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VI. 代 表 的 論 文

VOLUME 27 · NUMBER 24 · AUGUST 20 2009

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Risk-Stratified Therapy and the Intensive Use of Cytarabine Improves the Outcome in Childhood Acute Myeloid Leukemia: The AML99 Trial From the Japanese Childhood AML Cooperative Study Group

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Submitted July 4, 2008; accepted February 23, 2009; published online ahead of print at www.jco.org on July 20, 2009.

Supported by a grant-in aid for cancer research and a grant for clinical cancer research from the Ministry of Health, Labor and Welfare, Japan.

Presented in part in the 47th Annual Meeting of the American Society of Hematology, Atlanta, GA, December 10-13, 2005.

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

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The Acknowledgment and Appendix are included in the full-text version of this article; they are available online at www.jco.org. They are not included in the PDF version (via Adobe® Reader®).

0732-183X/09/2724-4007/$20.00

DOI: 10.1200/JCO.2008.18.7948

ABSTRACT

Purpose
To improve the prognosis in children with newly diagnosed acute myeloid leukemia (AML) by introducing a dose-dense intensive chemotherapy regimen and an appropriate risk stratification system.

Patients and Methods
Two hundred forty children with de novo AML were treated with continuous cytarabine-based induction therapy and stratified to three risk groups based on the initial treatment response, age, and WBC at diagnosis and cytogenetics. All of the patients were treated with intensive consolidation chemotherapy including three or four courses of high-dose cytarabine. Allogeneic hematopoietic stem-cell transplantation (HSCT) was indicated for only the intermediate-risk patients with matched related donors and for all the high-risk subsets.

Results
Two hundred twenty-seven children (94.6%) achieved a complete remission (CR). Four children demonstrated induction death. The median follow-up of the live patients was 55 months (range, 37 to 73 months). The 5-year overall survival of all 240 children was 75.6% (95% CI, 70.3% to 81.4%) and event-free survival was 61.6% (95% CI, 55.8% to 68.1%). The 5-year disease-free survival in each risk group were 71.3% (95% CI, 63.4% to 80.2%) in the low-risk group (n = 112), 59.8% (95% CI, 50.6% to 70.7%) in the intermediate-risk group (n = 92), and 56.5% (95% CI, 39.5% to 80.9%) in the high-risk group (n = 23). Eight children died during the first CR, including four after HSCT.

Conclusion
A high survival rate, 75.6% at 5 years, was achieved for childhood with de novo AML in the AML99 trial. The treatment strategy was well tolerated with only 1.7% induction death rate and 3.5% remission death rate. Low-risk children were successfully treated with chemotherapy alone.

J Clin Oncol 27:4007-4013. © 2009 by American Society of Clinical Oncology

INTRODUCTION

The use of intensive chemotherapy and hematopoietic stem-cell transplantation (HSCT) with better facilities for supportive care over the last two decades has achieved dramatic improvements in the treatment outcome for children with acute myeloid leukemia (AML). Approximately 80% to 90% of these children now achieve a complete remission (CR) and the 5-year overall survival (OS) and event-free survival (EFS) rates are 50% to 60% and 40% to 50%, respectively.[1,2] However, when the results are compared with those of pediatric acute lymphoblastic leukemia (ALL), they are not so favorable and further improvements are necessary. HSCT may be the treatment of choice for improving the outcome in children with AML.[3,4] However, considering acute regimen-related toxicities and long-term adverse effects of HSCT, the indications for HSCT during the first CR should be restricted.[5,6]

We conducted a nationwide cooperative clinical protocol AML99 investigation, in which a risk-stratified strategy and dose-dense intensive chemotherapy were introduced. In risk stratification, low-risk patients were treated with chemotherapy alone and allogeneic (Allo) HSCT was indicated only for the intermediate-risk patients with a matched related donor and for all of the high-risk

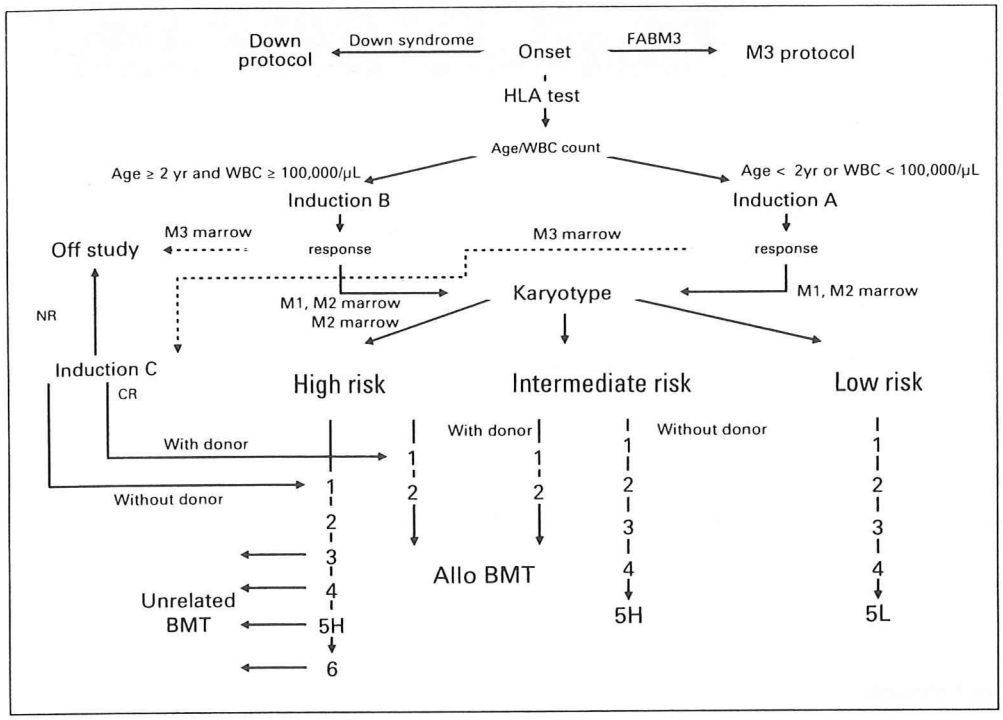

Fig 1. Scheme and details of the Japanese cooperative study AML99. Refer to the Appendix (online only) for further explanation. Abbreviations: FABM3, French-American-British classification M3; Allo, allogenic; NR, no response; CR, complete remission; BMT, bone marrow transplant; HLA, human leukocyte antigen.

patients. In dose-dense intensive chemotherapy, either continuous or high-dose cytarabine was adopted in all courses of chemotherapy. This report describes the improved treatment results of the AML99 protocol for children with de novo AML.

Between January 2000 and December 2002, a total of 260 children age 0 to 18 years with newly diagnosed AML, excluding children with Down's syndrome and acute promyelocytic leukemia, were enrolled in the AML99 trial by 98 centers, which covered approximately two thirds of the Japanese pediatric population. The French-American-British classification was used for the initial diagnosis of AML. Ten children were excluded from further analysis because of the following reasons: misdiagnosis (n = 4), natural killer (NK) cell/myeloid leukemia (n = 2), granulocytic sarcoma (n = 1), and death before initiation therapy (n = 3). Ten other children with secondary AML were also excluded from this analysis.

Treatment Design of the AML99 Trial

The schema and details of the AML99 protocol are shown in Figure 1. Children younger than age 2 years or those with a WBC lower than 100,000/μL at diagnosis were treated with induction A. Children older than age 2 years and with WBC of 100,000/μL or higher were treated with induction B. Induction C was a rescue regimen for children who showed M3 marrow after induction A. Consolidation therapy consisted of five (for low- and intermediate-risk group) or six (for high-risk group) courses and triple intrathecal therapy was given as a part of each course. After consolidation course 1 (the second course of therapy) or induction C, patients in remission were stratified into three risk groups: low-risk children were defined as those with t(8;21) and a WBC lower than 50,000/μL, inv(16), or an age younger than 2 years without high-risk factors; high-risk children were those with CR after consolidation course 1 or induction C or with abnormalities of monosomy 7,[7] 5q-[7], t(16;21),[8] t(9;22) (Philadelphia chromosome [Ph1])[9]; intermediate-risk children were those who were not in either a low-risk or high-risk group. Low-risk children were treated only with chemotherapy, regardless the availability of a suitable HSCT donor. All high-risk children were allocated to Allo-HSCT in the first remission, including unrelated bone marrow transplantation (BMT). Matched related BMT was recommended for intermediate-risk children with a HLA-matched-related donor (MRD), whereas the remainder of the children was randomly assigned between four courses of consolidation chemotherapy plus autologous BMT (A-BMT) versus five courses of chemotherapy. However, the random assignment was stopped and the protocol was amended to eliminate the A-BMT arm in June 2002, because of a very low consent rate for this random assignment. Only five patients underwent A-BMT and these patients were included in the chemotherapy group in the current analysis. No prophylactic cranial irradiation was included in the protocol. Patients were treated on an inpatient basis during each treatment phase. The protocol was approved in

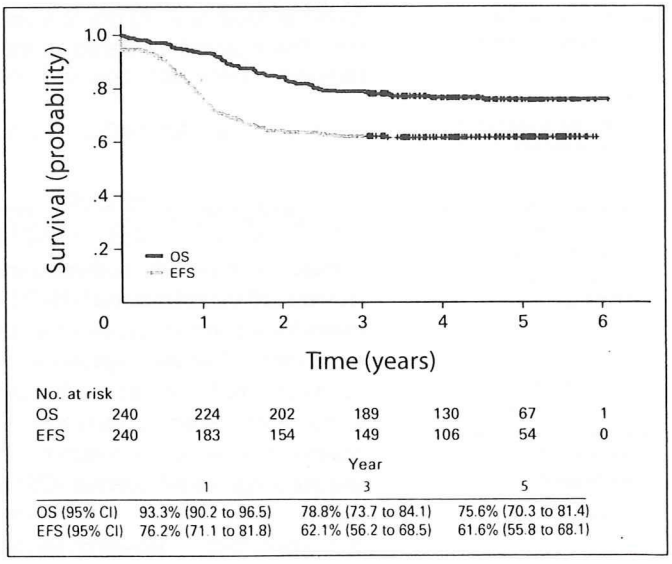

Fig 2. Probability of overall survival (OS) and event-free survival (EFS) in the Japanese cooperative study AML99.

Table 1. Patient and Disease Characteristics

Characteristic	Patients	
	No.	%
Patients enrolled	260	
Patients analyzed	240	100
Age, years		
< 2	45	19
2-9	116	48
≥ 10	79	33
Sex		
Male	128	53
Female	112	47
WBC, $\times 10^3/\mu L$		
< 20	115	48
20-< 50	60	25
50-< 100	29	12
≥ 100	36	15
CNS involvement		
Yes	7	3
No	233	97
FAB type		
M0	10	4
M1	36	15
M2	84	35
M4	39	16
M5a	27	11
M5b	17	7
M6	3	1
M7	20	8
Unclassifiable/not known	4	2
Cytogenetics		
t(8;21)	77	32
inv16	12	5
11q23 abnormalities	41	17
t(9;11)	15	6
Other 11q23 abnormalities	26	11
Normal	53	22
Others	56	23
Unknown	1	< 1

Abbreviation: FAB, French-American-British.

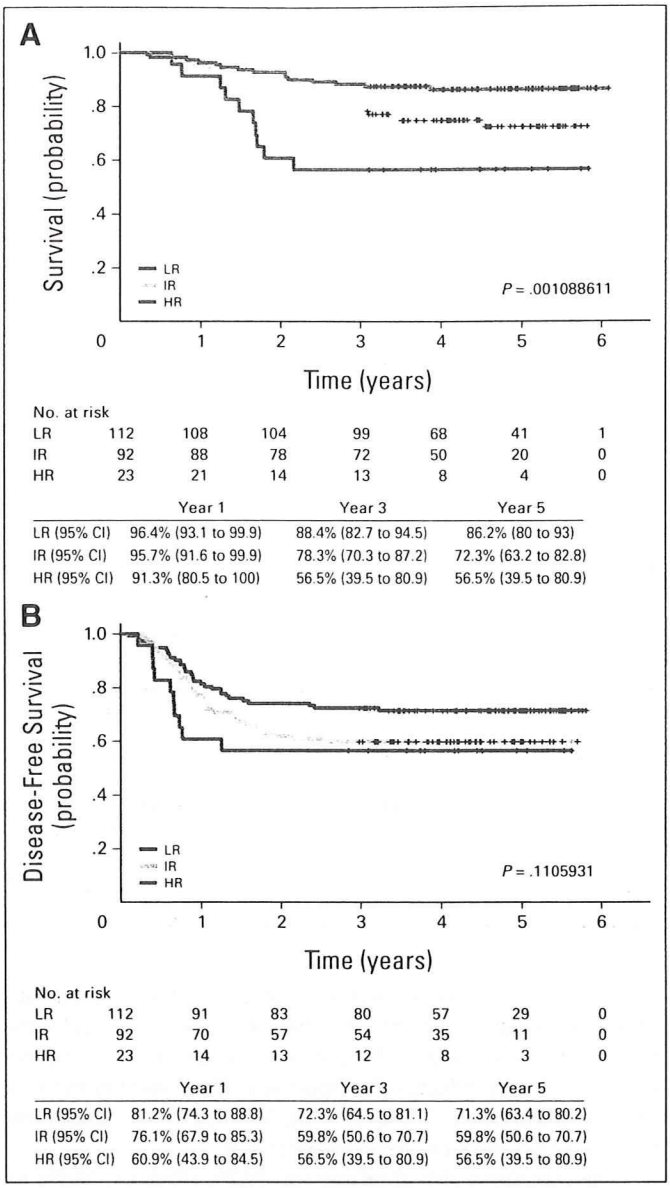

Fig 3. Probability of survival by risk group in the Japanese cooperative study AML99: (A) overall survival and (B) disease free survival. LR, low risk; IR, interediate risk; HR, high risk.

the institutional review board and written informed consent was obtained from the parents or guardians of all patients.

Statistical Analysis

CR was defined by fewer than 5% blast cells in the bone marrow aspirate and the absence of extramedullary involvement (EMI) and had to be achieved before starting of consolidation course 2. CR rates were compared between induction A and B using the Mantel-Haenzel test for trend and Fisher's exact test. The estimation of survival was performed using the Kaplan-Meier method and the curves were compared by means of the log-rank test. The OS was defined as time from the start of treatment to death from any cause or last follow-up. The EFS was defined as time from the start of treatment to first event (induction failure, relapse, or death from any cause) or the last follow-up. The disease-free survival (DFS) was defined as time from the date of remission to first event (relapse or death from any cause) or last follow-up. The CIs were calculated according to Greenwood's formula.

A total of 240 children with newly diagnosed de novo AML, excluding children with Down's syndrome and acute promyelocytic leukemia, were eligible in the current analysis. The median follow-up of the surviving patients was 55 months (range, 37 to 73 months). The characteristics of the patients and the diseases are listed in Table 1.

Overall Results

The bone marrow response rate (< 5% blasts in bone marrow after initial induction course) was 87.1% (209 of 240) and the CR rate (after the first consolidation course or induction C) was 94.6% (227 of 240). Four patients demonstrated induction death (1.7%) and eight children had resistant disease. Eight children with resistant disease were treated with Allo-HSCT, and four of these patients were still alive at the first CR. In one patient, induction chemotherapy was stopped because of toxicity, and this patient was treated with chemotherapy only and still alive in the first CR. Of the 240 children, 214 children were treated with induction A and 26 were treated with induction B.

Table 2. Outcome Data of the Recent Studies for Pediatric AML From Major Groups

Study Group	No. of Patients	Early Death Rate (%)	CR Rate (%)	Time of Evaluation	CR Rate (%) After One Course of Chemotherapy	Induction Regimen (/m^2)	No. of Courses
EORTC-CLG 58,921[11,12]	177	2	84	After 2 courses	69	Ara-C 100 mg 24 hours cont IV days 1-2, 100 mg/12 hours days 3 to 8; VP-16 150 mg IV day 3-5; MIT or IDA 10 mg days 6 to 8	4 Maintenance
LAME-91[13,14]	247	4	91	After 2 courses	84	Ara-C 200 mg 24h cont IV days 1 to 7; MIT 12 mg IV days 1 to 5	3 Maintenance
BFM-93[15-17]	427	7	83	After 4 courses	ND	Ara-C 100 mg 24 hours cont IV days 1 to 2, 100 mg/12 hours days 3 to 8; VP-16 150 mg IV days 6 to 8; DNR 60 mg or IDA 12 mg IV days 3 to 5	4 Maintenance
BFM-98[18,19]	473	3	88	After 4 or 5 courses	ND	Ara-C 100 mg 24 hours cont IV days 1 to 2, 100 mg/12 hours days 3 to 8; VP-16 150 mg IV days 6 t o 8; IDA 12 mg IV days 3 to 5	4 or 5 Maintenance
MRC-AML10[20,21]	303	4	93	After 4 courses	68	Ara-C 100 mg/12 hours IV days 1 to 10; DNR 50 mg IV days 1, 3, 5; 6-TG 75 mg/12 hours PO days 1 to 10 or VP-16 100 mg IV days 1 to 5	4
MRC-AML12[22,23]	455	4	92	After 4 courses	ND	Ara-C 100 mg/12 hours IV days 1 to 10; VP-16 100 mg IV days 1 to 5; DNR 50 mg IV days 1, 3, 5 or MIT 12 mg IV days 1, 3, 5	4 or 5
NOPHO-AML93[24,25]	223	2	92	After 2 or 3 courses	65	Ara-C 200 mg 24 hours cont IV days 1 to 4; VP-16 100 mg 24 hours cont IV days 1 to 4; DOX 75 mg 8 hours IV day 5; 6-TG 100 mg/12 hours PO days 1 to 4	6-8
POG-8821[26,27]	511	4	77	After 2 courses	ND	Ara-C 100 mg 24 hours cont IV days 1 to 7; DNR 45 mg IV days 1 to 3; 6-TG 100 mg PO days 1 to 7	9
CCG-2891[28,29]	750	4	78	After 2 courses	74	DEX 6 mg/12 hours; Ara-C 200 mg cont IV; 6-TG 100 mg/12 hours; VP-16 100 mg cont IV; DNR 20 mg cont IV days 0 to 4, 10 to 14, or 14 to 18	8
TCCSG AML M91-13 and M96-14[10]	192	3.6	88	ND	ND	Ara-C 200 mg 12 hours cont IV days 6 to 12; VP-16 150 mg 2 hours IV days 1 to 5; MIT 5 mg IV days 6 to 10	7 or 9
AML99	240	1.7	94	After 2 courses	86	Ara-C 200 mg 12 hours cont IV days 6 to 12; VP-16 150 mg 2 hours IV days 1 to 5; MIT 5 mg IV days 6 to 10	6

(continued on following page)

The bone marrow response rate, the CR rate, and induction death rate of these two groups were 88.8% (n = 190), 95.8% (n = 205) and 1.4% (n = 3) with induction A, and 73.1% (n = 19), 84.6% (n = 22), and 3.9% (n = 1) with induction B, respectively. The 5-year OS and EFS for all 240 children was 75.6% (95% CI, 70.3% to 81.4%) and 61.6% (95% CI, 55.8% to 68.1%), respectively (Fig 2).

The cumulative risk of relapse was 32.2% (95% CI, 38.1% to 25.7%). The relapse sites were predominantly (86.3%; 63 of 73) located in the bone marrow (BM). Ten patients suffered from EMI or combined BM plus EMI. Although no prophylactic cranial irradiation was included in this protocol, CNS relapses occurred only in three patients (three of 227; 1.3%). One patient suffered a CNS relapse with a BM relapse, one patient a BM relapse and a skin relapse, and one patient a testicular relapse. Although AML99 was a highly intensive protocol, only eight children (3.5%) died in the first CR, four during chemotherapy and four after HSCT.

Results According to Risk Stratification

Among those who achieved first remission, 112 children were stratified to the low-risk group, 92 to the intermediate-risk group, and 23 to the high-risk group. The 5-year OS and DFS in each of the risk groups were 86.2% (95% CI, 80.0% to 93.0%) and 71.3% (95% CI, 63.4% to 80.2%) in the low-risk group, 72.3% (95% CI, 63.2% to 82.8%) and 59.8% (95% CI, 50.6% to 70.7%) in the intermediate-risk group, and 56.5% (95% CI, 39.5% to 80.9%) and 56.5% (95% CI, 39.5% to 80.9%) in the high-risk group (Fig 3).

Among the low-risk children, 96 of 112 underwent five courses of consolidation chemotherapy without any event. Six patients relapsed and three died of infection in CR during chemotherapy. In seven patients, chemotherapy was stopped because of other reasons (three for infectious complications, three for protocol violation including one who underwent Allo-BMT, and one for a parent's refusal).

Among the intermediate-risk children, 22 had a matched related donor and 70 had no donor. Of 22 patients with a donor, 21 received MRD HSCT and one did not because of a fungal infection. After HSCT, two died in CR (one of respiratory distress and one of acute graft-versus-host disease). Among the 70 patients without a donor, 62 received chemotherapy only, three received Allo-HSCT, and five received auto HSCT. Of the 62 patients who received chemotherapy, seven relapsed, one died of infection during chemotherapy, and chemotherapy was stopped in two patients because of infectious complications. The 5-year DFS in the matched donor group and the no donor group were 81.8% (95% CI, 67.2% to 99.6%) versus 52.9% (95%CI, 42.4% to 65.9%; $P = .029$), respectively. However, there was no statistical difference in terms of OS in the matched donor group versus the no donor group (81.8%, 95% CI, 67.2% to 99.6% *v* 69.2%, 95% CI, 58.3% to 82.1%; $P = .380$).

Sixteen of the 23 children in the high-risk group received HSCT in the first CR (six related BMT, six unrelated BMT, and four cord blood stem-cell transplantation). Two patients who received cord blood stem-cell transplantation died in CR (one of fungal infection

Table 2. Outcome Data of the Recent Studies for Pediatric AML From Major Groups (continued)

Study Group	Cumulative Doses				5-Year EFS		5-Year OS	
	Anthracyclines (mg/m^2)	Cytarabine (g/m^2)	High-Dose Cytarabine (dose [$/m^2$] × times/course × number of courses)	Etoposide (mg/m^2)	%	SE (%)	%	SE (%)
EORTC-CLG 58,921[11,12]	380	23.32-29.32	3 g × 6 × 1 or 3 g × 8 × 1 or 3 g × 10 × 1; 2 g × 6 × 1	1,350	48	4	62	4
LAME-91[13,14]	460 Amsacrine 450	9.8-13.4	1 g × 8 × 1	400	48	4	62	4
BFM-93[15-17]	300-400	23.1-41.1	3 g × 6 × 1 or 3 g × 6 × 2	950	51	3	58	2
BFM-98[18,19]	420	41-47	3 g × 6 × 2 or 3 g × 6 × 2, 1 g × 6 × 1	950	49	3	62	3
MRC-AML10[20,21]	550 Amsacrine 500	10.6	1 g × 6 × 1	500-1,500	49		58	
MRC-AML12[22,23]	300-610 Amsacrine 500	4.6-34.6	(−) or 1 g × 6 × 1 or 3 g × 8 × 1 or both	1,500	56		66	
NOPHO-AML93[24,25]	300-375	49.6-61.3	1 g × 6 × 1; 2 g × 6 × 2 or 3; 3 g × 6 × 1	1,600	50	3	66	3
POG-8821[26,27]	360	55.7	3 g × 6 × 3	2,250	32	2	42	2
CCG-2891[28,29]	350	28.3	3 g × 4 × 2	1,900	34	3	45	3
TCCSG AML M91-13 and M96-14[10]	495	69.4-99.4	3 g × 6 × 2; 3 g × 5 × 4 or 2	3,750-5,750	56		62	
AML99	300-375	59.4-78.4	3 g × 6 × 2; 2 g × 10 × 1 or 2	3,150-3,200	61	3	75	3

Abbreviations: AML, acute myeloid leukemia; CR, complete remission; EFS, event-free survival; OS, overall survival; EORTC-CLG, European Organization of Research and Treatment of Cancer–Children Leukemia Group; Ara-C, cytarabine; cont, continuous; IV, intravenously; VP-16, etoposide; MIT, mitoxantrone; IDA, idarubicin; LAME, French Leucemie Aigue Myeloblastique Enfant; BFM, Berlin-Frankfurt-Munster; ND, no data; DNR, daunorubicin; MRC, Medical Research Council; PO, orally; DOX, doxorubicin; NOPHO, Nordic Society of Pediatric Hematology and Oncology; POG, Pediatric Oncology Group; CCG, Children's Cancer Group; TCCSG-AML, Tokyo Children's Cancer Study Group–Acute Myeloid Leukemia.

and one of acute graft-versus-host disease). The 5-year OS of these 16 patients was 68.8%. Of seven patients who did not received Allo-HSCT in the first CR, five patients relapsed and died despite receiving Allo-HSCT after the first relapse, and two patients were still alive in the first CR with chemotherapy only.

DISCUSSION

The 5-year EFS of 61.6% and 5-year OS of 75.6% achieved in the AML99 is better than those reported in the Tokyo Children's Cancer Study Group (TCCSG) study (from August 1991 to September 1998) conducted preceding to the AML99 (5-year EFS, 56%; 5-year OS, 67%).[10] The chemotherapy regimens in TCCSG AML M91-13 and M96-14 comprised a total nine and seven courses, respectively. In these two studies, the remission induction course was the same as that of induction A course in the AML99 protocol and six of eight consolidation courses included high-dose cytarabine in M91-13 and four of six in M96-14. Since the reduction on consolidation chemotherapy courses from eight to six did not compromise the treatment results in this TCCSG studies, the chemotherapy regimen in the AML99 protocol comprised five consolidation courses. In TCCSG studies, only two high-dose cytarabine courses administered at 12-hour intervals and in the AML99 protocol, three or four high-dose cytarabine courses administered at 12-hour intervals including one or two courses of $2g/m^2$ cytarabine every 12 hours for 5 days. This dose dense use of cytarabine in the AML99 protocol may be one of the main explanations for the superior outcome.

Table 2[10-29] presents that the results achieved in the Japanese AML99 protocol is currently the best among the large-scale studies reported from other major childhood AML study groups.

The induction regimen of AML99 is quite unique regarding its 12-day long duration of treatment and the precedent setting administration of etoposide. When comparing the marrow response rate after one course of chemotherapy, AML99 has a rate of 86% and this result is better than those of other studies (Table 2). This good marrow response rate may explain one of the reasons for the superior outcome observed in AML99.

Table 2 presents cumulative doses of drugs, the number of chemotherapy courses, and the survival rates in the major study groups. In comparison to other studies, AML99 used much more cumulative doses of cytarabine including two or three courses of high-dose cytarabine, more doses of etoposide, and moderate doses of anthracyclines

during total six courses of chemotherapy. The good survival rates achieved by incorporating high cumulative doses of anthracyclines in the French Leucémie Aiguë Myéloblastique Enfant study[13,14] and in the Medical Research Council (MRC) study,[20-23] or the frequent use of high-dose cytarabine in the Nordic Society of Pediatric Hematology and Oncology (NOPHO) study[24,25] shows that these strategies may improve the outcome of children with AML. However, considering the long-term adverse effects of cardiotoxicity caused by anthracyclines, high-dose cytarabine plays a key role in postremission chemotherapy.[2,30] Cancer and Leukemia Group B showed that the higher postremission cytarabine dose was associated with a better 5-year continuous CR (3 g/m^2, 42%; 400 mg/m^2, 33%; 100 g/m^2, 17%; $P < .001$) especially in core binding factor (CBF) AML, such as AML with t(8;21) or inv(16) and AML with a normal karyotype.[31] Repetitive use of high-dose cytarabine based postremission chemotherapy in AML99 may be one of the main explanations for the superior outcome. The treatment of AML is usually very intensive and near-myeloablative and the hematologic toxicities and related complications, such as infections, are severe and sometimes fatal. In AML99, the early death rate was only 1.7% and the death rate in first CR was 3.5%. These rates were the lowest among the major group studies.[1]

In the AML99 protocol, 89 patients with CBF AML were included and the 37% incidence (89 of 239 patients) was higher than the 31% incidence observed in TCCSG studies,[10] 20% in MRC12,[22,23] or 22% in Berlin-Frankfurt-Munster 98.[18,19] The patients with CBF AML tend to show a relatively favorable outcome and appear to profit from the administration of high-dose cytarabine. This may be one of the reasons for the superior outcome in the AML99 protocol. In the AML99 trial, low-risk children were treated with chemotherapy alone and their 5-year EFS and OS was 71.3% and 86.2%, respectively. These results reveal that children with low-risk AML can therefore be cured with chemotherapy alone. In the low-risk group, six patients had severe adverse events in CR (three died of infection and three had cessation of the protocol due to infection). It may therefore be appropriate to reduce the course of treatment for low-risk children, because there was no difference in the survival or relapse rate between four and five courses of treatment by the randomized control trial in the MRC AML12 study.[22,23]

In AML99, the intermediate-risk children were genetically randomly assigned to receive MRD HSCT during the first CR. Patients with MRD had a significantly better DFS, but the OS between the donor group and no-donor group did not differ significantly. These results suggest that matched related BMT should be reserved for the second CR in intermediate-risk children. MRC AML10 revealed that in patients treated with Allo-HSCT, there was a decrease in the relapse rate (donor 26% *v* no donor 42% at 7 years; $P = .02$), but no significant OS advantage (donor 70% *v* no donor 60% at 7 years; $P = .1$).[21,23] In the NOPHO-AML93, the 7-year DFS was higher in children treated with Allo-BMT in comparison to those treated with chemotherapy (64% *v* 51%; $P = .04$), but an analysis of the OS showed no difference (71% *v* 69%).[24,25] This good result in the chemotherapy group can be explained by the good results in the relapsed patients treated with HSCT in the second CR.[32] Since the outcome of pediatric AML treated only with intensive chemotherapy has been improved and relapsed children are still alive at the first CR after a successful subsequent HSCT, the indications for HSCT during the first remission should therefore be limited to high-risk children.

Based on these considerations, the following AML-05 study conducted by the Japanese Leukemia/Lymphoma Study Group, which covers almost all Japanese children with leukemia or lymphoma, is presently in progress to assess the validity of the reduced number of consolidation courses with more restrictive indications for HSCT.

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

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bjh research paper

FBXW7 and *NOTCH1* mutations in childhood T cell acute lymphoblastic leukaemia and T cell non-Hodgkin lymphoma

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Received 19 November 2008; accepted for publication 28 December 2008

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Summary

Mutation analysis of *FBXW7* and *NOTCH1* genes was performed in 55 T cell acute lymphoblastic leukaemia (T-ALL) and 14 T cell non-Hodgkin lymphoma (T-NHL) patients who were treated on the Japan Association of Childhood Leukaemia Study (JACLS) protocols ALL-97 and NHL-98. *FBXW7* and/or *NOTCH1* mutations were found in 22 (40·0%) of 55 T-ALL and 7 (50·0%) of 14 T-NHL patients. *FBXW7* mutations were found in 8 (14·6%) of 55 T-ALL and 3 (21·4%) of 14 T-NHL patients, and *NOTCH1* mutations in 17 (30·9%) of 55 T-ALL and 6 (42·9%) of 14 T-NHL patients. Three (5·4%) T-ALL and two (1·4%) T-NHL patients had mutations in both *FBXW7* and *NOTCH1*. *FBXW7* mutations included one insertion, one deletion, one deletion/insertion and nine missense mutations. *NOTCH1* mutations were detected in the heterodimerization domain (HD) in 15 cases, in the PEST domain in seven cases, and in both the HD and PEST domains in one case. Five-year event-free survival and overall survival for patients with *FBXW7* and/or *NOTCH1* mutations were 95·5% (95% CI, 71·9–99·4%) and 100% respectively, suggesting that T-ALL patients with *FBXW7* and/or *NOTCH1* mutation represent a good prognosis compared to those without *FBXW7* and/or *NOTCH1* mutations (63·6%, $P = 0·007$ and 78·8%, $P = 0·023$, respectively).

Keywords: ALL, childhood, prognostic factors, genetic analysis, T cells, molecular diagnosis.

The outcomes of paediatric T cell acute lymphoblastic leukaemia (T-ALL) have improved in recent years as a result of intensified therapies, with 5-year relapse-free survival rates in the range of about 60–85% (Gaynon *et al*, 2000; Maloney *et al*, 2000; Moghrabi *et al*, 2007; Pui *et al*, 2004), which are relatively low compared to those of B-precursor ALL. A stringent assessment of the risk of relapse is critical in determining which patients need to receive more effective therapy. In T-ALL, it has been reported that the abnormal expression of *TLX1* (*HOX11*) is associated with a favourable prognosis, although the prognostic significance of this finding has yet to be determined (Ferrando *et al*, 2004; Ferrando *et al*, 2002). On the other hand, a few reports have suggested that microarray analysis could distinguish high-risk cases in T-ALL (Ferrando & Look 2003; Winter *et al*, 2007).

Recently, activating mutations of *NOTCH1* gene have been reported in more than half of T-ALL cases (Weng *et al*, 2004). *NOTCH1*, previously termed *TAN1*, was discovered as a

First published online 24 February 2009
doi:10.1111/j.1365-2141.2009.07607.x

partner gene in T-ALL with a *t*(7;9)(q34;q34·3), and was found in <1% of T-ALLs (Ellisen *et al*, 1991). A good clinical outcome for T-ALL patients with *NOTCH1* mutations was reported in the paediatric ALL-BFM 2000 study (Breit *et al*, 2006). On the contrary, other papers reported that *NOTCH1* mutations were not associated with good clinical outcome in T-ALL (van Grotel *et al*, 2008; Zhu *et al*, 2006). Thus, clinical significance of *NOTCH1* mutation in T-ALL still remains controversial.

F-box and WD40 domain protein 7 (FBXW7; previously termed FBW7, CDC4, or Archipelago), is also considered a candidate prognostic factor in T-ALL. FBXW7 was originally isolated as a Lin12/NOTCH-negative regulator in Caenorhabditis elegans (Hubbard *et al*, 1997), and plays a critical role in intracellular NOTCH1 degradation which depends on an intact NOTCH1 PEST domain (Fryer *et al*, 2004; Tetzlaff *et al*, 2004). Recently, it was reported that the *FBXW7* gene was mutated in various tumours including breast, ovarian, and endometrial cancers and T-ALL cell lines (Moberg *et al*, 2001).

In this study, we analyzed the frequencies and clinical significance of *FBXW7* and *NOTCH1* mutations in paediatric T-ALL and T cell non-Hodgkin lymphoma (T-NHL). *FBXW7* as well as *NOTCH1* was found to be frequently mutated in paediatric T-ALL and T-NHL. We firstly described that mutations of either *FBXW7* or *NOTCH1* genes, rather than *FBXW7* or *NOTCH1* alone, were associated with good clinical outcome in T-ALL.

Methods

Patients and treatments

All children with T-ALL or T-NHL, aged under 15 years were enrolled into the Japan Association of Childhood Leukaemia Study (JACLS) protocol ALL-97 between 1997–2001 and JACLS trial NHL-T98 between 1998–2002 (Oda *et al*, 2006) (Fig S1). All T-NHL patients were pathologically diagnosed as having lymphoblastic lymphoma. Patients who failed to obtain complete remission (CR) with risk adapted induction chemotherapy were scheduled to undergo F-protocol at 6 weeks following the start of their initial induction chemotherapy. Samples from 55 newly diagnosed T-ALL and 14 T-NHL patients were examined in this study. At the time of diagnosis, bone marrow (BM) and/or peripheral blood (PB) cells were obtained from T-ALL patients and lymph nodes and/or pleural effusions were obtained from T-NHL patients. T-lineage immunophenotypic subtype was defined as simultaneous expression of two or more T-lineage associated molecules including CD2, CD3, CD5, CD7, and CD8, on at least 20% of lymphoblasts. T-ALL was characterized by definition as the presence of more than 25% bone marrow involvement of lymphoblasts. Cytogenetic studies were performed on 60 patients by using regular G-banding method. Advanced stage (stages 3 and 4) T-NHL patients were enrolled in this protocol, and the histopathology of specimens was reviewed by central pathology reviewers. A total of 69 patients were included in the present study; 49 were male and 20 female; 55 were children diagnosed with T-ALL (median age of 9·5 years; range: 2·0–15·0 years) and 14 with T-NHL (median age of 11·0 years; range: 3·7–15·0 years). The basic clinical and immunological characteristics of this patient subgroup did not differ from those of the entire group. The two-year treatment regimen consisted of induction therapy (vincristine sulfate, high dose-methotrexate, cytarabine, prednisone, L-asparaginase), five drug consolidation therapy A and B including high doses of L-asparaginase, and maintenance therapy with block-rotated treatment using the drugs listed above. Informed consent was obtained from the patients or their parents, according to guidelines based on the tenets of the revised Helsinki protocol. The institutional review board of Gunma Children's Medical Centre approved this project.

DNA and RNA preparation

DNA and RNA were prepared from samples of BM, PB, lymph nodes, and pleural effusions containing tumour cells of patients with primary T-ALL and T-NHL, by using the AllPrep DNA/RNA Mini Kit (Qiagen, Valencia, CA, USA).

Detection of FBXW7 and NOTCH1 mutations

Mutation detection was performed by polymerase chain reaction (PCR)-based denaturing high-performance liquid chromatography (dHPLC) using a WAVE DNA fragment analysis system (Transgenomic, Omaha, NE, USA) equipped with a DNASep HT cartridge (Weng *et al*, 2004). The PCR products of positive cases detected by PCR-based dHPLC were purified using the QIAquick PCR Purification Kit (Qiagen). Sequencing by means of fluorescent-dye chemistry was performed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) (Shimada *et al*, 2006; Taketani *et al*, 2004). For further confirmation of insertion and deletion mutations, the purified PCR products were subcloned using a TOPO-TA Cloning Kit (Invitrogen, Carlsbad, CA, USA) and then sequenced (Taketani *et al*, 2004). *FBXW7* mutations were screened from exons 2 to 12 using primers described previously (Cassia *et al*, 2003). *NOTCH1* mutations in the N-terminal region and the C-terminal region of the HD domain (exons 26 and 27), the transcriptional activation domain (TAD) (exon 34), and the PEST domain (exon 34) were screened by using primers described previously (Weng *et al*, 2004).

Statistical analyses

Proportional differences between groups were analyzed by chi-squared or Fisher's exact tests. The Kaplan–Meier method was used to estimate survival rates. Differences in prognosis between groups were evaluated using the log-rank test. Event-free survival (EFS) was measured from the time of diagnosis

to the time of analysis or first event. Failure to achieve remission, relapse or death that occurred during continuous complete remission were evaluated as events. Overall survival (OS) was defined as the time from diagnosis to death. Multivariate survival analysis was performed using the Cox proportional-hazards model. A P value of less than 0·05 (two-sided) was considered statistically significant. All statistical analyses were performed using STATA 8·1 (STATACORP LP, College Station, TX, USA).

Results

FBXW7 and NOTCH1 mutations in T-ALL and T-NHL patients

FBXW7 and/or *NOTCH1* mutations were found in 22 (40·0%) of 55 T-ALL and 7 (50·0%) of 14 T-NHL patients (Tables I–III). *FBXW7* mutations were found in 8 (14·6%) of 55 T-ALL and 3 (21·4%) of 14 T-NHL patients, and *NOTCH1*

Table I. *FBW7* and *NOTCH1* mutations in T-ALL and T-NHL patients.

	FBXW7 mutation		*NOTCH1* mutation	
Patient no.	Nucleotide*	Amino acid	Nucleotide†	Amino acid
T-ALL 4	–	–	4778T > C	L1593P
T-ALL 5	1662C > T	R505C	4817_4818insGCCCCC	1606delinsLPP
T-ALL 8	1450_1451ins AGCTGTT GTCTCTCATCATATG CCTTCTCAC	435AVVSHHMPSHHfsX	–	–
T-ALL 20	1542C > T	R465C	4847T > A	I1616N
T-ALL 22	–	–	4775_4776insGAC	1592delinsLT
T-ALL 23	–	–	7355_7356insCTGGC	2453WRCTLFCPRKAPPCP RRCHPRWSHPfsX
T-ALL 26	–	–	4818_4819insCTTTATCTC	1606_1607insHYL
T-ALL 30	–	–	4732_4734del	1578delV
T-ALL 31	–	–	4732_4734del	1578delV
T-ALL 32	2029T > C	V627A	–	–
T-ALL 33	–	–	4754T > C	L1585P
T-ALL 34	1543G > A 715_718delinsGAC	R465H 189RPQNIQVPLGLYHV QQHQQLLGTSEQPM AKGNNDAELHLSSHL QASRNGfsX	–	–
T-ALL 35	–	–	7412delinsAG	S2471X
T-ALL 37	–	–	4732-4734del	1578delV
T-ALL 38	1585G > A	R479Q	–	–
T-ALL 41	–	–	4754T > C	L1585P
T-ALL 46	–	–	7318C > T	Q2440X
T-ALL 49	1585G > A	R479Q	4732-4734del	1578delV
T-ALL 50	–	–	7330C > T	Q2444X
T-ALL 65	–	–	4814_4815delinsCCCCCCCCGA CCATAAGCC	1606PPDHKPSVTHTASRfsX
T-ALL 67	1543G > A	R465H	–	–
T-ALL 75	–	–	4818_4822delinsAGCACACCA GCCCAAGC	1606delinsLAHQP
T-NHL 18	–	–	4709_4718del	1570_1573delinsVDK
T-NHL 25	–	–	7541_7542del	2515RVPfsX
T-NHL 54	–	–	4793G>C	R1598P
			7541_7542del	2515RVPfsX
T-NHL 55	1543G > A	R465H	–	–
T-NHL 58	1543G > A	R465H	4845_4847delinsCCCCTCGAA	1615_1617delinsIPSNF
T-NHL 59	–	–	7326_7327insCGCGGAGGTGC	2443RGGACSHWAPAAWRC TLFCPRRAPPCP RRCHPR WSHPfsX
T-NHL 61	2107del	653RVNLFETfsX	7403_7404insGGGGG	2469GGHPRWSHPfsX

*Nucleotide number is according to the GenBank accession number NM_033632.
†Nucleotide number is according to the GenBank accession number NM_017617.

Table II. Association of *NOTCH1* and *FBXW7* mutations with clinical characteristics in 55 T-ALL patients.

	NOTCH1			*FBXW7*			*FBXW7* and/or *NOTCH1*		
	Mutation (+)	Mutation (−)		Mutation (+)	Mutation (−)		Mutation (+)	Mutation (−)	
Patient characteristics	*n* (%)	*n* (%)	*P*	*n* (%)	*n* (%)	*P*	*n* (%)	*n* (%)	*P*
Overall	17	38		8	47		22	33	
Gender									
Male	12 (70·6)	25 (65·8)	0·726	7 (87·5)	30 (63·8)	0·250	16 (72·7)	21 (63·6)	0·481
Female	5 (29·4)	13 (34·2)		1 (12·5)	17 (36·2)		6 (27·3)	12 (36·4)	
Age at diagnosis (years)				3 (37·5)	27 (57·4)	0·295	12 (54·5)	18 (54·5)	1·0
<10	10 (58·8)	20 (52·6)	0·670	5 (62·5)	20 (42·6)		10 (45·5)	15 (45·5)	
≥10	7 (41·2)	18 (47·4)							
Presenting at diagnosis WBC ($x10^9$/l)									
<100	12 (70·6)	18 (47·4)	0·110	17 (89·5)	26 (72·2)	0·238	16 (72·7)	14 (42·4)	0·027
≥100	5 (29·4)	20 (52·6)		5 (9·1)	13 (36·1)		6 (27·3)	19 (57·6)	
Mediastinal involvement									
Yes	12 (70·6)	22 (57·9)	0·371	4 (50·0)	30 (63·8)	0·464	14 (63·6)	20 (60·6)	0·821
No	5 (29·4)	16 (42·1)		4 (50·0)	17 (36·2)		8 (36·4)	13 (39·4)	
T cell immunophenotype									
Pro and Pre	3 (17·6)	5 (13·2)	0·665	0 (0)	8 (17·0)	0·287	3 (13·6)	5 (15·2)	0·164
Cortical	8 (47·1)	14 (36·8)		5 (62·5)	17 (36·2)		12 (54·5)	10 (30·3)	
Mature	6 (35·3)	19 (50·0)		3 (37·5)	22 (46·8)		7 (31·8)	18 (54·5)	
Chromosomal abnormalities*									
No	11 (68·8)	21 (55·3)	0·749	8 (100·0)	24 (52·2)	0·031	16 (76·2)	16 (48·5)	0·172
Yes									
Abnormalities involving TCR locus† (+)	1 (6·3)	5 (13·2)		0 (0·0)	6 (13·0)		4 (19·0)	12 (36·4)	
Abnormalities involving TCR locus (−)	4 (25·0)	12 (31·6)		0 (0·0)	16 (34·8)		1 (4·8)	5 (15·2)	
Relapse									
Yes	0 (0)	10 (26·3)	0·022	1 (12·5)	9 (19·1)	1·0	1 (4·5)	9 (27·3)	0·039
No	17 (100)	28 (73·7)		7 (87·5)	38 (80·9)		21 (95·5)	24 (72·7)	

Pro and Pre ($CD7^+$ and $CD1^-$), Corical ($CD1^+$), Mature ($CD1^+$, $sCD3^+$).
P, χ^2 or Fisher's exact test; TCR, T cell receptor.
*Total $n = 54$.
†Chromosomal abnormalities including 14q11, 7p15, and 7q35.

mutations in 17 (30·9%) of 55 T-ALL and 6 (42·3%) of 14 T-NHL patients. Three (5·4%) T-ALL and two (1·4%) T-NHL patients presented mutations in both *FBXW7* and *NOTCH1* (Table I).

The 12 *FBXW7* mutations detected included nine missense mutations, one 31 bp insertion, one single nucleotide deletion, and one deletion/insertion mutation (Table I). Seven of nine missense mutations were clustered in a 'hot spot' encoding arginines 465 and 479 residues which are highly conserved in the WD40 (tryptophan–aspartic-acid) repeat of FBXW7 (Fig S2A). Of the 12 identified *FBXW7* mutations, one insertion (T-ALL 8), one deletion/insertion (T-ALL 34), and one single nucleotide deletion (T-NHL 61) have not been previously described in T-ALL or other cancers (Fig S2B–D). *FBXW7* missense mutation encoding V627A (T-ALL 32) was also a novel mutation. V627 of FBXW7 is evolutionarily conserved, and V627A was not detected in normal lymphocytes from 20 healthy volunteers. One patient (T-ALL 34) had a *FBXW7* deletion/insertion mutation and a missense mutation that encoded FBXW7 residue R465H (Table I, Fig S2C).

Of the 24 *NOTCH1* mutations detected in 23 cases, 16 (66·7%) were located in sequences encoding the HD domain, 8 (33·3%) in the PEST domain (Table I). In one case (T-NHL 54), mutations were detected in both the HD and PEST domains. Of these 24 mutations, 17 (70·9%) were short in-frame insertion or deletions, 5 (20·8%) were missense mutations, and 2 (8·3%) were nonsense mutations in sequences encoding the HD or PEST domains, respectively. Furthermore, a single nucleotide polymorphism C5097T was observed in the sequence encoding the C-terminal region of the HD domain in 63 (91·3%) of 69 patients, as previously reported for Japanese adult patients with mature T cell malignancies (Shimizu *et al*, 2007).

Table III. Association of *NOTCH1* and *FBXW7* mutations with clinical characteristics in 14 T-NHL patients.

	NOTCH1			*FBXW7*			*FBXW7* and/or *NOTCH1*		
	Mutation (+)	Mutation (−)		Mutation (+)	Mutation (−)		Mutation (+)	Mutation (−)	
Patient characteristics	*n* (%)	*n* (%)	*P*	*n* (%)	*n* (%)	*P*	*n* (%)	*n* (%)	*P*
Overall	6	8		3	11		7	7	
Gender									
Male	5 (83·3)	7 (87·5)	1·0	2 (66·7)	10 (90·9)	0·396	6 (85·7)	6 (85·7)	1·0
Female	1 (16·7)	1 (12·5)		1 (33·3)	1 (9·1)		1 (14·3)	1 (14·3)	
Age at diagnosis (years)									
<10	4 (66·7)	2 (25·0)	0·277	2 (66·7)	4 (36·4)	0·538	4 (57·1)	2 (28·6)	0·592
≥10	2 (33·3)	6 (75·0)		1 (33·3)	7 (63·6)		3 (42·9)	5 (71·4)	
Mediastinal involvement									
Yes	0 (0·0)	1 (12·5)	1·0	0 (0·0)	1 (9·1)	1·0	0 (0·0)	1 (14·3)	1·0
No	6 (100·0)	7 (87·5)		3 (100·0)	10 (90·9)		7 (100·0)	6 (85·7)	
T cell immunophenotype									
Pro and Pre	0 (0·0)	0 (0·0)	1·0	0 (0·0)	0 (0·0)	1·0	0 (0·0)	0 (0·0)	1·0
Cortical	2 (33·3)	2 (28·6)		1 (33·3)	3 (30·0)		2 (28·6)	2 (33·3)	
Mature	4 (66·7)	5 (71·4)		2 (66·7)	7 (70·0)		5 (71·4)	4 (66·7)	
Chromosomal abnormalities*									
No	4 (66·7)	3 (42·9)	0·755	3 (100·0)	4 (40·0)	0·217	5 (71·4)	2 (33·3)	0·470
Yes									
Abnormalities involving TCR locus† (+)	1 (16·7)	1 (14·3)		0 (0·0)	2 (20·0)		1 (14·3)	1 (16·7)	
Abnormalities involving TCR locus (−)	1 (16·7)	3 (42·9)		0 (0·0)	4 (40·0)		1 (14·3)	3 (50·0)	
Relapse									
Yes	0 (0·0)	2 (25·0)	0·473	0 (0·0)	2 (18·2)	1·0	0 (0·0)	1 (14·3)	1·0
No	6 (100·0)	6 (75·0)		3 (100·0)	9 (81·8)		7 (100·0)	6 (85·7)	

Pro and Pre ($CD7^+$ and $CD1^-$), Corical ($CD1^+$), Mature ($CD1^+$, $sCD3^+$).

P, χ^2 or Fisher's exact test; TCR, T cell receptor.

*Total $n = 13$.

†Chromosomal abnormalities including 14q11, 7p15, or 7q35.

Clinical characteristics of FBXW7 and NOTCH1 mutations

The clinical and biological characteristics of the patients in this study are shown in Tables II and III. *FBXW7* and/or *NOTCH1* mutations were associated only with white blood cell (WBC) counts. *FBXW7* and/or *NOTCH1* mutations, but not *FBXW7* or *NOTCH1* alone, were found more frequently in T-ALL patients with low WBC count, $<10 \times 10^9$/l, than in those with higher WBC count, $>10 \times 10^9$/l ($P = 0{\cdot}027$). *FBXW7* mutations, but not *NOTCH1* mutations, were negatively associated with chromosome abnormalities in both T-ALL and T-NHL. All T-ALL and T-NHL patients having *FBXW7* mutation lacked a chromosome abnormality (100% vs. 52·2%, $P = 0{\cdot}031$ in T-ALL and 100% vs. 40·0%, $P = 0{\cdot}217$ in T-NHL).

Prognostic significance of FBXW7 and NOTCH1 mutations

We next analyzed the correlation between *FBXW7* and/or *NOTCH1* mutations and clinical outcome. T-ALL patients with *NOTCH1* mutation had a better clinical outcome than those without *NOTCH1* mutation {100% vs. 65·8% [95% confidence interval (CI), 48·5–78·5%]; $P = 0{\cdot}008$ for 5-year EFS and 100% vs. 81·6% [95% CI, 65·2–90·8%]; $P = 0{\cdot}065$ for 5-year OS, respectively} (Fig S3), while the prognostic difference between patients with and without *FBXW7* mutation was not significant [87·5% (95% CI, 38·7–98·1%) vs. 74·5% (95% CI, 59·4–84·6%); $P = 0{\cdot}400$ for 5-year EFS and 100% vs. 85·1% (95% CI, 71·3–92·6%); $P = 0{\cdot}259$ for 5-year OS, respectively] (Fig S4). The 5-year EFS and OS for T-ALL patients with *FBXW7* and/or *NOTCH1* mutations were extremely high, suggesting a good prognosis for patients with *FBXW7/NOTCH1* mutation compared to those without [95·5% (95% CI, 71·9–99·4%) vs. 63·6% (95% CI, 45·0–77·5%); $P = 0{\cdot}007$ and 100% vs. 78·8% (95% CI, 60·6–89·3%); $P = 0{\cdot}023$, respectively] (Fig 1). Notably, all three patients with both *FBXW7* and *NOTCH1* mutations were alive without relapse.

Multivariate analysis of prognostic factors adjusted for gender, age at diagnosis, and WBC count presented at diagnosis revealed that *FBXW7* and/or *NOTCH1* mutation status, risk group for treatment, and chromosomal abnormalities retained

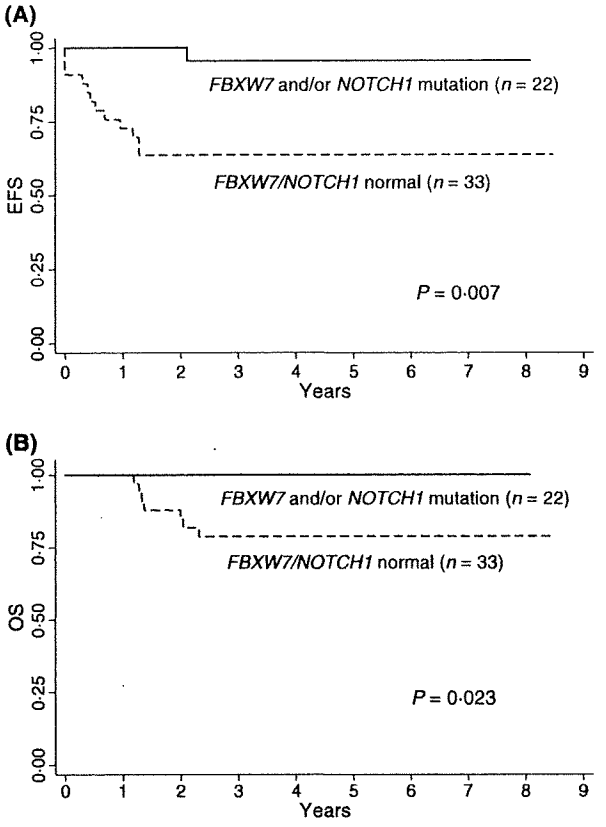

Fig 1. Kaplan–Meier estimate of (A) event-free survival and (B) overall survival of T-ALL patients with or without *FBXW7* and/or *NOTCH1* mutation.

their significant effects on EFS (Table IV). On the other hand, multivariate analysis adjusted for *NOTCH1* and/or *FBXW7* mutation status, risk group for treatment, and chromosomal abnormalities, in addition to gender, age at diagnosis, and WBC count presented at diagnosis, revealed that none of them retained EFS significance (Table IV).

In T-NHL, patients with *NOTCH1* and/or *FBXW7* mutation also had a good prognosis, although the differences in 5-year EFS and OS for patients with and without *NOTCH1* and/or *FBXW7* mutations were not significant [EFS, 85·7% (95% CI, 33·4–97·9%) vs. 57·1% (95% CI, 17·2–83·7%), $P = 0·313$; OS, 85·7% (95% CI, 33·4–97·9%) vs. 53·6% (95% CI, 13·2–82·5%), $P = 0·286$].

Discussion

In this study, we found 14·6% *FBXW7* mutations and 30·9% *NOTCH1* mutations in T-ALL patients, and 21·4% *FBXW7* mutations and 42·3% *NOTCH1* mutations in T-NHL patients. Frequencies of *FBXW7* and *NOTCH1* mutations in T-ALL in this study were similar to those in other recent studies (8·6–30·8% for *FBXW7* mutations, and 30·8–70·8% for *NOTCH1* mutations) (Akhoondi *et al*, 2007; Lee *et al*, 2005; Malyukova *et al*, 2007; Mansour *et al*, 2006; O'Neil *et al*, 2007; Thompson *et al*, 2007; van Grotel *et al*, 2008). This is the first report describing high frequencies of *FBXW7* and *NOTCH1* mutations in T-NHL as well as in T-ALL. The types of mutations identified were similar in T-ALL and T-NHL patients (Table I), although it was previously reported that gene expression profiling revealed intrinsic differences between T-ALL and T-NHL (Raetz *et al*, 2006).

Our results demonstrated that *FBXW7* and/or *NOTCH1* mutations as well as *NOTCH1* mutations alone had a good prognosis in T-ALL patients. The *P* value regarding the significant difference in prognosis for patients with *FBXW7* and/or *NOTCH1* status ($P = 0·007$ for EFS) was less than for those with *NOTCH1* status alone ($P = 0·008$), although the difference in prognosis for *FBXW7* status alone was not significant ($P = 0·397$). All T-ALL and T-NHL patients with

Table IV. Multivariate analysis of effects of *FBXW7* and/or *NOTCH1* mutations on EFS in 55 T-ALL patients.

	Crude HR		Adjusted HR1*		Adjusted HR2†	
	(95% CI)	*P*‡	(95% CI)	*P*‡	(95% CI)	*P*‡
FBXW7 and/or *NOTCH1* mutation						
Negative	1·00§		1·00§		1·00§	
Positive	0·10 (0·01–0·78)	0·028	0·10 (0·01–0·77)	0·027	0·24 (0·05–1·13)	0·071
Chromosomal abnormalities						
No	1·00§		1·00§		1·00§	
Yes						
Abnormalities involving TCR locus (+)	5·99 (1·55–23·22)	0·010	6·04 (1·54–23·70)	0·010	6·41 (1·35–30·58)	0·020
Abnormalities involving TCR locus (−)	7·63 (1·53–38·11)	0·013	10·80 (2·03–57·57)	0·005	3·22 (0·89–11·67)	0·076

HR, hazard ratio; CI, confidence interval.

*Adjusted for sex, age at diagnosis and presenting white blood cell count (categorical: see Table I).

†Adjusted for sex, age at diagnosis, presenting white blood cell count, *FBXW7* and/or *NOTCH1* mutations category, determined risk and chromosomal abnormalities) (categorical: see Table I).

‡*P*, X^2 test.

§Reference category.

FBXW7 mutations, with the exception of one T-ALL patient, have survived without relapse. One patient (T-ALL 38) had an isolated CNS relapse; however, the patient had survived 2 years after the relapse episode.

The paediatric ALL-BFM 2000 study reported the good clinical outcome for T-ALL patients with *NOTCH1* mutations (Breit *et al*, 2006), however, other two reports described results that were not compatible with this (van Grotel *et al*, 2008; Zhu *et al*, 2006). One possible explanation for this discrepancy of prognostic impact is the different treatment protocols used; the survival rates reported in other papers were apparently lower [28·8% 3-year relapse free survival (Zhu *et al*, 2006) and 65% 5-year disease-free survival (van Grotel *et al*, 2008)] for T-ALL patients with *NOTCH1* mutation than those of the ALL-BFM 2000 study (90% relapse-free survival) and our study (100% 5-year EFS). On the other hand, there was no statistically significant impact of *NOTCH1* mutations on prognosis in T-NHL patients, perhaps because the number of T-NHL patients was small in this study. Further study of T-NHL patients is needed to clarify the association of *FBXW7* and *NOTCH1* mutations with T-NHL prognosis.

Four novel mutations were found, and two of the four, V627A in T-ALL 32 and a frame shift mutation at codon 653 in T-NHL 61, were positioned outside of a 'hot spot' region. Codon 627 is localized in the seventh β-propeller blade (β-PB7) of FBXW7 (Orlicky *et al*, 2003), and a R689W mutation in the β-PB8 was also reported in T-ALL cases (Malyukova *et al*, 2007). C-terminal truncation of FBXW7 observed in T-NHL 61 was also reported in an endometrial tumour (nonsense mutation of codon 658) (Akhoondi *et al*, 2007), and these mutations result in the absence of a portion of β-PB7 and all of β-PB8. These findings suggested that a structural change of any β-propeller blades may have similar effects on FBXW7 function. Furthermore, it was also demonstrated that *Fbxw7* deficiency in adult haematopoietic cells leads to T-ALL in mice (Matsuoka *et al*, 2008), suggesting that inactivation of FBXW7 plays a critical role in T-ALL leukaemogenesis.

Chromosomal abnormalities of the *TLX3* (5q35) and *TLX1* (10q24) locus have been reported to be associated with poor and good outcome (van Grotel *et al*, 2008). In this study, chromosomal abnormalities involving the *TLX1* locus were found in one patient and chromosomal abnormalities involving the breakpoint at 5q35·1 (*TLX3*) were not found in any patients. *t*(10;11)(q13;q14) [*PICALM-MLLT10* (previously termed *CALM-AF10*)] was not found. The prognostic significance of these cytogenetic abnormalities was not clear because the number of patients was small.

Notably, *FBXW7* mutations were only observed in T-ALL and T-NHL patients lacking chromosomal abnormalities. *FBXW7* is considered to be a haplo-insufficient tumour suppressor gene (Mao *et al*, 2004). Inactivation of FBXW7 has been reported to cause chromosomal instability in karyotypically stable colorectal cancer cells, resulting in a striking phenotype associated with micronuclei and chromosomal instability (Rajagopalan *et al*, 2004). On the contrary, *FBXW7* mutation has been reported to lack association with chromosomal instability in colorectal cancer (Kemp *et al*, 2005), which was compatible with the present results for T-ALL. Further studies are needed to clarify this issue.

In conclusion, *FBXW7* and *NOTCH1* are functionally related each other, and the mutations of either *FBXW7* or *NOTCH1* genes rather than *FBXW7* or *NOTCH1* alone were associated with good clinical outcome in T-ALL, suggesting that the status of both *FBXW7* and *NOTCH1*, rather than *FBXW7* or *NOTCH1* alone, is a useful prognostic factor in T-ALL.

Acknowledgements

We thank all the participants of the JACLS ALL-97 and NHL-98 study. We also thank Professor Masafumi Taniwaki, Department of Haematology and Oncology, Kyoto Prefectural University of Medicine Graduate School of Medical Science for his help, and Miss Chisato Kubota and Mr Yoshiharu Tokita for technical assistance. This work was supported by a grant for Cancer Research, and a grant for Research on Children and Families from the Ministry of Health, Labour, and Welfare of Japan, a Grant-in-Aid for Scientific Research (C) and Exploratory Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and by a Research grant for Gunma Prefectural Hospitals.

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