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# VI. 代 表 的 論 文

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ORIGINAL REPORT

# 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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Clinical Trials repository link available on JCO.org.

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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.

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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 impact 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,



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methotrexate was administered at a dose of 3 g/m$^2$ 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/m$^2$ over 24 hours and IT chemotherapy (MTX1 arm) with six courses of methotrexate 3 g/m$^2$ 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/m$^2$ on days 1 and 2; 10 mg/m$^2$ on days 3 to 5 |
| Cyclophosphamide | 200 mg/m$^2$ on days 1 and 2 |
| Triple intrathecal injection | Day 1 |
| Course A | |
| Dexamethasone | 10 mg/m$^2$ on days 1 to 5 |
| Methotrexate | Random assignment* on day 1 |
| Ifosfamide | 800 mg/m$^2$ on days 1 to 5 |
| Cytarabine | 150 mg/m$^2$ × 2 on days 4 and 5 |
| Etoposide | 100 mg/m$^2$ on days 4 and 5 |
| Course B | |
| Dexamethasone | 10 mg/m$^2$ on days 1 to 5 |
| Methotrexate | Random assignment* on day 1 |
| Cyclophosphamide | 200 mg/m$^2$ on days 1 to 5 |
| Doxorubicin | 25 mg/m$^2$ on days 4 and 5 |

*Arm MTX1 included methotrexate 1 g/m$^2$ in 24-hour infusion with triple intrathecal injection at day 1 and leucovorin rescue (15 mg/m$^2$) at 42, 48, and 54 hours. Arm MTX3 included methotrexate 3 g/m$^2$ in 3-hour infusion with no intrathecal injection and leucovorin rescue (15 mg/m$^2$ 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/m$^2$) 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.



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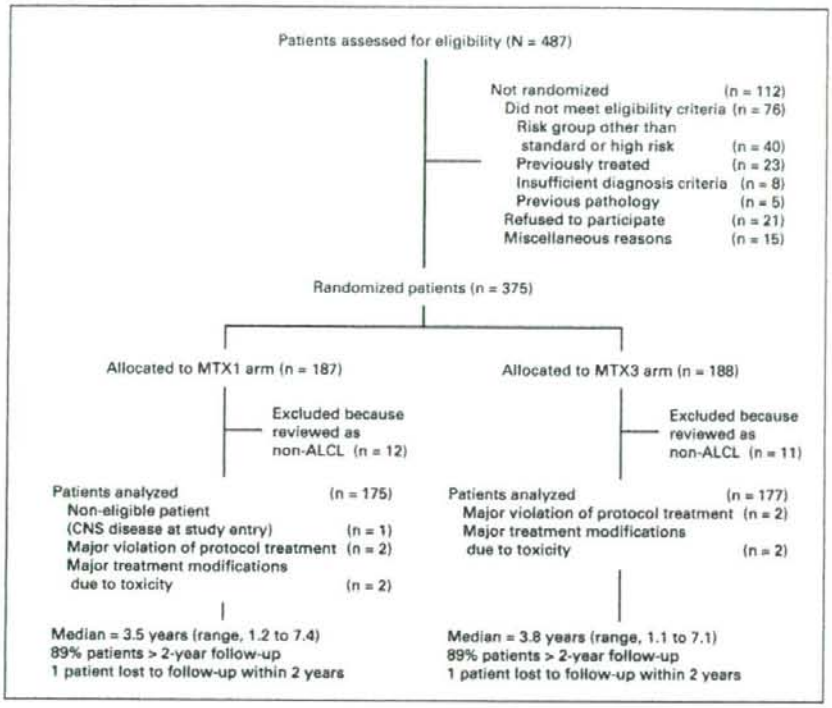


**Fig 1.** Participant flow CONSORT diagram. ALCL, anaplastic large-cell lymphoma.

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- *v* 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.



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**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.



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Among the 43 remaining patients, 14 experienced early progression on treatment, one was not assessable because of an early change of treatment, two died of treatment-related toxicity before achieving a complete remission, and 26 presented with a residual abnormality after the sixth course. Overall, 102 events were reported (treatment-related death, n = 4; early progression, n = 14; and relapse, n = 84). Seventy-three of the 84 relapses occurred during the first 2 years after random assignment. Progression and relapses occurred most frequently at the site of the primary tumor (69%) and were associated or not with new tumor site(s). Only two patients had a CNS relapse as the first event. The 2-year EFS rate of the 352 patients was 74.1% (95% CI, 69.2% to 78.4%).

Overall, 32 deaths were reported (21 as a result of disease progression and 11 as a result of toxicity), including seven deaths after progression or relapse. The 2-year OS rate of the 352 patients was 92.5% (95% CI, 89.3% to 94.8%).

### *Comparison of Outcome Between Treatment Arms*

The outcome results by treatment arm are listed in Table 3. There was no significant difference between the two randomized groups for any of the main and secondary efficacy end points.

The complete remission rates were 89% and 87% in the MTX1 and MTX3 arms, respectively (difference = −2%; 91.76% CI, −8% to 4%). As shown in Figure 2B, the EFS curves were superimposed, with 2-year EFS rates of 73.7% and 74.5% in the MTX1 and MTX3 arms, respectively. The 2-year EFS difference was +0.8% (91.76% CI, −7.3% to 9.0%). The HR for events in the MTX3 arm compared with the MTX1 arm was 0.98 (91.76% CI, 0.69 to 1.38). This result was similar when the strict intent-to-treat population (HR = 1.02; 91.76% CI, 0.74 to 1.42) or the per-protocol population (HR = 1.02; 91.76% CI, 0.72 to 1.45) was considered.

There was no significant heterogeneity in treatment effects in terms of EFS according to country ($P = .86$), risk group ($P = .15$), or the treatment allocated by the second random assignment ($P = .41$). The 2-year OS rates were 90.1% and 94.9% in the MTX1 and MTX3 arms, respectively (Fig 2C). The HR for death in the MTX3 arm compared with the MTX1 arm was 0.67 (91.76% CI, 0.36 to 1.25).

**Table 3.** Outcome by Treatment Arm

| Outcome | No. of Patients | |
|---|---|---|
| | MTX1 Arm (n = 175) | MTX3 Arm (n = 177) |
| Response to chemotherapy | | |
| Complete remission* | 155 | 154 |
| Residual abnormality | 10 | 16 |
| Progressive disease | 8† | 6† |
| Not assessed | 2 | 1 |
| Event | 51 | 51 |
| Progression on treatment | 8† | 6† |
| Relapse | 42 | 42 |
| Toxic death as first event | 1 | 3 |
| CNS relapse | 2 | 0 |
| Deaths | 19 | 13 |

Abbreviation: MTX, methotrexate.
*Complete remission was defined as the disappearance of disease from all initially involved sites lasting for at least 4 weeks.
†The eight and six patients with progression on treatment are the same as those listed as having progressive disease under the Response to chemotherapy heading.

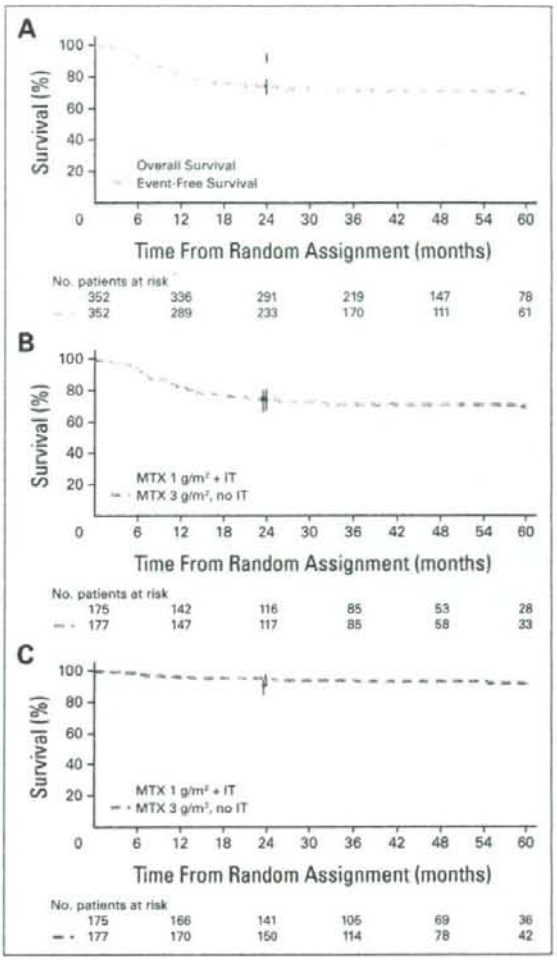


**Fig 2.** (A) Event-free survival (EFS) and overall survival (OS) of the whole study population. (B) EFS by treatment. (C) OS by treatment. MTX, methotrexate.

### *Toxicity*

Toxicity results are listed in Table 4. Severe toxicity was reported after 75% of courses and consisted mostly of grade 4 hematologic toxicity (72% courses) and grade 3 to 4 mucositis (13%). These toxicities were significantly more frequent after MTX1 courses than after MTX3 courses. The incidence of grade 3 to 4 infection was low (5%) and comparable for both types of courses. However, if all grades of infection are considered, the incidence was significantly higher after MTX1 courses (50%) compared with MTX3 courses (32%; $P < .0001$). No severe complications related to the IT injections were reported.

In this trial, to our knowledge the largest ever conducted in ALCL, we observed that the EFS curve for patients treated with chemotherapy


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**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/m$^2$ 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/m$^2$ 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/m$^2$ 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/m$^2$ in a 24-hour infusion plus an IT injection with methotrexate 3 g/m$^2$ 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/m$^2$ of doxorubicin) and alkylating agents (3.4 g/m$^2$ of cyclophosphamide and 12 g/m$^2$ 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,[6] 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



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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.



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# The Role of Hematopoietic Stem Cell Transplantation With Relapsed or Primary Refractory Childhood B-Cell Non-Hodgkin Lymphoma and Mature B-Cell Leukemia: A Retrospective Analysis of Enrolled Cases in Japan

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***Background.*** There have been excellent treatment results for children with B-cell non-Hodgkin lymphoma (B-NHL) and mature B-cell leukemia (B-ALL) in the last few decades. However, a small subset of relapsed or refractory patients, after first-line therapy, still have a poor prognosis. ***Procedure.*** Thirty-three patients with relapsed or primary refractory B-NHL/B-ALL among 327 newly diagnosed patients between 1996 and 2004 were analyzed retrospectively. ***Results.*** After salvage therapy, 18 patients were chemotherapy-sensitive and 15 patients suffered from progression. Among 18 patients who had a chemotherapy-sensitive disease, 4 of 5 patients who underwent hematopoietic stem cell transplantation (HSCT) during remission survived without progression, while 3 of 12 patients who did not receive HSCT were alive without disease progression. Fifteen patients never sensitive to salvage therapy died. ***Conclusions.*** Patients with relapsed/primary refractory B-NHL/B-ALL have a poor prognosis with current treatment approaches, while the patients sensitive to salvage therapy have a respectable chance to achieve a sustained complete second remission with HSCT. Pediatr Blood Cancer 2008;51:188–192. © 2008 Wiley-Liss, Inc.

**Key words:** childhood; mature B-cell leukemia (B-ALL); non-Hodgkin lymphoma; refractory; relapsed; stem cell transplantation

## INTRODUCTION

There have been excellent treatment results for children with B-cell non-Hodgkin lymphoma (B-NHL) and mature B-cell leukemia (B-ALL) in the last few decades along with the assignment of highly intensive and sequential chemotherapeutic regimens stratified according to risk [1–5]. However, patients with relapsed or refractory disease still have a poor prognosis, particularly in patients treated with intensive first-line therapy. And there are few reports on treatment in relapsed or refractory pediatric B-NHL/B-ALL. It is, therefore, very difficult to assess the role of megatherapy or other treatment. In this study, we summarized the results of 33 pediatric patients who had relapsed or primary resistant disease after first-line therapy with B-NHL/B-ALL enrolled in a national survey of Japan, and validate the availability of hematopoietic stem cell transplantation (HSCT) for these patients.

In Japan, there have been four study groups for pediatric hematological tumors; such as, the Japan Children's Cancer and Leukemia Study Group (JCCLSG), the Japan Association of Childhood Leukemia Study (JACLS), the Kyushu-Yamaguchi Children's Cancer Study Group (KYCCSG) and the Tokyo Children's Cancer Study Group (TCCSG). Treatment protocols of these groups for B-NHL modified French LMB89 [2] or German BFM90 [3] consist of short-duration, intensive, alkylating agent therapy (i.e., cyclophosphamide) coupled with other agents, such as intermediate- or high-dose methotrexate, vincristine, anthracyclines, etoposide and cytarabine. Result in survival rates of these collaborative groups with each chemotherapy regimens were 70–80% in stages III–IV.

## PATIENTS AND METHODS

We analyzed the data on all children with relapsed/refractory B-NHL/B-ALL have been enrolled in four multicenter trials of childhood NHL. JCCLSG, JACLS, KYCCSG and TCCSG had enrolled 54 patients (JCCLSG NHL-960 study; 1996–2004), 125 patients (JACLS NHL-98 study; 1998–2002), 9 patients (KYCCSG NHL 96 study; 1996–2004) and 139 patients (TCCSG NHL 96 study; 1996–2001) respectively. The first-line treatments used in each study differed, however there were no considerable differences in therapeutic results. Of the 327 patients included in these series, 26 patients relapsed after achieving first complete remission (CR) and 7 patients did not achieve first CR. CR and partial remission (PR) were defined as previously described [6]. The medical records for these 33 patients were retrospectively collected from each study group. Details of salvage or second-line treatment are shown in Table I. NHL-B02 pilot regimen is now used for the patients with childhood B-NHL/B-ALL in Japan, and in other cases childhood ALL regimen of each group was used for relapsed B-NHL/B-ALL. Several patients were treated with regimens published in parts elsewhere [7–10]. Overall survival rate (OS) and progression-free survival rate (PFS) were estimated using the Kaplan–Meier method and data were compared by the log-rank test. The prognostic analysis was based on PFS. Multivariate Cox model was also fitted to adjust the potential effects of the baseline characteristics. Results were analyzed as of January 31, 2006.

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**TABLE I. Salvage TherapyDose/Schedule**

| | Therapy dose/schedule |
|---|---|
| NHL-B02 pilot | HDMTX 5,000 mg/m$^2$ day 1 in 24 hr infusion + rescue |
| A | Dexamethasone 10 mg/m$^2$ (divided doses) days 1–7 then reduce over 4 days to 0 |
| | Vincristine 1.5 mg/m$^2$ day 2 (maximum: 2.0 mg) |
| | Cyclophosphamide 1,000 mg/m$^2$ days 4, 5 |
| | Pirarubicin 30 mg/m$^2$ days 4, 5 (maximum: 45 mg) |
| | IT MTX 3–12 mg/m$^2$ days 1, 8 |
| | IT hydrocortisone 10–25 mg/m$^2$ days 1, 8 |
| | IT cytarabine 6–30 mg/m$^2$ days 1, 8 |
| B | Vincristine 1.5 mg/m$^2$ day 1 (maximum: 2.0 mg) |
| | Dexamethasone 10 mg/m$^2$ (divided doses) days 1–5 then reduce over 3 days to 0 |
| | HD Ara-C 2,000 mg/m$^2$ every 12 hr days 2–4 |
| | Etoposide 150 mg/m$^2$ days 2–5 |
| | IT MTX 3–12 mg/m$^2$ days 1, 8 |
| | IT hydrocortisone 10–25 mg/m$^2$ days 1, 8 |
| | IT cytarabine 6–30 mg/m$^2$ days 8 |
| JCCLSG NHL 960 | HD Ara-C 2,000 mg/m$^2$ days 1–4 |
| | Etoposide 200 mg/m$^2$ days 1–4 |
| | IT MTX 7.5–1.5 mg/m$^2$ day 2 |
| | IT hydrocortisone 30–50 mg/m$^2$ day 2 |
| JACLS ALL-97 | Vincristine 1.5 mg/m$^2$ days 1, 8, 15, 22, 29 (maximum: 2.0 mg) |
| HR | Dexamethasone 10 mg/m$^2$ days 1–7 |
| | Pirarubicin 25 mg/m$^2$ days 2, 4 |
| | Prednisone 40 mg/m$^2$ (divided doses) days 8–29 then reduce over 5 days to 0 |
| | L-asparaginase 10,000 U/m$^2$ days 9, 11, 13, 16, 18, 20 |
| | IT MTX 8–12 mg/m$^2$ days 1, 29 |
| | IT hydrocortisone 15–25 mg/m$^2$ days 1, 29 |
| | IT cytarabine 20–30 mg/m$^2$ days 1, 29 |
| F | Mitoxantorone 8 mg/m$^2$ days 1–3 |
| | Cytarabine 500 mg/m$^2$ days 1–3, 8–10 |
| | Prednisone 40 mg/m$^2$ (divided doses) days 1–3, 8–10 |
| | Etoposide 200 mg/m$^2$ days 8–10 |
| | IT MTX 8–12 mg/m$^2$ day 1 |
| | IT hydrocortisone 15–25 mg/m$^2$ day 1 |
| | IT cytarabine 20–30 mg/m$^2$ day 1 |
| TCCSG L99 | Vincristine 1.5 mg/m$^2$ days 1, 8, 15, 22, 29 (maximum: 2.0 mg) |
| HEX | Cyclophosphamide 1,000 mg/m$^2$ days 1, 30 |
| | Prednisone 60 mg/m$^2$ (divided doses) days 1–28 then reduce over 7 days to 0 |
| | Pirarubicin 20 mg/m$^2$ days 2, 3, 31, 32 |
| | Prednisone 60 mg/m$^2$ (divided doses) days 8–29 then reduce over 5 days to 0 |
| | L-asparaginase 6,000 U/m$^2$ days 1, 3, 5, 7, 8, 10, 12, 14, 15, 17, 19, 21 |
| | IT MTX 6–12.5 mg/m$^2$ days 8, 15, 22 |
| | IT hydrocortisone 12–25 mg/m$^2$ days 8, 15, 22 |
| | IT cytarabine 12–25 mg/m$^2$ days 8, 15, 29 |
| Rituximab | Rituximab 375 mg/m$^2$ days 1, 8, 15, 22 |

HDMTX, high dose methotrexated; IT, intrathecal.

## RESULTS

### Characteristics of Patients

Twenty-three were males and 10 were females with a median age at onset of 13 years (range 1–16 years). Histological classification showed 20 Burkitt lymphoma/leukemia (BL), 12 diffuse large B-cell lymphoma (DLBCL) and one mature B-ALL not further classified. The diagnosis of B-NHL/B-ALL was based on histopathology and immunohistochemistry. From 24 of these 33 patients, the histopathological material was reviewed centrally by a reference laboratory utilized by the study [11]. Cytogenetic studies were performed in 14 patients and showed no abnormality in 5 patients, t(8;14) in 5 patients, and other abnormalities in 4. Murphy's stage was stage I or II in 3 cases and stage III or IV in 30 cases. Sites of relapse/progress included the primary sites in 23 and new sites in 10 cases. Fifteen had bone marrow (BM) involvement (include 6 cases with new BM lesion) and 6 had central nervous system (CNS) disease (include one case with new CNS lesion). Twenty-eight patients progressed or relapsed in the first 12 months from first diagnosis. Characteristics, details of treatment and follow-up of the patients with relapsed or refractory conditions are shown in the Tables II and III.

### Patient Outcome After First Relapse/Progress

After a median follow-up period of 48 months, the 4-year OS and PFS rates for these patients were 20.8 ± 8.2% and 20.5 ± 7.2%,

**TABLE II. Characteristics, Treatment, and Outcome of the Patients With Relapsed B-NHL**

| Patient number | Stage | Time to relapse (months) | Histology | Site of relapse | Treatment of relapse (Table I) | Outcome |
|---|---|---|---|---|---|---|
| 1 | I | 67 | DLBCL | Abdomen | CHOP [7] + Rit + operation | Alive |
| 2 | III | 18 | DLBCL | Bone + spleen | NHL-B02 pilot: A, B + Rit + RT | Alive |
| 3 | III | 35 | DLBCL | Primary site (abdomen) + Neck | NHL-B02 pilot: A, B | Alive |
| 4 | III | 25 | BL | Primary site (neck) | NHL-B02 pilot: A, B + Rit + auto PBSCT | Alive |
| 5 | III | 23 | DLBCL | Primary site (abdomen + neck) | JACLS ALL97 HR + auto PBSCT | Alive |
| 6 | IV | 5 | BL | Primary site (abdomen + CNS) | JCCLSG NHL 960 | Alive |
| 7 | III | 12 | DLBCL | Primary site (mediastinum) | NHL-B02 pilot: A, B | Died |
| 8 | IV | 6 | BL | Primary site (BM) | CA 100 mg/m$^2$ + VP 100 mg/m$^2$ days 1–3 | Died |
| 9 | IV | 5 | BL | Primary site (BM) | ICE [8] + Rit + CBT | Died |
| 10 | IV | 7 | BL | BM | CBT | Died |
| 11 | IV | 3 | BL | Primary site (abdomen + CNS) | ALL-REZ BFM 90[9] + RT | Died |
| 12 | III | 4 | BL | BM | Palliatve | Died |
| 13 | IV | 6 | BL | Primary site (BM) | Not available | Died |
| 14 | III | 4 | DLBCL | Primary site (mediastinum + abdomen) | ICE [8] + Rit + related PBSCT | Died |
| 15 | IV | 2 | BL | Primary site (bone + BM) | HD-CA + VP + VCR + Dex + RT + related BMT | Died |
| 16 | III | 8 | DLBCL | CNS | Related PBSCT | Died |
| 17 | IV | 8 | B-ALL | Primary site (CNS) + BM | JACLS ALL97 F + related BMT | Died |
| 18 | III | 7 | BL | Primary site (mediastinum + abdomen) | ESHAP [10] + Rit + related PBSCT | Died |
| 19 | IV | 6 | BL | Primary site (BM) | TCCSG L99 HEX | Died |
| 20 | IV | 5 | BL | Primary site (BM) | TCCSG L99 HEX + related PBSCT | Died |
| 21 | III | 5 | BL | BM + abdomen | VP + VCR + PSL | Died |
| 22 | IV | 4 | BL | Primary site (neck + BM) | NHL-BFM 90[3] | Died |
| 23 | II | 7 | DLBCL | Neck | TCCSG L99 HEX | Died |
| 24 | III | 6 | BL | Primary site (abdomen) + BM | RT | Died |
| 25 | IV | 5 | BL | Primary site (BM) | TCCSG L99 HEX + related PBSCT | Died |
| 26 | III | 4 | BL | BM | Not available | Died |

DLBCL, diffuse large B-cell lymphoma; BL, Burkitt lymphoma; CNS, central nervous system; BM, bone marrow; Rit, Rituximab; RT, radiation therapy; PBSCT, peripheral blood stem cell transplantation; CBT, cord blood transplantation; BMT, bone marrow transplantation; CA, cytarabine; VP, etoposide; HD-CA, high dose cytarabine; VCR, vincristine; Dex, dexamethasone; PSL, prednisolone.

respectively. Nine of 33 patients are alive and 24 patients died. Twenty-one patients died of their primary disease, and 3 patients died of therapy-related toxicity. Outcomes according to the kinetics of response to therapy are depicted in Figure 1. All of 15 cases never reaching CR or PR died after salvage therapy with or without HSCT. Ten cases achieved CR and 8 cases achieved PR.

## HSCT and Outcome

Among the patients achieving CR or PR, 4 of 5 patients who underwent HSCT and 3 of the 12 patients who did not receive HSCT were alive without disease progression. The other one patient who underwent HSCT with progression died of lymphoma (Fig. 1).

**TABLE III. Characteristics, Treatment, and Outcome of the Patients With Primary Refractory B-NHL**

| Patient number | Stage | Histology | Site of progress | Treatment of progress | Outcome |
|---|---|---|---|---|---|
| 27 | II | DLBCL | Abdomen | Continuation of 1st-line treatment | Alive in CR |
| 28 | IV | DLBCL | CNS | Continuation of 1st-line treatment + auto PBSCT | Alive in PR |
| 29 | IV | BL | BM | Continuation of 1st-line treatment + related BMT | Alive in CR |
| 30 | IV | BL | BM + head + abdomen | Continuation of 1st-line treatment | Died |
| 31 | III | DLBCL | Abdomen | ICE[8] + Rituximab | Died |
| 32 | III | BL | Abdomen | Not available | Died |
| 33 | IV | DLBCL | Bone + CNS + abdomen | Continuation of 1st-line treatment | Died |

CR, complete remission; PR, partial remission.

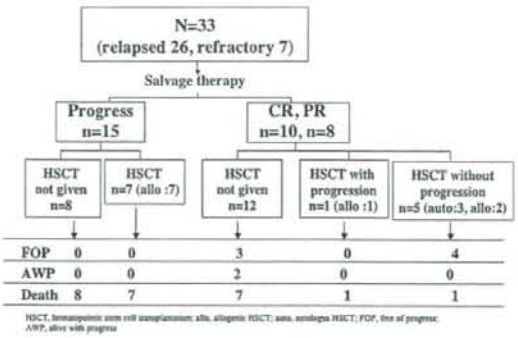


**Fig. 1.** Outcome in patients with relapsed/refractory B-NHL/B-ALL.

Details of treatment and outcome of the patients with HSCT are shown in Table IV. Disease status at the time of HSCT had an influence on prognosis, however, neither high-dose chemotherapy (HDC) regimens nor kind of graft related to. There were three survivors without disease progression who were not given HSCT. Two of the survivors had stage I or II at initial diagnosis and achieved second remissions after short intensive courses of chemotherapy. Another patient had stage IV DLBCL and relapsed at 18 months after diagnosis, he received a second-line treatment consisting of an intensive chemotherapy according to the NHL-B02 pilot regimen. The patient achieved a CR, however he could not receive HSCT because of contracting by Aspergillus pneumonia. Rituximab and local radiotherapy were successful and he continues in remission 38 months from diagnosis.

## Prognostic Factors

In the multivariate analysis, response to salvage therapy was the only significant prognostic factor. PFS was worse among patients with poor response to salvage therapy as compared to the other ($P = 0.037$). On the contrary the PFS was not associated with histologic type, time to relapse, BM or CNS involvement.

## DISCUSSION

Outcome of children with B-cell NHL/B-ALL has dramatically improved, while, for relapsed or primary resistant patients, the chance of cure with currently available therapy is low [12,13]. Also in this analysis, the 4-year OS and PFS rates for these patients is about 20%. None of the 15 patients who never reached CR or PR after salvage therapy was alive whereas, 9 of 18 children undergoing salvage therapy in CR or PR were alive. In our series, various retrieval chemotherapy regimens were used, making it difficult to make efficacy comparisons; however, the results of this are in line with previous reports [14,15] showing that chemoresistance is associated with a very poor outcome.

For the patients with second CR or PR, HSCT seems to be an effective strategy, as shown that 4 of 5 patients who received HSCT after having achieved a second CR or PR without progress were PFS, while only 3 of 12 not given HSCT were alive without disease. However, there was no theoretic influence of HDC regimens and previous reports observed in a small group of pediatric patients [16,17], so the optimum conditioning regimen in children is under discussion [18,19]. Previous reports [15,20,21] showed that the major determinant of survival was the remission status of patients before HDC, neither HDC regimens nor type of graft, and our results showed similar findings. In our study, 7 cases received chemotherapy combined with rituximab but there was no significant contribution to their response rate (data not shown).

Another finding from this analysis of factors contributing to PFS reveals that response to salvage therapy was the only significant prognostic factor. It appears important to focus on the salvage therapy. The schedule of a salvage therapy should be tailored to the known features of the tumor (e.g., cell resistance) and be selected of drugs for use nonoverlapping first-line therapy.

In summary, this study demonstrates that the prognosis for patients with relapsed/refractory childhood B-NHL/B-ALL was poor. However, for the patients sensitive to salvage therapy, HSCT seems to be an effective strategy.

**TABLE IV. Details of the 13 Patients Treated With HSCT**

| Patient number | Status before HDC | HDC regimen | Graft (match of HLA) | HSCT | Outcome |
|---|---|---|---|---|---|
| 4 | CR | BU + L-PAM | Autologous | PBSCT | Alive in CR |
| 5 | CR | CY + VP + TBI | Autologous | PBSCT | Alive in CR |
| 9 | Progress | CY + TEPA + TBI | Unrelated (4/6) | CBT | Died of lymphoma |
| 10 | Progress | L-PAM + CA + TBI | Unrelated (6/6) | CBT | Died of HDC |
| 14 | Progress | Flu + ATG + L-PAM + TBI | Related (4/6) | PBSCT | Died of lymphoma |
| 15 | Progress | BU + L-PAM | Unrelated (6/6) | BMT | Died of HDC |
| 16 | Progress | CY + VP + CBDCA + MCNU | Allogeneic (not available) | PBSCT | Died of lymphoma |
| 17 | CR | Not available | Sibling (6/6) | BMT | Died of lymphoma |
| 18 | Progress | VP + TBI | Sibling (6/6) | PBSCT | Died of lymphoma |
| 20 | Progress | Flu + ALG + L-PAM + IDA | Related (4/6) | PBSCT | Died of lymphoma |
| 25 | Progress | Not available | Sibling (6/6) | PBSCT | Died of lymphoma |
| 28 | PR | TEPA + L-PAM | Autologous | PBSCT | Alive in PR |
| 29 | PR | CY + TBI | Sibling (6/6) | BMT | Alive in CR |

HDC, high-dose chemotherapy; BU, busulfan; L-PAM, melphalan; CY, cyclophosphamide; VP, etoposide; TBI, total body irradiation; TEPA, thiotepa; CA, cytarabine; Flu, fludarabine; ATG, anti-thymocyte globuline; CBDCA, carboplatin; MCNU, ranimustine; ALG, anti-lymphocyte globuline; IDA, idarubicin hydrochloride; PBSCT, peripheral blood stem cell transplantation; CBT, cord blood transplantation; BMT, bone marrow transplantation.

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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

*Abbreviations:* ALL, acute lymphoblastic leukemia; *MLL*, *mixed lineage leukemia*; MLL-R, *MLL* gene rearranged; FISH, fluorescence in situ hybridization; ACA, additional chromosomal abnormalities other than 11q23 translocation; EFS, event-free survival; OS, overall survival; SEs, standard errors; CIs, confidence intervals.

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## 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 (≥300,000/μL), 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 ($n = 83$).

Nine patients with normal karyotype were excluded from the study, because these patients had *MLL* gene rearrangements that were not detected by conventional cytogenetics. The remaining 74 were therefore classified into two subgroups: "ACA group", comprising those with additional chromosomal abnormalities other than 11q23 translocation, and "non-ACA group", comprising patients with sole 11q23 translocation with *MLL* gene rearrangements. Three-way 11q23 translocations and simple or complex structural chromosomal changes other than 11q23 abnormalities were also included in the additional chromosomal abnormalities (ACA) group, because several genetic changes in addition to *MLL* could be involved in these cases, as described in previous reports [16,17].

### 2.4. Statistical analysis

The analysis of treatment outcome was updated on 30 September 2007. Event-free survival (EFS) and Overall survival (OS) rates were estimated by the method of Kaplan–Meier and standard errors (SEs) with the Greenwood formula, and then were compared with the log-rank test. Confidence intervals (CIs) were computed with a 95% confidence level. The clinical and biologic features of patients in the two different subgroups were compared with $\chi^2$ tests for homogeneity. A Cox regression model was used for the multivariate analysis. *P*-values, when cited, are two sided, with a value of 0.05 or less taken to indicate statistical significance.

Table 1
Eighteen *MLL* rearranged ALL infants with additional chromosomal abnormalities

| Patient # | Karyotype | Sex | Age (month) | WBC, $\times 10^6$/L | CNS[a] | HSCT in CR1 | Outcome |
|---|---|---|---|---|---|---|---|
| 1 | 46,XX,add(11)(q25) [6]/46,XX [11] | F | 4 | 193.8 | – | No | BM relapse. DOD (2nd relapse) after UBMT |
| 2 | 46,XY,t(4;11)(q21;q23),t(2;4)(q31;q32) [20]<br>46,XY,t(4;11)(q21;q23),t(2;4)(q31;q32) (2qter → 2q31::4q32 → 4q21::11q23 → cen → 11pter) | M | 3 | 169.9 | – | No | BM relapse. TRD after BMT |
| 3[b] | 46,XX[18].ish ins(4;11)(q21;q23.3q23.3)(RP11-216H7+, MLL5′+; MLL5′–,MLL3′+)[10] | F | 2 | 953.0 | + | No | BM relapse. DOD (2nd relapse) after UCBT |
| 4 | 46,XX,t(4;11;15)(q21;q23;q22) [9]/46,XX [1] | F | 0 | 121.6 | + | RBMT | Death in CCR (TRD) |
| 5 | 46,XX,add(1)(q32),der(2)t(2;4)(p1?;q21),add(4)(q21), del(11)(q?),add(16)(p11) [20] | F | 8 | 7.7 | – | No | CCR |
| 6[b] | 46,XX[20]. ish ins(4;11)(q21;q23.3q23.3)(RP11-216H7+, MLL5′+,MLL3′+; MLL5′-,MLL3′-) | F | 2 | 500.0 | – | RBMT | CCR |
| 7 | 48,XX,+X,t(4;11)(q21;q23),+der(4)t(4;11)(q21;q23) [20] | F | 0 | 421.5 | – | No | BM relapse. DOD |
| 8 | 46,XY,der(9)t(9;11)(p22;q13),add(11)(q13) [20] | M | 1 | 473.5 | + | No | Induction failure. TRD after RBMT |
| 9 | 46,XY,t(4;11;5)(q21;q23;p11) [20] | M | 3 | 1000.0 | – | UCBT | Death in CCR (TRD after 2nd UCBT because of rejection) |
| 10 | 46,XX,t(2;9)(p10;q10),add(7)(p22),add(9)(p13), add(11)(p11) [20] | F | 7 | 1.7 | – | UCBT | CCR |
| 11 | 46,XX,t(4;11;9)(q21;q23;q22) [20] | F | 9 | 250.7 | + | UCBT | Death in CCR (TRD) |
| 12 | 46,XX,add(4)(q11) [4]/46,XX [6] | F | 5 | 12.1 | + | ABMT | Relapse. TRD after UCBT |
| 13 | 46,XY, t(6;11)(p10;q10),add(11)(q23) [20] | M | 5 | NA | NA | No | CNS relapse. TRD after RBMT |
| 14 | 48,XY,+X,add(2)(p21),del(2)(p?),+6,der(7)add(7)(p11) add(7)(q32),del(11)(q?),add(12)(q13),-17,-17,add(19)(p13), +der(?)t(?;17)(?;q21),+mar1 [20] | M | 2 | 25.6 | + | No | DOD before initial therapy |
| 15[b] | 46,XY[20].ish ins(10;11)(p12;q23.3q23.3) (MLL5′+,MLL3′+;MLL5′, MLL3′–) | M | 2 | 537.0 | – | No | BM relapse. CCR after UBMT in CR2 |
| 16 | 47XX,t(4;11)(q21;q23),+?i(8)(q10) [20] | F | 5 | 59.0 | NA | No | BM relapse. DOD |
| 17 | 47XX,+5,t(9;11)(p22;q23) [5]/46,XX [2] | F | 3 | 22.8 | – | UCBT | CCR |
| 18[b] | 46,XX,t(4;11)(q21;q23)[20].ish t(4;11;21)(q21;q23;q22) (216H7+; 216H7+,MLL5′+,MLL3′-; MLL3′+) | F | 0 | 198.2 | – | No | Induction failure. CCR after UBMT |

F, female; M, male; WBC, white blood cell; BM, bone marrow; CNS, central nervous system; CR1, first complete remission; CCR, continuous complete remission; ABMT, autologous bone marrow transplantation; RBMT, related donor bone marrow transplantation; UCBT, unrelated cord blood transplantation; UBMT, unrelated bone marrow transplantation; DOD, death of disease; TRD, treatment-related death; NA, data not available.

[a] CNS disease was diagnosed if more than five leukemic cells/μl were found in cerebrospinal fluid.

[b] FISH analysis has proven complex chromosomal abnormality in these patients. Cloning of the breakpoint regions revealed that patient #6 had 46,XX, ins(4;11)(4pter → 4q21::11q24.1 → 11q23.3(MLL3′)::11q23.3 → 11q23.3(MLL5′)::4q21 → 4qter;11pter → 11q23.3:: 11q24.1 → 11qter), and patient #15 had 46,XY, ins(10;11)(10pter → 10p12::11q23.3 → 11q23.3(MLL3′)::11q23.3 → 11q23.3(MLL5′)::10p12 → 10qter;11pter → 11q23.3:: 11q23.3 → 11qter).

## 3. Results

Among the 74 eligible infants, 18 (24.3%) were classified as the ACA group, as shown in Table 1. Four patients (patients #4, #9, #11, and #18) had three-way 11q23 translocation. Other novel translocations were also observed in four patients: t(2;4)(q31;q32) in patient #2, t(9;11)(p22;q13) in patient #8, t(2;9)(p10;q10) in patient #10, and t(6;11)(p10;q10) in patient #13. FISH analysis confirmed complex structural chromosomal changes in four patients including insertion of 4q21 fragment to 11q23 locus and *vice versa* resulting in *MLL-AF4* fusion gene (patients #3, #6, and #18) or insertion of 10p12 to 11q23 locus resulting in *MLL-AF10* fusion gene (patient #15). Other frequent chromosomal changes were +X in two patients, involvement in chromosome 4 in two, chromosome 5 in two, chromosome 7 in two, and chromosome 11 except 11q23 in four.

The clinical and biologic findings were compared between the ACA and non-ACA groups, including age at disease onset, sex, initial white blood cell (WBC) count, central nervous system (CNS) involvement, and type of 11q23 translocation. As shown in Table 2, the frequency of sole t(4;11)(q21;q23) was significantly higher in the non-ACA group than in the ACA group. The frequency of positive central nervous system leukemia or young age at onset also tended to be higher in the ACA group than the non-ACA group, although the difference was not statistically significant.

Among the 18 patients in the ACA group, a total of 14 events were observed: one leukemic death before initiating therapy (patient #14); two induction failure (patients #8, and #18); eight relapses (patients #1, #2, #3, #7, #12, #13, #15, and #16); three treatment-related deaths (patients #4, #9, and #11). Only four patients in this group survived without any evidence of disease (patients #5, #6, #10, and #17) (Table 1).

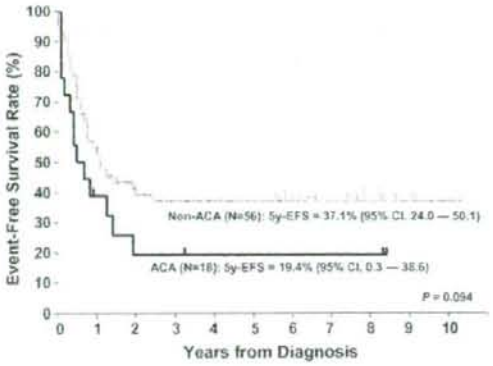


Fig. 1. Event-free survival estimates for 74 infants with ALL and *MLL* gene rearrangements in the MLL96 and MLL98 studies: a comparison between patients with additional chromosomal abnormalities and patients with sole 11q23 abnormality excluding normal karyotype with *MLL* gene rearrangements. Median follow-up period: 78 months (range, 8–124 months).

The EFS and OS rates were also compared between two groups. The 5-year EFS rate in the ACA group tended to be worse than that in the non-ACA group, without a statistically significant difference between two groups (Fig. 1). The 5-year OS in the ACA group was significantly worse than that in the non-ACA group; 26.7% (95% CI, 4.7–48.8%) *vs.* 52.1%

Table 2
Comparison in clinical and laboratory findings between the ACA and non-ACA groups

| | Total number of Pt. (%) | ACA group number of Pt. (%) | Non-ACA group number of Pt. (%) | *P*-value[a] |
|---|---|---|---|---|
| Total number of patients | 74 | 18 | 56 | |
| Age, month | | | | 0.136 |
| <3 | 21 (28.4) | 8 (44.4) | 13 (23.2) | |
| ≥ 3, <6 | 29 (39.2) | 7 (38.9) | 22 (39.3) | |
| ≥ 6 | 24 (32.4) | 3 (16.7) | 21 (37.5) | |
| Sex | | | | 0.650 |
| Male | 28 (37.8) | 6 (33.3) | 22 (39.3) | |
| Female | 46 (62.2) | 12 (66.7) | 34 (60.7) | |
| WBC count, $\times 10^9$/L | | | | 0.599 |
| <100 | 23 (31.1) | 6 (33.3) | 17 (30.3) | |
| ≥ 100, <300 | 29 (39.2) | 5 (27.8) | 24 (42.9) | |
| >300 | 21 (28.4) | 6 (33.3) | 15 (26.8) | |
| NA | 1 (1.3) | 1 (5.6) | 0 (0.0) | |
| CNS disease[b] | | | | 0.131 |
| Positive | 16 (21.6) | 6 (33.3) | 10 (17.9) | |
| Negative | 52 (70.3) | 10 (55.6) | 42 (75.0) | |
| Unknown | 6 (8.1) | 2 (11.1) | 4 (7.1) | |
| Karyotype | | | | 0.012 |
| t(4;11)(q21;q23) | 47 (63.5) | 7 (38.9) | 40 (71.4) | |
| Other 11q23 | 27 (36.5) | 11 (61.1) | 16 (28.6) | |

ACA, additional chromosomal abnormalities other than 11q23 translocation; Pt., patients; WBC, white blood cell; CNS, central nervous system; NA, data not available.

[a] Comparison between two different groups.

[b] CNS disease was diagnosed if more than five leukemic cells/μL were found in cerebrospinal fluid.