Brief report

Infants with acute lymphoblastic leukemia and a germline *MLL* gene are highly curable with use of chemotherapy alone: results from the Japan Infant Leukemia Study Group

Jun Nagayama, Daisuke Tomizawa, Katsuyoshi Koh, Yoshihisa Nagatoshi, Noriko Hotta, Tomoko Kishimoto, Yoshihiro Takahashi, Tomoko Kuno, Kanji Sugita, Takashi Sato, Kohji Kato, Atsushi Ogawa, Tatsutoshi Nakahata, Shuki Mizutani, Keizo Horibe, and Eiichi Ishii

Although infants with acute lymphoblastic leukemia (ALL) and a germline *MLL* gene have a better prognosis than comparable infants with a rearranged *MLL* gene, their optimal therapy is controversial. In 2 consecutive studies, conducted between 1996 and 2002, we treated 22 cases of infant ALL with germline *MLL* using chemotherapy alone. The 5-year event-free survival rate was 95.5% with a 95% confidence interval of 86.9 to 100%. All 21 infants with precursor B-cell ALL have been in first complete remission for 3.5 to 8.8 years. Most treatment-related toxicities were predictable and well tolerated, and neither secondary malignancies nor physical growth impairments have been observed. These results indicate that chemotherapy of the type described here is both safe and highly effective against infant precursor B-cell ALL with *MLL* in the germline configuration. (Blood. 2006;107:4663-4665)

Introduction

Infants younger than 1 year of age with acute lymphoblastic leukemia (ALL), who represent 2.5% to 5% of all childhood ALL cases, still show generally poor responses to treatment.[1,2] This inferior outcome is closely associated with young age, negative CD10 on leukemic cells, and positive *MLL* gene rearrangements.[3,4] Whether infants with germline *MLL* can be treated less aggressively than those with rearrangement of this gene is still unclear, because most study groups have enrolled infants on the same therapeutic protocol regardless of their *MLL* gene status.[5-11] In those trials, the event-free survival rate for infants with ALL and positive CD10 expression or lack of 11q23 abnormalities ranged from 52% to 79%, suggesting a worse outcome than seen in childhood ALL in general, even though some infants with a rearranged *MLL* gene might have been inadvertently included in the better-risk cohort.[10-13]

The Japan Infant Leukemia Study Group segregated infants with ALL into 2 subgroups according to their *MLL* gene status in 2 consecutive studies. Infants with a rearranged *MLL* gene received intensive chemotherapy followed by hematopoietic stem cell transplantation, whereas those with a germline *MLL* were treated with chemotherapy alone.[14,15] As reported here, a highly promising outcome was obtained in the latter subgroup, providing a rationale for the design of future studies focusing on infant ALL.

Study design

Between December 1995 and December 2002, all consecutive infants with ALL and age younger than 12 months were registered and treated on 2 protocols designated MLL96 and MLL98. Written informed consent, provided according to the Declaration of Helsinki, was obtained from the parents or guardians of the patients, and the institutional review boards approved all aspects of this investigation. Each patient was evaluated with respect to the characteristics of leukemic cells, including immunophenotype, cytogenetics, and *MLL* gene rearrangement. Each patient with positive CD10 expression was assigned to the chemotherapy subgroup, after confirmation of the *MLL* gene status by Southern blot analysis or fluorescence in situ hybridization. If a rearrangement was found, the patient was excluded from the chemotherapy subgroup. The treatments used in these 2 studies were identical, consisting of induction, consolidation, and central nervous system (CNS) prophylaxis, intensification, reinduction, and maintenance phases (Table 1). The total duration of therapy was 83 to 85 weeks.

The present analysis was performed on October 31, 2005. Overall survival (OS) was defined as the time from diagnosis to death due to any cause or to the date of last contact. Event-free survival (EFS) was defined as the time from diagnosis until the date of an adverse event or, if no such event occurred, until the date of last contact. Induction failure (including early death or resistant leukemia), relapse, death during complete remission, and the development of a second malignancy were considered adverse events. OS and EFS rates were estimated by the Kaplan-Meier method. The 95% confidence intervals (CIs) for Kaplan-Meier estimates of survival were calculated by the use of standard errors.

From the Section of Pediatrics, National Kyushu Cancer Center, Fukuoka; Department of Pediatrics and Developmental Biology, Tokyo Medical and Dental University, Tokyo; Department of Pediatrics, University of Tokyo, Tokyo; Department of Pediatrics, Yamaguchi University, Yamaguchi; Department of Pediatrics, Nara Medical University, Nara; Department of Pediatrics, Hirosaki University, Hirosaki; Department of Pediatrics, Osaka Medical College, Suita; Department of Pediatrics, University of Yamanashi, Chuo; Department of Pediatrics, Hiroshima University, Hiroshima; Division of Pediatric Hematology/Oncology, Nagoya Red Cross 2nd Hospital, Nagoya; Division of Pediatrics, Niigata Cancer Center Niigata Hospital, Niigata; Department of Pediatrics, Kyoto University, Kyoto; Clinical Research Center, National Nagoya Hospital, Nagoya; and Department of Pediatrics, Saga University, Saga, Japan.

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A complete list of the participating members of the Japan Infant Leukemia Study Group appears in the "Appendix."

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An Inside *Blood* analysis of this article appears at the front of this issue.

Reprints: Eiichi Ishii, Department of Pediatrics, Faculty of Medicine, Saga University, 5-1-1 Nabeshima, Saga 849-8501, Japan; e-mail: ishiei@med.saga-u.ac.jp.

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Table 1. Treatment plan for infant ALL with a germline *MLL* gene

Phase and drug	Site, duration	Dosage	Time of dose(s)
Induction			
DEX	Intravenous	10 mg/m²	Days 1-14
PSL	By mouth or intravenous	60 mg/m²	Days 15-28
VCR	Intravenous	0.05 mg/kg	Days 1, 8, 15, 22
CPA	Intravenous, 1-2 h	1200 mg/m²	Day 2
DXR	Intravenous, 1 h	25 mg/m²	Days 3, 5
ASP	Intravenous, 3-4 h	10 000 U/m²	Days 16, 18, 20, 23, 25, 27
TIT	Intrathecal	Age-adjusted†	Days 1, 15, 29
VP-16	Intravenous, 1-2 h	100 mg/m²	Days 29-32
Ara-C	Intravenous, 4 h	500 mg/m²	Days 29-32
Consolidation and CNS prophylaxis			
MTX	Intravenous, 24 h	3 g/m²	Days 1, 15, 29
TIT	Intrathecal	Age-adjusted†	Days 1, 15, 29
CPA	Intravenous, 1-2 h	500 mg/m²	Days 2, 16, 30
ASP	Intravenous or intramuscular	10 000 U/m²	Days 2, 16, 30
PSL	By mouth or intravenous	60 mg/m²	Days 1-3
Intensification			
VCR	Intravenous	0.05 mg/kg	Days 1, 8, 15
DNR	Intravenous	25 mg/m²	Days 1, 8, 15
Ara-C	Intravenous, 1 h	60 mg/m²	Days 2-7, 9-14
6-MP	By mouth	75 mg/m²	Days 1-14
TIT	Intrathecal	Age-adjusted†	Days 1, 15
Maintenance*			
Regimen A			
6-MP	By mouth	75 mg/m²	Days 1-14
MTX	By mouth	30 mg/m²	Days 1, 8
VP-16	Intravenous, 1-2 h	150 mg/m²	Day 14
Ara-C	Intravenous, 4 h	200 mg/m²	Day 14
Regimen B			
6-MP	By mouth	75 mg/m²	Days 1-14
MTX	By mouth	30 mg/m²	Days 1, 8
PSL	By mouth	60 mg/m²	Days 15-28
VCR	Intravenous	0.05 mg/kg	Days 15, 22, 29
MTX	Intravenous, 5 h	300 mg/m²	Day 15
TIT	Intrathecal	Age-adjusted†	Every 6 weeks

Reinduction regimen is the same as that for induction.

DEX indicates dexamethasone; PSL, prednisolone; VCR, vincristine; CPA, cyclophosphamide; DXR, doxorubicin; ASP, L-asparaginase; TIT, triple intrathecal therapy; VP-16, etoposide; Ara-C, cytarabine; MTX, methotrexate; DNR, daunorubicin; 6-MP, 6-mercaptopurine. The dose of each drug except VCR was reduced by one third in patients younger than 2 months and by one fourth in those 2 to 4 months of age.

*Each cycle consisted of two courses of regimen A, followed by regimen B. Each regimen was given over 2 weeks. The 12-week course was repeated 4 times. The total period of maintenance therapy becomes almost 56 weeks.

†Doses were adjusted according to the patient's age at administration as follows: 90 days old or younger, MTX 3 mg, hydrocortisone (HDC) 10 mg, Ara-C 6 mg; younger than 1 year old, MTX 6 mg, HDC 10 mg, Ara-C 12 mg; 1 year and older, MTX 8 mg, HDC 15 mg, Ara-C 20 mg.

Results and discussion

A total of 101 infants with ALL were registered in the MLL96 or MLL98 study; 79 with rearranged *MLL* were assigned to the hematopoietic stem cell transplantation (HSCT) subgroup and 22 with germline *MLL* to the chemotherapy subgroup. In the latter, all but one patient, who had been treated on an acute myeloid leukemia (AML)–oriented protocol, received chemotherapy alone (Table 1). The male-female ratio was 20:2, and the median age at diagnosis was 9.8 months (range, 3.8-12.0 months). Only 3 patients were younger than 6 months old at diagnosis. The median white blood cell count was 21.8×10^9/L (range, 2.8-574.1 $\times 10^9$/L). Neither CNS involvement nor severe hepatosplenomegaly was observed. By immunophenotyping, 21 patients had precursor B-cell phenotype with positive CD10 antigen expression; one infant with T-lineage ALL (T-ALL) had hyperleukocytosis at diagnosis (574.1×10^9/L). By cytogenetic analysis, 15 of the patients including the infants with T-ALL had normal karyotypes, whereas one had hyperdiploidy, one had inv(11)(p13q23), one had t(1;19)(q32;p13) and 4 had other chromosomal abnormalities without an 11q23 translocation.

All 22 patients achieved complete remission (CR) after induction therapy. Subsequently, the 20 patients with precursor B-cell ALL remained in first CR for 3.5 to 8.8 years (median, 7 years). The 5-year EFS and OS rates for the 21 patients who were treated on the same protocol were identical, 95.2% (95% CI, 86.7%-100%). By the intent-to-treat convention, adding the patient who received AML-oriented chemotherapy and remains in CR, the EFS and OS estimates are 95.5% (95% CI, 86.9%-100%). The infant with T-ALL suffered a relapse and died after HSCT. Comparison of EFS rates by *MLL* gene status demonstrated a significantly better result for the patients with germline *MLL* ($P < .001$; Figure 1).

The principal grade 3 nonhematologic toxicities (National Cancer Institute–CTCAE [Common Terminology Criteria for Adverse Events] system) were as follows: induction phase—liver dysfunction (n = 11), bacterial infection (n = 8), convulsion (n = 3), diarrhea (n = 3), and allergic reaction to L-asparaginase (n = 1); consolidation phase—liver dysfunction (n = 2), bacterial infection (n = 5), and diarrhea (n = 2); intensification phase—

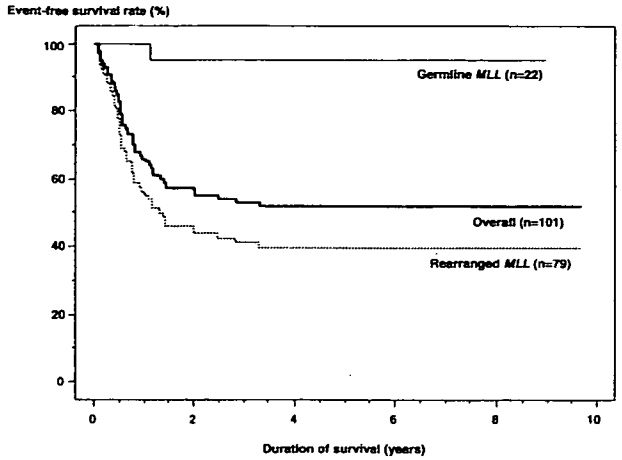

Figure 1. Event-free survival rates for infants with ALL treated in the MLL96 or MLL98 study. Outcome was significantly better in patients with germline *MLL* (95.5%) than in those with rearranged *MLL* (39.7%; $P < .001$). The overall result was 52.0%.

liver dysfunction (n = 2) and bacterial infection (n = 3); reinduction phase—liver dysfunction (n = 6) and bacterial infection (n = 7); and maintenance phase—liver dysfunction (n = 7) and bacterial infection (n = 1). Grade 4 liver dysfunction and hematologic toxicity were observed in 1 and 4 patients during maintenance phase, respectively. Long-term sequelae were also evaluated. Body heights and weights reached the normal ranges in all patients; median standard deviation (SD) scores for height and weight were 0.1 SD (−1.0 to 0.9 SD) and −0.1 SD (−1.1 to 1.0 SD), respectively. A second malignancy was not detected in any patient.

Our results demonstrate the efficacy of chemotherapy alone in infants with ALL and a germline *MLL* gene. In previous studies with a less favorable outcome, an 11q23 translocation or negative CD10 expression was substituted for a demonstrated *MLL* gene rearrangement.[10-13] More precise determination of *MLL* gene status in the present study may have enabled us to select a "true" germline *MLL* subgroup, contributing to the excellent results. Hilden et al,[16] using reverse transcription-polymerase chain reaction to detect gene rearrangement, also reported a superior outcome in infants with germline *MLL* treated with chemotherapy alone, as did Pui et al[17] in infants without the t(4;11). These results support our conclusion that infant ALL with germline *MLL* may be highly curable with chemotherapy alone. Two large multicenter trials of chemotherapy for infant ALL (INTERFANT99 and POG/COG9407) are nearing completion, and it will be important in the future to compare their experience with ours to identify the protocol elements that are most critical in securing a high EFS rate with acceptable toxicity.

Despite the relatively small number of patients in this analysis, the plan of chemotherapy we described appears to be well tolerated and to yield a very high survival rate. Although rare, infant ALL carries one of the highest risks for treatment failure among all lymphoid leukemias. Thus, international cooperation is needed to compare the advantages and disadvantages of emerging therapies in controlled clinical trials for this disease.

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Appendix

The members of the Japan Infant Leukemia Study Group are as follows: Hokkaido Children's Hospital and Medical Center (Takanori Oda); Hirosaki University (Yoshihiro Takahashi); Chiba University (Takeyuki Sato); Gunma Children's Hospital (Yasuhide Hayashi); Yamanashi University (Kanji Sugita); Kanagawa Children's Medical Center (Tsuyuko Hayashi); Tokyo Medical and Dental University (Daisuke Tomizawa, Shuki Mizutani); University of Tokyo (Katsuyoshi Koh); Showa University (Keiichi Isoyama); Keio University (Tetsuya Mori); Niigata Cancer Center Niigata Hospital (Atsushi Ogawa); Kanazawa University (Takahiro Uehara); National Nagoya Hospital (Keizo Horibe); Japanese Red Cross Nagoya First Hospital (Kohji Kato); Mie University (Masahiro Hirayama); Shiga Medical School (Shigeru Ohta); Kyoto Katsura Hospital (Yoshihiro Wakazono); Kyoto University (Tatsutoshi Nakahata); Osaka Medical College (Tomoko Kuno); Hyogo Children's Hospital (Yoshiyuki Kosaka); Okayama University (Megumi Oda); Hiroshima University (Takashi Sato); National Kyushu Cancer Center (Jun Nagayama); University of Miyazaki (Hiroshi Moritake); and Saga University (Eiichi Ishii, Chairman).

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Brief report

KIT mutations, and not *FLT3* internal tandem duplication, are strongly associated with a poor prognosis in pediatric acute myeloid leukemia with t(8;21): a study of the Japanese Childhood AML Cooperative Study Group

Akira Shimada, Tomohiko Taki, Ken Tabuchi, Akio Tawa, Keizo Horibe, Masahiro Tsuchida, Ryoji Hanada, Ichiro Tsukimoto, and Yasuhide Hayashi

Patients with t(8;21) acute myeloid leukemia (AML) are considered to have a good prognosis; however, approximately 50% of them relapse. The genetic alterations associated with a poor outcome in t(8;21) AML remain unknown. Recently, aberrations of receptor tyrosine kinases (RTKs) were frequently found in patients with AML. However, the prevalence and prognostic impact of RTK aberrations in pediatric t(8;21) AML remains undetermined. Here, we found the kinase domain mutations of the *KIT* gene in 8 (17.4%) of 46 patients with t(8;21) AML among newly diagnosed pediatric patients with AML treated on the AML99 protocol in Japan. Significant differences between patients with or without *KIT* mutations were observed in the 4-year overall survival (50.0% versus 97.4%, $P = .001$), disease-free survival (37.5% versus 94.7%, $P < .001$) and relapse rate (47.0% versus 2.7%, $P < .001$). Furthermore, *FLT3* internal tandem duplication was found in only 2 (4.3%) patients. These results suggested that *KIT* mutations are strongly associated with a poor prognosis in pediatric t(8;21) AML. (Blood. 2006; 107:1806-1809)

Introduction

Patients with t(8;21) acute myeloid leukemia (AML) have been reported to have a good prognosis; however, approximately 50% of them relapse.[1,2] A high presenting leukocyte count, CD56 expression, or extramedullary disease has been reported to be associated with a poor prognosis in t(8;21) AML.[1,3,4] However, the genetic alterations associated with a poor outcome in patients with t(8;21) AML remain unknown. Recent studies revealed that internal tandem duplication (ITD) of *FLT3* is considered to be one factor predicting poor prognosis in adult and pediatric patients with AML.[5-9] More recently, *KIT* mutations were found in 12.7% to 48.1% of adult patients with AML with t(8;21)[10-12] and were reported to be associated with a poor prognosis.[13,14] The prevalence and prognostic impact of *KIT* mutations in pediatric t(8;21) AML remain unknown. We performed the mutational analysis of *KIT* and *FLT3* in pediatric patients with t(8;21) AML who were treated on the Japanese Childhood AML Cooperative Study Group Protocol, AML99. We report here that *KIT* mutations are strongly associated with a poor prognosis in pediatric patients with t(8;21) AML.

Study design

Patients and samples

The diagnosis of AML was based on the French-American-British (FAB) classification, and cytogenetic analysis was performed using a routine G-banding method. From January 2000 to December 2002, 318 patients were newly diagnosed as having de novo AML. Of 240 patients, 77 (32.1%), except for 29 AML-M3 and 49 Down syndrome, had t(8;21)(q22; q22) according to cytogenetics or *AML1-MTG8* fusion transcript with the reverse-transcriptase–polymerase chain reaction (RT-PCR) (Figure S1; see the Supplemental Materials link at the top of the online article, at the *Blood* website). Samples were available from 135 (56.3%) of 240 patients with AML, including 46 (59.7%) of 77 patients with t(8;21) AML. Of 46 patients with t(8;21) AML, 3 patients were classified into M1, 39 into M2, and 4 into

From the Department of Hematology/Oncology, Gunma Children's Medical Center, Gunma; the Department of Molecular Laboratory Medicine, Kyoto Prefectural University of Medicine Graduate School of Medical Science, Kyoto; the Department of Hematology, Kanagawa Children's Medical Center, Yokohama, Kanagawa; the Department of Pediatrics, National Hospital Organization Osaka National Hospital, Osaka; the Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya; the Department of Pediatrics, Ibaraki Children's Hospital, Ibaraki; the Division of Hematology/Oncology, Saitama Children's Medical Center, Saitama; and the Department of First Pediatrics, Toho University School of Medicine, Omori, Tokyo, Japan.

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A.S. performed genetic analysis and wrote the paper; T.T. assisted with the genetic analysis; K.T. performed the statistical analysis; A.T., K.H., M.T., and R.H. arranged the clinical data; I.T. designed the AML cooperative study in Japan; and Y.H. designed the study and wrote the paper.

The online version of this article contains a data supplement.

Reprints: Yasuhide Hayashi, Director, Gunma Children's Medical Center, 779 Shimohakoda, Kitatachibana, Gunma 377-8577, Japan; e-mail: hayashiy-tky @umin.ac.jp.

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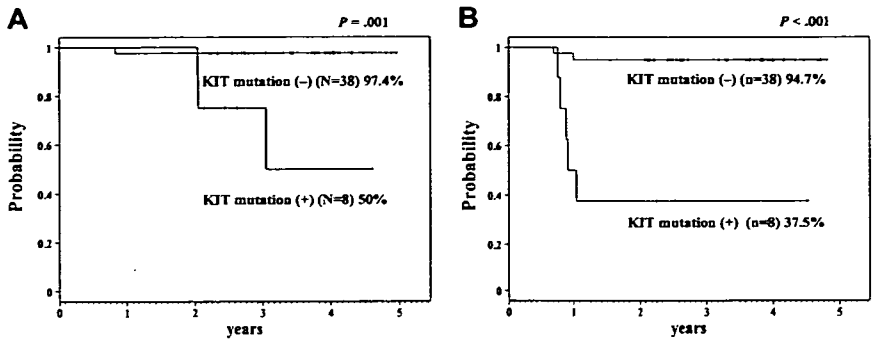

Figure 1. Kaplan-Meier analysis. This analysis shows 4-year overall survival (A) and disease-free survival (B) of the patients with or without *KIT* mutation. The difference is statistically significant (A: $P = .001$; B: $P < .001$).

M4. There were no statistical differences between 46 analyzed patients with t(8;21) AML and the 31 nonanalyzed patients in age (median 7.5 years [range: 2-15 years] versus 9 years [range: 1-15 years]), initial white blood cell (WBC) count (median: 14.4 × 10^9/L; range: 1.65 × 10^9/L-107.7 × 10^9/L; versus 9.1 × 10^9/L; range: 1.4 × 10^9/L-136 × 10^9/L), induction rate (100% versus 93.5%), relapse rate (15.2% versus 19.4%), and 4-year overall survival rate (4y-OS; 87% versus 91%). In the AML99 protocol, patients with t(8;21) with initial WBC count lower than 50 × 10^9/L were categorized into a low-risk group. Thus, after patients with t(8;21) AML obtained complete remission (CR) with induction chemotherapy (cytarabine, etoposide, and mitoxantrone), they were treated with 5 additional courses of intensive chemotherapy (high-dose cytarabine [HDCA], etoposide, idarubicine, and mitoxantron; Figure S2 and Tsukimoto et al[15]). If the initial WBC count was greater than 50 × 10^9/L, patients were categorized into an intermediate-risk group and received allogeneic stem cell transplantation (allo-SCT) in the case of the presence of a donor. Informed consent was obtained from the patients or patients' parents, according to guidelines based on the tenets of the revised Helsinki protocol. The institutional review board of Gunma Children's Medical Center approved this project.

KIT mutation analysis

Mutational analysis of the extracellular (EC) domain (exons 8, 9), transmembrane (TM) domain (exon 10), juxtamembrane (JM) domain (exon 11), and the second intracellular kinase (TK) 2 domain (exons 17 and 18) of *KIT* gene was performed with RT-PCR followed by direct sequencing. Primers used are shown in Table S1.

FLT3 mutation analysis

Mutational analysis of ITD within the JM domain and D835 mutation (D835Mt) within the TK2 domain of the *FLT3* gene was performed as previously reported.[16-18]

Statistical analysis

Estimation of survival distributions was performed using the Kaplan-Meier method and the differences were compared using the log-rank test. Disease-free survival (DFS), event-free survival (EFS), and overall survival (OS) were defined as the times from diagnosis to relapse, from diagnosis to event (relapse or death of any cause), and from diagnosis to death of any cause or the last follow-up. Statistical difference analysis was performed using the χ^2 test.

Results and discussion

KIT and *FLT3* expressions were found in all of the 46 t(8;21) AML samples. Although *KIT* mutations have been reported in a small number of pediatric patients with t(8;21) AML,[8,19] TK2 domain mutations of the *KIT* gene were found in 8 (17.4%) of 46 patients in this study (Table 1). However, we could not find any mutation other than the TK2 domain. The N822K mutation, which has been frequently reported so far,[12] was found in 3 of 8 patients in this study.

The statistical differences between patients with or without *KIT* mutations were not significant in age (median 8 years [range: 1-15 years] versus 7 years [range: 2-15 years]), and the initial WBC count (median: 20.65 × 10^9/L; range: 4.6 × 10^9/L-66.2 × 10^9/L; versus 14.3 × 10^9/L; range: 1.65 × 10^9/L-107.7 × 10^9/L). Interestingly, *KIT* mutations were observed only in M2 patients according to FAB classification. Another report also suggested that *KIT* mutations were frequently found in M2 patients with t(8;21).[19] Significant differences between patients with or without *KIT* mutations were observed in 4-year OS (50.0% versus 97.4%, $P = .001$, Figure 1), DFS (37.5% versus 94.7%, $P < .001$), and relapse rate (47.0% versus 2.7%, $P < .001$). Short CR duration and high relapse rate were more significant than those of the previous report in adults.[14] *KIT* mutations have recently been reported not to influence the clinical outcome in pediatric core-binding factor (CBF) leukemia patients.[20] Although they found *KIT* mutations in 5 of 16 cases of t(8;21) AML, they did not describe the clinical outcome of patients with t(8;21) AML with or without *KIT* mutations. Furthermore, the clinical outcome of the patients

Table 1. Clinical characteristics of patients with t(8;21) AML with *KIT* mutations

Patient no.	Age, y	Sex	WBC count, × 10^9 cells/L	Additional chromosome abnormalities	Time of relapse, mo	Status of allo-SCT	Survival, mo	*KIT* mutation
1	8	F	14.10	None	12	Second CR	37	A814S
2	8	M	27.60	−Y	14	Second CR	47*	N822K
3	8	F	10.77	−X	10	Second CR	25	D816H
4	6	M	34.50	−Y, +4	12	Second CR	26*	N822K
5	3	F	20.50	None	11	—	25	N822K
6	1	F	4.60	−X, t(7;9)	—	—	32*	N822T
7	15	M	20.80	−Y	—	First CR	56*	D816V
8	13	M	66.20	None	—	First CR	30*	V825A

— indicates not applicable.
*Patient still alive.

without *KIT* mutations in their study was poorer than the outcome of those in our study (EFS 63% versus 92.1%). Our result may depend on our good clinical outcome of patients with t(8;21) AML without *KIT* mutations.

Except for 2 patients who received allo-SCT in first CR (patients no. 7 and no. 8 in Table 1), 5 of 6 (83.3%) patients with the mutation relapsed within 14 months after diagnosis. Allo-SCT was performed in 6 of 8 patients with t(8;21) AML with *KIT* mutations (2 in first CR, 4 in second CR) and 4 patients are still alive. In contrast, allo-SCT was also performed in only 1 of 38 patients with t(8;21) AML without *KIT* mutation in second CR, and this patient is still alive.

A high presenting leukocyte count and extramedullary disease were not associated with the poor prognosis in this study. Notably, *KIT* was mapped to chromosome 4 at band q11 and trisomy 4 was reported to be associated with *KIT* mutation.[21] One patient with trisomy 4 in addition to t(8;21) had N822K mutation (patient no. 4). As for additional chromosome abnormality, loss of sex chromosome was observed in 5 (62.5%) of 8 patients with *KIT* mutation and 14 (37%) of 38 patients without mutations, although the difference between them was not statistically significant. Recently, it has been reported that AML blasts with N822K mutation are sensitive to the tyrosine kinase inhibitor Gleevec/STI571/imatinib mesylate.[12] The effectiveness of imatinib mesylate for the patient with AML with *KIT* mutation was also reported.[22] Thus, tyrosine kinase inhibitors may be applicable for these patients in the future.

Two samples examined at relapse showed the same mutations as those at diagnosis (patients no. 3 and no. 5), and these *KIT* mutations disappeared in samples in remission, suggesting that *KIT* mutation was not a constitutional abnormality.

Recently, clonal leukemic cells with *AML1-MTG8* fusion transcript have been reported to arise in utero.[23] Moreover, it was reported that this fusion transcript was not sufficient for full leukemogenesis, and that additional genetic events were required.[24,25] *KIT* mutations may be one of the secondary genetic events of the stepwise leukemogenesis of t(8;21) AML.

FLT3-ITD was found in only 2 (4.6%) of 46 patients with t(8;21). One patient died during chemotherapy, and the other patient was disease free for 42 months from diagnosis. *FLT3*-ITD is considered to be strongly associated with a poor prognosis in AML.[6,7] However, *FLT3*-ITD was rarely reported in patients with t(8;21) AML.[8,9,13,14,20] Our data also confirmed the low incidence of *FLT3*-ITD in patients with t(8;21) AML. As for D835Mt of the *FLT3* gene, we found the mutation in 1 of 46 patients, who was alive for 31 months after diagnosis.

In total, 11 (23.9%) of 46 patients with t(8;21) AML in this study had *KIT* or *FLT3* mutations, suggesting that the pediatric patients with t(8;21) AML had genetic heterogeneity. In conclusion, *KIT* mutations are considered to be strongly associated with poor prognosis in pediatric t(8;21) AML.

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Appendix

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事務局：独立行政法人国立病院機構
名古屋医療センター臨床研究センター内
〒460-0001　名古屋市中区三の丸4丁目1番1号
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