A retrospective analysis of gene fusions and treatment outcomes in pediatric acute megakaryoblastic leukemia without Down syndrome

Acute megakaryoblastic leukemia (AMKL) is a specific subtype of acute myelogenous leukemia (AML) with unique clinical and biological features. In sharp contrast to myeloid leukemia associated with Down syndrome (DS), AMKL of children without DS is associated with poor outcomes.[1-4] Hematopoietic cell transplantation (HCT), particularly with allogeneic (allo) grafts, represents a curative treatment option for a subset of high-risk AMKL; however, currently there is no specific consensus on indications.[1,5] Although some studies proposed HCT as a post-remission consolidative therapy, its clinical benefit remains controversial.[5,6]

Previous studies unveiled an array of recurrent genetic alterations in AMKL and demonstrated their prognostic implications.[4,7-11] These genetic lesions might be utilized in risk-adopted therapies to improve the currently unsatisfactory treatment outcomes of this rare malignancy. In the search for precise risk stratification and optimal therapeutic interventions, we conducted a retrospective study of pediatric AMKL which included laboratory testing for gene fusion detection and survival analysis according to biological and clinical factors.

In the present study, we retrospectively analyzed bone marrow or blood samples containing leukemic blasts from pediatric *de novo* AMKL patients who were referred to study hospitals in Japan and South Korea between 1990 and 2017. AMKL diagnosis required positivity for platelet-associated antigens (CD36, CD41, CD42b, or CD61) in addition to cell morphology, and constitutional +21 was excluded by clinical phenotype and metaphase analysis.[12] Reverse transcription-polymerase chain reaction (RT-PCR) was used to screen for recurrent gene fusions observed in AMKL (*Online Supplementary Table S1*). All samples without these lesions were analyzed with RNA sequencing to search for other gene rearrangements.[13] In a final analysis, correlations among the clinical data, therapeutic interventions, cytogenetic findings, and detected gene fusions were explored. The Institutional Review Board of Nagoya University Graduate School of Medicine approved this study, and written informed consent was obtained from participants or their guardians.

The probability of overall survival (OS) and leukemia-free survival (LFS) was calculated using the Kaplan-Meier method, and the distribution of groups was compared using the log-rank test. Cumulative incidence of relapse (CIR) was calculated using the Gray's method, in which any death during remission was set to competing risk. All *P* values were two-sided; $P<0.05$ was considered statistically significant.

A summary of key information of 30 patients is shown in Figure 1A and *Online Supplementary Table S2*. The median age at diagnosis was 1.3 years (interquartile range [IQR], 0.3-1.8). Metaphase analysis revealed -7 in 3 (10%). All patients received AML-oriented high-dose cytarabine and anthracycline-based chemotherapy. Regarding treatment responses, 20 (67%) and 7 (23%) achieved hematologic complete remission (HCR), defined as <5% bone marrow blasts, after the first and second chemotherapy courses, respectively, whereas 2 (7%) did not respond to the initial induction therapies. The remaining one patient (3%) died soon after presentation from pneumonia.

Sixteen patients underwent HCT during the first complete remission (CR1), whereas 2 did so in the later disease stages. In the 1990s, autologous (auto) grafts were utilized in cases without HLA-matched sibling donor; in this study, allo- and auto-HCT were administrated in 14 (78%) and 4 (22%) patients, respectively. Indications of transplantation were determined by local clinicians, principally based on treatment response (e.g., not achieving remission after induction chemotherapy), donor availability (e.g., the presence of a family donor), and high-risk cytogenetics already identified at the time. Most of the patients undergoing both allo- and auto-HCT received busulfan-based myeloablative conditioning (Table 1).

The median follow-up period was 91 months (IQR, 12-145) and 20 patients were doing well at the final visit. In the study cohort, 7 and 3 died of leukemia and accompanied complications, respectively. The 5-year OS, LFS, and CIR were 66% (95% confidence interval [CI]: 47-80%), 63% (95% CI: 43-78%), and 29% (95% CI: 15-49%), respectively (Figure 1B). Univariate analysis of prognostic factors revealed that the 5-year LFS was significantly worse in patients who did not achieve HCR after the first course of induction chemotherapy than in those who did (30% [95% CI: 5.2-61%] *vs.* 76% [95% CI: 52-89%]; $P=0.02$) (Table 2). Of note, all 8 who experienced relapse died of leukemia or treatment-related complications with active disease.

Gene fusions of any type were detected by RT-PCR in 19 patients (63%). These fusions were mutually exclusive and included *CBFA2T3::GLIS2*, *RBM15::MRTFA*, *NUP98::KDM5A*, *FUS::ERG*, and *KMT2A::MLLT3* in 9, 5, 3, 1, and 1 patient, respectively (Figure 1A). Comprehensive transcriptome analysis by RNA sequencing in the remaining 11 patients did not reveal any apparent driver gene rearrangements. Based on previous reports, we considered *CBFA2T3::GLIS2*, *NUP98::KDM5A*, *FUS::ERG*, *KMT2A::MLLT3*, and -7 as high-risk genetic/cytogenetic aberrations, which were identified in 16 patients

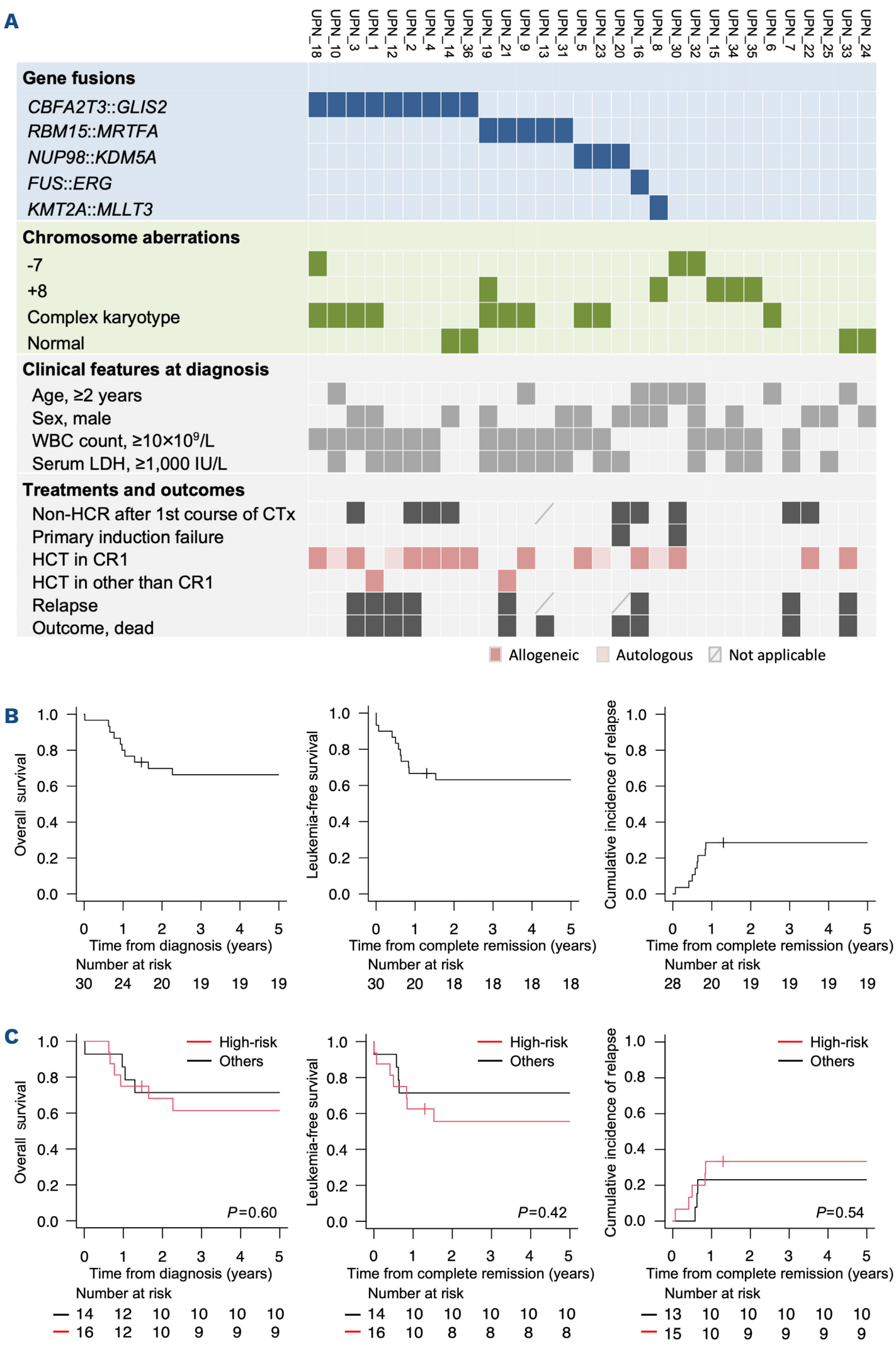

Figure 1. Clinical features and outcomes of the patients. (A) A summary of gene fusions detected, chromosome aberrations, and key clinical information on each patient. (B) Overall survival (OS), leukemia-free survival (LFS), and cumulative incidence of relapse (CIR) estimates in the overall cohort (N=30). (C) OS, LFS, and CIR estimates in patients with (red lines) or without (black lines) high-risk aberrations defined in this study: *CBFA2T3::GLIS2*, *NUP98::KDM5A*, *FUS::ERG*, *KMT2A::MLLT3*, and -7. CR1: first complete remission; CTx: chemotherapy; HCR: hematologic complete remission; HCT: hematopoietic cell transplantation; LDH: lactate dehydrogenase; WBC: white blood cell.

Table 1. Key characteristics of allogeneic and autologous hematopoietic cell transplantation.

Characteristic	Allogeneic, N=14		Autologous, N=4	
	N	%	N	%
Disease status at transplantation				
CR1	12	86	4	100
Other than CR1	2	14	0	0
HCT donor				
HLA-matched sibling	4	29	NA	
HLA-haploidentical family	1	7	NA	
Unrelated	9	64	NA	
Stem cell graft				
Bone marrow	6	43	3	75
PBSC	3	21	1	25
Cord blood	4	29	0	0
Bone marrow plus PBSC	1	7	0	0
Conditioning regimen				
Busulfan-based myeloablative	13	93	3	75
Others	1	7	1	25

CR1: first complete remission; HCT: hematopoietic cell transplantation; HLA: human leukocyte antigen; N: number; NA: not applicable; PBSC: peripheral blood stem cell.

(53%) in the study cohort.[9,14,15] Five of 8 who experienced relapse had high-risk gene fusions (*CBFA2T3::GLIS2*, N=4; *FUS::ERG*, N=1), whereas another harbored *RBM15::MRTFA*. The remaining 2 did not harbor any predefined genetic/cytogenetic abnormalities. There was no significant difference in 5-year OS, LFS, and CIR between the high-risk and non-high-risk patients (OS 61% [95% CI: 33-81%] *vs.* 71% [95% CI: 41-88%], *P*=0.60; LFS 56% [95% CI: 29-76%] *vs.* 71% [95% CI: 41-88%], *P*=0.42; CIR 33% [95% CI: 15-63%] *vs.* 23% [95% CI: 8.1-56%], *P*=0.54) (Figure 1C). The 5-year OS, LFS, and CIR of patients with *CBFA2T3::GLIS2* (N=9), which is strongly associated with adverse outcomes, were 53% (95% CI: 18-80%), 56% (95% CI: 20-81%), and 44% (95% CI: 20-80%), respectively. These rates were not statistically inferior to those of patients without *CBFA2T3::GLIS2* (Table 2). In contrast to non-high-risk patients (3/14, 21%), more than half of the high-risk patients (9/16, 56%), particularly those with *CBFA2T3::GLIS2* (6/9, 67%), underwent allo-HCT during CR1. In our small cohort, the survival rates of high-risk patients who underwent allo-HCT in CR1 (N=9) did not differ from those of patients who received only chemotherapy or auto-HCT as their first-line treatment (N=7) (5-year LFS, 67% [95% CI: 28-88%] *vs.* 57% [95% CI: 17-84%], *P*=0.65).

Recent advances in diagnostics and intensive chemotherapy have improved outcomes in children with AMKL; however, survival rates remain extremely low in patients who experience relapse. Indeed, all 8 patients with recurrence of leukemia in the present study did not survive. Thus, identification of patients at high-risk of relapse is crucial to increase the chance of cure. Early response to conventional chemotherapy is one of the most powerful indicators, and consistent with previous reports, we demonstrated that the 5-year LFS was significantly worse in patients who were not in HCR after the first course of induction chemotherapy.[3,6] On the other hand, several studies reported that a subset of patients with AMKL harbored recurrent genetic alterations which were implicated in prognosis.[4,7-11] In particular, *CBFA2T3::GLIS2*, *NUP98::KDM5A*, and *KMT2A* rearrangements were associated with adverse outcomes, with OS rates of 14-42%, 35-36%, and 27-36%, respectively.[4,9,10] Furthermore, *FUS::ERG* fusion was also distinctly associated with poor prognosis in pediatric AML.[15]

A few studies reported that HCT as post-remission consolidative treatment provided better outcomes. A representative study demonstrated that estimate of 2-year event-free survival was higher in patients who underwent allo-HCT compared to those who received chemotherapy alone (26% *vs.* 0%).[1] Furthermore, a recent study reported that the 5-year OS was significantly higher for patients who underwent HCT in CR1 (72%) than for those who did in the second CR (23%) and non-CR (16%).[16] Conversely, other studies documented no benefit of HCT, including that administrated during CR1; however, their risk stratification criteria did not include most of the recently identified genetic alterations specific to AMKL.[2,3,14] While novel molecular targets in AMKL such as FOLR1 have emerged, clinically available treatments remain limited.[17] Moreover, given the extremely low survival rates for relapsed patients, considering HCT as a first-line treatment for high-risk patients with AMKL is viable and reasonable.

In the present study, the 5-year OS, LFS, and CIR in high-risk patients, especially in those with *CBFA2T3::GLIS2*, did not significantly differ from those in non-high-risk or *CBFA2T3::GLIS2*-negative patients. Furthermore, these rates appeared to be favorable compared to those reported in previous studies.[10,11] Most of the high-risk patients (13/16 [81%], including allogeneic: 9/16 [56%]) and *CBFA2T3::GLIS2*-positive patients (8/9 [89%], including allogeneic: 6/9 [67%]) underwent HCT during CR1, which might be associated with better survival.

Table 2. Univariate analysis of prognostic factors for leukemia-free survival and relapse rates.

Prognostic factors	Leukemia-free survival			Cumulative incidence of relapse		
	N	Probability at 5 years, %	*P*	N	Probability at 5 years, %	*P*
Age at diagnosis, years						
<2	22	63.6	0.82	20	30.0	0.70
≥2	8	62.5		8	25.0	
Sex						
Male	14	71.4	0.47	13	23.1	0.61
Female	16	55.6		15	33.3	
Year of diagnosis						
1990–1999	6	66.7	0.85	5	20.0	0.77
2000–2017	24	62.2		23	30.4	
Hepatosplenomegaly						
Absent	9	55.6	0.70	8	25.0	0.70
Present	21	66.7		20	30.0	
WBC count, x10^9/L						
<10	11	63.6	0.85	10	0.20	0.40
≥10	19	63.2		18	33.3	
Serum LDH, IU/L						
<1,000	13	69.2	0.43	13	23.1	0.51
≥1,000	17	58.8		15	33.3	
HCR after 1st course of CTx						
Yes	21	76.2	0.02	20	20.1	0.11
No	9	29.6		8	56.0	
CBFA2T3::GLIS2						
Negative	21	66.7	0.50	19	21.1	0.16
Positive	9	55.6		9	44.4	
RBM15::MRTFA						
Negative	25	63.8	0.75	24	29.2	0.89
Positive	5	60.0		4	25.0	
NUP98::KDM5A						
Negative	27	62.7	0.97	26	30.8	0.37
Positive	3	66.7		2	0	
-7						
Negative	27	63.0	0.75	25	32.0	0.27
Positive	3	66.7		3	0	

CTx: chemotherapy; HCR: hematologic complete remission; LDH: lactate dehydrogenase; N: number; WBC: white blood cell.

In a previous study, 9 of 12 patients with *CBFA2T3::GLIS2* achieved HCR after induction therapy. In that cohort, 3 of 4 who received allo-HCT at CR1 survived whereas 4 of the remaining 5 who received chemotherapy alone as initial treatment died after relapse.[4] The small number of patients included in the present study does not allow us to conduct detailed analysis or draw solid conclusions. Additionally, our practice did not entirely exclude rare cases of cryptic DS. Further studies in large cohorts are essential to clarify the clinical utility of AMKL-specific risk stratification that includes genetic alterations, and to assess the feasibility of allo-HCT during CR1 for high-risk patients.

In conclusion, the present study identified recurrent gene rearrangements found in pediatric AMKL, with favorable survival even in patients with high-risk genetic/cytogenetic features. More precise risk stratification, incorporating treatment response and genetic features in AMKL, could help identify those who may benefit from HCT as a first-line consolidative treatment, thereby leading to improved outcomes.

Authors

Kyogo Suzuki,[1] Asahito Hama,[1] Yusuke Okuno,[2] Yinyan Xu,[1] Atsushi Narita,[1] Nao Yoshida,[3] Hideki Muramatsu,[1] Nobuhiro Nishio,[4] Koji Kato,[3] Seiji Kojima,[1] Keon Hee Yoo[5#] and Yoshiyuki Takahashi[1#]

[1]Department of Pediatrics, Nagoya University Graduate School of Medicine, Aichi, Japan; [2]Department of Virology, Nagoya City University Graduate School of Medical Sciences, Aichi, Japan; [3]Department of Hematology and Oncology, Children's Medical Center, Japanese Red Cross Aichi Medical Center Nagoya First Hospital, Aichi, Japan; [4]Department of Advanced Medicine, Nagoya University Hospital, Aichi, Japan and [5]Department of Pediatrics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea

[#]KHY and YT contributed equally as senior authors.

Correspondence:
Y. TAKAHASHI - ytakaha@med.nagoya-u.ac.jp
K.H. YOO - hema2170@skku.edu

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Contributions
KS designed and performed the research, analyzed the data, and wrote the manuscript. YO and YX designed and performed the research and analyzed the data. AN, HM, NN and SK designed the research and analyzed the data. NY and KK provided samples and patient data. KHY provided samples and patient data, and supervised the project. AH and YT led the entire project, designed and performed the research, analyzed the data, and wrote the manuscript. All authors critically reviewed and approved the final version of the manuscript for publication.

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Data-sharing statement
Detailed data are available from the first author upon reasonable request: kyogo1116@gmail.com

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