Infant acute lymphoblastic leukemia with *MLL* gene rearrangements: outcome following intensive chemotherapy and hematopoietic stem cell transplantation

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Forty-four infants with acute lymphoblastic leukemia (ALL) characterized by *MLL* gene rearrangements were treated on a protocol of intensive chemotherapy followed by hematopoietic stem cell transplantation (HSCT) between November 1998 and June 2002. The remission induction rate was 91.0%, and the 3-year overall survival and event-free survival (EFS) rates, with 95% confidence intervals, were 58.2% (43.5%-72.9%) and 43.6% (28.5%-58.7%), respectively. Univariate analysis of EFS by presenting features indicated a poorer outcome in patients younger than 6 months of age with high white blood cell counts ($\geq 100 \times 10^9$/L; EFS rate, 9.4% versus 55.1% for all others, *P* = .0036) and in those with central nervous system invasion (EFS rate, 10.0% versus 56.9% for all others, *P* = .0073). The 3-year posttransplantation EFS rate for the 29 patients who underwent HSCT in first remission was 64.4% (46.4%-82.4%). In this subgroup, only the timing of HSCT (first remission versus others) was a significant risk factor by multivariate analysis (*P* < .0001). These results suggest that early introduction of HSCT, possibly with a less toxic conditioning regimen, may improve the prognosis for infants with *MLL*+ ALL. Identification of subgroups or patients who respond well to intensified chemotherapy alone should have a high priority in future investigations. (Blood. 2004;104:3527-3534)

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Introduction

Despite remarkable advances in the treatment of childhood acute lymphoblastic leukemia (ALL), especially with the introduction of intensive multiagent chemotherapy, infants with this disease continue to have a poor prognosis.[1,2] Their leukemic cells are characterized biologically by a lack of the early lymphocyte antigen CD10 and by 11q23 translocations/*MLL* gene rearrangements, the latter feature being associated with a highly distinct gene expression profile and a poor outcome in most treatment programs.[3,4] Especially dire is the prognosis for infants with the 4;11 translocation, the vast majority of whom have a relapse and die of progressive disease.[5,6] On the other hand, several groups have indicated that a good initial steroid response and 11q23 translocations other than the 4;11 translocation predict a more favorable outcome in infants with ALL.[7,8] Recently, Pui et al[9] reported that age under 1 year and *MLL* rearrangements are the most adverse risk factors in childhood ALL, underscoring the clinical significance of these 2 characteristics. Thus, improving the prognosis of infant ALL will likely require innovative strategies in which patients with or without *MLL* gene rearrangements are regarded as discrete subgroups requiring different treatment protocols.

The role of hematopoietic stem cell transplantation (HSCT) in the management of infants with ALL is still unclear,[10] primarily because only small numbers of patients with documented *MLL*+ ALL have been treated with this modality in reported series.[11-13] Between 1996 and 1998, we implemented a prospective treatment protocol (MLL96) that stratified infant ALL patients according to the presence or absence of *MLL* gene rearrangements; infants with *MLL*− ALL were treated with standard chemotherapy only, whereas those with *MLL*+ disease were given intensive chemotherapy followed by HSCT.[14] Unfortunately, the results were not satisfactory, as demonstrated by a 3-year event-free survival (EFS) rate of only 34.0% in the *MLL*+ subgroup, reflecting a high relapse rate during the first 6 months of chemotherapy and before HSCT. However, the outcome in patients undergoing HSCT in first remission was superior to that in patients receiving transplants later, suggesting an advantage from early introduction of HSCT.
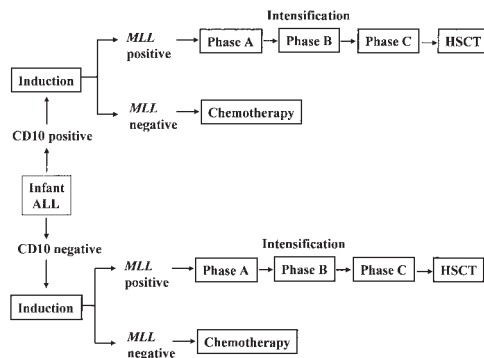
Figure 1. Flow diagram illustrates the design of the MLL98 protocol. Each infant presenting with ALL was assigned to one of 2 subgroups at diagnosis according to the expression of CD10 antigen on leukemic blast cells. Patients were reassigned to subgroups based on the detection of *MLL* gene rearrangements after remission induction. Those with *MLL*+ blasts received 3 courses of intensification therapy followed by HSCT. Chemotherapy regimens for *MLL*+ cases are reported in Table 1.

Consequently, to prevent early relapse in this subgroup, we instigated treatment protocol MLL98, which specified more intensive chemotherapy and HSCT early in first remission (3-5 months after diagnosis). Here we describe the effectiveness and toxicity of this protocol, as well as the prognostic factors that appear to discriminate between subgroups of infants with a better or worse risk of treatment failure.

Patients, materials, and methods

Patients

Between November 1998 and June 2002, 54 infants younger than 12 months of age at the time of diagnosis of ALL were registered with the Japan Infant Leukemia Study Group and treated on the MLL98 protocol. In a nationwide surveillance study, the incidence rate of new infant ALL cases was calculated as 20/y (96 cases between 1997 and 2001; K.H., unpublished data, March 2003). Therefore, the current study included approximately 80% of all infants diagnosed with ALL in Japan over the 3.5-year enrollment period. Written informed consent was obtained from the parents of all patients at the time of enrollment, and all aspects of this investigation were approved by the appropriate institutional review boards. Prior to treatment, each patient was evaluated with respect to the characteristics of the leukemic cells, including immunophenotype, cytogenetics, and *MLL* gene rearrangement. The diagnosis of ALL was based on more than 30% lymphoblasts in the bone marrow (BM), with less than 3% staining positively for myeloperoxidase (MPO). Central nervous system (CNS) invasion was defined as more than 5 mononuclear cells per microliter of cerebrospinal fluid with obvious lymphoblasts.

Immunophenotype, cytogenetics, and detection of *MLL* gene rearrangements

Cell surface markers were analyzed with an EPICS-PROFILE flow cytometer (Coulter Electronics, Hialeah, FL) at our central laboratory. Surface antigens studied in this investigation were as follows: CD10, CD19, and CD20 (B-cell markers); CD2, CD3, cytoplasmic CD3, CD4, CD5, CD7, and CD8 (T-cell markers); and CD13, CD14, CD33, cytoplasmic MPO, CD41, and glycophorin A (myeloid-cell markers). HLA-DR and CD56 served as additional markers. A positive reading for each antigen was defined as reactivity by 25% or more of the leukemic cells. Cytogenetic analysis of leukemic cells was performed by a G-banding technique. The presence of *MLL* gene rearrangements was determined by Southern blot analysis, as described previously.[14] Briefly, DNA extracted and digested with *Bam*HI and *Hin*dIII was hybridized with a cDNA probe covering the breakpoint cluster region of the *MLL* gene.

Treatment protocol

All infants enrolled in this study were treated according to the MLL98 protocol (Figure 1; Table 1). Each patient was assigned to a subgroup

according to the presence or absence of CD10 expression at diagnosis (CD10 negativity correlates closely with *MLL* gene rearrangement[6]). The subgroup assignments were reassessed when the presence or absence of an *MLL* gene rearrangement was determined by Southern blot analysis.

Table 1 presents the details of the protocol for infants with *MLL*+ALL. Those with CD10+ ALL received an induction regimen of vincristine (VCR), doxorubicin (DXR), cyclophosphamide (CPA), L-asparaginase (ASP), and dexamethasone (DEX) or prednisolone (PSL) for 4 weeks, followed by etoposide (VP-16) and cytarabine (Ara-C) for 4 days. Those with CD10− ALL received VCR, DXR, CPA, and DEX for 2 weeks followed by VP-16 and Ara-C for 4 days. Depending on the results of Southern blot analysis, the subgroup with *MLL*− ALL was then given 2 years of standard chemotherapy that included consolidation, intensification, reinduction, and maintenance phases. With the exception of VCR, drug dosages were based on body surface area rather than body weight, the method used our previous investigation.[14] This change increased the dosage of all antileukemic drugs by 1.2- to 2-fold. Thus, total doses of drugs other than VCR were reduced by one third in patients younger than 2 months and by one fourth in those 2 to 4 months of age. Patients with molecularly confirmed *MLL*+ALL received 3 courses of intensification chemotherapy, as described in Table 1, and then underwent HSCT. The intent was to perform transplantation in all patients in first remission preferentially within 3 to 5 months after diagnosis; however, those who had relapses before HSCT underwent the procedure at relapse or in second remission. The conditioning regimen consisted of a combination of total body irradiation (TBI; 2 Gy, twice a day on days −7 to −5 for a total of 12 Gy), VP-16 (60 mg/kg on day −4), and CPA (60 mg/kg on days −3 to −2 for a total of 120 mg/kg) or a combination of busulfan (BU; 35 mg/m,^{2} 4 times a day on days −8 to −5 for a total of 560 mg/m^2), VP-16, and CPA.

Table 1. Pretransplant chemotherapy for patients with *MLL*+ ALL*

Regimen	Dosing schedule
Induction therapy	
VCR	0.05 mg/kg, IV, days 1 and 8
DEX	10 mg/m^2/d in 2 divided doses, IV, days 1-14
CPA	1200 mg/m^2, DIV, day 2
DXR	25 mg/m^2, IV or DIV, days 3 and 5
VP-16	100 mg/m^2, DIV, days 15-18
Ara-C	500 mg/m^2, DIV, days 15-18
TIT	Days 1 and 15
Intensification therapy	
Phase A	
VP-16	100 mg/m^2, DIV, days 1-3
Ara-C	200 mg/m^2, DIV, days 4-8
THP-DXR	30 mg/m^2, IV or DIV, days 4 and 5
PSL	60 mg/m^2/d in 3 divided doses, PO, days 4-9
ASP	20 000 U/m^2, DIV, day 9
TIT	Day 1
Phase B	
VCR	0.05 mg/kg, IV, day 1
DEX	10 mg/m^2/d in 2 divided doses, PO, days 1-7
MTX	3000 mg/m^2/24 h, DIV, day 1
Folinic acid	15 mg/m^2 (36 hours after the start of MTX, 7 times)
CPA	600 mg/m^2/d in 2 divided doses, DIV, days 2 and 3
TIT	Day 1
Phase C	
MIT	10 mg/m^2, IV or DIV, day 1
VP-16	100 mg/m^2, DIV, days 1-5
Ara-C	3000 mg/m^2, DIV, days 1-5
TIT	Day 1

VCR, vincristine; DEX, dexamethasone; CPA, cyclophosphamide; DXR, doxorubicin; VP-16, etoposide; Ara-C, cytarabine; TIT, triple intrathecal therapy (age < 3 months: MTX, 3 mg; hydrocortisone, 10 mg; Ara-C, 6 mg; age ≥ 3 months: MTX, 6 mg; hydrocortisone, 10 mg; Ara-C, 12 mg); THP-DXR, tetrahydropyranyl doxorubicin; PSL, prednisolone; ASP, L-asparaginase; MTX, methotrexate; MIT, mitoxanthrone; IV, intravenously; DIV, drip intravenously; PO, by mouth.

*The dose of each drug except VCR was reduced by one third in patients younger than 2 months and by one fourth in those 2 to 4 months of age.

Table 2. Presenting characteristics and initial outcome in 44 infants with *MLL*⁺ ALL

Feature with subcategories	No.
Gender	
Male	19
Female	25
CD10 antigen	
Positive	3
Negative	41
Age at diagnosis, mo	
Median	5
Range	0-11
WBC count, ×10⁹/L	
Median	129
Range	1.7-1524
CNS invasion	10/41
Karyotype	
4;11 translocation	27
11;19 translocation	4
9;11 translocation	3
Other	2
Normal	8
Outcome	
Induction failure	1
Death during induction	2
Relapse	17
BM	12
CNS	4
BM/CNS	1
Death in CCR after HSCT	4
Refusal of treatment after CR	1
CCR	19

CCR indicates continuous complete remission.

Prophylaxis for graft-versus-host disease (GVHD) consisted of either cyclosporin A (CSA) or FK506 (FK) combined with short-term methotrexate (MTX).

Statistical analysis

Comparisons of continuous variables (eg, age, white blood cell [WBC] count, time to transplantation) were made with the Mann-Whitney *U* test. Differences in the distribution of categorical variables (eg, gender, WBC count, age, CNS invasion at diagnosis, karyotypes, and remission status at HSCT) were analyzed with Fisher exact test. Overall survival (OS) and event-free survival (EFS) rates with 95% confidence intervals (CIs) were estimated by the Kaplan-Meier method and compared with use of the log-rank test. Multivariate analysis of survival was performed with the Cox proportional hazard model and a stepwise regression method. OS was calculated for the period from the day of diagnosis until the day of death due to any cause. EFS was calculated from the day of diagnosis until the date of an adverse event: relapse, diagnosis of a secondary cancer, or death due to any cause. Patients failing to respond to induction therapy were assigned an EFS time of zero. In comparisons of EFS between patients undergoing HSCT in first remission or in some other state, the duration of EFS, considered as posttransplantation time, was calculated from the day of transplantation until the earliest date of any adverse event, as defined. All results were updated to April 30, 2004.

Results

Patient characteristics

Altogether, 54 infants were enrolled in the MLL98 study. Among the 44 *MLL*⁺ patients, 3 were CD10⁺ and therefore received induction therapy specified for patients with that marker. Each of
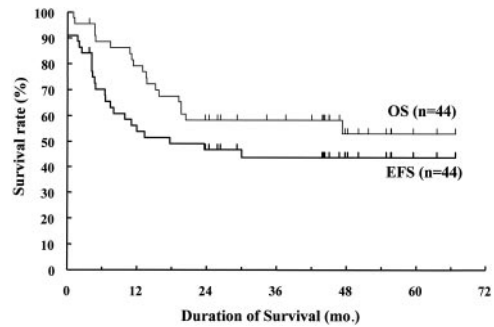

Figure 2. OS and EFS rates for 44 infants with *MLL*⁺ ALL. The estimated rates at 3 years were 58.2% (95% CI, 43.5%-72.9%) and 43.6% (28.5%-58.7%), respectively. Tick marks represent patients still at risk of death or other adverse event.

the remaining 41 patients, all of whom were CD10⁻, received the more aggressive induction therapy described in Table 1. These 44 patients were given 3 courses of intensification therapy after molecular confirmation of *MLL* positivity. Table 2 presents the clinical characteristics of the 44 *MLL*⁺ ALL infants (19 boys and 25 girls). The median WBC count at diagnosis was 129×10^9/L, and there was a high incidence (24.4%) of CNS involvement. The 4;11 translocation was detected in 27 cases (61.4%) by chromosomal analysis, and 8 patients had normal karyotypes despite the presence of *MLL* gene rearrangements by Southern blot analysis.

Treatment results

Remission induction and 3-year OS and EFS rates among the infants with *MLL*⁺ ALL were 91.0%, 58.2% (95% CI, 43.5%-72.9%), and 43.6% (28.5%-58.7%; Figure 2), respectively; the median duration of observation was 785 days. Among the 41 infants who achieved complete remission (CR), 12 had relapses in the BM, 4 in the CNS, and 1 in the BM/CNS; 19 remained in remission (Table 2). Relapse occurred before HSCT in 11 patients and later in 7 (including 1 patient who failed to respond to induction therapy). Of the 5 patients who were saved, 2 continued in remission after first HSCT and 3 after a second HSCT.

Log-rank comparison of EFS rates by selected clinical and biologic features of the *MLL*⁺ infants (Table 3) generally failed to

Table 3. Comparison of 3-year EFS rates by presenting features in infants with *MLL*⁺ ALL

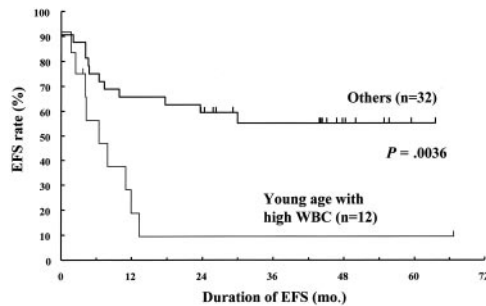| Feature	No. of patients	EFS (%)	P
Age, mo			
Younger than 6	25	28.0	.0499
6 or older	19	63.2	
Gender			
Male	19	44.9	.8470
Female	25	42.7	
WBC count, ×10⁹/L			
Less than 100	20	53.3	.1867
100 or higher	24	35.2	
Less than 200	30	46.9	.5861
200 or higher	14	35.7	
Less than 300	35	46.1	.7018
300 or higher	9	33.3	
CNS involvement			
Positive	10	10.0	.0073
Negative	31	56.9	
Karyotype			
4;11 translocation	27	37.0	.1763
Other	17	56.3	

Figure 3. Comparison of EFS rates for infants with *MLL*+ ALL stratified by age and WBC count at diagnosis. The outcome for patients younger than 6 months with high WBC count ($\geq 100 \times 10^9$/L) was significantly worse than that associated with the corresponding favorable characteristics ($P = .0036$). Tick marks represent patients still at risk of death or other adverse events.

demonstrate statistically significant relationships. However, patients with CNS invasion at diagnosis and those younger than 6 months had a significantly poorer prognosis than those without these characteristics. An especially poor outcome was noted in infants younger than 6 months who presented with a WBC count of $\geq 100 \times 10^9$/L: 3-year EFS, 9.4% versus 55.1%, $P = .0036$ (Figure 3).

Of the 38 infants who underwent HSCT, 29 were in first remission and 9 in second remission or relapse (Table 4). Eighteen in first remission underwent transplantation within the specified 3- to 5-month window after diagnosis. Six patients did not undergo HSCT, 3 because of very early relapse and 1 because of parental refusal (these 4 infants died as a result of disease progression), and 2 because of fatal viral infections that developed during induction therapy. Unrelated cord blood transplantation (UCBT), related

Table 4. Characteristics and outcome of HSCT in 38 patients with *MLL*+ ALL

| Age, mo/gender | WBC count, ×10⁹ | HSCT | | | | Conditioning regimen | GVHD prophylaxis | Outcome |
		Type	Status	HLA*	Time, mo†			
5/F	260	UCBT	CR1	4/6	4	BU	CSA	CCR
6/M	21	UCBT	CR1	5/6	5	TBI	CSA	CCR
7/F	24	UCBT	CR1	5/6	4	BU	FK	CCR
7/F	668	UCBT	CR1	5/6	5	TBI	FK	CCR
6/F	420	UCBT	CR1	5/6	6	TBI	CSA	CCR
4/F	7	UCBT	CR1	5/6	4	TBI	CSA	Rel‡
11/M	119	UCBT	CR1	6/6	5	TBI	FK	CCR
4/F	5	UCBT	CR1	5/6	6	TBI	CSA	Rel§
6/M	39	UCBT	CR1	5/6	5	BU	CSA	CCR
4/M	1000	UCBT	CR1	4/6	5	BU	CSA	Fail″
5/M	26	UCBT	CR1	6/6	6	BU	CSA	CCR
1/M	39	UBMT	CR1	6/6	5	TBI	FK	CCR
3/F	143	RPBSCT	CR1	5/6	7	BU	CSA	Rel§
7/F	2	UCBT	CR1	4/6	8	TBI	FK	CCR
2/F	48	RBMT	CR1	6/6	5	BU	CSA	CCR
4/M	552	UCBT	CR1	5/6	6	TBI	FK	Rel‡
4/F	35	RBMT	CR1	4/6	4	TBI	FK	CCR
6/M	1524	UBMT	CR1	6/6	6	BU	FK	CCR
11/F	299	UCBT	CR1	5/6	5	BU	CSA	CCR
9/F	251	UCBT	CR1	4/6	3	TBI	FK	D¶
9/F	732	RBMT	CR1	6/6	4	TBI	CSA	Rel‡
3/M	54	UCBT	CR1	5/6	5	TBI	FK	Rel#
7/M	1252	UCBT	CR1	6/6	6	BU	CSA	Rel#
1/M	44	UCBT	CR1	5/6	6	TBI	FK	D¶
10/M	31	UBMT	CR1	6/6	8	Other	FK	CCR
4/F	23	UCBT	CR1	5/6	5	BU	FK	CCR
11/F	143	RBMT	CR1	5/6	5	TBI	CSA	CCR
4/F	50	RPBSCT	CR1	5/6	5	BU	FK	CCR
7/M	124	UCBT	CR1	4/6	6	BU	CSA	CCR
6/M	47	UCBT	CR2	6/6	4	TBI	CSA	Rel#
3/F	288	UCBT	CR2	5/6	5	TBI	CSA	CCR
0/M	24	UCBT	CR2	5/6	3	BU	FK	D¶
3/F	134	UCBT	IF	4/6	4	TBI	CSA	Rel‡
4/F	225	RBMT	Rel	6/6	8	BU	CSA	CCR
7/F	11	UCBT	Rel	5/6	6	TBI	CSA	Rel§
2/M	54	RBMT	Rel	5/6	11	Other	FK	Rel‡
2/F	188	UBMT	Rel	6/6	5	BU	FK	Rel‡
1/M	900	UCBT	Rel	6/6	5	TBI	FK	Rel‡

CR1 indicates first remission; FK, FK506; Rel, relapse; Fail, failure of transplantation; D, death; CR2, second remission; IF, induction failure. Other abbreviations are explained in the text and Tables 1 and 2.

*HLA results: 6/6, complete match; 5/6, 1-locus mismatch; 4/6, 2-loci mismatch.

†Time from diagnosis to HSCT.

‡Death due to disease progression without second HSCT.

§Death due to disease progression after second HSCT.

″Toxic death after second HSCT.

¶Toxic death without second HSCT.

#CCR after second HSCT.

bone marrow transplantation (RBMT), unrelated bone marrow transplantation (UBMT), and related peripheral blood stem cell transplantation (RPBSCT) were performed in 26, 6, 4, and 2 patients, respectively (Table 4). The median time to transplantation, regardless of the source of stem cells, was 5 months. The type of HSCT was decided on by each participating investigator, based on the availability of suitable donors at the time of transplantation. The HLA disparities at low resolution in the patients who received UCBT were complete match in 5, 1-locus mismatch in 15, and 2-loci mismatch in 6. Among those undergoing RBMT, 3 had a complete match, 2 a 1-locus mismatch, and 1 a 2-loci mismatch; in the RPBSCT subgroup, both patients had a 1-locus mismatch. All 4 patients receiving UBMT had a complete match. The conditioning regimen consisted of TBI in 20 patients, BU in 16, and other agents in 2. CSA and FK were used as GVHD prophylaxis in 20 and 18 patients, respectively (Table 4).

The 3-year posttransplantation EFS rate for all 38 patients undergoing HSCT was 54.4% (38.1%-70.7%) when the duration of EFS was calculated from the day of transplantation. For the 29 patients undergoing transplantation in first remission, it was 64.4% (46.4%-82.4%) compared with 22.2% (0%-49.4%) for the 9 patients undergoing transplantation in second remission or after relapse ($P = .0044$; Figure 4A). The difference in outcome between patients treated with HSCT in the 3- to 5-month window after diagnosis and those treated later in remission was not statistically significant. These results suggested that the timing of HSCT for infants with MLL^+ALL exerts a critical influence on outcome, prompting us to test this factor against other potentially important covariates. Stepwise multivariate analysis of posttransplantation EFS rates indicated independent predictive strength for remission status at HSCT (first remission versus other) in the context of age (younger versus older than 6 months at diagnosis), gender (male versus female), CNS invasion at diagnosis, initial WBC count (< 100 versus $\geq 100 \times 10^9$/L), and karyotype (4;11 translocation versus other; hazard ratio, 0.0268; $P < .0001$).

Patients undergoing UCBT in first remission tended to fare worse than those receiving other types of donor cells (3-year posttransplantation EFS rate, 58.7% versus 77.8%, $P = .3716$; Figure 4B), but the difference was not statistically significant; the numbers of patients in the RBMT, UBMT, and RPBSCT subgroups were too small to allow further comparisons. When the 20 patients with UCBT in first remission were stratified by HLA disparity, TBI and BU conditioning regimens, or CSA- and FK-based methods of GVHD prophylaxis, we found no significant differences in posttransplantation EFS, using Kaplan-Meier methods and log-rank test (data not shown). A repeated stepwise multivariate survival analysis, using the same risk factors as just described with the addition of HLA disparity (complete match versus 1-locus mismatch versus 2-loci mismatch), conditioning regimen (TBI versus BU), and method of GVHD prophylaxis (CSA versus FK), again demonstrated independent prognostic strength for time of HSCT (first remission versus other; hazard ratio, 0.2717; $P = .0226$).

Because it is possible that the patients undergoing HSCT in first remission had more favorable prognostic factors than others in the series, we compared selected clinical characteristics of these 2 groups. Apart from an older age at diagnosis for infants given transplants in first remission ($P = .0132$), there were no significant differences in gender, WBC count, time to transplantation, CNS invasion, and karyotype (data not shown).

Seven patients had relapses and one had graft failure following the first HSCT (Table 4). After subsequent UCBT (n = 2), RPBSCT (n = 1), or UBMT (n = 2), 3 infants undergoing UCBT or UBMT attained new CRs without further adverse events as of April 30, 2004. Consequently, 3 infants having relapses, including 2 undergoing HSCT in first remission and one in second remission, have been in CR after the second HSCT (Table 4).

Toxicity

A major consideration in the treatment of ALL in infants is the significant potential for toxicity due to myeloablative chemotherapy and ionizing radiation. Two patients in our series died as a result of adenoviral infection or interstitial pneumonia during induction therapy. Fatal complications were not observed during the intensification phase of chemotherapy. Table 5 reports the toxicities associated with HSCT. Four patients had transplantation-related deaths due to sepsis, graft rejection, respiratory failure, or veno-occlusive disease of the liver (VOD)/thrombotic microangiopathy (TMA). Seven others, 5 of whom had undergone UCBT, developed grade II or III acute GVHD, whereas 3 others had chronic limited GVHD. Of the 7 patients presenting with VOD, 5 had received the BU-containing conditioning regimen. This complication was fatal in one infant and severe in another, who eventually required liver transplantation. Long-term side effects remain to be analyzed, although the late sequelae of the MLL96 protocol,[14] which relied on the same regimens used in MLL98, do not suggest any prohibitive complications. However, as preliminary results, growth impairment has been reported by several investigators who registered patients in this study undergoing HSCT with a TBI-conditioning regimen (E. I., unpublished observations, April 2004).
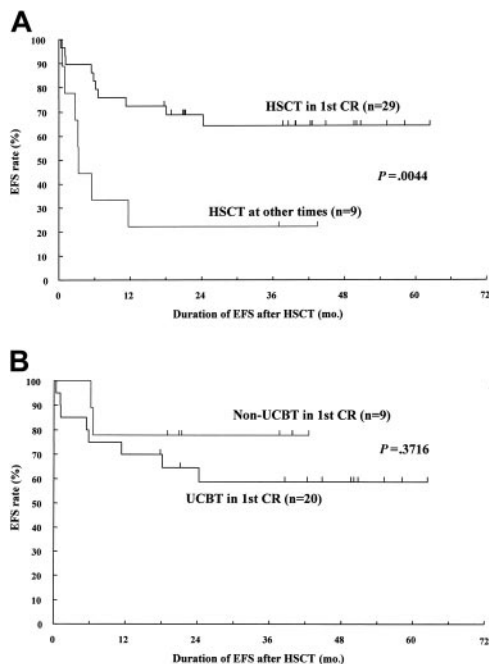

Figure 4. Comparison of EFS rates. Comparison of EFS rates for infants with MLL^+ ALL by remission status at the time of HSCT (A) and by the source of donor cells (B). Patients receiving transplants in first remission fared significantly better than those undergoing HSCT at other times (3-year posttransplantation EFS, 64.4% versus 22.2%, $P = .004$). There was no statistically appreciable difference in outcome between patients receiving cord blood or non–cord blood in first remission. Tick marks represent patients still at risk of death or other adverse events.

Discussion

Historically, the results of chemotherapy for infants with ALL, particularly those with 4;11 translocation/MLL^+or CD10^{−} disease,

Table 5. Toxicities associated with HSCT

Deaths	No.
Total	14
Relapse	10
Transplantation-related death	4
Rejection	1
Sepsis	1
Respiratory failure	1
VOD/TMA	1
Complications	
Mucous membrane damage	19
VOD	7
Diarrhea/vomiting	5
Sepsis	5
Liver dysfunction	5
Interstitial pneumonia	3
Acute GVHD	14
Grade I	7
Grade II	5
Grade III	2
Chronic GVHD	3
TTP/TMA	3
Fungal infection	2
Rejection	2
Renal failure	1

TMA indicates thrombotic microangiopathy; TTP, thrombotic thrombocytopenic purpura.

have been poor, with EFS rates ranging from 17% to 43%.[5,8,15-21] The best current results are those reported from the Dana-Farber Cancer Institute, where an intensified drug regimen yielded a 4-year EFS rate of 54% in all infants with ALL and 43% in those with MLL^+ disease; however, the patient sample was too small to permit firm conclusions.[22] Considered together, these findings suggest that the outcome for MLL^+ infant ALL is unlikely to be improved with use of intensive chemotherapy alone. This interpretation is supported by the experience in Japan. Between 1996 and 1998, we treated infant ALL according to the presence (HSCT) or absence (intensive chemotherapy only) of MLL gene rearrangements, securing a 3-year EFS rate of 34.0% for MLL^+ cases, which did not represent an improvement over previous reports.[14] Most of these patients had relapses within 6 months after the initiation of chemotherapy; however, the 3-year posttransplantation EFS rate for MLL^+ patients undergoing HSCT in first remission was 58.0%, suggesting a therapeutic advantage for early use of transplantation.[14]

Thus, in the current study, we elected to initiate HSCT within 3 to 5 months after diagnosis as a means to reduce the relapse rate in infants with MLL^+ALL who had achieved remission. The 3-year postdiagnosis EFS rate for these patients increased to 43.6%, but the gain over our previous experience was not statistically significant.[14] Nonetheless, the relatively high 3-year posttransplantation EFS rate for patients undergoing HSCT in first remission (64.4%) is encouraging and supports the working hypothesis of MLL98. Failure to produce a significant improvement over the postdiagnosis HSCT results in MLL96 can be attributed, in part, to the dismal prognosis of infants with CNS invasion or age younger than 6 months, many of whom had a disease relapse before HSCT could be attempted. Clearly, the development of more effective induction and intensification therapies for these subgroups should be a priority of future investigations.

Marco et al[13] performed allogeneic or autologous HSCT in 26 patients with infant leukemia, including AML. They noted that patients given transplants earlier than 4 months after remission induction had a significantly better prognosis than those undergo-

ing the procedure at later times. Moreover, the period from remission induction to transplantation was the sole prognostic factor identified by multivariate analysis. Similarly, Leung et al[23] studied 22 infant leukemia patients treated with HSCT, reporting that disease status at transplantation was the only prognostic factor with significant predictive strength in patients in remission. These findings, together with our similar result by multivariate analysis, suggest that the early introduction of HSCT could save a proportion of patients who otherwise would have relapses relatively soon after induction therapy. On the other hand, Pui et al[9] evaluated a substantial number of childhood ALL patients presenting with 11q23 abnormalities and concluded that HSCT was not effective in this series. In this retrospective analysis, however, the treatment method, source of donor cells, and conditioning regimen varied widely among the patients. In particular, the EFS rate for infants was too low (only 19%) to permit an accurate assessment of the effect of HSCT, in contrast to our MLL98 study, in which all MLL^+ cases were assigned to the HSCT arm.

The majority of patients in the current investigation underwent UCBT rather than UBMT, for the following reasons. First, cord blood can be obtained within 2 months from donors in Japan, whereas it usually takes 5 to 6 months to identify a suitable unrelated marrow donor through the Japan Marrow Donor Program (JMDP) and proceed to transplantation. Second, cord blood can provide sufficient numbers of donor cells for patients with a low body weight, such as infants. Third, a nationwide network of cord blood banks has been established in Japan (approximately 16 000 cord blood units had been stored as of October 2003), permitting the procurement of adequate quantities of cord blood mismatched at 2 or fewer loci in nearly all cases.[24-26] Fourth, cord blood transplants are associated with a relatively low risk of GVHD,[26-30] although this benefit may be offset by a diminished graft-versus-leukemia (GVL) effect and a higher risk of relapse.[31] In our study, the EFS of patients undergoing UCBT tended to be lower than that associated with other sources of donor cells. Thus, in future studies, the induction of GVL reactions using ubenimex or granulocyte-macrophage colony-stimulating factor (GM-CSF) should be considered as a means to prevent relapse.[32,33]

Two patients who underwent UCBT following treatment with the BU-containing regimen had complications associated with graft rejection. Although the pharmacokinetics of BU in infants are unknown, the absorption and metabolism of oral BU vary widely among individual patients,[34-36] indicating a need to develop an effective BU pharmacokinetics monitoring system for younger children.[37] In addition, the incidence of VOD was higher than in previous reports on the outcome of HSCT in infants, despite the administration of ursodeoxycholic acid and heparin to most patients in this investigation. The factors contributing to this unexpectedly high frequency of VOD remain unclear, and this issue must be addressed in the future.

A major concern with the use of HSCT in infants is the possibility of late adverse sequelae, including growth impairment. Pirich et al[12] performed allogeneic HSCT in 7 infants with ALL using a conditioning regimen that incorporated TBI, CPA, and VP-16; this protocol contributed to short stature in 3 patients, with TBI implicated as the principal risk factor.[38] TBI was also reported to be a risk factor for hypopituitarism and cataract, and these toxicities were inversely correlated with age.[13,39] We analyzed growth and development in 34 infants with ALL followed for 5 to 8 years who had been treated on MLL96 protocol,[14] which included intensive chemotherapy and HSCT administered in much the same way as in the MLL98 study. Growth (body height and body weight)

was compared by analysis of variance among patients receiving chemotherapy alone (chemotherapy group, n = 19), HSCT with a TBI-conditioning regimen (TBI group, n = 9), and HSCT with a BU-conditioning regimen (BU group, n = 6) at the onset of leukemia, at the time of treatment cessation, and at 1, 2, and 3 years after treatment. At 3 years after the completion of therapy, patients in the TBI group showed marked growth impairment (mean body height, −2.2 SD; mean body weight, −0.9 SD) that tended to be more pronounced but not significantly different from results in the chemotherapy (−0.3 SD; +0.3 SD) and BU (−1.2 SD; −0.6 SD) groups ($P = .063$ and $P = .071$, respectively). As for other late sequelae, psychosomatic deterioration was observed in 3 patients (1 in the BU and 2 in the chemotherapy groups), hypothyroidism in one TBI-treated patient, and immunodeficiency in one patient receiving chemotherapy only. Neither lung nor cardiac dysfunctions nor second malignancies were detected in any patient in this series. These findings support the recommendation that TBI be eliminated from future studies of HSCT in infants with *MLL*+ALL, particularly in view of the similar EFS rates for the TBI- and BU-treated subgroups.

Recently, reduced-intensity hematopoietic stem cell transplantation (RIST) using nonmyeloablative conditioning has become available for leukemia patients.[40,41] Because the effects of HSCT are partly attributable to an antileukemic immune reaction mediated by donor lymphocytes, the aim of RIST is to promote a GVL effect, thus contributing to an improved survival rate and fewer transplant-related complications. Recent reports indicate that RIST could be effective for high-risk ALL[42] or non-Hodgkin lymphoma.[43,44] Barker et al[45] also performed UCBT with RIST in adults, obtaining a high engraftment rate and satisfactory end results. Another report noted that an infant with AML survived free of disease despite episodes of grade II GVHD following UCBT with RIST.[46] Consequently, RIST may replace conventional myeloablative HSCT for infants with *MLL*+ALL, especially those who are ineligible for high-dose chemoradiotherapy because of immature organs or the risk of late toxicities. However, the number of infants who have received RIST is too small to allow definite recommendations.

Although the present investigation tested HSCT in all infants with *MLL*+ALL, it may be possible to identify subgroups that would fare well without transplantation. The Berlin-Frankfurt-Munster (BFM) group,[7] for example, reported that the early response to prednisone was the strongest prognostic factor in infant ALL, so that poor responders to this agent may be the only group requiring HSCT in first remission. Two large cooperative groups (Children's Oncology Group and Interfant) initiated international studies to test the efficacy of intensified chemotherapy for infant ALL. A preliminary study by the Children's Oncology Group yielded a 2-year EFS rate of 60% in infants with *MLL*+ALL[47]; results of the Interfant99 study have not yet been published, although initial analyses indicate an improvement in outcome. Thus, a major challenge for the future will be to confirm the efficacy of intensified chemotherapy for selected subgroup of infants. Recently, the level of minimal residual disease (MRD) has emerged as a powerful prognostic factor in childhood ALL. In patients with t(4;11)+ ALL, the detection of MRD with the polymerase chain reaction (PCR) was significantly related to outcome.[48] Indeed, in our preliminary study, MRD was present after induction or intensification chemotherapy in most *MLL*+ patients (unpublished data, November 2003). Hence, monitoring of MRD by PCR should be considered as an additional means to discriminate among subgroups with a favorable or unfavorable prognosis.

This investigation suggests that the early introduction of HSCT, preferably within 3 to 5 months after diagnosis, can be an effective strategy in terms of preventing relapse in infants with *MLL*+ ALL. Two subgroups in our study, patients younger than 6 months with high WBC counts ($\geq 100 \times 10^9$/L) and those with CNS invasion at diagnosis, continued to fare poorly, indicating a need to consider more frequent intrathecal chemotherapy or intensified high-dose Ara-C therapy before HSCT for these patients. For all infants undergoing HSCT, less toxic conditioning regimens might be introduced without any appreciable loss of efficacy. Finally, the identification of subgroups who may respond well to intensified chemotherapy alone should be given a high priority in future investigations.

Appendix

The institutions and investigators who registered patients in this study were as follows: Hirosaki University (Etsuro Ito); National Kyushu Cancer Center (Yoshihisa Nagatoshi); Chiba University (Takeyuki Sato); Saga University (Eiichi Ishii); Yokohama City University (Koichiro Ikuta); Toho University (Akira Ohara); Kanagawa Children's Medical Center (Hisato Kigasawa); Japanese Red Cross Nagoya First Hospital (Kohji Kato); Ehime University (Yasushi Ishida); Gunma University (Chitose Ogawa); Hukui Medical College (Akihiko Tanizawa); Jikei University (Kohji Fujisawa); Sapporo Medical University (Nobuhiro Suzuki); Kyoto University (Tatsutoshi Nakahata); Tohoku University (Masue Imaizumi); National Center for Child Health and Development (Yukiko Tsunematsu); Tokyo Metropolitan Kiyose Children's Hospital (Tatashi Kaneko); Juntuedo University (Masahiro Saito); Japanese Red Cross Hiroshima Hospital (Kazuko Hamamoto); Okayama University (Megumi Oda); Chiba Children's Hospital (Yuri Okimoto); Saitama Children's Medical Center (Ryoji Hanada); Osaka Medical Center and Research Institute for Maternal and Child Health (Keisei Kawa); Tokai University (Miharu Yabe); Osaka City General Hospital (Masahiro Sako); Tokyo Metropolitan Komagome Hospital (Hidefumi Kaku); Kurume University (Haruhiko Eguchi); Kitazato University (Naoya Nakadate); Keio University (Eisuke Takahashi); Asahikawa Medical College (Makoto Yoshida); Ibaragi Children's Medical Center (Masahiro Tsuchida); Anjou Health and Welfare Hospital (Yuichi Miyajima); Yamanashi University (Kanji Sugita); Wakayama Medical Center (Keigo Hamahata); and National Seiroka Hospital (Ryota Hosoya)

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