk patients (86% and 57%, respectively; $P = .03$). Difference was not significant between patients in the intermediate risk group and t(8;21) undergoing allogeneic SCT in second or third CR ($P = .36$). The OS of inv(16) patients undergoing allogeneic SCT not in CR was 70% at 3 years, which was also significantly better than that of t(8;21) (18%; $P = .03$) and the intermediate risk group (25%; $P = .045$).

In addition, the OS of t(8;21) undergoing allogeneic SCT in first CR was significantly better than that of the unfavorable risk group (84% and 53%, respectively; $P < .001$), but the difference between the 2 groups was not significant among patients undergoing allogeneic SCT in second or third CR. In contrast, OS was not different between inv(16) and unfavorable groups undergoing allogeneic SCT in first CR, but it was significantly different when they underwent allogeneic SCT in second or third CR (86% and 44%, for inv(16) and unfavorable groups, respectively; $P = .01$) or allogeneic SCT in non-CR (70% and 15%, respectively; $P = .006$).

Survival curves of patients who underwent autologous SCT in first CR, second or third CR, and not in CR are shown in Figure 2A, 2B, and 2C, respectively. The overall survival of patients with t(8;21), inv(16), and intermediate, unfavorable, and unknown cytogenetic risks in first CR was 77%, 59%, 74%, 38%, and 71%, respectively ($P = .049$), while that of patients undergoing autologous SCT in second or third CR was 43%, 50%, 59%, 44%, and 42%, respectively ($P = .8$). The OS of patients undergoing autologous SCT not in CR with t(8;21), inv(16), intermediate, and

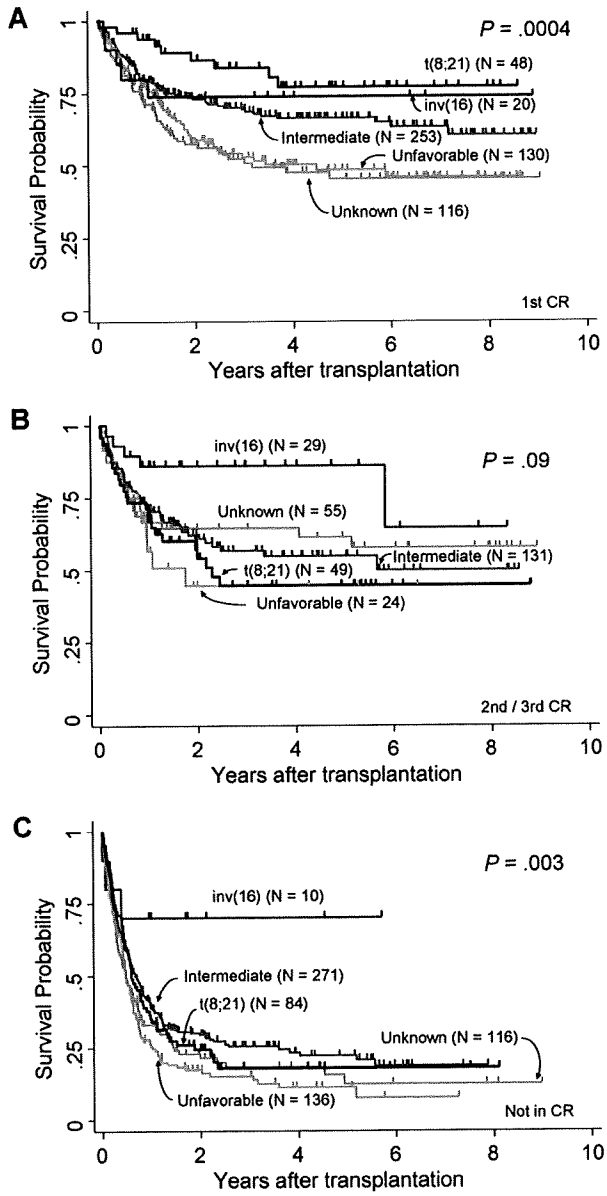

Figure 1. OS difference of patients undergoing allogeneic SCT between cytogenetic subgroups. (A) Survival curves of patients undergoing allogeneic SCT in first CR. (B) Survival curve of patients undergoing allogeneic SCT in second or third CR. (C) Survival curves of patients undergoing allogeneic SCT not in CR. Each are categorized by cytogenetic risk groups, respectively.

unknown risks was 17%, 100%, 25%, and 13%, respectively, and the survival curve of patients in the unfavorable risk group did not reach 3 years ($P = .35$).

Figure 3A and B focus on t(8;21) and inv(16) patients, stratified according to the type of (allogeneic or autologous) and disease status at the time of transplantation (first CR, second or third CR, and not in CR). The 3-year overall survival of t(8;21) patients in first CR was not different between allogeneic and autologous transplantation (84% and 77%, respectively), as well as that of patients in second or third CR (45% and 43%, respectively) and patients not in CR (18% and 17%, respectively). Similarly, the 3-year OS of inv(16) patients was not different between allogeneic and autologous transplantation when they underwent transplantation in first CR (74% and 59%). A significant difference was observed among the 3 disease status groups of t(8;21) patients ($P < .001$; Figure 3A), but not inv(16) patients ($P = .75$; Figure 3B).

The OS of allogeneic SCT, excluding cord blood transplantation, was not different from the analysis presented here, including bone marrow, peripheral blood, and cord blood transplantation (Table S2; Figures S1,S2).

DFS after SCT was also different among cytogenetic risk groups ($P < .001$). DFS of patients with inv(16) (69% at 3 years) was better compared with t(8;21) (49%), intermediate (46%), unfavorable (31%), and unknown (41%) risk groups. Among patients undergoing allogeneic SCT in first CR, DFS was also different among cytogenetic subgroups ($P < .001$). When t(8;21), inv(16), and intermediate cytogenetic subgroups undergoing allogeneic SCT in first CR were compared, the difference was not statistically significant between t(8;21) and inv(16) (78% and 73% at 3 years; $P = .58$), between t(8;21) and intermediate risk group (78% and 63%; $P = .1$), nor between inv(16) and intermediate risk group (73% and 63%; $P = .65$). DFS of patients with t(8;21) undergoing allogeneic SCT in first CR was better than that of the unfavorable risk group (78% and 47%, respectively; $P < .001$), but the difference was not significant between inv(16) and unfavorable risk groups (73% and 47%, respectively; $P = .16$).

DFS was not significantly different when 5 cytogenetic subgroups among patients undergoing allogeneic SCT in second or third CR were compared ($P = .32$). The DFS of patients undergoing allogeneic SCT in second or third CR was not significantly different between t(8;21) and inv(16) (43% and 71% at 3 years; $P = .053$), t(8;21) and the intermediate group (43% and 47%; $P = .76$), or inv(16) and the intermediate group (71% and 47%; $P = .06$). The difference was also not significant between t(8;21) and unfavorable risk groups (43% and 42%; $P = .7$), nor between inv(16) and unfavorable risk groups (71% and 42%; $P = .06$). The DFS of patients undergoing allogeneic SCT who were not in CR was significantly different among the 5 cytogenetic subgroups ($P = .005$), and that of inv(16) (75% at 3 years) was significantly better than t(8;21) (18%; $P = .02$), the intermediate risk group (22%; $P = .03$) and the unfavorable risk group (10%; $P = .003$).

Relapse and TRM

The relapse rate (RR) after SCT also differed among cytogenetic subgroups ($P < .001$). The RR of patients with inv(16) (18% at 3 years) was lower than t(8;21) (38%), intermediate (38%), and unfavorable (56%) risk groups. The RR of t(8;21) and inv(16) after allogeneic SCT was not statistically different in either first CR (16% and 6%; $P = .45$) or second or third CR (34% and 16%, respectively; $P = .09$).

Transplantation-related mortality (TRM) of all patients with AML was 22% at 3 years. The TRM of t(8;21) (18%), inv(16) (11%), and intermediate (21%), unfavorable (24%), and unknown risk groups (27%) was significantly different among cytogenetic risk groups ($P = .02$).

Evaluation of prognostic variables in CBF

Univariate analyses of t(8;21) showed that age ($P = .004$), not in CR at transplantation ($P < .001$), allogeneic SCT ($P = .01$), and TBI regimen ($P = .006$) were significant prognostic factors indicating poor OS (Table 5). Multivariate analysis for OS revealed older age ($P = .01$) and not in CR at transplantation ($P < .001$) as the independent prognostic variables. Univariate analyses of t(8;21) patients who received allogeneic SCT in CR showed that age ($P = .02$), TBI regimen ($P = .01$), and second and third CR at

Table 4. Outcome of the AML patient population by cytogenetic risk groups

	t(8;21)		inv(16)		Intermediate		Unfavorable		Unknown		
	%	N	%	N	%	N	%	N	%	N	*P*
OS											
Allogeneic SCT											
CR1	84	48	74	20	69	253	53	130	52	116	< .001
CR2/CR3	45	49	86	29	57	131	44	24	64	55	.09
Non-CR	18	84	70	10	25	271	15	136	18	116	.003
Autologous SCT											
CR1	77	42	59	13	74	89	38	15	71	39	.05
CR2/CR3	43	7	50	2	59	15	44	6	42	18	.8
Non-CR	17	6	100	1	25	16	0	10	13	8	.35
DFS											
Allogeneic SCT											
CR1	78	48	73	19	63	249	47	129	48	113	< .001
CR2/CR3	43	48	71	27	47	129	42	22	57	54	.32
Non-CR	18	81	75	8	22	255	10	128	16	107	.005
Autologous SCT											
CR1	73	41	62	13	64	81	33	15	61	36	.09
CR2/CR3	43	7	50	2	36	14	50	6	39	18	.89
Non-CR	17	6	100	1	25	16	0	10	17	6	.45

transplantation ($P < .001$) were also significantly prognostic for poor OS. These variables remained significant after multivariate analysis. Univariate analyses for inv(16) patients showed only age ($P = .009$) to be a significant prognostic factor (Table 5). The univariate analysis of inv(16) patients who underwent allogeneic SCT in CR showed only additional karyotype abnormalities to be an unfavorable prognostic variable ($P = .009$).

Additional cytogenetic abnormalities to CBF

A total of 49 patients with t(8;21) and 14 with inv(16) had additional cytogenetic abnormalities. Data for additional cytogenetic abnormalities were obtained in 42 patients with t(8;21) and 13 patients with inv(16) (Table 6). Additional abnormalities were selected that have been reported to be prognostic by others, including loss of sex chromosome (X or Y), trisomy 8, trisomy 4, del(7q), and del(9q) for the t(8;21) group, and trisomy 22, trisomy 8, trisomy 21, del(7q), and del(9q) for the inv(16) group.[14,15,20,22,23] There were no patients with trisomy 21 in the data of patients with CBF. Patients with t(8;21) and patients with inv(16) were analyzed separately. Among t(8;21) patients undergoing allogeneic SCT, survival was not different between patients with and without additional karyotype abnormalities. When patients with inv(16) were analyzed, the survival was not different between patients with (n = 13) and without (n = 67) additional abnormalities (61% and 74%, respectively; $P = .07$). The survival of patients undergoing allogeneic SCT without additional abnormality (n = 52) was significantly better than that with additional abnormality (n = 11), (85% and 53%, respectively; $P = .004$). When analysis was restricted to patients in CR with inv(16) undergoing allogeneic SCT, a similar difference was observed (86% without additional abnormality [n = 42], and 60% with additional abnormality [n = 8], respectively; $P = .03$). Difference in OS was observed among non-CR patients with (n = 9) and without (n = 1) additional abnormality, but this difference may not be relevant with too few patients in the analysis. We further analyzed subgroups of additional abnormalities of the patients with inv(16). Although the number of patients were limited, significant difference was found among 3 groups of patients; trisomy 8 or trisomy 22 as a sole abnormality (n = 4), without additional abnormality (n = 69), and other additional abnormality to inv(16) (n = 10). The OS at 3 years were 100%, 74%, and 42%, respectively ($P = .002$). The OS of patients undergoing allogeneic SCT was also different among these 3 groups (100%, n = 3; 85%, n = 52; and 33%, respectively; $P < .001$).

Discussion

We analyzed the outcome of a large group of patients with adult CBF AML in Japan who were treated with SCT. The current study focused on the different outcome of the 2 different cytogenetic subgroups of patients with CBF AML undergoing SCT. Our study demonstrated a comparable outcome between patients with t(8;21) and inv(16) undergoing SCT in first CR, but the prognosis between these 2 cytogenetic subgroups was different beyond first CR.

In the literature, there have been several reports showing inferior survival of patients with t(8;21) compared with inv(16) patients undergoing induction chemotherapy and SCT.[14,15,20] Other studies categorized both patients with t(8;21) and inv(16) undergoing allogeneic SCT together as good-risk CBF AML,[1,21] with a relatively comparable prognosis. In our study, OS of patients with t(8;21) undergoing allogeneic SCT in first CR was not statistically different from intermediate cytogenetic subgroup (84% and 79% at 3 years, respectively; $P = .058$). Moreover, the survival of inv(16) (74% at 3 years) and intermediate cytogenetic subgroups showed no statistically significant difference.

In contrast, we have here demonstrated that the prognosis of patients with t(8;21) undergoing allogeneic SCT with second or third CR disease was significantly poor compared with those with inv(16). This finding is consistent with those of other studies reporting differences between the 2 types of CBF AML.[14,15] In the present study, non-CR disease with t(8;21) was also significantly poor compared with patients with inv(16). The Acute Leukemia French Association reported that allogeneic donor availability among patients with CBF AML who were in second CR was a prognostic factor for better survival.[16] We believe that different treatment strategies should be applied for patients with t(8;21) and those with inv(16) other than first CR.

Patients with t(8;21) undergoing allogeneic SCT and autologous SCT had a similar survival rate when they underwent transplantation in first CR, and in further CR. No survival difference between allogeneic SCT and autologous SCT was also

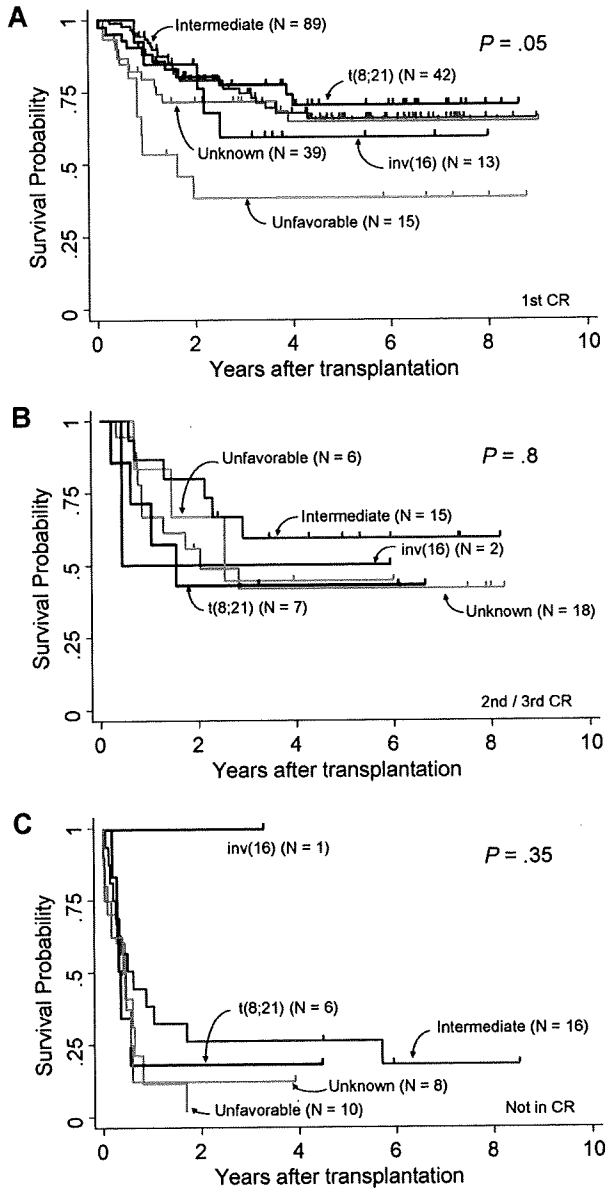

Figure 2. OS difference of patients undergoing autologous SCT between cytogenetic subgroups. (A) Survival curves of patients undergoing autologous SCT in first CR. (B) Survival curves of patients undergoing autologous SCT in second or third CR. (C) Survival curves of patients undergoing autologous SCT not in CR. Each are categorized by cytogenetic risk groups, respectively.

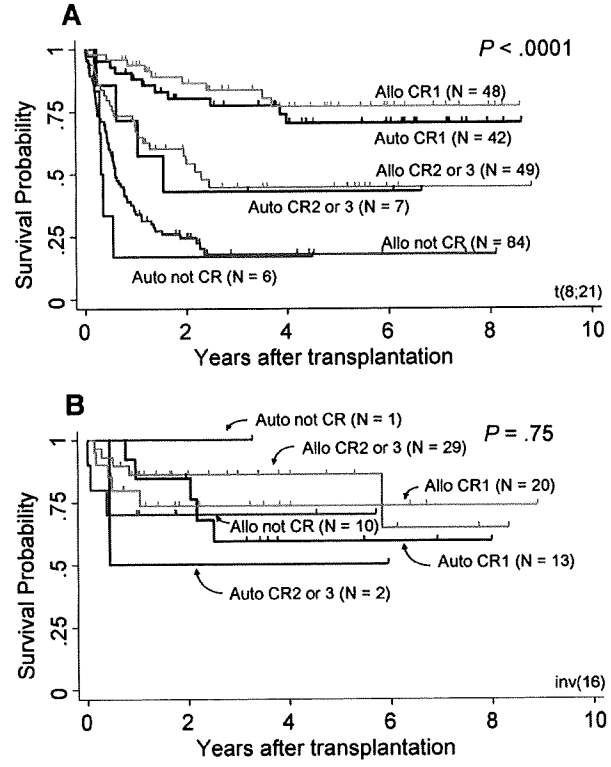

Figure 3. OS of patients with CBF. Survival curves of patients with t(8;21) (A) and with inv(16) (B). Both are stratified according to the type of transplantation (allogeneic or autologous) and disease status at the time of transplantation (first CR, second or third CR, and not in CR).

observed among inv(16) patients receiving SCT in first CR (74% and 59%, respectively). The University of California, San Francisco (UCSF) group described the good results of patients with advanced AML undergoing autologous SCT in second or third remission, including patients with CBF.[24] As in our study, the European Group for Blood and Marrow Transplantation (EBMT) reported that the survival rate of t(8;21) patients who received allogeneic bone marrow transplantation was not significantly different from that of patients who received autologous SCT.[1] Results by others showed that allogeneic SCT in first CR did not benefit good-risk cytogenetic subgroups.[3,25,26] Schlenk et al also demonstrated that t(8;21) patients receiving allogeneic SCT or chemotherapy showed no difference in outcome.[23] These results suggest that autologous SCT can be considered as postremission therapy for patients with CBF AML, but it remains unclear whether SCT is more beneficial for patients with CBF than high-dose cytarabine. Survival of patients with inv(16) was favorable beyond first CR. Patients with inv(16) in second or third CR, or even non-CR patients, are good candidates for allogeneic SCT. There are long-term survivors after allogeneic SCT in non-CR disease, so t(8;21) patients with no other choice of treatment, such as those in further CR or non-CR, can proceed to allogeneic SCT. In order to confirm the appropriate treatment for t(8;21) patients in first CR, a prospective trial is needed to compare the results of autologous SCT for t(8;21) in first CR with standard chemotherapy. t(8,21) patients with suitable related or well-matched donors should be recommended to participate in a risk-adopted prospective trial when they receive allogeneic SCT in first CR.

There were differences between the 2 types of CBF AML with respect to prognostic valuables. Age was a significant and independent prognostic variable in both t(8;21) and inv(16) patients, a finding in agreement with reports from some,[14,27] but not all,

Table 5. Prognostic factors affecting overall survival of patients with t(8;21)

Variables	Unfavorable factors	Hazard ratio	95% CI	P
t(8;21)				
Age		1.02	1.01-1.04	.004
Disease status at SCT	Not in CR	4.4	3.1-6.5	< .001
Transplantation	Allo-SCT	1.9	1.2-3.0	.01
Conditioning regimen	TBI	1.7	1.2-2.5	.005
inv(16)				
Age		1.1	1.0-1.1	.009

CI indicates confidence interval.

Table 6. Additional cytogenetic abnormalities among patients with CBF

Additional cytogenetic abnormalities	t(8;21), no.	inv(16), no.
None	206	69
With additional abnormalities	49	14*
−Y	10	0
−X	5	0
Trisomy 22	0	3†
Trisomy 8	0	2†
Trisomy 4	2*	0
Complex	7	4
del(7q)	1†	2
del(9q)	6	0
Other abnormalities	27	9‡
Unknown	7	1

*Patients with additional change to inv(16) and trisomy 4 with t(8;21) tended to show poor survival tendency, with $P < .1$.

†All patients with trisomy 22, trisomy 8 with inv(16), and del(7q) with t(8;21) were alive and censored at survival analysis.

‡Other abnormalities with inv(16) was poorly prognostic, with $P < .001$.

investigators.[28] Transplantation in CR was a significant and independent prognostic factor for patients with t(8;21), but not for those with inv(16). The Cancer and Leukemia Group B (CALGB) also reported differences between t(8;21) and inv(16) in prognostic factors, in terms of race, sex, and secondary cytogenetic abnormalities.[14] Among patients with CBF AML, t(8;21) and inv(16) patients undergoing SCT should be considered 2 separate clinical entities in future clinical studies.

Several specific additional karyotype abnormalities have been reported to be prognostic in patients with CBF AML. Among t(8;21) patients, no specific additional karyotype abnormality was prognostic for overall survival. The poor prognosis of t(8;21) patients with trisomy 4 has been reported by others,[22] but the survival difference was not statistically significant ($P = .085$) in our case series. Since there were limited numbers of patients with additional abnormalities, the real significance of each additional abnormality should be investigated in large numbers of patients.

The reason for the different survival results between patients with t(8;21) and inv(16) undergoing allogeneic SCT in our study remains unclear. The impact of additional mutational events such as c-Kit, FLT3, RAS, and gene-expression profiles was reported to be associated with the clinical outcome of patients with CBF AML.[29-34] The effects of these additional mutational events and gene-expression profiles on the clinical outcome of autologous and allogeneic SCT have not yet been studied. Which proportion of the patients with CBF AML benefited from earlier SCT remains to be identified in future clinical studies. Recent studies by others also suggested that prognosis of CBF AML could differ among different ethnic groups or races.[14,35-37] The background molecular basis among the Japanese population must also be taken into account in future studies.

In conclusion, the survival outcome of patients with CBF AML was similar when they received allogeneic or autologous SCT in first CR. However, the outcomes were significantly different between t(8;21) and inv(16) when they received allogeneic SCT beyond first CR. Therefore, these 2 kinds of CBF AML should be managed differently when applying SCT.

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Authorship

Contribution: Y. Kuwatsuka, K.M., and R.S. contributed to data collection, designed and performed the study, analyzed the data, and wrote the manuscript; M.K., A.M., H.O., R.T., S.T., K.K., K.Y., Y.A., T.Y., and H.S. contributed to data collection and analysis and writing of the paper; and Y. Kodera contributed to data collection and writing of the paper, conceived the study, and provided intellectual input.

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Engraftment syndrome following allogeneic hematopoietic stem cell transplantation in children

Nishio N, Yagasaki H, Takahashi Y, Hama A, Muramatsu H, Tanaka M, Yoshida N, Yoshimi A, Kudo K, Ito M, Kojima S. Engraftment syndrome following allogeneic hematopoietic stem cell transplantation in children.
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Abstract: ES is a complication that occurs immediately before or at the timing of neutrophil engraftment following autologous or allogeneic SCT. It is characterized by fever, skin rash, and non-cardiac pulmonary infiltrates. We evaluated the incidence, risk factors, and outcomes of ES following allogeneic SCT in children. Of 100 pediatric patients, 20 (20%) developed ES occurring at a median of 14 days (range 8–27 days) post-transplant. Patients presented with fever (100%), skin rash (100%), diffuse pulmonary infiltration (25%), and body weight gain (85%). On multivariate analysis, significant risk factors for ES included younger age (< 8 yr old) and human leukocyte antigen disparity between donors and recipients. Univariate analysis showed that patients with ES had a higher incidence of developing chronic graft-versus-host disease and ES was not associated with other complications. Event-free survival did not significantly differ between patients with and without ES regardless of the presence of malignant or non-malignant diseases.

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ES is a complication that occurs immediately before or at the time of neutrophil engraftment following autologous or allogeneic SCT (1). It is characterized by fever, skin rash, and non-cardiac pulmonary infiltrates. Although the pathophysiology of ES is poorly understood, pro-inflammatory cytokines such as interleukin-1, tumor necrosis factor-alpha and interferon gamma and subsequent cellular and cytokine interactions might play major roles (1).

ES has been identified in several series of adults who received various conditioning regimens and stem cell sources (2–15). Information about ES in pediatric patients following allogeneic SCT is required because the incidence and severity of other complications such as GVHD are quite different between children and adults. Although several reports have described ES following autologous SCT in children (12, 16, 17), only one report has published in an allogeneic setting (18). Therefore, we retrospectively analyzed the incidence, risk factors, and clinical outcome of ES in pediatric patients after allogeneic SCT.

Abbreviations: ATG, anti-thymocyte globulin; BM, bone marrow; CMV, cytomegalovirus; EFS, event-free survival; MTX, methotrexate; ES, engraftment syndrome; G-CSF, granulocyte colony-stimulating factor; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HR, hazard ratio; PBSC, peripheral blood stem cells; PSL, prednisolone; SCT, stem cell transplantation; TBI, total body irradiation; VOD, veno-occlusive disease.

Materials and methods

Between April 1997 and December 2006, 109 patients underwent allogeneic hematopoietic SCT at the Department of Pediatrics, Nagoya University Hospital. Patients who were > 18 yr old or who did not receive any conditioning before transplantation (e.g., patients with severe immunodeficiency syndrome) were excluded. For patients who received multiple transplants, only the first transplant was included for this analysis. We assessed the remaining 100 pediatric patients with hematological malignancies (n = 43), non-malignant hematological disorders (n = 53), and solid tumors (n = 4) who met the inclusion criteria.

Among the malignant diseases, hematological malignancies and solid tumors not in remission were defined as high risk, while all other conditions were defined as low risk. Conditioning regimens were classified into TBI-based myeloablative regimens, high-dose chemotherapy-based myeloablative regimens, and reduced-intensity regimens defined as those in which hematopoietic recovery was expected within 28 days without transplantation (19). All patients received standard doses of oral amphotericin B and acyclovir as fungal and viral prophylaxis. Blood was obtained for weekly CMV antigenemia testing from engraftment to discharge, and patients who developed CMV antigenemia received preemptive therapy with ganciclovir. G-CSF was started from day five to neutrophil engraftment in patients excluding those with myeloid malignant diseases. ATG was used in patients with non-malignant diseases, mainly, aplastic anemia who were transplanted from unrelated donor and in patients transplanted from haplo-identical related donor. Engraftment day was defined as the first of three consecutive days in which the patient had an absolute neutrophil count greater than 0.5×10^9/L. Failure to engraft by day 30 was considered as primary graft failure.

Acute GVHD was graded according to the established criteria (20). GVHD prophylaxis comprised tacrolimus (intravenous infusion of 0.02 mg/kg/day starting on day −1, with dose adjustments to maintain blood levels of 5–15 ng/dL) or cyclosporine (intravenous infusion of 3 mg/kg/day starting on day −1, with dose adjustments to maintain trough blood levels at 100–200 ng/dL) with or without methotrexate (15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, and +11). When patients had recovered from gastrointestinal toxicity, the route of both drugs was switched to oral administration.

Diagnosis and management of ES

We defined ES according to the diagnostic criteria described by Spitzer (1). The major criteria were as follows: (i) fever of ≥38.3 °C without identifiable infectious etiology, (ii) erythrodermatous rash involving more than 25% of the body surface area and not attribute to medication, and (iii) non-cardiogenic pulmonary edema manifested as diffuse pulmonary infiltrates consistent with this diagnosis, and hypoxia. The minor criteria were as follows: (i) hepatic dysfunction with either total bilirubin ≥2 mg/dL or transaminase levels greater than or equal to double the normal level, (ii) renal insufficiency (serum creatinine of greater than or equal to double the baseline value), (iii) weight gain ≥2.5% of baseline body weight, and (iv) transient encephalopathy unexplainable by other causes. A diagnosis of ES was established based on the presence of all three major criteria or of two major criteria and one or more of the minor criteria arising within 96 h after engraftment. Additional clinical and pathologic symptoms and signs of GVHD should at least initially be absent.

After a diagnosis of ES, patients were treated with 2 mg/kg/day of PSL. If the patient had no response or disease progressed, second-line treatment with high dose methylprednisolone was applied. Once symptoms improved, corticosteroids were gradually tapered over 3–4 wk.

Statistical methods

We statistically analyzed factors associated with the development of ES. Pretransplantation variables included patient age, gender, and underlying disease. Transplantation-related variables included ABO blood type incompatibility, use of amphotericin B, busulfan, fludarabine, ATG and G-CSF, intensity of conditioning regimen, GVHD prophylaxis, stem cell source, and HLA disparity. For risk factor analysis, we considered HLA disparity as a binary variable. The analysis of HLA matching for transplants using unrelated donors was basically based on serological typing. The high-risk group included patients transplanted with BM from related donors or cord blood from unrelated donors with greater than or equal to two serological locus mismatches, or BM from unrelated donor greater than or equal to one serological locus mismatch. Serological mismatches at two HLA-DR loci were included. Patients transplanted from other donors were defined as low risk.

Predictors for the occurrence of ES were evaluated by univariate analysis using the Mann–Whitney *U*-test for continuous variables, the chi-squared test for categorical variables, and multivariate analysis using a logistic regression model. Complications in patients with and without ES were also evaluated by univariate analysis. Values of $p < 0.05$ were considered statistically significant. Survival was estimated by the Kaplan–Meier method and differences were assessed using the log-rank test. The cumulative incidence of non-relapse mortality was estimated considering death because of relapse or progression of underlying disease as competing risk (21). All analyses were performed using SPSS 14.0 (SPSS, Chicago, IL, USA).

Results

Table 1 shows the patients' characteristics. The median age was 8.8 years (range, 0.4–18 yr). The risk of underlying disease was high in 26 patients. The sources of stem cells were BM (n = 82), cord blood (n = 15), PBSC (n = 1), and BM combined with PBSC (n = 2). The conditioning regimen was myeloablative for 78 patients, and 48 of them received a TBI-based regimen. ATG and G-CSF was administered to 41 and 81 patients, respectively. GVHD prophylaxis was tacrolimus with MTX in 66 patients, cyclosporine with MTX in 26 and others in the remaining eight.

Twenty patients (median age, 6.5 yr; range 0.6–14.0 yr) developed ES, and their characteristics are shown in Table 2. Median time to neutrophil recovery above 500/μL was 16 days (range 13–26 days), and the onset of ES occurred at a median of 14 days (range 8–27 days). Patients presented with fever (100%), skin rash (100%), diffuse pulmonary infiltration (25%), hepatic dysfunction (10%), renal dysfunction (5%), and body weight gain (85%). None of the patients who developed pulmonary infiltration required mechanical ventilation. Risk factors for developing ES were evaluated (Table 3). High-risk HLA disparity was significant in univariate analysis ($p = 0.03$). Multivariate analysis identified HLA disparity and younger age (<8 yr old) as significant risk factors for developing ES (HR = 6.58, $p = 0.018$ and HR = 4.16, $p = 0.032$, respectively). Use of

Table 1. Patients and transplant characteristics

	n = 100
Patient age, years, median (range)	8.8 (0.4–18)
Gender, male/female	59/41
Donor/patient gender	
Male/male	30
Male/female	16
Female/female	24
Female/male	29
Unknown	1
Diagnosis	
ALL	13
AML	11
Lymphoma	5
CML	3
AA	34
MDS	6
Others	28
Risk of underlying malignancies	
High	26
Low	21
Cell source	
BM	
Matched related	29
Matched unrelated	27
Mismatched related	10
Mismatched unrelated	16
PBSC	
Mismatched related	1
Cord blood	
Matched unrelated	2
Mismatched unrelated	13
BM + PBSC	
Mismatched related	2
Conditioning regimen	
Myeloablative	
TBI	48
Non-TBI	30
Reduced intensity	22
Use of ATG	
Yes	41
No	59
GVHD prophylaxis	
Tacrolimus + sMTX	66
Cyclosporine + sMTX	26
Others	8

ALL, acute lymphoblastic leukemia; AML, acute myelogeneous leukemia; CML, chronic myelogeneous leukemia; MDS, myelodysplastic syndrome; AA, aplastic anemia; BM, bone marrow; PBSC, peripheral blood stem cells; TBI, total body irradiation; ATG, anti-thymocyte globulin; GVHD, graft-versus-host disease; sMTX, short-term methotrexate.

G-CSF and parental amphotericin B, and the infused cell dose were not significantly associated with the development of ES in our series. A skin biopsy was taken from 14 patients with ES. Pathologic findings of skin had no evidence of apoptosis and intraepithelial lymphocytosis in all patients. Epidermo-dermal junction showed mild edematous changes without lymphocytic infiltration. Small venules and capillaries contained neutrophils in these lumens. Histopathologic findings were different from acute GVHD of the skin.

Table 2. Clinical characteristics of patients with ES and symptoms of ES

Median age, years (range)	6.5 (0.6–14.0)
Gender, male/female	14/6
Diagnosis	
AA	7
ALL	5
Others	8
Median days of engraftment (range)	16 (13–26)
Median days of onset of ES, (range)	14 (8–27)
Symptoms of ES (%)	
Fever	20 (100)
Skin rash	20 (100)
Pulmonary infiltrate	5 (25)
Hepatic dysfunction	2 (10)
Renal dysfunction	1 (5)
Body weight gain	17 (85)
Encephalopathy	0 (0)
Diarrhea	6 (30)

AA, aplastic anemia; ALL, acute lymphoblastic leukemia; ES, engraftment syndrome.

The first-line therapy of PSL achieved complete resolution of the ES symptoms within three days in nine of 14 patients. Resolution was not achieved in the remaining five patients even at seven days after starting therapy. Two patients reached complete resolution of ES after the administration of high dose methylprednisolone and one did so after undergoing second-line therapy with etoposide. One patient received high dose methylprednisolone as a first-line therapy and reached complete resolution. The remaining four patients achieved complete resolution within 28 days after the onset of ES without specific treatment. Transplant-related complications in patients with and without ES are shown in Table 4. Acute GVHD developed in eight of 20 patients with ES. Among the eight patients, ES progressed and merged with acute GVHD in two patients, while acute GVHD developed after resolution of ES in six patients. Eight of evaluable 17 patients with ES later developed chronic GVHD and three of these eight patients had acute GVHD prior to developing chronic GVHD. Univariate analysis revealed that patients with ES more frequently developed chronic GVHD ($p = 0.038$).

Fig. 1 shows the EFS rates of patients with malignancies. The EFS of patients with and without ES in low-risk group did not significantly differ ($75 \pm 21\%$ vs. $69 \pm 13\%$, respectively, $p = 0.98$). In high risk group, EFS of patients with ES were likely to be lower than that of patients without ES ($42 \pm 22\%$ vs. $62 \pm 19\%$, respectively) but the difference did

Table 3. Risk factors for developing ES

Variable	ES, n = 20 (%)	No ES, n = 80 (%)	Univariate p-value	Multivariate HR	95% CI	p-value
Age, <8 yr	13 (65)	32 (40)	0.11	4.17	1.13–15.3	0.032
Female gender	6 (30)	34 (43)	0.31			NS
High risk underlying disease	6 (30)	20 (25)	0.44			NS
Malignancy	10 (50)	37 (46)	0.76			NS
Blood type mismatch	12 (60)	44 (55)	0.69			NS
Median days of engraftment (range)	16.5 (13–26)	17 (11–34)	0.51			NE
Infused NCC from BM or PBSC, 10^8/kg (range)	4.3 (1.1–30)	3.3 (0.85–11)	0.19			NE
Infused NCC from CB, 10^7/kg (range)	4.0 (3.5–33)	5.8 (1.1–14)	0.45			NE
Use of parental amphotericin B	1 (5)	4 (5)	1.0			NS
Use of busulfan	6 (30)	24 (30)	1.0			NS
Use of fludarabine	5 (25)	28 (35)	0.39			NS
Use of ATG	9 (45)	32 (40)	0.68			NS
Use of G-CSF	18 (90)	63 (79)	0.25			NS
Reduced intensity regimen	2 (10)	20 (25)	0.15			NS
GVHD prophylaxis						
Cyclosporine + sMTX	5 (25)	21 (26)	0.91			NS
Cord blood	5 (25)	10 (13)	0.16			NS
HLA disparity						
High risk	9 (45)	17 (21)	0.030	6.58	1.38–31.5	0.018

ES, engraftment syndrome; HR, hazard ratio; CI, confidential interval; NCC, nuclear cell count; BM, bone marrow; PBSC, peripheral blood stem cells; CB, cord blood; ATG, anti-thymocyte globulin; G-CSF, granulocyte colony stimulating factor; GVHD, graft versus host disease; sMTX, short-term methotrexate; HLA, human leukocyte antigen; NS, not significant; NE, not evaluable.

Table 4. Complications in patients with and without ES

Variable	ES, n = 20 (%)	No ES, n = 80 (%)	p-value
Grade II–IV aGVHD	8/20 (40)	23/78 (29)	0.33
cGVHD	8/17 (47)	16/72 (22)	0.038
IP	0/20 (0)	8/80 (10)	0.12
VOD	1/20 (5)	3/80 (3.8)	0.80

ES, engraftment syndrome; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; IP, idiopathic pneumonia; VOD, veno-occlusive disease.

not reach statistical significance (p = 0.29). The overall survival rates of patients with non-malignant disease were excellent (Fig. 2) and there were no significant differences between patients with and without ES (100% vs. 95 ± 3%, respectively, p = 0.50). Fig. 3 shows transplant-related mortality rates. Patients with and without ES did not significantly differ with respect to these rates (5 ± 5% vs. 11 ± 3%, respectively, p = 0.56).

The causes of deaths in three of the 20 patients with ES were because of relapse of underlying disease in two and VOD in one. None of the patients died of ES. Among those without ES, six died of underlying disease, four of idiopathic pneumonia, one of encephalitis, one of VOD, one of multi organ failure, and one of fungal infection.

Discussion

ES is a clinical entity described in both autologous and allogeneic settings and comprises symptoms or signs including fever, skin rash, and non-cardiac pulmonary edema (1, 2, 4, 7–9, 11, 12, 16, 17, 22). Several studies have examined ES in pediatric patients with autologous transplants (7, 12) but only one has published in those with allogeneic SCT (18). We found that the incidence of ES was 20% using Spitzer's criteria (1) and that HLA disparity and younger age were risk factors for developing ES. To date, consensus has not been reached regarding the definition of ES, which make it difficult to compare the incidence, risk factors, and outcome of ES in our study with those reported by others.

The original definition proposed by Spitzer was not uniformly applied in subsequent published studies. Several investigators have proposed their own criteria with which to evaluate the incidence of ES. Maiolino et al. (7) evaluated the incidence of ES in children who received autologous transplants and pointed out that Spitzer's criteria were stricter than others. Applying the criteria proposed by Spitzer reduced the incidence of ES in their series from 20% to 11%. The criteria of Maiolino et al. mainly comprised non-infectious fever and skin rash, and they noted that the risk of under-diagnosis and delay in starting treatment might increase if Spitzer's criteria

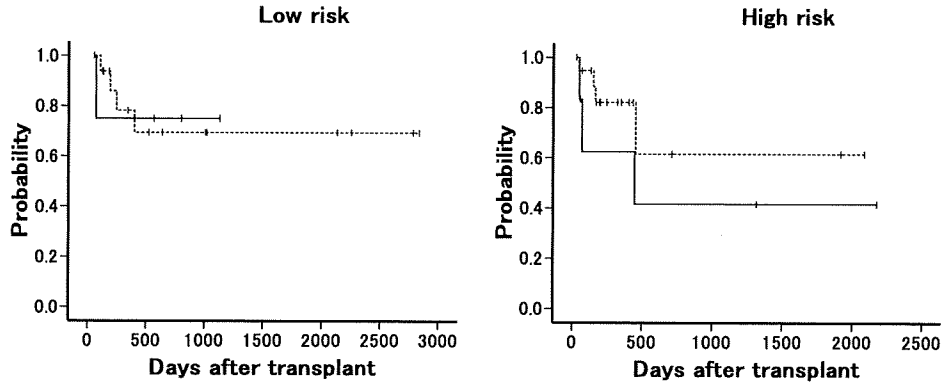

Fig. 1. EFS among patients with low and high risk of malignant diseases. ES (+), solid line; ES (−), dotted line. (a) Low risk of underlying malignant disease. EFS was similar for patients with or without ES (75 ± 21% vs. 69 ± 13%, respectively, p = 0.98). (b) High risk of underlying malignant disease. EFS was lower for patients with, than without ES, but the difference did not reach statistical significance (42 ± 22% vs. 62 ± 19%, respectively; p = 0.29).

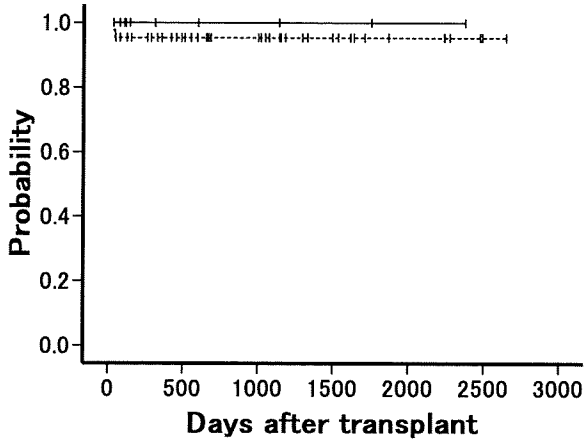

Fig. 2. Overall survival of patients with non-malignant diseases. ES (+), solid line; ES (−), dotted line. Overall survival did not significantly differ between patients with and without ES (100% vs. 95 ± 3%, respectively; p = 0.50).

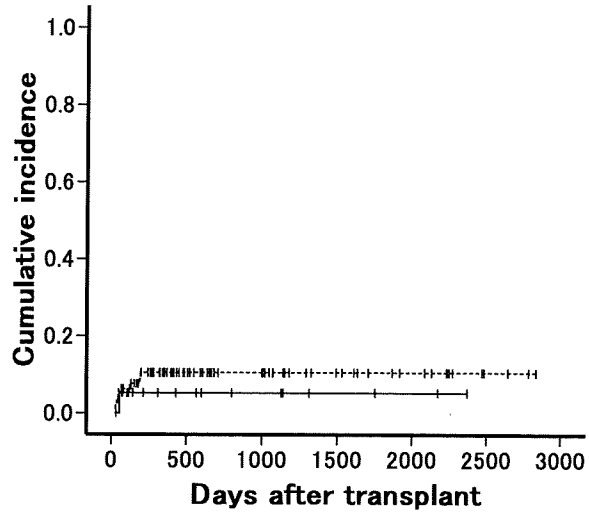

Fig. 3. Cumulative incidence of transplant-related mortality. ES (+), solid line; ES (−), dotted line. Transplant-related mortality did not significantly differ between patients with and without ES (5 ± 5% vs. 11 ± 3%, respectively; p = 0.56).

were applied. Recently, Schmid et al. (18) reported the incidence of ES in pediatric patients who received allogeneic transplants, using their own criteria. The incidence of ES was 48% according to their criteria, which decreased to 20% according to Spitzer's criteria. The incidence of ES was 20% in our study using the Spitzer's criteria, which was comparable with that of Schmid's study when they applied Spitzer's criteria in their cohort. When we applied the criteria used by Schmid's study, the incidence increased to 33%. It is quite important to know which criteria were used in evaluating the incidence of ES. We classified our series of patients using Spitzer's criteria because we thought it was better to use stricter criteria to define the true patients with ES.

Compared with autologous SCT, only a few studies have addressed ES in allogeneic SCT (2–4). The terminology between ES and hyper-acute GVHD in the allogeneic setting is confusing. Hyper-acute GVHD has been used to describe the symptoms of unexplained fever, skin rash, hepatic dysfunction, and diarrhea that often accompany non-cardiac pulmonary edema and renal dysfunction before and immediately after engraftments. The timing of onset and clinical symptoms seem to overlap between ES and hyper-acute GVHD. Originally, hyper-acute GVHD was described following haplo-identical transplantation or transplantation without GVHD prophylaxis during the 1980s. Powles et al. (23)

described patients who developed hyper-acute GVHD after haplo-identical transplantation; the symptoms were skin rash, fever, massive non-cardiac pulmonary edema, renal failure, and seizure. Early reports from Seattle indicated that hyper-acute GVHD was characterized by early onset (median eight days post-transplant), severe skin rash, fever, and liver or gut involvement (24). Most tissue biopsies confirmed the diagnosis of acute GVHD. The patients described in these two series seem to meet Spitzer's criteria for ES. Perhaps because hyper-acute GVHD was described in extraordinary situations, these reports have not received much attention by investigators. In 2004, Kim et al. (25) defined hyper-acute GVHD as unexplained fever in addition to skin rash, hepatic dysfunction and diarrhea occurring before engraftment. The overall incidence of hyper-acute GVHD was 36.7% and an alternative donor source was the only significant risk factor for developing hyper-acute GVHD. Most biopsy findings were compatible with those of acute GVHD.

Traditionally, acute GVHD has been considered to develop after neutrophil engraftment. However, symptoms or signs of acute GVHD often occur early, even before engraftment. Saliba et al. (26) recently defined hyper-acute GVHD as occurring within the first 14 days after SCT and evaluated its incidence, clinical manifestation, outcome and risk factors. According to their findings, hyper-acute GVHD developed in 9% of all patients, which accounted for 27% of biopsy-proven patients with grade II–IV acute GVHD. HLA disparity was a significant risk factor for developing hyper-acute GVHD, and the overall response rate to methylprednisolone was significantly lower in the hyper-acute, than in other acute GVHD groups. In contrast, the Boston group applied Spitzer's criteria to an evaluation of 73 adult patients who received non-myeloablative SCT (27). Thirty-five (50%) of 70 evaluable patients met the definition of ES, presenting with fever (100%), skin rash (100%), hepatic dysfunction (74%), fluid retention/weight gain (60%), non-cardiac pulmonary edema (23%), renal dysfunction (23%), and transient encephalopathy (6%). The distribution of the incidence of these symptoms was similar in the current study and that of the Boston group. In our study, all of the skin biopsy findings were not compatible with acute GVHD. The Boston group previously reported that skin biopsies showed changes consistent with acute GVHD in two of 20 patients with ES (28).

Although histological evaluation is routinely applied, the sensitivity and specificity of skin biopsy for the diagnosis of acute GVHD has not been established (29, 30). During the early post-transplant period, detecting infiltrating lymphocytes within recipient skin is difficult. So, it seems impossible to discriminate ES and hyper-acute GVHD only on the basis of histological findings. Although the clinical symptoms of ES and hyper-acute GVHD overlap, the responses to treatment differ. Patients with ES in most series, including ours, responded better to steroid than those with hyper-acute GVHD (4, 26).

We found that HLA disparity is a significant risk factor for developing ES. Moreover, patients with ES had a higher incidence of chronic GVHD, suggesting that ES is caused by immunological alloreactivity and that it may represent the early phase of acute GVHD. The pathophysiology of ES and hyper-acute GVHD are poorly understood. Elucidating the pathophysiology of both conditions will require understanding whether ES is indeed the early manifestation of acute GVHD.

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Correlation of Clinical Features With the Mutational Status of GM-CSF Signaling Pathway-Related Genes in Juvenile Myelomonocytic Leukemia

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ABSTRACT: Mutations in *RAS*, neurofibromatosis type 1 (*NF1*), and *PTPN11*, constituents of the granulocyte-macrophage colony-stimulating factor signaling pathway, have been recognized in patients with juvenile myelomonocytic leukemia (JMML). We assessed 71 children with JMML for *NRAS*, *KRAS*, and *PTPN11* mutations and evaluated their clinical significance. Of the 71 patients, three had been clinically diagnosed with neurofibromatosis type 1, and *PTPN11* and *NRAS/KRAS* mutations were found in 32 (45%) and 13 (18%) patients, respectively. No simultaneous aberrations were found. Compared with patients with *RAS* mutation or without any aberrations, patients with *PTPN11* mutation were significantly older at diagnosis and had higher fetal Hb levels, both of which have been recognized as poor prognostic factors. As was expected, overall survival was lower for patients with the *PTPN11* mutation than for those without (25 *versus* 64%; $p = 0.0029$). In an analysis of 48 patients who received hematopoietic stem cell transplantation, *PTPN11* mutations were also associated with poor prognosis for survival. Mutation in *PTPN11* was the only unfavorable factor for relapse after hematopoietic stem cell transplantation ($p = 0.001$). All patients who died after relapse had *PTPN11* mutation. These results suggest that JMML with *PTPN11* mutation might be a distinct subgroup with specific clinical characteristics and poor outcome. **(*Pediatr Res* 65: 334–340, 2009)**

Juvenile myelomonocytic leukemia (JMML) is a rare clonal myelodysplastic/myeloproliferative disorder that affects young children. It is characterized by specific hypersensitivity of JMML cells to granulocyte-macrophage colony-stimulating factor (GM-CSF) *in vitro*, but is thought to be a genetically and phenotypically heterogeneous disease (1–4). JMML seems to have its genesis in dysregulation of GM-CSF signal transduction, and gene mutations interfering with downstream components of the GM-CSF signaling pathway can be identified in approximately 70% of children with this disorder (2,5–11). Constitutional mutations of *NF1* occur in approximately 10% of patients with JMML (2,5,12). *NF1* is known to be the causative gene of neurofibromatosis type 1 (NF1), an autosomal dominant cancer predisposition syndrome. *NF1* codes for neurofibromin, a GTPase activating protein for Ras, and acts as a tumor suppressor (13). Similarly, oncogenic *RAS* mutations at codons 12, 13, and 61 have been identified in approximately 20–25% of patients with JMML (2,6–8). These mutations lead to elevated levels of Ras-GTP, the active form of Ras, resulting in constitutive activation of the signal transduction pathway (14).

Somatic mutations in *PTPN11*, which encodes the protein tyrosine phosphatase SHP-2, a molecule that also relays the signal from the GM-CSF receptor to Ras, have been reported in approximately 35% of patients with JMML (9–11). Germline mutations in *PTPN11* were first observed in Noonan syndrome (NS) (15), and somatic mutations have also been identified in hematological malignancies (9–11,16,17). SHP-2 is a positive regulator in this signal transduction pathway and *PTPN11* mutations cause gain of function in SHP-2, resulting in inappropriate activation of the GM-CSF pathway (10). *PTPN11* mutations have been found without coexisting *NRAS*, *KRAS*, or *NF1* mutations (9,11,16). These alterations are thought to be responsible for GM-CSF hypersensitivity and the clinical features associated with this condition. Given this information, mutational analysis of *PTPN11* and *RAS*, or

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Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; **HbF,** fetal hemoglobin; **HSCT,** hematopoietic stem cell transplantation; **JMML,** juvenile myelomonocytic leukemia; **NF1,** neurofibromatosis type 1; **NS,** Noonan syndrome; **OS,** Overall survival

either identification of an *NF1* mutation or a clinical diagnosis of NF1, are currently key diagnostic procedures for JMML. However, we have a poor understanding of the relationship between mutational status and the clinical features of JMML.

Most patients with JMML usually experience an aggressive clinical course and die from progressive disease unless treated with hematopoietic stem cell transplantation (HSCT) (1,4,18,19). Recent studies have shown that children with JMML have better outcomes when they undergo HSCT early in the course of the disease (20,21). In contrast, there is a certain proportion of patients who have a stable clinical course for a considerable period of time, and disease that sometimes spontaneously resolves without any treatment (1,22,23). Therefore, information on prognostic factors that can be used to identify patients requiring early HSCT is important in developing a treatment plan.

Although several clinical characteristics have been reported as prognostic factors for JMML, including age at diagnosis, sex, fetal Hb (HbF) level, platelet count, and cytogenetic abnormality (1,19–21,23–26), the relationship between prognosis and particular genetic aberrations is unclear and needs to be clarified. Thus, in the current study, we assessed 71 children with JMML for *NRAS*, *KRAS*, and *PTPN11* mutations and analyzed the association between mutational status and previously recognized prognostic factors for JMML, then evaluated the clinical significance of these mutations to clarify whether genotype-phenotype correlations exist.

MATERIALS AND METHODS

Patients. A total of 71 children with JMML diagnosed between 1987 and 2006 in 30 institutions throughout Japan were studied retrospectively. The diagnosis of JMML was based on the internationally accepted criteria previously published (27). We excluded patients with NS, a JMML-like myeloproliferative disease characterized by spontaneous regression of the disease. The clinical and hematological characteristics of the 71 patients are summarized in Table 1. The median age at diagnosis was 24 mo (range, 1–69 mo).

Table 1. *Patients characteristics*

No. of patients	71
Median age at diagnosis, mo (range)	24 (1–69)
Male/female	43/28
Peripheral blood	
Median Hb at diagnosis, g/dL (range)	9.3 (4.9–13.0)
Percentage of HbF at diagnosis (range)	19.0 (1.0–78.0)
Median WBC count at diagnosis, $\times 10^9$/L (range)	31.8 (7.6–563.0)
Median monocyte count at diagnosis, $\times 10^9$/L (range)	4.2 (1.0–84.5)
Median platelets count at diagnosis, $\times 10^9$/L (range)	42.0 (1.4–320.0)
Hepatomegaly (yes/no)	67/4
Splenomegaly (yes/no)	68/3
Cytogenetic study, no.of patients	
Normal	55
Monosomy 7	9
Other abnormalities	2
+8	1
−Y	1
+X,+13	1
Inv(4)(p14p16)	1
t(3;18)(q25;q21)	1
Del(6)(q?),−20	
No. of patients with clinical evidence of NF1	3
No. of patients received HSCT	48

WBC, white blood cell.

Karyotypic abnormalities were detected in 16 patients, including nine patients with monosomy 7. Three children had clinical evidence of NF1. Treatment was planned in the institute responsible for each child and 48 of 71 patients had been treated with HSCT. The source of grafts was bone marrow from a related donor for 15 patients, bone marrow from an unrelated donor for 20, unrelated cord blood for 11, and related peripheral blood for two. Total body irradiation, TBI, was used in half of the patients and the remainder underwent a non-TBI regimen in which the drug dosage varied widely. Approval for this study was obtained from the Ethics Committee of Nagoya University Graduate School of Medicine.

Screening for mutations of the PTPN11, NRAS, and KRAS genes. Written informed consent was obtained from the parents of each patient, and bone marrow or peripheral blood samples were obtained at initial diagnosis. Mononuclear cells were isolated using Ficoll-Hypaque density gradient centrifugation and they were cryopreserved until use. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Chatsworth, CA). To screen for *PTPN11* mutations, we amplified genomic DNA corresponding to exons 2, 3, 4, 7, 8, 12, and 13 of *PTPN11* using the PCR with cycling parameters and primers as previously reported (28,29). The PCR products were purified and directly sequenced on an ABI Prism 3100 DNA Analyzer (Applied Biosystems, Foster City, CA) using a BigDye terminator cycle sequencing kit (Applied Biosystems).

NRAS and *KRAS* mutations of codons 12, 13, and 61 were identified as previously described (11,30) and were confirmed by sequencing.

Analysis of correlations between clinical characteristics and mutational status. To assess the correlations between clinical characteristics and mutational status, patients were subdivided into four groups: those with a *PTPN11* mutation, a *RAS* mutation, a clinical diagnosis of NF1, or none of these. However, the number of patients with NF1 was too small to allow subgroup analysis, so these patients were excluded from this analysis. Then, for the three remaining groups, we performed a comparison of clinical and laboratory findings.

Statistical analysis. The date of analysis was January 30, 2008. Survival probabilities were estimated by the Kaplan-Meier method and comparisons between probabilities for different patient groups were performed using the two-sided log-rank test. All results are expressed as 5-y probabilities with a 95% confidence interval (CI). Overall survival (OS) for all patients was defined as the time from diagnosis to death or last follow-up. OS in patients who received HSCT was defined as the time from transplantation to death or last follow-up. Relapse incidence was defined as the probability of experiencing a relapse and death without relapse was considered a competing event. The parameters for univariate analyses of OS and relapse incidence included age at diagnosis, sex, platelet count, percentage of HbF, karyotype, and mutational status. For multivariate analyses, the Cox proportional hazard regression model was used. To evaluate correlations between clinical characteristics and mutational status, differences in continuous variables were analyzed using the Mann-Whitney U test and differences in frequencies were tested using the χ^2 test. When appropriate (because of small sample size), Fisher's exact test was used. p values less than 0.05 were considered statistically significant. These statistical analyses were performed with StatView-J 5.0 software (Abacus Concepts Inc., Berkeley, CA).

RESULTS

Mutation analysis. The results of the *PTPN11* and *NRAS/KRAS* mutational screening for the 71 Japanese children with JMML are listed in Table 2. We found *PTPN11* mutations in 32 of 71 (45%) patients. All mutations were missense changes, 30 of which were in exon 3 and two of which were in exon 13. Thirteen of 71 (18%) patients had *RAS* mutations, 11 of which were in *NRAS* and two of which were in *KRAS*. Ten of 13 patients with *RAS* mutations had been reported in a previous study (22). Three (4%) patients were clinically diagnosed with NF1. No patient with NF1 was found to have mutations in *PTPN11* or *RAS*, and 23 (32%) patients had neither a *PTPN11* mutation nor a *RAS* mutation, nor a clinical diagnosis of NF1.

Comparison of clinical characteristics of patients according to mutational status. We compared the laboratory and clinical parameters for patients with a *PTPN11* mutation, with a *RAS* mutation, and without any aberration (Table 3). In the

group corresponding to individuals with a *PTPN11* mutation, quite distinct characteristic features were evident, whereas there was no difference in the clinical characteristics displayed by individuals in the groups with a *RAS* mutation and no aberration. Patients with *PTPN11* mutations were significantly older at diagnosis (median: 35 mo) than those with *RAS* mutations (median: 10 mo; $p < 0.0001$) or those without any aberrations (median: 10 mo; $p = 0.0037$), and the presence of the *PTPN11* mutation in infants was rare (only two of 32 patients). In addition, the HbF level was significantly higher in the *PTPN11* mutation group than in the *RAS* mutation group or the group with no aberration (25.6 versus 8.6%; $p = 0.0026$ or versus 9.8%; $p = 0.0014$). The patients with monosomy 7 are known to have normal or only slightly elevated HbF levels (1). When the patients with monosomy 7 were excluded from the analysis, strong correlations of higher HbF level with *PTPN11* mutation compared with *RAS* mutation or no aberration were still observed ($p = 0.0004$ or $p = 0.0014$, respectively). Even after the groups other than the *PTPN11* group were combined (including the patients with NF1), the factors of older age at diagnosis and higher HbF level were still significantly different between the *PTPN11* mutation group and the other group. Karyotypic aberrations other than monosomy 7 occurred only in patients with the *PTPN11* mutation. Patients with a *PTPN11* mutation were more likely to receive HSCT than those with a *RAS* mutation or without any aberrations. As shown in Table 3, no correlation was observed between mutational subgroup and sex ratio, white blood cell count, or platelet count.

Because of the small number of patients in the NF1 group, we excluded these three patients from subgroup analysis. However, consistent with previous findings (1), children with NF1 had been given a diagnosis at an older age except for one patient with a family history (JMML was diagnosed in two girls with NF1 at 7 and 47 mo and in one boy with NF1 at 69 mo) but no other clinical parameters differed from those of the other mutational subgroups.

Prognostic impact of the GM-CSF signaling pathway-related genes. For all 71 children, the OS probability at 5 y was 43% (95% CI: 35–51), and the median follow-up time for all living patients was 59 mo (range, 13–240 mo). Given the quite distinct clinical characteristics of the *PTPN11* mutation group, which associated with recognized poor prognostic factors, we compared the clinical outcomes for patients with or without a *PTPN11* mutation. The survival of patients with a *PTPN11* mutation was significantly inferior to survival of patients without (25 *versus* 64%; $p = 0.0029$) as shown in Figure 1. Of the patients without *PTPN11* mutation, survival

Table 2. *PTPN11, NRAS, and KRAS mutations in 71 children with JMML*

Gene	No. of patients	Nucleotide substitution	Amino acid substitution
PTPN11	2	179G>T	Gly60Val
	4	181G>T	Asp61Tyr
	3	182A>T	Asp61Val
	1	214G>A	Ala72Thr
	5	215C>T	Ala72Val
	11	226G>A	Glu76Lys
	1	226G>C	Glu76Gln
	3	227A>G	Glu76Gly
	2	1508G>C	Gly503Ala
NRAS	3	34G>A	Gly12Ser
	2	34G>T	Gly12Cys
	1	35G>A	Gly12Asp
	3	38G>A	Gly13Asp
	1	181C>A	Gln61Lys
	1	182A>T	Gln61Leu
KRAS	1	35G>A	Gly12Asp
	1	35G>T	Gly12Val

Table 3. *Correlation between mutational status and clinical characteristics in JMML*

	Mutational group				
	PTPN11, $n = 32$ (45%)	*NRAS/KRAS*, $n = 13$ (18%)	p^*	No aberrations, $n = 23$ (32%)	p^*
Median age at diagnosis, mo	35	10	<0.0001	10	0.0037
Older than 24 mo, no.	24	1	<0.0001	8	0.0067
24 mo or younger, no.	8	12		15	
Gender, male/female					
Male, no.	22	8	NS	12	NS
Female, no.	10	5		11	
Median HbF level, %	25.6	8.6	0.0026	9.8	0.0014
More than 10%, no.	29	5	0.0008	11	0.0023
10% or less, no.	3	8		12	
Median WBC count, $\times 10^9$/L	27.7	29.4	NS	36.0	NS
Median platelets count, $\times 10^9$/L	38.5	55.0	NS	45.0	NS
Less than 40 $\times 10^9$/L, no.	17	5	NS	10	NS
40 $\times 10^9$/L or more, no.	15	8		13	
Cytogenetics					
Abnormal karyotype, no.	11	2	NS	2	NS
Monosomy 7, no.	4	2	NS	2	NS
Other abnormalities, no.	7	0		0	
Normal karyotype, no.	21	11		21	
No. of patients received HSCT	28	6	0.0107	11	0.0023

* These were compared with those of *PTPN11* group.
WBC, white blood cell; NS, not significant.