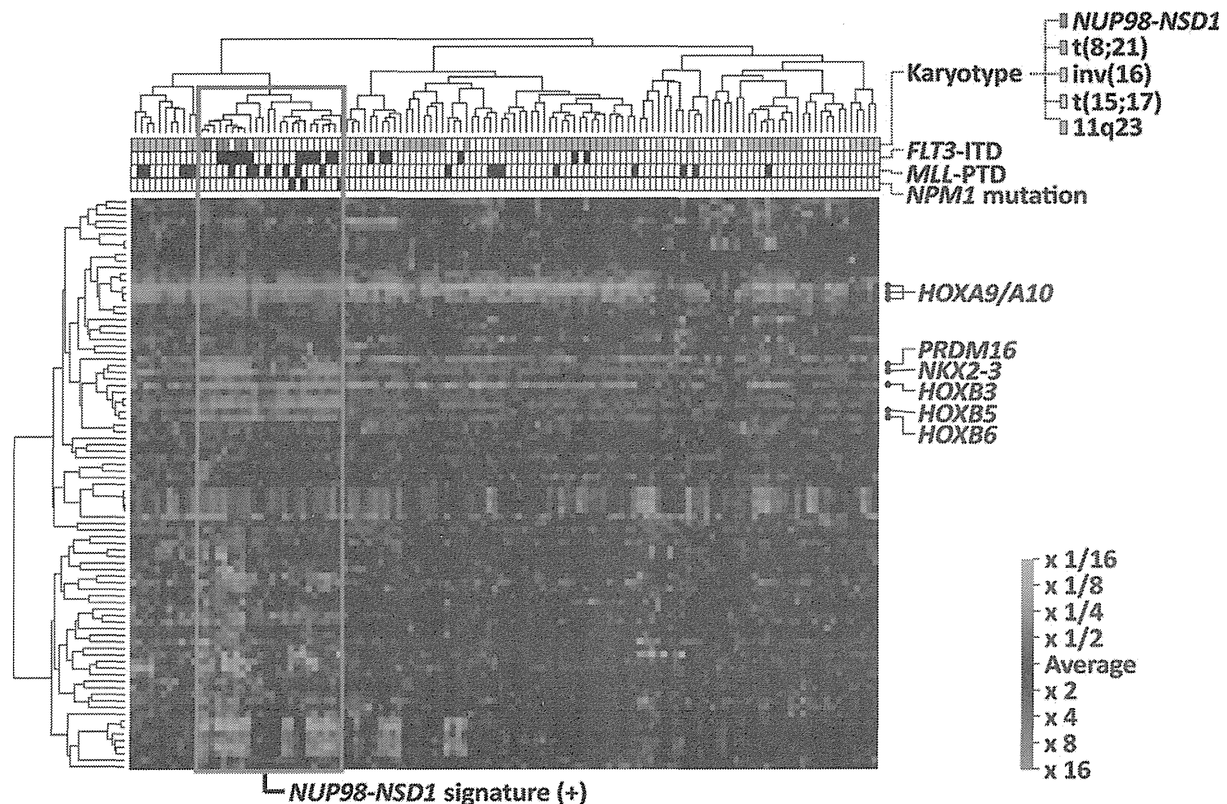

Figure 1. Supervised hierarchical clustering analysis of the 124 patients using differentially expressed genes between *NUP98-NSD1*-positive and -negative patients. Two-dimensional hierarchical clustering analysis of the 124 patients was performed using 87 probe sets that were differentially expressed between the *NUP98-NSD1*-positive and -negative patients (Table S3). Each column represents a patient and each row represents a probe set. The karyotype and the *FLT3*-ITD, *MLL*-PTD, and *NPM1* mutation status of each patient are indicated. Relative expression levels normalized to the average for each probe set are indicated by color, where red and green represent high and low expressions, respectively.

HOXB5, and *HOXB6*), another homeobox gene *NKX2–3*, a zinc finger transcription factor gene *PRDM16*, and a noncoding RNA gene *LOC404266* was particularly marked (Fig. 1). Although *HOXA9* and *HOXA10* were also overexpressed in these patients, their overexpression was shared by many patients with 11q23 abnormalities (Fig. 1).

Clinical and Molecular Characteristics of *NUP98-NSD1*-Positive and *NUP98-NSD1*-Like Patients

We compared the clinical and molecular features of the *NUP98-NSD1*-positive and *NUP98-NSD1*-like patients; there were no significant differences in terms of age, initial WBC count, gender, FAB subtypes, and cytogenetics (Table 1). Frequent gene mutations were also detected in both the *NUP98-NSD1*-positive and -like patients. All six *NUP98-NSD1*-positive patients and 16 *NUP98-NSD1*-like patients had one or several of the mutations analyzed (Table 2). *NUP98-NSD1*-positive patients frequently had class I aberrations, such as *FLT3*-ITD (4/6), and *NRAS* (1/6), *KRAS* (1/6), and *KIT* (1/6) mutations; however, no class II aberrations (*NPM1* or *MLL*-PTD) were detected in *NUP98-NSD1*-positive patients. On the other hand, *NUP98-NSD1*-like patients frequently had class II aberrations [*MLL*-PTD (7/18) and *NPM1* (3/18)] in addition to class I aberrations [*FLT3*-ITD (8/18), *KRAS* (3/18), and *KIT* (2/18)] (Table 2). Furthermore, we identified *DEK-NUP214*, *NUP98-HOXA13* and *MLL-MLLT4* fusion genes, which were generated by t(6;9)(p22;q34), t(1;7;11)(q32;p15;p15), and t(6;11)(q27;q23), respectively, in *NUP98-NSD1*-like patients (Table 2). In addition, we identified another type of *NUP98-NSD1* fusion transcript using another forward primer of *NUP98*-F', located on the 5′ side further from the known *NUP98* junction point (Supporting Information Table S2), in a *NUP98-NSD1*-like patient (Supporting Information Fig. S4).

Clinical and Molecular Characteristics of *NUP98-NSD1* Signature-Positive Patients

When compared with 100 *NUP98-NSD1* signature-negative patients, age (median 8.8 years vs.

TABLE 1. Clinical and Molecular Characteristics of the *NUP98-NSD1*-Positive and *NUP98-NSD1*-Like Patients

	NUP98-NSD1-positive	*NUP98-NSD1*-like	P-value
Total (n = 24)	6	18	
Age (range), y	7.2 (2–15)	9.3 (0–15)	0.28
Mean WBC ($\times 10^9$/l) (range)	115.9 (9.0–329.0)	60.5 (2.3–255.0)	0.31
Gender			0.34
Male	5 (83.3%)	9 (50.0%)	
Female	1 (16.7%)	9 (50.0%)	
FAB subtype			
M0	0 (0.0%)	1 (5.6%)	
M1	1 (16.7%)	4 (22.2%)	
M2	0 (0.0%)	2 (11.1%)	
M3	0 (0.0%)	0 (0.0%)	
M4	2 (33.3%)	6 (33.3%)	
M5	2 (33.3%)	4 (22.2%)	
M6	0 (0.0%)	0 (0.0%)	
M7	0 (0.0%)	1 (5.6%)	
Unclassified	1 (16.7%)	0 (0.0%)	
Cytogenetics			
Normal	4 (66.7%)	11 (61.1%)	1.00
t(8;21)(q22;q22)	0 (0.0%)	0 (0.0%)	1.00
t(15;17)(q22;q12)	0 (0.0%)	0 (0.0%)	1.00
inv(16)(p13q22)	0 (0.0%)	0 (0.0%)	1.00
abnormal 11q23	0 (0.0%)	2 (11.1%)	1.00
del(9q)	2 (33.3%)	0 (0.0%)	0.054
Others	0 (0.0%)	5 (27.8%)	0.28
Gene mutations			
FLT3-ITD	4 (66.7%)	8 (44.4%)	0.64
DNMT3A	0 (0.0%)	0 (0.0%)	1.00
NPM1	0 (0.0%)	3 (16.7%)	0.55
KIT	1 (16.7%)	2 (11.1%)	1.00
NRAS	1 (16.7%)	0 (0.0%)	0.25
KRAS	1 (16.7%)	3 (16.7%)	1.00
WT1	2 (33.3%)	3 (16.7%)	0.57
MLL-PTD	0 (0.0%)	7 (38.9%)	0.13

Abbreviations: y, years; WBC, white blood cell count; FAB,French-American-British subtype.

6.7 years; $P = 0.069$) and initial WBC count (median 74.4 $\times$ 10^9/ L vs. 49.7 $\times$ 10^9/ L; $P = 0.025$) were higher in the 24 *NUP98-NSD1* signature-positive patients. They frequently had a normal karyotype (62.5%) or del(9q) (8.3%), but did not have the favorable chromosomal translocations t(8;21), t(15;17), and inv(16) (Tables 2 and 3). The frequencies of the M4 and M5 subtypes (14/24; $P < 0.001$) were higher than in *NUP98-NSD1* signature-negative patients.

Prognosis of *NUP98-NSD1*-Positive and *NUP98-NSD1*-Like Patients

Both the 4-year OS and EFS were 33.3% in *NUP98-NSD1*-positive patients and 38.9% in *NUP98-NSD1*-like patients, which is significantly worse than for those with *NUP98-NSD1* signature-negative patients (86.0% in OS and 72.0% in EFS; Figs. 2A and 2B). Five of the six *NUP98-NSD1*-positive and 8 of the 18 *NUP98-NSD1*-like patients received allogeneic-stem cell transplantation (allo-SCT) (Table 2). Of the *NUP98-NSD1*-positive patients, only two of the three who underwent allo-SCT in first CR are still alive and, in the *NUP98-NSD1*-like patients, all 4 who received SCT in first CR and 3 of 10 who were treated with only chemotherapy are still alive without relapse. All 12 relapsed patients and two who did not achieve CR died (Table 2). Multivariate Cox regression analysis of OS was used to construct a model including the *NUP98-NSD1* signature, *FLT3*-ITD, *WT1*, *NPM1*, t(8;21), del(9q), and initial WBC, which were statistically significant in univariate analysis. In this model, the *NUP98-NSD1* signature and *FLT3*-ITD were independent poor prognostic factors (Table 4). Among the 24 patients displaying the *NUP98-NSD1* signature, the outcome of the 12 *FLT3*-ITD-positive patients was worse than that of the 12 *FLT3*-ITD-negative

TABLE 2. Individual Characteristics of the *NUP98-NSD1* Signature-Positive Patients

ID	Age (y)	Sex	FAB	Cytogenetic aberrations	*NUP98-NSD1*	Risk	CR	Relapse	SCT	Class I mutations	Class II mutations	Other mutations	Survival (m)
A106	5	F	M1	50,XX,+6,+8,del (9q?),+21,+22	+	Off study	+	–	CR 1	*FLT3*-ITD	–	–	63+
A188	10	M	U/C	Normal	+	High	+	+	CR 2	*FLT3*-ITD	–	*WT1*	21
A282	2	M	M5	46,XY,del(9)(q13q22)	+	Intermediate	+	–	CR 1	*FLT3*-ITD	–	–	43+
A325	5	M	M5	Normal	+	High	+	+	CR 2	*FLT3*-ITD	–	–	15
A333	15	M	M4	Normal	+	Intermediate	+	+	–	*NRAS*	–	–	31
A335	6	M	M4	Normal	+	Intermediate	+	–	CR 1	*KRAS+KIT*	–	*WT1*	7[a]
A044	6	M	M2	Normal	–	Intermediate	+	+	–	–	–	–	30
A059	12	F	M7	Normal	–	Intermediate	+	+	CR 2	*FLT3*-ITD	–	*WT1*	16
A089	9	F	M4	Normal	–	Off study	–	–	Non-CR	*FLT3*-ITD	*MLL*-PTD	–	25
A154	8	F	M0	Normal	–	High	+	+	CR 2	*FLT3*-ITD	–	–	20
A167	2	M	M5	Normal	–	High	+	–	CR 1	–	*MLL*-PTD	–	55+
A171	12	M	M1	46,XY,t(1;7;11) (q32;p15;p15)	–	Intermediate	+	–	CR 1	–	*MLL*-PTD	*WT1*	55+
A173	13	M	M1	Normal	–	Intermediate	+	–	CR 1	*FLT3*-ITD +*KIT*	*MLL*-PTD	*WT1*	54+
A199	10	F	M5	46,XX,add(10)(p11.2), del(11)(q13q23)	–	Intermediate	+	+	–	*KIT+KRAS*	–	–	41
A202	15	F	M4	Normal	–	Intermediate	+	–	–	–	*NPM1*	–	50+
A211	14	F	M1	47,XX,+8	+[b]	Intermediate	+	+	CR 2	*FLT3*-ITD	*MLL*-PTD	–	14
A234	13	M	M4	46,XY, t(6;11) (q27;q23)	–	Intermediate	+	+	–	*KRAS*	*MLL*-PTD	–	29
A243	5	F	M1	Normal	–	Intermediate	+	+	–	*FLT3*-ITD	–	–	8
A245	11	F	M4	Normal	–	Intermediate	+	–	–	*FLT3*-TK (D835)	*NPM1*	–	46+
A249	0	M	M4	46,XY,t(5;6) (q33;q22)	–	Low	+	–	–	–	–	–	46+
A259	5	M	M5	46,XY,t(6;9) (p23;q34)	–	Intermediate	+	–	CR 1	*FLT3*-TK (D835) + *KRAS*	–	–	48+
A297	6	M	M5	Normal	–	Off study	–	–	–	*FLT3*-ITD	*NPM1*	–	6
A299	13	F	M4	Normal	–	Intermediate	+	+	–	–	*MLL*-PTD	–	27
A355	13	M	M2	46,XY,t(15;17) (q13;q11)	–	Intermediate	+	+	–	*FLT3*-ITD	–	–	15

Abbreviations: y, years; F, female; M, male; FAB, French-American-British subtype; U/C, unclassified; CR, complete remission; SCT, stem cell transplantation; CR 1, first CR; CR 2, second CR; m, month; +, alive. The *NUP98-NSD1*-positive cases are indicated in bold type.

[a]A335 died of severe GVHD and acute pneumonia.

[b]Another type of *NUP98-NSD1* fusion transcript was identified by additional RT-PCR using another forward primer.

TABLE 3. Clinical and Molecular Characteristics of the *NUP98-NSD1* Signature Positive and Negative Cases

	NUP98-NSD1 signature (+)	*NUP98-NSD1* signature (−)	*P*-value
Total ($n = 124$)	24	100	
Age (range), y	8.8 (0−15)	6.7 (0−15)	0.069
Mean WBC (-10^9/l) (range)	74.4 (2.3–329.0)	49.7 (1.0–440.0)	0.025
Gender			1.00
Male	14 (58.3%)	57 (57.0%)	
Female	10 (41.7%)	43 (43.0%)	
FAB subtype[a]			
M0	1 (4.2%)	3 (3.0%)	
M1	5 (20.8%)	14 (14.0%)	
M2	2 (8.3%)	39 (39.0%)	
M3	0 (0.0%)	10 (10.0%)	
M4	8 (33.3%)	9 (9.0%)	
M5	6 (25.0%)	11 (11.0%)	
M6	0 (0.0%)	1 (1.0%)	
M7	1(4.2%)	12 (12.0%)	
Unclassified	1 (4.2%)	1 (1.0%)	
Cytogenetics			
Normal	15 (62.5%)	11 (11.0%)	<0.001
t(8;21)(q22;q22)	0 (0.0%)	41 (41.0%)	<0.001
t(15;17)(q22;q12)	0 (0.0%)	10 (10.0%)	0.21
inv(16)(p13q22)	0 (0.0%)	6 (6.0%)	0.60
abnormal 11q23	2 (8.3%)	9 (9.0%)	1.00
del(9q)	2 (8.3%)	0 (0.0%)	0.036
Others	5 (20.8%)	23 (23.0%)	1.00
Gene mutations			
FLT3-ITD	12 (50.0%)	6 (6.0%)	<0.001
DNMT3A	0 (0.0%)	0 (0.0%)	1.00
NPM1	3 (12.5%)	0 (0.0%)	0.007
KIT	3 (12.5%)	12 (12.0%)	1.00
NRAS	1 (4.2%)	9 (9.0%)	0.69
KRAS	4 (16.7%)	9 (9.0%)	0.28
WT1	5 (20.8%)	6 (6.0%)	0.037
MLL-PTD	7 (29.2%)	14 (14.0%)	0.12

[a]*NUP98-NSD1* signature-positive patients were significantly associated with the M4 and M5 subtypes (14/24; $P < 0.001$) when compared with *NUP98-NSD1* signature-negative patients.

patients (4-year OS: 25% vs. 50%), although the difference was not significant ($P = 0.400$).

We further analyzed the prognostic significance of *NUP98-NSD1*-positive patients and *NUP98-NSD1*-like patients other than those with t(15;17) and Down syndrome because they represent distinct AML entities. All 10 patients with t(15;17) and all six patients with Down syndrome were *NUP98-NSD1* signature negative. The outcome of the six patients with *NUP98-NSD1* gene fusion was significantly worse than that of the *NUP98-NSD1* signature-negative patients in OS ($P < 0.001$; 4-year OS: 33.3% vs. 85.7%; Fig. 2C) and in EFS ($P = 0.022$; 4-year EFS: 33.3% versus 70.2%; Fig. 2D). Furthermore, the outcome of the 18 *NUP98-NSD1*-like AML patients was significantly worse than that of the *NUP98-NSD1* signature-negative patients in OS ($P < 0.001$; 4-year OS: 38.9% versus 85.7%; Fig. 2C) and in EFS ($P = 0.002$; 4-year EFS: 38.9% versus 70.2%; Fig. 2D).

DISCUSSION

In this study, we found 24 patients with *NUP98-NSD1*-related gene expression signature, including six with the *NUP98-NSD1* gene fusion (*NUP98-NSD1*-positive) and 18 without (*NUP98-NSD1*-like). This signature represented 19% (24/124) of all pediatric AML patients and 58% (15/26) of all cytogenetically normal cases (Fig. 3). Our results also revealed that the *NUP98-NSD1* signature, irrespective of the presence of the *NUP98-NSD1* fusion, is a novel poor prognostic factor in AML.

The relationship between *NUP98-NSD1*-positive AML and *NUP98-NSD1*-like AML resembles that of *BCR-ABL*-positive acute lymphoblastic

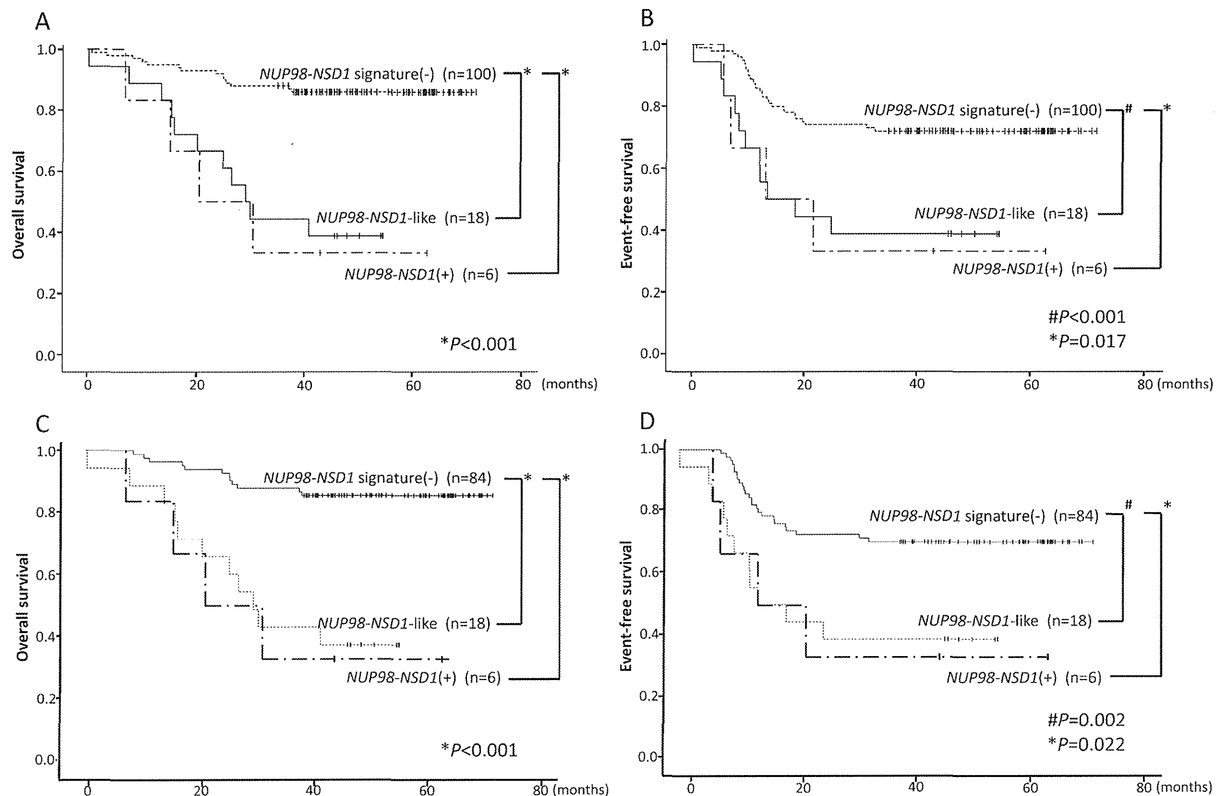

Figure 2. Survival based on *NUP98-NSD1* status by Kaplan-Meier method. Comparison of *NUP98-NSD1*-positive, *NUP98-NSD1*-like, and *NUP98-NSD1* signature-negative patients as regards OS (A) and EFS (B). Comparison of *NUP98-NSD1*-positive, *NUP98-NSD1*-like patients, and *NUP98-NSD1* signature-negative patients other than t(15;17) and Down syndrome, as regards OS (C) and EFS (D).

TABLE 4. Multivariate Analysis of OS of the Pediatric AML Patients

	P-value	SE	HR	Lower CI	Upper CI
NUP98-NSD1 signature	0.005	0.38	2.89	1.38	6.02
FLT3-ITD	0.005	0.40	3.06	1.41	6.63
WT1	0.20	0.54	0.50	0.18	1.43
NPM1	0.49	1.05	0.48	0.06	3.77
t(8;21)	0.18	0.46	0.54	0.22	1.32
del(9q)	0.97	371.69	<0.001	<0.001	–
Initial WBC	0.10	0.002	1.00	1.00	1.01

Abbreviations: SE, standard error; HR, hazard ratio; CI, confidence interval.

leukemia (ALL) and *BCR-ABL*-like ALL (Den Boer et al., 2009; Mullighan et al., 2009). In their gene expression analyses, a significant number of genetically unclassified B-cell precursor ALL patients clustered together with *BCR-ABL*-positive patients, and these *BCR-ABL*-like patients had a poor prognosis, similar to *BCR-ABL*-positive patients. Recently, it was reported that some *BCR-ABL*-like patients express tyrosine kinase/cytokine receptor gene-related fusion genes, such as *NUP214-ABL1*, *EBF1-PDGFRB*, *BCR-JAK2*, and *STRN3-JAK2* (Roberts et al., 2012). Thus, as potentially important fusions might be detected in *NUP98-NSD1*-like patients, we performed RT-PCR using various primer sets for detecting *NUP98-HOXA9*, *NUP98-HOXA11*, *NUP98-HOXA13*, *NUP98-TOP1*, *NUP98-PRRX1*, *NUP98-DDX10*, *NUP98-MLL*, *NUP98-NSD3*, *DEK-NUP214*, *MLL-MLLT4*, and other junction points of *NUP98-NSD1* (Supporting Information Table S2). As a result, t(6;9)/*DEK-NUP214*, t(1;7;11)/*NUP98-HOXA13*, and t(6;11)/*MLL-MLLT4*, which are well-known poor prognostic markers, were found in some *NUP98-NSD1*-like patients in our study (Table 2). Furthermore, another type of *NUP98-NSD1* fusion transcript was identified in a *NUP98-NSD1*-like patient (Supporting Information Fig. S4). This fusion transcript has previously been reported in an adult patient with refractory anemia with excess blasts (La Starza et al., 2004). It is likely that other *NUP98-NSD1*-like patients also have unknown fusion genes with the same functions as *NUP98-NSD1* gene fusion.

It has been reported that hematological malignancies with *NUP98*-fusion genes are strongly

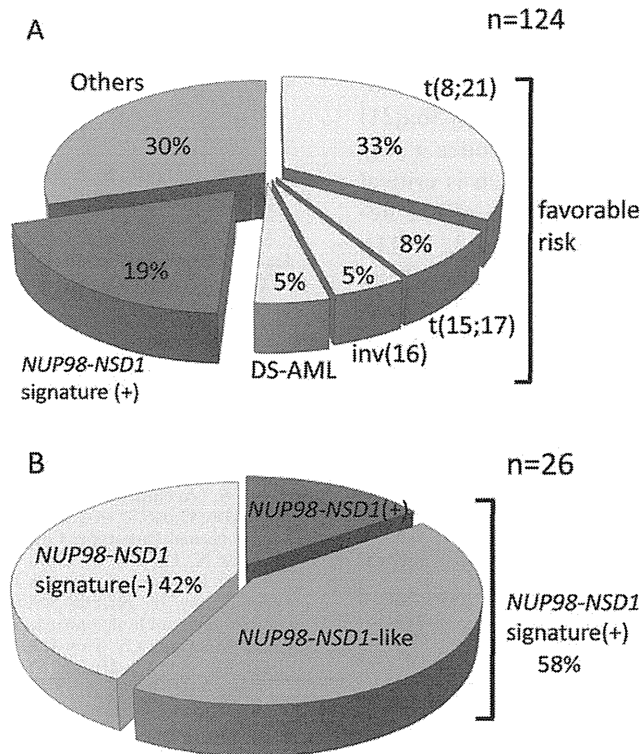

Figure 3. The *NUP98-NSD1* signature is frequent in pediatric AML. Frequencies of *NUP98-NSD1* signature-positive patients in the total pediatric AML cohort (A) and in cytogenetically normal AML (B).

associated with class I mutations (Chou et al., 2009; Taketani et al., 2010). Interestingly, *NUP98-NSD1*-positive patients had only class I aberrations, except for *WT1* mutations, although *NUP98-NSD1*-like patients frequently had Class II aberrations (*NPM1* mutations, *MLL*-PTD, and the *NUP98-HOXA13*, *MLL-MLLT4*, and *DEK-NUP214* fusion genes) in addition to class I aberrations. Because the *NUP98-NSD1* fusion is considered to act as a Class II aberration, no additional Class II aberrations might be necessary for leukemogenesis in *NUP98-NSD1*-positive patients.

The frequency of *FLT3*-ITD was higher than other mutations in both *NUP98-NSD1*-positive and *NUP98-NSD1*-like patients. Many studies have shown that *FLT3*-ITD has a negative impact on outcome in both adult and pediatric AML patients. In fact, in this study, *FLT3*-ITD was an independent poor prognostic factor in addition to the *NUP98-NSD1* signature (Table 4). This suggests that the *NUP98-NSD1* signature and *FLT3*-ITD play a key potential role in AML patients with poor prognosis.

Among the 100 *NUP98-NSD1* signature-negative patients, 23 patients relapsed and of those patients, nine died. On the other hand, of the 24 *NUP98-NSD1* signature-positive patients, 13 patients relapsed and all of those died. Except for 2 patients who did not achieve CR, 12 of the 22 *NUP98-NSD1* signature-positive patients received allo-SCT. Six of the seven patients (86%) who received allo-SCT in first CR were still alive without relapse; however, all five patients who received allo-SCT in second CR died. Only three patients were alive among the 10 patients who were treated with chemotherapy alone. Thus, allo-SCT is recommended in first CR of *NUP98-NSD1* signature-positive patients.

In the *NUP98-NSD1* signature-positive patients, the *HOXA9*, *HOXA10*, *HOXB3*, *HOXB5*, and *HOXB6* genes were up-regulated (Fig. 1). *NUP98* is frequently fused to homeobox genes, and some *NUP98*-homeobox and *NUP98*-non-homeobox fusion genes were revealed to activate *HOXA* cluster genes in hematopoietic cells (Gough et al., 2011). Overexpression of some *HOX* genes is known to enhance the self-renewal of hematopoietic stem and progenitor cells and to perturb differentiation (Grier et al., 2005). It is expected that the aberrant expression of *HOX* genes plays an important role in the leukemogenesis of AML displaying the *NUP98-NSD1* signature. In addition,

two transcription factor genes, *PRDM16* and *NKX2–3*, were markedly up-regulated (Fig. 1). *PRDM16* (also known as *MEL1*) was originally isolated as a translocated gene in t(1;3)(p36;q21) AML (Mochizuki et al., 2000) and encodes a zinc finger protein with a PR domain, which is critical for the establishment and maintenance of the hematopoietic stem cell pool (Agulio et al., 2011). *NKX2–3* is an NKX family homeobox gene (Pabst et al., 1999), whose involvement in leukemogenesis has not been reported; however, its highly homologous paralog *NKX2–5* is rearranged and ectopically expressed in T-cell ALL with t(5;14)(q35;q32) and t(5;14)(q35;q11.2) (Nagel et al., 2003; Przybylski et al., 2006). Taken together, these data suggest that both *PRDM16* and *NKX2–3* play an important role in leukemogenesis.

Our results indicate that the *NUP98-NSD1*-related gene expression signature is associated with a poor outcome in addition to the *NUP98-NSD1* gene fusion in pediatric AML. Most of the patients displaying the *NUP98-NSD1* signature were classified into an intermediate risk group, but their unfavorable outcome suggests that a high-risk group is a more suitable stratification. Although further investigations are necessary, we believe that our work contributes to improving the risk stratification of pediatric AML.

ACKNOWLEDGMENTS

The authors would like to thank the following Japanese childhood AML Cooperative Study Group institutions and investigators that participated in the study: Akira Morimoto, Department of Pediatrics, Kyoto Prefectural University of Medicine; Ryoji Kobayashi, Department of Pediatrics, Hokkaido University School of Medicine; Hiromasa Yabe, Department of Pediatrics, Tokai University School of Medicine; Kazuko Hamamoto, Department of Pediatrics, Hiroshima Red Cross Hospital; Shigeru Tsuchiya, Department of Pediatric Oncology, Institute of Development, Aging and Cancer, Tohoku University; Yuichi Akiyama, Department of Pediatrics, National Hospital Organization Kyoto Medical Center; Hisato Kigasawa, Department of Hematology, Kanagawa Children's Medical Center; Akira Ohara, Department of First Pediatrics, Toho University School of Medicine; Hideki Nakayama, Department of Pediatrics, Hamanomachi Hospital; Kazuko Kudo, Department of Pediatrics, Nagoya University Graduate School of Medicine; and Masue Imaizumi, Department of Hematology/Oncology, Miyagi Prefectural Children's Hospital.

REFERENCES

Agulio F, Avagyan S, Labar A, Sevilla A, Lee DF, Kumar P, Lemischka IR, Zhou BY, Snoeck HW. 2011. Prdm16 is a physiologic regulator of hematopoietic stem cells. Blood 117:5057–5066.

Arai Y, Hosoda F, Kobayashi H, Kawakami K, Takahara J. 1997. The inv(11)(p15q22) chromosome translocation of de novo and therapy-related myeloid malignancies results in fusion of the nucleoporin gene, NUP98, with the putative RNA helicase case, DDX10. Blood 89:3936–3944.

Brown J, Jawad M, Twigg SR, Saracoglu K, Sauerbrey A, Thomas AE, Eils R, Harbott J, Kearney L. 2002. A cryptic t(5;11)(q35;p15.5) in 2 children with acute myeloid leukemaia with apparently normal karyotypes, identified by a multiplex fluorescence in situ hybridization telomere assay. Blood 99:2526–2531.

Casas S, Aventín A, Nomdedéu J, Sierra J. 2003. Cryptic t(5;11)(q35;p15.5) in adult de novo acute myelocytic leukemia with normal karyotype. Cancer Genet Cytogenet 145:183.

Cerveira N, Correia C, Dória S, Bizarro S, Rocha P, Gomes P, Torres L, Norton L, Borges BS, Castedo S, Teixeira MR. 2003. Frequency of NUP98-NSD1 fusion transcript in childhood acute myeloid leukaemia. Leukemia 17:2244–2247.

Chou WC, Chen CY, Hou HA, Lin LI, Tang JL, Yao M, Tsay W, Ko BS, Wu SJ, Huang SY, Hsu SC, Chen YC, Huang YN, Tseng MH, Huang CF, Tien HF. 2009. Acute myeloid leukemia bearing t(7;11)(p15;p15) is a distinct cytogenetic entity with poor outcome and a distinct mutation profile: comparative analysis of 493 adult patients. Leukemia 23:1303–1310.

Den Boer ML, van Slegtenhorst M, De Menezes RX, Cheok MH, Buijs-Gladdines JG, Peters ST, Van Zutven LJ, Beverloo HB, Van der Spek PJ, Escherich G, Horstmann MA, Janka-Schaub GE, Kamps WA, Evans WE, Pieters R. 2009. A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. Lancet Oncol 10:125–134.

Döhner K, Schlenk RF, Habdank M, Scholl C, Rücker FG, Corbacioglu A, Bullinger L, Fröhling S, Döhner H. 2005. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. Blood 106:3740–3746.

Eisen MB, Spellman PT, Brown PO, Botstein D. 1998. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci USA 95:14863–14868.

Frohling S, Scholl C, Gilliland DG, Levine RL. 2005. Genetics of myeloid malignancies: Pathogenetic and clinical implications. J Clin Oncol 23:6285–6295.

Gough SM, Slape CI, Aplan PD. 2011. NUP98 gene fusions and hematopoietic malignancies: Common themes and new biological insights. Blood 118:6247–6257.

Grier DG, Thompson A, Kwasniewska A, McGonigle GJ, Halliday HL, Lappin TR. 2005. The pathophysiology of HOX genes and their role in cancer. J Pathol 205:154–171.

Hollink IH, van den Heuvel-Eibrink MM, Arentsen-Peters ST, Pratcorona M, Abbas S, Kuipers JE, van Galen JF, Beverloo HB, Sonneveld E, Kaspers GJ, Trka J, Baruchel A, Zimmermann M, Creutzig U, Reinhardt D, Pieters R, Valk PJ, Zwaan CM. 2011. NUP98/NSD1 characterizes a novel poor prognostic group in acute myeloid leukemia with a distinct HOX gene expression pattern. Blood 118:3645–3656.

Ichikawa H, Tanabe K, Mizushima H, Hayashi Y, Mizutani S, Ishii E, Hongo T, Kikuchi A, Satake M. 2006. Common gene expression signatures in t(8;21)- and inv(16)-acute myeloid leukemia. Br J Haematol 135:336–347.

Imaizumi M, Tawa A, Hanada R, Tsuchida M, Tabuchi K, Kigasawa H, Kobayashi R, Morimoto A, Nakayama H, Hamamoto K, Kudo K, Yabe H, Horibe K, Tsuchiya S, Tsukimoto I. 2011. Prospective study of a therapeutic regimen with all-trans retinoic acid and anthracyclines in combination of cytarabine in children with acute promyelocytic leukaemia: the Japanese childhood acute myeloid leukaemia cooperative study. Br J Haematol 152:89–98.

Jaju RJ, Fidler C, Haas OA, Strickson AJ, Watkins F, Clark K, Cross NC, Cheng JF, Aplan PD, Kearney L, Boultwood J,

Wainscoat JS. 2001. A novel gene, NSD1, is fused to NUP98 in the t(5;11)(q35;p15.5) in de novo childhood acute myeloid leukemia. Blood 98:1264–1267.

Jo A, Tsukimoto I, Ishii E, Asou N, Mitani S, Shimada A, Igarashi T, Hayashi Y, Ichikawa H. 2009. Age-associated difference in gene expression of paediatric acute myelomonocytic lineage leukaemia (FAB M4 and M5 subtypes) and its correlation with prognosis. Br J Haematol 144:917–929.

Kudo K, Kojima S, Tabuchi K, Yabe H, Tawa A, Imaizumi M, Hanada R, Hamamoto K, Kobayashi R, Morimoto A, Nakayama H, Tsuchida M, Horibe K, Kigasawa H, Tsukimoto I. 2007. Prospective study of a pirarubicin, intermediate-dose cytarabine, and etoposide regimen in children with Down syndrome and acute myeloid leukemia: The Japanese Childhood AML Cooperative Study Group. J Clin Oncol 25:5442–5447.

La Starza R, Gorello P, Rosati R, Riezzo A, Veronese A, Ferrazzi E, Martelli MF, Negrini M, Mecucci C. 2004. Cryptic insertion producing two NUP98/NSD1 chimeric transcripts in adult refractory anemia with an excess of blasts. Genes Chromosomes Cancer 41:395–399.

Marcucci G, Haferlach T, Döhner H. 2011. Molecular genetics of adult acute myeloid leukemia: Prognostic and therapeutic implications. J Clin Oncol 29:475–486.

Mochizuki N, Shimizu S, Nagasawa T, Tanaka H, Taniwaki M, Yokota J, Morishita K. 2000. A novel gene, MEL1, mapped to 1p36.3 is highly homologous to the MDS1/EVI1 gene and is transcriptionally activated in t(1;3)(p36;q21)-positive leukemia cells. Blood 96:3209–3214.

Mullighan CG, Su X, Zhang J, Radtke I, Phillips LA, Miller CB, Ma J, Liu W, Cheng C, Schulman BA, Harvey RC, Chen IM, Clifford RJ, Carroll WL, Reaman G, Bowman WP, Devidas M, Gerhard DS, Yang W, Relling MV, Shurtleff SA, Campana D, Borowitz MJ, Pui CH, Smith M, Hunger SP, Willman CL, Downing JR. 2009. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med 360:470–480.

Nagel S, Kaufmann M, Drexler HG, MacLeod RA. 2003. The cardiac homeobox gene NKX2–5 is deregulated by juxtaposition with BCL11B in pediatric T-ALL cell lines via a novel t(5;14)(q35.1;q32.2). Cancer Res 63:5329–5334.

Nakamura T. 2005. NUP98 fusion in human leukemia: Dysregulation of the nuclear pore and homeodomain proteins. Int J Hematol 82:21–27.

Nakamura T, Largaespada DA, Lee MP, Johnson LA, Ohyashiki K, Toyama K, Chen SJ, Willman CL, Chen IM, Feinberg AP, Jenkins NA, Copeland NG, Shaughnessy JD Jr. 1996. Fusion of the nucleoporin gene NUP98 to HOXA9 by the chromosome translocation t(7;11)(p15;p15) in human myeloid leukaemia. Nature Genet 12:154–158.

Nebral K, König M, Schmidt HH, Lutz D, Sperr WR, Kalwak K, Brugger S, Dworzak MN, Haas OA, Strehl S. 2005. Screening for NUP98 rearrangements in hematopoietic malignancies by fluorescence in situ hybridization. Haematologica 90:746–752.

Pabst O, Zweigerdt R, Arnold HH. 1999. Targeted disruption of the homeobox transcription factor Nkx2–3 in mice results in postnatal lethality and abnormal development of small intestine and spleen. Development 126:2215–2225.

Panarello C, Rosanda C, Morerio C. 2002. Cryptic translocation t(5;11)(q35;p15.5) with involvement of the NSD1 and NUP98 genes without 5q deletion in childhood acute myeloid leukemia. Genes Chromosomes Cancer 35:277–281.

Przybylski GK, Dik WA, Grabarczyk P, Wanzeck J, Chudobska P, Jankowski K, von Bergh A, van Dongen JJ, Schmidt CA, Langerak AW. 2006. The effect of a novel recombination between the homeobox gene NKX2–5 and the TRD locus in T-cell acute lymphoblastic leukemia on activation of the NKX2–5 gene. Haematologica 91:317–321.

Pui CH, Carroll WL, Meshinchi S, Arceci RJ. 2011. Biology, risk stratification, and therapy of pediatric acute leukemias: An update. J Clin Oncol 29:551–565.

Roberts KG, Morin RD, Zhang J, Hirst M, Zhao Y, Su X, Chen SC, Payne-Turner D, Churchman ML, Harvey RC, Chen X, Kasap C, Yan C, Becksfort J, Finney RP, Teachey DT, Maude SL, Tse K, Moore R, Jones S, Mungall K, Birol I, Edmonson MN, Hu Y, Buetow KE, Chen IM, Carroll WL, Wei L, Ma J, Kleppe M, Levine RL, Garcia-Manero G, Larsen E, Shah NP, Devidas M, Reaman G, Smith M, Paugh SW, Evans WE, Grupp SA, Jeha S, Pui CH, Gerhard DS, Downing JR, Willman CL, Loh M, Hunger SP, Marra MA, Mullighan CG. 2012. Genetic alterations activating kinase and cytokine receptor signaling in high-risk acute lymphoblastic leukemia. Cancer Cell 22:153–166.

Romana SP, Radford-Weiss I, Ben Abdelali R, Schluth C, Petit A, Dastugue N, Talmant P, Bilhou-Nabera C, Mugneret F, Lafage-Pochitaloff M, Mozziconacci MJ, Andrieu J, Lai JL, Terre C, Rack K, Cornillet-Lefebvre P, Luquet I, Nadal N, Nguyen-Khac F, Perot C, Van den Akker J, Fert-Ferrer S, Cabrol C, Charrin C, Tigaud I, Poirel H, Vekemans M, Bernard OA, Berger R. 2006. NUP98 rearrangements in hematopoietic malignancies: a study of the Groupe Francophone de Cytogénétique Hématologique. Leukemia 20:696–706.

Sano H, Shimada A, Taki T, Murata C, Park MJ, Sotomatsu M, Tabuchi K, Tawa A, Kobayashi R, Horibe K, Tsuchida M, Hanada R, Tsukimoto I, Hayashi Y. 2012. RAS mutations are frequent in FAB type M4 and M5 of acute myeloid leukemia, and related to late relapse: A study of the Japanese Childhood AML Cooperative Study Group. Int J Hematol 95:509–515.

Shiba N, Taki T, Park MJ, Shimada A, Sotomatsu M, Adachi S, Tawa A, Horibe K, Tsuchida M, Hanada R, Tsukimoto I, Arakawa H, Hayashi Y. 2012. DNMT3A mutations are rare in childhood acute myeloid leukaemia, myelodysplastic syndromes and juvenile myelomonocytic leukaemia. Br J Haematol 156:413–414.

Shimada A, Taki T, Tabuchi K, Tawa A, Horibe K, Tsuchida M, Hanada R, Tsukimoto I, Hayashi Y. 2006. KIT mutations, and not FLT3 internal tandem duplication, are strongly associated with a poor prognosis in pediatric acute myeloid leukemia with t(8;21): a study of the Japanese Childhood AML Cooperative Study Group. Blood 107:1806–1809.

Shimada A, Taki T, Tabuchi K, Taketani T, Hanada R, Tawa A, Tsuchida M, Horibe K, Tsukimoto I, Hayashi Y. 2008. Tandem duplications of MLL and FLT3 are correlated with poor prognoses in pediatric acute myeloid leukemia: a study of the Japanese childhood AML Cooperative Study Group. Pediatr Blood Cancer 50:264–269.

Taketani T, Taki T, Shibuya N, Ito E, Kitazawa J, Terui K, Hayashi Y. 2002a The HOXD11 gene is fused to the NUP98 gene in acute myeloid leukemia with t(2;11)(q31;p15). Cancer Res 62:33–37.

Taketani T, Taki T, Ono R, Kobayashi Y, Ida K, Hayashi Y. 2002b. The chromosome translocation t(7;11)(p15;p15) in acute myeloid leukemia results in fusion of the NUP98 gene with a HOXA cluster gene, HOXA13, but not HOXA9. Genes Chromosomes Cancer 34:437–443.

Taketani T, Taki T, Shibuya N, Kikuchi A, Hanada R, Hayashi Y. 2002c Novel NUP98-HOXC11 fusion gene resulted from a chromosomal break within exon 1 of HOXC11 in acute myeloid leukemia with t(11;12)(p15;q13). Cancer Res 62: 4571–4574.

Taketani T, Taki T, Nakamura T, Kobayashi Y, Ito E, Fukuda S, Yamaguchi S, Hayashi Y. 2010. High frequencies of simultaneous FLT3-ITD, WT1 and KIT mutations in hematological malignancies with NUP98-fusion genes. Leukemia 24: 1975–1977.

Tsukimoto I, Tawa A, Horibe K, Tabuchi K, Kigasawa H, Tsuchida M, Yabe H, Nakayama H, Kudo K, Kobayashi R, Hamamoto K, Imaizumi M, Morimoto A, Tsuchiya S, Hanada R. 2009. Risk-stratified therapy and the intensive use of cytarabine improves the outcome in childhood acute myeloid leukemia: The AML99 trial from the Japanese Childhood AML Cooperative Study Group. J Clin Oncol 27:4007–4013.

Walter MJ, Payton JE, Ries RE, Shannon WD, Deshmukh H, Zhao Y, Baty J, Heath S, Westervelt P, Watson MA, Tomasson MH, Nagarajan R, O'Gara BP, Bloomfield CD, Mrózek K, Selzer RR, Richmond TA, Kitzman J, Geoghegan J, Eis PS, Maupin R, Fulton RS, McLellan M, Wilson RK, Mardis ER, Link DC, Graubert TA, DiPersio JF, Ley TJ. 2009. Acquired copy number alterations in adult acute myeloid leukemia genomes. Proc Natl Acad Sci USA 106:12950–12955.

Pediatr Blood Cancer 2013;60:1513–1519

PBSCT Is Associated With Poorer Survival and Increased Chronic GvHD Than BMT in Japanese Paediatric Patients With Acute Leukaemia and an HLA-Matched Sibling Donor

Aki Shinzato, MD,[1] Ken Tabuchi, MD,[2] Yoshiko Atsuta, MD,[3] Masami Inoue, MD,[4] Jiro Inagaki, MD,[5] Hiromasa Yabe, MD,[6] Katsuyoshi Koh, MD,[7] Koji Kato, MD,[8] Hideaki Ohta, MD,[9] Hisato Kigasawa, MD,[10] Toshiyuki Kitoh, MD,[11] Atsushi Ogawa, MD,[12] Yoshiyuki Takahashi, MD,[13] Yoji Sasahara, MD,[14] Shun-Ichi Kato, MD,[15] and Souichi Adachi, MD[1,16]*

Background. Peripheral blood stem cells (PBSC) may be used as an alternative to bone marrow (BM) for allogeneic transplantation. Since peripheral blood stem cell bank from unrelated volunteer donor has been started in Japan, use of PBSC allografts may be increased. Therefore we surveyed the outcomes of Japanese leukemia children after PBSC and BM transplantation. ***Procedure.*** This retrospective study compared the outcomes of 661 children (0–18 years) with acute lymphoblastic leukaemia (ALL) or acute myeloid leukaemia (AML) who received their first allogeneic peripheral blood stem cell transplantation (PBSCT; n = 90) or bone marrow transplantation (BMT; n = 571) from HLA-matched siblings between January 1996 and December 2007. ***Result.*** Neutrophil recovery was faster after PBSCT than after BMT (ALL: $P < 0.0001$; AML: $P = 0.0002$), as was platelet recovery (ALL: $P = 0.0008$; AML: $P = 0.0848$). However, the cumulative incidence of chronic graft-versus-host disease (GvHD) was higher after PBSCT than after BMT (ALL: 26.0% vs. 9.9%, $P = 0.0066$; AML: 41.6% vs. 11.1%, $P < 0.0001$). The 5-year disease-free survival (DFS) was lower after PBSCT than after BMT for ALL (40.6% vs. 57.1%, $P = 0.0257$). The 5-year overall survival (OS) was lower after PBSCT than after BMT for ALL (42.4% vs. 63.7%, $P = 0.0032$) and AML (49.8% vs. 71.8%, $P = 0.0163$). Multivariate analysis revealed the use of PBSC was a significant risk factor for DFS and OS. PBSCT and BMT did not differ in relapse rate, acute GvHD for ALL and AML, or in DFS for AML. ***Conclusion.*** PBSC allografts in Japanese children engraft faster but are associated with poorer survival and increased chronic GvHD. Pediatr Blood Cancer 2013; 60:1513–1519. © 2013 Wiley Periodicals, Inc.

Key words: acute leukaemia; bone marrow transplantation; children; chronic graft-versus-host disease; peripheral blood stem cell transplantation

INTRODUCTION

Allogeneic peripheral blood stem cell (PBSC) transplantation (PBSCT) was established along with allogeneic bone marrow (BM) transplantation (BMT) in the last decade [1–7]. In October 2010, a bank that stores PBSC from unrelated volunteer donors was established in Japan. Other progress in this area in Japan relates to the registration of haematopoietic stem cell transplantation (HSCT), which, until five years ago, involved four separate registry organisations. However, in 2006, the registers of these organisations were computerised and unified under the Transplantation Registry Unified Management Program (TRUMP) [8]. In 2008, the HSCT data of paediatric patients that had been stored on paper in the four registries were entered into TRUMP electronically. TRUMP has thus made it possible to analyse the paediatric HSCT data with greater accuracy.

While several prospective and retrospective randomised controlled trials (RCTs) and meta-analyses that compared BMT and PBSCT have been published [3,4,6,9–15], most have focused on adult patients only. To survey the outcomes of Japanese children after allogeneic HSCT from related donors, TRUMP data were used to conduct a retrospective, multi-centre study that compared the outcomes of 661 paediatric patients with leukaemia after their transplantation with allogeneic PBSC or BM from HLA-matched siblings. The impact of chronic graft-versus-host disease (GvHD) after transplantation was also examined.

PATIENTS AND METHODS

Study Population

The Japan Society for Haematopoietic Cell Transplantation uses a standardised reporting form to collect the data of individual transplant

Additional Supporting Information may be found in the online version of this article.

[1]Department of Pediatrics, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [2]Department of Haematology, Kanagawa Children's Medical Centre, Yokohama, Japan; [3]Department of Hematopoietic Stem Cell Transplantation Data Management, Nagoya University, School of Medicine, Nagoya, Japan; [4]Department of Haematology and Oncology, Osaka Medical Centre and Research Institute for Maternal and Child Health, Izumi, Japan; [5]Section of Pediatrics, National Kyushu Cancer Centre, Fukuoka, Japan; [6]Department of Pediatrics and Cell Transplantation, Tokai University School of Medicine, Isehara, Japan; [7]Department of Haematology and Oncology, Saitama Children's Medical Centre, Saitama, Japan; [8]Division of Pediatric Haematology/Oncology, Children's Medical Centre, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [9]Department of Pediatrics, Graduate School of Medicine, Osaka University, Suita, Japan; [10]Department of Haemato-Oncology/Regeneration Medicine, Kanagawa Children's Medical Centre, Yokohama, Japan; [11]Department of Pediatrics, Shiga Medical Centre for Children, Moriyama, Japan; [12]Department of Pediatrics, Niigata Cancer Centre Hospital, Niigata, Japan; [13]Department of Pediatrics, Graduate School of Medicine, Nagoya University, Nagoya, Japan; [14]Department of Pediatrics and Pediatric Oncology, Graduate School of Medicine, Tohoku University, Sendai, Japan; [15]Department of Cell Transplantation and Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan; [16]Human Health Sciences Department, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Conflict of interest: Nothing to declare.

*Corresponding to: Souichi Adachi, Human Health Sciences, Graduate School of Medicine, Kyoto University, 53 Kawahara-cho, Syogoin, Sakyo-ku, Kyoto 606-8507, Japan. E-mail: adachiso@kuhp.kyoto-u.ac.jp

Received 28 August 2012; Accepted 12 February 2013

© 2013 Wiley Periodicals, Inc.
DOI 10.1002/pbc.24524
Published online 19 March 2013 in Wiley Online Library (wileyonlinelibrary.com).

patients from each transplant centre. Follow-up reports are also submitted annually after transplantation. Between January 1996 and December 2007, the data of 1,048 paediatric patients with acute lymphoblastic leukaemia (ALL) or acute myeloid leukaemia (AML) who underwent a myeloablative preparative regimen and allogeneic BMT or PBSCT from a family donor were reported to the Japan Society for Haematopoietic Cell Transplantation. Patients were excluded from the study if their data were incomplete (n = 20), if they had received BM together with PBSC (n = 15), if they received grafts from father or mother (n = 322), or if they received grafts from anyone other than an HLA-identical sibling or a sibling whose HLA matched in all but one antigens (n = 30). As a result, 661 patients were included in this study. This study was approved by the Data Management Committee for the Nationwide Survey of the Japan Society for Haematopoietic Cell Transplantation.

Methods of HLA Typing

The method of HLA typing varied from time to time among participated institutes. In general, serological or low-resolution HLA typing was performed by lymphocyte cytotoxicity test until 2003 and reversed SSO after 2003. High-resolution HLA typing was done for class II alleles by PCR-RFLP from 1992 to 2000, and done by SBT for class I and II after 2000.

Engraftment Evaluation

Neutrophil engraftment was defined as an absolute neutrophil count greater than 0.5×10^9/L occurring on the first of three consecutive days. Platelet engraftment was defined as the first day with a platelet count greater than 50×10^9/L without further need for platelet transfusions.

End Points and Definition

End points were assessed on the date of last patient contact and were analysed on the 31 October 2009. The study end points were relapse rate (RR), disease-free survival (DFS), overall survival (OS), acute and chronic GvHD and transplantation-related mortality (TRM). Acute GvHD was graded according to standard criteria by the attending physicians of each hospital [16]. Chronic GvHD was graded as limited (localised skin or single organ involvement) or clinically extensive [17]. The reported causes of death were reviewed and categorised and TRM was defined as death with no evidence of disease. DFS was defined as survival without evidence of relapse, the event under study being death or relapse. Patients who died as a result of relapse or disease progression after transplantation were considered to have died of their original disease.

Risk status at transplantation was categorised as either standard or high. Standard-risk (SR) disease included ALL in first or second complete remission (CR1, CR2) except when it was Philadelphia chromosome-positive [Ph (+)] or exhibited mixed-lineage leukaemia gene (MLL) rearrangement. SR disease also included AML in CR1 or CR2. All other diseases status were categorised as high-risk (HR) disease.

Statistical Analysis

The cumulative incidences of neutrophil and platelet recovery, acute and chronic GvHD, relapse, survival and TRM of the PBSCT and BMT groups were compared by using the log-rank test to compare Kaplan and Meier curves. The 95% confidence intervals (CIs) were calculated by using the Greenwood formula [18]. The significance of all other differences between both groups was estimated by using the Chi-square test for categorical variables and the Kruskal–Wallis test for continuous variables. Multivariate Cox analysis [19] was used to study the potential effect of the following factors on OS and DFS: age, diagnosis, disease risk, stem cell source and donor-recipient HLA compatibility.

RESULTS

Patient Characteristics

The data of 661 paediatric patients with acute leukaemia who underwent their first stem cell transplantation were analysed. The patients were divided according to whether they had ALL (n = 411) or AML (n = 250). The characteristics of these two groups are shown in Table I. Of the ALL group, 60 (15%) received PBSCT and 351 (85%) received BMT. Of the AML group, 30 (12%) received PBSCT and 220 (88%) received BMT. The ages of both the ALL and AML groups ranged from 0 to 18 years and the median ages were both 9.0 years. For the ALL group, 247 (60%) and 164 (40%) had SR and HR disease respectively, while 202 (81%) and 48 (19%) of the AML group had SR and HR disease, respectively. The PBSC and BM recipients in the ALL and AML groups did not differ in terms of gender (ALL, $P = 0.28$; AML, $P = 0.23$) or conditioning regimen (ALL, $P = 0.37$; AML, $P = 0.42$). But for the ALL group, the PBSC recipients were significantly older than the BM recipients (ALL, $P < 0.01$; AML, $P = 0.20$).

These ALL and AML groups were also divided further according to the disease risk into SR and HR subgroups. For all four subgroups, the gender ratios of the PBSC and BM recipients did not differ significantly (ALL SR, $P = 0.29$; ALL HR, $P = 0.93$; AML SR, $P = 0.64$; AML HR, $P = 0.23$). The ages of the PBSC and BM recipients did not differ significantly either except for in the ALL HR subgroup, where PBSCT was associated with a significantly higher median age than BMT (ALL SR, $P = 0.13$; ALL HR, $P < 0.01$; AML SR, $P = 0.12$; AML HR, $P = 0.89$).

Engraftment Associated With PBSCT and BMT in ALL and AML

In the ALL group, 59/60 and 350/351 patients after PBSCT and BMT survived more than 28 days, while in the AML group, 30/30 and 220/220 patients after PBSCT and BMT survived more than 28 days. Of these surviving patients in ALL, engraftment occurred in 58 (98.3%) patients after PBSCT and 346 (98.9%) patients after BMT. Of the surviving patients in AML, 29 (96.7%) patients after PBSCT and 219 (99.5%) patients after BMT exhibited engraftment.

The median times to recovering a neutrophil count of $>0.5 \times 10^9$/L for the ALL and AML groups and their SR and HR subgroups are shown in Figure 1. For the ALL group, PBSCT and BMT were associated with median times of 13 and 16 days ($P < 0.0001$). Similarly, the median times after PBSCT and BMT in the ALL SR subgroup were 13 and 16 days ($P < 0.0001$), respectively. For the ALL HR subgroup, these times were 13 and 16 days ($P = 0.0003$), respectively. The median times after PBSCT and BMT in the AML group were 12.5 and 17 days, respectively ($P = 0.0002$), while for the AML SR subgroup, they were 12 and 17 days, respectively ($P < 0.0001$). However, in the AML HR subgroup, PBSCT (14.5 days), did not differ significantly from BMT (18 days) in terms of neutrophil recovery ($P = 0.1795$). Thus, PBSCT was associated with

TABLE I. Patient Characteristics

	ALL (n=411)				AML (n=250)			
	PBSC	BM	*P*-value	Total	PBSC	BM	*P*-value	Total
Number of patients	60 (15)	351 (85)		411	30 (12)	220 (88)		250
Median age, years [range]	11.0 [4–18]	8.0 [0–18]	<0.01	9.0 [0–18]	11.5 [0–17]	9.0 [0–18]	0.20	9.0 [0–18]
Sex (male/female)	33/27	222/129	0.28	255/156	14/16	132/88	0.23	146/104
Risk group			0.03				<0.01	
Standard risk	28 (47)	219 (62)		247 (60)	18 (60)	184 (84)		202 (81)
High risk	32 (53)	132 (38)		164 (40)	12 (40)	36 (16)		48 (19)
Conditioning regimen			0.37				0.42	
TBI-based	44 (73)	279 (79)		323 (79)	11 (37)	102 (46)		113 (45)
Chemotherapy-based	16 (27)	72 (21)		88 (21)	19 (63)	118 (54)		137 (55)
GVHD prophylaxis								
CSA+short MTX	32 (53)	167 (48)		199 (48)	18 (60)	108 (49)		126 (50)
CSA alone	9 (15)	49 (14)		58 (14)	5 (17)	23 (10)		28 (11)
Short MTX alone	7 (12)	94 (27)		101 (25)	1 (3)	65 (30)		66 (26)
FK506+short MTX	5 (8)	15 (4)		20 (5)	0 (0)	8 (4)		8 (3)
Standard risk								
Median age, years [range]	10.5 [4–17]	8.0 [1–18]	0.13	8.0 [1–18]	12.0 [0–17]	9.0 [0–18]	0.12	9.0 [0–18]
Sex (male/female)	15/13	144/75	0.29	159/88	9/9	108/76	0.64	117/85
High risk								
Median age, years [range]	11.0 [5–18]	8.0 [0–18]	<0.01	9.0 [0–18]	9.0 [1–16]	10.0 [0–17]	0.89	10.0 [0–17]
Sex (male/female)	18/14	78/54	0.93	96/68	5/7	24/12	0.23	29/19

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; BM, bone marrow; PBSC, peripheral blood stem cell; TBI, total body irradiation; CSA, cyclosporine; MTX, methotrexate. Standard-risk disease includes ALL in first or second complete remission (unless Philadelphia chromosome positivity or mixed-lineage leukemia gene rearrangement was present) and AML in first or second complete remission. High-risk disease includes all other diseases statuses. Values are given as n (%).

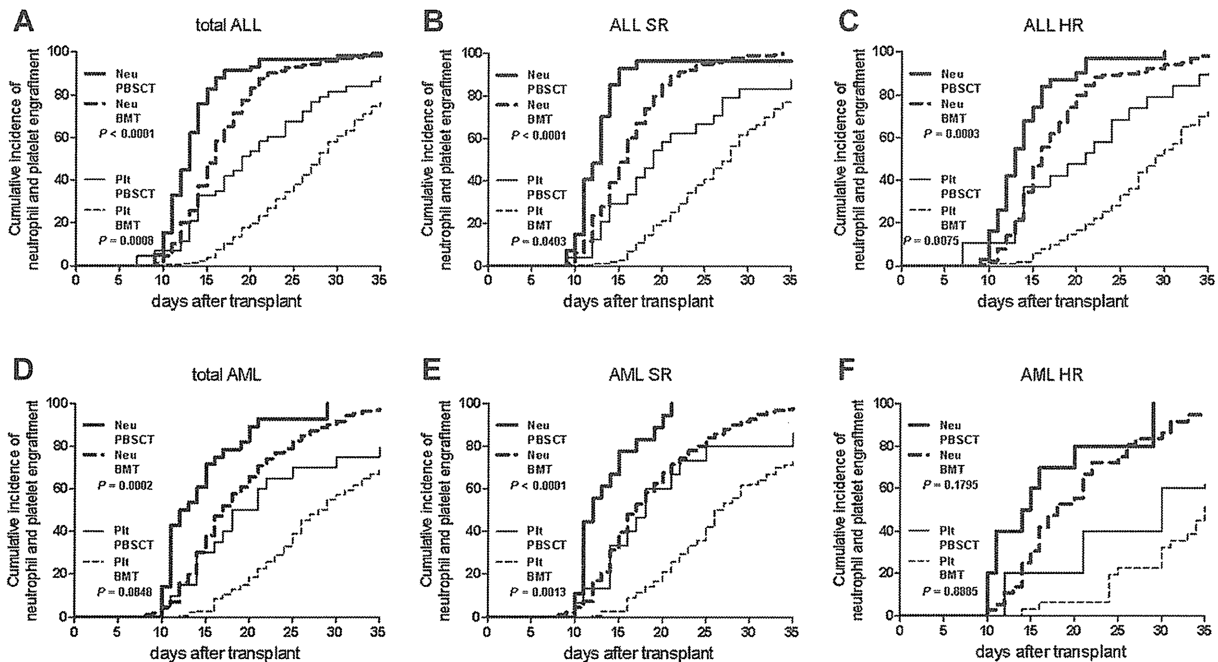

Fig. 1. Engraftment to neutrophil count $>0.5 \times 10^9$/L and engraftment to platelet count $>50 \times 10^9$/L. **A**: total ALL; (**B**) ALL standard-risk; (**C**) ALL high-risk; (**D**) total AML; (**E**) AML standard-risk; and (**F**) AML high-risk.

significantly faster neutrophil recovery than BMT in total ALL, ALL SR, ALL HR, total AML and AML SR.

As shown in Figure 1, for the ALL group, the median times to recovering a platelet count of $>50 \times 10^9$/L after PBSCT and BMT were 19 and 28 days, respectively ($P = 0.0008$). For the ALL SR subgroup, the times after PBSCT and BMT were 19 and 28 days, respectively ($P = 0.0403$), while for the ALL HR subgroup, the times were 21 and 29 days, respectively ($P = 0.0075$). For the AML group, these times after PBSCT and BMT were 19.5 and 28 days, respectively ($P = 0.0848$), while the times for the AML SR subgroup were 18 and 26.5 days, respectively ($P = 0.0013$). For the AML HR subgroup, these times were 30 and 35 days, respectively ($P = 0.8885$). Thus, PBSCT was associated with significantly faster platelet recovery than BMT in the ALL group, ALL SR, ALL HR and AML SR.

Relapse Rate, Disease-Free Survival and Overall Survival Associated With PBSCT and BMT in ALL and AML

As shown in Supplementary Figure 1, the RR after PBSCT and BMT did not differ significantly for ALL and AML or their SR and HR subgroups (total ALL, $P = 0.0663$; ALL SR, $P = 0.0977$; ALL HR, $P = 0.7708$; total AML, $P = 0.1549$; AML SR, $P = 0.4334$; AML HR, $P = 0.9871$).

PBSCT and BMT in the ALL group were associated with 5-year DFS values of 40.6% and 57.1%, respectively ($P = 0.0257$; Fig. 2A) and with OS values of 42.4% and 63.7%, respectively ($P = 0.0032$; Fig. 2A). In the AML group, PBSCT and BMT were associated with 5-year DFS values of 52.9% and 67.4%, respectively ($P = 0.0677$; Fig. 2D) and OS values of 49.8% and 71.1%, respectively ($P = 0.0163$; Fig. 2D). In ALL SR, PBSCT and BMT were associated with 5-year DFS values of 59.1% and 70.5%, respectively ($P = 0.2584$; Fig. 2B) and OS values of 58.2% and 76.9%, respectively ($P = 0.0579$; Fig. 2B). In ALL HR, PBSCT and BMT were associated with 5-year DFS values of 23.0% and 34.0%, respectively ($P = 0.2930$; Fig. 2C) and OS values of 28.8% and 40.1%, respectively ($P = 0.2507$; Fig. 2C). In AML SR, PBSCT and BMT were associated with 5-year DFS values of 71.8% and 72.5%, respectively ($P = 0.8519$; Fig. 2E) and OS values of 68.6% and 77.3%, respectively ($P = 0.6297$; Fig. 2E). In AML HR, PBSCT and BMT were associated with 5-year DFS values of 25.0% and 40.0%, respectively ($P = 0.4275$; Fig. 2F) and OS values of 22.2% and 43.2%, respectively ($P = 0.1339$; Fig. 2F).

As shown in Table II, multivariate Cox analysis revealed that PBSCT was significant risk factor for a poorer DFS (HR = 1.37; 95% CI, 1.01–1.88; $P = 0.044$) and OS (HR = 1.51; 95% CI, 1.09–2.09; $P = 0.013$). In addition, having HR disease was found to be a significant adverse risk factor for DFS (HR = 3.32; 95% CI, 2.59–4.26; $P < 0.001$) and OS (HR = 3.58; 95% CI, 2.73–4.70; $P < 0.001$).

Acute GvHD Associated With PBSCT and BMT in ALL and AML

As shown in Supplementary Figure 2, PBSCT was associated with higher cumulative incidences of grades II–IV acute GvHD than BMT only in AML HR (57.6% vs. 23.2%, $P = 0.0264$) but not in the ALL group (31.2% vs. 21.8%, $P = 0.0826$), ALL SR (26.0% vs. 19.7%, $P = 0.4255$), ALL HR (34.9% vs. 25.4%, $P = 0.1784$), the AML group (33.9% vs. 18.0%, $P = 0.0506$) and AML SR (15.4% vs. 17.1%, $P = 0.8503$). Six patients died of grade IV acute GvHD: one patient with ALL after PBSCT, two patients with ALL after BMT and three patients with AML after BMT.

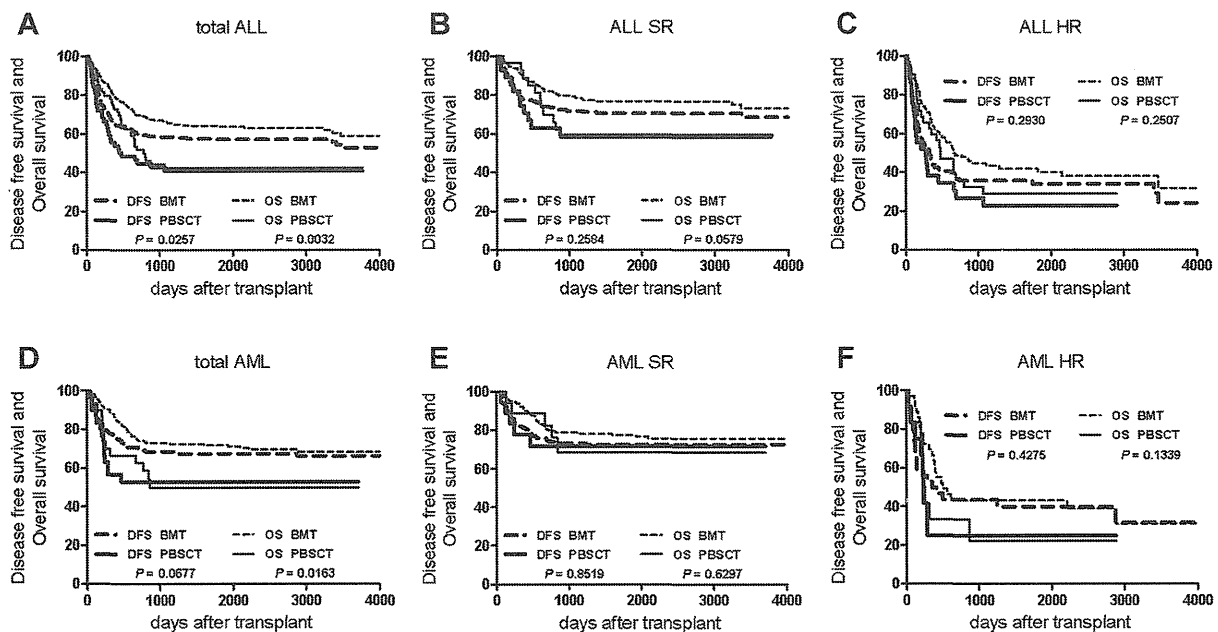

Fig. 2. Disease free survival and overall survival. **A**: total ALL; (**B**) ALL standard-risk; (**C**) ALL high-risk; (**D**) total AML; (**E**) AML standard-risk; and (**F**) AML high-risk.

TABLE II. Multivariate Model of Prognostic Risk Factors for Disease Free Survival and Overall Survival

	Disease free survival			Overall survival		
Variable	Hazard ratio	95% conf. interval	*P*-value	Hazard ratio	95% conf. interval	*P*-value
Diagnosis: AML vs. ALL	0.91	0.70–1.18	0.470	1.03	0.78–1.36	0.849
Risk group: HR vs. SR	3.32	2.59–4.26	<0.001	3.60	2.74–4.72	<0.001
Stem cell source: peripheral blood vs. bone marrow	1.38	1.01–1.88	0.044	1.51	1.09–2.09	0.013

AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; HR, high-risk; SR, standard-risk; HLA, human leucocyte antigen.

As shown in Supplementary Figure 2, the cumulative incidences of grades III–IV acute GvHD was similar for PBSCT and BMT in the ALL group (9.1% vs. 7.7%, $P = 0.7741$), ALL SR (0% vs. 4.8%, $P = 0.3027$), ALL HR (16.2% vs. 12.6%, $P = 0.5896$), the AML group (15.7% vs. 8.5%, $P = 0.3268$), AML SR (9.1% vs. 8.3%, $P = 0.9534$) and AML HR (27.1% vs. 9.8%, $P = 0.1729$).

Chronic GvHD Associated With PBSCT and BMT in ALL and AML

Shown in Figure 3, PBSCT was associated with a higher cumulative incidence of any grade of chronic GvHD than BMT for the ALL group (52.6% vs. 19.6%, $P = 0.0002$), ALL SR (39.7% vs. 18.3%, $P = 0.0007$), ALL HR (48.8% vs. 21.6%, $P = 0.0949$), the AML group (56.3% vs. 23.1%, $P = 0.0002$), AML SR (40.2% vs. 21.0%, $P = 0.0905$) and AML HR (81.8% vs. 39.7%, $P = 0.0027$). Similarly, as shown in Figure 3, the cumulative incidence of extensive chronic GvHD was significantly higher in PBSCT than BMT for the ALL group (26.0% vs. 9.9%, $P = 0.0066$), ALL SR (24.3% vs. 8.3%, $P = 0.0059$), the AML group (41.6% vs. 11.1%, $P < 0.0001$), AML SR (30.6% vs. 9.9%, $P = 0.0215$) and AML HR (56.4% vs. 23.5%, $P = 0.0046$). However, the difference observed in ALL HR did not achieve statistical significance (36.4% vs. 12.7%, $P = 0.3225$). In addition, as shown in Figure 3, the cumulative incidence of limited chronic GvHD was higher after PBSCT than after BMT for the ALL group (34.6% vs. 10.6%, $P = 0.0172$) and ALL SR (39.0% vs. 10.8%, $P = 0.0419$), but it was similar for ALL HR (18.1% vs. 9.9%, $P = 0.1812$), the AML group (23.1% vs. 13.2%, $P = 0.3273$), AML SR (12.8% vs. 12.0%, $P = 0.9658$) and AML HR (54.5% vs. 20.6%, $P = 0.2462$).

Transplantation-Related Mortality Associated With PBSCT and BMT in ALL and AML

As indicated by Figure 4, for the ALL group, cumulative incidences of TRM in PBSCT and in BMT were 5.1% and 4.0% at

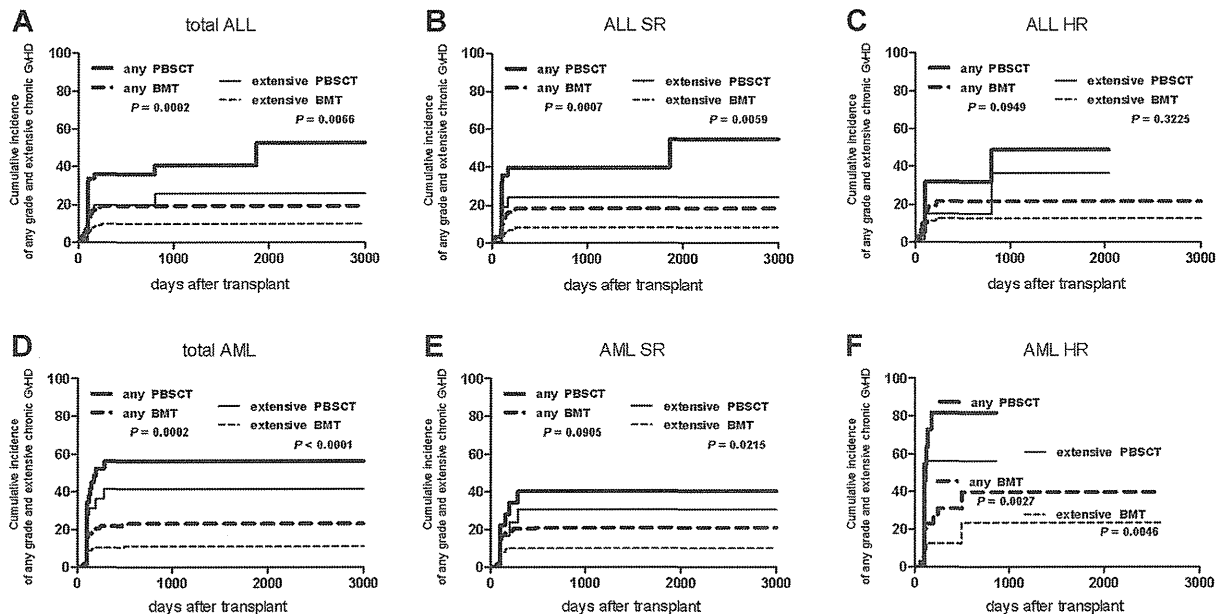

Fig. 3. Cumulative incidence of any grade and extensive chronic GvHD. **A**: total ALL; (**B**) ALL standard-risk; (**C**) ALL high-risk; (**D**) total AML; (**E**) AML standard-risk; and (**F**) AML high-risk.

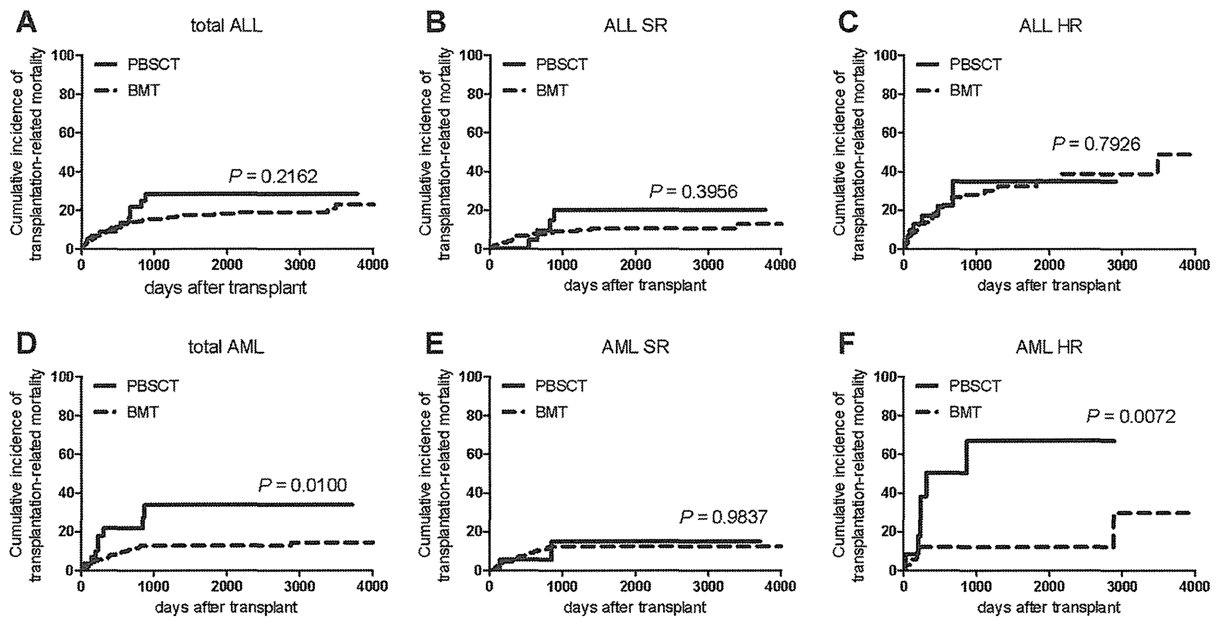

Fig. 4. Cumulative incidence of transplantation-related mortality. **A**: total ALL; (**B**) ALL standard-risk; (**C**) ALL high-risk; (**D**) total AML; (**E**) AML standard-risk; and (**F**) AML high-risk.

100 days, 8.8% and 9.6% at 1 year and 28.2% and 15.3% at 3 years, respectively ($P = 0.2162$). For the AML group, cumulative incidences of TRM in PBSCT and in BMT were 3.3% and 1.8% at 100 days, 21.6% and 6.0% at 1 year and 33.7% and 12.7% at 3 years, respectively ($P = 0.0100$). Thus, for both ALL and AML, PBSCT and BMT were associated with similar TRM incidences early after transplantation, PBSCT is associated with higher TRM incidences later after transplantation, only for the AML group and AML HR (ALL SR, $P = 0.3956$; (ALL SR, $P = 0.3956$; ALL HR, $P = 0.7926$; AML SR, $P = 0.9837$; AML HR, $P = 0.0072$; Supplementary Table I).

DISCUSSION

This retrospective study compared the outcomes of allogeneic HSCT using two stem cell sources, namely BM or PBSC, in 661 Japanese children with acute leukaemia. The stem cell donors were HLA-matched sibling donors and the study was based on data acquired between January 1996 and December 2007. It was observed that PBSCT was associated with more rapid haematopoietic recovery than BMT, as has also been shown in most previous studies with adult patients [3,4,9,11–15] and paediatric patients [20]. The OS and DFS were significantly lower after PBSCT than after BMT.

Multivariate analysis revealed that the use of peripheral blood allografts was an adverse risk factor for OS and DFS. Similarly, while previous studies of adults with acute leukaemia suggest that PBSCT and BMT were associated with equivalent survival rates [9,11,15], the IBMRT study of children and adolescents reported that PBSCT was associated with lower survival and higher TRM [21]. The higher mortality observed in children after PBSCT is likely to be due to the higher incidences of chronic GvHD. This has been reported in younger patients with acquired severe aplastic anemia [22]. Thus, peripheral blood allografts in younger recipients may be associated with a higher risk of mortality and chronic GvHD, which suggests that caution should be exercised when considering the use of PBSC in paediatric patients.

It should be noted that this is a retrospective study, which has a number of limitations. In particular, we could not exclude the possibility that unidentified confounding variables could affect the transplant outcomes and could not adjust the data for unknown or unmeasured factors. Thus, the results presented here should be interpreted with caution. Nevertheless, these observations suggest that BM should be the preferred stem cell source for children with acute leukaemia who have HLA-matched sibling donors. It remains to be seen whether these conclusions can be extrapolated to alternative donors, namely HLA-mismatched related donors and HLA-identical unrelated donors. At present, there are no guidelines regarding the preferred source of stem cells (PBSC or BM) from unrelated volunteer donors. However, the biggest risk associated with selecting PBSC over BM concerns the fact that higher numbers of T cells are infused, which increases the risk of chronic GvHD and transplantation-related mortality. This risk may be greater with unrelated volunteer donors than with sibling donors. Indeed, it has been shown that when using unrelated donors, a single HLA mismatch increases the risk of GvHD more significantly if the transplant involves PBSC than if it involves BM [23]. Since a bank that stores PBSC from unrelated volunteer donors is now available in Japan, it is likely that peripheral blood allografts will be used more frequently for allogeneic transplantation in children. However, before such widespread clinical changes take place, the risks and benefits of the various allografts that are available should be considered. In cases of PBSC will be used more frequently such as active infections at SCT, mycophenolate

mofeti of GvHD prophylaxisis. More detailed analyses and future trials may reveal that BM stem cells and PBSC are suitable for different situations.

PBSCT did not differ from BMT in terms of the incidence of grades III–IV acute GvHD for the total ALL and total AML groups (and all of their SR and HR subgroups). This has also been observed in several studies with adult patients [3,12–14] and paediatric patients [20], even though there are 10-fold more T cells in the peripheral blood than in the BM. This may relate to the use of G-CSF [24,25]. However, the AML HR subgroup was observed to develop grades II–IV acute GvHD significantly more often after PBSCT than after BMT. It is possible that the acute GvHD experienced by the PBSC recipients in the AML HR subgroup reflects the induction of a graft-versus-leukemia effect. However, arguing against this is that the RR after PBSCT and BMT in the AML HR subgroup was similar. The observation that allogeneic PBSCT is not associated with a higher incidence of acute GvHD does not appear to extend to chronic GvHD. As observed in other studies with adult patients [3,9,11,13,26,27], PBSCT was associated with a significantly higher incidence of chronic GvHD than BMT in the total ALL and total AML groups (and the ALL SR and AML HR subgroups). In particular, PBSCT was associated with a significantly higher incidence of extensive chronic GvHD in the total ALL and total AML groups along with all SR and HR subgroups apart from ALL HR. Although we were not able to investigate organ injury in detail, another study has shown that chronic GvHD after PBSCT involves higher numbers of organs and requires longer and multiple courses of immunosuppressive therapy [28].

While a meta-analysis has demonstrated that allogeneic PBSCT is associated with a significant decrease in relapse in both early and late stage patients [29], our study found that the RR after PBSCT and BMT did not differ significantly for ALL and AML. This was also observed by a study analysing the IBMTR/EBRT registry data of adult patients with leukaemia [10], as well as by the IBMTR study of children and adolescents with acute leukaemia [21]. The allogeneic graft-versus-leukaemia effect varies from one disease to another and depends on the stage of the disease and donor histocompatibility. In our study, which only assessed paediatric patients with acute leukaemia, increases in the incidence of extensive chronic GvHD did not lead to a concomitant decrease in the RR. It will be necessary to examine the relationship between GvHD and relapse in the future.

In summary, our study demonstrates that while the use of peripheral blood allografts from HLA-matched sibling donors in Japanese paediatric patients with ALL or AML leads to faster engraftment, it is also associated with poorer survival and quality of life due to chronic GvHD.

ACKNOWLEDGEMENTS

We thank all the staff and resident members of the transplant centres in Japan. We also thank Mrs. Rie Goi for technical assistance.

REFERENCES

1. Kessinger A, Smith DM, Strandjord SE, et al. Allogeneic transplantation of blood-derived T cell-depleted hemopoietic stem cells after myeloablative treatment in a patient with acute lymphoblastic leukemia. Bone Marrow Transplant 1989;4:643–646 [PubMed: 2573397].
2. Russel NH, Hunter AE, Rogers S, et al. Peripheral blood stem cells as an alternative to marrow for allogeneic transplantation (letter). Lancet 1993;341:1482 [PubMed: 8099182].
3. Heldal D, Tjonnfjord G, Brinch L, et al. A randomised study of allogeneic transplantation with stem cells from blood or bone marrow. Bone Marrow Transplant 2000;25:1129–1136 [PubMed: 10849524].
4. Schmitz N, Bacigalupo A, Hasenclever D, et al. Allogeneic bone marrow transplantation vs filgrastim-mobilised peripheral blood progenitor cell transplantation in patients with early leukaemia: First results of a randomised multicentre trial of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant 1998;21:995–1003 [PubMed: 9632272].
5. Vigorito AC, Azevedo WM, Marques JFC, et al. A randomized prospective comparison of allogeneic bone marrow and peripheral blood progenitor cell transplantation in the treatment of haematological malignancies. Bone Marrow Transplant 1998;22:1145–1151 [PubMed: 9894716].
6. Blaise D, Kuentz M, Fortanier C, et al. Randomized trial of bone marrow versus lenograstim-primed blood cell allogeneic transplantation in patients with early-stage leukemia: A report from the Societe Francaise de Greffe de Moelle. J Clin Oncol 2000;18:537–546 [PubMed: 10653869].
7. Gratwohl A, Baldomero H, Horisberger B, et al. Current trends in hematopoietic stem cell transplantation in Europe. Blood 2002;100:2374–2386 [PubMed: 12239145].
8. Atsuta Y, Suzuki R, Yoshimi A, et al. Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP System. Int J hematol 2007;86:269–274 [PubMed: 17988995].
9. Schmitz N, Beksac M, Hasenclever D, et al. Transplantation of mobilized peripheral blood cells to HLA-identical siblings with standard-risk leukemia. Blood 2002;100:761–767 [PubMed: 12130483].
10. Schmitz N, Eapen M, Horowitz MM, et al. Long-term outcome of patients given transplants of mobilized blood or bone marrow: A report from the International Bone Marrow Transplant Registry and the European Group for Blood and Marrow Transplantation. Blood 2006;108:4288–4290 [PubMed: 16946302].
11. Champlin RE, Schmitz N, Horowitz MM, et al. Blood stem cells compared with bone marrow as a source of hematopoietic cells for allogeneic transplantation. IBMTR Histocompatibility and Stem Cell Sources Working Committee and the European Group for Blood and Marrow Transplantation (EBMT). Blood 2000;95:3702–3709 [PubMed: 10845900].
12. Powles R, Mehta J, Kulkarni S, et al. Allogeneic blood and bone-marrow stem-cell transplantation in haematological malignant diseases: A randomized trial. Lancet 2000;355:1231–1237 [PubMed: 10770306].
13. Bensinger WI, Martin PJ, Storer B, et al. Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. N Engl J Med 2001;344:175–181 [PubMed: 11172139].
14. Couban S, Simpson DR, Barnett MJ, et al. A randomized multicenter comparison of bone marrow and peripheral blood in recipients of matched sibling allogeneic transplants for myeloid malignancies. Blood 2002;100:1525–1531 [PubMed: 12176866].
15. Ringden O, Labopin M, Bacigalupo A, et al. Transplantation of peripheral blood stem cells as compared with bone marrow from HLAidentical siblings in adult patients with acute myeloid leukemia and acute lymphoblastic leukemia. J Clin Oncol 2002;20:4655–4664 [PubMed: 12488410].
16. Przepiorka D, Weisdorf D, Martin P, et al. 1994; Consensus conference on acute GVHD grading. Bone Marrow Transplant 1995:825–828 [PubMed: 7581076].
17. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med 1980;69:204–217 [PubMed: 6996481].
18. Kaplan EL, Meier P. Nonparametric estimation from imcomplete observations. J Am Stat 1958;53:457–481.
19. Cox DR. Regression models and life tables (with discussion). J Royal Stat, Series B 1972;34: 187–220.
20. Lin YF, Lairson DR, Chan W, et al. Children with acute leukemia: A comparison of outcomes from allogeneic blood stem cell and bone marrow transplantation. Pediatr Blood Cancer 2011;56:143–151 [PubMed: 21108446].
21. Eapen M, Horowitz MM, Klein JP, et al. Higher mortality after allogeneic peripheral-blood transplantation compared with bone marrow in children and adolescents: The Histocompatibility and Alternate Stem Cell Source Working Committee of the International Bone Marrow Transplant Registry. J Clil Oncol 2004;22:4872–4880 [PubMed: 15520055].
22. Schrezenmeier H, Passweg JR, Marsh JC, et al. Worse outcome and more chronic GVHD with peripheral blood progenitor cells than bone marrow in HLA-matched sibling donor transplants for young patients with severe acquired aplastic anemia. Blood. 2007;110:1397–1400 [PubMed: 17475907].
23. Garderet L, Labopin M, Gorin NC, et al. Patients with acute lymphoblastic leukaemia allografted with a matched unrelated donor may have a lower survival with a peripheral blood stem cell graft compared to bone marrow. Bone Marrow Transplant 2003;31:23–29 [PubMed: 12621503].
24. Pan L, Delmonte JJ, Jalonen CK, Ferrara JL. Pretreatment of donor mice with granulocyte colony-stimulating factor polarizes donor T lymphocytes toward type-2 cytokine production and reduces severity of experimental graft-versus-host disease. Blood 1995;86:4422–4429 [PubMed: 8541530].
25. Zeng D, Dejbakhsh JS, Strober S. Granulocyte colony-stimulating factor reduces the capacity of blood mononuclear cells to induce graft-versus-host disease: Impact on blood progenitor cell transplantation. Blood 1997;90:453–463 [PubMed: 9207483].
26. Cutler C, Giri S, Jeyapalan S, et al. Acute and chronic graft-versus-host disease after allogeneic peripheral-blood stem-cell and bone marrow transplantation: A meta-analysis. J Clin Oncol 2001;19:3685–3691 [PubMed: 11504750].
27. Tanimoto TE, Yamaguchi T, Tanaka Y, et al. Comparative analysis of clinical outcomes after allogeneic bone marrow transplantation versus peripheral blood stem cell transplantation from a related donor in Japanese patients. Br J Haematol 2004;125:480–493 [PubMed: 15142119].
28. Mohty M, Kuentz M, Michallet M, et al. Chronic graft-versus-host disease after allogeneic blood stem cell transplantation: Long-term results of a randomized study. Blood 2002;100:3128–3134 [PubMed: 12384409].
29. Stem Cell Trialists' Collaborative Group. Allogeneic peripheral blood stem-cell compared with bone marrow transplantation in the management of hematologic malignancies: An individual patient data meta-analysis of nine randomized trials. J Clin Oncol 2005;23:5074–5087 [PubMed: 16051954].

Int J Hematol (2013) 98:437–445
DOI 10.1007/s12185-013-1409-6

ORIGINAL ARTICLE

WT1 mutation in pediatric patients with acute myeloid leukemia: a report from the Japanese Childhood AML Cooperative Study Group

Hirozumi Sano · Akira Shimada · Ken Tabuchi · Tomohiko Taki · Chisato Murata · Myoung-ja Park · Kentaro Ohki · Manabu Sotomatsu · Souichi Adachi · Akio Tawa · Ryoji Kobayashi · Keizo Horibe · Masahiro Tsuchida · Ryoji Hanada · Ichiro Tsukimoto · Yasuhide Hayashi

Received: 29 March 2013 / Revised: 29 July 2013 / Accepted: 6 August 2013 / Published online: 27 August 2013
© The Japanese Society of Hematology 2013

Abstract Mutations in *Wilms tumor 1* (*WT1*) have been reported in 10–22 % of patients with cytogenetically normal acute myeloid leukemia (CN-AML), but the prognostic implications of these abnormalities have not been clarified in either adults or children. One hundred and fifty-seven pediatric AML patients were analyzed for *WT1* mutations around hotspots at exons 7 and 9; however, amplification of the *WT1* gene by the reverse transcriptase-polymerase chain reaction was not completed in four cases (2.5 %). Of the 153 evaluable patients, 10 patients (6.5 %) had a mutation in *WT1*. The incidence of *WT1* mutations was significantly higher in CN-AML than in others (15.2 vs. 4.5 %, respectively, $P = 0.03$). Of the 10 *WT1*-mutated cases, eight (80 %) had mutations in other genes, including *FLT3*-ITD in two cases, *FLT3*-D835 mutation in two, *KIT* mutation in three, *MLL*-PTD in three, *NRAS* mutation in one, and *KRAS* mutation in two (in some cases, more than one additional gene was mutated). The incidences of *KIT* and *FLT3*-D835 mutations were significantly higher in patients with than in those without *WT1* mutation. No significant differences were observed in the 3-year overall survival and disease-free survival; however, the presence

H. Sano · A. Shimada · C. Murata · M. Park · K. Ohki · M. Sotomatsu · Y. Hayashi (✉)
Department of Hematology/Oncology, Gunma Children's Medical Center, 779 Shimohakoda, Hokkitsu, Shibukawa, Gunma 377-8577, Japan
e-mail: hayashiy-tky@umin.ac.jp

H. Sano · R. Kobayashi
Department of Pediatrics, Sapporo Hokuyu Hospital, 6-6 Higashi-Sapporo, Shiroishi-ku, Sapporo 003-0006, Japan

A. Shimada
Department of Pediatrics/Pediatric Hematology & Oncology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikatacho, Kita-ku, Okayama 700-8558, Japan

K. Tabuchi
Department of Hematology, Kanagawa Children's Medical Center, 2-138-4 Mutsugawa, Minami-ku, Yokohama, Kanagawa 232-8555, Japan

T. Taki
Department of Molecular Diagnostics and Therapeutics, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, 465 Kajii-cho, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan

S. Adachi
Department of Human Health Sciences, Graduate School of Medicine, Kyoto University, 53 Shougoin, Kawaharacho, Sakyo-ku, Kyoto 606-8507, Japan

A. Tawa
Department of Pediatrics, National Hospital Organization Osaka National Hospital, 2-1-14 Hoenzaka, Chuo-ku, Osaka 540-0006, Japan

K. Horibe
Clinical Research Center, National Hospital Organization Nagoya Medical Center, 4-1-1 Sannomaru, Naka-ku, Nagoya, Aichi 460-0001, Japan

M. Tsuchida
Department of Pediatrics, Ibaraki Children's Hospital, 3-3-1 Futabadai, Mito, Ibaraki 311-4145, Japan

R. Hanada
Division of Hematology/Oncology, Saitama Children's Medical Center, 2100 Oaza Magome, Iwatsuki-ku, Saitama 339-8551, Japan

I. Tsukimoto
First Department of Pediatrics, Toho University School of Medicine, 6-11-1, Omori-nishi, Ota-ku, Tokyo 143-8541, Japan

Springer

of *WT1* mutation was related to a poor prognosis in patients with CN-AML, excluding those with *FLT3*-ITD and those younger than 3 years.

Keywords Acute myeloid leukemia · *WT1* mutation · Cytogenetically normal acute myeloid leukemia · Prognosis

Introduction

The prognosis of pediatric acute myeloid leukemia (AML) patients has improved markedly over the past decade, with an overall survival rate of about 60–70 % according to the results of various clinical trials; however, relapse remains a major cause of treatment failure, occurring in 30–40 % of patients in their first complete remission (CR) [1–6]. Several study groups have shown that chromosome abnormalities are independent and strong predictors of the outcome in both childhood and adult AML [1, 4, 7]. On the other hand, cytogenetically normal acute myeloid leukemia (CN-AML) is the largest cytogenetic subgroup of AML, representing approximately 40 % of pediatric AML patients [7]. Recently, CN-AML has been recognized as highly heterogeneous molecularly, since several abnormalities were discovered, including mutations in *FLT3*, *NPM1*, *CEBPA*, and *MLL* genes and aberrant expression of *BAALC*, *ERG*, and *MN1* genes [8]. These alterations have been associated with the treatment outcome and serve as a basis for risk assessment in CN-AML [8, 9]. Discovering novel genetic markers may lead to an improvement in molecular risk stratification and allow a more accurate prediction of the response to therapy.

Wilms tumor 1 (*WT1*) is located at chromosome 11p13 [10] and encodes a transcription factor capable of activating or repressing gene transcription, depending on the cell type, WT1 protein isoform, and target gene [11]. Although initially considered a tumor suppressor gene [12], *WT1* has also been demonstrated to act as an oncogene [11, 13–15]. Mutations of the *WT1* gene have been reported in 10–22 % of cases of CN-AML in both adults [16–18] and children [19]. *WT1* gene mutations cluster to exons 7 and 9, and are associated with induction failure and/or relapse in adults and children [16–22]. However, there have been few reports on *WT1* gene mutation in pediatric AML patients. Thus, we performed mutational analysis of *WT1* in pediatric AML patients who were treated on the Japanese Childhood Cooperative Study Group Protocol, AML99 [5], and demonstrated that *WT1* mutations were related to a poor prognosis in patients older than 2 years with CN-AML excluding those with *FLT3*-ITD. Furthermore, we analyzed the association between *WT1* mutations and other gene aberrations including *RAS* and *KIT* mutations, *FLT3*-ITD, *FLT3*-D835, and *MLL*-PTD.

Materials and methods

Patients

The diagnosis of AML was based on the FAB classification, and cytogenetic analysis was performed using a routine G-banding method. From January 2000 to December 2002, 318 patients were newly diagnosed with de novo AML. Of these, samples from 157 patients were available for molecular analysis, including 13 with FAB-M3 and 10 with Down syndrome (DS), who were treated on different treatment protocols [5, 23–25]. There were no significant differences between the 134 patients without FAB-M3 or DS and the 106 non-analyzed patients in terms of the age [median 6 years (range 0–15 years) vs. 6 years (range 0–15 years), respectively] and initial WBC count [median 24.8×10^9/L (range 1.65-621.0 $\times 10^9$/L) vs. 13.8×10^9/L (range 1.0-489.0 $\times 10^9$/L, $P = 0.08$), respectively]. Patients who were younger than 2 years or had an initial WBC count <100,000/μL were treated using the induction A regimen [etoposide (VP-16), cytarabine, and mitoxantrone (MIT), (ECM)]. Patients who were older than 2 years and had an initial WBC count > 100,000/μL were treated using the induction B regimen [VP-16, cytarabine, and idarubicin (IDA), (ECI)]. If patients achieved a complete remission (CR), they were classified into three risk groups (62 low, 57 intermediate, and 10 high) according to the results of cytogenetic analyses or the achievement of CR after the 2 initial courses of chemotherapy [5, 23–25]. AML patients with t(8;21)(q22;q22) (except for those with WBC counts >50,000/μL) or inv(16)(p13q22) were classified into the low-risk (LR) group. Patients with monosomy 7, 5q-, t(16;21), or Philadelphia (Ph) chromosome were classified into the high-risk (HR) group. Patients were treated with additional chemotherapy or allogeneic hematopoietic stem cell transplantation (allo-HSCT) in each risk group.

Informed consent was obtained from the patients or their parents, according to guidelines based on the tenets of the revised Helsinki protocol. The institutional review board of Gunma Children's Medical Center approved this project.

Detection of *WT1* mutations

Total RNA (4 μg) extracted from the bone marrow or peripheral blood samples at diagnosis was reverse transcribed to cDNA with a cDNA Synthesis Kit (GE Healthcare Japan Corporation, Tokyo, Japan). Mutations of exons 7 and 9 of the *WT1* gene were directly sequenced using the following primers: exon 7 WT1-1s 5′-TACG AGAGCGATAACCACAC-3′; exon 7 WT1-4as 5′-GTC CTTGAAGTCACACTGGT-3′; exon 9 WT1-3s 5′-ACCA GTGTGACTTCAAGGAC-3′; exon 9 WT1-2as 5′-TCAA AGCGCCAGCTGGAGTT-3′.

Table 1 Ten cases with *WT1* mutation in this study

No.	Age (year)	Sex	Karyotype	*WT1* mutation	*FLT3*-ITD or Mt	*KIT* Mt	*NRAS* Mt	*KRAS* Mt	*MLL*-PTD	*WT1* mRNA expression (copies/μgRNA)	Outcome [observation period (year)]
1	14	M	Normal	(1) 7 bp ITD in exon 7[a] (2) H357R	D835Y	Wt	Wt	G12D	Wt	65,000	5.6+[b]
2	6	F	Normal	(1) R462W	Wt	Wt	Wt	Wt	PTD	21,000	1.4
3	8	F	t(8;21)	(1) W395X (2) 101 bp deletion in exons 9 to 10[a]	Wt	A814S	Wt	Wt	Wt	1,400	3.0
4	13	F	t(8;21)	(1) 16 bp insertion in exon 9[a]	Wt	Wt	Wt	Wt	Wt	32,000	4.7+
5	14	M	Other[c]	(1) 115 bp deletion in exons 7 to 8[a] (2) 137 bp insertion and 110 bp deletion in exon 7[a] (3) S381X	D835Y	Wt	Wt	Wt	Wt	16,000	4.2+
6	12	M	Other[d]	(1) 11 bp ITD in exon 7[a]	Wt	Wt	Wt	Wt	PTD	7,000	4.5+
7	13	M	Normal	(1) P376L and 3 bp insertion and 8 bp ITD in exon 7[a]	ITD	V474L	Wt	Wt	PTD	18,000	4.4+
8	10	M	Normal	(1) 3 bp deletion and 4 bp insertion in exon 7[a]	ITD	Wt	Wt	Wt	Wt	14,000	1.7
9	3	F	Inv(16)	(1) 7 bp deletion and 2 bp insertion in exon 7[a]	Wt	Wt	G12D	Wt	Wt	12,000	3.6+
10	6	M	Normal	(1) 157 bp deletion in exons 8 to 10[a]	Wt	V540L	Wt	G13D	Wt	23,000	0.6

M male, *F* female, *Mt* mutation, *Wt* wild-type

[a] Resulting in the frameshift and premature stop codon

[b] Non-CR- > off study, but surviving

[c] 46,XY[2/8]/ 46,XY,del(6)(q15q21),-7,-9,-10,+3mar[1/8]/ 46,XY,?del(3)(p25)[1/8]/ 47,XY,-5,-8,-10,add(12)(q24.1),-16,-18,+6mar[1/8]/ 46,XY,-2,-6,-8,+3mar[1/8]/ 46,XY,-8,+mar[1/8]/ 46,Y,?add(X)(p11.2)[1/8]

[d] 46,XY,t(1;7;11)(q32;p15;p15)[14/20]/ 46,XY[6/20]

Detection of *FLT3*-ITD, *FLT3*-D835, *MLL*-PTD, *KIT*, and *RAS* mutations

Mutation analysis of internal tandem duplication (ITD) within the JM domain and D835 mutation (D835Mt) within the TK2 domain of *FLT3* was performed as previously reported [23, 25–27]. Mutation analysis of partial tandem duplication (PTD) of *MLL* was performed using the primer pair 6.1 (located in exon 9) and E3AS (located in exon 4), as previously reported [25]. Mutation analysis of the kinase domain, extracellular domain, and transmembrane domain of the *KIT* gene was performed with the reverse transcriptase-polymerase chain reaction (RT-PCR) followed by direct sequencing, as previously reported [23]. Mutation analysis of the *RAS* gene around hot spots at codons 12, 13, and 61 was performed as previously reported [28].

Detection of *WT1* mRNA expression by quantitative RT-PCR (qRT-PCR)

WT1 expression at diagnosis was measured using the qRT-PCR system, as previously reported [29]. We determined the cut-off value of *WT1* expression to be 2,500 copies/μg RNA, because the value for the 90th percentile of *WT1* expression in normal bone marrow samples was 2,519 copies/μg RNA [29].

Statistical analysis

The χ^2 test was used to compare the frequencies of mutations. Fischer's exact test was used when data were sparse. The survival distribution was assessed using the Kaplan–Meier method, and differences were compared using the log-rank test [30]. Overall survival (OS) was defined as the time from diagnosis until death owing to any cause or the last follow-up. Disease-free survival (DFS) was defined as the time from the date of complete remission until relapse or death; patients alive and relapse-free at last follow-up were censored. Multivariate analyses were performed to investigate whether *WT1* mutation might serve as a prognostic factor in 130 AML patients, excluding those with FAB-M3 and Down syndrome. *FLT3*-ITD, *FLT3*-D835, *MLL*-PTD, *KIT*, *RAS*, and *WT1* mutation were examined whether these alterations influenced about the 3-year OS and DFS. Karyotypic abnormalities were not included in analytic variables since they were apparently confounded with aforementioned genomic alterations. These statistical analyses were based on Dr. SPSS II for Windows (release 11.0.1J, SPSS; Japan, Inc.).

Results

WT1 mutations

Of the 157 analyzed pediatric AML patients, amplification of the *WT1* gene by RT-PCR was not completed in 4 (2.5 %) cases. Therefore, the following analyses were conducted with the 153 evaluable cases excluding these four.

Ten (6.5 %) of the 153 cases had an activating mutation (Table 1). In 7 cases (70 %) with *WT1* mutation, two or more mutations were detected in the *WT1* gene (Table 1). There was no significant difference in the age, sex, WBC count at diagnosis, or frequency of extramedullary infiltration of leukemic cells between patients with and without *WT1* mutations; however, the frequency of allo-HSCT was significantly higher in patients with than in those without *WT1* mutation (70.0 vs 35.7 %, respectively, $P = 0.03$) (Table 2).

Table 2 Clinical characteristics of 153 evaluable patients with and without *WT1* mutations

	All patients	*WT1*-Mt	*WT1*-Wt
Age, median (years)	6 (0–15)	11 (3–14)	6 (0–15)
≥3	113	10	103
<3	40	0	40
WBC count, median ($\times 10^9$/L)	20.7 (1.0–620.0)	35.1 (3.6–440.0)	20.5(1.0–620.0)
Male/female	87/66	6/4	81/62
Patients with Down syndrome	10 (6.5 %)	0	10 (7.0 %)
Patients who underwent allo-HSCT	58 (37.9 %)	7 (70.0 %)	51 (35.7 %)
Total	153	10	143
Risk group (excluding FAB-M3 and Down syndrome)			
Low	58 (44.6 %)	2 (20.0 %)	56 (46.7 %)
Intermediate	57 (42.5 %)	6 (60.0 %)	51 (41.1 %)
High	10 (7.5 %)	1 (10.0 %)	9 (7.3 %)
Non-CR	5 (3.7 %)	1 (10.0 %)	4 (3.2 %)
Total	130	10	120

WT1-Mt patients with *WT1* mutation, *WT1-Wt* patients lacking *WT1* mutation, *HSCT* hematopoietic stem cell transplantation

Table 3 FAB classification, other genomic alterations, and karyotypic abnormalities of 153 evaluable patients with *WT1* mutations

	All patients	*WT1*-Mt
FAB classification		
M0	5	0
M1	24	3 (12.5 %)
M2	44[a]	4 (9.1 %)
M3	13	0
M4	21	1 (4.8 %)
M5	24	1 (4.2 %)
M6	1	0
M7	19[a]	0
Unclassified	2	1 (50.0 %)
Other genomic alterations		
FLT3-ITD	17	2 (11.8 %)
FLT3 D835 mutation	8	2 (25.0 %)
KIT mutation	11	3 (27.3 %)
MLL-PTD	21	3 (14.3 %)
NRAS	11	1 (9.1 %)
KRAS	18	2 (11.1 %)
Total	153	10 (6.5 %)
Karyotypic abnormalities (excluding Down syndrome)		
Normal	33	5 (15.2 %)
t(8;21)	44	2 (4.5 %)
11q23 abnormality	19	0
t(15;17)	13	0
inv(16)	7	1 (14.3 %)
Others	25	2 (8.0 %)
Unknown	2	0
Total	143	10 (7.0 %)

WT1-Mt patients with *WT1* mutation

[a] Of 10 cases with Down syndrome, 9 were classified into FAB-M7 and 1 into FAB-M2. None of them had *WT1* mutations

The incidence of mutations in *WT1* was significantly higher in pediatric CN-AML (15.2 vs. 4.5 %, respectively, $P = 0.04$) (Table 3, in which DS patients were not included in karyotypic abnormalities).

Correlations between *WT1* mutations and other gene aberrations

The incidence of mutations in *KIT* was significantly higher in patients with than in those without the *WT1* mutation (30 vs. 5.6 %, respectively, $P < 0.01$). Moreover, the incidence of *FLT3*-D835 mutation was also significantly higher in patients with than in those without the *WT1* mutation (20.0 vs. 4.2 %, respectively, $P = 0.03$). The distribution of *FLT3*-ITD, *MLL*-PTD, and mutations in *NRAS* and *KRAS* was not different from those without *WT1* mutation (Table 3).

Correlation between *WT1* mutation and *WT1* mRNA expression

A higher *WT1* expression (≥2,500 copies/μg RNA) was detected in 9 (90 %) of 10 cases with *WT1* mutation (Table 1). On the other hand, a higher *WT1* expression was detected in 113 (77 %) of 147 cases without *WT1* mutation. The difference was not significant ($P = 0.33$).

Clinical outcome and prognostic significance of *WT1* mutations

There were no differences in the 3-year OS and DFS between those with and without *WT1* mutation in 130 evaluable AML patients, excluding those with FAB-M3 and DS (Fig. 1). The frequency of *WT1* mutation was not different between patients with and without CR after induction therapy (6.7 vs. 20.0 %, respectively, $P = 0.13$). Among patients with a normal karyotype, *WT1* mutation tended to be related to a poorer 3-year OS and DFS than those without *WT1* mutation, although the differences were not significant ($P = 0.38$ and $P = 0.45$, respectively) (Fig. 2).

WT1 mutations were not randomly distributed over the different cytogenetic subgroups. The frequency of *WT1* mutation in CN-AML was higher than in other cytogenetic subgroups ($P = 0.04$). This trend was similar to previous pediatric reports [19, 21, 22]. Moreover, the frequency of *WT1* mutation in patients <3 years was lower than in patients aged 3 years or older; however, this difference was not significant (0 vs. 8.6 %, respectively, $P = 0.06$). In other pediatric reports, the frequency of *WT1* mutation was significantly lower in patients <3 years old than in patients aged 3 years or older [19, 21, 22]. Furthermore, *FLT3*-ITD in AML was too strong a prognostic factor to assess whether or not *WT1* mutation has a prognostic impact [25]. Thus, we analyzed the clinical impact of *WT1* mutation in patients with CN-AML excluding those with *FLT3*-ITD and <3 years. In patients with a normal karyotype, aged 3 years or older, and showing no evidence of *FLT3*-ITD, *WT1* mutation was related to a poorer prognosis based on the 3-year OS and DFS ($P = 0.17$ and $P < 0.01$, respectively) (Fig. 3). *WT1* mutation was not a significant risk factor on 3-year OS and DFS by multivariate analyses (Tables 4, 5).

Discussion

Although several papers reported the differences in clinical outcome between patients with and without *WT1* mutation, we could not identify any differences between them in this study. However, other studies demonstrated that these