Adverse prognostic impact of *KIT* exon 17 mutations despite negative flow cytometric measurable residual disease in pediatric acute myeloid leukemia with *RUNX1::RUNX1T1*

Pediatric patients with acute myeloid leukemia (AML) with t(8;21)(q22;q22)/*RUNX1::RUNX1T1* are classified as a favorable risk with an excellent 3-year event-free survival (EFS) of approximately 70-80%, while some of them have refractory diseases after relapse.[1,2] Prognostic factors in *RUNX1::RUNX1T1*-positive AML include secondary genetic abnormalities[3-5] and treatment responses.[1,6,7] *KIT* mutations are observed almost exclusively in core-binding factor AML[8] and many pediatric and adult studies have revealed that *KIT* mutations, particularly in exon 17, are associated with poor prognosis in AML with *RUNX1::RUNX1T1*.[9-11] Also, flow cytometry-based measurable residual disease (flow-MRD) has emerged as a robust prognostic predictor for pediatric AML.[1,6,7,12,13] However, the combined prognostic impact of *KIT* mutations and flow-MRD remains to be examined. Herein, we investigated how *KIT* exon 17 mutations and flow-MRD status coordinately affected the prognosis of children with *RUNX1::RUNX1T1*-positive AML who were enrolled in the Japan Children's Cancer Group (JCCG) trial, JPLSG-AML-12, and revealed that *KIT* exon 17 mutations were associated with a significantly poor prognosis even among patients with negative MRD.

Patients were recruited to the AML-12 trial from March 2014 to February 2018. The AML-12 trial randomly assigned patients to receive initial induction therapy, including standard-dose cytarabine (ECM) or high-dose cytarabine (HD-ECM).[12] Flow-MRD was centrally monitored at the end of inductions 1 (EOI1) and 2 but did not guide toward subsequent therapies. Gemtuzumab ozogamicin (GO) was not involved in the treatment plan. Targeted capture sequencing with a custom gene panel for mutation profiling of pediatric AML was used to analyze DNA extracted from leukemic samples. The institutional review board of each participating institution approved the treatment methods and data and sample collection protocols in the clinical trial, and written informed consent was obtained from all patients or their parents/guardians. This study was approved by the Institutional Review Board of Yokohama City University Hospital and the Ethical Review Board of the JCCG, and conducted under the Declaration of Helsinki.

The AML-12 trial included 101 pediatric patients with AML with *RUNX1::RUNX1T1* who were 0-17 years old. Six patients who were treated in the non-selected phase II institutions were excluded.[12] Hence, 95 patients were included in the analysis with a median of 9.7 (range, 2.2-17.9) years and 45 (47.4%) female patients (*Online Supplementary Table S1*). In the targeted capture sequencing analysis, *KIT* was the most affected gene detected in 37 (38.9%) patients, with 29 and ten patients with exon 17 and 8 mutations, respectively. The 29 (30.5%) patients with *KIT* exon 17 mutations had a lower frequency of CD19 expression (P=0.006) and a higher frequency of CD56 expression (P=0.026) in the flow cytometry analysis for diagnostic samples than those without the mutations.

The 5-year EFS and OS (95% confidence interval [CI]) from registration were 67.4% (95% confidence interval: 57.0-75.8) and 82.6% (95% CI: 73.1-89.0) in the entire cohort (*Online Supplementary Figure S1A*). The 5-year EFS and OS of patients with *KIT* exon 17 mutations (44.8% [95% CI: 26.5-61.6] and 61.7% [95% CI: 41.6-76.7], respectively) were significantly inferior to those without (77.3% [95% CI: 65.2-85.6] and 92.0% [95% CI: 81.8-96.6], respectively; both, P<0.001) (*Online Supplementary Figure S1B, C*). *KIT* exon 8 mutations did not show a significant prognostic impact (*Online Supplementary Figure S1D, E*). As well as in the entire cohort of the AML-12 trial,[12] HD-ECM induction treatment did not show a prognostic superiority over ECM induction treatment in the entire *RUNX1::RUNX1T1* cohort (5-year EFS of 73.1% [95% CI: 58.8-83.1] and 60.5% [95% CI: 44.3-73.3] in the ECM and HD-ECM group, respectively; P=0.206; and 5-year OS of 90.3% [95% CI: 78.1-95.8] and 72.5% [95% CI: 55.4-83.9] in the ECM and HD-ECM group, respectively; P=0.027) and in the patients with or without *KIT* exon 17 mutations (*Online Supplementary Figure S1F, G*). Multivariable Cox regression analyses adjusted by treatment arms and previously investigated prognostic factors[12] revealed that *KIT* exon 17 mutations remained significantly associated with inferior EFS and OS from registration (Table 1).

Next, we analyzed EFS and OS from EOI1 in 82 patients whose flow-MRD data at EOI1 were available to evaluate the association of both *KIT* exon 17 mutations and flow-MRD status with prognosis. *KIT* exon 17 mutations still demonstrated an adverse effect on EFS and OS from EOI1 (Figure 1A, B). Also, the 5-year EFS and OS of patients achieving negative MRD with a cutoff at 0.1% (71.6% [95% CI: 59.9-80.5] and 85.9% [95% CI: 75.2-92.2], respectively) were significantly better than those with positive MRD (12.5% [95% CI: 0.7-42.3] and 37.5% [95% CI: 8.7-67.4], respectively) (both P<0.001; Figure 1C, D). In the combined analysis of *KIT* exon 17 status and flow-MRD levels (Figure 1E, F), patients with both unmutated *KIT* exon 17 and negative MRD

Table 1. Multivariable Cox regression analyses on event-free survival and overall survival in the AML-12 cohort.

Multivariable	From registration							From EOI1						
	N	EFS			OS			N	EFS			OS		
		HR	95% CI	*P*	HR	95% CI	*P*		HR	95% CI	*P*	HR	95% CI	*P*
KIT exon 17														
WT	66	1			1			57	1			1		
MT	29	3.56	1.72-7.36	<0.001	7.17	2.31-22.29	<0.001	25	3.45	1.50-7.96	0.004	7.14	2.10-24.29	0.002
MRD at EOI1[a]														
<0.1%	-	-	-	-	-	-	-	74	1			1		
≥0.1%								8	2.53	0.96-6.65	0.060	3.24	1.01-10.47	0.049
Treatment arm														
ECM	52	1			1			44	1			1		
HD-ECM	43	1.29	0.62-2.66	0.493	2.35	0.79-7.00	0.123	38	1.37	0.63-2.99	0.432	3.54	1.07-11.76	0.039
Age in years at Dx														
1-9	49	1			1			44	1			1		
≥10	46	1.10	0.54-2.23	0.800	1.62	0.59-4.44	0.351	38	1.38	0.63-3.02	0.416	2.10	0.70-6.31	0.188
WBC $\times 10^9$/L in PB at Dx														
<50	86	1			1			74	1			1		
≥50	9	0.25	0.03-1.86	0.177	0.59	0.08-4.57	0.613	8	0.40	0.05-3.03	0.376	1.69	0.20-14.40	0.632
FLT3-ITD														
Negative	90	1			1			77	1			1		
Positive	5	2.40	0.70-8.20	0.161	11.20	2.65-47.37	0.001	5	1.93	0.51-7.36	0.336	11.00	2.25-53.64	0.003

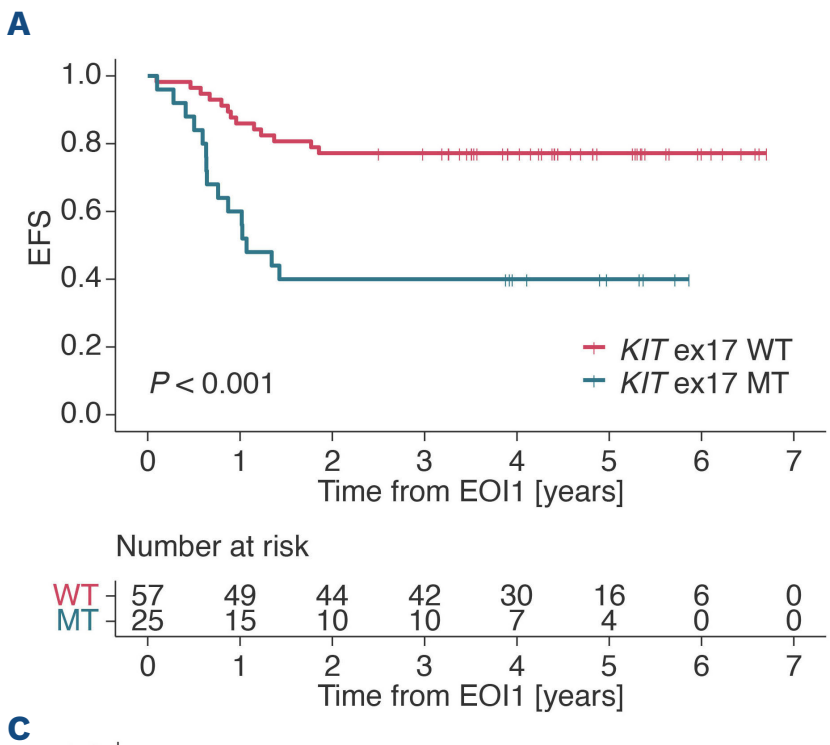
Multivariable Cox regression analyses were conducted with treatment arms, *KIT* mutations, and previously investigated prognostic factors (age, white blood cell counts [WBC] counts at diagnosis, and *FLT3*-internal tandem duplication[ITD]) as covariates. [a]Measurable residual disease (MRD) values at end of induction 1 (EOI1) were used as a covariate only for the analysis of event-free survival (EFS) and overall survival (OS) from EOI1. HR: hazard ratio; CI: confidence interval; WT: wild-type; MT: mutated; ECM: etoposide, standard-dose cytarabine, and mitoxantrone; HD-ECM: etoposide, high-dose cytarabine, and mitoxantrone; Dx: diagnosis; PB: peripheral blood.

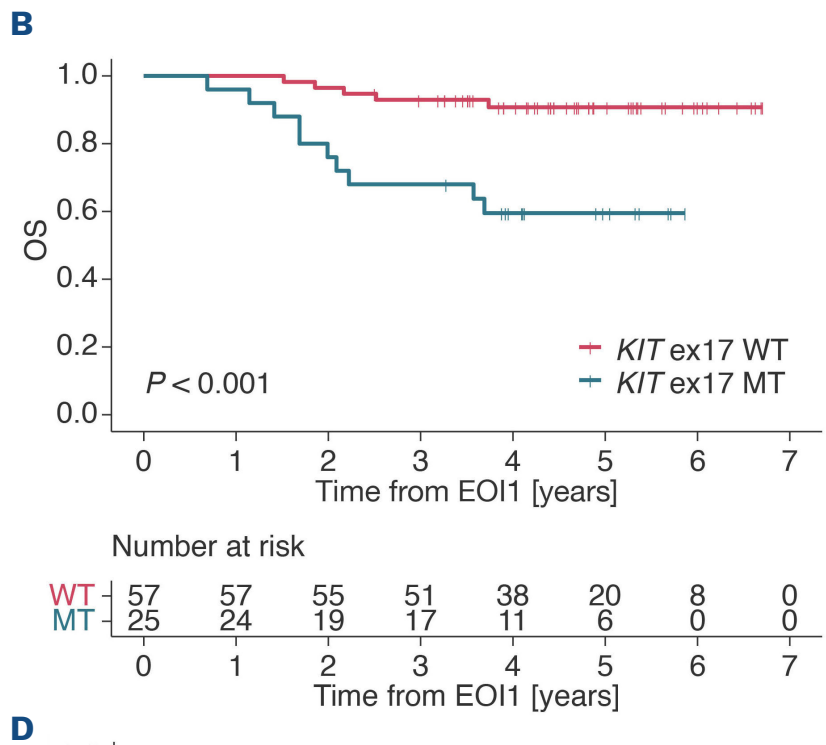
achieved 5-year EFS and OS of 80.0%, (95% CI: 66.8-88.4) and 92.2% (95% CI: 80.4-97.0), respectively. Positive MRD adversely affected prognosis irrespective of *KIT* exon 17 status. Moreover, in patients who achieved negative MRD levels, those with *KIT* exon 17 mutations demonstrated significantly worse 5-year EFS and OS compared with those without *KIT* exon 17 mutations, with a 5-year EFS of 47.4% (95% CI: 24.4-67.3; *P*=0.003) and OS of 68.0% (95% CI: 42.1-84.2; *P*=0.006). Multivariable Cox regression analyses adjusted by covariates including *KIT* exon 17 mutational status and flow-MRD levels revealed that positive MRD was associated with significantly inferior OS and a clear trend of inferior EFS but with no statistical significance (Table 1). Even with an adjustment by MRD levels, *KIT* exon 17 mutations were still associated with significantly inferior EFS. HD-ECM treatment and *FLT3*-internal tandem duplication were also significantly associated with inferior OS.

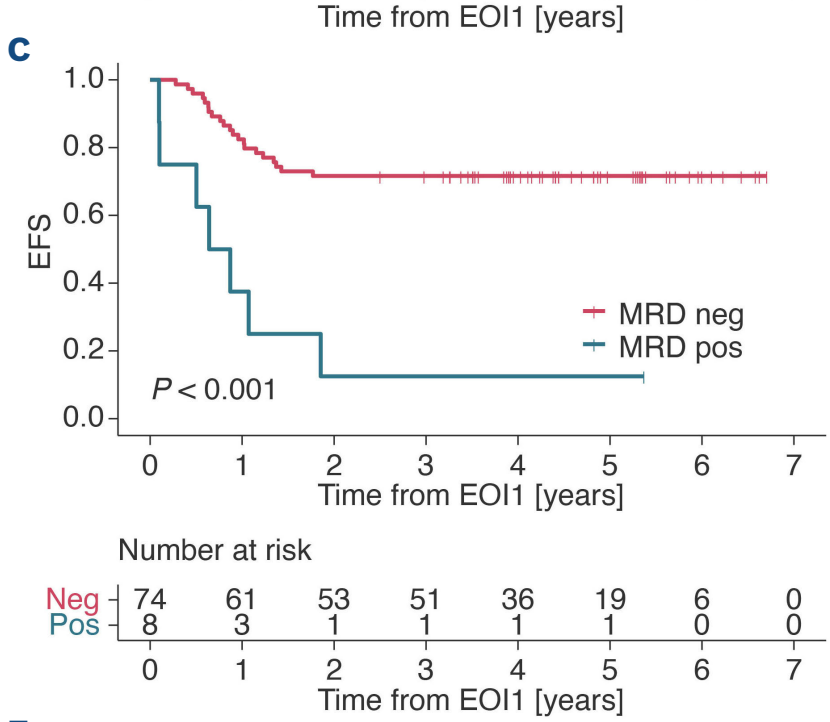
Then, we analyzed the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) dataset and compared the results with those from the AML-12 cohort. We obtained datasets of the AAML0531 trial conducted by the Children's Oncology Group, where patients were randomly assigned to a standard therapy arm or an experimental therapy arm with GO treatment.[14] The TARGET cohort covered 87.0% (114/131 patients) of all patients with *RUNX1::RUNX1T1* in the AAML0531 trial. *KIT* exon 17 mutations were less frequent (N=16) among patients with *RUNX1::RUNX1T1* in the TARGET cohort than among those in the AML-12 cohort (14.0% *vs*. 30.5%; *P*=0.006).

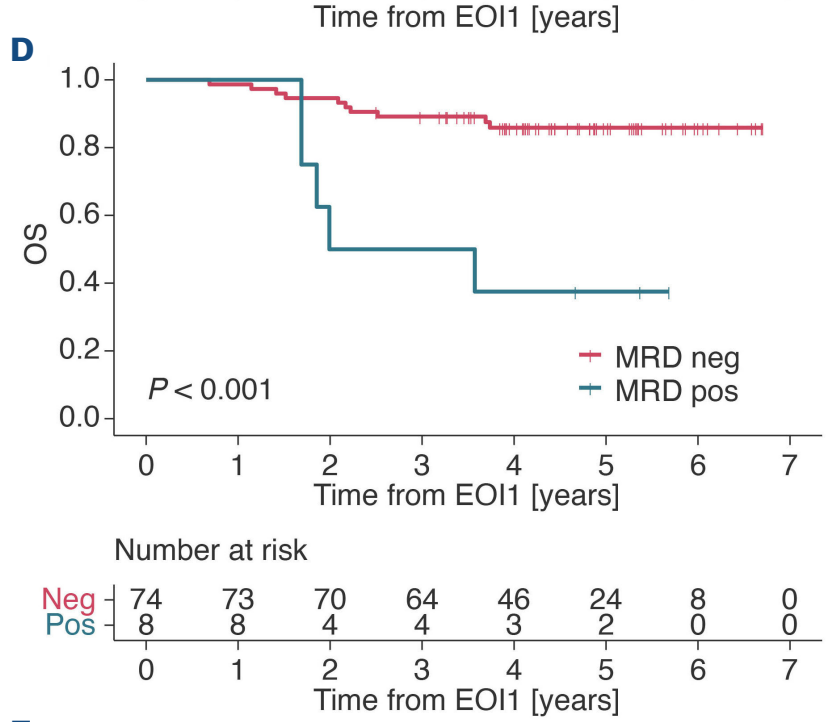
Patients with *RUNX1::RUNX1T1*-positive AML in the TARGET cohort demonstrated 5-year EFS and OS of 70.7% (95% CI: 61.4-78.2) and 81.0% (95% CI: 72.3-87.2%), respectively, similar to the results in our cohort (*Online Supplementary Figure S2A*). However, no significant difference in the prognosis was observed between patients with and without KIT exon 17 mutations (Figure 2A, B). *KIT* exon 17 mutations did not serve as a determinant for prognostic outcomes in patients positive or negative for MRD (*Online Supplementary Figure S2B*,C).

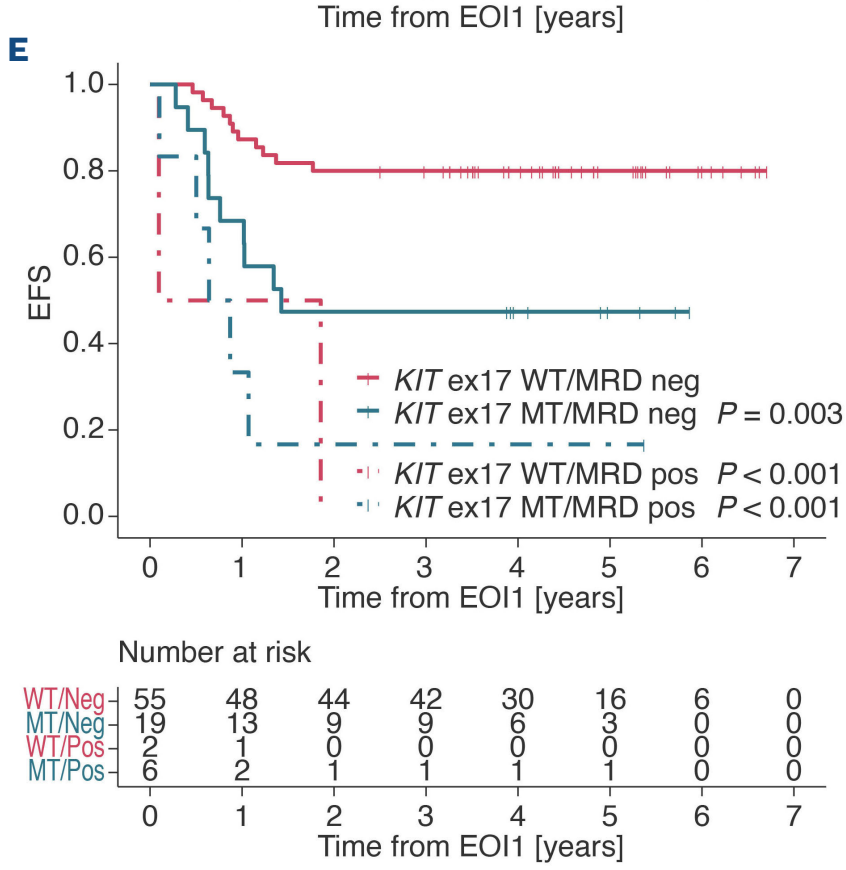
As a previous study demonstrated a therapeutic benefit of GO in core-binding factor AML,[15] we investigated the association between GO treatment and prognosis in *RUNX1::RUNX1T1* AML in the TARGET cohort. In patients with *KIT* exon 17 mutations, the GO treatment group demonstrated a clear trend of better 5-year EFS than the no GO treatment group, without a statistical significance probably due to the low number of cases (87.5% [95% CI: 38.7-98.1] *vs*. 37.5% [95% CI: 8.7-67.4]; *P*=0.059); conversely, patients without *KIT* exon 17 mutations demonstrated almost identical 5-year EFS regardless of GO treatment administration (74.9% [95% CI: 60.0-84.9] *vs*. 69.4% [95% CI: 54.5%–80.3%]; *P*=0.650) (Figure 2C, D). The 5-year OS according to *KIT* mutation status and GO treatment was











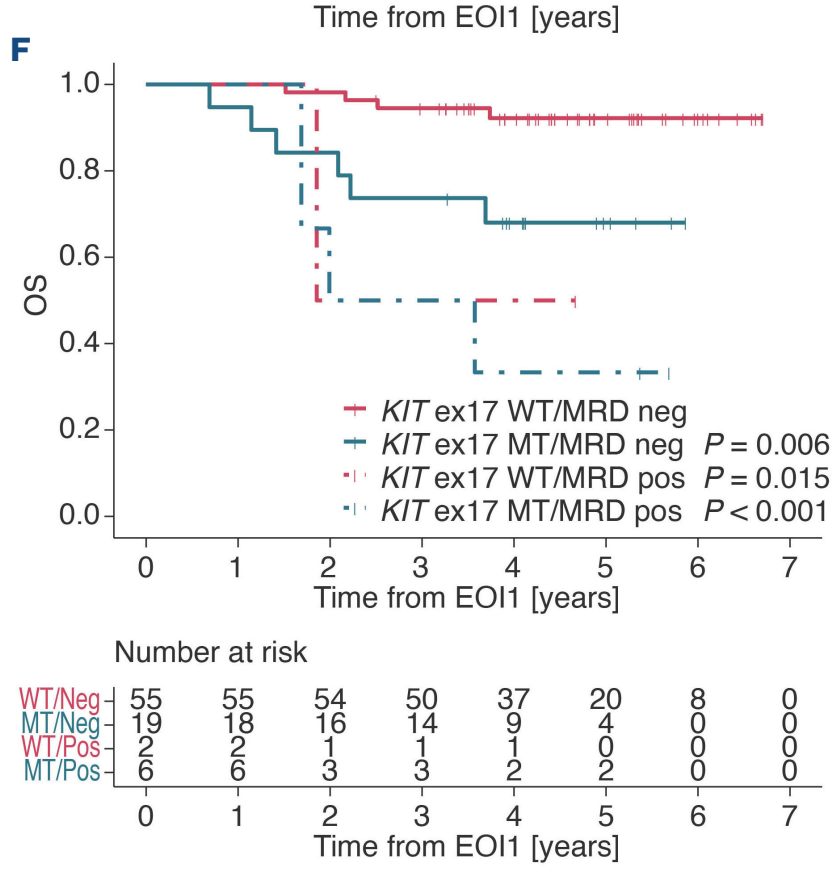


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Figure 1. Survival curves from end of induction 1 of the patients with *RUNX1::RUNX1T1*-positive acute myeloid leukemia in the AML-12 cohort. (A) Event-free survival (EFS) according to *KIT* exon 17 mutational status. The 5-year EFS was 77.2% (95% confidence interval [CI]: 64.0-86.1) and 40.0% (95% CI: 21.3-58.1%) in patients without and with *KIT* exon 17 mutations, respectively (*P*< 0.001). (B) Overall survival (OS) according to *KIT* exon 17 mutational status. The 5-year OS was 90.7% (95% CI: 79.1-96.1) and 59.5% (95% CI: 37.8-75.8) in patients without and with *KIT* exon 17 mutations, respectively (*P*<0.001). (C) EFS according to flow cytometry-measurable residual disease (flow-MRD) levels at end of induction 1 (EOI1). The 5-year EFS was 71.6% (95% CI: 59.9-80.5) and 12.5% (95% CI: 0.7-42.3) in patients with negative and positive MRD at EOI1, respectively (*P*<0.001). (D) OS according to flow-MRD levels at EOI1. The 5-year OS was 85.9% (95% CI: 75.2-92.2) and 37.5% (95% CI: 8.7-67.4) in patients with negative and positive MRD at EOI1, respectively (*P*<0.001). (E) EFS according to *KIT* exon 17 mutational status and flow-MRD levels at EOI1. The 5-year EFS was 80.0% (95% CI: 66.8-88.4) in patients without *KIT* exon 17 mutations and with negative MRD at EOI1, 47.4% (95% CI: 24.4-67.3) in those with the mutations and with negative MRD (*P*=0.003), 0.0% in those without the mutations and with positive MRD (*P*<0.001), and 16.7% (95% CI: 0.8-51.7) in those with the mutations and with positive MRD (*P*<0.001). (F) OS according to *KIT* exon 17 mutational status and flow-MRD levels at EOI1. The 5-year OS was 92.2% (95% CI: 80.4-97.0) in patients without *KIT* exon 17 mutations and with negative MRD at EOI1, 68.0% (95% CI: 42.1-84.2) in those with the mutations and with negative MRD (*P*=0.006), 50.0% (95% CI: 0.6-91.0) in those without the mutations and with positive MRD (*P*=0.015), and 33.3% (95% CI: 4.6-67.6) in those with the mutations and with positive MRD (*P*<0.001). Panels (E and F) present *P* values compared to patients without *KIT* exon 17 mutations and with negative MRD at EOI1. Ex17: exon 17; WT: wild-type; MT: mutated; neg: negative; pos: positive.

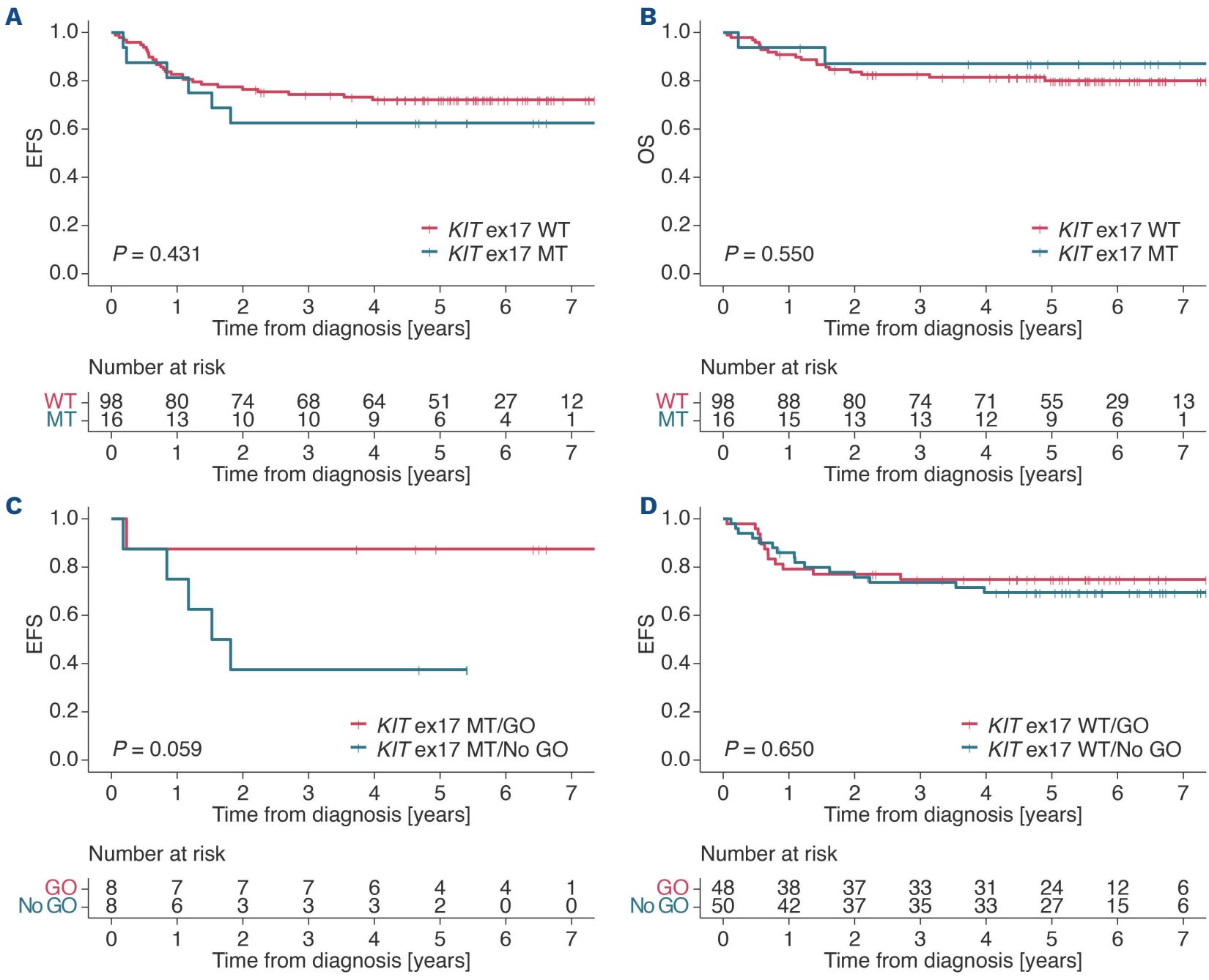

Figure 2. Survival curves of the patients with *RUNX1::RUNX1T1*-positive acute myeloid leukemia in the TARGET cohort. (A) Event-free survival (EFS) according to *KIT* exon 17 mutational status. The 5-year EFS was 72.1% (95% confidence interval [CI]: 62.0-79.9) and 62.5% (95% CI: 34.9-81.1) in patients without and with *KIT* exon 17 mutations, respectively (*P*=0.431). (B) Overall survival (OS) according to *KIT* exon 17 mutational status. The 5-year OS was 80.0% (95% CI: 70.4-86.8) and 87.1% (95% CI: 57.3-96.6) in patients without and with *KIT* exon 17 mutations, respectively (*P*=0.550). (C) EFS according to gemtuzumab ozogamicin (GO) treatment in the patients with *KIT* exon 17 mutations. The 5-year EFS was 87.5% (95% CI: 38.7-98.1) and 37.5% (95% CI: 8.7-67.4) in patients who were treated and not treated with GO, respectively (*P*=0.059). (D) EFS according to GO treatment in the patients without *KIT* exon 17 mutations. The 5-year EFS was 74.9% (95% CI: 60.0-84.9) and 69.4% (95% CI: 54.5-80.3) in patients who were treated and not treated with GO, respectively (*P*=0.650). Ex17: exon 17; WT: wild-type; MT: mutated.

not significantly different (*Online Supplementary Figure S2D, E*). No significant interaction between *KIT* exon 17 mutations and GO treatment was observed either in EFS (*P*=0.159) or OS (*P*= 0.966).

This study provided a new insight into the combined influence of *KIT* exon 17 mutations and flow-MRD levels on prognosis in pediatric AML with *RUNX1::RUNX1T1*. Patients with positive MRD had a dismal prognosis, regardless of the presence or absence of *KIT* exon 17 mutations. Further, even when limited to the MRD-negative group, patients with *KIT* exon 17 mutations had a significantly worse prognosis compared to those without the mutations. In multivariable analysis, regardless of whether MRD levels were included as a covariate, *KIT* exon 17 mutations were associated with a significantly inferior prognosis. These results highlight that the prognostic impact of *KIT* exon 17 mutations should be prioritized even under MRD-guided therapy and patients with *KIT* exon 17 mutations require treatment intensification irrespective of MRD levels.

In contrast, no significant association of *KIT* exon 17 mutations on prognosis in pediatric AML with *RUNX1::RUNX1T1* was revealed by public data from the TARGET dataset. This discrepancy between the two cohorts may be attributed to GO treatment. Therapeutic benefits of GO treatment in pediatric core binding factor AML with *KIT* exon 17 mutations were revealed in a previous study.[15] Further, studies reporting a poor prognosis of patients with *KIT* mutations have adopted treatment regimens without GO.[3,9-11] These observations indicated that GO treatment may improve the prognosis of AML with *RUNX1::RUNX1T1* and *KIT* mutations. Adding GO to the treatment of patients with *KIT* mutations might demonstrate a significant influence on the prognosis of pediatric patients in Japan, considering the higher prevalence of *KIT* exon 17 mutations in children with *RUNX1::RUNX1T1*-positive AML than patients in the TARGET cohort and in other countries or regions.[4,9,10]

In conclusion, pediatric AML with *RUNX1::RUNX1T1* and *KIT* exon 17 mutations demonstrated a poor long-term prognosis even among patients with negative MRD, thereby requiring treatment intensification for these patients regardless of MRD levels. The comparison between the AML-12 and TARGET cohorts indicated GO as a potential candidate for future therapeutic development, although a prospective study is warranted to confirm this finding.

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Disclosures

No conflicts of interest to disclose.

Contributions

SK, S-IT, JM, ST, DT, and NS designed the study. S-IT, ST, SA, DT, and NS acquired financial support. SK, S-IT, SI, HH, TK, MK, JT, SA, DT and NS collected materials and data. All authors analyzed and interpreted data. SK, S-IT, JM, ST, and NS wrote the manuscript. SI, HH, YO, TK, KO, TD, NK, MK, JT, SA, and DT critically edited the manuscript; and all authors approved the final version of the manuscript and are accountable for all aspects of the work.

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Data-sharing statement

Japan Children's Cancer Group (JCCG) is committed to sharing, with qualified external researchers, access to patient-level data and supporting clinical documents from eligible studies. The JCCG steering committee reviewed and approved these requests based on scientific merit. All data provided are anonymized to respect the privacy of patients who participated in the trial under applicable laws and regulations.

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