また施策面では教育が重要であり，これに関してMertensら[11]は長期フォローの有識者17名に対してアンケート調査を行い，「概して一次診療医が小児がん経験者の晩期合併症の問題を十分理解していない」点と「小児がん経験者が将来自分の健康上に起こる合併症に無頓着で定期受診の必要性を認識していない」以上の2点が長期フォロー推進の障害になることを指摘している．この対策として一次診療医には教科書をはじめとした出版物や講演会活動で啓発を行い，患者教育においてはまず両親に長期フォローの必要性を認識してもらい，患者にはその年齢と理解できる基準に合わせて教育を行ってゆくことを推奨している．

わが国においては，小児慢性特定疾患の制度改定も経済的な側面から今後長期フォローに多大な影響を与える要素になると考える．アンケート結果からは「改定後の状態でも致し方ない」という意見は少数にとどまったことは，現場においても長期フォローの将来に対する危機感がにじみ出ている．今後はリスクベースの長期フォローの実現に向け，治療内容に応じた晩期合併症のフォローに関しては，投薬が必要なくても公費負担を申請できるよう国に働きかけてゆくことも必要であると考える．

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The Status Quo of Long-term Follow Up for Survivors of Childhood Cancer—A Nationwide Survey in Japan

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The importance of a long-term follow-up (LTFU) for childhood cancer survivors is increasing, as the number of long-term survivors continues to grow and many researchers describe the late effects that sometimes occur among such survivors. The purpose of this study is to assess the status quo of LTFU for childhood cancer survivors in Japan. Among the 211 registered institutions of the Japan Pediatric Leukemia/Lymphoma Study Group (JPLSG), 145 (68.7%) replied our questionnaire. The following factors related to LTFU were assessed : the outline of institution, the level of cooperation with the co-medical staff, the degree of experience in treating late effects and the management strategies, the methods of trying to contact survivors who were lost to follow-up, the management of survivors after they come of age, and ideal LTFU system. The results showed that most institutions had difficulty in managing such survivors due to their limited resources and the lack of any established network. Four suggestions were indicated to provide high quality LTFU ; i) establishing LTFU guidelines based on the optimal risk-based late effects care, ii) preparing a comprehensive phamphlet to be handed out to each survivor, iii) establishing a LTFU national center and regional network, iv) Try to get increased public funding for such programs.

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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

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Background. *Mixed-lineage leukemia* (*MLL*)-partial tandem duplication (PTD) is associated with poor prognosis in adult acute myeloid leukemia (AML), but its relationship to pediatric AML is unknown. ***Procedure.*** One hundred fifty-eight newly diagnosed AML patients, including 13 FAB-M3 and 10 Down syndrome (DS) patients, who were treated on the Japanese Childhood AML Cooperative Treatment Protocol AML 99 were analyzed for *MLL*-PTD, as well as internal tandem duplication (ITD) and the kinase domain mutation (D835Mt) in the *FLT3* gene. ***Results.*** We found *MLL*-PTD in 21 (13.3%) of 158 AML patients, but not in FAB-M3 or DS patients. The differences between patients with and without *MLL*-PTD were significant for 3-year overall survival (OS) (56.3% vs. 83.2%, $P=0.018$), disease-free survival (DFS) (41.7% vs. 69.6%, $P=0.010$), and relapse rate (RR) (54.3% vs. 27.6%, $P=0.0085$) of 135 AML patients excluding the FAB-M3 and DS patients. Furthermore, ITD and D835Mt in the *FLT3* gene were found in 17 (12.6%) and 8 (5.9%) of these 135 patients, respectively. The differences between patients with *FLT3*-ITD and the wild-type allele were significant for 3-year OS (35.3% and 84.3%, $P<0.0000001$), DFS (40.0% and 66.9%, $P<0.003$), and RR (52.4% and 30.3%, $P<0.005$). Coduplication of both genes was found in only 3 (1.9%) patients. ***Conclusion.*** AML patients with *FLT3*-ITD, but not D835Mt, showed a poor prognosis. AML patients with *MLL*-PTD were also correlated with poor prognosis in this study. Pediatr Blood Cancer 2008;50:264–269. © 2007 Wiley-Liss, Inc.

Key words: AML; childhood; cytogenetics; *FLT3*; *MLL*; *tandem duplication*

INTRODUCTION

Risk classification of acute myeloid leukemia (AML) patients based on cytogenetic abnormalities has been widely accepted in adult and pediatric AML studies [1–4]. AML patients with t(8;21), inv(16), and t(15;17) have been classified into a low risk (LR) group, those with monosomy 5 and monosomy 7 into a high risk (HR) group, and others into an intermediate risk (IR) group [2–4]. Patients with normal karyotype were classified into the IR group and showed various prognoses. Classification by gene alterations other than karyotypic abnormalities would be preferable for improving the treatment outcome of pediatric AML patients.

Chromosome 11q23 abnormalities involving the *mixed-lineage leukemia* (*MLL*) gene are found in about 5% of adult AML patients and in ~50% of infants with AML [5–7]. *MLL*-partial tandem duplication (PTD) is reported in ~10% of adult AML patients, but in 20–50% of adult AML patients with a normal karyotype and trisomy 11 [8–13]. *MLL*-PTD is associated with a poor prognosis in adult AML patients and a high relapse rate (RR) [10–13]. On the other hand, the prevalence and prognosis of *MLL*-PTD in pediatric AML patients remains obscure, although a relatively high prevalence of *MLL*-PTD has been reported in a few articles [14,15].

Fms-related tyrosine kinase 3 (*FLT3*) is one of the class III receptor tyrosine kinases that is normally expressed in hematopoietic stem cells and early progenitor cells [16,17]. Internal tandem duplication (ITD) of the juxtamembrane domain (JM) of the *FLT3* gene occurs in approximately 30% of adult AML patients [18–20] and in ~20% of pediatric AML patients [21–23]. *FLT3*-ITD is strongly associated with poor prognosis, especially in patients with a normal karyotype [18–23]. Furthermore, ~10% of adult AML patients have an activating loop mutation in the kinase domain specifically, a point mutation in aspartic acid residue at codon 835 (D835Mt). These patients show a poor prognosis [19,20,24]. The prevalence and prognostic significance of *FLT3*-D835Mt in pediatric AML patients are controversial [21,23].

We have previously reported the existence of the coduplication of *MLL* and *FLT3* in pediatric AML patients who had poor prognoses [25]. These results were confirmed in adult patients with a normal karyotype and trisomy 11 [12,13,26,27]. We here performed mutation analysis of both *MLL* and *FLT3* genes in 158 unselected pediatric AML patients treated on the Japanese pediatric AML collaborative treatment protocol AML99. These data suggest that *FLT3*-ITD and *MLL*-PTD are both important markers of poor prognosis in pediatric AML patients.

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PATIENTS AND METHODS

Patients

The diagnosis of AML was made according to the French–American–British (FAB) classification. Cytogenetic analysis was performed using the G-banding method. Among 318 newly diagnosed de novo AML patients enrolled from January 2000 to December 2002, 158 samples were available for molecular analysis (Table I). Among the 158 patients, there were 13 patients with FAB-M3 and 10 patients with Down syndrome (DS) who were treated with different treatment protocols [28–30]. There were no significant differences between the 135 analyzed patients without FAB-M3 and DS and the 105 non-analyzed patients in terms of age (median 6 years (range: 0–15 years) vs. 6 years (range: 0–15 years)) and initial WBC count (median 24.8×10^9/L (range: 1.65–621.0×10^9/L) vs. 13.8×10^9/L (range: 1.0–489.0×10^9/L, $P = 0.0764$)). Patients who were younger than 2 years old or had an initial WBC count <100,000/μl were treated with the Induction A regimen (etoposide (VP16), cytarabine (CA) and mitoxantrone (MIT), (ECM)). Patients who were older than 2 years old and had an initial WBC count >100,000/μl were treated with the Induction B regimen (VP16, CA and idarubicin (IDA), (ECI)). If patients achieved complete remission (CR), the patients were classified into three risk groups (62 in low, 57 in intermediate and 10 in high) according to the results of cytogenetic analyses or the achievement of CR after initial 2 courses of chemotherapy [28–30] (Supplemental Fig. 1 which has been reported in Blood [30], http://bloodjournal.hematologylibrary.org/cgi/data/2005-08-3408/DC1/2). AML patients with t(8;21) (except for those with WBC counts >50,000/μl) or inv(16)(p11q22) were classified into the LR group. Patients with monosomy 7, 5q-, t(16;21), or Ph1 were classified into the HR group. Patients were treated with additional chemotherapy or allogeneic stem cell transplantation (allo-SCT) in each risk group (Supplemental Fig. 1).

Informed consent was obtained from the patients or patients' 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 *MLL*-PTD

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 (Amersham Bioscience, Tokyo, Japan). *MLL*-PTD was examined by simple first round reverse transcriptase-polymerase chain reaction (RT-PCR) with 35 cycles using the primer pair 6.1 (located on exon 9) and E3AS (located on exon 4), according to the conditions previously reported [10,25,31]. We did not use the nested RT-PCR method because a previous report suggested that the *MLL*-PTD transcripts were highly detected in the healthy controls [31]. We used the CTS cell line as a positive control for *MLL*-PTD and water as a negative control for RT-PCR analysis

TABLE I. Clinical Characteristics of Patients With *MLL* or *FLT3* Gene Alterations

	All patients	*MLL*-PTD	*FLT3*-ITD	*FLT3*-D835Mt
Age, median (year)	6 (0–15)	10 (2–15)	9 (2–15)	11 (2–14)
WBC count, median ($\times 10^9$/L)	20.7 (1.0–620.0)	31.4 (3.6–343.4)	33.2 (3.0–620.0)	45.0 (3.3–440.0)
Male/female	89/69	12/9	8/12	7/4
FAB classification				
M0	6	1	1	0
M1	24	7(2[a])	4(2[a])	2
M2	46	5	4	2
M3	13	0	3	3
M4	22	4(1[a])	1(1[a])	1
M5	25	3	5	3
M6	1	0	0	0
M7	19	1	1	0
Unclassified	2	0	1	0
Karyotypic abnormalities				
Normal	33	8(2[a])	9(2[a])	2
t(8;21)	46	4	2	1
11q23 abnormalities	20	5	0	1
t(15;17)[b]	13	0	3	3
inv(16)	7	0	0	2
DS[b]	10	0	0	0
Others[c]	27	4(1[a])	5(1[a])	2
Unknown	2	0	1	0
Total	158	21	20	11
Risk group				
Low	62	4	2	3
Intermediate	57	13(2[a])	8(2[a])	4
High	10	3	2	0
Non-CR	6	1(1[a])	5(1[a])	1
Total	135	21	17	8

[a]Cases who showed *MLL*-PTD and *FLT3*-ITD simultaneously; [b]DS—Down syndrome, patients with FAB-M3 or DS were treated with the different protocol; [c]others contain −7, +8 or complex karyotypes.

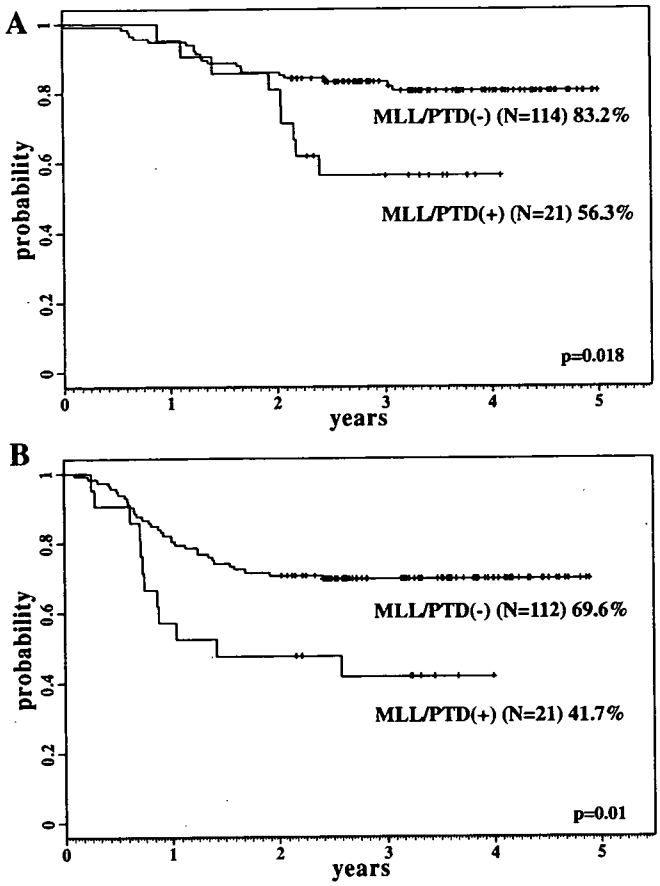

Fig. 1. Probabilities of 3-year OS (A) and 3-year DFS (B) in 135 AML patients excluding those with FAB-M3 and Down syndrome. Kaplan–Meier method estimates for patients with and without *MLL*-PTD are shown. The difference in patient numbers between OS and DFS resulted from the death of two patients during induction therapy.

[32,33]. Furthermore, we analyzed *MLL*-PTD in 10 normal bone marrow samples. Five microliter of the PCR products were electrophoresed in a 3% agarose gel. The amplified products were purified and directly sequenced.

Detection of *FLT3*-ITD and D835Mt

Using 1 μl of the cDNA, PCR amplification was performed for the JM and tyrosine kinase domain of the *FLT3* gene. The PCR procedure has been reported previously using primer pairs R5, R6, and 17F, TKR [30,34,35]. If a longer size product was found, the product was cut from the gel, purified with a QIAquick gel extraction kit (Qiagen, Chatsworth, CA), and directly sequenced on a DNA sequencer (ABI PRISM 310 Genetic Analyzer; Applied Biosystems, Foster City, CA) using a BigDye terminator cycle sequencing kit (Applied Biosystems). D835Mt was confirmed using *Eco*RV digestion and followed by direct sequencing as previously reported [24,30,34,35].

Statistical Analysis

Estimation of the survival distributions was performed using the Kaplan–Meier method and the differences were compared using the log-rank test. Disease-free survival (DFS) was defined as the time from diagnosis until the date of relapse. Overall survival (OS) was defined as the time from diagnosis until death owing to any cause or the last follow-up. Statistical difference analysis was performed using the χ^2 test. The prognostic significance of the clinical variables was assessed by using Cox proportional hazards model. These statistical analyses were performed with statistical software R. For all analyses, the *P*-values were two-tailed, and a *P*-value of less than 0.05 was considered statistically significant.

RESULTS

MLL-PTD

MLL-PTD was found in 21 (13.3%) of 158 pediatric AML patients (Table I). One type of fusion transcript (exon 9 and exon 3) was found in 10 patients, and the other type (exon 10 and exon 3) was found in 11 patients. Only one patient showed both fusion transcripts corresponding to alternatively spliced exons 10 and 11 to exon 3 (Supplemental Fig. 2). Furthermore, 10 normal bone marrow samples did not show *MLL*-PTD transcripts. *MLL*-PTD was frequently found in FAB-M1, M4 and patients with normal karyotype or 11q23 abnormalities (Table I). *MLL*-PTD was not found in FAB-M3 and DS patients. Patients with trisomy 11 were not found in this study. Remarkably, more than half of the patients with *MLL*-PTD were classified into the IR group (13 of 21 (61.9%)). The median age of patients with *MLL*-PTD was 10 years old (2–15) and no patients with *MLL*-PTD under 2 years old were found. Excluding the FAB-M3 and DS patients, the statistical differences in the clinical outcome between patients with and without *MLL*-PTD were significant for 3-year OS (56.3% vs. 83.2%, $P = 0.018$), DFS (41.7% vs. 69.6%, $P = 0.01$), and RR (54.3% vs. 27.6%, $P = 0.0085$) (Fig. 1). Allo-SCT was performed in 18 (85.7%) of 21 *MLL*-PTD patients, and 9 (50.0%) of them have been alive for a median of 42.0 months. The three patients without allo-SCT are all alive. Notably, six of the eight patients who received allo-SCT in the 1st CR and three of four patients who received allo-SCT in the 2nd CR are still alive.

FLT3-ITD and D835Mt

FLT3-ITD was found in 20 (12.7%) of 158 patients (Table I). All patients except for one showed both *FLT3*-ITD and *FLT3*-WT transcripts by RT-PCR. Half of the *FLT3*-ITD consisted of an in-frame tandem repeat of exon 11 (12–147 bp). The other half of *FLT3*-ITD showed insertions of 1–15 bp between the duplicated regions. *FLT3*-D835Mt was found in 11 (7.0%) of 158 patients. D835Mt consisted of D835Y (seven patients), D835V (two patients) and D835H (two patients). Differences in the median age of patients with *FLT3*-ITD, D835Mt, and the wild-type gene (WT) were not statistically significant (9, 11, and 5 years old, respectively). All patients with *FLT3*-ITD or D835Mt were older than 2 years old. The difference in the median initial WBC count between patients with *FLT3*-ITD and WT was significant ($P = 0.014$). Excluding FAB-M3 and DS patients, the differences between AML patients with *FLT3*-ITD, D835Mt, and WT were significant for the 3-year OS (35.3%, 100% and 84.3%, $P < 0.0000001$), DFS (40.0%, 87.5%, and 66.9%, $P < 0.003$), and RR (52.4%, 11.8% and 30.3%, $P < 0.005$) (Fig. 2). *FLT3*-ITD was found in five (83.3%) of six patients who did not

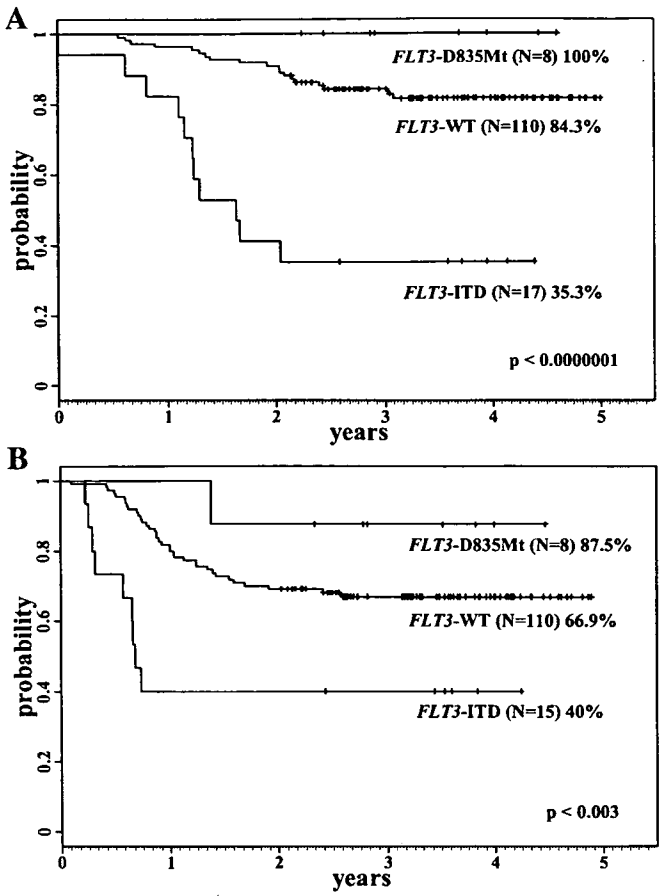

Fig. 2. Probabilities of 3-year OS (**A**) and 3-year DFS (**B**) in 135 AML patients, excluding those with FAB-M3 and Down syndrome. The Kaplan–Meier method for patients with *FLT3*-ITD, D835Mt, and WT is shown. The difference in patient numbers between OS and DFS resulted from the death of two patients during induction therapy.

attain CR. Allo-SCT was performed in 12 (70.6%) of 17 *FLT3*-ITD patients; of whom 4 (33.3%) were still alive for a median 43.5 months. The remaining eight patients died. Furthermore, four of seven patients who received allo-SCT in the 1st CR are still alive. Three of five patients without allo-SCT are also alive.

FLT3-ITD and D835Mt were found in 3 (23.1%) of 13 patients with FAB-M3. Both alterations of the *FLT3* gene did not influence the clinical outcome of FAB-M3 patients. Furthermore, these *FLT3* alterations were not found in DS patients.

Coduplication of the *MLL* and *FLT3* Genes

Coduplication of the *MLL* and *FLT3* genes were found in only 3 (1.9%) of 158 patients (Table I). Two patients had normal karyotype and one patient had +8. All three patients received allo-SCT, and two of them died because of disease progression.

Multivariate Analysis of Clinical Outcome

Multivariate analysis of *FLT3*-ITD, *MLL*-PTD, M1 marrow after induction therapy and initial high WBC count (more than 100×10^9/L) was carried out for 3-year OS and DFS data from 135 AML patients. Only *FLT3*-ITD was significant for 3-year OS (hazard ratio 8.4, 95% confidence interval (95% CI) 3.2–21.8, $P < 0.0001$). For 3-year DFS, *FLT3*-ITD, and M1 marrow after induction therapy were significant (hazard ratio 3.6 and 3.1, 95% CI 1.7–7.6 and 1.4–7.0, $P < 0.001$ and 0.007). Moreover, multivariate analysis was performed for 108 patients excluding those who received allo-SCT in 1st CR. Only *FLT3*-ITD was significant for 3-year OS (hazard ratio 16.0, 95% CI 4.7–54.7, $P < 0.00001$) (Table II). On the other hand, *MLL*-PTD was significant for 3-year DFS (hazard ratio 3.2, 95% CI 1.3–7.7, $P < 0.01$) (Table III).

DISCUSSION

In this study, *MLL*-PTD was found in 21 (15.6%) of 135 pediatric AML patients excluding those with FAB-M3 and DS. We used the simple first round RT-PCR method and not the nested RT-PCR method to minimize the possibility of detecting false positive *MLL*-PTD transcripts. *MLL*-PTD in pediatric AML has been reported at a relatively high frequency in a small number of patients: 2 (20%) of 10 patients [14] and 5 (9.4%) of 53 patients [15]. These data are compatible with our results. However, Shih et al. [36] have recently reported that *MLL*-PTD was rarely found in pediatric AML patients (one of 123, 0.8%). The difference of these frequencies in pediatric AML remains unknown but it may be partially due to the patient's age; although the median age of 16 patients with *MLL* rearrangements, including one *MLL*-PTD, is 1.3 years (1 day to 5.5 years) in the paper by Shih et al. [36], that of 21 patients with *MLL*-PTD is 10 years (2–15 years), and 17 of 21 patients with *MLL*-PTD is more than 6 years old in our study.

Patients with *MLL*-PTD showed a poor prognosis, a short duration of remission, and a high RR, as previously reported for adult AML patients [10–14,26]. Multivariate analysis suggested that *MLL*-PTD was a marker of poor prognosis for 3-year DFS, but not for 3-year OS, in AML patients excluding those who received allo-SCT in 1st CR in this study. This result may be explained by the effectiveness of allo-SCT in 2nd CR for patients with *MLL*-PTD. Indeed, four patients received allo-SCT in 2nd CR, and three of these patients are still alive.

Regarding karyotypic abnormalities, our results also confirmed that *MLL*-PTD was frequently found in AML patients with a normal karyotype as reported for adult patients [10–14]. Interestingly, *MLL*-PTD was found in AML patients with 11q23 translocations in this study. Moreover, *MLL*-PTD was also found in AML patients with

TABLE II. Prognostic Factors for 3-year Overall Survival in 108 AML Patients Treated on AML99 Protocol, Excluding Those Who Received Allo-SCT in 1st CR

Variable	*P*-values	Hazard ratio	95% CI
FLT3-ITD	<0.00001	16.0	4.7–54.7
MLL-PTD	0.25	2.1	0.6–7.4
M1 marrow after induction therapy	0.092	5.3	0.8–37.3
WBC > 100×10^9/L	0.14	0.19	0.02–1.7

TABLE III. Prognostic Factors for 3 Year Disease-Free Survival in 108 AML Patients Treated on AML99 Protocol, Excluding Those Who Received Allo-SCT in 1st CR

Variable	*P*-values	Hazard ratio	95% CI
FLT3-ITD	<0.0001	7.7	2.9–20.6
MLL-PTD	0.0099	3.2	1.3–7.7
M1 marrow after induction therapy	0.028	9.3	2.1–40.1
WBC > 100×10^9/L	0.013	3.1	1.3–7.5

t(8;21), which has not previously been reported in adult AML [10–14,26]. Unfortunately, we could not analyze DNA because genomic samples were not available. Two of the 4 t(8;21)-AML patients with *MLL*-PTD were also found to have *KIT* mutations in our previous study [30], suggesting that some patients possibly had genetic instability. We must await further studies to clarify these issues.

As for *FLT3* gene, multivariate analysis also strongly suggested that *FLT3*-ITD was an independent marker of poor prognosis in pediatric AML as previously reported [18,20,22]. D835Mt did not represent a poor prognosis in this study, confirming a previous report of pediatric AML [21], although D835Mt has been reported to be associated with poor prognosis in adult AML [18–20,24]. The difference between adult and pediatric AML remains unknown.

The coduplication of both genes was found in 3 (1.9%) of 158 patients in this study, which is compatible with previous reports (4 (1.6%) of 250 and 16 (1.7%) of 956 adult AML patients) [12,26]. The mechanism of formation of *MLL*-PTD and *FLT3*-ITD remains unknown. *MLL* and *FLT3* loci demonstrate similar susceptibilities to agents that modify chromatin configuration, including topoisomerase II inhibitors [27]. We conclude that the coduplication of *MLL* and *FLT3* genes is rare in pediatric AML as well as adult AML.

There was no definitive result as to the effectiveness of allo-SCT for the pediatric patients with *MLL*-PTD or *FLT3*-ITD. In this study, the majority of patients received allo-SCT due to the protocol agreement or relapse (18 (85.7%) of 21 *MLL*-PTD and 12 (70.6%) of 17 *FLT3*-ITD). Eight *MLL*-PTD patients and seven *FLT3*-ITD patients received allo-SCT in the 1st CR. Although similar results for 3-year DFS were found in patients with *MLL*-PTD (41.7%) and *FLT3*-ITD (40.0%), there was a difference in the 3-year OS between *MLL*-PTD (56.3%) and *FLT3*-ITD (35.3%) ($P = 0.024$). This difference was possibly due to the effectiveness of allo-SCT for the patients with *MLL*-PTD rather than those with *FLT3*-ITD as a lack of effectiveness of allo-SCT has been recently reported for patients with *FLT3*-ITD [37].

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ORIGINAL REPORT

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

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ABSTRACT

Purpose
To evaluate a less intensive chemotherapeutic regimen specifically designed for patients with Down syndrome (DS) and acute myeloid leukemia (AML), and to determine the prognostic factors for event-free survival.

Patients and Methods
Seventy-two patients with AML-DS were treated with remission induction chemotherapy consisting of pirarubicin (25 mg/m^2/d for 2 days), cytarabine (100 mg/m^2/d for 7 days), and etoposide (150 mg/m^2/d for 3 days). Patients received four courses of intensification therapy of the same regimen. Prophylaxis for CNS leukemia was not included.

Results
All but two patients were younger than 4 years, and 67 of the 72 patients (93%) were diagnosed as acute megakaryoblastic leukemia (AMKL). Seventy of the 72 patients (97.2%) achieved a complete remission (CR), and the estimated 4-year event-free survival (EFS) rate was 83% ± 9%. Nine patients relapsed, and one died as a result of pneumonia during CR. Multivariate analysis revealed that the presence of monosomy 7 was a greater risk factor of adverse outcome (odds ratio = 5.67; P = .027).

Conclusion
A less intensive chemotherapeutic regimen produces excellent outcomes in standard-risk AML-DS patient. Risk-oriented therapy should be considered for future trials in AML-DS.

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INTRODUCTION

Patients with Down syndrome (DS) have a 10- to 20-fold increased risk of developing acute leukemia.[1,2] In particular, the relative risk of developing acute megakaryoblastic leukemia (AMKL) is estimated to be 500 times higher in children with DS than in those without DS.[3]

Before the 1990s, most patients with acute myeloid leukemia (AML)-DS were treated outside clinical trials and received suboptimal therapy, resulting in dismal outcomes.[4,5] The first report that there was a significantly better outcome for children with AML-DS came from the Pediatric Oncology Group (POG) in 1992.[6] After recognition of the favorable outcome when patients were treated with protocols of the cooperative study groups for childhood AML, there has been an increase in recruitment to collaborative protocol studies.[7-10] However, it has become apparent that induction failure and relapse are rare, but treatment-related deaths are frequent in most series. Since then, several collaborative groups have adapted their AML protocols for AML-DS by reducing the dose of chemotherapeutic agents.[11,12] In recent reports, the 5-year event-free survival (EFS) has exceeded 80%, largely because of the reduction in treatment-related deaths, with a fall from 30% to 40% in the early 1990s to 3% to 5% in recent studies.[13-15]

A treatment regimen specifically designed for AML-DS has been used in Japan since the mid-1980s.[16,17] The regimen is less intensive and does not include high-dose cytarabine and prophylaxis against CNS leukemia. On the basis of encouraging results, since 1999, we have conducted a prospective multi-institutional study for AML-DS using a

slightly modified protocol. In the present study, we present the outcome of this study in 72 patients with AML-DS and analyze the prognostic risk factors for survival.

PATIENTS AND METHODS

Patients with AML-DS aged less than 18 years were eligible for treatment according to the DS protocol proposed by the Japanese Childhood AML Cooperative Study Group. Neonates with transient myeloproliferative disorder (TMD), defined as appearance of myeloid blasts within the first months of life, and those with spontaneous remission were not included. The protocol was approved by the institutional review board, and written informed consent was obtained from the parents of all patients.

The diagnosis of AML was classified according to the French-American-British (FAB) cooperative group criteria.[18] The diagnosis of AMKL, corresponding to FAB M7, was made irrespective of the initial blast count, which was often less than 30%. For the assessment of megakaryocytic lineage, at least 10% of the blast cells needed to be positive for one or more of the platelet-specific antigens such as CD36, CD41, CD42, and CD61. Immunophenotyping was performed at the reference laboratories. Morphology of bone marrow or peripheral blood smears and results of karyotyping were centrally reviewed.

Remission induction chemotherapy consisted of pirarubicin (25 mg/m^2/d, on days 1 and 2), which was estimated to be equivalent as 25 mg/m^2/d of daunomycin (DNR), cytarabine (100 mg/m^2/d on day 1 through 7), and etoposide (150 mg/m^2/d on day 3 through 5). Each drug was administered over a 1-hour infusion. Patients who achieved complete remission (CR) received four courses of intensification therapy of the same regimen. Prophylactic therapy for CNS leukemia was not included in the protocol.

Estimation of survival was performed using the Kaplan-Meier method, and the differences were compared using the log-rank test.[19] EFS was defined as time from diagnosis to any event (induction failure, relapse, or death) and overall survival (OS) was defined as the time from diagnosis to death from any cause. Statistical differences were analyzed using the χ^2 test. We used the Cox regression model in a multivariate analysis of predictive factors for EFS. *P* values less than.05 were considered significant.

Table 1. Clinical and Laboratory Characteristics of AML Patients With Down Syndrome (N = 72)

Characteristic	No.	%
Age, months		
Median, months	12	
0-12	8	11
12-24	34	47
24-36	23	32
36-48	5	7
≥ 48	2	3
Sex		
Male	44	61
Female	28	39
WBC, ×10^9/L		
Median	5.8	
Range	1.9-107.0	
Hb, g/dL		
Median	8.4	
Range	3.5-15.7	
Pl, ×10^9/L		
Median	31	
Range	1.0-240.0	
FAB classification		
M1	1	1.4
M2	1	1.4
M5	1	1.4
M6	1	1.4
M7	67	93.0
Unclassified	1	1.4
Cytogenetics		
Trisomy 8	10	14
Monosomy 7	6	8
Additional 21	5	7
t(9;11)	1	1.4

Abbreviations: AML, acute myeloid leukemia; FAB, French-American-British.

RESULTS

Between January 2000 and June 2004, 72 patients with AML-DS were enrolled onto the study. The interim analysis was performed in December 2005. The median follow-up period was 44 months, ranging from 19 months to 70 months. Characteristics of the 72 patients are summarized in Table 1. Their median age was 22 months (range, 7 to 88 months). All but two patients were younger than 4 years. Forty-four patients were male and 28 were female. Major congenital heart anomalies were present in eight patients. Nine patients had a history of TMD as neonates, and 8 had a history of myelodysplastic syndrome. The WBC counts ranged from 1.9 to 107 × 10^9/L (median, 5.8 × 10^9/L), the hemoglobin levels from 3.5 to 15.7 g/dL (median: 8.4 g/dL), and the platelet counts from 1 to 240 × 10^9/L (median, 31 × 10^9/L). At the time of initial presentation, 38 patients had less than 30% of leukemic blasts in the bone marrow. However, the percentage of leukemic blasts increased within a short period, and they were finally diagnosed as FAB M7. The distribution of FAB subtypes showed a predominance of M7 (93%). One patient each was classified as FAB M1, M2, M5a, or M6. One patient with low blast counts could not be classified. Cytogenetic results were available for 71 patients. In addition to the constitutional aberrations, an additional chromosome was found, involving chromosomes 8 (n = 10) and 21 (n = 5). Six patients had monosomy 7.

A total of 70 of the 72 patients (97.2%) achieved a CR: 65 patients after one cycle of the induction course; four patients after receiving a further cycle of consolidation after the induction course; and one of the three patients with M3 marrow after induction course who then achieved a CR after intensified reinduction therapy containing high-dose cytarabine. Two patients received unrelated cord blood transplantation (UCBT) during the first CR; both survived. These two patients had a chromosomal abnormality of t(9;11) or monosomy 7 and both were excluded at the time of transplantation.

Nine patients relapsed in the bone marrow and one of these nine had a CNS relapse simultaneously. The patient who had CNS relapse had megakaryoblastic leukemia. No patient suffered from isolated CNS relapse. Eight of the nine patients relapsed during chemotherapy. All patients with relapse during chemotherapy and resistant disease died without achieving a second CR. One patient who relapsed after cessation of the chemotherapy received bone marrow transplantation (BMT) from an human leukocyte antigen–matched sibling donor and successfully achieved a second CR. However, he died of pneumonia 2 years after BMT. Chemotherapy-related mortality was low; only one patient died as a result of pneumonia during the second course of intensification. Fifty-eight patients remain in the first CR, with a median duration of 43 months (range, 18 to 69 months). The 4-year EFS was 83.3% ± 9.1% and the 4-year OS was 83.7% ± 9.5% (Fig 1).

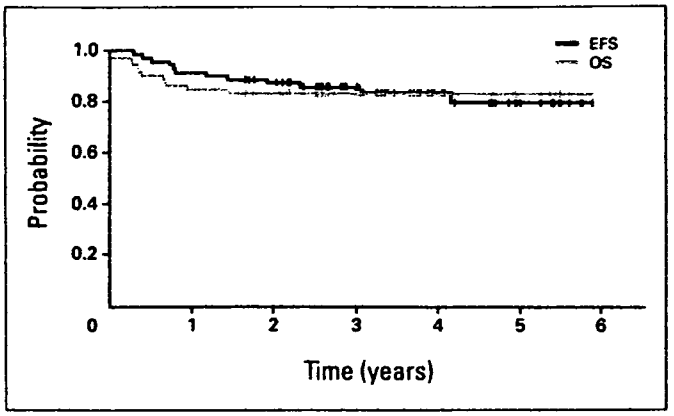

Fig 1. Actuarial survival rate for the AML99 Down syndrome protocol. Among the 72 patients, 70 achieved complete remission (CR). Nine patients relapsed. One patient died as a result of pneumonia during the first CR. Two patients received cord blood transplantation in first CR. Fifty-eight patients remained in first CR. The 3-year overall survival (OS) was 84% and the 3-year event-free survival (EFS) 83%, respectively.

We evaluated the predictive factors for EFS in 70 patients, excluding the two patients who received UCBT during the first CR. There was no difference in outcome with respect to age group. The 40 patients who were aged 2 years or younger had a 4-year EFS of 85% ± 11.8%, compared with the 30 patients older than 2 years, who had a 4-year EFS of 80% ± 15.7% ($P = .478$). We also compared the EFS of the 63 patients who were aged 3 or younger and the seven patients older than 3 years. The 4-year EFS was 84.1% ± 9.6% and 71.4% ± 28.6%, respectively ($P = .282$). Only two patients were older than 4 years: one with FAB M5a and t(9;11), who received UCBT and is still living, and the other with FAB M2, who also remains in the first CR.

Table 2 shows the outcomes for the six patients with monosomy 7. Five of these six patients were diagnosed as FAB M7 and the other as FAB M1. Five of the six patients achieved a CR, but two then relapsed and received hematopoietic stem-cell transplantation (HSCT); however, both of them died. One patient who received UCBT in the first CR is alive and well. Only two of the five patients who did not undergo HSCT are alive with no evidence of disease. Excluding the patient who received UCBT in the first CR, three of the other five patients experienced induction failure or relapse. The 3-year EFS in the five patients with monosomy 7 was significantly worse than in the 65 patients without monosomy 7 (40,0% ± 26.3% *v* 86.2% ± 8.8%; $P = .007$; Fig 2).

In the multivariate analysis, we evaluated the predictive factors for EFS (Table 3). The presence of monosomy 7 was a greater risk factor of adverse outcome (odds ratio = 5.67; $P = .027$). There was no difference in outcome between the patient groups of age older than 1.8 years and 0 to 1.8 years (odds ratio = 2.640; $P = .170$).

The regimen-related toxicities were relatively tolerable. Table 4 shows the duration of neutropenia and incidence of grade 3 or 4 toxicity during induction and each intensification phase of therapy. Only one patient died as a result of pneumonia in the second course of intensification. Subclinical cardiac dysfunction was revealed by echocardiography in four patients after the induction therapy. Reduction of chemotherapy dose was required in one of them. All but one patient received all scheduled courses of therapy without dose reduction. Although the patients were treated with the same regimen, the duration of neutropenia was longer during the induction phase than during the other courses of intensification.

DISCUSSION

During the last decade, several large collaborative studies have reported the experience of AML-DS children treated using standard AML protocols.[6-10] The most remarkable finding of these studies has been the lower incidence of induction failure and relapse compared with non-DS children with AML. Consistent with the clinical results, in vitro studies have demonstrated that DS leukemic cells are more sensitive to several chemotherapy drugs compared with non-DS leukemic cells.[20-22] Zwaan et al demonstrated a 12-fold increase in sensitivity to cytarabine in DS-AML cells compared with non-DS AML cells, as well as increased sensitivity to anthracyclines (two- to seven-fold) and etoposide (20-fold).[23] Mutation of the *GATA-1* gene is the hallmark of DS-AML.[24] Taub and Ge[25] provided the evidence for the potential linkage of the *GATA-1* mutation and the increased sensitivity to cytarabine resulting from effects on *cytidine deaminase* gene expression.

Conventional treatment of AML-DS has been associated with excessive treatment-related mortality (TRM). Thus, several collaborative study groups have adapted their standard AML protocol for AML-DS by reducing the dose of drugs or prolonging the interval between chemotherapy courses. In the Children's Oncology Group

Table 2. Outcome of AML-DS Patients With Monosomy 7

UPN	Age (months)	Sex	Karyotype	CR	Relapse	HSCT	Duration of Survival (months)	Cause of Death
A097	20	Female	47,XX,−7,−16, +21, +r1, +mar	Yes	Yes	UCBT (2nd CR)	27	Leukemia
A357	32	Male	47,XY,−4,−7,−13,−16, +21, add(22)(p13), +r1, +r2, +mar	No	NA	No	3	Leukemia
A408	21	Female	90,XXXX,−3,−7,−9, del(11)(q?),−18, +21, +21	Yes	No	No	> 30	
A425	22	Female	48,XX,−7, +21, +21, +r1	Yes	NA	UCBT (1st CR)	> 30	
A467	39	Male	48,XY,−7, +8, +21, +r1	Yes	No	No	> 29	
A538	15	Male	48,XY,−7, +21	Yes	Yes	Sib-BMT (non-CR)	49	Leukemia

Abbreviations: AML-DS, acute myeloid leukemia–Down syndrome; UPN, unique patient number; CR, complete remission; HSCT, hematopoietic stem-cell transplantation; UCBT, unrelated cord blood transplantation; Sib-BMT, bone marrow transplantation from human leukocyte antigen–matched sibling; NA, not assessable.

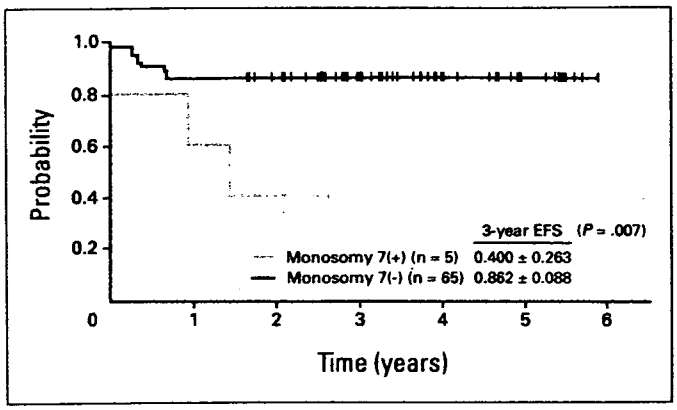

Fig 2. Actuarial survival rate by monosomy 7 status. The 3-year event-free survival (EFS) in the five patients with monosomy 7 was 40.0%, compared with 86.2% in the 65 patients without monosomy 7 (P = .007). Multivariate analysis revealed that the presence of monosomy 7 was a greater risk factor of adverse outcome (odds ratio: 5.67; P = .027).

(COG) trial A2971 (n = 130),[12] etoposide, dexamethasone, and the maintenance course were eliminated from the previous CCG2891 protocol. The outcome in COG A2971 was not different to that in CCG2891: the 3-year EFS was 79% versus 77%. In the AML-BFM98 study (n = 66),[11] AML-DS patients were treated with reduced doses of anthracyclines and cytarabine compared with the previous AML-BFM93 protocol (n = 44). The cumulative doses of anthracyclines and cytarabine were 220 to 240 mg/m^2 and 23 to 29 g/m^2 in the BFM98 study, and 440 mg/m^2 and 23.3 g/m^2 in the BFM93 study, respectively. Outcome improved significantly for patients treated in the BFM98 study, with a 3-year EFS of 91% ± 4% versus 70% ± 7% in the BFM93 study (P = .001). In the BFM98 study, three patients died in CR and three relapsed. The treatment related deaths equaled those caused by disease, which suggested that further reduction in dose-intensity may be justified. Accordingly, the major question in therapy of AML-DS is the intensity of chemotherapy that may be further reduced while maintaining the current high cure rate.

Among the several multi-institutional studies of AML-DS, the Japanese trial is unique in that it has been specifically designed for AML-DS from the beginning using the least dose-intensive regimen. Originally, each chemotherapy course consisted of daunorubicin (25 mg/m^2/d for 2 days), cytarabine (100 mg/m^2/d for 7 days), and etoposide (150 mg/m^2/d for 3 days). The protocol did not include prophylaxis against CNS leukemia. Between 1987 and 1997, 33 patients were treated with the same regimen in 12 hospitals.[17] The CR and 8-year EFS rates were 100% and 80% ± 7%, respectively, which is comparable to the outcome reported in other recent studies. During remission, two patients died of cardiac toxicity and one died of septicemia. Three children relapsed, one of whom was rescued by reinduction therapy containing high-dose cytarabine. Of the 28 patients with cytogenetic analysis, four had monosomy 7. It is of note that two of the three patients who relapsed had monosomy 7.

Table 3. Multivariate Analysis of Prognostic Factors Without Planned-SCT Patients (disease-free survival, n = 68)

Variable	Hazard Ratio	95% CI	P
Age > 1.8 years	2.640	0.6642 to 10.49	.170
Log_{10} (WBC)	1.020	0.1990 to 5.23	.980
FAB M7	0.264	0.0529 to 1.31	.100
Female sex	2.399	0.6845 to 8.40	.170
Monosomy 7	5.672	1.2209 to 26.35	.027

Abbreviations: SCT, stem-cell transplantation; FAB, French-American-British classification.

The main aims of the present study were to reduce regimen-related toxicity, in particular cardiotoxicity, and to identify prognostic factors for EFS with a uniform treatment. The present AML 99 DS protocol used pirarubicin instead of the original daunorubicin and fixed the number of treatment courses to five. Pirarubicin is much less cardiotoxic and more myelosuppressive than daunorubicin.[26-28] The cardiotoxicity of pirarubicin should be calculated as 0.8× compared with daunorubicin.[28]

Cytarabine and anthracyclines have been key drugs for the AML. However, the use of anthracyclines is limited by cardiomyopathy, which is irreversible with both acute and subacute manifestations.[26,27,29] One of the problems associated with the treatment of AML-DS is the high frequency of congenital heart anomalies. In a recent report from POG,[30] 57 patients with AML-DS were enrolled in the 9421 protocol, which includes 135 mg/m^2 of daunorubicin and 60 to 100 mg/m^2 of mitoxantrone. Twelve of the 57 patients (21%) had documented congestive heart failure (CHF) requiring chronic diuretics and/or inotropes with diminished fractional shortening on echocardiogram. In the study, four patients died of CHF. In the present study, cardiac dysfunction was observed in four patients (5.9%) during the induction therapy, but all recovered after the induction therapy. Thus, a cumulative pirarubicin dose of 250 mg/m^2 may be tolerable for patients with AML-DS, even those with congenital heart anomalies.

As for other types of leukemia, risk-oriented therapy is proposed if any prognostic factors are identified in AML-DS. In the CCG2891 study,[13] patients with AML-DS who were 2 years old or younger had a 6-year EFS of 86% compared with those older than 2 years, who had a 6-year EFS of 64% (P = .002). Multivariate analysis in that study showed that AML-DS patients older than 2 years had an increased risk of relapse (odds ratio = 4.9; P = .006). However, subsequent studies did not confirm these findings. In the BFM98 study, there was no difference in outcome between those 2 years or younger and those older than 2 years (EFS; 83% ± 4%, 81% ± 7%, respectively). The present study also did not identify age older than 2 years as a risk factor in the multivariate analysis. Even in the CCGA2971 study, which reduced the dose-intensity from the CCG2891 study, the 3-year EFS was 83% in those younger than 2 years and 79% in those older than 2 years, with no statistically significance difference.[12]

The distribution of age at diagnosis skews to a younger age in patients with AML-DS. Only 10% (seven of 72) and 3% (two of 72) of patients were older than 3 years and 4 years, respectively, in the present study. No detailed data have previously been available that separately analyzes the 2 to 3, 3 to 4, and older than 4 age groups. In the present study, two of five patients experienced induction failure or relapse in the 3 to 4 year age group. Only two patients were older than 4 years: one with FAB M5 who received UCBT and is alive, and the other with FAB M2 who remains in CR. In the CCG2891 study, the EFS was only 28% in nine patients older than 4 years.[13] In the BFM study, three of four patients older than 4 years were classified as FAB M1/M2, and only one patient with FAB M7 remained in the first CR.[11] A better age cut may be beyond 3 or 4 years to discriminate the prognosis in AML-DS patients.

Table 4. Incidence of GradeIII/IV Treatment-Related Toxicity During Induction and Intensification Phases of Therapy

Measure	Induction Phase	Intensification Phase			
		1	2	3	4
No. of assessed patients	68	65	64	62	59
Sepsis, %	7.3	7.7	1.6	6.5	3.4
Infection of any site, %	10.3	9.2	6.3	1.6	1.7
Pulmonary, %	3.0	1.5	3.0	0	1.7
Cardiac, %	5.9	0	0	0	0
Liver, %	5.9	3.1	6.3	3.2	0
GI, %	1.5	0	0	0	0
Patients who required reduction of drug dose, %	0	1.5	1.6	1.6	1.7
Duration of ANC $< 0.5\times10^9$, days	22.4	16.0	13.9	13.8	14.4
Duration of ANC $< 0.2\times10^9$, days	13.1	8.0	6.6	6.6	7.1

Abbreviation: ANC, absolute neutrophil count.

We would like to emphasize that the TRM in the current study was only 1.4% (one of 72 patients) which is much lower than those of previous reports.[7-9,16] On the other hand, relapse and induction failure were found in 11 of 72 patients (14%), which is more frequent than in other reports with intensive regimens.[6,8,13] On the basis of the results of the present study, we have designed a risk-oriented therapy protocol for our next trial with AML-DS. The patients with M1 marrow after induction therapy should be classified into a standard-risk group and receive the same dose of pirarubicin and cytarabine regimen. In POG 8498, daunorubicin was used only in induction, with a total dose of 135 mg/m^2, and high-dose cytarabine in consolidation. Although this study included only small cohort of 14 DS patients, all were alive as last updated in 2005.[31] We will include high-dose cytarabine for patients with M2 or M3 marrow after induction therapy, classified into a high-risk group, who might have adverse prognostic factors such as age older than 3 and the presence of monosomy 7.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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ORIGINAL ARTICLE

Outcome of risk-based therapy for infant acute lymphoblastic leukemia with or without an *MLL* gene rearrangement, with emphasis on late effects: a final report of two consecutive studies, MLL96 and MLL98, of the Japan Infant Leukemia Study Group

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We evaluated the efficacy of a treatment strategy in which infants with acute lymphoblastic leukemia (ALL) were stratified by their *MLL* gene status and then assigned to different risk-based therapies. A total of 102 patients were registered on two consecutive multicenter trials, designated MLL96 and MLL98, between 1995 and 2001. Those with a rearranged *MLL* gene (MLL-R, $n=80$) were assigned to receive intensive chemotherapy followed by hematopoietic stem cell transplantation (HSCT), while those with germline *MLL* (MLL-G, $n=22$) were treated with chemotherapy alone. The 5-year event-free survival (EFS) rate for all 102 infants was 50.9% (95% confidence interval, 41.0–60.8%). The most prominent late effect was growth impairment, observed in 58.9% of all evaluable patients in the MLL-R group. This plan of risk-based therapy appears to have improved the overall prognosis for infants with ALL, compared with previously reported results. However, over half the events in patients with *MLL* rearrangement occurred before the instigation of HSCT, and that HSCT-related toxic events comprised 36.3% (8/22) of post-transplantation events, suggesting that further stratification within the MLL-R group and the development of more effective early-phase intensification chemotherapy will be needed before the full potential of this strategy is realized.
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Keywords: infant acute lymphoblastic leukemia; *MLL* gene; hematopoietic stem cell transplantation; late effects

Introduction

The outcome of therapy for children with acute lymphoblastic leukemia (ALL) has markedly improved over the last four decades, to the extent that approximately 80% of affected patients are now cured.[1] However, infants with ALL, who represent 2.5–5% of all childhood ALL cases, continue to have high relapse rates and a dismal prognosis, as illustrated by recently published event-free survival (EFS) rates of 22–54%.[2–8] ALL in infancy has several distinctive clinical characteristics compared with common childhood ALL, including hyperleukocytosis, hepatosplenomegaly, central nervous system (CNS) disease and a high frequency of molecularly evident rearrangement of the *MLL* gene at chromosome band 11q23.[9–12] Among these features, *MLL* gene rearrangement, found in 70–80% of infant ALL cases studied with molecular techniques, is an independent risk factor most predictive of recurrent leukemia.[13–15]

The high failure rate in infants with ALL, especially those with *MLL* gene rearrangement, can be attributed to early relapse after remission rather than toxicity, and warrants consideration of intensified therapy. We therefore segregated infants with ALL into two subgroups according to their *MLL* gene status in two consecutive nationwide multicenter studies, designated MLL96 and MLL98. Infants with a rearranged *MLL* (MLL-R) gene received intensive chemotherapy followed by hematopoietic stem cell transplantation (HSCT), while those with a germline *MLL* gene were treated with standard intensive chemotherapy for ALL alone. This report updates findings published earlier[16–18] and extends the analysis to long-term side effects. By combining data from two studies with similar treatment strategies, we were able to analyze results for a relatively large cohort with this rare subtype of ALL. The additional detail on prognostic features should facilitate further risk stratification among *MLL*-rearranged cases, while outcome data on the increased number of patients undergoing HSCT should stimulate critical discussion of the role of this procedure in future treatment strategies for infants with ALL.

Materials and methods

Patients

Between December 1995 and December 2001, 102 consecutive infants with ALL, younger than 12 months, were registered and treated on two protocols, designated MLL96 (55 patients) and MLL98 (47 patients). These studies accrued more than 80% of all Japanese infants with ALL over the 6-year enrollment period, based on results of a nationwide surveillance study.[17] The

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diagnosis of ALL was based on bone marrow morphology and cytochemical staining results. Each patient was evaluated with respect to the characteristics of the leukemic cells, including immunophenotype, cytogenetics and *MLL* gene status. Written informed consent, provided according to the Declaration of Helsinki, was obtained from the guardians of the patients, with institutional review board approval obtained for all aspects of this investigation.

Detection of MLL *gene rearrangement and cytogenetic analysis*

The *MLL* gene status of each patient was determined by Southern blot analysis and/or split-signal fluorescence *in situ* hybridization (FISH) as previously described.[16] Leukemic cell karyotypes were determined by cytogenetic analysis with a G-banding technique.[16]

Treatment

The details of the therapeutic regimens used in the MLL96 and MLL98 studies are described in Supplementary Tables.[16–18] Briefly, patients with rearranged *MLL* (MLL-R) received induction therapy and three courses of postremission intensification therapy followed by HSCT if a suitable donor was available. Patients with germline *MLL* (MLL-G) were assigned to a chemotherapy arm consisting of induction, consolidation and CNS prophylaxis, intensification, reinduction and maintenance phases, administered over 83–85 weeks. Except for vincristine, drug dosages on the MLL98 protocol were calculated on the basis of body surface area, while dosages on the MLL96 protocol were based on body weight. This modification increased the dosages of all antileukemic drugs used in the MLL98 study by 1.2- to 2-fold over those in the MLL96 study.

Patients in the MLL-R group underwent HSCT in first remission (CR1), if a 5 to 6/6 human leukocyte antigen-matched related donor, 6/6-matched unrelated donor or 4 to 6/6-matched unrelated cord blood donor was available. The protocol-specified conditioning regimen comprised either a total-body irradiation (TBI; 12 Gy in six fractions, twice a day on days −7 to −5) or busulfan (BU; 35 mg/m^2/dose orally, 4 times a day on days −8 to −5) with a combination of etoposide (60 mg/kg intravenously on day −4) and cyclophosphamide (60 mg/kg intravenously on days −3 and −2). Prophylaxis for graft-vs-host disease (GVHD) consisted of either cyclosporine or tacrolimus combined with short-term methotrexate.

Evaluation of the late effects

Late effects studied included cardiac, pulmonary, renal, endocrine, dental, orthopedic, dermatologic, ophthalmologic, auditory, psychological, growth and development and occurrence of secondary malignancies. Medical records regarding these issues were reviewed by each physician of the participating centers, and these data were collected by questionnaire which was sent to each participating center.

Statistical considerations

The analysis of treatment outcome was updated on 30 September 2006, combining data from both the MLL96 and MLL98 studies because of their similar 5-year survival estimates (see Results). EFS and overall survival (OS) rates were estimated by the method of Kaplan–Meier and standard errors (s.e.) with the Greenwood formula, and then were compared with the log-rank test. Confidence intervals (CIs) were computed with a 95% confidence level. The clinical, demographic and biologic features of patients were compared with χ^2 tests for homogeneity. A Cox regression model was used for the multivariate analysis. *P*-values, when cited, are two-sided, with a value of 0.05 or less taken to indicate statistical significance.

Table 1 Characteristics of infants with ALL treated in the MLL96 and MLL98 studies

	Overall, n *(%)*	*MLL-R,* n *(%)*	*MLL-G,* n *(%)*	P-*value**
Total no. of patients	102	80	22	
Age (months)				<0.001
<3	19 (18.6)	19 (23.8)	0 (0.0)	
3 to <6	31 (30.4)	27 (33.7)	4 (18.2)	
⩾6	52 (51.0)	34 (42.5)	18 (81.8)	
Gender				<0.001
Male	52 (51.0)	32 (40.0)	20 (90.9)	
Female	50 (49.0)	48 (60.0)	2 (9.1)	
WBC count (× 10^9/l)				<0.001
<100	44 (43.2)	25 (31.3)	19 (86.4)	
100 to <300	34 (33.3)	32 (40.0)	2 (9.1)	
⩾300	24 (23.5)	23 (28.7)	1 (4.5)	
CNS disease[a]				0.05
Positive	15 (14.7)	15 (18.8)	0 (0.0)	
Negative	81 (79.4)	59 (73.8)	22 (100.0)	
Unknown	6 (5.9)	6 (7.4)	0 (0.0)	
CD10				<0.001
Positive	24 (23.5)	3 (3.8)	21 (95.5)	
Negative	78 (76.5)	77 (96.2)	1 (4.5)	
Karyotype[b]				<0.001
t(4;11)(q21;q23)	41 (40.1)	41 (51.2)	0 (0.0)	
t(11;19)(q23;p13)	7 (6.9)	7 (8.8)	0 (0.0)	
t(9;11)(p22;q23)	6 (5.9)	6 (7.5)	0 (0.0)	
Other 11q23	6 (5.9)	6 (7.5)	0 (0.0)	
No 11q23 rearrangement	35 (34.1)	13 (16.2)	22 (100.0)	
Unknown	7 (6.9)	7 (8.8)	0 (0.0)	

Abbreviations: ALL, acute lymphoblastic leukemia; CNS, central nervous system; FISH, fluorescence *in situ* hybridization; MLL-G, patients with germline *MLL*; MLL-R, patients with MLL gene rearrangement; WBC, white blood cell count.
*Comparison between the MLL-G and MLL-R subgroups.
[a]CNS disease was diagnosed if more than 5 cells/mm^3 with recognizable blasts were found in cerebrospinal fluid.
[b]All 80 patients in the MLL-R group, including the 13 cases with "No 11q23 rearrangement" and the 7 "Unknown" cases by normal karyotypic analysis, were confirmed as "*MLL* rearranged" by Southern blotting and/or split-signal FISH.

Results

Patient characteristics

The characteristics of the patients at diagnosis are reported in Table 1. We identified 80 patients with MLL-R and 22 with MLL-G by Southern blot analysis and/or FISH. Patients in the MLL-R group were significantly younger (median age, 4 vs 9 months, $P<0.001$), and had higher leukocyte counts (median, 168.4×10^9/l vs 21.8×10^9/l, $P<0.001$). The frequency of CNS disease (defined as more than 5 cells/mm^3 with recognizable blasts in cerebrospinal fluid) was also significantly higher in the MLL-R group. Expression of the CD10 antigen was closely

Table 2 Distribution of events by *MLL* gene status and treatment phase

	No. of patients	No. of events	No. of events during induction		No. of events before HSCT		No. of events after HSCT	
			No CR	Death	Relapse	Death in CR	Relapse	Death in CR
Overall	102	50	5	1	21	1	14	8
MLL-G	22	1	0	0	1	0	—	—
MLL-R	80[a]	49	5	1	20	1	14	8

Abbreviations: CR, complete remission; HSCT, hematopoietic stem cell transplantation; MLL-G, patients with germline *MLL*; MLL-R, patients with MLL gene rearrangement.
[a]No events were observed among all four patients in the MLL-R group who did not receive HSCT for lack of a suitable donor.

associated with MLL-R. By karyotyping analysis, 11q23 abnormalities were found in 60 of 73 evaluable cases (82.2%) in the MLL-R group, half of whom had the *t*(4;11)(q21;q23) abnormality. The clinical characteristics of patients enrolled in the MLL96 study were comparable to those in the MLL98 study (data not shown).

Treatment outcome

Remission induction and subsequent events. The numbers and types of events are summarized in Table 2. The overall remission induction rate was 94.1% (96/102): Remission induction rates were high in both the MLL-R and MLL-G groups: 92.5% (74/80 patients) in the MLL-R group and 100% (22/22 patients) in the MLL-G group. There was one induction death due to a fatal adenoviral infection and five induction failures in the MLL-R group. Two of the latter patients survived for 4.9 and 6.0 years, respectively, without evidence of disease after alternative therapies (either acute myeloid leukemia-directed chemotherapy or second HSCT).

Of the 74 patients in the MLL-R group who achieved CR1, 53 remained in continuous complete remission (CCR) during the postremission phase, 1 patient died of infectious pneumonia and 20 relapsed (19 with isolated marrow relapses and 1 with relapse site not specified) before reaching the timepoint of HSCT. Among these 20 relapsed patients, 12 underwent allogeneic HSCT in second remission (CR2) and 3 underwent HSCT without remission; the 5 of 12 who underwent HSCT in CR2 remain in remission for a median duration of 8.4 years (range, 5.7–10.2 years). Forty-nine of the 53 cases in CCR underwent HSCT in CR1: 2 autologous HSCT, 21 HSCT from a related ($n=12$) or unrelated donor ($n=9$) and 26-unrelated cord blood transplantation. The median time from remission to transplantation was 4 months (range, 0–9 months). Twenty-seven of the 49 patients with HSCT remained in CCR at the time of analysis, 8 died in CCR (four of veno-occlusive disease, one of cytomegalovirus infection, one of bacterial sepsis, one of gastrointestinal hemorrhage due to GVHD and thrombotic microangiopathy and one of an unspecified transplant-related complication) and 14 relapsed (nine with an isolated marrow relapses, one with combined marrow/CNS relapse, two with CNS relapse, one with testicular relapse and one with relapse site not specified). Among the 14 relapsed patients, 3 continue to survive for a median duration of 7.2 years (range, 5.0–10.1 years) after subsequent HSCT, while the remaining 11 patients eventually died, mostly of relapsed disease. The four patients who lacked a suitable donor received chemotherapy only as specified by the protocol and remained in CCR for median duration of 8.7 years (range, 3.6–10.8 years).

Analysis of overall outcome. The estimated 5-year OS and EFS rates for all 102 patients were 60.5% (95% CI, 50.7–70.2%) and 50.9% (95% CI, 41.0–60.8%), respectively, after a median follow-up of 7.1 years (range, 1.5–10.8 years). Patients in the MLL-R group had a significantly worse outcome than those in the MLL-G group: 5-year OS, 50.8% (95% CI, 39.6–62.0%) vs 95.5% (95% CI, 86.6–100%) (Figure 1, $P<0.001$) and 5-year EFS, 38.6% (95% CI, 27.7–49.5%) vs 95.5% (95% CI, 86.6–100%) (Figure 1, $P<0.001$).

The only difference between the MLL96 and MLL98 protocols was the higher dosages of antileukemic drugs in the latter study, which was not associated with improved outcome as demonstrated by 5-year EFS rates in the MLL-R group: 35.7% (95% CI, 21.0–50.4%) in MLL96 vs 41.8% (95% CI, 25.8–57.8%) in MLL98 ($P=0.67$). Neither conditioning regimen received nor donor source had a significant impact on post-transplantation EFS rates among the 49 patients with a rearranged *MLL* gene who underwent HSCT after CR1 (Table 3).

Treatment outcome in the MLL-R group according to prognostic factors. The prognostic impact of several potential risk factors (Table 3) was determined in the MLL-R group. Infants younger than 6 or 3 months and those with CNS disease at diagnosis had significantly worse 5-year EFS rates than did infants without these features. Gender, leukocyte count and karyotype lacked prognostic significance in this univariate analysis. Further analysis with a Cox regression model indicated that only age less than 6 months exerted independent predictive strength (data not shown).

Long-term side effects. It was possible to evaluate long-term sequelae among 57 of the all 62 survivors of infant ALL treated on the MLL96 and MLL98 studies: 39 in the MLL-R group and 18 in the MLL-G group (complete follow-up data were not available for the remaining five patients). The median age of the 57 patients at analysis was 7.7 years (range, 1.1–10.4 years). Thirty-six of the 57 patients, all in the MLL-R group, underwent HSCT. Twenty-two received the TBI-based conditioning regimen, while 14 received the non-TBI conditioning regimen. In the TBI group, four patients had undergone allogeneic HSCT twice.

Significant late effects were not observed among patients in the MLL-G group. By contrast, various late complications were observed in the MLL-R group as follows: chronic GVHD in 5; hypothyroidism in 5; short stature (defined as a height standard deviation (s.d.) score below −2.0 or a requirement for growth hormone therapy) in 23; skin abnormalities (alopecia, scleroderma, hyper- or hypo-pigmentation) in 12; fasciitis in 1; ophthalmologic complications (dry eye, corneal opacity, retinal vasculitis) in 5; pulmonary complications (interstitial pneumonia, bronchiolitis obliterans) in 6; chronic diarrhea with malnutrition in 1; dental abnormalities in 6; multiple exostosis in 1; epilepsy in 2 and neurocognitive deficits (learning

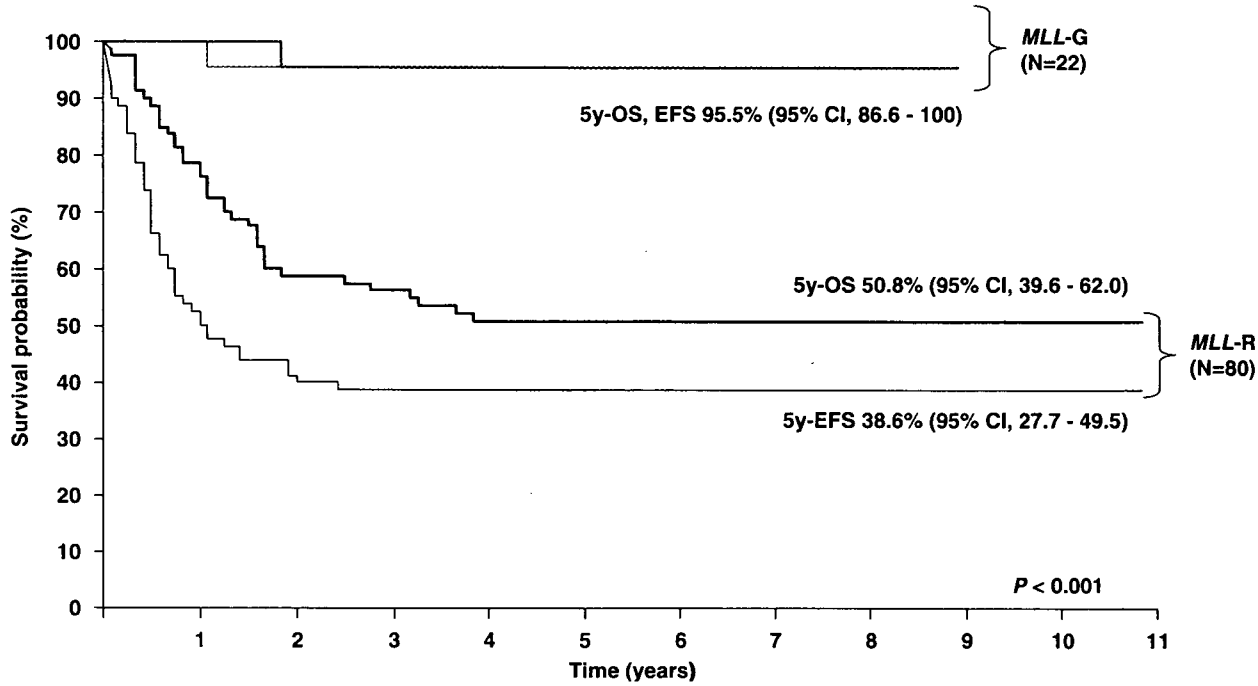

Figure 1 Overall survival (OS) and event-free survival (EFS) rates for infants with ALL treated in the MLL96 or MLL98 study by *MLL* status. Outcome was significantly better in patients with germline *MLL* (MLL-G) than in those with rearranged *MLL* (MLL-R) ($P<0.001$).

disability, intelligence impairment, autism) in 4. There were no cases of secondary malignancy or symptomatic chronic heart failure. Pubertal development could not be evaluated because all study patients were younger than 12 years old and had not entered puberty.

The distribution of height s.d. scores is shown in Figure 2. Median scores for the MLL-R and MLL-G subgroups were −2.49 (range, −8.05 to +2.92) and +0.05 (range, −1.00 to +0.90), respectively (Figure 2a, $P<0.001$). In contrast to the 36 patients who underwent HSCT, height s.d. scores for the three chemotherapy-only MLL-R patients were in the normal range (−1.88, −1.57, −1.48, respectively). The median height s.d. score for patients receiving TBI-based conditioning ($n=22$) was −3.07 (range, −8.05 to −0.08), which was significantly lower than the median value for those given non-TBI conditioning ($n=14$, −1.72 (range, −6.13 to +2.92)) (Figure 2b, $P=0.02$). However, 5 of the 14 patients in the latter group had height s.d. scores below −2.0.

Discussion

The Japanese MLL96 and MLL98 clinical trials are the first published prospective studies to stratify infants with ALL by their *MLL* gene status. The results demonstrated clear differences in clinical features, including treatment outcome, between patients with or without an MLL gene arrangement. In most previous reports of EFS rates for infants with ALL, these two subgroups have been combined,[2–8] when results are listed according to *MLL* gene status or CD10 expression, the EFS rates for those with a rearranged *MLL* gene or negative CD10 expression range from only 21 to 40%, and generally do not exceed 30%. Thus, our overall EFS rate of 50.9% justifies the decision to stratify infants with ALL by *MLL* gene status, so that appropriate risk-based treatments can be applied in each group. The method for determining *MLL* gene status is critical to segregating patients into 'true' *MLL* rearranged and non-rearranged cohorts. Because 16.2% of our *MLL*-rearranged patients lacked abnormalities in band 11q23 by normal karyotypic analysis, we conclude that molecularly based methods such as Southern blotting or split-signal FISH are essential in strategies to determine the accurate *MLL* gene status in infants with newly diagnosed ALL.

Whether allogeneic HSCT has an important role in the treatment of infants with ALL remains controversial because of the limited data on this issue.[19–27] Pui *et al.*[19] retrospectively analyzed cooperative group and individual transplant center data for children with ALL and 11q23 abnormalities, concluding that any type of HSCT was associated with a worse outcome than chemotherapy alone for *t*(4;11)-positive leukemia. In that study, the EFS rate for the 28 infants who underwent HSCT was only 19±3%, which is extremely low compared to our results and those of Sanders *et al.*,[27] who reported a 3-year disease-free survival rate of 42.2% among 40 infants with ALL following HSCT. However, this apparent improvement in outcome after HSCT must be interpreted with caution, since both the report of Sanders *et al.* and ours lack adequate retrospective or prospective control groups. Moreover, infants are the age group most vulnerable to intensive cytotoxic therapy, especially HSCT with a myeloablative preparative regimen, as illustrated by the high proportion of post-HSCT events in the current study (36.3%, 8/22) that were due to transplant-related toxicity. It is also notable that all four patients in the MLL-R subgroup, who did not receive HSCT for lack of a suitable donor, are alive without any subsequent events.

Allogeneic HSCT in infants always harbors the risk of late effects. In our analysis, 23 of 39 patients (58.9%) in the MLL-R group, especially those receiving TBI, had short stature after a median follow-up of 7.7 years. Sanders *et al.*[27] reported milder growth impairment in their series despite earlier treatment with a TBI-based conditioning regimen. This discrepancy may reflect the hyperfraction method of TBI used by Sanders *et al.*, such that 15.75 Gy was given three times a day over 7 days. Whatever the

Table 3 Five-year EFS by selected prognostic features for infants with a rearranged *MLL* gene

	No. of patients	*5-year EFS, % (s.e.)*	*P-value*
Age (months)			
<3	19	26.3 (10.1)	0.04
⩾3	61	42.4 (6.3)	
<6	46	27.8 (6.6)	0.02
⩾6	34	52.9 (8.5)	
Gender			
Male	32	37.5 (8.5)	0.74
Female	48	39.3 (7.0)	
WBC count ($\times 10^9$/l)			
<100	25	51.2 (10.1)	0.08
⩾100	55	32.7 (6.3)	
<300	57	41.9 (6.5)	0.28
⩾300	23	30.4 (9.6)	
CNS disease			
Positive	15	20.0 (10.3)	0.03
Negative	59	47.3 (6.5)	
Karyotype			
t(4;11)(q21;q23)	41	33.8 (7.4)	0.29
Others	39	46.8 (8.8)	
Conditioning regimen[a]			
TBI-based	26	47.1 (10.1)	
BU-based	23	65.2 (9.9)	0.24
Donor source[b]			
Unrelated cord blood	26	53.8 (9.7)	
Others	23	56.5 (10.3)	0.92

Abbreviations: BU, busulfan; CNS, central nervous system; EFS, event-free survival; HSCT, hematopoietic stem cell transplantation; s.e., standard error; TBI, total-body irradiation; WBC, white blood cell count.
[a]5-year EFS by conditioning regimen was compared among patients who had undergone HSCT in CR1.
[b]5-year EFS by donor source was compared among patients who had undergone HSCT in CR1.

explanation, we would stress that severe growth impairment was also observed among patients in the non-TBI group, which may indicate that any form of conditioning regimen for infants could increase the risk of growth retardation. We observed several other serious long-term side effects, but additional follow-up is needed before the impact of these complications can be fully assessed. Besides, caution is needed to evaluate some of the late effects reported here, because there is a certain methodological limitation in collecting these data, which is questionnaire-based, that may lead to an underestimation of these events.

Several steps will need to be taken to further improve the prognosis of infant ALL with *MLL* gene rearrangements. First, additional risk stratification may identify important subsets of patients who would benefit from alternative therapy. Our analysis indicated that age at diagnosis can be used to segregate patients into two subgroups with different 5-year EFS rates: 27.8% for infants younger than 6 months and 52.9% for infants 6 months or older. This approach would not only contribute to better control of the leukemic clone, but might also reduce treatment-related toxicity. Second, we would emphasize that nearly half of the events (21/49) in our MLL-R group occurred before the use of HSCT. Thus, despite an initial remission rate of more than 90%, effective strategies to prevent early relapse are urgently needed. Pieters *et al.*[28] described the *in vitro* drug-resistance data, which demonstrate greater sensitivity to cytarabine (Ara-C) and higher resistance to glucocorticoids and L-asparaginase by leukemic cells from infants. In fact, we did introduce intensive use of Ara-C and dexamethasone in our treatment, which may in part have contributed to improved outcome compared to the historical data. However, one might improve results by further intensifying the early phase of postremission intensification therapy with Ara-C and with intensive use of L-asparaginase, which was not used in the current therapy, to overcome the leukemic cell drug-resistance to this agent. Third, the toxic events and late effects related to HSCT are hardly acceptable for treating infants with ALL. Without clear evidence for benefit of HSCT, it will be necessary to devise an effective chemotherapy regimen without HSCT, at least for the 'lower-risk' MLL-R subgroup. Finally, two large

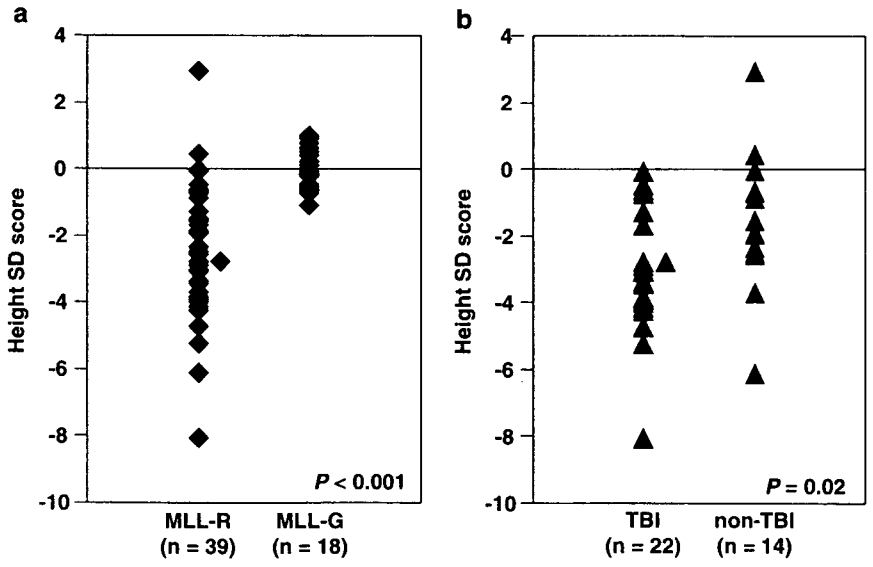

Figure 2 Distribution of height standard deviation (s.d.) scores among survivors of infant ALL in the MLL96 and MLL98 studies. (**a**) Comparison of scores for the *MLL*-rearranged (MLL-R) vs germline *MLL* (MLL-G) patients. (**b**) Comparison of scores between patients who received total-body irradiation (TBI)- or non-TBI-based conditioning regimens.

multicenter studies investigating effective chemotherapy regimens for infant ALL (Interfant 99 and POG/COG9407) have been completed in Europe and in the United States, and these results will be important in designing future protocols for this age group, as will studies to develop innovative targeted therapies for infants with *MLL*-rearranged ALL, now underway.[29,30]

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Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)