The outcomes of relapsed acute myeloid leukemia in children: Results from the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) AML-05R study

Hiroshi Moritake[1], Shiro Tanaka[2], Takako Miyamura[3], Hideki Nakayama[4], Norio Shiba[5], Akira Shimada[6], Kiminori Terui[7], Yuki Yuza[8], Katsuyoshi Koh[9], Hiroaki Goto[10], Harumi Kakuda[11], Akiko Saito[12], Daisuke Hasegawa[13], Shotaro Iwamoto[14], Takashi Taga[15], Souichi Adachi[16], and Daisuke Tomizawa[17]

[1]University of Miyazaki
[2]Graduate School of Medicine and Public Health, Kyoto University
[3]Osaka University Graduate School of Medicine
[4]Kyushu Cancer Center Institute for Clinical Research
[5]Yokohama City University
[6]Okayama University Hospital
[7]Hirosaki University Graduate School of Medicine
[8]Tokyo Metropolitan Children's Medical Center
[9]Saitama Children's Medical Center
[10]Kanagawa Childrens Medical Center
[11]Chiba Children's Hospital
[12]National Hospital Organization Nagoya Medical Center
[13]St. Luke's International Hospital
[14]Mie University Graduate School of Medicine
[15]Shiga University of Medical Science
[16]Kyoto University
[17]National Center for Child Health and Development

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Abstract

Background: The prognosis of children with acute myeloid leukemia (AML) has improved with the efficacy of hematopoietic cell transplantation as a second-line therapy and improvements in supportive care following anthracycline- and cytarabine-based chemotherapy; however, the outcomes of children with relapsed AML still remain unsatisfactory. Procedure: In order to identify prognostic factors and improve their prognosis, we analyzed 111 patients who relapsed after treatment with the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) AML-05 protocol and who were registered in the retrospective JPLSG AML-05R study. Results: The 5-year overall survival rate was 36.1%. The major determinant of survival was duration from the diagnosis to relapse. The mean duration in the non-surviving group (10.1 ± 4.1 months) was shorter than that in the surviving group (16.3 ± 8.3 months) ($p<0.01$). Moreover, achieving a second complete remission (CR2) prior to hematopoietic cell transplantation was associated with a good prognosis ($p<0.01$). Etoposide, cytarabine and mitoxantrone (ECM)- or fludarabine, cytarabine and granulocyte colony-stimulating factor (FLAG)-based regimens were therefore recommended for reinduction therapy ($p<0.01$). A genetic analysis also revealed the prognostic significance of FMS-like tyrosine kinase 3 (FLT3)-internal

tandem duplication as a poor prognostic marker (p=0.04) and core binding factor-AML, t(8;21) and inv(16), as good prognostic markers (p<0.01). Conclusions: Achieving a CR2 prior to HCT is important in order to improve the prognosis of relapsed pediatric AML. Recent molecular targeted therapies, such as FLT3 inhibitors, may contribute to overcome their prognoses. Larger prospective investigations are necessary to establish individualized treatment strategies for patients with relapsed childhood AML.

INTRODUCTION

The treatment of pediatric acute myeloid leukemia (AML) has been improved by intensifying the first-line treatment and improving supportive care; however, approximately 30% of the patients who successfully achieve remission subsequently relapse, and the outcomes are extremely poor, with overall survival (OS) rates of 16–38% during observation periods of 2–10 years.[1-11] The time to relapse after the diagnosis and the use of hematopoietic cell transplantation (HCT) are widely known as important prognostic factors that influence the outcome after relapse. Other factors, such as the reinduction therapy regimen, M5 or M7 morphology, response to reinduction therapy, *FMS-like tyrosine kinase 3* -internal tandem duplication (*FLT3* -ITD) positivity, and other cytogenetic and molecular features have been proposed as prognostic factors by several investigators.[2 3 4 6 10 11] In the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) AML-05 study, excessive treatment reduction was observed to induce a higher incidence of relapse in children with core binding factor (CBF)-AML.[12] However, two-thirds of children with relapsed CBF-AML could be salvaged with intensive reinduction therapy followed by allogeneic HCT[13]. We retrospectively investigated the outcomes of 111 patients with pediatric relapsed AML (relapsed CBF-AML, n=32; and relapsed non-CBF-AML, n=79) who were registered in the JPLSG AML-05R.

METHODS

Patients

From 2006 to 2010, 443 patients with AML who were [?]18 years of age, excluding those with Down syndrome or acute promyelocytic leukemia, were enrolled in the JPLSG AML-05 protocol.[12] Among 443 patients, 137 patients (30.9%) relapsed. We conducted a retrospective analysis (the JPLSG AML-05R study) of all relapsed patients after registration in the JPLSG-AML05 study using questionnaires that were distributed between 2012 and 2013. To clarify the prognosis of relapsed AML, we collected the following data: patient's age at relapse, time from the diagnosis to relapse, site of relapse, FAB classification, results of a chromosomal analysis, reinduction chemotherapy regimen, rate of achieving a second complete remission (CR2) after initial reinduction therapy, detailed HCT information, the outcome and the cause of death. The HCT regimens were defined as reduced intensity if the dose of busulfan was <8 mg/kg or the total body irradiation dose was <800 cGy. All studies were performed with the approval of the Ethics Board of the University of Miyazaki and the JPLSG steering committee.

Genetic analysis

Ninety-three leukemic samples of bone marrow (BM) or peripheral blood obtained at the time of the diagnosis of 111 patients were available for this analysis. DNA and total RNA were extracted using an AllPrep DNA/RNA Mini Kit (Qiagen, Hilden, Germany). Total RNA was reverse transcribed to cDNA with a cDNA Synthesis Kit (GE Healthcare, Tokyo, Japan). Mutational analyses of the *FLT3* -ITD, *KIT* (exons 8 and 17), *N*-and *K-RAS* (exons 1 and 2), *nucleophosmin 1 (NPM1)* (exon 12) and *WT1* (exons 7 and 9) genes were performed using DNA and cDNA in accordance with the previous reports.[14-17] The detection of *KMT2A*-partial tandem duplication (PTD) was performed by multiplex ligation-dependent probe amplification.[18] Screening of *NUP98-NSD1* was performed by reverse transcription-polymerase chain reaction (RT-PCR).[19] The high or low expression of the *MDS1 and EVI1 complex locus* (*MECOM*) and *PR domain containing 16* (*PRDM16*) were determined based on the *ABL1* ratio by quantitative RT-PCR.[20]

Statistical analyses

The primary endpoint of the study was OS after relapse, and the secondary endpoint was the CR2 rate. The OS curves after relapse were estimated by the Kaplan-Meier method. The prognostic factors for OS were identified through comparisons of survival curves with log-rank tests and a multivariate Cox regression analysis. The covariates in the multivariate Cox regression analysis were selected through backward variable selection using a critical value of p=0.15 from the following factors: age, relapse site, duration from the diagnosis to relapse, reinduction therapy, achievement of a CR2, CBF-AML, *KMT2A*rearrangement, *FLT3* -ITD, *N-* and *K-RAS* mutations,*NUP98-NSD1* , *KIT* mutation, *WT1* mutation,*KMT2A* -PTD, *NPM1* mutation, and the expression levels of*MECOM* and *PRDM16* . The prognostic factors for OS and a CR2 were described as the mean ± standard deviation and analyzed using at -test for continuous variables and described as the frequency and proportion and examined using Fisher's exact test for categorical variables. All reported p values are two-tailed, and p values of <0.05 were considered to indicate statistical significance. All of the statistical analyses were performed using the SAS version 9.4 software program (SAS Institute, Cary, NC, USA).

RESULTS

Patient characteristics

The information from 111 of 137 relapsed patients (81.0%) was collected using questionnaires. The characteristics of the 111 patients are shown in Table I. The median age at relapse was 8.2 ± 5.0 years. The median duration from the diagnosis to relapse was 12.7 ± 6.9 months. The sites of relapse were as follows: isolated BM (n=102), BM and central nervous system (CNS; n=3), BM, CNS and other sites (sinusoid or orbit; n=2), and BM and other sites (orbit, liver, spleen, subcutaneous, bone, or lymph node) without CNS involvement (n=4). Morphologically, 33 patients (29.7%) showed FAB M2 morphology, followed by M4 (n=21), M5 (n=20), M7 (n=13), M1 (n=11), M0 (n=4), M6 (n=4), M3 (n=1), and unclassified (n=4). The risk classifications in the AML-05 registry were as follows: high-risk (n=17); intermediate-risk (n=59), and low-risk (n=31). The protocol treatment was discontinued for the 3 remaining patients.

Mutation and expression analyses

We were able to perform molecular analyses for 93 patients (Table I). The t(8;21) and inv(16) mutations were detected in 27 patients and 5 patients, respectively[13]. *KMT2A* rearrangements were detected in 23 patients (24.7%). *KIT* mutations, excluding 7 patients with M541L polymorphism, were detected in 17 patients (17.5%). The following mutations were also detected: *FLT3* -ITD (n=10),*N-RAS* (n=10), *WT1* (n=7), *K-RAS* (n=4),*NUP98-NSD1* (n=2), *KMT2A* -PTD (n=2), and *NPM1* (n=2). High expression levels of *MECOM* and *PRDM16* were identified in 24 (25.8%) and 32 (34.4%) patients, respectively.

Salvage reinduction chemotherapy and response

The type of salvage reinduction chemotherapy depended on each investigator's choice. The salvage regimens were divided into three categories: etoposide, cytarabine and mitoxantron (ECM)-based regimens (n=54); fludarabine, cytarabine and granulocyte colony-stimulating factor (FLAG)-based regimens (n=38); and other regimens (n=14). Two patients immediately received HCT without reinduction chemotherapy. Three patients were treated with palliative therapy. Sixty-four of 106 patients who received reinduction achieved a CR2 after the initial reinduction (60.4%). Table II summarizes the CR2 rate according to the initial reinduction regimens. Thirty-four of 54 patients achieved a CR2 (63.0%) after ECM-based regimens. Twenty-five of 38 patients achieved a CR2 (65.8%) after FLAG-based regimens. Five of 14 patients who received other regimens achieved a CR2 (35.7%). Among patients who relapsed within 1 year from the diagnosis, the CR2 rates in the patients who received ECM- and FLAG-based regimens were 48.0% and 52.4%, respectively. In patients who relapsed more than 1 year after the diagnosis, the CR2 rates in the patients who received ECM- and FLAG-based regimens were 75.9% and 82.4%, respectively.

HCT characteristics

Among 111 patients, 103 patients received HCT. The characteristics of the 103 patients are shown in Table III. The disease status when receiving HCT was classified as CR in 71 patients and non-CR in 32 patients.

One-hundred two patients received allogeneic HCT (related, n=36; unrelated, n=66), while a single patient who relapsed at 17 months after the diagnosis received autologous HCT. BM was the most common source of stem cells (n=61: 59.2%), followed by cord blood (n=33: 32.0%) and peripheral blood (n=9: 8.7%). Thirty-one patients underwent HCT from human leukocyte antigen-matched donors (n=49: 30.1%) and 72 (n=54: 69.9%) underwent HCT from mismatched donors. The conditioning regimens were as follows: myeloablative (n=90 [busulfan-based, n=32; total body irradiation-based, n=58), reduced-intensity (n=5), and others (n=8). The following drugs were administered as prophylaxis against graft-versus-host disease: tacrolimus with short-term methotrexate (n=68), cyclosporin with short-term methotrexate (n=27), methotrexate (n=3), tacrolimus (n=2), and cyclosporine (n=2).

Factors predicting survival after relapse

The causes of death in the 64 patients who died were classified as follows: AML (n=43), treatment-related toxicity (n=20), and accidental death (n=1). The 5-year probability of overall survival (pOS) was 36.1% (95% confidence interval, 25.6–46.7%) (Figure 1). The results of a univariate analysis of prognostic factors related to survival after relapse are shown in Table IV. The mean duration from diagnosis to relapse in the non-surviving group (10.1 ± 4.1 months) was significantly shorter than that in the surviving group (16.3 ± 8.3 months) ($p<0.01$). Patients who relapsed within 12 months definitely showed a poorer prognosis in comparison to those who relapsed at more than 12 months from the diagnosis ($p<0.01$) (Figure 2A). The sites of relapse and FAB classification were not relevant to the survival prognosis ($p=0.91$; $p=0.76$). On a further time-to-event analysis of pOS, failure to achieve a CR2 after initial reinduction chemotherapy was identified as a significant prognostic factor by a log-rank tests ($p<0.01$) (Figure 2B). The five-year overall survival rates according to the AML-05 risk groups were as follows: high-risk, 14.7%; intermediate-risk, 32.3%; low-risk, 61.7%; and 0% for patients who discontinued the protocol treatment ($p<0.01$) (Figure 2C). The presence of the *FLT3* -ITD had a marked disadvantageous effect on the pOS ($p=0.04$). Moreover, relapsed patients with CBF-AML had a significant survival advantage in comparison to patients without CBF-AML ($p<0.01$) (Figure 2D). Relapse within 12 months ($p<0.01$), failure to achieve a CR2 ($p<0.01$) and*FLT3* -ITD ($p=0.01$) were also identified as significant prognostic factors in the multivariate Cox regression analysis.

DISCUSSION

We retrospectively investigated the characteristics of 111 patients with relapsed AML after the JPLSG AML05 protocol. Among the patients with relapsed childhood AML in the JPLSG AML-05R study, the 5-year pOS rate after relapse was 36.1%. These data show a slightly better outcome in comparison to other historical studies[1-11]. One possible reason for this is that 111 relapsed patients in the AML-05R study included a high percentage of patients with relapsed CBF-AML (n=32: 28.8%) due to excessive treatment reduction in the AML-05 study. Among these 32 patients, 21 patients (65.6%) were salvaged by HCT[13]. In non-CBF-AML, the 5-year pOS rate after relapse was 25.4%.

Allogeneic HCT after reinduction therapy is generally selected as a curative strategy after relapse. In our analyses, achieving a CR2 prior to HCT was associated with a good prognosis. FLAG- or ECM-based regimens are therefore recommended for intensive reinduction therapy. Based on the results of our statistical analysis, ECM-based treatment was considered to be as effective as FLAG-based treatment. In our analysis, the FLAG-based regimen included the addition of idarubicin, a single dose of gemtuzumab ozogamicine (GO), or both. In order to improve the CR2 rate, the FLAG-based regimen was modified based on a clinical study and each institution's experience for pediatric AML. Liposomal daunorubicin, which shows less cardiotoxicity, is an attractive drug and its addition to FLAG-based therapy was reported, by the international Berlin-Frankfurt-Munster study group, to achieve a CR rate of 64% in patients with relapsed AML[10]. A French study, based on a single institution's experience reported that the addition of three fractionated doses of GO to FLA resulted in a CR or disease control in 6 of 8 children with refractory AML[21].

To identify the genetic risk factors, we conducted genetic analyses of 10 genes. *FLT3* -ITD was identified as a significantly poor prognostic factor, while CBF-AML was found to be a good prognostic factor. Patients with *FLT3* -ITD showed a dismal prognosis, which was consistent with previous studies. Recently, selective

FLT3 inhibitors, such as gilteritinib and quizartinib, have been demonstrated to be effective as a single agent in adult patients with relapsed or refractory AML[22 23]. In children, quizartinib combined chemotherapy in relapsed AML patients with*FLT3* -ITD showed was associated with an encouraging response with a favorable toxicity profile in a phase I study by the Therapeutic Advances in Childhood Leukemia & Lymphoma Study group[24]. Further improvements of the treatment strategy for pediatric AML patients with *FLT3* -ITD are expected in the near future.

In conclusion, we analyzed 111 pediatric patients with relapsed AML. Achieving a CR2 through ECM- or FLAG-based regimens prior to HCT was important for achieving a good outcome. *FLT3* -ITD and CBF-AML were identified as prognostic factors. The development of molecular targeted therapy or immunotherapy is necessary to establish individualized treatment strategies for patients with relapsed childhood AML.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest in association with the present study.

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FIGURE LEGENDS

Figure 1. Overall survival curve of 111 patients with relapsed acute myeloid leukemia. The 5-year probability of overall survival was 36.1%.

Figure 2 A. Overall survival (OS) after recurrence in patients who relapsed within 12 months of the prognosis and >12 months from the diagnosis (p<0.01). The 5-year probability of OS (pOS) was 22.1% and 54.6%, respectively. B. OS after recurrence in patients who achieved a second complete remission (CR2) and those without a CR2 (p<0.01). The 5-year pOS was 49.7% and 18.4%, respectively. C. OS according to the AML-05 risk group (p<0.01). The 5-year pOS was as follows: high-risk, 14.7%; intermediate-risk, 32.3%; low-risk, 61.7%, and patients who discontinued the protocol treatment, 0% . D. OS after recurrence in patients with core-binding factor (CBF)-AML and non-CBF-AML (p<0.01). The 5-year pOS was 62.1% and 25.4%, respectively.

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Tables20200708.docx available at https://authorea.com/users/346455/articles/472460-the-outcomes-of-relapsed-acute-myeloid-leukemia-in-children-results-from-the-japanese-pediatric-leukemia-lymphoma-study-group-jplsg-aml-05r-study

Figure 1

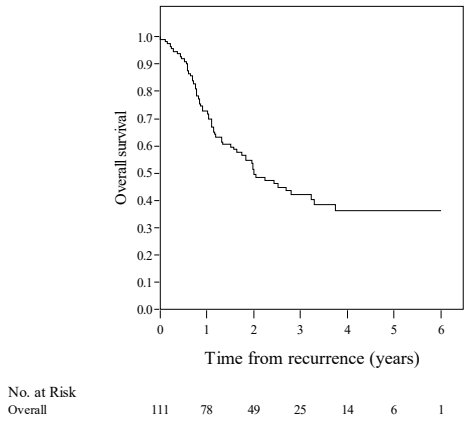
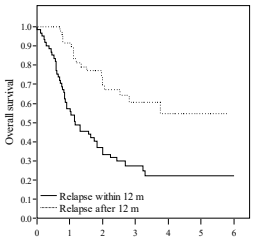


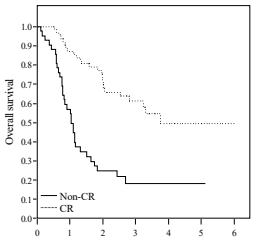
Figure 2

A



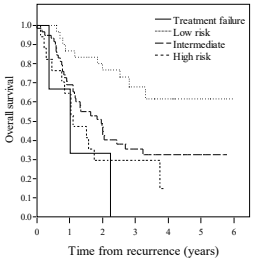
No. at Risk							
Relapse within 12 m	61	34	21	10	5	3	1
Relapse after 12 m	50	44	28	15	9	3	

B



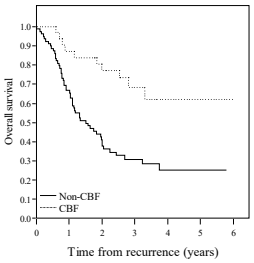
No. at Risk							
Non-CR	42	23	10	5	4	1	
CR	64	54	38	20	10	5	1

C



No. at Risk							
Treatment failure	3	2	1				
Low risk	31	26	23	12	7	3	1
Intermediate	59	39	22	11	7	3	
High risk	17	11	3	2			

D



No. at Risk							
Non-CBF	79	51	26	13	7	3	
CBF	32	27	23	12	7	3	1