REGULAR ARTICLE

blood advances

Prospective evaluation of prognostic impact of *KIT* mutations on acute myeloid leukemia with *RUNX1-RUNX1T1* and *CBFB-MYH11*

Yuichi Ishikawa,[1,*] Naomi Kawashima,[1,*] Yoshiko Atsuta,[2] Isamu Sugiura,[3] Masashi Sawa,[4] Nobuaki Dobashi,[5] Hisayuki Yokoyama,[6] Noriko Doki,[7] Akihiro Tomita,[8] Toru Kiguchi,[9] Shiro Koh,[10] Heiwa Kanamori,[11] Noriyoshi Iriyama,[12] Akio Kohno,[13] Yukiyoshi Moriuchi,[14] Noboru Asada,[15] Daiki Hirano,[16] Kazuto Togitani,[17] Toru Sakura,[18] Maki Hagihara,[19] Tatsuki Tomikawa,[20] Yasuhisa Yokoyama,[21] Norio Asou,[22] Shigeki Ohtake,[23] Itaru Matsumura,[24] Yasushi Miyazaki,[25] Tomoki Naoe,[16] and Hitoshi Kiyoi,[1] for the Japan Adult Leukemia Study Group

[1]Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [2]Japanese Data Center for Hematopoietic Cell Transplantation, Nagoya, Japan; [3]Division of Hematology and Oncology, Toyohashi Municipal Hospital, Toyohashi, Japan; [4]Department of Hematology and Oncology, Anjo Kosei Hospital, Anjo, Japan; [5]Division of Clinical Oncology and Hematology, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan; [6]Department of Hematology, National Hospital Organization Sendai Medical Center, Sendai, Japan; [7]Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo, Japan; [8]Department of Hematology, Fujita Health University School of Medicine, Toyoake, Japan; [9]Department of Hematology, Chugoku Central Hospital, Fukuyama, Japan; [10]Department of Hematology, Fuchu Hospital, Izumi, Japan; [11]Department of Hematology, Kanagawa Cancer Center, Yokohama, Japan; [12]Division of Hematology and Rheumatology, Nihon University School of Medicine, Tokyo, Japan; [13]Department of Hematology and Oncology, JA Aichi Konan Kosei Hospital, Konan, Japan; [14]Department of Hematology, Sasebo City General Hospital, Sasebo, Japan; [15]Department of Hematology and Oncology, Okayama University Hospital, Okayama, Japan; [16]Department of Hematology, National Hospital Organization Nagoya Medical Center, Nagoya, Japan; [17]Department of Hematology and Respiratory Medicine, Kochi Medical School, Kochi, Japan; [18]Leukemia Research Center, Saiseikai Maebashi Hospital, Maebashi, Japan; [19]Department of Hematology and Clinical Immunology, Yokohama City University Hospital, Japan; [20]Department of Hematology, Saitama Medical Center, Saitama Medical University, Kawagoe, Japan; [21]Department of Hematology, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan; [22]Department of Hematology, International Medical Center, Saitama Medical University, Hidaka, Japan; [23]Kanazawa University, Kanazawa, Japan; [24]Department of Hematology and Rheumatology, Kindai University Faculty of Medicine, Osaka, Japan; and [25]Department of Hematology, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Key Points

- *KIT* exon 17 mutation is a poor prognostic factor in AML patients with *RUNX1-RUNX1T1*, but not in those with *CBFB-MYH11*.
- *NRAS* mutation is a poor prognostic factor in AML patients with *CBFB-MYH11*.

The prognostic impact of *KIT* mutation on core-binding factor acute myeloid leukemia (CBF-AML) remains controversial. We registered 199 newly diagnosed de novo CBF-AML patients, aged 16 to 64 years, who achieved complete remission. They received 3 courses of high-dose cytarabine therapy and no further treatment until hematological relapse. Mutations in exons 8, 10-11, and 17 of the *KIT* gene were analyzed. Furthermore, we analyzed mutations in 56 genes that are frequently identified in myeloid malignancies and evaluated minimal residual disease (MRD). The primary end point was relapse-free survival (RFS) according to *KIT* mutations. The RFS in *KIT*-mutated patients was inferior to that in unmutated patients (hazard ratio, 1.92; 95% confidence interval, 1.23-3.00; $P = .003$). Based on subgroup analysis, *KIT* mutations had a prognostic impact in patients with *RUNX1-RUNX1T1*, but not in those with *CBFB-MYH11*, and only exon 17 mutation had a significant prognostic impact. Multivariate Cox regression analysis with stepwise selection revealed that the *KIT* exon 17 mutation and the presence of extramedullary tumors in patients with *RUNX1-RUNX1T1*, and loss of chromosome X or Y and *NRAS* mutation in patients with *CBFB-MYH11* were poor prognostic factors for RFS. MRD was evaluated in 112 patients, and it was associated with a poorer RFS in the patients with *CBFB-MYH11*, but not in those with *RUNX1-RUNX1T1*. These results suggested that it is necessary to separately evaluate AML with *RUNX1-RUNX1T1* or *CBFB-MYH11* according to appropriate prognostic factors. This study was registered at www.umin.ac.jp/ctr/ as #UMIN000003434.

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*Y.I. and N.K. contributed equally to this study as first authors.

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Send data sharing requests to the corresponding author at kiyoi@med.nagoya-u.ac.jp.

The full-text version of this article contains a data supplement.

Introduction

Acute myeloid leukemia (AML) with *RUNX1-RUNX1T1* or *CBFB-MYH11* is categorized into a favorable cytogenetic risk group, and allogeneic hematopoietic stem cell transplantation (HSCT) is not generally recommended during the first complete remission (CR).[1] However, several prognostic factors, including genetic alterations, have been demonstrated.[2-8] In particular, *KIT* mutation has been suggested to be associated with a poor prognosis in AML patients with *RUNX1-RUNX1T1* or *CBFB-MYH11*.[3,5,9-12] Many types of *KIT* mutations have been identified in cancer cells, but there are 3 mutation hot-spots (exon 8, exon 10-11, and exon 17) in AML.[3,10,12-14] Several groups previously reported that *KIT* mutation was a poor prognostic factor for overall survival (OS), event-free-survival, and/or relapse-free-survival (RFS) in AML with *RUNX1-RUNX1T1* or *CBFB-MYH11*.[3,10,12,15,16] On the other, some groups reported that *KIT* mutation was not associated with the long-term prognosis.[1,17-19] This controversy may be caused by several study limitations such as the prognostic relevance being mostly evaluated retrospectively, not all types of *KIT* mutations being evaluated, and the analyzed patient number being insufficient for statistical power. Furthermore, the prognostic impact of recently identified recurrent mutations, such as *ASXL1, ASXL2*, and *ZBTB7A*, on AML with *RUNX1-RUNX1T1* or *CBFB-MYH11* remains unclear. We therefore conducted a prospective, multicenter cooperative study (Japan Adult Leukemia Study Group [JALSG] core-binding factor [CBF]-AML209-KIT) to evaluate the prognostic impact of *KIT* mutation in AML patients with *RUNX1-RUNX1T1* or *CBFB-MYH11* who were treated using the same high-dose cytarabine (HiDAC) regimen. Furthermore, we evaluated the frequency and clinical relevance of other gene mutations and prognostic impact of minimal residual disease (MRD).

Methods

Patients

Patients aged 16 to 64 years old with newly diagnosed de novo AML according to the World Health Organization 2008 classification, and an Eastern Cooperative Oncology Group performance status of 2 or lower were eligible for enrollment if they had a *RUNX1-RUNX1T1* or *CBFB-MYH11* chimeric transcript and achieved CR within 2 courses of standard induction therapy. All patients in this study were registered in the JALSG registration study after being diagnosed with AML and were treated using a standard dose of idarubicin + cytarabine or daunorubicin + cytarabine for induction therapy, as shown in supplemental Table 1. Other inclusion criteria were: serum alanine aminotransferase or serum aspartate aminotransferase level up to 2.5 times the institutional upper limit of normal; serum bilirubin up to 2.0 mg/dL; serum creatinine level up to 1.5 times the institutional upper limit of normal; left ventricular ejection fraction greater than 50% on ultrasound echocardiography; or Pa_{O_2} greater than 60 Torr or Sp_{O_2} greater than 90% under room air. We excluded patients with secondary AML, those with a history of hematological abnormalities before registration, those with other types of malignant tumors, those with a history of craniotomy, and those with a history of receiving whole brain radiation to have a more uniform patient background and to exclude safety concerns associated with previous treatments.

We also excluded patients with cardiac dysfunction corresponding to either of the following: Patients who need to use cardiac pacemakers, those with a complete left bundle branch block, those with 2 branch blocks, those with ventricular or atrial tachyarrhythmia requiring treatment, those with a digestive tract ulcer of A2 stage or higher, those with uncontrolled diabetes mellitus, those with a fasting blood glucose level ≤200 that could not be maintained by insulin administration, and those with active uncontrolled infections. Written informed consent was received from all patients. The protocol was approved by the ethics committees of all participating institutions. This study was registered in the UMIN Clinical Trials Registry (UMIN000003434, http://www.umin.ac.jp/ctr/).

Treatments

All patients received 3 courses of HiDAC therapy (2 g/m^2 by 3-hour infusion every 12 hours for 5 days), as previously reported.[20,21] For patients older than 60 years of age, 1 dose of cytarabine was reduced to 1.5 g/m^2. We recommended that patients be hospitalized in the lower than NASA Class 10 000 clean room during treatment. Best supportive care, including administration of antibiotics and platelet transfusion, was performed if indicated. When patients had life-threatening documented infections during neutropenia, the use of granulocyte colony-stimulating factor was permitted. Bone marrow (BM) examination was performed to confirm CR before each course and at the end of the last course. After the completion of 3 courses of HiDAC therapy, patients did not receive further chemotherapy, immunotherapy, or HSCT until hematological relapse was observed. If patients developed hematological relapse, the best treatment, including HSCT, was applied at each institute.

Cytogenetic and molecular analyses

Cytogenetic G-banding analysis was performed using standard methods at each institute. Chimeric gene transcripts of *RUNX1-RUNX1T1* and *CBFB-MYH11* were centrally quantified by the real-time quantitative polymerase chain reaction (RT-qPCR) method using BM or peripheral blood samples at diagnosis according to a previous report.[22] *FLT3*-ITD mutation was centrally examined by the PCR method; genomic PCR was performed and the amplified products were subjected to agarose gel electrophoresis as previously reported.[23] These results were immediately reported to each institute. Residual DNA and RNA samples were preserved at the JALSG sample storage center. Mutations in exons 8, 10, 11, and 17 in the *KIT* gene were analyzed using the preserved DNA extracted from AML cells at diagnosis, as previously reported.[24] We also analyzed mutations in *FLT3, NPM1, CEBPA, NRAS, TP53, WT1,* and *IDH1* genes, and partial tandem duplication of the *KMT2A* gene (*KMT2A*-PTD) in 198 patients (supplemental Table 2). In addition, we analyzed mutations in 49 other genes in 170 patients using the TruSight Myeloid Sequencing Panel (Illumina, San Diego, CA), as previously reported (supplemental Table 2).[8,25]

Assessment of MRD

The chimeric transcript level of *RUNX1-RUNX1T1* or *CBFB-MYH11* using BM samples was evaluated after the hematological recovery from the third course of HiDAC therapy by RT-qPCR, as previously reported.[22] Because the lower detection limit of *RUNX1-RUNX1T1* and *CBFB-MYH11* transcripts was 50 copies/μg RNA in our system, we defined MRD as positive if each transcript was ≥50 copies/μg RNA. To avoid interfering with protocol treatment,

the results of gene mutations and MRD levels were not disclosed to institutes until 2 years after registration.

Definitions and study end points

Relapse after CR was defined as the presence of at least 1 of the following: reappearance of leukemic blasts in the peripheral blood, recurrence of more than 5% blasts in BM not attributable to any other cause, such as BM regeneration after chemotherapy, and development of extramedullary leukemia. We did not include chimeric transcript levels of *RUNX1-RUNX1T1* or *CBFB-MYH11* in the definition of CR or relapse. OS was defined as the time from the start date of induction therapy to death from any cause or last follow-up. RFS was defined as the time from the date of CR to relapse or death of any cause or the last follow-up.

The primary end point was RFS in AML patients with *RUNX1-RUNX1T1* or *CBFB-MYH11* according to *KIT* mutations. Secondary end points were OS according to *KIT* mutations, clinical relevance of the genetic alterations, prognostic impact of known prognostic factors, and MRD levels after the completion of therapy.

Statistical analysis

This study was prospectively powered to demonstrate a lower RFS of AML patients with *RUNX1-RUNX1T1* or *CBFB-MYH11* harboring *KIT* mutations than in those without them. With a sample size of 175 patients, the study had a power >90% at a 5% level of significance by the log-rank test if the incidence of *KIT* mutation in AML with *RUNX1-RUNX1T1* or *CBFB-MYH11* was 25%, and the 2-year RFS of patients with and without *KIT* mutations was 35% and 60%, respectively, according to previous reports.[9,10,15,26] The RFS and OS were estimated by the Kaplan-Meier method; differences in survival distributions were evaluated using the log-rank test. Differences in continuous variables were analyzed by the Mann-Whitney *U* test for distribution between 2 groups. Analysis of frequencies was performed using Fisher's exact test for 2 × 2 tables or Pearson's χ^2 test for larger tables. The prognostic significance of the clinical variables was assessed using the Cox proportional hazards model. We also attempted to construct a prognostic factor model in CBF-AML by adding a molecular type that stratifies the prognosis of CBF-AML. Factors that independently affect disease-free survival were narrowed down using the stepwise method and multivariate analysis using the Cox proportional hazard model. Factors that were candidates for model construction were factors whose $P < .1$ in the univariate analysis. Two-sided $P < .05$ was considered significant. Analyses were performed using Stata version 13·1 (StataCorp, College Station, TX).

One planned interim analysis for the primary end point was to be performed 1 year after the 100th patient was enrolled; this analysis took place independently of the study secretariat in March 2014, and the JALSG data and safety monitoring board made the decision to continue this study. Significance for the primary end point followed the O'Brien-Fleming method to maintain a 5% level of significance. Allogeneic transplantation was performed during the first remission period for 3 patients, and they were treated as deviations and censored at the time of transplantation in RFS analysis, including the multivariate analysis.

Results

Enrollment

Between May 2010 and September 2014, 203 patients from 85 institutes were enrolled. Four patients were excluded: 3 did not fulfill the eligibility criteria and 1 received other treatment (Figure 1). Thus, 199 patients consisting of 132 (66.3%) patients with *RUNX1-RUNX1T1* and 67 (33.7%) with *CBFB-MYH11* were included in the primary analysis.

KIT mutations and patient characteristics

KIT mutations were identified in 63 of the 199 patients (31.7%): 42 of 132 (31.8%) and 21 of 67 (31.3%) patients with *RUNX1-RUNX1T1* and *CBFB-MYH11*, respectively (Table 1; supplemental Table 3). A total of 68 mutations were identified in the 63 patients with *KIT* mutations and mutation in exon 17 was the most frequently identified (50/68, 73.5%), followed by that in exon 8 (14/68, 20.6%) and in exons 10-11 (4/68, 5.9%) (supplemental Table 4). *KIT* mutation in exon 8 was more frequent in AML with *CBFB-MYH11* (9/24, 37.5%) than in that with *RUNX1-RUNX1T1* (5/44, 11.4%) ($P = .014$). Although mutation at the N822 residue in exon 17 was identified in 13 of 44 (29.5%) *KIT* mutations of the patients with *RUNX1-RUNX1T1*, no patient with *CBFB-MYH11* had this mutation ($P = .008$); however, mutation at the D816 residue was equally identified in patients with *RUNX1-RUNX1T1* (21/44, 47.7%) and *CBFB-MYH11* (13/24, 54.1%). Patient characteristics according to *KIT* mutation are presented in Table 1 and supplemental Table 4. The median BM blast percentage and white blood cell (WBC) index[27] in the patients with *RUNX1-RUNX1T1* were higher in the *KIT*-mutated patients than in the unmutated patients. G-banding karyotype analysis was performed on 197 patients. An additional cytogenetic abnormality was observed in 126 patients. However, there was no significant difference in additional cytogenetic abnormalities between *KIT*-mutated and unmutated patients (supplemental Table 5).

Landscape of gene mutations in CBF-AML

Identified gene mutations in analyzed AML patients are shown in Figure 2A. *KIT* mutation (31.7%) was the most frequently identified, followed by *NRAS* (21.7%), *FLT3* (12.1%), and *ASXL2* (11.8%) mutations in AML with *RUNX1-RUNX1T1* or *CBFB-MYH11*; however, the mutation status was different between AML with *RUNX1-RUNX1T1* and *CBFB-MYH11* (Figure 2B). *ASXL2, ASXL1, RAD21,* and *ZBTB7A* mutations were more frequent in AML with *RUNX1-RUNX1T1* than in that with *CBFB-MYH11*. In contrast, *NRAS, KRAS,* and *FLT3*-TKD mutations were more frequent in AML with *CBFB-MYH11* than in that with *RUNX1-RUNX1T1* (Figure 2B). Significantly overlapping mutations were observed between *KIT* and *ASXL2*, *NRAS* and *KRAS*, and *CSF3R* and *ASXL1*. Mutually exclusive mutations were observed between *KIT* and *NRAS* and *KIT* and *ZBTB7A* (Figure 2C-E; supplemental Figure 1).

Prognostic impact of *KIT* mutation

The median follow-up period was 1566 days (range, 356-2453), and the 2-year RFS and OS in the entire cohort were 61.31% (95% confidence interval [95% CI]: 54.11-67.72) and 85.79% (95% CI: 80.09-89.97), respectively. By chimeric transcripts, the RFS and OS of patients with *RUNX1-RUNX1T1* were not significantly different

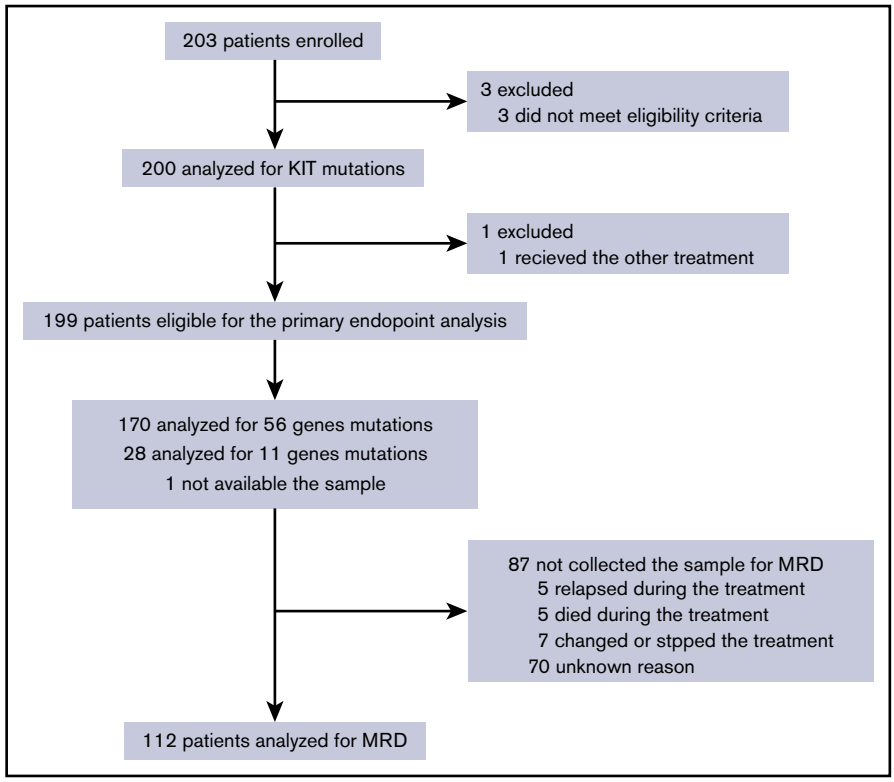

Figure 1. CONSORT flow diagram. The primary end point, relapse-free survival, was evaluated in 199 eligible patients. Prognostic analysis of MRD was performed on 112 patients whose samples were collected.

from those of patients with *CBFB-MYH11*: the 2-year RFS rates were 62.3% (95% CI, 53.3-70.0) and 59.6% (95% CI, 46.8-70.2) for *RUNX1-RUNX1T1* and *CBFB-MYH11*, respectively ($P = .88$) (supplemental Figure 4).

The 2-year RFS rates were 48.6% (95% CI, 35.7-60.3) and 67.1% (95% CI, 58.5-74.4) in *KIT*-mutated and unmutated patients, respectively (hazard ratio [HR], 1.92; 95% CI, 1.23-3.00; $P = .003$ by log-rank test) (Figure 3A). Among the 3 types of *KIT* mutations, only the mutation in exon 17 had a lower prognostic impact on the RFS of CBF-AML patients (HR, 2.30; 95% CI, 1.45-3.64; $P < .001$) (Figure 3B). Furthermore, mutations at D816 and N822 residues had a significant prognostic impact, whereas the prognostic impact of other mutations in exon 17 was unclear because of the small number of patients (supplemental Figure 2).

Although there was no significant difference in RFS between the patients with *RUNX1-RUNX1T1* and *CBFB-MYH11* (supplemental Figure 3), based on subgroup analysis, *KIT* mutations had a prognostic impact on RFS only in patients with *RUNX1-RUNX1T1*: the 2-year RFS rates were 39.5% (95% CI, 24.7-53.9) and 72.8% (95% CI, 62.2-80.9) in *KIT*-mutated and unmutated patients, respectively (HR, 3.27; 95% CI, 1.90-5.64; $P < .001$) (Figure 3C). Furthermore, only the *KIT* exon 17 mutation had a lower prognostic impact on the RFS of AML patients with *RUNX1-RUNX1T1* (HR, 3.82; 95% CI, 2.21-6.60; $P < .001$) (Figure 3D). In contrast, no *KIT* mutations affected the RFS of patients with *CBFB-MYH11* (Figure 3E, F). *KIT* mutation was also associated with a poorer OS for AML with *RUNX1-RUNX1T1,* but not for that with *CBFB-MYH11* (supplemental Figure 4), and the prognostic impact of each *KIT* mutation on OS was the same as that on RFS.

Prognostic factors in CBF-AML

We examined prognostic factors for RFS in 199 patients who were eligible for analysis of the primary end point. Multivariate Cox regression analysis with stepwise selection demonstrated that only the *KIT* exon 17 mutation was an independent poor prognostic factor for RFS in CBF-AML patients (HR, 2.42; 95% CI, 1.52-3.85; $P < .001$).

By CBF-subtype, *KIT* exon 17 mutation (HR, 4.17; 95% CI, 2.38-7.34; $P < .001$) and the presence of extramedullary tumors (HR, 3.85; 95% CI, 1.35-10.9; $P = .011$) in patients with *RUNX1-RUNX1T1*, and loss of chromosome X or Y (HR, 5.79; 95% CI, 1.21-27.6; $P = .03$) and *NRAS* mutation (HR, 2.38; 95% CI, 1.03-5.53; $P = .04$) in those with *CBFB-MYH11* were identified as poor prognostic factors for RFS by multivariate analysis (Table 2).

We also analyzed the prognostic impact of gene mutation in 170 patients in whom 56 gene mutations were examined. By multivariate analysis, *KIT* mutation (HR, 3.56; 95% CI, 1.97-6.44; $P < .001$) and *TET2* mutation (HR, 2.53; 95% CI, 1.37-11.5; $P = .01$) in patients with *RUNX1-RUNX1T1*, and *NRAS* mutation (HR, 2.36; 95% CI, 1.00-5.58; $P = .05$) in patients with *CBFB-MYH11* were found to be poor prognostic factors for RFS (supplemental Table 5).

MRD analysis

We evaluated the MRD level after the completion of 3 courses of HiDAC therapy in 112 patients. MRD was positive in 32 of 75 (42.7%) and 16 of 37 (43.2%) patients with *RUNX1-RUNX1T1* and *CBFB-MYH11*, respectively (Figure 4A). The RFS of patients

Table 1. Patient characteristics according to *KIT* mutation

Characteristic	All patients (n = 199)	*KIT* unmutated (n = 136)	*KIT* mutated (n = 63)	*P*
Chimera transcript type, n (%)				1.00
RUNX1-RUNX1T1	132 (66)	90 (66)	42 (67)	
CBFB-MYH11	67 (34)	46 (34)	21 (33)	
Sex, n (%)				.53
Male	125 (63)	81 (61)	42 (67)	
Female	74 (37)	55 (39)	21 (33)	
Age, y				.72
Median	41	41	41	
Range	16-64	17-64	16-64	
WBC, $\times 10^9$/L				.12
Median	9.6	8.5	12.1	
Range	0.80-287.0	0.80-287.0	1.84-192.4	
BM blasts, %				<.001
Median	61.2	53.8	73.5	
Range	10.8-97.0	10.8-96.5	26.1-97.0	
WBC index*				.002
Median	4.73	3.35	6.11	
Range	0.35-41.3	0.35-41.3	0.93-29.6	
Extramedullary tumor, n (%)	20 (10)	13 (10)	7 (11)	.80
CD19 expression, n/N (%)	68/192 (35)	49/129 (38)	19/63 (30)	.34
CD56 expression, n/N (%)	91/193 (47)	56/130 (43)	35/63 (56)	.13
Induction therapy, n (%)				.27
Daunorubicin base	68 (34)	43 (32)	25 (40)	
Idarubicin base	131 (66)	93 (68)	38 (60)	
Induction cycle, n (%)				1.00
1 course	187 (94)	128 (94)	59 (93)	
2 courses	12 (6)	8 (6)	4 (6)	
Additional cytogenetic abnormalities (n = 197), n/N (%)				
Loss of X/Y	78/197 (40)	55/135 (40)	23/62 (38)	.64
Trisomy 8	7/197 (4)	4/135 (3)	3/62 (5)	.68
Trisomy 22	18/197 (9)	11/135 (8)	7/62 (11)	.60
del(9q)	14/197 (7)	12/135 (9)	2/62 (3)	.23
del(7q)/-7	4/197 (2)	3/135 (2)	1/62 (2)	1.00
Complex	14/197 (7)	7/135 (5)	7/62 (11)	.14

*WBC index calculated in patients with *RUNX1-RUNX1T1*.

with MRD was lower than that of those without MRD (HR, 2.39; 95% CI, 1.24-4.61; $P = .009$) (Figure 4B). Of note, the presence of MRD was associated with a poorer RFS in patients with *CBFB-MYH11* (HR, 4.55; 95% CI, 1.20-17.2; $P = .03$), but not in those with *RUNX1-RUNX1T1* ($P = .11$) (Figure 4C-D). The presence of MRD was significantly associated with *KIT* exon 17 mutation in the patients with *RUNX1-RUNX1T1* (supplemental Table 6). Multivariate analysis of 112 patients demonstrated the presence of MRD (HR, 5.49; 95% CI, 1.43-21.0; $P = .01$) and *NRAS* mutation (HR, 3.93; 95% CI, 1.03-15.0; $P = .05$) to be poor prognostic factors for RFS in patients with *CBFB-MYH11*. In contrast, WBC count ($>50 \times 10^9$/L) (HR, 5.57; 95% CI, 1.24-15.0; $P = .03$), *KIT* exon17 mutation (HR, 3.71; 95% CI, 1.60-8.64; $P = .002$), and *FLT3*-TKD mutation (HR, 3.39; 95% CI, 1.13-10.2; $P = .03$) were poor prognostic factors in patients with *RUNX1-RUNX1T1* (supplemental Table 7).

Discussion

The prognostic impact of *KIT* mutation is a major clinical concern in AML patients with *RUNX1-RUNX1T1* and *CBFB-MYH11* because controversial results were reported by several groups. In this large prospective study, we demonstrated that the adverse effects of *KIT* mutation were observed only in AML patients with *RUNX1-RUNX1T1* and not in AML patients with *CBFB-MYH11*, although our study included a small number of patients with *CBFB-MYH11* compared with the previous study.[3] Furthermore, there was no significant difference in the RFS or OS between patients with

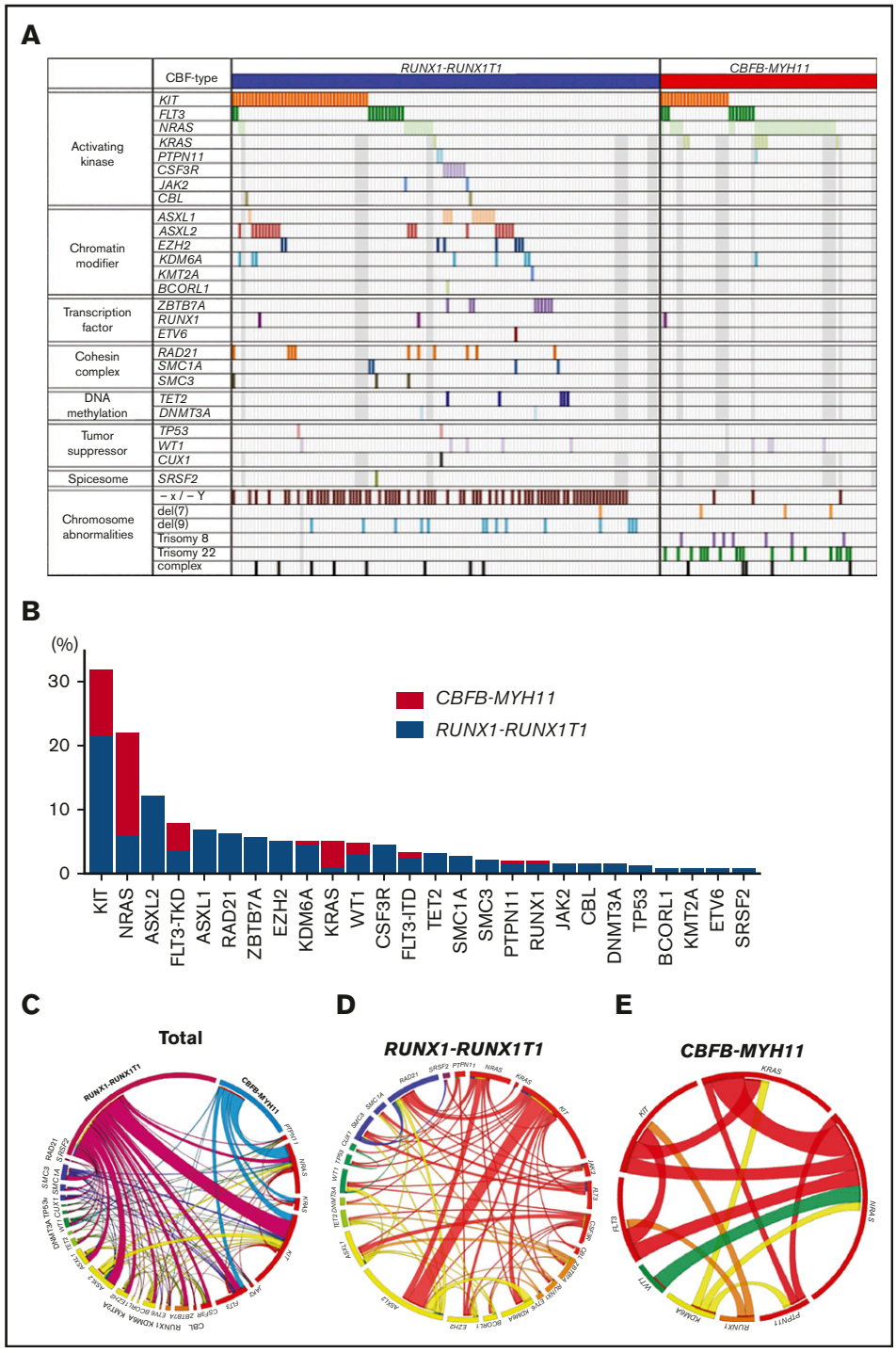

Figure 2. Mutation landscape of AML with *RUNX1-RUNX1T1* or *CBFB-MYH11*. (A) Identified mutations in analyzed patients are shown. Gray boxes indicate the patients whose samples were not analyzed. (B) The frequency of recurrently mutated genes by CBF-AML fusion type is shown. (C) Circos plots illustrate the association of mutated genes in AML with *RUNX1-RUNX1T1* or *CBFB-MYH11*. (D-E) Circos plots illustrate the association of mutated genes in AML with *RUNX1-RUNX1T1* and AML with *CBFB-MYH11*. The width of the arches indicates the percentage of mutations.

RUNX1-RUNX1T1 and *CBFB-MYH11* in this study. Because the results of mutation analysis were not reported to each institute until the completion of the protocol therapy and any further intervention was prohibited until hematological relapse, the present results are sufficient to evaluate the clinical relevance of *KIT* mutations and other molecular abnormalities in adult patients with CBF-AML treated using HiDAC. Moreover, in the patients with *CBFB-MYH11*, *NRAS* mutation was preferentially identified in *KIT*-unmutated patients, and had an adverse effect on RFS, whereas *NRAS* mutation did not affect the RFS of patients with *RUNX1-RUNX1T1* (Table 2). The fusion transcripts *RUNX1-RUNX1T1* and *CBFB-MYH11* are not sufficient for leukemia development and additional driver mutations, such as *KIT, FLT3,* and *RAS* mutations, are required for its onset.[28] However, the present study suggested that the prognostic impact of these driver mutations differs between patients with *RUNX1-RUNX1T1* and *CBFB-MYH11*.

Several groups previously reported that the MRD level examined by the chimeric transcripts using RT-qPCR was useful for predicting

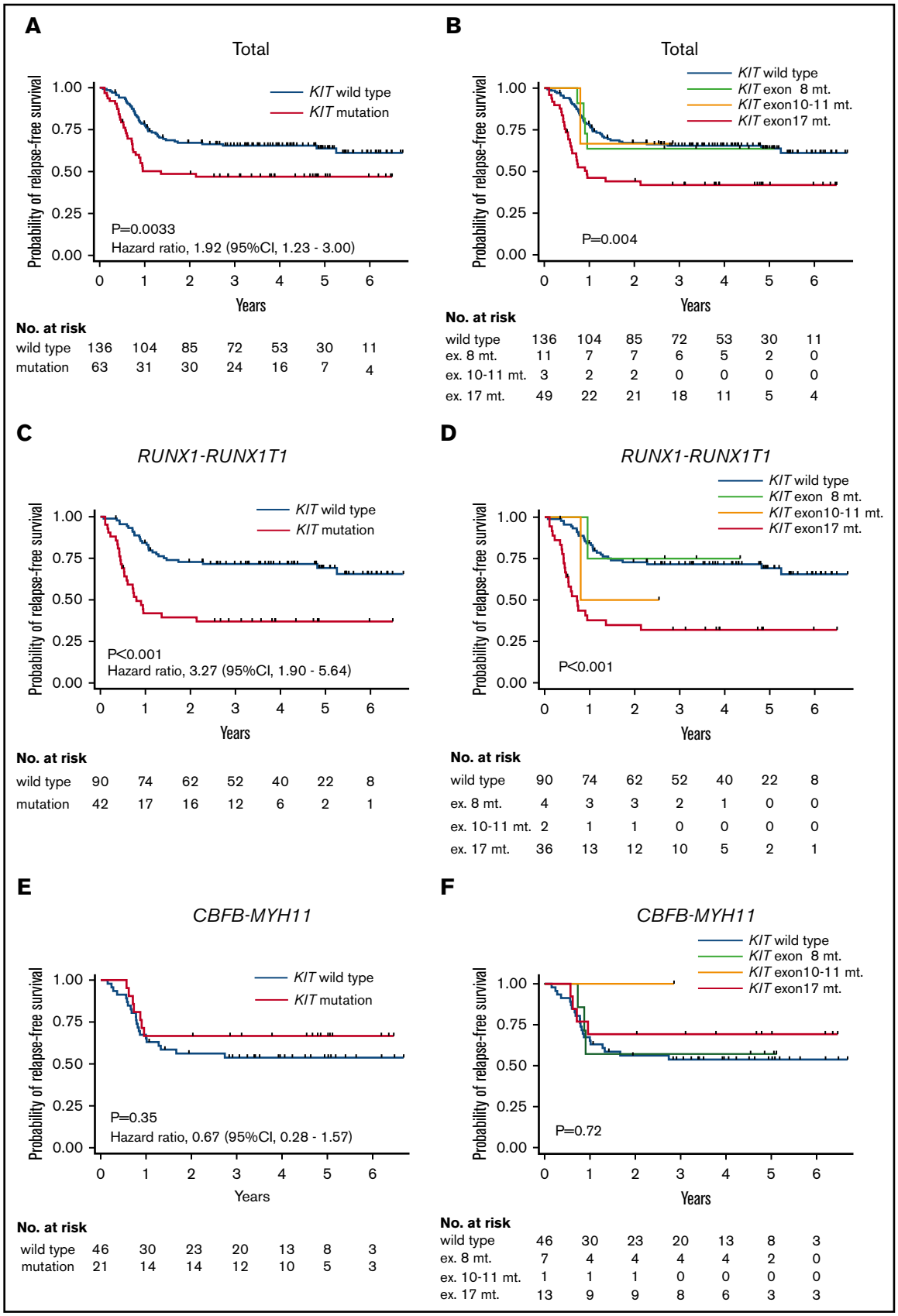

Figure 3. RFS according to *KIT* mutation. (A) Kaplan-Meier estimates of RFS according to *KIT* mutation in 199 CBF-AML patients. Kaplan-Meier estimates of RFS in patients with (C) *RUNX1-RUNX1T1* or (E) *CBFB-MYH11*. (B,D,F) Kaplan-Meier estimates of RFS according to the *KIT* mutation type.

Table 2. Multivariate analysis for RFFS

	RUNX1-RUNX1T1			*CBFB-MYH11*		
Variables	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
KIT exon17 mutation	4.17	2.38-7.34	<.001			
Extramedullary tumor	3.85	1.35-10.93	.011			
Loss of X/Y				5.79	1.21-27.6	.03
NRAS mutation				2.38	1.03-5.53	.04

the long-term prognosis of CBF-AML patients; however, there are several opinions regarding thresholds and time points for MRD assessment.[1,19,29-32] Although we evaluated MRD after completing the 3-course consolidation therapy, MRD samples were not collected from 87 patients for several reasons, including disease progression (Figure 1). The RFS was significantly lower in the sample-uncollected patients than the collected patients among those with either *RUNX1-RUNX1T1* or *CBFB-MYH11*. Therefore, the present study evaluated the clinical significance of MRD in patients who were able to maintain CR during consolidation therapy. Although further studies are required to clarify when MRD should be evaluated, our study demonstrated the importance of MRD for evaluating the prognosis of AML patients with *CBFB-MYH11*.

In conclusion, we clarified the prognostic impact of *KIT* mutation and the MRD status in adult AML patients with *RUNX1-RUNX1T1* or *CBFB-MYH11* who were treated using HiDAC, refining the concept of risk stratification of AML patients with *RUNX1-RUNX1T1* and *CBFB-MYH11*. Other treatment strategies, including allogeneic HSCT during the first remission, or addition of gemtuzumab or ozogamicin to chemotherapy, should be considered for patients with a high risk of relapse identified by this study.[33] The molecular risk groups presented in this study are amenable to routine diagnostic assessment, and provide a foundation for future clinical trials and research.

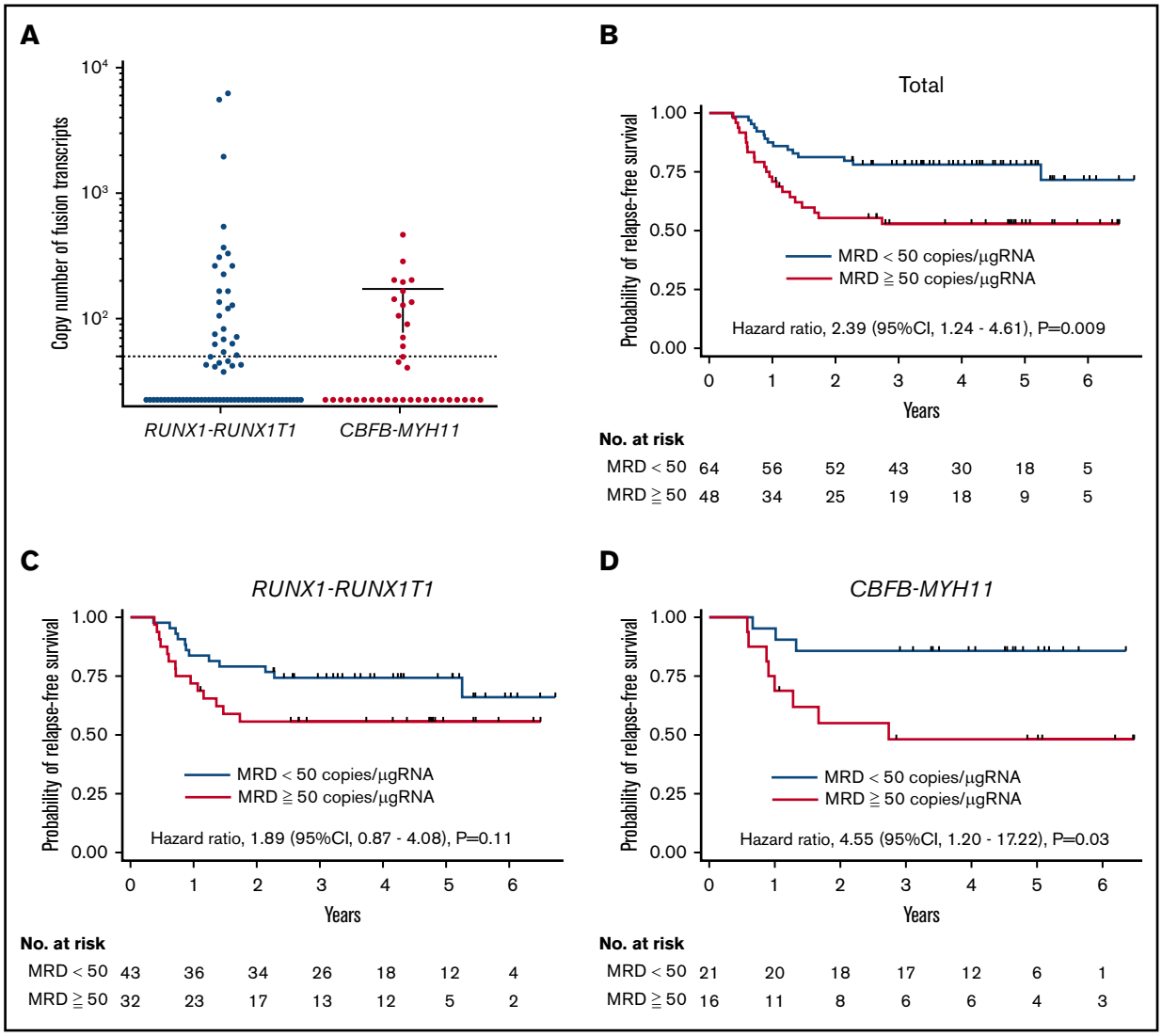

Figure 4. RFS according to the MRD level. (A) The *RUNX1-RUNX1T1* or *CBFB-MYH11* chimeric transcript level in each patient after the completion of 3 courses of HiDAC therapy is shown. (B) Kaplan-Meier estimates of RFS according to the MRD status in 112 CBF-AML patients. Kaplan-Meier estimates of RFS in patients with (C) *RUNX1-RUNX1T1* or (D) *CBFB-MYH11*.

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Authorship

Contribution: H. Kiyoi was the chief investigator of the trial; H. Kiyoi, Y.I., N.K., Y.A., I.M., Y. Miyazaki, and T.N. were involved in conception and study design; I.S., M.S., N. Dobashi, H.Y., N. Doki, A.T., T.K., S.K., H. Kanamori, N.I., A.K., Y. Moriuchi, N. Asada, D.H., K.T., T.S., M.H., T.T., Y.Y., and S.O. were involved in patient accrual and data acquisition; H. Kiyoi, Y.I., N.K., and N. Asou performed laboratory experiments and analysis; Y.I. and Y.A. performed the statistical analysis; H. Kiyoi, Y.I., N.K., S.O., Y. Miyazaki, and Y.A. were responsible for data analysis and interpretation; H. Kiyoi, Y.I., and N.K. were responsible for the preparation and writing of the manuscript; and all authors contributed to and approved the final manuscript.

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A list of participating investigators and institutes of Japan Adult Leukemia Study Group, in addition to the authors, appears in the supplemental appendix.

ORCID profiles: Y.I., 0000-0001-6024-6617; I.S., 0000-0002-4430-7544; H.Y., 0000-0001-7658-0687; S.K., 0000-0002-1732-2220; N.I., 0000-0001-9176-1988; Y.Y., 0000-0003-0366-4147; S.O., 0000-0003-0112-6564; I.M., 0000-0003-2818-4270; H.K., 0000-0001-6382-9498.

Correspondence: Hitoshi Kiyoi, Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Tsurumai-cho 65, Showa-ku, Nagoya 466-8550, Japan; e-mail: kiyoi@med.nagoya-u.ac.jp.

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