REGULAR ARTICLE

blood advances

Fusion partner–specific mutation profiles and *KRAS* mutations as adverse prognostic factors in *MLL*-rearranged AML

Hidemasa Matsuo,[1,2,*] Kenichi Yoshida,[3,*] Kana Nakatani,[1] Yutarou Harata,[1] Moe Higashitani,[1] Yuri Ito,[1] Yasuhiko Kamikubo,[1] Yusuke Shiozawa,[4] Yuichi Shiraishi,[5] Kenichi Chiba,[5] Hiroko Tanaka,[6] Ai Okada,[5] Yasuhito Nannya,[3] June Takeda,[3] Hiroo Ueno,[3] Nobutaka Kiyokawa,[7] Daisuke Tomizawa,[8] Takashi Taga,[9] Akio Tawa,[10] Satoru Miyano,[6] Manja Meggendorfer,[11] Claudia Haferlach,[11] Seishi Ogawa,[3,12,13] and Souichi Adachi[1]

[1]Department of Human Health Sciences, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [2]Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya, Japan; [3]Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [4]Department of Pediatrics, The University of Tokyo, Tokyo, Japan; [5]Division of Genome Analysis Platform Development, National Cancer Center Research Institute, Tokyo, Japan; [6]Laboratory of DNA Information Analysis, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [7]Department of Pediatric Hematology and Oncology Research, National Research Institute for Child Health and Development, Tokyo, Japan; [8]Division of Leukemia and Lymphoma, Children's Cancer Center, National Center for Child Health and Development, Tokyo, Japan; [9]Department of Pediatrics, Shiga University of Medical Science, Otsu, Japan; [10]Higashiosaka Aramoto Heiwa Clinic, Higashiosaka, Japan; [11]MLL Munich Leukemia Laboratory, Munich, Germany; [12]Institute for the Advanced Study of Human Biology (WPI ASHBi), Kyoto University, Kyoto, Japan; and [13]Department of Medicine, Center for Hematology and Regenerative Medicine, Karolinska Institute, Stockholm, Sweden

Key Points

- Mutations in *MLL*-rearranged AML are associated with *MLL* fusion partners, and *KRAS* mutations frequently coexist with high-risk *MLL* fusions.
- *KRAS* mutations are novel adverse prognostic factors in *MLL*-rearranged AML, regardless of fusion partner-based risk subgroup.

Mixed-lineage leukemia (*MLL*) gene rearrangements are among the most frequent chromosomal abnormalities in acute myeloid leukemia (AML). *MLL* fusion patterns are associated with the patient's prognosis; however, their relationship with driver mutations is unclear. We conducted sequence analyses of 338 genes in pediatric patients with *MLL*-rearranged (*MLL*-r) AML (n = 56; JPLSG AML-05 study) alongside data from the TARGET study's pediatric cohorts with *MLL*-r AML (n = 104), non–*MLL*-r AML (n = 581), and adult *MLL*-r AML (n = 81). *KRAS* mutations were most frequent in pediatric patients with high-risk *MLL* fusions (*MLL-MLLLT10*, *MLL-MLLT4*, and *MLL-MLLT1*). Pediatric patients with *MLL*-r AML (n = 160) and a *KRAS* mutation (*KRAS*-MT) had a significantly worse prognosis than those without a *KRAS* mutation (*KRAS*-WT) (5-year event-free survival [EFS]: 51.8% vs 18.3%, $P < .0001$; 5-year overall survival [OS]: 67.3% vs 44.3%, $P = .003$). The adverse prognostic impact of *KRAS* mutations was confirmed in adult *MLL*-r AML. *KRAS* mutations were associated with adverse prognoses in pediatric patients with both high-risk (*MLLT10*+*MLLT4*+*MLLT1*; n = 60) and intermediate-to-low–risk (*MLLT3*+*ELL*+others; n = 100) *MLL* fusions. The prognosis did not differ significantly between patients with non–*MLL*-r AML with *KRAS*-WT or *KRAS*-MT. Multivariate analysis showed the presence of a *KRAS* mutation to be an independent prognostic factor for EFS (hazard ratio [HR], 2.21; 95% confidence interval [CI], 1.35-3.59; $P = .002$) and OS (HR, 1.85; 95% CI, 1.01-3.31; $P = .045$) in *MLL*-r AML. The mutation is a distinct adverse prognostic factor in *MLL*-r AML, regardless of risk subgroup, and is potentially useful for accurate treatment stratification. This trial was registered at the UMIN (University Hospital Medical Information Network) Clinical Trials Registry (UMIN-CTR; http://www.umin.ac.jp/ctr/index.htm) as #UMIN000000511.

Introduction

Acute myeloid leukemia (AML) is a genetically and clinically heterogeneous disease.[1,2] Mixed-lineage leukemia (*MLL*; gene symbol, *KMT2A*) gene rearrangements are among the most common chromosomal abnormalities in AML.[3,4] The proportion of *MLL*-rearrangements in AML is higher in younger patients:

Submitted 26 May 2020; accepted 17 August 2020; published online 29 September 2020. DOI 10.1182/bloodadvances.2020002457.

Original data may be obtained by e-mail request to the corresponding author Souichi Adachi (e-mail: adachiso@kuhp.kyoto-u.ac.jp).

*H.M. and K.Y. contributed equally to this study.

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

© 2020 by The American Society of Hematology

~40% in infant AML, 20% in pediatric AML, and 5% to 10% in adult AML.[5-8] *MLL*-rearranged (*MLL*-r) AML is heterogeneous, with more than 60 different fusion partner genes identified.[9] Morphologically, most *MLL*-r AML is classified as the French-American-British (FAB)-M4 or FAB-M5 type.[10,11] Although all patients with *MLL*-r AML had long been thought to have a poor prognosis, a study showed that each patient's prognosis varies considerably according to the *MLL* fusion partner.[12] For example, t(9;11)(p22;q23)/*MLL-MLLT3*(*AF9*), the most common fusion pattern, is associated with intermediate risk, whereas t(1;11)(q21;q23)/*MLL-MLLT11* is associated with low risk, and t(10;11)(p12;q23)/*MLL-MLLT10*(*AF10*) and t(6;11)(q27;q23)/*MLL-MLLT4*(*AF6*) are associated with high risk. Therefore, several fusion genes are currently used for risk stratification of AML treatment.[3,4,13]

Compared with other AML subtypes, the number of coexisting driver mutations is lower in *MLL*-r AML.[1,8] RAS pathway genes are frequently mutated.[14-16] Other pathways associated with epigenetic regulation, transcription factors, the cohesin complex, and the cell cycle have also been identified in *MLL*-r AML[17]; however, little is known about the distribution and prognostic significance of driver mutations, according to specific *MLL* fusion partner genes, because the cohorts affected have been too small to allow for detailed analysis. In this study, we examined data from 160 pediatric and 81 adult patients with *MLL*-r AML, as well as control data from 581 patients with pediatric non–*MLL*-r AML, and we identified fusion partner–specific mutation patterns and *KRAS* mutations as distinct adverse prognostic factors in *MLL*-r AML, regardless of risk subgroup.

Methods

Patients and study protocol

The AML-05 study is a Japanese nationwide multi-institutional study of children (age, <18 years) with de novo AML, conducted by the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG). Patients (n = 485) were enrolled from 1 November 2006 through 31 December 2010. Details of the schedules and treatment regimens in this trial have been described previously.[18] Among the 485 enrolled patients, 56 with *MLL*-r AML were available for this study, all of whom were the same as those analyzed in a previous study.[17]

In addition, mutation and clinical data were collected from patients with *MLL*-r AML in the TARGET cohort.[8] Of those, 104 and 581 patients with *MLL*-r AML and non–*MLL*-r AML, respectively, were identified, and their clinical data were obtained from the TARGET Data Matrix (https://ocg.cancer.gov/programs/target/data-matrix). In addition, mutation data and clinical information from adult *MLL*-r AML patients, collected at the MLL Munich Leukemia Laboratory, were analyzed. Among 85 adult patients with de novo *MLL*-r AML in the previous study,[14] 4 without survival data were excluded, leaving 81 available for this study. The survival data were updated from the publication date.

This study was conducted in accordance with the principles set down in the Declaration of Helsinki and approved by the ethics committees of all participating institutions. All patients, or their parents or guardians, provided written informed consent.

DNA sequencing and mutation calling

As previously reported,[17] 338 target genes (supplemental Table 1) in 56 *MLL*-r AML samples from the AML-05 study were screened for mutations by targeted capture sequencing. Target genes were selected based on the 4 criteria. They must be (1) known driver genes in myeloid malignancies or other neoplasms; (2) associated with myeloid malignancies; (3) mutated, as detected by whole-exome sequencing in the previous study[17]; and (4) therapeutically targetable genes.

Sample preparation, sequencing, and data analyses were performed as previously reported.[17] Target enrichment was conducted with a SureSelect custom kit (Agilent, Santa Clara, CA), which was designed to capture all coding exons of the 338 target genes and 1216 single-nucleotide polymorphisms for copy number analysis. Candidate somatic mutations were selected using the following parameters: (1) supported by ≥5 reads in tumor samples; (2) a variant allele frequency in tumor samples >0.02; (3) $P < 0.0001$ (calculated using EBCall[19]); and (4) found in both positive- and negative-strand reads. Single-nucleotide polymorphisms with minor allele frequencies >0.001, those with synonymous mutations, and those in nontargeted genes were excluded, as were mutations with variant allele frequency 0.4 to 0.6, unless the same mutations were reported in the COSMIC database (Catalogue Of Somatic Mutations In Cancer; https://cancer.sanger.ac.uk), as identified in hematological malignancies, or were nonsense/frameshift mutations in known tumor-suppressor genes in myeloid malignancies.

Mutation data for samples from 104 and 581 TARGET cohort patients with *MLL*-r AML and non–*MLL*-r AML, respectively, were collected as described in a previous publication.[8] The methods of sequencing and data analysis of the samples from 85 adult patients with *MLL*-r AML at the MLL Munich Leukemia Laboratory have been reported.[14]

Lollipop plots were generated using ProteinPaint (https://pecan.stjude.org/proteinpaint/) to visualize the *KRAS* mutations.[20]

Statistical analysis

Survival analyses were performed by the Kaplan-Meier method in Prism 5 (GraphPad Software, San Diego, CA), and groups were compared by using an unstratified log-rank test or the Gehan-Breslow-Wilcoxon test. Categorical variables were compared by using Fisher's exact test. Multivariate analysis was performed with JMP Pro 14 (SAS Institute, Cary, NC). A 2-sided $P < .05$ was considered statistically significant.

Results

Patient characteristics

The characteristics of 160 pediatric patients with *MLL*-r AML (56 and 104 from the AML-05 and TARGET cohorts, respectively) are detailed in Table 1. Sex, age, and white blood cell count (WBC) were similar between the AML-05 and TARGET cohorts. Median age at diagnosis for the entire cohort was 3.9 years (range, 0.0-18.2). In both cohorts, most cases were classified as FAB-M5 (AML-05, 58.9%; TARGET, 63.5%) or FAB-M4 (AML-05, 25.0%; TARGET, 15.4%). The frequency of fusion partners was also similar; in both cohorts, the most frequent partner was *MLLT3* (AML-05, 44.6%; TARGET, 36.5%), followed by *MLLT10* (AML-05,

Table 1. Characteristics of patients with *MLL*-r AML in the AML-05 and TARGET cohorts

	AML-05 (n = 56)		TARGET (n = 104)		Total (N = 160)	
	n	%	n	%	n	%
Sex						
Male	24	42.9	48	46.2	72	45.0
Female	32	57.1	56	53.8	88	55.0
Median age (range), y	3.0	0.0-15.1	4.2	0.1-18.2	3.9	0.0-18.2
Median WBC (range), $\times 10^9$/L	27.4	1.1-459.0	34.0	1.3-610.0	32.6	1.1-610.0
FAB subtype						
M0	0	0.0	3	2.9	3	1.9
M1	4	7.1	3	2.9	7	4.4
M2	1	1.8	2	1.9	3	1.9
M4	14	25.0	16	15.4	30	18.8
M5	33	58.9	66	63.5	99	61.9
M7	0	0.0	1	1.0	1	0.6
RAEB-T	1	1.8	0	0.0	1	0.6
NOS	0	0.0	1	1.0	1	0.6
Unknown	3	5.4	12	11.5	15	9.4
Fusion partner						
MLLT3	25	44.6	38	36.5	63	39.4
MLLT10	11	19.6	26	25.0	37	23.1
ELL	10	17.9	13	12.5	23	14.4
MLLT1	5	8.9	6	5.8	11	6.9
MLLT4	3	5.4	9	8.7	12	7.5
Others	2	3.6	12	11.5	14	8.8

Data are number (percentage) of patients, unless otherwise stated.
NOS, not otherwise specified.

19.6%; TARGET, 25.0%) and *ELL* (AML-05, 17.9%; TARGET, 12.5%).

Distribution of driver mutations in *MLL*-r AML

The landscape of driver mutations in 160 pediatric patients with *MLL*-r AML is shown in Figure 1. The most frequent mutations were identified in activated signaling pathway genes (*FLT3*, *KRAS*, *NRAS*, *PTPN11*, *CBL*, and *BRAF*; 114 of 160 patients; 71.3%). Mutations in genes associated with epigenetic regulation (*SETD2*, *ASXL1*, *ASXL2*, *BCOR*, *CREBBP*, *EP300*, and *KDM6A*; 27 of 160 patients; 16.9%), transcription factors (*WT1*, *SPI1*, *GATA2*, and *RUNX1*; 12 of 160 patients; 7.5%), and the cohesin complex (*STAG2* and *SMC3*; 12 of 160 patients; 7.5%) were also recurrently detected.

The frequencies of the driver mutations differed according to *MLL* fusion partner genes. *FLT3* mutations (n = 42), including *FLT3* internal tandem duplications (*FLT3*-ITD; n = 7) and *FLT3* tyrosine kinase domain mutations affecting codons 835 and 836 (n = 16), were more frequent in patients with *MLL-MLLT3* (27 of 63; 42.9%) and *MLL-MLLT1* (4 of 11; 36.4%). RAS pathway genes were more frequently mutated in specific groups, such as *KRAS* in those with *MLL-MLLT10* (17 of 37; 45.9%), *MLL-MLLT4* (5 of 12; 41.7%), *MLL-MLLT1* (3 of 11; 27.3%), and other (4 of 14; 28.6%) fusions, and *NRAS* in those with *MLL-ELL* (10 of 23; 43.5%) and *MLL-MLLT4* (4 of 12; 33.3%) fusions. Other pathway mutations also coexisted with specific partner genes; *SETD2* mutations were frequent in patients with *MLL-MLLT4* fusions (4 of 12; 33.3%), and *STAG2* mutations were frequent in those with *MLL-ELL* (6 of 23; 26.1%). These data suggest that each *MLL* fusion group has a unique pattern of driver mutations and, interestingly, that *KRAS* mutations are more frequent in patients with high-risk translocations (*MLL-MLLT10* and *MLL-MLLT4*).

Prognostic significance of *KRAS* mutations in *MLL*-r AML

Next, we examined the prognostic significance of each driver mutation in patients with *MLL*-r AML. Among 8 frequently mutated genes (≥5%: ≥8 patients with mutations among the 160 patients) and 1 copy number change (trisomy 8), only *KRAS* mutations were associated with an adverse prognosis for event-free survival (EFS) and overall survival (OS; Figure 2A; supplemental Figure 1). Compared with patients without *KRAS* mutations (*KRAS*-WT; n = 118), those with *KRAS* mutations (*KRAS*-MT; n = 42) had significantly inferior prognoses (5-year EFS: 51.8% vs 18.3%, $P < .0001$; 5-year OS: 67.3% vs 44.3%, $P = .003$). Type and distribution of *KRAS* mutations were similar in the TARGET and AML-05 cohorts; most mutations were missense and located in the G12/G13 hotspots (supplemental Figure 2). Detailed information on *KRAS* mutations according to *MLL* fusion type is provided in supplemental Table 2. Double *KRAS* mutations were identified in 3 patients (2 with *MLL-MLLT10* fusions and 1 with an *MLL-MLLT3* fusion). There were no obvious correlations between the location of *KRAS* mutations and

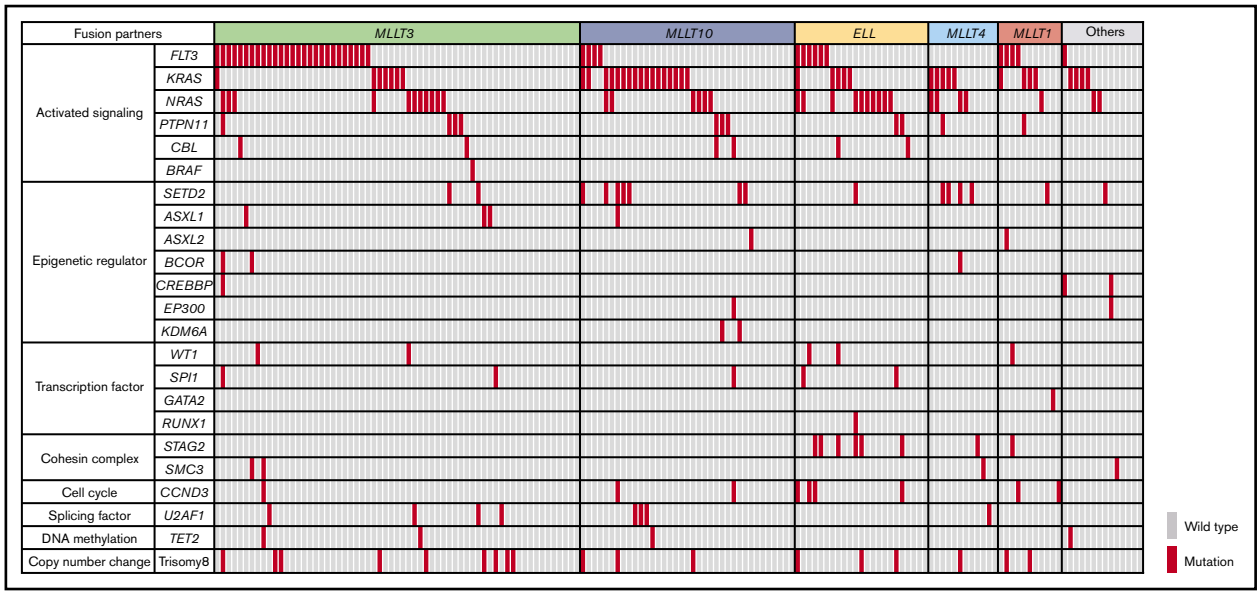

Figure 1. Mutational landscape of *MLL*-r AML. Distribution of driver mutations in patients with *MLL*-r AML (n = 160), according to *MLL* fusion partner.

MLL fusions, except that the *KRAS* G13 mutation was not detected in *MLL-ELL* leukemias.

We also examined whether the adverse prognostic significance of *KRAS* mutations is abrogated by allogeneic stem cell transplantation (SCT) in first complete remission (CR1). Among the 42 pediatric patients with *MLL*-r AML and *KRAS* mutations, SCT data were available for 39. Compared with patients who did not undergo allo-SCT in CR1 (n = 35), those who did (n = 4) had a favorable prognosis; however, the difference was not statistically significant (non-SCT vs SCT: 5-year EFS: 16.1% vs 50.0%, $P = .24$; 5-year OS: 38.9% vs 50.0%, $P = .49$; supplemental Figure 3).

In AML, *FLT3* mutations (especially *FLT3*-ITD) are important adverse prognostic indicators[21]; therefore, we analyzed their prognostic significance relative to *KRAS* mutations. Survival was compared in patients with *FLT3*-ITD (n = 4); other *FLT3* mutations, including those in the tyrosine kinase domain (indicated as *FLT3*-MT, n = 31); *KRAS* mutations (*KRAS*-MT, n = 37); both *FLT3* and *KRAS* mutations (*FLT3&KRAS*-MT, n = 4); and neither *FLT3* nor *KRAS* mutations (others; n = 81) (supplemental Figure 4). Two patients with *FLT3*-ITD and other *FLT3* mutations and 1 patient with *FLT3*-ITD and *KRAS* mutations were excluded because of the small samples. The results showed that the 2 groups with *KRAS* mutations (*KRAS*-MT and *FLT3&KRAS*-MT) had a poorer prognosis than the other 3 groups without *KRAS* mutations (*FLT3*-ITD, *FLT3*-MT, and others).

KRAS mutations have not been reported as a prognostic factor in pediatric AML; therefore, we examined their prognostic significance in patients with non–*MLL*-r AML (n = 581; Figure 2B). There were no significant differences in 5-year EFS (50.6% vs 54.5%; $P = .55$) or 5-year OS (67.1% vs 64.9%; $P = .84$) between patients with *KRAS*-WT (n = 533) or *KRAS*-MT (n = 48) non–*MLL*-r AML.

In addition, to confirm the adverse prognostic significance of *KRAS* mutations in *MLL*-r AML, we analyzed adult patients with *MLL*-r AML (n = 81; Figure 2C). The locations and types of *KRAS* mutations in adult patients with *MLL*-r AML were similar to those in pediatric patients with *MLL*-r AML (supplemental Figure 2). Compared with adult patients with *KRAS*-WT (n = 61), patients with *KRAS*-MT (n = 20) had a significantly adverse 5-year OS (33.4% vs 27.3%; $P = .02$), but not 5-year EFS (17.3% vs 20.5%; $P = .10$); however, patents carrying *KRAS*-MT had shorter median survival times for both EFS and OS (*KRAS*-WT vs *KRAS*-MT; EFS: 308 days vs 89 days; OS: 528 days vs 89 days). Therefore, rather than use the log-rank test, we analyzed the data by using the Gehan-Breslow-Wilcoxon test, which gives more weight to deaths at early time points and found significant differences in both EFS ($P = .009$) and OS ($P = .007$). Overall, these results suggest that *KRAS* mutations can be an adverse prognostic factor in *MLL*-r AML and that the prognostic significance may be limited to this disease subgroup.

Prognostic significance of *KRAS* mutations according to *MLL* fusion partner

We also analyzed the prognostic significance of *KRAS* mutations according to *MLL* fusion partner. First, the prognosis of patients was compared for each fusion partner gene (Figure 3A). Consistent with a previous report,[12] patients with *MLL-MLLT10* (n = 37), *MLL-MLLT4* (n = 12), or *MLL-MLLT1* (n = 11) had poor prognoses compared with those with other fusion types. Therefore, we dichotomized patients into high-risk (*MLLT10*+*MLLT4*+*MLLT1*; n = 60) and intermediate-to-low-risk (*MLLT3*+*ELL*+others; n = 100) groups. The frequency of *KRAS* mutation was significantly elevated in the high-risk group (26 of 60; 43.3%) relative to the intermediate-to-low–risk group (16 of 100; 16.0%; $P = .0002$; Figure 3B). Further, in the high-risk group, patients with *KRAS*-MT (n = 26) had significantly more adverse prognoses than did patients with *KRAS*-WT (n = 34; 5-year EFS: 31.5% vs 10.3%, $P = .007$; 5-year OS: 52.4% vs 40.5%, $P = .16$; Figure 3C). Moreover, patients with *KRAS*-MT in the intermediate-to-low-risk group (n = 16) had significantly more adverse prognoses than did patients with *KRAS*-WT (n = 84; 5-year EFS: 60.3% vs 31.3%, $P = .02$; 5-year OS: 73.4% vs 50.0%, $P = .04$; Figure 3D). When we analyzed the prognosis of patients for each *MLL* fusion group, patients with *KRAS*-MT generally had inferior prognoses vs those with

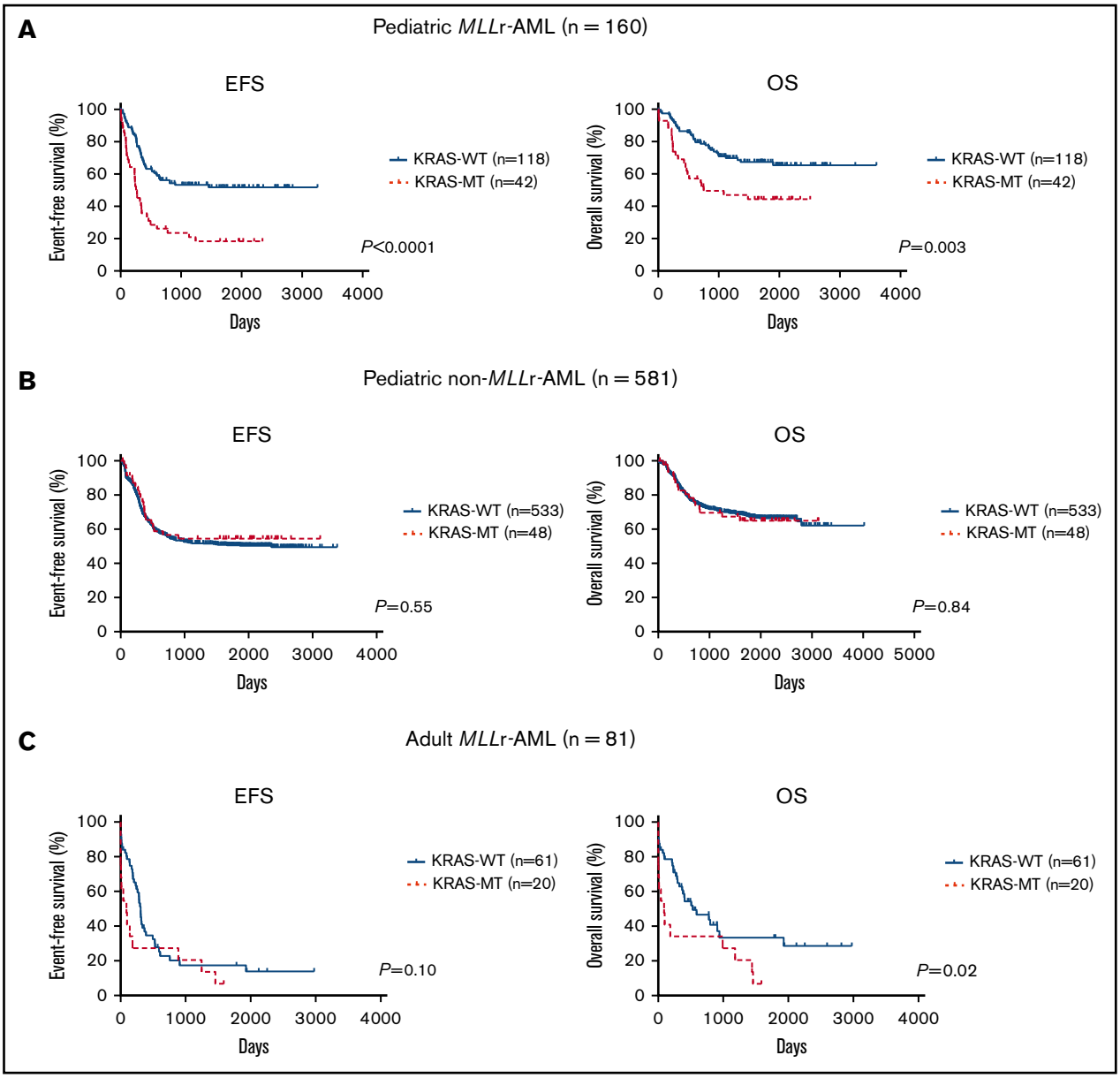

Figure 2. Prognostic significance of *KRAS* mutations. (A) Prognostic significance of *KRAS* mutations in pediatric patients with *MLL*-r AML who were in the TARGET and AML-05 cohorts (n = 160). (B) Prognostic significance of *KRAS* mutations in pediatric patients with non–*MLL*-r AML enrolled in the TARGET cohort (n = 581). (C) Prognostic significance of *KRAS* mutations in adult patients with *MLL*-r AML in the MLL laboratory (n = 81). The log-rank test was used for survival estimates.

KRAS-WT; however, the difference was significant for only a few *MLL* fusion groups because of the small subgroup sample sizes (supplemental Figure 5). These results suggest that *KRAS* mutations can be adverse prognostic factors in patients with *MLL*-r AML, regardless of the risk of *MLL* fusion types.

Multivariate analysis

Finally, we investigated whether *KRAS* mutation is an independent prognostic factor in patients with *MLL*-r AML. We performed a multivariate Cox regression analysis that included the following variables: age, WBC, *MLL* fusion gene, driver mutations, and trisomy 8 (Table 2). Mutations with lower frequencies (<5%, <8 mutations among 160 cases), which made the Cox regression model unstable, were excluded from this analysis. The results indicated that *KRAS* mutation was the only prognostic factor predicting both poor EFS (hazard ratio [HR], 2.21; 95% confidence interval [CI], 1.35-3.59; $P = .002$) and poor OS (HR, 1.85; 95% CI, 1.01-3.31; $P = .045$). These results suggest that *KRAS* mutation is a prognostic factor in patients with *MLL*-r AML, independent of age, WBC, *MLL* fusion partner, and other driver mutations.

Discussion

In this study, we analyzed the distribution of driver mutations in *MLL*-r AML carrying each *MLL* fusion by mutation profiling, revealing several associations between *MLL* fusions and driver mutations, as follows:

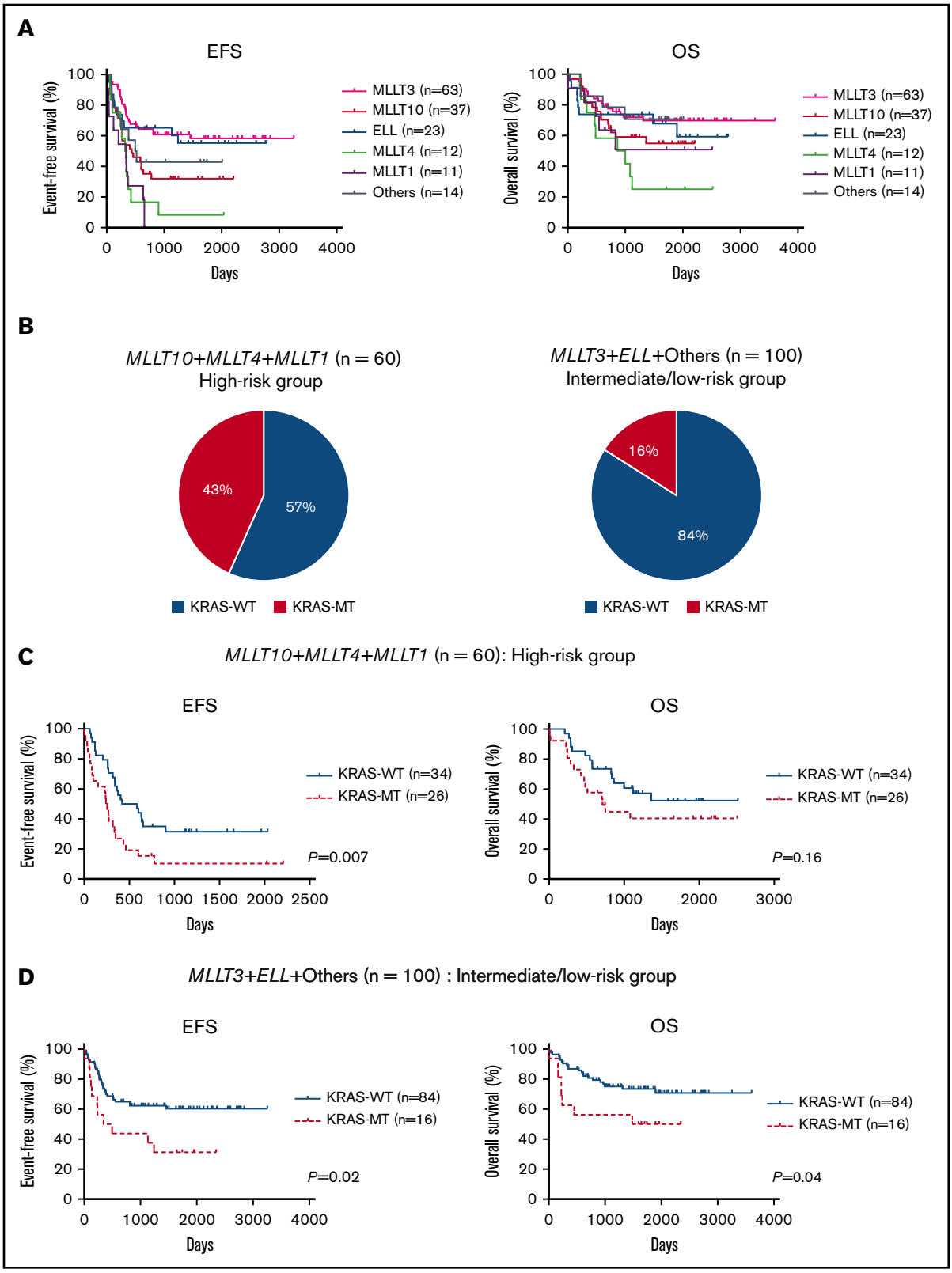

Figure 3. Prognostic significance of fusion partners and *KRAS* mutations according to risk subgroup based on fusion patterns in *MLL*-r AML. (A) Comparison of EFS and OS in patients, according to *MLL* fusion partners. (B) Frequency of *KRAS* mutations in the high-risk group (*MLLT10*+*MLLT4*+*MLLT1*; n = 60) and intermediate-to-low–risk group (*MLLT3*+*ELL*+others; n = 100). (C-D) Prognostic significance of *KRAS* mutations in pediatric patients with *MLL*-r AML with high-risk fusion partners (*MLLT10*+*MLLT4*+*MLLT1*; n = 60) (C) and with intermediate-to-low–risk fusion partners (*MLLT3*+*ELL*+others; n = 100) (D).

Table 2. Multivariate analysis of patients with *MLL*-r AML

	EFS			OS		
	HR	95% CI	*P*	HR	95% CI	*P*
Age (≥10 y)	1.24	0.72-2.11	.43	2.13	1.15-3.90	**.02**
WBC (≥50 × 10^9/L)	1.46	0.91-2.35	.12	1.37	0.76-2.47	.29
MLL-MLLT3	0.70	0.31-1.74	.43	1.08	0.38-3.88	.89
MLL-MLLT10	1.07	0.48-2.60	.88	1.29	0.45-4.67	.65
MLL-ELL	0.70	0.24-2.03	.51	1.77	0.52-6.97	.37
MLL-MLLT4	1.63	0.62-4.48	.32	1.99	0.60-7.78	.27
MLL-MLLT1	2.61	0.96-7.27	.06	1.64	0.41-7.03	.48
FLT3 mutation	0.76	0.39-1.45	.41	0.52	0.23-1.13	.10
KRAS mutation	2.21	1.35-3.59	**.002**	1.85	1.01-3.31	**.045**
NRAS mutation	1.02	0.56-1.76	.95	0.89	0.44-1.68	.73
PTPN11 mutation	1.41	0.57-3.02	.43	0.89	0.26-2.30	.83
SETD2 mutation	1.09	0.54-2.04	.80	0.94	0.39-2.04	.88
STAG2 mutation	1.03	0.27-3.26	.96	0.17	0.01-0.93	**.04**
CCND3 mutation	0.80	0.26-2.04	.66	1.58	0.43-4.57	.46
U2AF1 mutation	1.62	0.59-3.75	.32	1.21	0.40-3.02	.71
Trisomy 8	1.46	0.67-2.88	.32	2.50	1.07-5.32	**.04**

Bold indicates statistical significance ($P < .05$).

FLT3 mutations were frequent in *MLL-MLLT3* and *MLL-MLLT1* AML; *KRAS* mutations were frequent in *MLL-MLLT10*, *MLL-MLLT4*, and *MLL-MLLT1*; *NRAS* mutations were frequent in *MLL-ELL* and *MLL-MLLT4*; *SETD2* mutations were frequent in *MLL-MLLT4*; and *STAG2* mutations were frequent in *MLL-ELL* leukemia. These patterns may reflect the cooperative mechanisms involved in *MLL* fusion and driver mutation–induced leukemogenesis.

In *MLL*-r AML, the number of cooperating driver mutations has been reported to be fewer than in other disease subtypes,[1,8] and the prognostic significance of driver mutations has not been fully elucidated. Our previous study revealed that patients with *MLL*-r AML with driver mutations may have an adverse prognosis,[17] although the impact of each driver mutation was unclear because of the small samples. In this study, by analyzing data from 160 pediatric and 81 adult patients with *MLL*-r AML, we found that *KRAS* mutations were associated with adverse prognoses in pediatric and adult patients. Interestingly, *KRAS* mutations significantly coexisted with high-risk *MLL* fusions, such as *MLL-MLLT10*, *MLL-MLLT4*, and *MLL-MLLT1*. In a multivariate analysis, *KRAS* mutation was an independent adverse prognostic factor, whereas there were no statistically significant findings for *MLL* fusions. Therefore, the adverse prognoses of patients with high-risk *MLL* fusions may be explained by the frequency of *KRAS* mutations. According to the recommendations from an international expert pediatric AML panel, patients with several *MLL* fusions, including *MLL-MLLT4* and *MLL-MLLT10*, are categorized as an adverse prognostic group, whereas those with other *MLL* fusions are categorized as an intermediate prognostic group; no driver mutations were used for risk stratification.[3] In our data, patients with *KRAS*-MT and intermediate-to-low-risk *MLL* fusions had worse prognoses, comparable with those of patients with high-risk *MLL* fusions without *KRAS* mutations. Moreover, among patients with high-risk *MLL* fusions, those with *KRAS*-MT had extremely poor prognoses. These data suggest that *KRAS* mutations are useful for accurate risk stratification and should be considered for use as a screening test, in addition to identification of the *MLL* fusion type. We also found that the adverse prognostic significance of *KRAS* mutations may be abrogated by allo-SCT in CR1. Analysis of a larger cohort is needed to validate our findings in a future study.

KRAS mutations were not associated with adverse prognosis in non–*MLL*-r AML, and the importance of *KRAS* examination for risk stratification may be higher in patients with *MLL*-r AML than in those with non–*MLL*-r AML. AML is a heterogeneous disease and RAS pathway mutations are frequent events in several disease subtypes, including core binding factor AML.[1,2,22] Therefore, there may be some disease subtypes that can be risk stratified by *KRAS* mutations. A detailed analysis of a large number of patients with non–*MLL*-r AML should also be included in future studies.

Our findings may be applicable not only for risk stratification but could also suggest novel treatments. Although *KRAS* mutations are among the most frequent genetic aberrations in cancer, effective treatments targeting *KRAS* have yet to be developed[23,24]; however, several *KRAS* inhibitors specific for the G12C mutant have been developed recently and show remarkable results for treatment of solid tumors with *KRAS* mutations.[25-28] A pan-*KRAS* inhibitor, targeting both G12 and G13 mutations, has also entered clinical study.[29] In our study, *KRAS* mutations were frequent (>40%) in patients with AML with high-risk *MLL* fusions, and most of the mutations detected were in G12 and G13, suggesting that inhibitors targeting these mutations may be promising treatments for these patients.

Acknowledgments

The authors thank the Human Genome Center, Institute of Medical Science, The University of Tokyo, for providing Supercomputing resources.

This work was supported by a Grant in-Aid from the Agency for Medical Research and Development (Project for Development of Innovative Research on Cancer Therapeutics [P-DIRECT]), the Project for Cancer Research and Therapeutic Evolution

[P-CREATE], and Practical Research for Innovative Cancer Control) and JSPS KAKENHI (JP19K16832).

Authorship

Contribution: H.M. and K.Y. analyzed the clinical and sequencing data; K.N., Y.H., M.H., and Y.I. helped with the analysis; Y. Shiozawa, Y. Shiraishi, K.C., H.T., A.O., and S.M. developed the sequence data processing pipelines; H.M., K.Y., Y.N., J.T., and H.U. performed the sequencing; N.K., D.T., T.T., A.T., and S.A. collected clinical samples; M.M., and C.H. provided the adult patient sequencing data and clinical information; Y.K., S.O., and S.A. supervised the project; and H.M. and K.Y. wrote the manuscript.

Conflict-of interest disclosure: The authors declare no competing financial interests.

ORCID profiles: H.M., 0000-0002-7578-006X; H.T., 0000-0001-9634-8922; A.O., 0000-0002-9782-5900; D.T., 0000-0003-1520-7007; A.T., 0000-0003-4520-7905; S.O., 0000-0002-7778-5374.

Correspondence: Souichi Adachi, Department of Human Health Sciences, Graduate School of Medicine, Kyoto University, 53 Kawahara-cho, Shogoin, Sakyoku, Kyoto 606-8507, Japan; e-mail: adachiso@kuhp.kyoto-u.ac.jp.

References

1. Ley TJ, Miller C, Ding L, et al; Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia [published correction appears in *N Engl J Med*. 2013;369:98]. *N Engl J Med*. 2013;368(22):2059-2074.
2. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. *N Engl J Med*. 2016;374(23):2209-2221.
3. Creutzig U, van den Heuvel-Eibrink MM, Gibson B, et al; AML Committee of the International BFM Study Group. Diagnosis and management of acute myeloid leukemia in children and adolescents: recommendations from an international expert panel. *Blood*. 2012;120(16):3187-3205.
4. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424-447.
5. Grimwade D, Walker H, Oliver F, et al; The Medical Research Council Adult and Children's Leukaemia Working Parties. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. *Blood*. 1998;92(7):2322-2333.
6. Lie SO, Abrahamsson J, Clausen N, et al. Treatment stratification based on initial in vivo response in acute myeloid leukaemia in children without Down's syndrome: results of NOPHO-AML trials. *Br J Haematol*. 2003;122(2):217-225.
7. Raimondi SC, Chang MN, Ravindranath Y, et al. Chromosomal abnormalities in 478 children with acute myeloid leukemia: clinical characteristics and treatment outcome in a cooperative pediatric oncology group study-POG 8821. *Blood*. 1999;94(11):3707-3716.
8. Bolouri H, Farrar JE, Triche T Jr., et al. The molecular landscape of pediatric acute myeloid leukemia reveals recurrent structural alterations and age-specific mutational interactions [published correction appears in *Nat Med*. 2018;24(4):526]. *Nat Med*. 2018;24(1):103-112.
9. Meyer C, Burmeister T, Gröger D, et al. The MLL recombinome of acute leukemias in 2017. *Leukemia*. 2018;32(2):273-284.
10. Matsuo H, Kajihara M, Tomizawa D, et al. EVI1 overexpression is a poor prognostic factor in pediatric patients with mixed lineage leukemia-AF9 rearranged acute myeloid leukemia. *Haematologica*. 2014;99(11):e225-e227.
11. Balgobind BV, Zwaan CM, Pieters R, Van den Heuvel-Eibrink MM. The heterogeneity of pediatric MLL-rearranged acute myeloid leukemia. *Leukemia*. 2011;25(8):1239-1248.
12. Balgobind BV, Raimondi SC, Harbott J, et al. Novel prognostic subgroups in childhood 11q23/MLL-rearranged acute myeloid leukemia: results of an international retrospective study. *Blood*. 2009;114(12):2489-2496.
13. Tomizawa D, Tanaka S, Hasegawa D, et al. Evaluation of high-dose cytarabine in induction therapy for children with de novo acute myeloid leukemia: a study protocol of the Japan Children's Cancer Group Multi-Center Seamless Phase II-III Randomized Trial (JPLSG AML-12). *Jpn J Clin Oncol*. 2018;48(6):587-593.
14. Grossmann V, Schnittger S, Poetzinger F, et al. High incidence of RAS signalling pathway mutations in MLL-rearranged acute myeloid leukemia. *Leukemia*. 2013;27(9):1933-1936.
15. Sandhöfer N, Metzeler KH, Rothenberg M, et al. Dual PI3K/mTOR inhibition shows antileukemic activity in MLL-rearranged acute myeloid leukemia. *Leukemia*. 2015;29(4):828-838.
16. Lavallée VP, Baccelli I, Krosl J, et al. The transcriptomic landscape and directed chemical interrogation of MLL-rearranged acute myeloid leukemias. *Nat Genet*. 2015;47(9):1030-1037.
17. Matsuo H, Yoshida K, Fukumura K, et al. Recurrent *CCND3* mutations in *MLL*-rearranged acute myeloid leukemia. *Blood Adv*. 2018;2(21):2879-2889.
18. Tomizawa D, Tawa A, Watanabe T, et al. Excess treatment reduction including anthracyclines results in higher incidence of relapse in core binding factor acute myeloid leukemia in children. *Leukemia*. 2013;27(12):2413-2416.
19. Shiraishi Y, Sato Y, Chiba K, et al. An empirical Bayesian framework for somatic mutation detection from cancer genome sequencing data. *Nucleic Acids Res*. 2013;41(7):e89.
20. Zhou X, Edmonson MN, Wilkinson MR, et al. Exploring genomic alteration in pediatric cancer using ProteinPaint. *Nat Genet*. 2016;48(1):4-6.
21. Daver N, Schlenk RF, Russell NH, Levis MJ. Targeting FLT3 mutations in AML: review of current knowledge and evidence. *Leukemia*. 2019;33(2):299-312.

22. Bowen DT, Frew ME, Hills R, et al. RAS mutation in acute myeloid leukemia is associated with distinct cytogenetic subgroups but does not influence outcome in patients younger than 60 years. *Blood*. 2005;106(6):2113-2119.

23. Sanchez-Vega F, Mina M, Armenia J, et al; Cancer Genome Atlas Research Network. Oncogenic Signaling Pathways in The Cancer Genome Atlas. *Cell*. 2018;173(2):321-337.e10.

24. Drosten M, Barbacid M. Targeting the MAPK Pathway in KRAS-Driven Tumors. *Cancer Cell*. 2020;37(4):543-550.

25. Janes MR, Zhang J, Li LS, et al. Targeting KRAS Mutant Cancers with a Covalent G12C-Specific Inhibitor. *Cell*. 2018;172(3):578-589.e17.

26. Lanman BA, Allen JR, Allen JG, et al. Discovery of a Covalent Inhibitor of $KRAS^{G12C}$ (AMG 510) for the Treatment of Solid Tumors. *J Med Chem*. 2020; 63(1):52-65.

27. Canon J, Rex K, Saiki AY, et al. The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity. *Nature*. 2019;575(7781):217-223.

28. Hallin J, Engstrom LD, Hargis L, et al. The $KRAS^{G12C}$ Inhibitor MRTX849 Provides Insight toward Therapeutic Susceptibility of KRAS-Mutant Cancers in Mouse Models and Patients. *Cancer Discov*. 2020;10(1):54-71.

29. News in brief. Race for undruggable KRAS speeds up. *Nat Biotechnol*. 2019;37(11):1247.