Clinical significance of RAS pathway alterations in pediatric acute myeloid leukemia

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 doi:10.3324/haematol.2020.269431

Received: August 28, 2020.

Accepted: March 12, 2021.

Pre-published: March 18, 2021.

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Supplementary Methods

Patients

The present study enrolled patients with de novo AML who participated in the Japanese AML-05 trial conducted by the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG). The AML-05 trial is registered with the UMIN Clinical Trials Registry [UMIN-CTR, (URL: http://www.umin.ac.jp/ctr/index.htm), number UMIN000000511]. Patients diagnosed with acute promyelocytic leukemia, Down syndrome-associated AML, and secondary AML were excluded from this study. Details pertaining to the diagnosis, and risk stratification have been previously reported in different studies.[1,2] The treatment protocols and procedures for data and sample collection were approved by the institutional review boards of each participating institution; written informed consent was obtained from all patients or their parents/guardians.

Treatment

After the second induction course, the patients were stratified to one of the three risk groups according to their cytogenetic characteristics and treatment responses following the induction therapy. All the patients received three

additional intensified chemotherapeutic courses. Patients who had failed to achieve a complete remission after the second induction course were identified with induction failure and removed from the study. Allogeneic hematopoietic stem cell transplantation was indicated for all the high-risk patients after three or more treatment courses (Supplementary Figure S1).[1]

Data Collection

Every 6 months, data forms were forwarded to the JPLSG data coordination center at the National Center of Child Health and Development. These data were reviewed for internal consistency and face validity and transferred into an Excel database (Microsoft Corporation, Redmond, WA, USA). The clinical data of patients in each risk group were followed until December 2013 (censored for 3 years from the date of final registration). The JPLSG performed a central review of morphologic classification and karyotyping based on the World Health Organization Classification and cytogenetic analysis using conventional G-banding.

Supplementary Tables

Table S1.

Clinical characteristics of the patients between 328 available samples and 115 unavailable samples

	Unavailable (n = 115)	Available (n = 328)	*p*-value
Age median at diagnosis, y (range)	7.3 (0.1-16.7)	8.0 (0.0-17.9)	0.645
Gender, Male, n (%)	61 (53)	177 (54)	0.914
WBC median, $\times 10^9$/L (range)	7.6 (0.80-380.5)	21.8 (0.62-985.0)	**<0.001**
Stem cell transplantation, n (%)	59 (51)	151 (46)	0.385
Risk classification, n (%)			
Low risk	27 (23)	111 (34)	**0.046**
Intermediate risk	63 (55)	126 (38)	**0.003**
High risk	13 (11)	42 (13)	0.745
Induction failure	12 (10)	49 (15)	0.272
Cytogenetic feature n (%)			
Normal karyotype	24 (21)	61 (19)	0,585
Complex karyotype	17 (15)	36 (11)	0.316
RUNX1-RUNX1T1	29 (25)	93 (28)	0.546
CBFB-MYH11	5 (4)	27 (8)	0.211
KMT2A-rearrangement	22 (19)	49 (15)	0.303
Prognosis			
2-year Overall survival (%)	83.2	81.5	0.633
2-year Event-free survival (%)	70.1	58.5	0.642

WBC, white blood cell count

Table S2. Summary of characteristics of pediatric AML patients with *NRAS* mutation.

UPN	Amino acid change*	Gender	Age, y	WBC, ×10⁹/L	Cytogenetics	Additional genetic aberrations	CR	Relapse	Event	SCT	Prognosis
17	p.Q61H	F	6.5	13.2	46,XX,t(8;21)(q22;q22)[4]/47,sl,add(15)(p11.2),+21[16]	-	+	+	+	+	Alive
24	p.Q61K	M	0.9	20.2	46,XY,add(5)(p11),add(7)(p11.2),?t(13;19)(q11;p13)[20}	*NPM1*	+	-	-	-	Alive
26	p.G13V	M	10.9	9.9	46,XY[20]	-	+	-	-	-	Alive
33	p.G12S, p.G13R	F	10.6	21.1	48,XX,+8,inv(16)(p13q22),+22,[7]	–	+	-	-	-	Alive
36	p.Q61K	M	16.2	100.7	46,XY[20]	*CEBPA* (biallelic)	+	-	-	-	Alive
48	p.G13R	M	11.8	56.8	47,XY,+21[3]/46,XY[17]	*CEBPA* (monoallelic)	+	-	-	-	Alive
61	p.G12S, p.G13R	F	10.1	64.1	46,XX,inv(9)(p12q13),inv(16)(p13.1q22)[20]	-	+	-	-	-	Alive
78	p.G12D	M	13.8	12	46,XY[10]	*KMT2A-MLLT3*	+	-	-	-	Alive
83	p.G12D, p.G13V	F	12.1	10.3	46,XX[20]	*CEBPA* (biallelic)	+	-	-	-	Alive
95	p.G13D	F	6	14.7	45,X,-X,t(8;21)(q22;q22)[20]	-	+	-	-	-	Alive
107	p.Q61R	F	6.2	162.4	45,XX,t(8;21)(q22;q22)[20]	*ASXL1*, *ASXL2, SMC3*	+	-	-	-	Alive
111	p.G12D	M	5.6	547	46,XY,del(9)(q?)[20]	*NUP98-NSD1*, *WT1*, *CEBPA* (monoallelic)	+	+	+	+	Death
148	p.Q61R	M	8.6	121.6	47,XY,t(6;11)(q27;q23),+8[19]	*KMT2A-AFDN*	+	+	+	+	Death
151	p.G12D	M	4.1	38.6	46,XY,t(8;21)(q22;q22)[20]	-	+	-	-	-	Alive
152	p.Q61L	M	0.9	9.0	46,XY,-3,add(3)(p13),-7,-9,add(16)(q12.1),add(17)(p11.2),add(19)(p11), add(21)(q22),+r1,+mar1,+mar2[14]/46,XY[5]	-	+	-	-	+	Alive
173	p.G13D	M	12.3	17.2	45,X,-Y,t(8;21)(q22;q22),inv(9)(p12q13)[20]	-	+	-	-	-	Alive
190	p.Q61H	M	8.6	42.9	46,XY,add(7)(q11.2),t(8;21)(q22;q22)[18]/46,XY[2]	-	+	+	+	+	Alive
196	p.G12N	M	1.2	100.2	46,XY,inv(16)(p13.1q22)[20]	-	+	-	-	-	Alive
227	p.G12D	F	4.4	5.0	46,XX,t(16;21)(q24;q22)[4]/46,sl,t(1;16)(q32;p13.3)[16]	-	+	-	-	-	Alive
229	p.G13R	F	14.9	8.1	45,XX,inv(3)(q21q26.2),-7[20]	-	-	+	+	+	Death
255	p.G12D	M	11.7	18.6	46,XY,inv(16)(p13.1q22)[20]	-	+	-	-	-	Alive
262	p.G12D	M	12.3	159.3	46,XY,inv(16)(p13q22)[20]	*CBL*, *NF1*	+	-	-	-	Alive
268	p.G13D	F	15.3	9.1	46,XX[20]	*NPM1*	-	-	-	-	Alive
273	p.Q61K	M	1.5	43.3	47,XY,+8,t(14;20)(q11.2;q11.2),del(21)(q21q22)[20]	-	+	-	-	-	Alive
274	p.G13V	F	8.2	0.9	46,XX,?t(10;11)(p12;q14)[19]/46,XX[1]	-	+	+	+	+	Death

286	p.Q61K	F	5.8	16.4	46,XX,t(8;21)(q22;q22)[20]	-	+	-	-	-	Alive
289	p.G12V	F	2.3	49.9	46,X,-X,-2,-7,add(17)(q25),del(20)(q11.2),+r1,+mar1,+mar2[20]	-	+	-	-	-	Alive
292	p.Q61K	M	1	55.5	46,XY,t(8;21)(q22;q22)[9]/46,sl,add(22)(p11.2)[8]/46,XY[2]	-	+	+	+	+	Alive
322	p.G12D, p.G13D	M	13.2	91.7	46,XY,t(8;12;21)(q22;p13;q22)[8]/46,XY[1]	-	+	+	+	+	Alive
328	p.G13D	M	3.2	5.2	48,XY,+8,inv(16)(p13.1q22),+21[19]/48,XY,?	-	+	-	-	-	Alive
358	p.Q61R	F	4.3	10.4	46,XX,t(8;21)(q22;q22)[15]/46,XX[5]	*ASXL1*	+	-	-	-	Alive
362	p.G13D, p.Q61K	F	6.7	65.1	46,XX,t(8;21)(q22;q22)[20]	-	+	-	-	-	Alive
363	p.G13R, p.Q61K	M	1.3	119.2	46,XY,inv(16)(p13.1q22)[20]	-	+	-	-	-	Alive
379	p.Q61K	M	1.6	12.7	46,XY,-7[20]	-	+	-	-	+	Alive
384	p.G13D	M	9.3	5.8	45,X,-Y,t(8;21)(q22;q22)[18]/46,idem,+8[1]/46,XY[1]	-	+	-	-	-	Alive
390	p.Q61H	F	2.8	168.7	46,XX,del(16)(q22q24)[18]/47,XX,del(16)(q22q24),+22[2]	-	+	-	-	-	Alive
393	p.G13D	F	13.3	28.8	46,XX,?t(5;6)(p15;q24)[6]/47,idem,+mar,inc[10]	-	+	+	+	-	Alive
399	p.Q61R	F	12.8	68.9	46,XX,inv(16)(p13.1q22)[10]/47,idem,+8[9]/48,idem,+8,+22[1]	-	+	-	-	-	Alive
410	p.G12D	F	11.2	43.9	46,XX,t(8;21)(q22;q22)[20]	-	+	-	-	-	Alive
422	p.G13R	F	3.2	56.2	46,XX,del(9)(q?),t(16;16)(p13.1;q22)[20]	*WT1*	+	-	-	-	Alive
423	p.Q61K	M	14.9	159.5	46,XY[20]	*CEBPA* (biallelic), *CSF3R*	+	+	+	+	Alive
430	p.G12D	F	11	430.1	46,XX[20]	*KIT*	+	+	+	+	Alive
436	p.G12D	M	10.2	29.9	46,XY,add(1)(p36.1),add(5)(q31),add(7)(q22),del(9)(q?),t(10;11)(p12;q14),add(17)(p11.2)[1]/ 46,sl,add(3)(q11.2),-9,+mar1[4]/46,sdl1,-4,-15,+der(?)t(?;4)(?;q12),+mar2[11]/46,XY]4]	*RAD21*	+	+	+	+	Alive
437	p.G12V	F	0.6	19.3	46,XX,der(4)(4pter→4q33::11q21→11q23::19p13→19pter),der(11) t(4;11)(q33;q21),der(19)t(11;19)(q23;p13)[20]	*KIT*, *KMT2A*-rearrangement (partner undetermined)	+	-	-	-	Alive

UPN, unique patient number; WBC, white blood cell count; CR, complete remission; SCT, stem cell transplantation

*NCBI reference sequence; NM_002524

Table S3. Summary of characteristics of pediatric AML patients with *KRAS* mutation.

UPN	Amino acid change*	Gender	Age, y	WBC, ×10⁹/L	Cytogenetics	Additional genetic aberrations	CR	Relapse	Event	SCT	Prognosis
100	p.G60R	F	10.9	25.2	46,XX,t(8;21)(q22;q22)[1]/45,sl,-X[18]/46,XX[1]	*KIT*	+	-	-	-	Alive
122	p.G13D	F	0.6	58.0	48,XX,+8,+18,-19,+20[19]/45,XX,-2,-17,+18,-19,+20[1]	*BCOR*	-	-	+	-	Death
194	p.G13D	F	7.8	33.1	46,XX,t(8;21)(q22;q22)[19]/46,XX[1]	*CSF3R*	+	-	-	-	Alive
203	p.G13D	F	16.8	20.8	46,XX,t(7;21;8)(q22;q22;q22)[20]	*CSF3R*	+	-	-	-	Alive
259	p.G13D	M	10.6	85.3	46,XY[20]	-	+	+	+	+	Death
297	p.G13D	F	10.8	128.9	46,XX,t(6;11)(p21;q23)[25]	*KMT2A*-rearrangement (partner undetermined)	+	+	+	+	Death
303	p.G12V	M	11.9	3.0	46,XY,t(8;21)(q22;q22)[17]/46,XY[3]	-	+	+	+	+	Alive
308	p.G12V	F	1.6	35.4	46,XX,add(2)(q31),t(16;21)(q24;q22)[5]/ 45,sl,+2,-add(2),-11,add(18)(p11.2)[12]/ 91,sl,×2,-11[2]	-	+	-	-	-	Alive
339	p.G13D	M	4.1	7.9	45,X,-Y,t(8;21)(q22;q22)[20]	-	+	-	-	-	Alive
360	p.G13D	F	10.3	6.7	46,XX,t(8;21)(q22;q22)[20]	-	+	-	-	-	Alive
394	p.G12D	F	2.2	60.0	51,XX,+X,+6,add(7)(p11.2),+8,del(12)(p?),+13,+19[19]	-	+	-	-	+	Alive
439	p.G12D	F	8.9	321.7	46,XX,t(11;19)(q23;p13.1)[20]	*KMT2A-MLLT1*	+	+	+	+	Alive

UPN, unique patient number; WBC, white blood cell count; CR, complete remission; SCT, stem cell transplantation

*NCBI reference sequence; NM_004985

Table S4. Clinical characteristics between patients with or without RAS pathway alterations.

	All RAS pathway genes			*NF1*			*PTPN11*			*CBL*			*NRAS*			*KRAS*		
	Wild-type (n=248)	Alteration (n=80)	*p*-value	Wild-type (n=321)	Alteration (n=7)	*p*-value	Wild-type (n=313)	Mutation (n=15)	*p*-value	Wild-type (n=322)	Mutation (n=6)	*p*-value	Wild-type (n=284)	Mutation (n=44)	*p*-value	Wild-type (n=316)	Mutation (n=12)	*p*-value
Age median at diagnosis, years (range)	7.5 (0.0-17.9)	9.1 (0.4-16.8)	0.719	7.80 (0.0-17.9)	12.30 (7.0-15.2)	0.059	8.20 (0.0-17.9)	7.00 (0.4-14.1)	0.927	7.80 (0.0-17.9)	10.75 (2.3-15.1)	0.367	7.80 (0.0-17.9)	8.40 (0.6-16.2)	0.862	7.80 (0.0-17.9)	8.40 (0.6-16.8)	0.891
Gender, Male, n (%)	136 (54.8)	41 (51.2)	0.607	172 (53.6)	5 (71.4)	0.459	169 (54.0)	8 (53.3)	1.000	172 (53.4)	5 (83.3)	0.223	154 (54.2)	23 (52.3)	0.871	174 (55,1)	3 (25.0)	0.073
WBC median, $\times 10^9$/L (range)	20.6 (0.62-985.0)	29.4 (0.90-430.1)	0.132	22.4 (0.62-985.0)	15.5 (1.9-159.3)	0.450	21.9 (0.62-985.0)	17.8 (1.9-190.5)	0.912	21.3 (0.62-985.0)	106.7 (20.5-172.0)	**0.026**	21.6 (0.62-985.0)	29.4 (0.90-430.1)	0.295	21.3 (0.62-985.0)	34.3 (3.0-321.7)	0.434
Stem cell transplantation, n	117	34	0.520	147	4	0.707	140	11	**0.035**	149	2	0.691	138	13	**0.022**	146	5	1.000
Relapse, n	84	28	0.892	110	2	1.000	104	8	0.161	109	3	0.414	100	12	0.393	108	4	1.000
Risk classification, n																		
Low risk	80	31	0.342	110	1	0.430	110	1	**0.024**	108	3	0.411	89	22	**0.017**	105	6	0.231
Intermediate risk	96	30	0.895	122	4	0.435	120	6	1.000	125	1	0.412	109	17	1.000	122	4	0.773
High risk	35	7	0.252	42	0	0.601	38	4	0.111	42	0	1.000	40	2	0.091	41	1	1.000
No complete remission	37	12	1.000	47	2	0.281	45	4	0.254	47	2	0.221	46	3	0.116	48	1	1.000
Cytogenetic features, n																		
Normal karyotype	50	11	0.248	61	0	0.356	57	4	0.493	61	0	0.598	55	6	0.414	60	1	0.704
Complex karyotype	25	11	0.410	33	3	**0.031**	35	1	1.000	36	0	1.000	31	5	1.000	33	3	0.134
RUNX1-RUNX1T1	72	21	0.671	93	0	0.198	92	1	0.076	92	1	1.000	80	13	0.858	87	6	0.106
CBFB-MYH11	16	11	0.058	26	1	0.455	27	0	0.622	25	2	0.080	17	10	**0.001**	27	0	0.609
KMT2A-rearrangement	40	9	0.368	49	0	0.600	46	3	0.477	48	1	1.000	46	3	0.116	47	2	0.696
Monosomy 7	1	7	**<0.001**	7	1	0.160	6	2	**0.047**	8	0	1.000	4	4	**0.013**	8	0	1.000
FLT3-ITD	38	2	**0.001**	40	0	1.000	39	1	1.000	39	1	0.545	40	0	**0.005**	40	0	0.374
KIT mutations	42	6	0.045	47	1	1.000	48	0	0.140	47	1	1.000	46	2	**0.040**	46	2	0.690
WT1 mutations	20	4	0.464	24	0	1.000	22	2	0.301	24	0	1.000	22	2	0.754	24	0	1.000
KMT2A-PTD	9	2	1.000	11	0	1.000	9	2	0.085	11	0	1.000	11	0	0.372	11	0	1.000
NPM1 mutations	11	5	0.552	16	0	1.000	14	2	0.162	15	1	0.261	14	2	1.000	16	0	1.000

CEBPA biallelic mutations	18	3	0.430	21	0	1.000	21	0	0.610	21	0	1.000	18	3	1.000	21	0	1.000
RUNX1 mutations	4	4	0.103	7	1	0.160	5	3	**0.004**	8	0	1.000	8	0	0.604	8	0	1.000
CSF3R mutations	5	3	0.409	8	0	1.000	8	0	1.000	8	0	1.000	7	1	1.000	6	2	**0.030**
5q deletion	2	0	1.000	2	0	1.000	2	0	1.000	2	0	1.000	2	0	1.000	2	0	1.000
FUS-ERG	3	1	1.000	4	0	1.000	3	1	0.171	4	0	1.000	4	0	1.000	4	0	1.000
NUP98-NSD1	10	1	0.307	11	0	1.000	11	0	1.000	11	0	1.000	11	0	1.000	11	0	1.000
ASXL1 mutations	6	2	1.000	7	1	0.160	8	0	1.000	8	0	1.000	7	1	1.000	8	0	1.000
ASXL2 mutations	12	2	0.531	14	0	1.000	14	0	1.000	14	1	1.000	12	2	1.000	14	0	1.000
BCOR mutations	2	2	0.251	4	0	1.000	4	0	1.000	4	0	1.000	3	1	0.440	3	1	0.139
BOCRL1 mutations	3	4	0.063	4	2	**0.006**	6	0	1.000	6	0	1.000	5	1	0.582	6	0	1.000
RAD21 mutations	5	3	0.409	8	0	1.000	6	2	**0.047**	8	0	1.000	7	1	1.000	8	0	1.000
SMC3 mutations	4	2	0.636	6	0	1.000	6	0	1.000	6	0	1.000	4	2	0.186	6	0	1.000
STAG2 mutations	2	3	0.096	5	0	1.000	3	2	**0.018**	5	0	1.000	5	0	1.000	4	1	0.171
PRDM16 high expression	57	20	0.762	75	2	0.669	68	9	**0.002**	75	2	0.628	70	7	0.253	76	1	0.307
MECOM high expression	36	15	0.377	47	4	**0.013**	44	7	**0.004**	50	1	1.000	47	4	0.268	50	1	0.700

WBC, white blood cell count

Table S5. Summary of references for *NF1* alterations in AML.

Reference	Number of patients	Frequency of deletion	Frequency of mutation	Prognosis	Other results	Somatic or germline
Eisfeld AK, et al. *Leukemia* (2018)	1021 de novo AML (adult)	(LOH was detected in patients with VAF>0.70)	5.2%	Poor in patients aged <60	Significant association with complex karyotype	NA
Boudry-Labis E, et al. *Am J Hematol.* (2013)	485 de novo AML (adult)	3.5%	1/5 (20%) patients with *NF1* deletion	No significant difference but tendency for lower CR rate and shorter OS	Significant association with unfavorable cytogenetics	One mutation (c.2027dupC) in a patient with *NF1* deletion was determined as a somatic mutation
Harferlach C, et al. *Leukemia* (2012)	1161 myeloid malignancies (adult)	23/315 (7.3%) in de novo AML	15/29 (51.7%) patients with myeloid malignancies and *NF1* deletion	NA	-	NA
Parkin B, et al. *Clin cancer res.* (2010)	95 AML* (adult)	10.5%	2/10 (20%) of patients with *NF1* deletion	NA	High frequency (7/10) in complex karyotype	Determined as somatic mutations
Harferlach C, et al. *Leukemia* (2010)	37 AML with *CBFB/MYH11* ** (adult)	16%	NA	NA	-	NA
Suela J, et al. *J Clin Oncol.* (2007)	120 de novo AML (adult)	5.8%	NA	NA	High frequency (5/7) in complex karyotype	NA
Balgobind BV, et al. *Blood* (2008)	71 AML with *KMT2A*-r (pediatric)	2.80%	1/2 (50%) of patients with *NF1* deletion	1 patient with *NF1* deletions relapsed	-	NA (Both patients with *NF1* deletion had no clinical characteristics of neurofibromatosis)

AML, acute myeloid leukemia; LOH, loss of heterozygosity; VAF, variant allele frequency; NA, not analyzed; CR, complete remission; OS, overall survival.

* Including de novo, relapse, primary, secondary, therapy-related AML

** Including de novo and therapy-related AML

Table S6. Summary of references for *CBL* alterations in AML.

Reference	Number of patients	Frequency of mutation	Mutation/splice variant	Prognosis	Other results	Somatic or germline
Sargin B, et al. *Blood* (2007)	150 de novo AML (adult)	1 (0.7%)	Missense mutation at exon 9	NA	Normal karyotype	Somatic
Caligiuri MA, et al. *Blood* (2007)	12 de novo AML (adult)	4 (30%)	1 exon 8 deleted variant 3 missense mutations at exon 8	NA	-	NA
Abbas S, et al. *Haematologica* (2008)	319 de novo AML additional 79 CBF-AML (adult)	2/319 (0.6%) in AML 3/79 in CBF-AML	5 exon 8 deleted variants	NA	Significant association with CBF-AML	1 of 5 patients was analyzed and determined as somatic
Sanada, et al. *Nature* (2009)	222 myeloid malignancies (adult)	18/222 in all patients 2/24 (8%) in AML with MRC	16 missense mutations at exon 8 or exon 9 2 exon 8 deleted variants	NA	15/18 in patients had 11q UPD 1/2 in AML with MRC had 11q UPD	3 of 18 patients were analyzed and determined as somatic.
Reindl C, et al. *Clin Cancer Res*. (2009)	279 AML/MDS (adult)	3 (1.1%)	2 exon 8 deleted variants 1 exon 8+9 deleted variants	NA	2 in patients with CBF-AML 1 in patients with 11q aberration	All mutations were determined as somatic
Coenen EA, et al. *Br J Haematol*. (2012)	277 de novo AML (pediatric)	2/277 (0.7%)	1 exon 8 deleted variants 1 missense mutation at exon 8-intron 8 splice site (not affected the splicing)	NA	Exon 8 deleted variant was detected in patient with *KMT2A*-r	Exon 8 deleted variant was determined as somatic. Missense mutation at splice site was determined as germline

AML, acute myeloid leukemia; NA, not analyzed; CBF, core binding factor; MRC, myelodysplasia-related changes; MDS, myelodysplastic syndrome; UPD, uniparental disomy

Supplementary Figures

Figure S1. Treatment schedule of the AML-05 study

ECM comprised etoposide (150 mg/m2/d on days 1–5), cytarabine (200 mg/m2/d via 12-h continuous intravenous [CIV] infusion on days 6–12), mitoxantrone (5 mg/m2/d on days 6–10), and an age-adjusted dose of triple intrathecal chemotherapy (TIT) on day 6. HCEI comprised high-dose cytarabine (HDCA; 3 g/m2 every 12 h on days 1–3), etoposide (100 mg/m2/d on days 1–5), idarubicin (10 mg/m2 on day 1), and TIT on day 1. HCE comprised HDCA (2 g/m2 every 12 h on days 1–5), etoposide (100 mg/m2/d on days 1–5), and TIT on day 1. HCI comprised HCEI without etoposide. HC comprised HCE without etoposide. HCM comprised HDCA (2 g/m2 every 12 h on days 1–5), mitoxantrone (5 mg/m2/d on days 1–3), and TIT on day 1. CR, complete remission; Allo-HSCT, allogeneic hematopoietic stem cell transplantation. Asterisks indicate patients in the intermediate-risk or high-risk groups who experienced Grade 4 infection during intensification course 1 with HCM and received HC for intensification course 3. (Modified from Tomizawa D, et al. *Leukemia.* 2013).

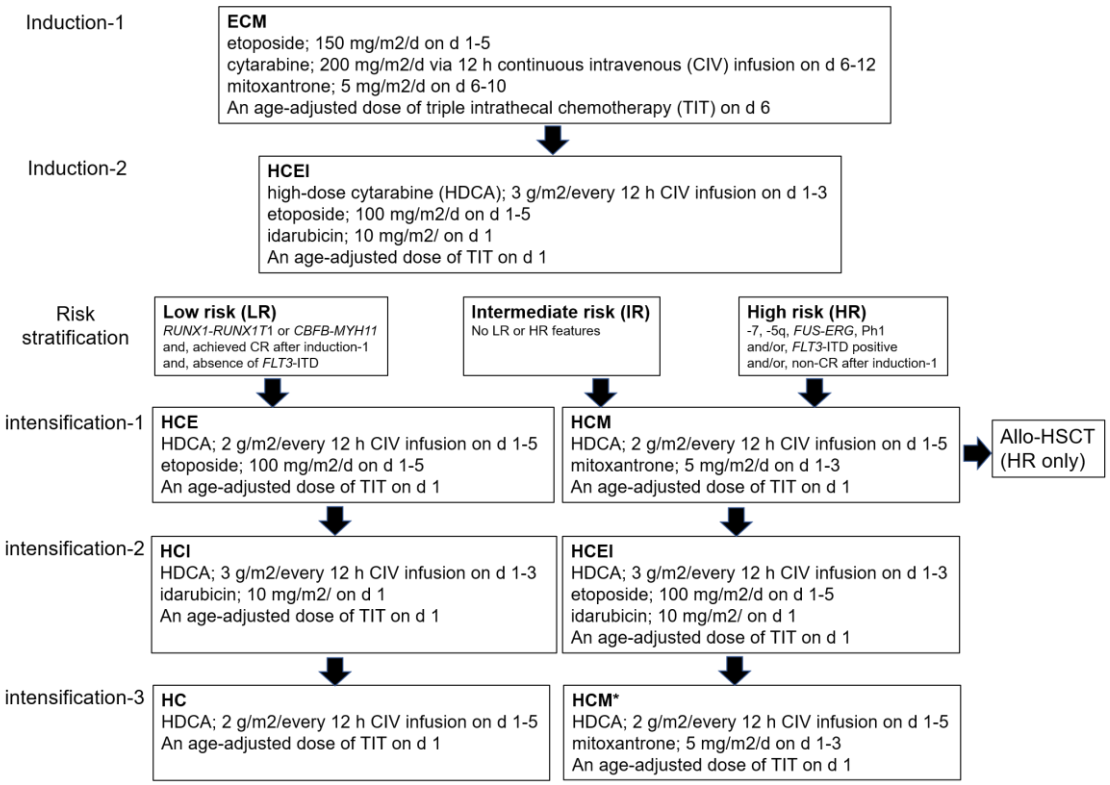


Figure S2. Copy number analysis by CNACS

Upper blue plots indicate total copy number (CN). Middle green bars indicate heterozygous single nucleotide polymorphisms used for CN analysis. Lower red and green plots indicate allele ratio. (A)-(D) Results of patients with *NF1* deletions; (E) Results of UPN 50 having *NF1* mutation with 17q uniparental disomy (UPD); (F) Results of UPN 97 having *CBL* mutation with 11q UPD.

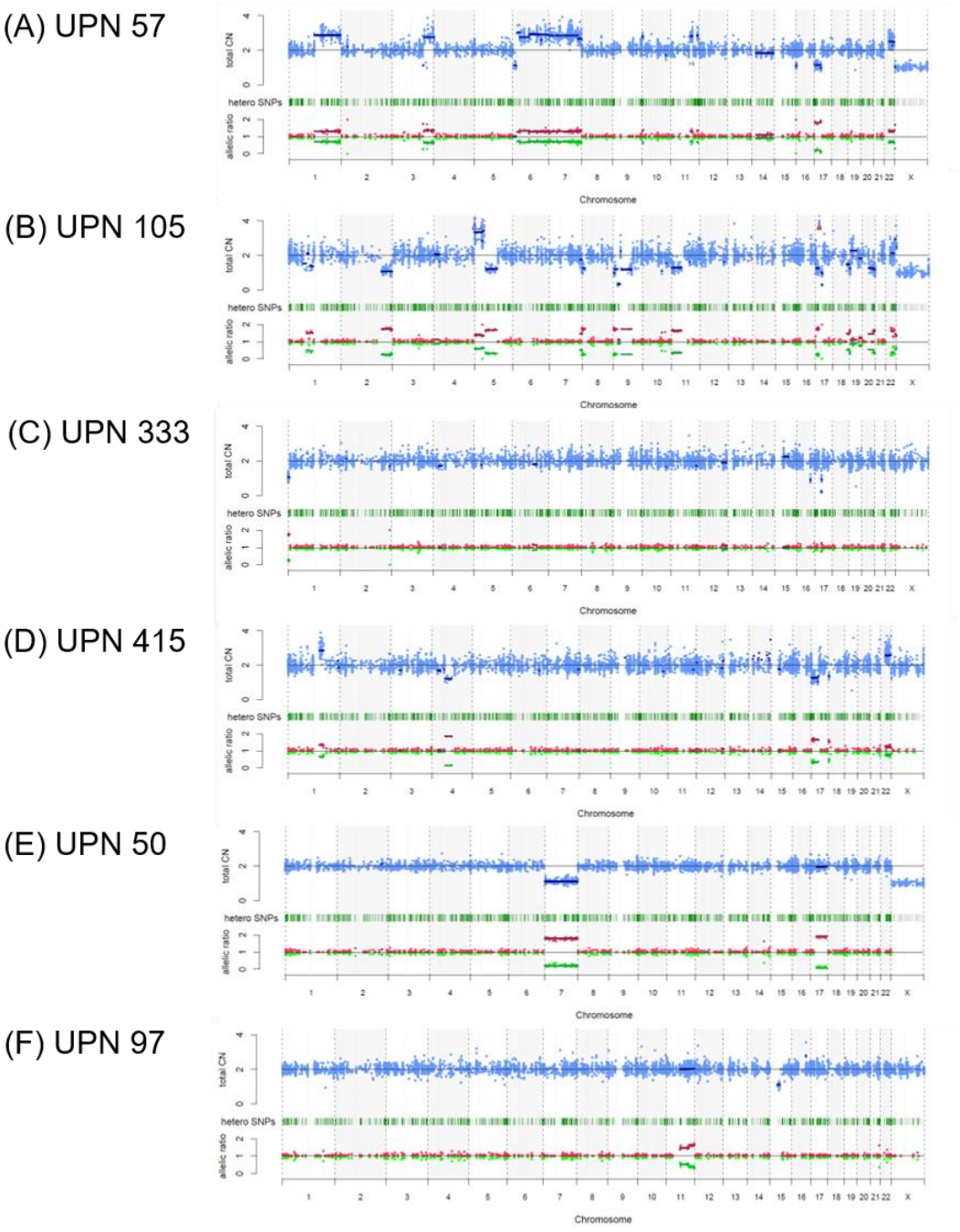


Figure S3. Comparison of white blood cell count at diagnosis

This figure shows white blood cell (WBC) count at diagnosis of patients with RAS pathway alterations. All RAS pathway genes, 29.4 (0.90–430.1) × 10^9/L; *CBL*, 106.7 (20.5–172.0) × 10^9/L; *NRAS*, 29.4 (0.90–430.1) × 10^9/L; *NF1*, 15.5 (1.9–159.3) × 10^9/L; *PTPN11*, 17.8 (1.9–190.5) × 10^9/L, *KRAS*, 34.3 (3.0–321.7) × 10^9/L. WBC count at diagnosis was significantly higher in patients with *CBL* mutations (p = 0.026).

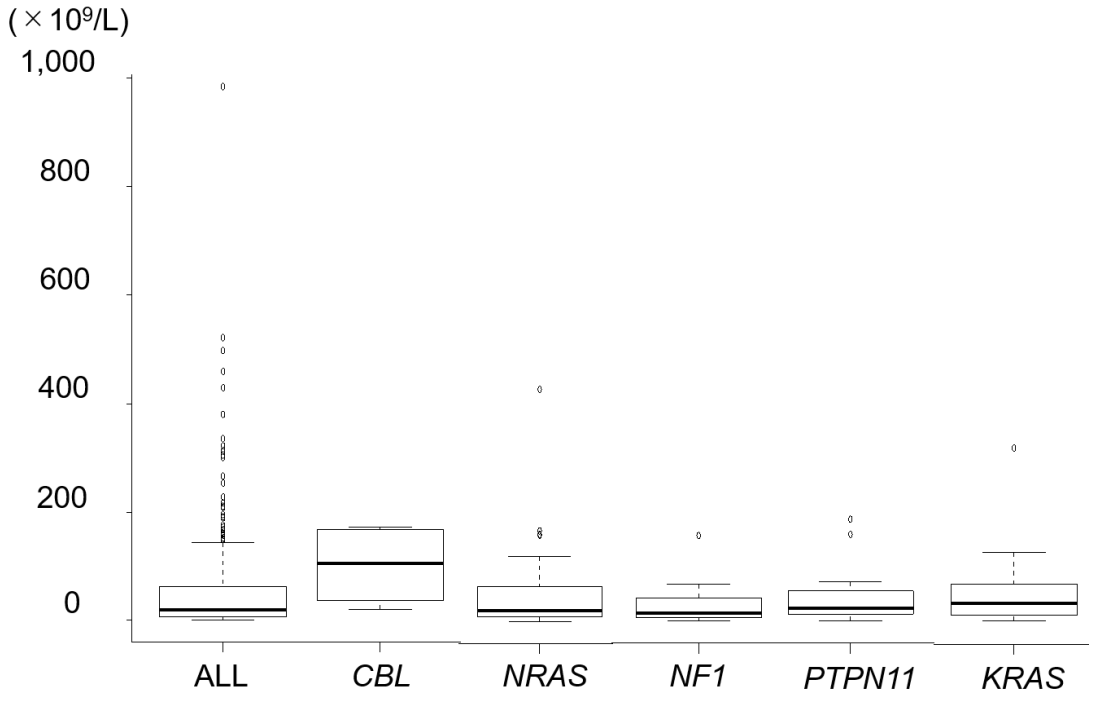


Figure S4. Prognostic significance of *CBL* and *KRAS* mutations in pediatric patients with AML

(A) and (C) show Kaplan–Meier curves of the overall survival of patients with and without *CBL* and *KRAS* alterations. (B) and (D) show the Kaplan–Meier curves of the event-free survival of patients with and without *CBL* and *KRAS* alterations.

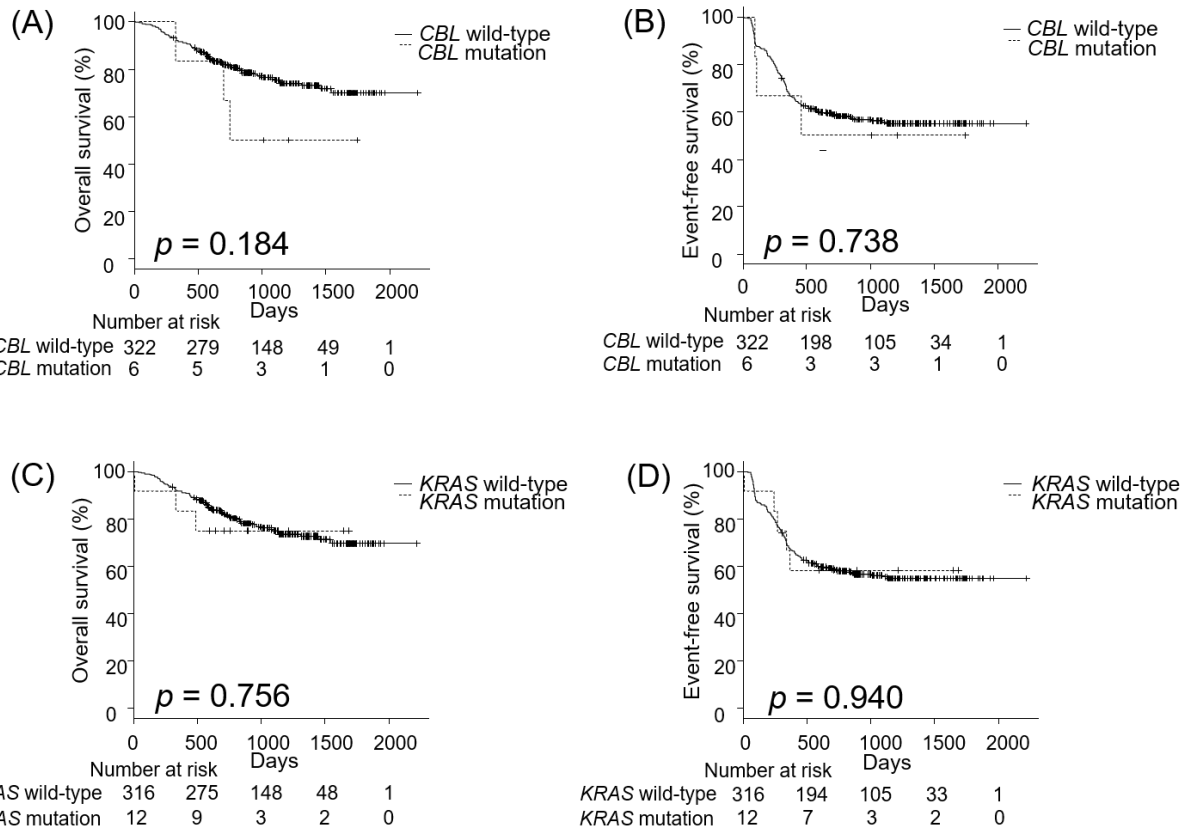
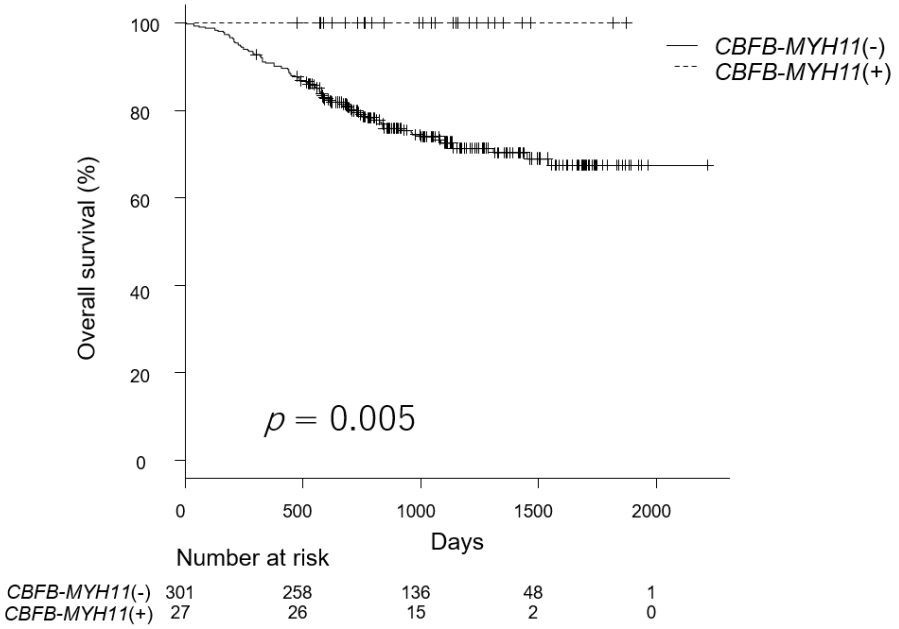


Figure S5. Kaplan–Meier curves of the overall survival of pediatric AML patients with and without *CBFB-MYH11*

Overall survival of patients with *CBFB-MYH11* was significantly better than that of patients without *CBFB-MYH11* (p = 0.005).



Supplementary References

1. 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.

2. Kinoshita A, Miyachi H, Matsushita H, et al. Acute myeloid leukaemia with myelodysplastic features in children: a report of Japanese Paediatric Leukaemia/Lymphoma Study Group. *Br J Haematol.* 2014;**167**(1):80-86.