Fig. 1.   Clinical courses and final outcomes of primary refractory patients. Tx denotes therapy; CR. complete remission; PR, partial remission; HDC/SCT, high dose chemotherapy and stem cell transplantation; BMT, bone marrow transplantation; Sib, sibling; PBSCT, peripheral blood stem cell transplantation; UCBSCT, unrelated cord blood transplantation.

Based on results of the univariate study (Table III), multivariate analysis was done with an adjustment for relapse within 12 months (vs. beyond), absence of HDC/SCT (vs. presence). Stage III/IV (vs. I/II), cell phenotype (T cell vs. B), and age at diagnosis (10 years and older vs. younger than 10). Histological immunophenotype lost its significance ($P = 0.25$) as a prognostic factor, while relapse within 12 months and absence of HDC/SCT were found to be statistically significant (Table III).

DISCUSSION

Although the prognosis of patients with LBL has been greatly improved, relapsed or primary refractory disease remains difficult to cure. According to the Children's Cancer Group's 5912 study, the survival rate was 33% at 2 years after relapse for 68 patients with non-Hodgkin lymphoma, including 26 LBL [3]. Nationwide studies performed in Austria (one patient survived among four relapsed/progressed LBL) and Germany (4 patients survived among 29 progressive T-LBL) also show poor prognosis of refractory/relapsed LBL [4,8]. In our present study, 3-year OS 43.2% was not satisfactory. Therapy for these patients remains poorly defined. Results of some studies suggest some roles of HDC/SCT for these patients [4,8,9]. Results of the present study show a significant hazard ratio for OS in absence of HDC/SCT. Although some selection bias might affect this retrospective study, it is interesting that it identified the possibility of a benefit of HDC/SCT for relapsed LBL. For acute lymphoblastic leukemia, which shares some characteristics with LBL, HDC/SCT reportedly results in longer
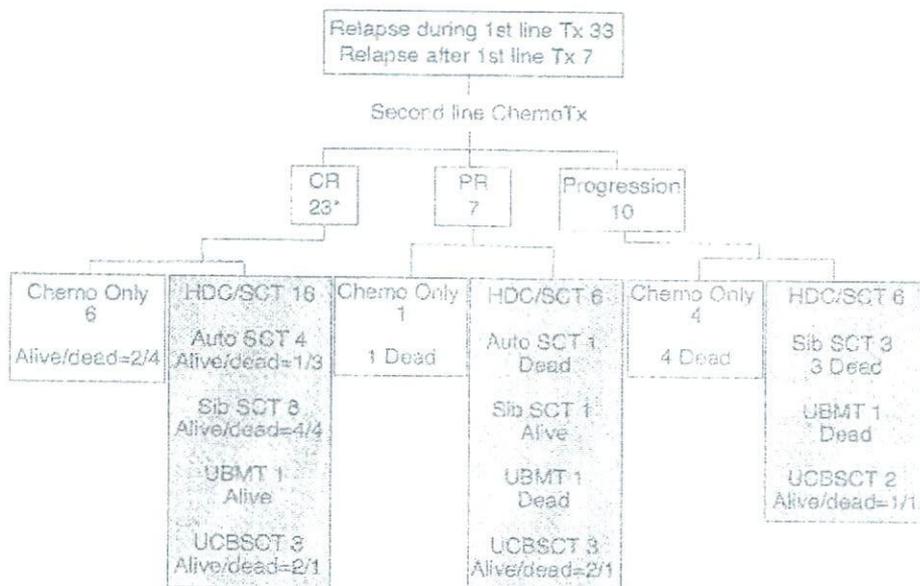

Fig. 2.   Clinical courses and final outcomes of recurrent patients. *This includes a patient with unknown subsequent therapy.
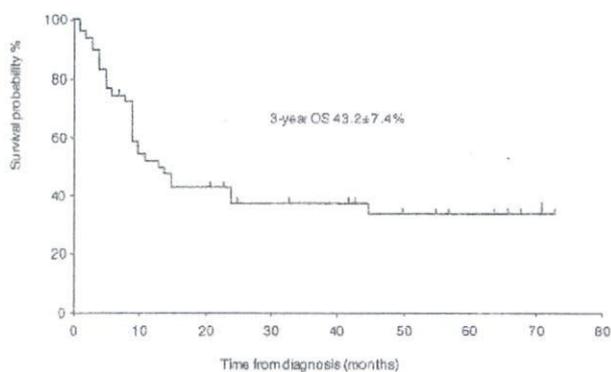
Fig. 3. Kaplan–Meier estimate of overall survival. The median follow-up period was 27.5 months.

leukemia-free survival when compared to outcomes of chemotherapy, especially for patients with poor prognoses [10,11]. However, a CCG1941 prospective study comparing chemotherapy versus HDC/SCT showed no significant advantage for HDC/SCT against chemotherapy for patients relapsing less than 12 months after completion of primary therapy [12]. The UK R1 study also showed that the related allograft was not significantly better than chemotherapy, although there was a moderate EFS benefit, especially in patients with a short first remission [13]. In our study, among 20 patients who relapsed within 12 months from initial diagnosis, 15 received HDC/SCT and 6 got CCR. The remaining 5 patients, all of whom had received chemotherapy, did not survive.

Nevertheless, no significance for OS was found between these groups of patients ($P = 0.052$).

For relapsed LBL patients, it is unknown which of allogeneic or autologous is the better donor source. Two recent reports described that allogeneic transplants engendered fewer recurrences but had higher related mortality in LBL patients [14,15]. Burkhardt reported that HDC followed by allogeneic SCT might have beneficial effects in refractory/relapsed cases of T-lymphoblastic lymphoma [8]. Although our study showed no significant difference of survival rates between allogeneic and autologous groups among all transplanted-patients, allogeneic SCT resulted in fewer relapses and progressive diseases than autologous SCT (8 patients among 26 showed relapse or progression after allogeneic transplantation, while 4 among 7 showed relapse or progression after autologous transplantation). Recent progress in supportive therapy during the SCT phase and adaptation to reduced intensity stem cell transplants might reduce transplantation-related mortality and lead to better outcomes [16].

Among the eight patients with primary refractory disease, only three achieved CR under ICE regimen or AML type therapy. For these patients, HDC/SCT was not a suitable salvage therapy. Six patients received allogeneic HDC/SCT as salvage therapy. Among them, only one patient who was on second relapse at the time of this analysis remained alive; all other patients died of recurrence or regimen-related toxicity. Three patients who showed progression after both first and second line chemotherapy died within 6 months from the initial diagnosis. Among 10 patients who showed progression of the disease in spite of second line therapy after relapse, only one patient was alive after UCBSCT. These

TABLE III. Analysis of Variable Factors Against Overall Survival

Factor	3-year OS	P for OS*	Hazard ratio	Confidence interval	P for OS**
Time of relapse, months					
≤12 (20)	31.9 ± 10.7	0.03	3.60	1.41–9.22	0.007
>12 (20)	51.6 ± 11.7				
Immunophenotype					
T cell (32)	32.8 ± 8.6	0.03	2.45	0.52–11.47	0.25
B cell (9)	72.9 ± 16.5				
Stage					
III/IV (39)	32.1 ± 7.9	0.07	1.55	0.41–5.86	0.51
I/II (9)	88.9 ± 10.5				
Age at diagnosis, years					
≥10 (22)	34.0 ± 10.5	0.09	2.08	0.88–4.92	0.10
<10 (26)	50.9 ± 10.2				
HDC/SCT					
Absence (14)	28.6 ± 12.1	0.10	2.64	1.07–6.52	0.035
Presence (33)	47.2 ± 9.3				
BM+					
Absence (29)	49.5 ± 9.6	0.42	NA	NA	NA
Presence (19)	34.0 ± 11.2				
CNS+					
Absence (40)	44.8 ± 8.2	0.40	NA	NA	NA
Presence (8)	37.5 ± 17.1				
Sex					
Male (32)	39.2 ± 9.3	0.58	NA	NA	NA
Female (16)	50.0 ± 12.5				

BM+, BM involvement at refractory/relapsed phase; CNS+, CNS involvement at refractory/relapsed phase; NA, not applicable. Number of patients are enclosed in parentheses. *P value is calculated using a double-sided log rank test; **Hazard ratio and P value is calculated using a Cox proportional-hazard model.

expressly refractory patients require novel therapeutic agents and strategies.

Predictive factors of poor survival are important when selecting patients for a new experimental therapy. Our data show that the time to relapse has prognostic importance. A BFM group study of relapsed T-cell LBL (29 patients) showed that age, gender, and localization of relapse had no prognostic value, which were same findings of our study. In contrast, they reported that the time of relapse was not associated with the outcome [8].

The outcomes of patients with relapsed/ primary refractory LBL were not satisfactory. However, those who responded to second line chemotherapy showed a respectable chance of survival. For these patients, HDC/SCT was associated with good prognosis. The relative rarity of refractory LBL patients highlights the need for carefully designed clinical trials through multicenter/international collaboration to answer issues of efficient second line chemotherapy, prognostic factors for these refractory patients, and SCT efficacy.

ACKNOWLEDGMENT

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JOURNAL OF CLINICAL ONCOLOGY

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Submitted May 30, 2008; accepted September 19, 2008; published online ahead of print at www.jco.org on January 12, 2009.

Supported by the Association Cent pour Sang la Vie and by the Institut Gustave-Roussy, France; the Forschungshilfe Station Peiper and the Berlin-Frankfurt-Muenster Study Centre, Giessen, Germany, the Associazione Italiana Contro le Leucemie and the Fondazione Città della Speranza, Padova, Italy; and the Ministry of Health, Labor and Welfare, Japan.

Presented in part at the 48th Annual Meeting of the American Society of Hematology, December 9-12, 2006, Orlando, FL and the 39th Annual Meeting of the International Society of Pediatric Oncology, October 30-November 3, 2007, Mumbai, India.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/09/2706-897/$20.00

DOI: 10.1200/JCO.2008.18.1487

Impact of the Methotrexate Administration Dose on the Need for Intrathecal Treatment in Children and Adolescents With Anaplastic Large-Cell Lymphoma: Results of a Randomized Trial of the EICNHL Group

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A B S T R A C T

Purpose

To compare the efficacy and safety of two methotrexate doses and administration schedules in children with anaplastic large-cell lymphoma (ALCL).

Patients and Methods

This randomized trial for children with ALCL was based on the Non-Hodgkin's Lymphoma–Berlin-Frankfurt-Muenster 90 (NHL-BFM90) study protocol and compared six courses of methotrexate 1 g/m^2 over 24 hours and an intrathecal injection (IT) followed by folinic acid rescue at 42 hours (MTX1 arm) with six courses of methotrexate 3 g/m^2 over 3 hours followed by folinic acid rescue at 24 hours without IT (MTX3 arm). This trial involved most European pediatric/lymphoma study groups and a Japanese group.

Results

Overall, 352 patients (96% ALK positive) were recruited between 1999 and 2005; 175 were randomly assigned to the MTX1 arm, and 177 were assigned to the MTX3 arm. Ninety-two percent of patients received protocol treatment. Median follow-up time is 3.7 years. Event-free survival (EFS) curves were superimposed with 2-year EFS rates (73.6% and 74.5% in the MTX1 and MTX3 arms, respectively; hazard ratio = 0.98; 91.76% CI, 0.69 to 1.38). Two-year overall survival rates were 90.1% and 94.9% in MTX1 and MTX3, respectively. Only two CNS relapses occurred (both in the MTX1 arm). Toxicity was assessed after 2,050 courses and included grade 4 hematologic toxicity after 79% and 64% of MTX1 and MTX3 courses, respectively ($P < .0001$); infection after 50% and 32% of courses, respectively ($P < .0001$); and grade 3 to 4 stomatitis after 21% and 6% of courses, respectively ($P < .0001$).

Conclusion

The results of the NHL-BFM90 study were reproduced in this large international trial. The methotrexate schedule of the NHL-BFM90 protocol including IT therapy can be safely replaced by a less toxic schedule of methotrexate 3 g/m^2 in a 3-hour infusion without IT therapy.

J Clin Oncol 27:897-903. © 2009 by American Society of Clinical Oncology

Anaplastic large-cell lymphoma (ALCL) is a rare disease in children.[1] Most European pediatric groups recommend a treatment with short-pulse chemotherapy based on high-dose methotrexate, cyclophosphamide, vincristine, doxorubicin, and corticosteroids,[2-5] whereas in North America, ALCL patients receive prolonged repeated pulse chemotherapy without high-dose methotrexate.[6] The 2-year relapse rate is approximately 30% with most of these regimens.[2-10] Although the CNS relapse rate is low in previous series of pediatric ALCL, most groups still recommend CNS prophylaxis based on high-dose methotrexate and/or an intrathecal (IT) injection of chemotherapy.[2-6,10] However, the im-

pact of the dose and mode of administration of methotrexate on the risk of systemic and CNS relapses in ALCL patients is unclear.

The Non-Hodgkin's Lymphoma–Berlin-Frankfurt-Muenster 90 (NHL-BFM90) protocol[4] is one of the most attractive treatments in ALCL as a result of the good results obtained in terms of event-free survival (EFS; 5-year EFS, 76%; 95% CI, 67% to 85%) and the lower cumulative doses of drugs, such as alkylating agents, etoposide, and anthracyclines, known to be associated with a risk of long-term toxicity compared with other pediatric and adult protocols. In this protocol, methotrexate was administered at a dose of 0.5 g/m^2 in a 24-hour infusion with IT,[3,4] whereas in studies by other pediatric groups such as in France or the United Kingdom,

methotrexate was administered at a dose of 3 g/m² in a 3-hour infusion, with no IT in the French protocol.

Because IT injections impair the quality of life of patients during treatment[11] and may be associated with rare but major complications such as myelopathy, arachnoiditis, or leukoencephalopathy,[12,13] it was decided to ascertain whether the results of the NHL-BFM90 protocol would be maintained by substituting the standard treatment with IT for a higher dose of methotrexate in a shorter infusion without IT. This was the aim of this trial, which compares the efficacy and safety of two methotrexate doses and administration schedules in children with ALCL.

Study Design

This study was an international randomized trial comparing six courses of methotrexate 1 g/m² over 24 hours and IT chemotherapy (MTX1 arm) with six courses of methotrexate 3 g/m² over 3 hours without IT (MTX3 arm). The main objective of this trial was to estimate the differences in EFS between the MTX3 and the MTX1 arms. Additionally, high-risk patients (defined as patients with mediastinal and/or skin and/or visceral involvement) could enter a second random assignment before the second course that tested the impact on EFS of adding vinblastine during the five latter courses and then weekly for a total duration of treatment of 1 year (vinblastine trial using a factorial design). Only the results of the first random assignment (methotrexate trial) are reported here. Results of the second random assignment (vinblastine trial) will be the subject of a subsequent report.

Eligibility Criteria and Pretreatment Evaluation

This trial was conducted in 12 countries by 10 national or cooperative groups including most European pediatric/lymphoma study groups and a Japanese group. Eligible candidates were patients with biopsy-proven ALCL who were less than 22 years of age. Slides had to be available for a national pathology review. Patients with isolated skin disease, completely resected stage I disease, or CNS involvement were not eligible for the trial. Additional exclusion criteria were previous treatment, congenital immunodeficiency, AIDS, previous organ transplantation, or previous malignancy. Written informed consent had to be obtained. The local ethics committees approved the protocol according to current legislation in each country.

The diagnosis of ALCL was based on morphologic and immunophenotypic criteria and, if possible, on molecular criteria. Mandatory antibodies were CD30, CD15, EMA, ALK1, CD79a, CD20, CD3, CD43, and CD45RO. Slides were reviewed nationally and by an international panel of pathologists blinded to treatment allocation.

Pretreatment Evaluation

Patients underwent a physical examination, a full blood count and biochemical profile, chest/abdominal computed tomography and skeletal scintigraphy, bone marrow aspirate smears and bone marrow biopsies, cerebrospinal fluid cytospin examination, and biopsy of all skin lesions. Patients were staged according to the St Jude and Ann Arbor staging systems. Patients were classified as high risk if they had at least one risk factor, defined as the presence of skin and/or mediastinal and/or visceral involvement (defined as lung, liver, or spleen involvement), and as standard risk if they had no risk factors.

Treatment

Chemotherapy was based on the NHL-BFM90 protocol.[4] All patients received a 5-day prephase followed by six alternating courses (A and B) administered every 21 days (Table 1). The methotrexate dose and administration schedule in courses A and B were randomly allocated before the first course (course A). The duration of chemotherapy between the prephase and the sixth course was 4 months.

Table 1. Chemotherapy Doses and Schedule in Each Course

Course and Drug	Dose and Schedule
Prephase	
Dexamethasone	5 mg/m² on days 1 and 2; 10 mg/m² on days 3 to 5
Cyclophosphamide	200 mg/m² on days 1 and 2
Triple intrathecal injection	Day 1
Course A	
Dexamethasone	10 mg/m² on days 1 to 5
Methotrexate	Random assignment* on day 1
Ifosfamide	800 mg/m² on days 1 to 5
Cytarabine	150 mg/m² × 2 on days 4 and 5
Etoposide	100 mg/m² on days 4 and 5
Course B	
Dexamethasone	10 mg/m² on days 1 to 5
Methotrexate	Random assignment* on day 1
Cyclophosphamide	200 mg/m² on days 1 to 5
Doxorubicin	25 mg/m² on days 4 and 5

*Arm MTX1 included methotrexate 1 g/m² in 24-hour infusion with triple intrathecal injection at day 1 and leucovorin rescue (15 mg/m²) at 42, 48, and 54 hours. Arm MTX3 included methotrexate 3 g/m² in 3-hour infusion with no intrathecal injection and leucovorin rescue (15 mg/m² every 6 hours) starting at 24 hours and ending when the methotrexate level was < 0.15 μm/L. Additionally, high-risk patients could enter the second randomized trial before the first course B (vinblastine trial), which randomly assigned patients to receive or not receive a vinblastine injection (6 mg/m²) during the five latter courses and then weekly for a total duration of treatment of 1 year.

Response Criteria

Tumor response was evaluated after each course. A comprehensive evaluation had to be performed once all signs of disease had disappeared or no later than after the sixth course. A complete remission was defined as the disappearance of the disease for at least 4 weeks. A residual lesion at the end of treatment was not considered a treatment failure if the residual tumor volume was less than 30% of the initial tumor mass. Follow-up was performed every 2 to 4 months for the first 3 years, every 6 months during years 4 and 5, and then yearly. Relapses were to be confirmed by a biopsy.

Random Assignment

Overall, 175 centers participated in the trial. Random assignment was balanced and stratified according to country and risk group (standard- v high-risk group). Five different data centers managed the random assignment. A centralized randomization software was used in all five data centers except in Italy, with a minimization program (France) or stratified random assignment with permuted blocks of size four (Japan, Germany, and Sweden). In the Italian data center, predefined stratified balanced random assignment lists were used to allocate treatments.

Additionally, high-risk patients could enter a second random assignment before the first course B to receive or not receive vinblastine. This second random assignment was stratified according to country and to treatment allocated by the first random assignment (factorial design).

Statistical Considerations

The primary end point was EFS, which was defined as the time from random assignment to first failure (progression, relapse, second malignancy, or death) or to the last follow-up visit for patients in first complete remission. Secondary end points were overall survival (OS), complete remission, CNS relapse, and acute toxicity.

OS rates were estimated from the date of random assignment to the date of death, whatever the cause, or the date of the last follow-up visit for patients last seen alive. Survival rates (EFS and OS) were estimated using the Kaplan-Meier method with Rothman's 95% CIs.[14] Median follow-up time was estimated using Schemper's method.[15]

Acute toxicity was assessed using the National Cancer Institute Common Toxicity Criteria, version 2.0.[16] Grade 4 hematologic toxicity and grade 3 or 4 nonhematologic toxicity were classified as severe toxicity.
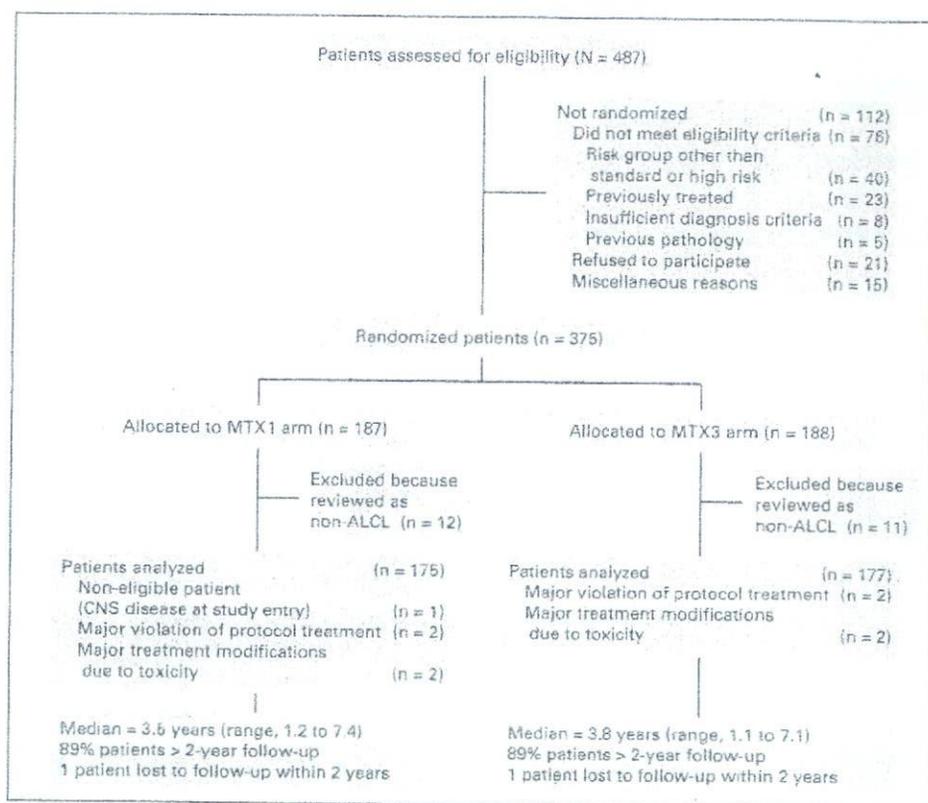
Fig 1. Participant flow CONSORT diagram. ALCL, anaplastic large-cell lymphoma

The issue raised in this trial was formulated as a noninferiority question in terms of EFS. Considering the factorial design of the trial, the sample size was determined for the vinblastine trial to demonstrate a reduction of the risk of events by adding vinblastine in high-risk patients. A total of 204 high-risk patients were required for the vinblastine trial. Assuming that the high-risk patients eligible for the vinblastine random assignment accounted for 64% of those eligible for the methotrexate random assignment, we expected to accrue 320 patients (204/0.64) onto the methotrexate trial during accrual onto the vinblastine trial. Given the expected sample size, it was recognized that a noninferiority conclusion could never be proven. Therefore, we planned to only provide CIs for differences in EFS in the two arms.

Planned Analysis

Three planned interim analyses were performed using Fleming's plan[17] and discussed with the independent data monitoring committee (IDMC). The present analysis, which is the final analysis, was performed with a one-sided $P = .0412$. The cutoff date of the present analysis was July 1, 2007.

The main analysis of EFS was to be performed on a modified intent-to-treat population, excluding only the patients for whom the diagnosis of ALCL had been rejected after review. Two secondary analyses were performed, one with no exclusions and the second on a per-protocol population that excluded patients who were not eligible for random assignment, patients for whom the diagnosis of ALCL had been rejected, and patients with a major modification of the allocated treatment.

The hazard ratios (HRs) for events (EFS) and death (OS) and their CIs were estimated using Cox models adjusted by the risk group (standard- high-risk group) and country and stratified by the treatment allocated by the second random assignment (not randomly assigned, no vinblastine, or vinblastine).

Prespecified secondary analyses, using Cox models, were performed to study variations in the treatment effect according to the risk group, treatment allocated by the second random assignment, and country. Heterogeneity in treatment effects according to country was assessed considering patients from Poland, Belgium, the Netherlands, and Sweden in a unique stratum because of a limited sample size in each of these countries. All reported P values for heterogeneity are two sided.

Toxicity rates between the MTX1 and MTX3 arms were compared using mixed models controlling for the number of the course (course 1 to 6) and the adjunction or not of vinblastine and considering the patient effect as a random effect (repetitive courses per patient). Data were entered and checked with the PIGAS software[18] and analyzed with SAS software (version 8.2; SAS Institute, Cary, NC).

Recruitment and Follow-Up

Between November 1999 and December 2005, 487 patients were screened for study entry. A total of 112 patients were not included in the trial (Fig 1). Thus, 375 patients (91% of the 411 potentially eligible patients) were included.

A central review of the slides was performed for 358 (95%) of 375 patients. The diagnosis of ALCL was rejected in 23 patients. Consequently, 352 patients were included in the main analysis; 175 were assigned to the MTX1 arm, and 177 were assigned to the MTX3 arm.

Baseline Data

The median age at diagnosis was 11.0 years (range, 4 months to 19.5 years). Baseline patient characteristics, overall and by treatment group, are listed in Table 2.

Table 2. Patient Characteristics by Treatment Arm

Characteristic	No. of Patients in MTX1 Arm (n = 175)	No. of Patients in MTX3 Arm (n = 177)	All Patients (N = 352)	
			No.	%
Male	103	108	211	60
Age, years				
< 3	10	9	19	5
3-16	151	157	308	88
> 16	14	11	25	7
Risk group				
Standard risk	65	68	133	38
High risk	109	109	218	62
CNS disease	1	0	1	0.3
"B" symptoms (MD, n = 2)	104	93	197	56
Site of disease				
Peripheral lymph node	150	158	308	88
Mediastinal involvement*	85	82	167	47
Lung lesion*	35	40	75	21
Liver involvement†	30	19	49	14
Spleen involvement†	39	25	64	18
Skin lesion‡	33	35	68	19
Soft tissue mass (MD, n = 1)	32	23	55	16
Bone lesion (MD, n = 43)	21/154	37/155	58/309	19
Bone marrow involvement§	28	14	42	12
St Jude stage				
1	14	10	24	7
2	29	37	66	19
3	106	116	222	63
4	26	14	40	11
Ann Arbor stage				
1	18	11	29	8
2	53	57	110	31
3	50	53	103	29
4	54	56	110	31
International Prognostic Index (MD, n = 73)				
0	22	30	52	19
1	39	40	79	28
2	45	45	90	32
3	34	24	58	21
Allocated treatment by the second random assignment				
No vinblastine	49	51	100	28
Vinblastine	49	47	96	27
Not randomly assigned in the R2 trial	77	79	156	44

Abbreviations: MTX, methotrexate; MD, missing data.
*Radiologic diagnosis by x-ray and/or computed tomography.
†Liver and spleen were considered involved if palpable clinically or enlarged on imaging > 5 cm below the costal margin or nodular on imaging
‡Skin involvement included biopsy-proven anaplastic large-cell lymphoma cutaneous involvement and clinically diagnosed skin lesions undoubtedly related to anaplastic large-cell lymphoma, with the exclusion of lesions limited to the skin overlying an involved node or a soft tissue mass.
§Bone marrow involvement was defined by the analysis of the bone marrow smears and trephine, using morphologic criteria.

All 352 patients were positive for CD30, 337 (96%) were positive for ALK, and 305 (87%) expressed at least one T-cell marker. According to the WHO classification,[19] which was available for 328 patients, the distribution of the subtypes was as follows: common type (n = 210, 64%), lymphohistiocytic (n = 10, 3%), small cell (n = 21, 6%), giant cell (n = 5, 1.5%), mixed (n = 76, 23%), and Hodgkin's-like (n = 6, 1.8%).

Treatment

Overall, 92% of the patients (162 patients in the MTX1 arm and 163 patients in the MTX3 arm) received protocol treatment of six courses with the planned methotrexate dose according to random

assignment. A major protocol violation was observed in four patients (two patients in both arms); the treatment was significantly modified as a result of toxicity in four additional patients (two patients in both arms). These eight patients are included in the main analysis but were excluded from the per-protocol analysis. A modification of the methotrexate dose or of the IT injection in less than three courses was also observed in nine and 10 patients in the MTX1 and MTX3 arms, respectively.

Outcome and Follow-Up

Median follow-up time was 3.8 years from random assignment. Only two patients were lost to follow-up. Disease disappeared completely from all initially involved sites in 309 (88%) of 352 patients.

Table 4. Acute Toxicity According to Treatment Arm

Reported Toxicity	Courses in MTX1 Arm (n = 1,025)			Courses in MTX3 Arm (n = 1,025)*			P†
	No. of Courses With Toxicity	No. of Courses Evaluated	% of Courses With Toxicity	No. of Courses With Toxicity	No. of Courses Evaluated	% of Courses With Toxicity	
All types, all grades	997	1,025	97	941	1,025	92	.002
Severe toxicity	846	1,025	83	701	1,025	68	< .0001
Hematologic grade 4 toxicity	812	1,024	79	655	1,022	64	< .0001
Neutropenia	794	1,024	78	639	1,023	62	< .0001
Anemia	83	1,024	8	50	1,023	5	.06
Thrombocytopenia	215	1,024	21	123	1,021	12	< .0001
Infection							
Grade 3-4	60	1,019	6	50	1,021	5	.32
All grades	508	1,019	50	331	1,021	32	< .0001
Other grade 3-4 toxicity	326	1,025	32	168	1,025	16	< .0001
Stomatitis	210	1,021	21	59	1,023	6	< .0001
Liver toxicity	128	955	13	97	977	10	.06
Miscellaneous	73	1,025	7	56	1,025	5	.13

Abbreviation: MTX, methotrexate.
*Detailed information on all courses (A and B) and toxicity observed after the courses was available for all patients except for one patient on the MTX3 arm.
†P value of the test comparing the toxicity rate between the two treatment groups by the means of mixed models controlling for the number of the course (course 1 to 6), the adjunction or not of vinblastine (treatment allocated by the second random assignment), the type of course (A v B), and the country, considering the patient effect as a random effect (repetitive courses per patient).

based on the NHL-BFM90 protocol with methotrexate at 3 g/m² in a 3-hour infusion without IT was superimposable on the EFS curve for patients treated with the same regimen but with methotrexate at 1 g/m² in a 24-hour infusion with IT. However, toxicity was significantly reduced in the MTX3 arm.

Conducting such a trial in this rare disease was only possible through the collaboration of European cooperative groups and a Japanese group. The external validity of this study is quite robust because, in all participating groups, most patients with childhood ALCL diagnosed between 1999 and 2006 were screened for trial entry with a random assignment rate of 91% among patients eligible for this trial. Furthermore, initial patient characteristics are those of the target population, as expected from previous reports.[20] The slides of the majority of patients were centrally reviewed, and the diagnosis of ALCL was rejected in only a small number of patients (23 of 358 patients).

The results of the NHL-BFM90 study[4] were reproduced in this international study. The 2-year EFS rate of 74% obtained for the whole trial population compares favorably with the results of previous reports on childhood ALCL.[2-6,9,10]

Although the EFS curves were superimposed, equivalence of the two arms in terms of EFS could not be statistically proven because of the limited number of patients. A total of 2,200 patients would have been required to prove noninferiority of MTX3 compared with MTX1, considering a 5% decrease in the 2-year EFS rate as the maximum allowable difference (limit HR = 1.23). Nevertheless, we were able to exclude the possibility that 2-year EFS of patients treated with MTX3 might be decreased by more than 7.3% compared with the EFS of patients treated with MTX1 with 95% confidence.

We demonstrated that the treatment in the MTX3 arm caused less hematologic and gut toxicity than the treatment in the MTX1 arm despite a higher dose of methotrexate. Decreased toxicity related to a shortened infusion of methotrexate has already been observed by the BFM group in the NHL-BFM95 study comparing methotrexate in a 4-hour infusion with methotrexate in a 24-hour infusion in childhood

B-cell non-Hodgkin's lymphoma.[21] In the present study, the interval between the end of the MTX infusion and folinic acid rescue was reduced in the short infusion arm. Therefore, the higher toxicity rate observed in the MTX1 arm may be a result of longer exposure to methotrexate as well as the delayed rescue.

In this study, only two patients had a CNS relapse as a first event. The low incidence of CNS relapses in ALCL has been evidenced in a number of previous reports in children[2,3,5,6,9,10,22-24] and adults.[25,26] However, most pediatric groups still recommend minimal CNS prophylaxis based either on high-dose methotrexate or on IT injections. In previous studies, the 3 g/m² dose of methotrexate in a 3-hour infusion was shown to provide potentially cytotoxic concentrations of the drug in CSF for several hours after the infusion.[27] The present study confirms that replacing methotrexate 1 g/m² in a 24-hour infusion plus an IT injection with methotrexate 3 g/m² in a 3-hour infusion is not associated with any excess CNS relapses in ALCL. Furthermore, the omission of triple IT therapy and the reduction in toxicity in the MTX3 arm should contribute to an improvement in the quality of life of the patients during treatment. Although toxicity was reduced in the MTX3 arm, this regimen still induces substantial acute toxicity. However, the low total doses of anthracyclines (150 mg/m² of doxorubicin) and alkylating agents (3.4 g/m² of cyclophosphamide and 12 g/m² of ifosfamide) in this regimen should avoid long-term complications.

Nevertheless, it is difficult to assess the exact role of high-dose methotrexate in the treatment of childhood ALCL. The results obtained in pediatric ALCL by the Pediatric Oncology Group,[5] with protocols based on doxorubicin, prednisone, and vincristine chemotherapy plus triple IT injections but without high-dose methotrexate, or in adults by several cooperative groups with the cyclophosphamide, doxorubicin, vincristine, and prednisone regimen are similar to those of our study.[28] These protocols are associated with less acute toxicity than the ones described in this study. However, the cumulative doses of anthracyclines and/or alkylating agents are significantly higher than those in the ALCL99 protocol and, therefore, may lead to long-term

adverse effects. Further trials are needed to assess whether methotrexate can be safely omitted from a short intensive treatment similar to the ALCL99 regimen for some subgroups of patients.

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Appendix

The following groups participated in this study: European Intergroup for Childhood Non-Hodgkin Lymphoma; Société Française de Lutte Contre les Cancers et Leucémies de l'Enfant; Associazione Italiana di Ematologia ed Oncologia Pediatrica; United Kingdom Children's Cancer and Leukaemia Group; Japanese Pediatric Leukemia/Lymphoma Study Group; Polish Pediatric Leukemia/Lymphoma Study Group; Austria–Berlin-Frankfurt-Muenster Group; Dutch Childhood Oncology Group; Belgian Society of Paediatric Haematology and Oncology; Nordic Society for Pediatric Hematology and Oncology; and Berlin-Frankfurt-Muenster Group.

ORIGINAL ARTICLE

Outcome of bone marrow transplantation from HLA-identical sibling donor in children with hematological malignancies using methotrexate alone as prophylaxis for graft-versus-host disease

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Abstract Most previous studies of graft-versus-host disease (GVHD) prophylaxis with methotrexate (MTX) alone in patients undergoing HLA-identical sibling donor bone marrow transplantation were performed in adults. With this background, we attempted to analyze the incidence and risk factors of GVHD in bone marrow transplantation (BMT) from an HLA-identical sibling donor in children with hematological malignancies using MTX alone as a prophylaxis for GVHD. Ninety-four patients received MTX by intravenous bolus injection, with a dose of 15 mg/m^2 on day +1, followed by 10 mg/m^2 on days +3, +6, and +11, and then weekly until day +60. The probability of developing grade II–IV acute GVHD and chronic GVHD was 19.1 and 31.8%, respectively. Age at transplantation and a female donor to male recipient were identified as risk factors for chronic GVHD in multivariate analysis, but no factors were identified for acute GVHD. The cumulative incidence of transplant-related mortality during the first 100 days was 9.6%. Disease-free survival at 5 years for standard- and high-risk patients was 82.1 and 39.5%, respectively. These results suggest that GVHD prophylaxis with MTX alone is safe and effective in young children under 10 years old at transplantation and in a setting other than female donor to male recipient.

Keywords HLA-identical sibling donor · GVHD prophylaxis · Methotrexate alone

1 Introduction

Allogeneic bone marrow transplantation (BMT) is an effective treatment for patients with hematologic malignancies, bone marrow failure syndromes, and congenital disorders of the lymphohematopoietic system. The transplant outcome depends on the severity of complications such as graft failure, infection, graft-versus-host disease (GVHD), organ damage, and the disease stage GVHD is a major complication of allogeneic BMT that results in significant morbidity and mortality. It occurs, despite prophylaxis, in 30–50% of patients undergoing transplantation from HLA-identical sibling donors [1] and in 50–80% of patients with transplants from HLA-matched unrelated donors [2]. Previous studies have shown that the combination of cyclosporine-A (CyA) and four doses of methotrexate (MTX) is more effective than either agent alone in the prevention of GVHD [1]. Thus, a regimen including CyA or FK506 plus short-term MTX (sMTX) was established in adults, even for unrelated donors [3, 4]. Although several investigators have reported data from multicenter randomized clinical trials to evaluate the effectiveness of GVHD prophylaxis regimens in adults,

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few data are available for pediatric patients, who usually show a lower incidence and less severe GVHD than adult patients. Ringden et al. [1] reported that the probability of developing acute GVHD did not differ between single or combined prophylaxis regimens in a pediatric population, and Locatelli et al. [5] reported that the incidence of GVHD in childhood was low compared to that in adults. Furthermore, Bacigalupo et al. [6] demonstrated in a randomized trial involving adults that GVHD prophylaxis with low-dose CyA (1 mg/kg per day) decreases the risk of relapse more than a higher dose (5 mg/kg per day), possibly because of a graft-versus-leukemia (GVL) effect. However, there is still a lack of data on pediatric patients, who usually show a different incidence and severity of GVHD than adult patients. Locatelli et al. confirmed that the use of low-dose CyA (1 mg/kg per day) led to a more favorable survival rate than regular-dose CyA (3 mg/kg per day) as a single prophylactic agent in pediatric patients [7]. However, in their report, almost all patients showed standard features, including acute leukemia in first or second complete remission (CR).

Herein, we report the effectiveness of MTX as a single agent for GVHD prophylaxis in 94 pediatric patients with hematological malignancies who underwent BMT from HLA-identical sibling donors including high-risk features. We also retrospectively analyzed the risk factors and incidence of GVHD.

2 Patients and methods

2.1 Patient characteristics

Ninety-four patients, aged 1–15 (median: 8 years old) received transplantations from HLA-identical sibling donors at the Japanese Red Cross Nagoya First Hospital between 1984 and 2000. The clinical characteristics of the patients are shown in Table 1.

All patients received MTX alone for GVHD prophylaxis. Patients were classified as having standard- or high-risk disease based on previously described criteria [8, 9]. Briefly, patients were categorized as standard-risk cases if they had acute lymphoblastic leukemia (ALL) in first or second complete remission (CR), acute myelogeneous leukemia (AML) in first CR, chronic myelogenous leukemia (CML) in the first chronic phase (CP), or malignant lymphoma in first CR. The other 38 patients, including those who received a second transplantation (five cases), were categorized as high-risk cases. Chromosomal abnormalities classified as standard risk included ALL with translocations of 9;22 (three cases) and 11q23 (three cases), as well as AML with translocations 8;21 (six cases) and 15;17 (two cases). ALL patients with 9;22 (four cases) and 11q23 (two

Table 1 Patient and donor characteristics

Patients	n = 94		%
Sex	Female	43	45.7
	Male	51	54.3
Age, median (range)		8 (1–15)	
	<10	56	59.6
	≥10	38	40.4
Disease	ALL	42	44.7
	CR1–2	27	
	CR3–5	3	
	Relapse	12	
	AML	35	37.2
	CR1	23	
	CR2	5	
	Relapse	7	
	AUL	4	4.3
	CR1	2	
	Relapse	2	
	CML	3	3.2
	CP1	2	
	BP	1	
	ML	5	5.3
	CR1	3	
	Relapse	2	
	MDS	5	5.3
Risk[a]	Standard risk	56	59.6
	High risk	38	40.4
Time at SCT	First	89	94.5
	Second	5	5.5
Conditioning	TBI	30	32
	Non-TBI	64	68
Post-BMT growth factor	None	43	45.7
	G-CSF	51	54.3
Donors	Age	9 (1–21)	
Donor sex	Female	45	47.9
	Male	49	52.1
Donor/patient sex	F to F	24	25.5
	F to M	21	22.3
	M to F	19	20.2
	M to M	30	31.9
ABO blood group	Compatible	63	67
	Minor mismatch	10	10.6
	Major mismatch	12	12.8
	Major and minor mismatch	9	9.6

ALL acute lymphoblastic leukemia, CR complete remission, AML acute myelogeneous leukemia, AUL acute unclassified leukemia, CML chronic myelocytic leukemia, CP chronic phase, BP blastic phase, ML malignant lymphoma, MDS myelodysplastic syndrome, SCT stem cell transplantation, TBI total body irradiation*standard risk; ALL CR1 or −2, AML CR1, AUL CR1, ML CR1, CML CP1, high risk; others

[a] Standard risk; ALL CR1 or −2, AML CR1, AUL CR1, ML CR1, CML CP1, high risk; others

cases) were included as high-risk patients because they received BMT at relapse. As of December 2005, the median follow-up duration was 161 (66–249) months. HLA typing of the donors and recipients was performed by serology. Previous chemotherapy regimens varied because the patients were treated at their referring institutions.

2.2 Pretransplant preparative regimens

The conditioning regimens are described in Table 1. Thirty-two patients received a preparative regimen consisting of busulfan (4 mg/kg per day × 4 days) and melphalan (LPAM) (180–210 mg/m^2), and 32 patients received busulfan (4 mg/kg per day × 2 days) in addition to LPAM + TBI (12–13.2 Gy). Thirty patients received other TBI-based regimens, such as cytarabine (CA) (4–6 g/m^2 per day × 2 days)/cyclophosphamide (CY) (60 mg/kg per day × 2 days)/TBI, CY/TBI, thiotepa (TEPA) (800 mg/m^2)/TBI, TEPA/CY/TBI, LPAM/TBI, and VP-16 (60 mg/kg per day)/LPAM/TBI.

2.3 Prophylaxis and treatment of GVHD

All patients received MTX alone as GVHD prophylaxis. MTX was scheduled to be given intravenously as a bolus injection at a dose of 15 mg/m^2 on day +1, followed by 10 mg/m^2 on days +3, +6, and +11, and then weekly until day +60, shorter than the Seattle protocol [10]. Folinic acid was given at 3 mg orally in divided doses on the next day of MTX injection to prevent mucositis caused by MTX. When patients developed acute GVHD of grade II or more, and extensive-type chronic GVHD, steroid therapy was started. If patients showed no improvement, CyA was added, according to the physician's assessment.

Acute GVHD was evaluated on an individual basis according to the standard criteria by Glucksberg [10]. Chronic GVHD was assessed as either limited or extensive, based on clinical and/or histological findings, as described by Glucksberg and Shulman, respectively [10, 11]. Mucositis and liver dysfunction were graded using the National Cancer Institute Common Toxicity Criteria (NCI-CTC). Interstitial pneumonia was diagnosed based on the clinical condition and computed tomography. If patients developed mucosal toxicity, liver/renal dysfunction, and interstitial pneumonia, the dose of MTX was withheld, at the physician's discretion.

2.4 Engraftment

Engraftment of neutrophils and platelets was defined as the first of three consecutive days with an absolute neutrophil count (ANC) >0.5 × 10^9/l and unsupported platelet count >50 × 10^9/l.

2.5 Statistical analysis

Acute and chronic GVHD, overall survival, disease-free survival (DFS), rate of relapse of malignant diseases, and transplant-related mortality (TRM) were assessed using the cumulative incidence and Kaplan–Meier product limit estimates. Significance between patient populations was tested using the log-rank test. In DFS analysis, both relapse and death in remission due to any cause were considered events, whereas, in relapse rate analysis, only disease relapse was considered as failure. In TRM analysis, all deaths not due to disease relapse were considered events. Risk factors of acute and chronic GVHD were analyzed using Cox proportional hazard analysis. Children showing sustained donor engraftment and surviving for more than 21 days and more than 100 days after the transplant were assessable for the occurrence and severity of acute and chronic GVHD, respectively. Factors that appeared to be predictive of developing grade II–IV acute GVHD and chronic GVHD in univariate analysis ($P < 0.10$) were considered for inclusion in multivariate Cox regression models. The likelihood ratio test was used to determine whether variables should be added or dropped from the multivariate model. The STATA package (STATACORP LP, College Station, TX, USA) was used for data analysis.

3 Results

3.1 Engraftment

The median amount of infused marrow-nucleated cell dose was 4.0 × 10^8/kg (range: 0.98–7.2 × 10^8/kg), and 92 patients (98%) showed neutrophil engraftment at a median of 17 days (range: 10–40), and 67 patients (71%) exhibited platelet engraftment at a median of 35 days. In patients receiving granulocyte colony-stimulating factor (G-CSF) after BMT, neutrophil engraftment was confirmed at 15 days, and that without G-CSF was confirmed at 20 days ($P < 0.01$). Three patients died before neutrophil engraftment of hepatic veno-occlusive disease (VOD) or invasive fungal infection with bacterial pneumonia, and 28 patients died prior to platelet engraftment.

3.2 Acute GVHD

In 91 evaluable patients, 30 (33%) developed grade I–IV acute GVHD. The cumulative incidence of grades II–IV and III–IV acute GVHD was 19.8 and 11%, respectively (Fig. 1). For 18 patients who developed acute GVHD (grade ≥II), MTX was replaced with prednisolone for the treatment of acute GVHD. CyA was added in 11 patients to treat GVHD, and ATG (anti-thymocyte globulin) was
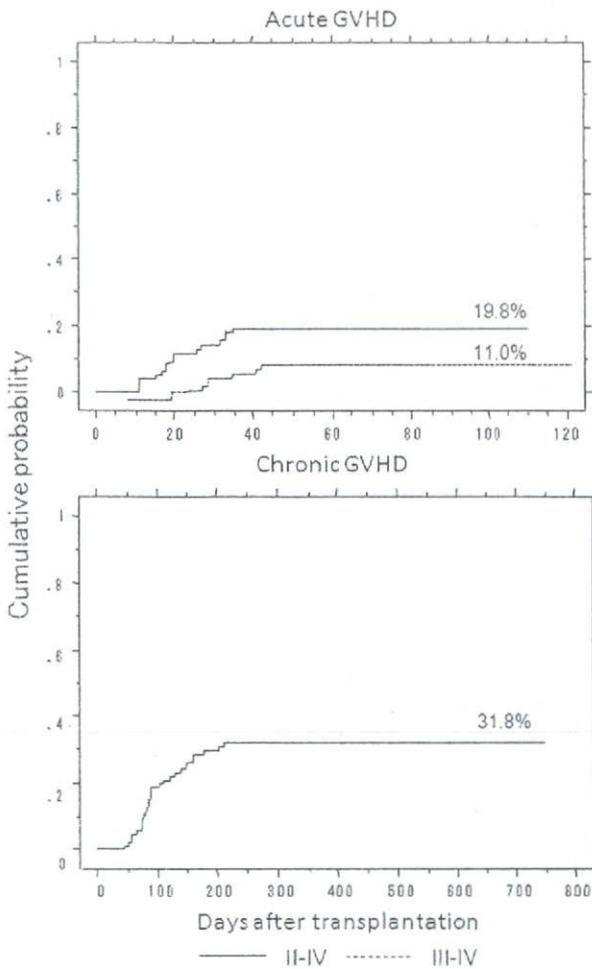
Fig. 1 Cumulative incidence of acute and chronic GVHD. *Upper* and *lower panels* show the cumulative incidence of acute and chronic GVHD. respectively

Table 2 Univariate analysis of potential risk factors for chronic GVHD

Factor	RR	95% CI	P value
Sex			
Male	1.00		
Female	1.12	0.53–2.38	0.77
Patient age (years)			
<10	1.00		
≥10	2.15	1.13 5.24	0.02
Risk			
Standard risk	1.00		
High risk	1.71	0.80–3.66	0.17
Conditioning			
TBI +	1.00		
TBI −	0.94	0.43–2.05	0.88
Busulfan +	1.00		
Busulfan −	0.86	0.38–1.97	0.73
Donors age			
<10	1.00		
≥10	1.14	0.54–2.43	0.73
Donor sex			
Male	1.00		0.26
Female	1.55	0.73–3.32	0.02
Donor/patient sex			
M to M	1.00		
F to F	1.80	0.48–6.70	0.38
M to F	2.99	0.90–9.93	0.07
F to M	3.87	1.21–12.34	0.02
ABO blood group			
Compatible	1.00		
Mismatch	1.12	0.51–2.45	0.77

RR indicates relative risk, *CI* confidence interval

given to four patients. Although the data are not shown, no risk factors for the development of grade II–IV acute GVHD were identified in univariate analysis.

3.3 Chronic GVHD

Although GVHD at 100 days or later after transplantation is defined as chronic GVHD by the classical criteria [10]. typical clinical and histological features of chronic GVHD could occur as early as 40 days post-transplantation. In our study. 27 of 85 assessable patients (31.8%) developed limited (seven patients) or extensive (20 patients) chronic GVHD (Fig. 1), and ten of 27 patients with cGVHD stopped receiving MTX. Sixteen of 27 patients developed cGVHD before 100 days after transplantation. including 11 patients diagnosed by histological examination and five patients with diagnostic signs based on the National Institutes of Health (NIH) consensus criteria [12]. On univariate

analysis, an older patient age (>10 years old) and female donor to male recipient were significantly associated with the risk of developing chronic GVHD (Table 2). Even in multivariate analysis, these two factors were identified as significant risk factors for chronic GVHD. and female donor to male recipient was the most significant predictive factor in different pairs of sex combinations (Table 3).

3.4 Compliance and toxicity of MTX administration

Twenty-three patients stopped receiving MTX by day −60. with a median of day +25 (range: 1–46). and a median of six doses (range: 1–9). The reasons for MTX discontinuation were the treatment of acute GVHD (nine patients). liver dysfunction (ten patients, including six patients with VOD and four patients with abnormal liver function test (grade 3 NCI-CTC)), two with respiratory failure, and two early deaths with severe infection. No patients stopped

Table 3 Multivariate analysis of potential risk factors for chronic GVHD

Factor	RR	95% CI	P value
Patient age (years)			<0.001
<10	1.00		
≥10	3.09	1.40–6.84	
Donor/patient sex			<0.001
M to M	1.00		
F to F	1.55	0.42–5.80	0.51
M to F	3.32	0.99–11.08	0.05
F to M	4.80	1.48–15.57	<0.001

receiving MTX because of grade IV mucositis of NCI-CTC. For these patients who stopped receiving MTX before day +60, prednisolone was started. The risk factors for MTX discontinuation were acute GVHD (≥grade 2) and second stem cell transplantation (SCT) (data not shown). Eighteen of 23 patients who stopped receiving MTX survived for more than 100 days after transplantation, and ten of 18 patients developed chronic GVHD. Thirteen patients (14.8%) developed interstitial pneumonia, and five of 13 patients died of respiratory failure (two cases) or other reasons (three cases).

3.5 Relapse and survival

The relapse rate for all patients was 22%, with a median of 5.73 months (range: 0.87–137). The relapse rates of standard-risk (SR) and high-risk (HR) patients were 11.6 and 36.8%, respectively, which were significant ($P = 0.002$) (Fig. 2).

The rate of transplant-related mortality (TRM) was 7.1 and 27.5% in SR and HR patients. respectively ($P = 0.01$).

Causes of death are listed in Table 4. Relapse was the most frequent cause of death. After relapse, respiratory failure (e.g., interstitial pneumonia, bronchiolitis obliterans) was the major cause of death. The probability of transplant-related mortality was 14.4% for all patients, and that of early (<100 days) TRM was 9.6%. The risk of transplant-related mortality was significantly greater in HR patients (TRM: 27.5%, early TRM: 18.4%) compared to SR patients (all TRM: 7.1%, early TRM: 3.6%) ($P = 0.01$). Disease-free survival (DFS) for all patients was 64.9% at 5 years, and was significantly higher in SR (82.1%) compared to HR (39.5%) patients ($P = 0.001$) (Fig. 2).

Stratifying the risk of disease, we analyzed the GVL effect with or without cGVHD. In fact, the relapse rate for SR patients with cGVHD was 6.7% compared with the 14.1% observed in patients without cGVHD ($P = 0.52$). For HR patients, the development of cGVHD was
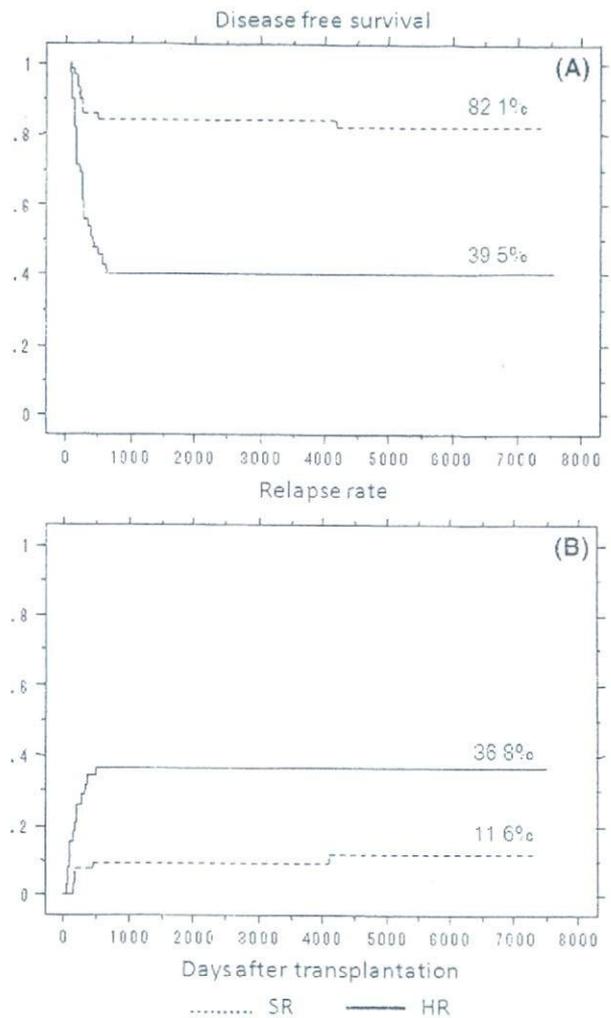

Fig. 2 a Disease-free survival. b Cumulative incidence of relapse. Standard-risk (*SR*) patients (*discontinuous line*), high-risk (*HR*) patients (*continuous line*)

Table 4 Cause of death ($n = 32$)

Cause	
Relapse	18
Rejection	1
Interstitial pneumonitis	2
Obstructive bronchiolitis	4
Infection	1
Acute GVHD	1
Veno occlusive disease	3
CNS toxicity	2

associated with a lower relapse rate (25%) than that of patients without cGVHD (47.4%), even though this was not significant ($P = 0.15$). In the same way, no significant
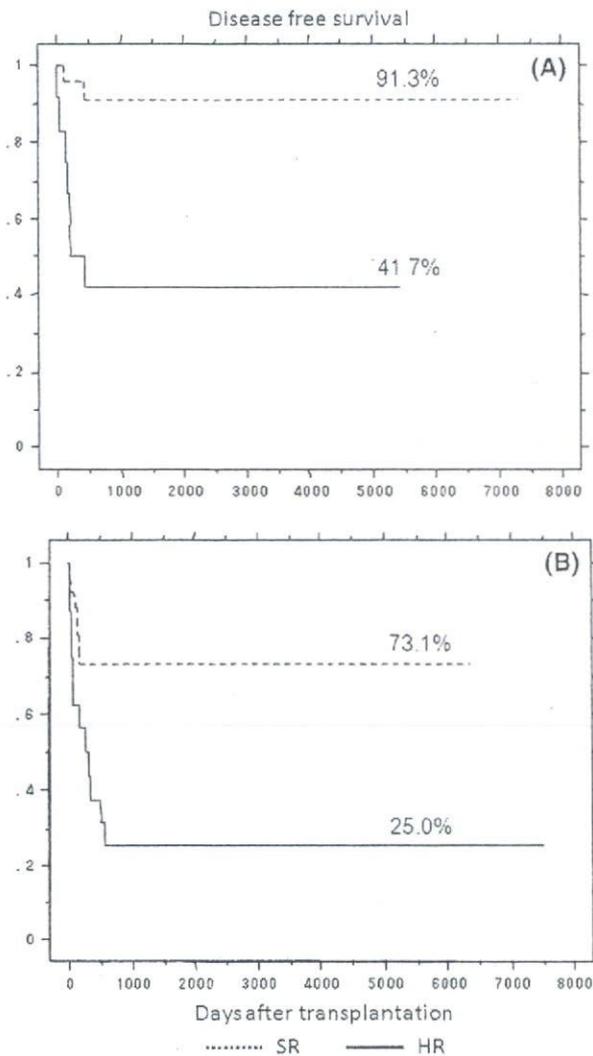
Disease free survival

Fig. 3 Disease-free survival in patients with (a) acute myelogenous leukemia (*AML*) and (b) acute lymphoblastic leukemia (*ALL*). Standard-risk (*SR*) patients (*discontinuous line*), high-risk (HR) patients (*continuous line*)

difference was observed for DFS between patients with or without cGVHD (64.2 vs. 66.7%, respectively, $P = $ NS). Meanwhile, stratifying the type of disease, DFS in AML patients was 91.3% in SR and 41.7% in HR patients, and the relapse rate was 4.3 and 41.7%, respectively. In ALL patients, DFS was 73.1% in SR and 25% in HR patients, and the relapse rate was 16 and 50%, respectively (Fig. 3).

4 Discussion

In this study, we analyzed the probability and risk factors of GVHD using MTX monotherapy as a prophylaxis in HLA-matched sibling bone marrow transplantation for

patients with hematological malignancies. In previous studies, the incidence of GVHD using MTX as a prophylaxis was 48–53% for grade II–IV acute GVHD and 9–36% for chronic GVHD [1, 13]. In a randomized study of patients with leukemia, the incidence and severity of acute GVHD was lower in patients receiving CyA + MTX than in those with CyA monotherapy [14]. Furthermore, compared with MTX alone, CyA was associated with lower rates of interstitial pneumonia, treatment-related mortality, and treatment failure [1]. However, these studies were exclusively performed in adult populations, and few reports have described the incidence and severity of GVHD using MTX monotherapy as a prophylaxis in a pediatric population. Aschan et al. [15], demonstrated that MTX combined with CyA increases leukemic relapse compared to monotherapy, even though it decreases GVHD, and the GVL effect is supported by studies that improved leukemia-free survival in adults with AML who had acute or chronic GVHD [16]. Based on previous experience, the risk of GVHD in a pediatric population has been considered to be lower than that in adults, and an older patient age is a risk factor for the development of GVHD [17]. For the above reasons, a single agent could be sufficient for the prevention of GVHD in pediatric patients. Koga et al. [8] reported no significant difference in the incidence of acute GVHD (grades II–IV) or any type of chronic GVHD between patients who received MTX or CyA (28.3 vs. 44% for acute GVHD and 19 vs. 20% for chronic GVHD, respectively).

In this study, we reported the feasibility of GVHD prophylaxis with MTX alone in 94 pediatric patients with hematological malignancies. Although the incidence of chronic GVHD was comparable with previous studies, the incidence of acute GVHD using MTX alone as a prophylaxis was lower in our study. This reason could be due to the genetic homogeneity of Japanese [9]. The relapse rate was 11.6% in standard-risk and 36.8% in high-risk patients. In the standard-risk setting, this result was superior to other reports [6, 7, 13]. The survival rate of all patients was 64.9%, which is also comparable to previous reports [7, 18, 19]. Especially, in standard-risk patients with AML, the DFS rate was higher than in previous reports [19, 20]. Neudorf et al. [19] reported the results of allogeneic bone marrow transplantation for children with AML in first CR using MTX alone as GVHD prophylaxis. The patients received 4 × 4 mg/kg of busulfan and 50 mg/kg × 4 of cyclophosphamide as a conditioning regimen and MTX alone as GVHD prophylaxis until day 100. In their study, the incidence of chronic GVHD, overall survival, and DFS rates were 21, 67, and 57%, respectively. In our study, AML patients received MTX until day 60 as GVHD prophylaxis, and the incidence of chronic GVHD in our patients was relatively higher (31%), but the 91% DFS rate

and 4.3% relapse rate in SR patients were superior to those of previous reports. Similarly to what Matsuyama et al. reported previously, almost all of our patients received busulfan (4 mg/kg per day × 4 days) and melphalan (LPAM) (180–210 mg/m²) as a conditioning regimen [21]. Probably, our results are dependent on the graft-versus-leukemia effect and eradication of leukemic cells by melphalan.

Based on karyotypic analysis at diagnosis, AML patients with translocations 8;21 and 15;17 are classified as having a favorable risk. Slovak et al. [22] observed superior overall survival after transplantation compared to chemotherapy among AML patients showing favorable chromosomal abnormalities. Conversely, Schlenk et al. [23] observed no difference between allogeneic stem cell transplantation (SCT) and intensive chemotherapy for this group of AML patients. Indeed, our current practice does not suggest that AML with an abnormal karyotype of t(8;21) and t(15;17) is an indication for sibling donor SCT in the first remission. However, in our study, among AML patients without these favorable abnormal karyotypes, DFS was 93% in standard-risk and 41.7% in high-risk patients (data not shown).

Although Horeowitz et al. [24] reported the direct antileukemic effect of MTX on relapse after transplantation for ALL, in our study, DFS for standard-risk ALL patients was not superior to that of AML patients. The reasons may be that, in our study, more AML patients received transplantation at first CR and the graft-versus-leukemia effect might occur more preferentially in AML patients [25].

Although one of the major toxicities of MTX is mucositis, it was not a reason for MTX cessation in this study. The major reason for its cessation was liver dysfunction because of GVHD or VOD, and predictive factors of MTX cessation were the development of acute GVHD ($\geq$ grade 2) and second transplantation. Ringden et al. [1] reported that MTX was associated with increased rate of interstitial pneumonia, treatment-related mortality, and treatment failure, compared with CyA in adult patients. However, in our study, the incidence of interstitial pneumonia was 14.8%, being lower than in previous reports [1, 24].

In the search for predictive factors of GVHD development, patient age and female donor to male recipient were found to be significant for the development of chronic GVHD, but no risk factors for acute GVHD were identified. Neudorf et al. [19] demonstrated that children older than 10 years are at a higher risk for developing severe acute GVHD, and others reported that age at transplantation and female donor to male recipient were risk factors for chronic GVHD in adult and pediatric populations [26]. Although Kollman et al. [27] demonstrated that donor age was a significant risk factor for GVHD, we did not document donor age as a risk factor of GVHD. Although the

data are not shown, patient age and female donor to male recipient were also significant risk factors for extensive chronic GVHD. In this study, the association of acute and chronic GVHD with a reduced risk of relapse was not documented, along with the association with overall survival, for patients with each high- or standard-risk malignancy. In the future, in addition to MTX, calcineurin inhibitors should be considered for patients undergoing bone marrow transplantation from an HLA-identical sibling in the setting of patients aged over 10 years old and a female donor to male recipient.

In this study, we reported the results of BMT from HLA-identical sibling donors in 94 pediatric patients with hematological malignancies using MTX alone as GVHD prophylaxis, and the relapse rate, OS, and DFS were found to be favorable compared to previous reports. In conclusion, we consider that the use of MTX alone is feasible to prevent severe acute GVHD and may reduce the risk of leukemia recurrence, possibly because of an enhanced GVL effect in the pediatric population, although the incidence of chronic GVHD was comparable to previous reports. In the future, a randomized control study should be considered to document the availability of MTX alone as GVHD prophylaxis in pediatric patients with hematological malignancies.

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Clinical features and outcome of *MLL* gene rearranged acute lymphoblastic leukemia in infants with additional chromosomal abnormalities other than 11q23 translocation

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Abstract

The treatment outcome for infant acute lymphoblastic leukemia (ALL) with positive *MLL* gene rearrangements remains poor. We analyzed whether additional chromosomal abnormalities (ACA) other than 11q23 translocation could affect the disease behavior and its prognosis.

Eighteen of seventy-four patients with infant acute lymphoblastic leukemia showed ACA, including three-way translocations in four, other novel translocations in four, and complex structural chromosomal changes in four. Only age less than 6 months and positive central nervous system leukemia were significant prognostic factors by multivariate analysis. However, overall survival rates were worse in patients with ACA compared to those with non-ACA. Genetic alterations induced by additional chromosomal changes may be associated with disease progression and poorer overall survival rates in infants with *MLL*-rearranged ALL.
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Keywords: Acute lymphoblastic leukemia; Infants; *MLL* gene rearrangements; Additional chromosomal abnormalities; Prognostic factor

1. Introduction

Efforts in clinical trials to improve the outcome for infants with acute lymphoblastic leukemia (ALL), one of the subtypes of childhood ALL with poor outcome, enabled overall survival rates of 40% or higher [1–3]. However, outcomes for infants with positive *mixed lineage leukemia* (*MLL*) gene rearrangements, found in 70–80% of infant ALL cases studied with molecular techniques, remain poor, despite the use of intensive multiagent chemotherapy in combination with hematopoietic stem cell transplantation [1,4,5]. Multivariate analyses on recently conducted large-scale clinical studies have revealed several risk factors among infants with ALL, including a rearranged *MLL* gene, younger age (<3 or 6 months), very high white blood cell count ($\geq 300,000/\mu L$), and poor response to initial prednisone therapy [2,3]. Among these factors, presence of *MLL* gene rearrangement is the most important, significantly correlated with both the adverse clinical features and the poor prognosis that is characteristic of this distinct subtype of childhood ALL [4].

The *MLL* gene is disrupted by 11q23 translocation and fuses to more than 55 different partner genes; mainly, *AF4/FEL* in 4q21, *AF9* in 9p22, *ENL* in 19p13, *AF6* in 6q27 and *ELL* in 19p13.1 [6,7]. The partner genes encode nuclear proteins with transcriptional activities or proteins with dimerization/oligomerization motifs, suggesting that the impaired transcriptional activity by the fusion with *MLL* gene could be associated with leukemogenesis in infant leukemia [8]. In addition to these translocations, partial duplication or deletion of the 11q23 locus disrupts the function of the *MLL* gene [9]. In fact, several previous studies demonstrated that different types of *MLL* gene rearrangements, especially the presence of t(4;11)(q21;q23), the most common *MLL* gene translocation in infant ALL, confer a poor outcome in infants [10–13]. However, we have demonstrated that different 11q23 translocations are not associated with inferior prognosis in *MLL* positive infant ALL [4,5].

Although the rearranged *MLL* gene plays an essential role in leukemogenesis of infant ALL, it is still obscure whether rearrangement of the *MLL* gene is sufficient for leukemic transformation. The murine knock-in model of t(9;11)(p22;q23) (*MLL-AF9*) required a long period to the onset of leukemia [14]. It has been known that some cases harbor additional chromosomal abnormalities other than 11q23 or complex chromosomal changes in *MLL* positive ALL infants [15,16]. Thus, it is possible that several unknown genes located in these chromosomal changes are disrupted, and are associated with leukemogenesis or progression of the disease. Recently, Moorman et al. has reported that no prognostic effect of additional chromosomal abnormalities was observed in a cohort of infants and children with ALL and 11q23 abnormalities in a large collaborative retrospective study [17]. On the other hand, to further improve the outcome of this subset of ALL, it is necessary to identify appropriate prognostic factors for additional risk stratification along with an improvement in anti-leukemic therapy. We therefore conducted a study investigating the prognostic relevance of complex chromosomal abnormalities in infants with ALL and a *MLL* gene rearrangement treated with Japanese MLL96 and MLL98 protocols.

2. Materials and methods

2.1. Patients

Between December 1995 and December 2001, 102 consecutive infants with ALL, younger than 12 months, were registered and treated with two protocols, designated MLL96 (55 patients) and MLL98 (47 patients). Five other patients were also treated with MLL98 protocol without registration in the study. Prior to treatment, each patient was evaluated with respect to the characteristics of their leukemic cells, including immunophenotype, cytogenetics, and *MLL* gene rearrangement. Among the enrolled patients, 86 were identified as *MLL* gene-rearranged (MLL-R). The details of the therapeutic regimens used in the MLL96 and MLL98 studies are described elsewhere [4,5]; briefly, all the 86 patients in the MLL-R group were assigned to receive induction therapy and three courses of postremission intensification therapy followed by allogeneic hematopoietic stem cell transplantation in first remission if a suitable donor was available [1,4,5]. Written informed consent, provided according to the Declaration of Helsinki, was obtained from the guardians of the patients, with institutional review board approval of the study enrollment.

2.2. Cytogenetics

The *MLL* gene status in each patient was determined by Southern blot analysis and/or fluorescence *in situ* hybridization (FISH) as previously published [4]. Two genomic probes were used to detect *MLL* gene rearrangement by FISH analysis: the S1363 probe located in the 5′ region of the *MLL* gene, including *MLL* exon 1, and the LB140 probe in the 3′ region of the *MLL* gene (kindly provided by Dr. Misao Ohki, National Cancer Institute, Japan). BAC clone 216H7 (Research Genetics, Huntsville, AL), which is located on 4q21 and covers introns 3 and 4 of the *AF4* gene, was used for the detection of a *MLL-AF4* fusion gene in combination with the S1363 and LB140 cosmid probes. The karyotypes of leukemic cells were determined by cytogenetic analysis performed by a G-banding technique, also as previously described [4]. Briefly, mononuclear cells were separated from the bone marrow or peripheral blood. After 24 h of incubation without external stimulation, the samples were fixed in Carnoy's fixative solution (3:1 methanol and acetic acid). Slides for cytogenetic analysis were prepared using the trypsin-G banding technique. Chromosomal abnormalities were described according to the International System for Human Cytogenetic Nomenclature (ISCN2005) [18].

2.3. Classification

Among the 86 MLL-R infants, only the patients with complete karyotypic data were included in the current analysis (n = 83).