Prognostic Impact of Specific Molecular Profiles in Pediatric Acute Megakaryoblastic Leukemia in Non-Down Syndrome

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Abstract

Pediatric acute megakaryoblastic leukemia with non-Down syndrome (AMKL) is a unique subtype of acute myeloid leukemia (AML). Novel *CBFA2T3-GLIS2* and *NUP98-KDM5A* fusions recurrently found in AMKL were recently reported as poor prognostic factors. However, their detailed clinical and molecular characteristics in patients treated with recent improved therapies remain uncertain. We analyzed molecular features of 44 AMKL patients treated on two recent Japanese AML protocols, the AML99 and AML-05 trials. We identified *CBFA2T3-GLIS2*, *NUP98-KDM5A*, *RBM15-MKL1*, and *KMT2A* rearrangements in 12 (27%), 4 (9%), 2 (5%), and 3 (7%) patients, respectively. Among 459 other AML patients, *NUP98-KDM5A* was identified in 3 patients, whereas *CBFA2T3-GLIS2* and *RBM15-MKL1* were only present in AMKL. *GATA1* mutations were found in 5 patients (11%). Four-year overall survival (OS) and event-free survival (EFS) rates of *CBFA2T3-GLIS2*-positive patients in AMKL were 41.7% and 16.7%, respectively. Three-year cumulative incidence of relapse in *CBFA2T3-GLIS2*-positive patients was significantly higher than that of *CBFA2T3-GLIS2*-negative patients (75.0% vs 35.7%, $P = 0.024$). In multivariate analyses, *CBFA2T3-GLIS2* was an independent poor prognostic factor for OS (HR, 4.34; 95% CI, 1.31–14.38) and EFS (HR, 2.95; 95% CI, 1.20–7.23). Furthermore, seven (54%) of 13 infant AMKL patients were *CBFA2T3-GLIS2*-positive. Notably, out of 7 *CBFA2T3-GLIS2*-positive infants, six (86%) relapsed and five (71%) died. Moreover, all of *CBFA2T3-GLIS2*-positive patients who experienced induction failure (n = 3) were infants, indicating worse prognosis of *CBFA2T3-GLIS2*-positive infants. These findings indicated the significance of *CBFA2T3-GLIS2* as a poor prognostic factor in AMKL patients,

particularly in infants.

Introduction

Pediatric acute megakaryoblastic leukemia in non-Down syndrome (AMKL) is a clinically and biologically distinct, FAB M7 subtype of acute myeloid leukemia (AML), accounting for approximately 5–15% of all pediatric AML patients (Athale et al., 2001; Dastugue et al., 2002; Reinhardt et al., 2005). AMKL was shown to have a poor prognosis with a survival rate of less than 40% (Athale et al., 2001; Barnard et al., 2007), however, recent advances in diagnostic techniques and intensive chemotherapy have led to improved long-term survival rates of over 60% (Hama et al., 2008; Schweitzer et al., 2015). Although the t(1;22)(p13;q13)/*RBM15-MKL1* molecular marker was repeatedly detected in 10%–25% of AMKL (Ma et al., 2001; Mercher et al., 2001; Inaba et al., 2015), information on cytogenetic and molecular pathogenesis in most AMKL patients was limited until the recent identification of novel cryptic translocations, inv(16)(p13.3q24.3) and t(11;12)(p15;p13) that encode *CBFA2T3-GLIS2* and *NUP98-KDM5A* fusion genes, respectively (Gruber et al., 2012; Thiollier et al., 2012; de Rooij et al., 2013). The frequencies of these fusion genes in AMKL patients were reported to be 13%–27% and 8%–10%, respectively (Gruber et al., 2012; Thiollier et al., 2012; de Rooij et al., 2013). Whereas *CBFA2T3-GLIS2* was demonstrated to be a poor prognostic factor in AMKL patients (Gruber et al., 2012), the correlation between *NUP98-KDM5A* and AMKL prognosis was unclear. However, a recent intergroup study has reported a poor prognosis with these fusion genes (de Rooij et al., 2016).

New biological insights into AMKL have been gradually accumulating; however, the prognostic significance and detailed characteristics of such novel fusion genes in patients treated with improved therapies in recent clinical trials have not been reported,

partially because of the small numbers of patients. Thus, in this study, we investigated the molecular and clinical features of 44 AMKL patients treated on two recent Japanese clinical trials, AML99 and AML-05.

Material and Methods

Patients and Samples

This present retrospective cohort study enrolled patients younger than 18 years who were diagnosed with de novo AML and participated in one of the two recent clinical trials in Japan, the AML99 trial by the Japanese Childhood AML Cooperative Study between January 2000 and December 2002 and the AML-05 trial by the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) between November 2006 and December 2010 (Tsukimoto et al., 2009; Tomizawa et al., 2013). The AML-05 trial is registered with UMIN Clinical Trials Registry (UMIN–CTR, URL: http://www.umin.ac.jp/ctr/index.htm), number UMIN000000511. A total of 503 patients whose leukemic samples were available were included in the present study; 134 from a total of 280 patients in the AML99 trial and 369 from a total of 443 patients in the AML-05 trial were eligible for this study, and patients with Down syndrome and acute promyelocytic leukemia were excluded. Among the eligible patients, 44 patients (9%) (10 from AML99 and 34 from AML-05) were diagnosed with AMKL; the remaining 459 patients were diagnosed with other FAB subtypes of AML (referred to as other AML). Extensive details on the diagnosis, risk-stratification, and treatment in these protocols were previously reported (Tsukimoto et al., 2009; Tomizawa et al., 2013; Kinoshita et al., 2014). Morphological, immunological, cytogenetic, and molecular characteristics of patients in the AML-05 trial were centrally reviewed,

whereas the diagnosis of patients in the AML99 trial was made by each hospital.

Treatment protocols and data and sample collections in both clinical trials were approved by the institutional review boards of each participating institution after written informed consent was obtained from patients or their parents/guardians. The present study was conducted in accordance with the Declaration of Helsinki and approved by the institutional review board of Gunma Children's Medical Center.

Cytogenetic and Molecular Characterization

Genomic DNA and total RNA were extracted from leukemic samples using the ALLPrep DNA/RNA Mini Kit (Qiagen, Hilden, Germany) and were reverse-transcribed to cDNA using the cDNA Synthesis Kit (GE Healthcare, Tokyo, Japan). Mutation analyses of *FLT3*-ITD, *NRAS*, *KRAS*, *KIT*, *WT1*, *NPM1*, and *GATA1* (Xu et al., 2003; Shimada et al., 2006; Sano et al., 2012; Shiba et al., 2013) and fusion gene analyses including *CBFA2T3-GLIS2*, *NUP98-KDM5A*, *KMT2A-MLLT3*, *KMT2A-MLLT10*, *RBM15-MKL1*, *RUNX1-RUNX1T1*, *CBFβ-MYH11*, *NUP98-NSD1*, and *FUS-ERG* were performed using polymerase chain reaction (PCR)/reverse transcription-PCR followed by Sanger sequencing, as previously reported (Gruber et al., 2012; Thiollier et al., 2012; de Rooij et al., 2013; Shiba et al., 2013). In this study, a complex karyotype was defined by three or more chromosome abnormalities (Slovak et al., 2000; Byrd et al., 2002; Schoch et al., 2005).

Statistics

Survival rates were estimated using the Kaplan–Meier method and compared using the log-rank test. Overall survival probability (OS) was defined as the time from diagnosis

to death by any cause, and event-free survival probability (EFS) was defined as the time from diagnosis to relapse, death by any cause, or induction failure (Cheson et al., 2003). Cumulative incidence of relapse (CIR) was defined as the time between diagnosis and relapse (induction failure was attributed to an event on day 0) and was analyzed by the Kalbfleisch and Prentice method that considered death and second malignancy as competing events. Groups were compared using the Gray's test. Data related to hematopoietic stem cell transplantation (HSCT) were restricted to all 34 AMKL patients in the AML-05 trial and seven of 10 AMKL patients in the AML99 trial. Statistical analyses were performed using the Fisher's exact test for categorical variables and Mann–Whitney U test for continuous variables (i.e., age and white blood cell [WBC] count). Independence of prognostic factors was examined using multivariate Cox regression analysis using age, WBC count at diagnosis, fusion genes, and gene mutations assessed in this study. For all analyses, *P* values of <0.05 were considered statistically significant with two-tailed testing. All analyses were performed using the SPSS® statistical package program version 22 (SPSS, Tokyo, Japan), GraphPad Prism® Version 6 (GraphPad Software, Tokyo, Japan), and EZR® version 1.20 (Saitama Medical Center, Jichi Medical University, Saitama, Japan).

Comparison of clinical features between eligible and ineligible patients in the AML99 trial is shown in Table S1, whereas that in the AML-05 trial was previously reported (Shiba et al, 2016). No significant differences in any features other than age at diagnosis (the AML 99 trial) and the frequency of RAEB-T (the AML-05 trial) were observed between the groups.

Results

Identification of Cytogenetic and Molecular Features of AMKL Patients

Among a total of 44 AMKL patients, five fusion gene patterns were identified in 21 patients (47.7%): *CBFA2T3-GLIS2*, *NUP98-KDM5A*, *RBM15-MKL1*, *KMT2A-MLLT3*, and *KMT2A-MLLT10* in 12 (27%), 4 (9%), 2 (5%), 2 (5%), and 1 (2%) patients, respectively (Fig. 1). Although t(1;22)(p13;q13)/*RBM15/MKL1* and t(9;11)(p22;q23)/*KMT2A-MLLT3* were found by conventional G-banding, inv(16)(p13.3q24.3)/*CBFA2T3-GLIS2*, t(11;12)(p15;p13)/*NUP98-KDM5A*, and t(10;11)(p12;q23)/*KMT2A-MLLT10* were not identified. One *CBFA2T3-GLIS2*-positive patient had a single-cell abnormality of t(15;16)(q24;q24), whereas none of the *NUP98-KDM5A*-positive patients had cytogenetic abnormalities involving 11p15 or 12p13 (Table 1). Detailed information on cytogenetic, molecular, and clinical features of all AMKL patients are shown in Tables 1 and S2.

Gene mutations, including *FLT3*-ITD, *NRAS*, *KRAS*, *KIT*, *WT1*, and *GATA1*, were detected in 17 patients (39%) (Fig. 1). *GATA1* mutation was the most frequent gene mutation (11.3%), whereas *NPM1* mutation was not found in any of the patients.

Complex karyotype, acquired trisomy 21, and hyperdiploidy were found in 22 (50%), 16 (36%), and 23 (52%) of 44 patients, respectively (Fig. 1). Only two patients did not have any cytogenetic features analyzed in the present study.

The differences in cytogenetic and molecular aberration frequencies between AMKL and other AML patients are shown in Table 2. In fusion gene analyses, *CBFA2T3-GLIS2* and *RBM15-MKL1* were only found in AMKL patients, whereas *NUP98-KDM5A* was detected in both groups. The remaining three AML patients with *NUP98-KDM5A* fusion gene were diagnosed with FAB M5, M6, and RAEB-T subtypes. Core binding factor-AML, *NUP98-NSD1*, and *FUS-ERG* were found in only

other AML patients.

Correlation of Fusion Genes with Gene Mutations and Cytogenetic Abnormalities

Assessment of cytogenetic features of 12 *CBFA2T3-GLIS2*-positive patients revealed that the complex karyotype was found in only two patients, which was significantly lower than in *CBFA2T3-GLIS2*-negative patients (P = 0.016) (Table 3). The normal karyotype, trisomy 21, and hyperdiploidy frequently coexisted with this fusion (33%, 50%, and 58%, respectively). Analysis of gene mutations in 12 *CBFA2T3-GLIS2*-positive patients identified *FLT3*-ITD, *KIT*, and *GATA1* in 2, 1, and 2 patients, respectively (Fig. 1).

No gene mutations were observed in other fusion-positive patients. Although *NUP98-KDM5A* was known as a cryptic fusion gene, three (75%) of the four patients with this fusion had a complex karyotype (Table 1).

Prognostic Relevance of Cytogenetic and Molecular Markers

No significant difference was observed in the 4-year OS between the AML99 (n = 134) and AML-05 (n = 369) trials (76.0% vs 66.9%, P = 0.202), whereas the 4-year EFS of the AML99 trial was significantly higher than that of the AML-05 trial (64.2% vs 52.4%, P = 0.016). Among AMKL patients, the 4-year OS and EFS of the AML99 trial were not significantly different than those of the AML-05 trial (60.0% vs 57.6%, P = 0.964, and 50.0% vs 30.9%, P = 0.305, respectively). Furthermore, 44 AMKL patients had significantly lower 4-year OS and EFS rates than those of 459 other AML patients (58.6% vs 71.8%, P = 0.019, and 36.6% vs 57.7%, P < 0.001, respectively). Analysis of survival rates in AMKL patients who received HSCT (n = 28) determined that six

(67%) of the nine patients with first complete remission (CR), three (30%) of the 10 relapsed patients, and four (44%) of the nine patients with induction failure finally survived.

CBFA2T3-GLIS2-positive patients (n = 12) tended to have lower 4-year OS and EFS rates than *CBFA2T3-GLIS2*-negative patients (n = 32) (41.7% vs 66.4%, P = 0.193, and 16.7% vs 44.1%, P = 0.068, respectively) (Fig. 2A and 2B). When the analysis was restricted to patients in the AML-05 trial (n = 34), the 4-year EFS of *CBFA2T3-GLIS2*-positive patients (n = 11) was significantly lower than that of *CBFA2T3-GLIS2*-negative patients (n = 23) (9.1% vs 41.9%, P = 0.030) (Fig. 2D). Furthermore, the 3-year CIR of *CBFA2T3-GLIS2*-positive patients was significantly higher than that of *CBFA2T3-GLIS2*-negative patients (75.0% vs 35.7%, P = 0.024) (Fig. 2E). Only two (17%) *CBFA2T3-GLIS2*-positive patients survived without relapse, and all of five *CBFA2T3-GLIS2*-positive patients who received chemotherapy alone in intensification therapy relapsed (Table 1). Eventually, all *CBFA2T3-GLIS2*-positive patients received HSCT, which was significantly more frequent than in *CBFA2T3-GLIS2*-negative patients (100% vs 57%, P = 0.014). Specifically, three of five *CBFA2T3-GLIS2*-positive patients who survived received HSCT at first CR.

Analysis of other cytogenetic and molecular features for prognosis demonstrated that patients with hyperdiploidy had a significantly better 4-year OS than those lacking hyperdiploidy (P = 0.048) (Table. S3). Notably, three of the six patients with hyperdiploidy who died were *CBFA2T3-GLIS*2-positive (Table 1). Patients with a complex karyotype also tended to have favorable 4-year OS, although the difference was not significant (P = 0.130) (Table S3). Among the 22 patients with a complex karyotype, four had induction failure and received HSCT, and three (75%) of those

survived without relapse. The prognosis of patients with trisomy 21 was not significantly different than that of those without trisomy 21 (Table S3).

Age Dependency of Molecular and Clinical Features in AMKL

The ages of AMKL patients were characterized by a bimodal distribution. When the patients were divided into early-onset (n = 41, 0–4 years) and late-onset (n = 3, 12–13 years) groups, fusion genes were observed in only early-onset patients, whereas all late-onset patients harbored gene mutations: two, one, and one patient with *FLT3*-ITD, *WT1*, and *KIT*, respectively (Tables 1 and S2).

Out of 41 early-onset patients, 13 (32%) were less than 1 year old (i.e., infants) and seven (54%) infants had *CBFA2T3-GLIS2*. Notably, among *CBFA2T3-GLIS2*-positive infants, six (86%) relapsed and five (71%) died (Table 1). Furthermore, all of *CBFA2T3-GLIS2*-positive patients who experienced induction failure (n = 3) were infants, indicating a worse prognosis of *CBFA2T3-GLIS2*-positive infants than *CBFA2T3-GLIS2*-positive older patients.

Finally, 3 late-onset patients tended to have a poor prognosis, although the number of patients was small; among these, two patients had induction failure, two patients relapsed, and all three patients died.

Cox Regression Analysis

Multivariate Cox regression analyses of OS and EFS (see Methods) in all AMKL patients (n = 44) using WBC count and age as continuous variables identified that *CBFA2T3-GLIS2* was an independent prognostic factor for poor OS and EFS and that *NUP98-KDM5A* was an independent prognostic factor for poor EFS (Table 4).

Whereas, multivariate Cox regression analyses of early-onset patients (n = 41), using age as a categorical variable (infants vs older patients), revealed that *CBFA2T3-GLIS2* and *NUP98-KDM5A* were independent prognostic factors for poor EFS (Table 4).

Discussion

Among 44 AMKL patients treated with two recent AML protocols in Japan, *CBFA2T3-GLIS2* fusion gene was the most frequently identified fusion gene (27%). In addition, *NUP98-KDM5A* (9%), *RBM15-MKL1* (5%), and *KMT2A* rearrangements (7%) were recurrently found. Gene mutations in AMKL tended to be less frequent in fusion-positive patients than in fusion-negative patients (14% vs 35%). Survival analyses indicated that *CBFA2T3-GLIS2* was a strong candidate for poor prognostic factor in AMKL patients, even in those treated with recent improved chemotherapies.

Our study included patients consecutively treated with either of the two recent clinical trials in Japan and revealed that AMKL had an improved OS at approximately 60%, which was consistent with a recent report by the Berlin-Frankfurt-Münster (BFM) study group that reported the OS as 70% (Schweitzer et al., 2015). Thus, our study might be able to identify relatively accurate frequencies as well as the clinical impact of genetic features in AMKL patients who were treated in recent clinical trials. The frequency of *CBFA2T3-GLIS2* in this study was either similar to or two-fold higher than those reported in three previous studies (27%, 13%, and 16%); additionally, the present study demonstrated that *CBFA2T3-GLIS2*-positive patients had a poor prognosis, in agreement with previous studies (Table 5).

In prognostic analyses of our cohort, the 4-year EFS was lower and the 3-year

CIR was higher in *CBFA2T3-GLIS2*-positive patients than in *CBFA2T3-GLIS2*-negative patients. One potential reason for this outcome is the high relapse rate of *CBFA2T3-GLIS2*-positive patients treated with chemotherapy alone as initial treatment. Among nine *CBFA2T3-GLIS2*-positive patients who achieved CR after induction therapy, four received HSCT at first CR, three of whom survived. In contrast, four of the remaining five patients who received chemotherapy alone as initial treatment died after relapse. Although the patient number was limited in the present study, and while a previous intergroup study reported that a benefit of HSCT for AMKL patients could not be demonstrated (de Rooij et al., 2016), these results indicated that HSCT at first CR should be considered in *CBFA2T3-GLIS2*-positive patients to avoid relapse.

The tendency of very poor prognosis observed in *CBFA2T3-GLIS2*-positive infants in this study raised the possibility that *CBFA2T3-GLIS2*-positive patients should be stratified into risk groups by age. The prognosis of infant AML patients was previously reported not to be poor, with a 5-year OS of 61%–75% and 5-year EFS of 44%–51% (Creutzig et al., 2012). Furthermore, in contrast to infant ALL (Pui et al., 2002), *KMT2A* rearrangements in infant AML were not associated with a poor prognosis (*KMT2A*-positive vs negative; 5-year OS, 71% vs 66%; 5-year EFS, 43% vs 52%) (Creutzig et al., 2012). High WBC counts (43.2×10^9/l) and high induction failure rate (43%) of *CBFA2T3-GLIS2*-positive infants in the present study might be indicators of hyper-proliferation of leukemic cells. Thus, future studies with a larger number of patients will be needed for further characterization of infant AMKL.

Although a previous intergroup study reported that *NUP98-KDM5A* was associated with poor prognosis in AMKL (Table 5), the present study could not

determine the prognostic power of *NUP98-KDM5A* due to the small number of *NUP98-KDM5A*-posiitve patients (n = 4). However, when prognostic analyses were performed in all 503 AML patients across both trials, including four AMKL and three other AML patients with *NUP98-KDM5A* (one of each with M5, M6, and RAEB-T subtypes), the 4-year OS and EFS of a total of seven *NUP98-KDM5A*-positive patients were significantly lower than those of *NUP98-KDM5A*-negative patients (28.6% vs 71.2%, $P = 0.003$; and 14.3% vs 56.9%, $P < 0.001$, respectively. data not shown). Furthermore, several studies reported that AML patients with other *NUP98*-rearrangements had a poor prognosis (Taketani et al., 2010; Hollink et al., 2011; Shiba et al., 2013). Thus, all together, these findings suggested that *NUP98-KDM5A* was potentially a poor prognostic factor in pediatric AML patients.

Hyperdiploidy was frequently observed in AMKL patients, consistent with a previous report (Sandahl et al., 2014), and was a significantly good prognostic factor in the present study. One reason for this finding was the high survival rate of patients with hyperdiploidy after induction failure and/or relapse. Their 5-year EFS was not significantly different than that of patients without hyperdiploidy (38.1% vs 33.1%, $P = 0.746$) (Table S3), suggesting that patients with hyperdiploidy could be salvaged by intensified chemotherapy instead of therapies used in intermediate-risk patients. Although the first option for patients who experienced induction failure or relapse is still HSCT, more intensified chemotherapy without HSCT might be a potential option for patients with hyperdiploidy.

The biology of leukemogenesis in fusion-negative patients is predicted to be heterogeneous. Interestingly, all late-onset patients were in the fusion-negative group (Table S2). Gene mutations such as *FLT3*-ITD, *WT1*, and *KIT* were found in all

late-onset patients, whereas a complex karyotype, found in 60% of fusion-negative patients, was not observed. Furthermore, cytogenetic aberrations that are frequently found in adult AMKL patients (Dastugue et al., 2002), such as t(9;22)(q34;q11), 3q21q26 changes, and -5/del(5q), were not observed in late-onset patients. A recent intergroup study reported the data from 82 pediatric AMKL patients lacking *CBFA2T3-GLIS2*, *NUP98-KDM5A*, *KMT2A* rearrangements or monosomy 7 (de Rooij et al., 2016). The age distribution of these patients was as follows: 0–4 years, n = 72 (88%); 5–7 years, n = 4 (5%); 8–10 years, n = 0; and 11–17, n = 6 (7%). Although molecular details of these patients were not investigated, this age distribution was similar to that observed in the present study and supported our findings. Thus, further analysis in a larger cohort is necessary to understand the heterogeneity of fusion-negative patients.

Molecular differences between AMKL and other AML were identified in the present study. *CBFA2T3-GLIS2* was not found in any of the 459 other AML patients, including 97 patients with a normal karyotype, although this fusion gene was reported in 4% (10/237) of other AML patients with a normal karyotype (Masetti et al., 2013). This discrepancy might be partially explained by the racial difference between the Japanese and American/European populations, which might be related to the relatively higher frequency of this fusion gene in AMKL patients in the present study. Several studies reported the possible differences of the relationship of FAB subtypes with certain fusion genes, such as *RUNX1-RUNX1T1* and *CBFβ-MYH11*, in the Japanese population compared with the American/European populations. A previous study from the BFM study group reported that all 57 *RUNX1-RUNX1T1*-positive patients and 41 of 42 *CBFβ-MYH11*-positive patients harbored FAB-M1/M2 and M4/M4Eo subtypes,

respectively (von Neuhoff et al., 2010). However, in the AML-05 trial, 3 of 86 *RUNX1-RUNX1T1*-positive patients and 5 of 30 *CBFβ-MYH11*-positive patients harbored non-M1/M2 and non-M4/M4Eo subtypes, respectively (data not shown). Otherwise, relatively small number of patients in the present study was associated with this discrepancy. Additionally, only 24% of fusion-positive AMKL patients had gene mutations, and all fusion-positive patients were early-onset. A recent study reported a very low frequency of gene mutations in infant ALL patients with *KMT2A* rearrangements (1.3 mutations/patient) (Andersson et al., 2015). Thus, the present study suggested a similarity of leukemogenesis between fusion-positive AMKL and infant ALL with *KMT2A* rearrangements.

In conclusion, the present study clarified the cytogenetic and molecular features and their clinical impact in pediatric AMKL patients treated in recent clinical trials. *CBFA2T3-GLIS2* was the most frequently identified fusion gene and might be a strong candidate for a poor prognostic factor in this disease, especially in infants. We propose that these findings will enable clinicians to design and administer appropriate risk-stratified therapies and develop new molecular-targeted therapies for this unique pediatric AML subtype.

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Authorship Contributions

Y.Hara and Y.Hayashi designed the study. Y.Hara, N.S., G.Y. and K.O. performed the experiments. H.A., T.Taki and Y.Hayashi supervised the work. Y.Hara and K.T. analyzed the results. Y.Hara, K.T. and T.Taki constructed the figures. M.P., D.T, A.K, A.M.S, N.K, A.T, K.H, T.Taga and S.A provided patient samples and data. Y.Hara, N.S., T.Taki and Y.Hayashi wrote the paper and all the authors critically reviewed and revised the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Table 1. Clinical and Cytogenetic/Molecular Profiles of Fusion-Positive Patients

ID	Protocol	WBC ($\times 10^9$/l)	Age (y)	Mutation	CK	Hyper -diploidy	CR after induction	Risk group	Relapse	HSCT	Outcome	Cytogenetics
CBFA2T3-GLIS2												
R-081	AML-05	48.2	0	-	No	Yes	No	N/A	Yes	Yes	dead	47,XY,+21[9]/46,XY[11]
R-116	AML-05	7.3	1	*FLT3*-ITD	No	No	Yes	High	Yes	Yes	dead	46,XY[20]
R-119	AML-05	11.2	1	*FLT3*-ITD	No	Yes	Yes	High	Yes	Yes	alive	47,XX,+3[11]/46,XX[9]
R-144	AML-05	20.0	1	*GATA1*	No	No	Yes	N/A	Yes	Yes	dead	46,XY,t(15;16)(q24;q24)[1]/47,XY,+Y[1]/46,XY
R-159	AML-05	30.5	0	-	No	No	Yes	Intermediate	Yes	Yes	dead	46,XY,[20]
R-192	AML-05	35.2	0	*GATA1*	No	Yes	Yes	Intermediate	Yes	Yes	alive	48,XX,+3,+21[9]/46,XX[11]
282-R	AML-05	62.8	0	*KIT*	Yes	Yes	Yes	Intermediate	Yes	Yes	dead	49,XY,+Y,+12,+21[2]/50,sl,+Y,+8,-12[18]
315-R	AML-05	52.8	1	-	No	Yes	Yes	Intermediate	No	Yes	alive	48,XY,+14,+21[20]
326-R	AML-05	30.7	0	-	No	No	No	N/A	No	Yes	dead	46,XX[20]
352-R	AML-05	75.3	0	-	No	No	No	N/A	Yes	Yes	alive	46,XY[20]
429-R	AML-05	73.6	0	-	Yes	Yes	Yes	Intermediate	Yes	Yes	dead	#1
A159	AML99	10.5	2	-	No	Yes	Yes	Intermediate	No	Yes	alive	46,XX[16]47,XX,+21[3]/48,ider,+4[1]
NUP98-KDM5A												
336-R	AML-05	7.0	1	-	Yes	No	Yes	Intermediate	Yes	Yes	dead	#2
368-R	AML-05	23.4	1	-	Yes	No	Yes	Intermediate	No	No	alive	#3
405-R	AML-05	11.0	2	-	Yes	No	No	N/A	No	Yes	alive	#4
A262	AML99	12.5	1	-	No	No	Yes	Low	Yes	N/A	dead	46,XY[20]
RBM15-MKL1												
R-005	AML-05	12.0	0	-	Yes	Yes	Yes	Intermediate	Yes	Yes	dead	#5
R-162	AML-05	42.2	0	-	No	No	Yes	Intermediate	No	No	alive	#6
KMT2A-MLLT3												
A093	AML99	24.1	2	-	Yes	Yes	Yes	Intermediate	No	Yes	alive	#7
A136	AML99	4.3	3	-	No	No	Yes	Intermediate	No	No	alive	46XX,t(9;11)(q22;q23)[20]
KMT2A-MLLT10												
A075	AML99	6.0	2	-	Yes	No	Yes	Intermediate	No	No	alive	#8

CK, complex karyotype; CR, complete remmision; HSCT, hematopoietic stem cell transplantation; N/A, not applicable

Outline of treatment was as follows: chemotherapy alone for low-risk in AML 99/AML-05 and intermediate-risk in AML-05; chemotherapy alone or HLA-matched-related HSCT for intermediate-risk in AML 99; allo-HSCT for high-risk in AML99/AML-05. R-144 withdrew AML-05 due to false positive *FLT3*-ITD. 315-R with intermediate-risk (AML-05) received HSCT at 1st CR due to doctor's decision.

#1: 48,XX,t(3;21)(q27;q22),+21,+21[11]/48,idem,der(19)(t(1;19)(q21;p13)[3]/90,idem×2,-4,-7,-9,-15,-18,-21[3]/46,XX[3]

#2: 45,XX,-15,add(18)(q21),add(19)(p13)[16]/46,sl,del(13)(q?)[2]/46,XX[2]

#3: 46,XX,add(6)(q23),der(8;15)(q10;q10),+mar[7]/46,XX,add(6)(q23),der(8;15)(q10;q10),del(13)(q12q14),+mar[4]/46,XX,add(11)(q13)[3]/46,XX[5]

#4: 46,XY,del(3)(q13.2),add(6)(p25),ins(11;?)(q13;?),ins(12;?)(q13;?),del(13)(q12q14)[10]/49,idem,+2,+9,+del(13)(q12q14),-17,+21[1]/46,XY[9]

#5: 61,XXX,der(1)t(1;22)(p13;q13),t(1;22)(p13;q13),-3,-4,-5,+7,-9,-11,-12,-13,-15,-18,+19,-22[10]/46,XX[10]

#6: 46,XY,der(1)t(1;22)(p13;q13)add(1)(q32),der(22)t(1;22)(add(1)(p22)[15]/46,XY[5]

#7: 61<2n>,XX,+2,+4,+6,+7,+8,+(9;11)(p22;q23),+10,+12,+14,+15,+17,+19,+20,+21,+22,+22[15/20]64,idem,+13,+15,+15,[1/20]46,XX [4/20]

#8: 46,XX,add(10)(p11),add(11)(q2?1)[5/20]46XX[15/20]

Table 2. Comparison of Patients with or without AMKL

	AMKL	Other FAB subtype	*P*
Total number of patients	44	459	
Age at diagnosis, n (%)			
Median age (range)	1 (0-13)	8 (0-17)	<0.001
0-4	41 (93)	144 (31)	<0.001
5-10	0 (0)	155 (34)	<0.001
10<	3 (7)	160 (35)	<0.001
Median WBC ($\times 10^9$/l) (range)	22.0 (4.3-191.6)	21.4 (0.6-985.0)	0.886
Gender			
Male	21 (48)	249 (54)	0.637
Female	23 (52)	210 (46)	
Fusion gene, n (%)	21 (48)	281(61)	0.106
inv(16)(p13.3q24.3)/*CBFA2T3-GLIS2*	12 (27)	0 (0)	<0.001
t(11;12)(p15;p13)/*NUP98-KDM5A*	4 (9)	3 (1)	0.002
abnormal 11q23/*KMT2A* rearrangement	3 (7)	67 (16)	0.125
t(1;22)(p13;q13)/*RBM15-MKL1*	2 (5)	0 (0)	0.008
t(8;21)(q22;q22)/*RUNX1-RUNX1T1*	0 (0)	151 (32)	<0.001
inv(16)(p13q22)/*CBFB-MYH11*	0 (0)	39 (8)	0.038
t(5;11)(p35;q15.5) /*NUP98-NSD1*	0 (0)	16 (3)	0.383
t(16;21)(p11; q22)/*FUS-ERG*	0 (0)	5 (1)	1.000
Cytogenetic feature, n (%)*	39 (89)	N/A	N/A
normal	7 (16)	97 (21)	0.559
monosomy 7	3 (7)	7 (2)	0.049
trisomy 21	16 (36)	N/A	N/A
complex karyotype	22 (50)	N/A	N/A
hyperdiploidy	23 (52)	N/A	N/A
Gene mutation, n (%)*	17 (39)	N/A	N/A
NRAS	3 (7)	57 (12)	0.339
KRAS	1 (2)	31 (7)	0.344
KIT	3 (7)	91 (20)	0.041
WT1	2 (5)	28 (6)	1.000
NPM1	0 (0)	16 (4)	0.383
FLT3-ITD	4 (9)	60 (13)	0.636
GATA1	5 (11)	N/A	N/A

non-DS-AMKL, non-Down syndrome acute megakaryoblastic leukemia; other FAB subtype, M0-M6, excluding M3 and Down syndrome; WBC, white blood cell; *FLT3*-ITD, *FLT3* internal tandem duplication; N/A, not applicable
*Number of patients who have any of these mutations.

Table 3. Comparison of AMKL Patients with or without *CBFA2T3-GLIS2*

	Positive	Negative	*P*
Total number of patients	12	32	
Age at diagnosis, n (%)			
Median age (range)	0 (0-2)	1.5 (0-13)	0.003
0-4	12 (100)	29 (91)	0.551
5-10	0 (0)	0 (0)	1.000
10<	0 (0)	3 (9)	1.000
Median WBC ($\times 10^9$/l) (range)	33.3 (7.3-75.3)	20.1 (4.3-191.6)	0.074
Gender			
Male	7 (58)	14 (44)	0.504
Female	5 (42)	18 (56)	
Cytogenetic feature, n (%)*	11 (92)	28 (88)	1.000
normal	4 (33)	3 (9)	0.075
monosomy 7	0 (0)	3 (9)	0.551
trisomy 21	6 (50)	10 (31)	0.303
complex karyotype	2 (17)	20 (63)	0.016
hyperdiploidy	7 (58)	16 (50)	0.740
Gene mutation, n (%)*	5 (42)	12 (38)	1.000
NRAS	0 (0)	3 (9)	0.551
KRAS	0 (0)	1 (3)	1.000
KIT	1 (8)	2 (6)	1.000
WT1	0 (0)	2 (6)	1.000
NPM1	0 (0)	0 (0)	1.000
FLT3-ITD	2 (17)	2 (6)	0.297
GATA1	2 (17)	3 (9)	0.603

WBC, white blood cell; *FLT3*-ITD, *FLT3* internal tandem duplication; N/A, not applicable
*Number of patients who have any of these mutations.

Table 4. Cox Regression Analyses for OS and EFS of AMKL Patients

Cohort	Number		Variable	Hazard ratio	95% interval	*P*
All patients	44	OS	*CBFA2T3-GLIS2*	4.34	1.31-14.38	0.016
			NUP98-KDM5A	4.99	0.90-27.78	0.066
			Age *	1.37	1.17-1.61	<0.001
			(Likelihood ratio test $P = 0.005$, Wald test $P = 0.003$, Score test $P < 0.001$)			
		EFS	*CBFA2T3-GLIS2*	2.95	1.20-7.23	0.018
			NUP98-KDM5A	3.99	1.07-14.91	0.040
			Age*	1.18	1.04-1.35	0.012
			(Likelihood ratio test $P = 0.035$, Wald test $P = 0.035$, Score test $P = 0.022$)			
Early-onset patients	41	OS	*CBFA2T3-GLIS2*	3.12	0.94-10.33	0.062
			NUP98-KDM5A	5.45	0.95-31.17	0.057
			Age#	0.75	0.23-2.43	0.630
			(Likelihood ratio test $P = 0.110$, Wald test $P = 0.123$, Score test $P < 0.630$)			
		EFS	*CBFA2T3-GLIS2*	2.73	1.13-6.64	0.026
			NUP98-KDM5A	4.14	1.08-15.89	0.038
			Age#	0.66	0.27-1.59	0.352
			(Likelihood ratio test $P = 0.048$, Wald test $P = 0.048$, Score test $P = 0.036$)			

OS, overall survival; EFS, event-free survival
*continuous variable, #categorical variable (infant patients vs older patients)

Table 5. Recent Studies for the Prognosis of *CBFA2T3-GLIS2* and *NUP98-KDM5A* in AMKL

Reference	Treatment	Variable	Number of patients	Overall survival*	Event-free survival*	Multivariate analysis
Gruber et al. (2012)	N/A	All patients	40	N/A	N/A	No
		CBFA2T3-GLIS2	12 (30%)	5 years: 28.1% vs 41.9%, $P = 0.05$	N/A	
		NUP98-KDM5A	N/A	N/A	N/A	
de Rooij et al. (2013)	N/A	All patients	73	5 years: 42%	5 years: 34%	No
		CBFA2T3-GLIS2	8 (11%)	5 years: 19% vs 35%, $P = 0.66$	5 years: 35% vs 42%, $P = 0.52$	
		NUP98-KDM5A	9 (12%)	5 years: 22% vs 45%, $P = 0.22$	5 years: 22% vs 36%, $P = 0.54$	
Masetti et al.** (2013)	AIEOP 2002/01 Protocol	All patients	N/A	N/A	N/A	No
		CBFA2T3-GLIS2	10 (N/A)	N/A	5 years: 26.6% vs 60.7%, $P = 0.046$	
		NUP98-KDM5A	N/A	N/A	N/A	
de Rooij et al. (2016)	N/A	All patients	153	4 years: 56%	4 years: 51%	Yes
		CBFA2T3-GLIS2	24 (16%)	4 years: 38%	4 years: 33%	
		NUP98-KDM5A	14 (9%)	4 years: 36%	4 years: 36%	
The present study	AML99 and AML-05 trials	All patients	44	4 years: 58.6%	4 years: 36.6%	Yes
		CBFA2T3-GLIS2	12 (27%)	4 years: 41.7% vs 66.4%, $P = 0.193$	4 years: 16.7% vs 44.1%, $P = 0.068$	
		NUP98-KDM5A	4 (9%)	4 years: 50.0% vs 60.4%, $P = 0.332$	5 years: 25.0% vs 38.2%, $P = 0.219$	

non-DS-AMKL, non-Down syndrome acute megakaryoblastic leukemial; N/A, not applicable.

*Outcome of fusion-positive patients was compared to that of fusion-negative patients.

**This study analyzed only cytogenetically normal patients.

Figure Legends

Figure 1. Correlation between fusion genes, gene mutations, and other cytogenetic features in AMKL patients

Green, red, and blue areas show fusion genes, gene mutations, and other cytogenetic abnormalities, respectively. Deeper colors indicate the presence of aberrations. *CBFA2T3-GLIS2*, *NUP98-KDM5A*, *RBM15-MKL1*, and *KTM2A* rearrangements were recurrent, and *RUNX1-RUNX1T1*, *CBFβ-MYH11*, *FUS-ERG*, and *NUP98-NSD1* were not found.

In 21 fusion-positive patients, gene mutations were restricted to *CBFA2T3-GLIS2* -positive patients. In 23 fusion-negative patients, gene mutations were observed in eight (34.8%) patients, including all patients with a normal karyotype (n = 2) or monosomy 7 (n = 3). Analysis for cytogenetic abnormalities determined that the complex karyotype was frequent regardless of the presence of fusion genes and gene mutations, whereas trisomy 21 was mutually exclusive with gene mutations other than *KIT*.

Figure 2. Kaplan–Meier analysis of *CBFA2T3-GLIS2* in AMKL patients

Panels show the survival rates and CIR of AMKL patients. Panels A and B show the 4-year OS and EFS of all patients (n = 44, AML-05 and AML99 trials). *CBFA2T3-GLIS2*-positive patients tended to have a poor prognosis, although it was not significantly different. Panels C and D show the results of analyses that were restricted to patients in the AML-05 trial (n = 34), which indicated that the 4-year EFS of *CBFA2T3-GLIS2*-positive patients was significantly lower than that of *CBFA2T3-GLIS2*-negative patients (P = 0.030). Panel E shows the 3-year CIR of all patients (n = 44). CIR of *CBFA2T3-GLIS2*-positive patients was significantly higher

than that of *CBFA2T3-GLIS2*-negative patients (75.0% vs 35.7%, $P = 0.024$).

Figure 1

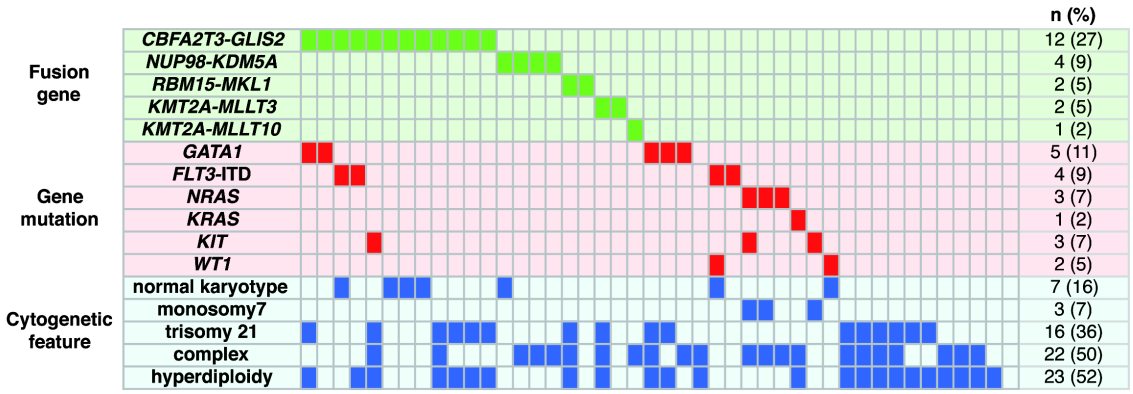
n (%)
Fusion gene
CBFA2T3-GLIS2
12 (27)
NUP98-KDM5A
4 (9)
RBM15-MKL1
2 (5)
KMT2A-MLLT3
2 (5)
KMT2A-MLLT10
1 (2)
Gene mutation
GATA1
5 (11)
FLT3-ITD
4 (9)
NRAS
3 (7)
KRAS
1 (2)
KIT
3 (7)
WT1
2 (5)
Cytogenetic feature
normal karyotype
7 (16)
monosomy7
3 (7)
trisomy 21
16 (36)
complex
22 (50)
hyperdiploidy
23 (52)

Figure 2

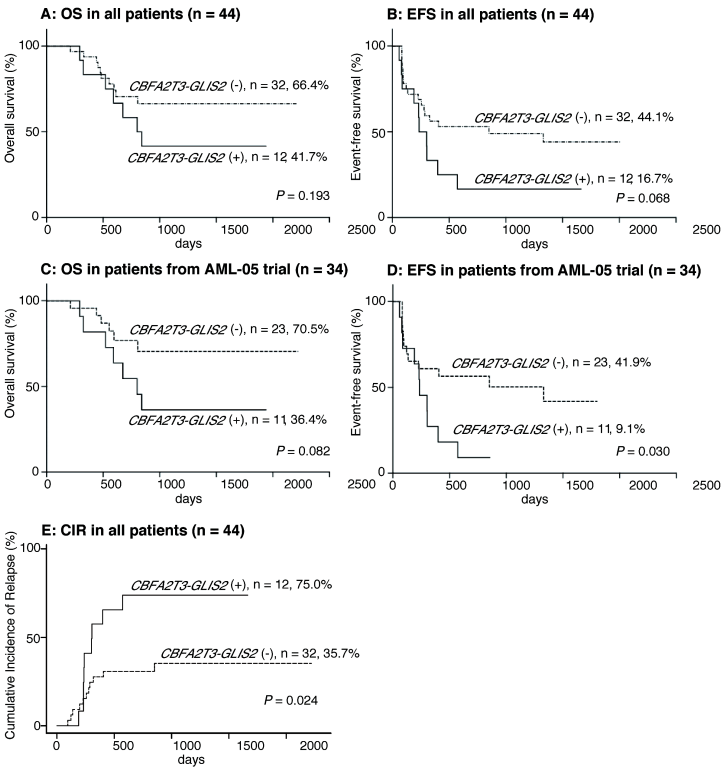
A: OS in all patients (n = 44)
Overall survival (%)
CBFA2T3-GLIS2 (-), n = 32, 66.4%
CBFA2T3-GLIS2 (+), n = 12 41.7%
P = 0.193
days
B: EFS in all patients (n = 44)
Event-free survival (%)
CBFA2T3-GLIS2 (-), n = 32, 44.1%
CBFA2T3-GLIS2 (+), n = 12 16.7%
P = 0.068
days
C: OS in patients from AML-05 trial (n = 34)
Overall survival (%)
CBFA2T3-GLIS2 (-), n = 23, 70.5%
CBFA2T3-GLIS2 (+), n = 11 36.4%
P = 0.082
days
D: EFS in patients from AML-05 trial (n = 34)
Event-free survival (%)
CBFA2T3-GLIS2 (-), n = 23, 41.9%
CBFA2T3-GLIS2 (+), n = 11, 9.1%
P = 0.030
days
E: CIR in all patients (n = 44)
Cumulative Incidence of Relapse (%)
CBFA2T3-GLIS2 (+), n = 12, 75.0%
CBFA2T3-GLIS2 (-), n = 32, 35.7%
P = 0.024
days

Table S1. Comparison of Eligible or Ineligible Patients in the AML99 Trial

	Eligible	Ineligible	*P*
Total number of patients	134	146	
Age at diagnosis (median)	6 (0-17)	4 (0-17)	0.017
Median WBC (×10^9/l) (range)	22.0 (4.3-191.6)	21.4 (0.6-985.0)	0.886
Gender			
Male	76 (57)	71 (49)	0.189
Female	58 (43)	75 (51)	
FAB classification, n (%)			
M0	5 (4)	6 (4)	1.000
M1	24 (18)	15 (10)	0.084
M2	44 (33)	41 (28)	0.436
M4	23 (17)	24 (16)	0.875
M5	25 (19)	36 (25)	0.248
M6	1 (1)	4 (3)	0.373
M7	10 (8)	18 (12)	0.232
others	2 (2)	2 (1)	1.000
4-year overall survival (%)	72.0	76.0	0.344

WBC, white blood cell

Table S2. Clinical and Cytogenetic/Molecular Profiles of Fusion-Negative Patients

ID	Protocol	WBC ($\times 10^9$/l)	Age (y)	Mutation	Hyper -diploidy	CR after induction	Risk	Relapse	HSCT	Reason for HSCT	Outcome	Cytogenetics
R-009	AML-05	50.7	3	-	Yes	Yes	HR	Yes	Yes	Relapse	alive	50,XY,del(5)(q13q31),+add(6)(q15),+8,del(15)(q11.2q15),+19,+21[8]/46,XY[12]
R-024	AML-05	20.2	0	*NRAS*	No	Yes	IR	No	No	N/A	alive	46,XY,add(5)(p11),add(7)(p11.2),?t(13;19)(q11;p13)[20]
R-043	AML-05	122.2	1	*GATA1*	Yes	Yes	IR	No	No	N/A	alive	51,XX,+8,+14,+19,+21,+21[20]
R-050	AML-05	19.9	13	*KIT*	No	No	N/A	No	Yes	Induction failure	dead	45,XY,-7[13]/46,XY[7]
R-066	AML-05	5.8	1	-	Yes	Yes	IR	Yes	Yes	Relapse	alive	47,XX,+8, del(12)(p?)[19]/46,XX[1]
R-071	AML-05	17.0	2	*GATA1*	No	Yes	IR	Yes	Yes	Relapse	dead	46,X,add(X)(p11.2), add(16)(q13),add(17)(q11.2), add(22)(q11.2)[13]/46, XX[7]
R-075	AML-05	28.1	1	-	Yes	N/A	N/A	No	No	N/A	alive	#
R-152	AML-05	9.0	0	*NRAS*, *KIT*	No	Yes	HR	No	Yes	High risk	alive	46,XY,-3,add(3)(p13),-7,-9,add(16)(q12.1),add(17)(p11.2),add(19)(p11), add(21)(q22),+r1,+mar1,+mar2[14]/46,XY[5]
R-165	AML-05	24.1	3	-	Yes	Yes	IR	No	No	N/A	alive	49,XX,t(2;7)(p13;p15),del(3)(q12),+18,+13,-14,add(19)(p13)×2,+mar1,+mar2[9] /46,XX[11]
217-R	AML-05	191.6	12	*FLT3*-ITD	No	No	N/A	Yes	Yes	Induction failure	dead	46,XX,del(9)(q11q22)[20]
245-R	AML-05	26.5	1	-	Yes	No	N/A	No	Yes	Induction failure	alive	48,XY,add(2)(q33),add(5)(q?22),add(7)(p13),-9,add,(11)(p11.2),-16,-17,+21, +4mar,inc[3]/46,XY[17]
289-R	AML-05	49.9	2	*NRAS*	No	Yes	IR	No	No	N/A	alive	46,X,-X,-2,-7,add(17)(q25),del(20)(q11.2),+r1,+mar1,+mar2[20]
327-R	AML-05	9.8	1	-	Yes	No	N/A	Yes	Yes	Induction failure	dead	50,XX,+X,add(1)(p34),t(2;5)(q31;p13),-3,add(8)(q24),del(8)(q22), add(9)(q11), del(10)(q22),add(16)(p13.1),add(18)(p11.2),+21,+21,+mar1,+mar2[8]/46, XX[12]
387-R	AML-05	6.8	2	*GATA1*	Yes	Yes	IR	No	No	N/A	alive	48,XX,+8,+21[16]/46,XX,[3]
394-R	AML-05	58.0	2	*KRAS*	Yes	Yes	HR	No	Yes	High risk	alive	51,XX,+X,+6,add(7)(p11.2),+8,del(12)(p?),+13,+19[19]
414-R	AML-05	11.0	2	*WT1*	No	Yes	IR	No	No	N/A	alive	46,XY[20]
416-R	AML-05	20.6	1	-	Yes	Yes	IR	No	No	N/A	alive	49,X ,add(1)(q21),add(2)(p21),+6,+7,add(7)(p13)×2,add(3)(q13),+19[13]/46,XX[7]
428-R	AML-05	44.3	1	-	Yes	No	N/A	No	Yes	Induction failure	alive	47,XY,?add(3)(q13),-7,add(9)(q34),+2mar,inc[1]/47,idem,add(7)(q32)[1]/50,XY, -7.+5mar,inc[1]/46,XY[17]
A059	AML99	6.7	12	*FLT3*-ITD, *WT1*	No	Yes	IR	Yes	Yes	N/A	dead	46,XX[20]
A109	AML99	13.6	0	-	Yes	Yes	LR	No	No	N/A	alive	48,XY,add(7)(p22),+21[20]
A187	AML99	38.1	0	-	Yes	Yes	LR	Yes	N/A	N/A	alive	47,XY,+21[2]47,idem,add(1)(p11),der(9)add(9)(p13),add(9)(q22),add(10)(q22)[7] 46,XY[10]
A303	AML99	29.6	2	-	Yes	Yes	IR	Yes	N/A	N/A	dead	47,XY,t(13;16)(q14;q24),+21[20]
A326	AML99	8.4	4	-	No	Yes	IR	No	Yes	N/A	dead	46,Y,t(X;10)(p11;p11)[5]/46,XY[15]

CR, complete remmision; HR, high risk; IR, intermediate risk; LR, low risk; HSCT, hematopoietic stem cell transplantation; N/A, not applicable

Outline of treatment was as follows: chemotherapy alone for low-risk in AML 99/AML-05 and intermediate-risk in AML-05; chemotherapy alone or HLA-matched-related HSCT for intermediate-risk in AML 99; allo-HSCT for high-risk in AML99/AML-05. R-075 withdrew the trial during induction therapy due to doctor's decision.

#: 51,X,add(X)(q22),+add(2)(q33),add(4)(p12),del(4)(q?),add(5)(q31),+6,del(6)(q?)×2,der(7)(add(7)(p11.2)add(7)(q32),del(8)(q24),-9,+10,+15,del(15)(q13q15)×2,-17,+19,+r1,+mar1[2]/52,sl, +21[2]/53,sl,+2,-add(2),+8,+r1[2]/46,XX[10]

Table S3. Survival Analyses of Cytogenetic Features in AMKL

Cytogenetics	Number (%)		Positive	Negative	*p*
Complex karyotype	22 (50)	4-year OS (%)	69.4	48.7	0.130
		4-year EFS (%)	42.9	31.2	0.373
		Induction failure (%)*	19.0	22.7	1.000
		HSCT (%)**	65.0	75.0	0.506
Trisomy 21	16 (36)	4-year OS (%)	60.6	57.5	0.509
		4-year EFS (%)	37.5	35.8	0.862
		Induction failure (%)*	18.8	22.2	1.000
		HSCT (%)**	78.6	63.0	0.482
Hyperdiploidy	23 (52)	4-year OS (%)	70.9	44.1	0.048
		4-year EFS (%)	33.1	38.1	0.746
		Induction failure (%)*	18.2	23.8	0.721
		HSCT (%)**	63.2	80.0	0.451

OS, overall survival probability; EFS, event-free survival probability; HSCT, hematopoietic stem cell transplantation
*43 patients were included in the analysis of induction failure rate because one patient withdrew the treatment during the induction therapy.
**The frequency of HSCT rate was analyzed in 41 patients due to the availability of the data.