Supplemental Figures and Tables for Shiba et al.

Transcriptome Analysis Offers a Comprehensive Illustration of the Genetic Background of Pediatric Acute Myeloid Leukemia.

Table S1: Clinical characteristics of 443 patients with de novo AML enrolled in AML-05 trial.

Table S2: The genetic background of 139 pediatric de novo AML patients performed RNA-sequencing.

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Figure S1: Comparison of cytogenetic alterations between Children's Oncology Group (COG) and Japan Children's Pediatric Leukemia/Lymphoma Study Group (JPLSG).

Figure S2: Treatment scheme in the AML-05 clinical trial.

Figure S3: Clinical and genetic profiles of 70 patients with AML with a normal karyotype.

Figure S4: Clinical and genetic profiles of 84 patients with AML with high *PRDM16* gene expression.

Table S1. Clinical characteristics of 443 patients with de novo AML enrolled in AML-05 trial.

		non-analyzed (n = 74)	analyzed (n = 369)	*P* value
Age at diagnosis	Mean ± SD	5.2 ±5.1	7.4 ± 5.1	<0.001
	Median (range)	3 (0–15)	7 (0–17)	
Gender male, n (%)		30 (41)	175 (47)	0.31
White cell count at diagnosis ($x10^3/mm^3$)	Mean ± SD	19.7 ± 38.1	54.0 ± 91.0	<0.001
	Median (range)	7.6 (1.0–247.6)	20.2 (0.6–985.0)	
Cytogenetic risk, n (%)				
Low risk		15 (20)	123 (33)	0.04¶
Intermediate risk		42 (57)	147 (40)	
High risk		6 (8)	50 (14)	
No complete remission		11 (15)	49 (13)	
AML subtype (FAB classification), n (%)				
M0		1 (1)	7 (2)	1
M1		9 (12)	48 (13)	1
M2		13 (17)	104 (28)	0.06
M3		0 (0)	1 (0.3)	1
M4		6 (8)	56 (15)	0.14
M5		21 (2)	73 (20)	0.12
M6		2 (3)	8 (2)	0.68
M7		15 (20)	33 (9)	0.007
RAEB		2 (3)	1 (0.3)	0.07
RAEB-T		5 (7)	34 (9)	0.65
Chromosomal abnormality or genetic mutation				
Normal karyotype, n (%)		15 (20)	70 (19)	0.75
Complex karyotype, n (%)		13 (18)	40 (11)	0.12
RUNX1-RUNX1T1		16 (22)	106 (29)	0.25
CBFB-MYH11		1 (1)	31 (8)	0.03
KMT2A rearrangement		16 (22)	55 (15)	0.17
Mortality, n (%)		18 (24)	56 (20)	0.84#

AML, acute myelogenous leukemia; SD, standard deviation.

P values were calculated using the Mann–Whitney U test for continuous values, and Fisher's exact test for categorical values.

¶ P values were calculated using the Cochrane-Armitage test.

 # P value was calculated using Log-rank test.

Table S2. The genetic background of 139 pediatric de novo AML patients performed RNA-sequencing.		
	examined	Not examined
Total	139	230
normal karyotype	60	10
monosomy7	3	5
trisomy8	18	10
RUNX1-RUNX1T1	0	106
CBFB-MYH11	0	31
NUP98-NSD1	7	4
FUS-ERG	3	2
DEK-NUP214	2	2
CEBPA double	21	2
NPM1	16	2
FLT3- ITD	33	14
KMT2A- PTD	12	1
CBFA2T3-GLIS2	3	8
KMT2A -rearrangement	14	47
NUP98-KDM5A	3	3
PRDM16 high	65	19
MECOM high	21	36
FAB-M7	9	25

Table S3. The clinical features of de novo pediatric AML patients with adverse fusions.

RNA No.	Fusions	sex	risk	Age (y)	FAB	chromosome	*FLT3-ITD*	*PRDM16 high*	*MECOM high*	relapse	outcome
147	*FUS-ERG*	F	HR	9.3	M1	47,XX,+8[12]/46,XX[8]	-	+	-	+	Dead
438	*FUS-ERG*	F	HR	13.6	M2	49,XX,+8,+10,+12[20]	-	+	-	+	Dead
234	*FUS-ERG*	M	HR	2.8	M5a	49,XY,+10,t(16;21)(p11.2;q22),+21,+22[19]/50,XY,+10,+13,t(16;21)(p11.2;q22),+21,+22[1]	-	-	-	+	Dead
310	*FUS-ERG*	F	HR	14.5	M5a	46,XX,t(16;21)(p11.2;q22)[20]	-	-	-	-	Dead
398	*FUS-ERG*	M	HR	4.7	M2	46,XY,t(2;11)(p21;p15),t(16;21)(p11.2;q22)[13]/46,XY[7]	-	+	-	+	Dead
115	*RPN1-MECOM*	F	non-CR	14.6	M5a	45,XX,inv(3)(q21q26),-7[16]/46,XX[4]	-	+	+	-	Dead
229	*RPN1-MECOM*	F	non-CR	14.9	RAEB-T	45,XX,inv(3)(q21q26.2),-7[20]	-	-	+	+	Dead
133	*DEK-NUP214*	M	IR	14.7	M2	46,XY,t(6;9)(p23;q34)[20]	-	+	-	+	Alive
280	*DEK-NUP214*	M	IR	11.2	M2	46,Y,der(X)add(X)(p11.2)add(X)(q22), t(6;9)(q15;p24)[20]	-	-	-	+	Dead
210	*DEK-NUP214*	M	non-CR	6.8	M5a	48,XY,der(4)t(4;6)(q21;p21)t(6;9)(p23;q34), der(6)t(4;6),+8, der(9)t(6;9), +13[1]/49, sl, +13[18]	+	+	-	-	Dead
420	*DEK-NUP214*	M	non-CR	6.1	M4	46,XY,t(6;9)(p23;q34)[19]/46,idem,add(20)(q11.2)[1]	+	+	-	+	Dead
148	*KMT2A-AFDN*	M	IR	8.6	M1	47,XY,t(6;11)(q27;q23),+8[19]	-	+	-	+	Dead
378	*KMT2A-AFDN*	M	IR	0.2	M5a	46, XY,t(6;11)(q27;q23)[3]/46,XY[17]	-	-	-	+	Alive
240	*KMT2A-AFDN*	F	IR	2.9	M4	46,XX,t(6;11)(q27;q23)[20]	-	-	+	+	Dead
297	*KMT2A-AFDN*	F	IR	10.8	M5a	46,XX,t(6;11)(p21;q23)[25]	-	-	-	+	Dead
232	*NUP98-KDM5A*	F	IR	1.7	M6a	46,XX[20]	-	+	+	+	Dead
413	*NUP98-KDM5A*	M	IR	1.3	RAEB-T	46, XY, del(13)(q?)[12]/46,XY[8]	-	+	+	+	Alive
353	*NUP98-KDM5A*	F	IR	14.3	M5a	48,XX,+6,+7[12]/48,idem,i(18)(q10)[7]/49,idem,+8,i(18)[1]	-	-	-	+	Dead
336	*NUP98-KDM5A*	F	IR	1.1	M7	45, XX,-15,add(18)(q21),add(19)(p13)[16]/46,sl,del(13)(q?)[2]/46,XX[2]	-	+	+	+	Dead
368	*NUP98-KDM5A*	F	IR	1.2	M7	46, XX, add(6)(q23),der(8;15)(q10;q10),+mar[7]/46,XX,add(6)(q23), der(8;15)(q10;q10), del(13)(q12q14), +mar[4]/46,XX,add(11)(q13)[3]/46,XX[5]	-	-	+	-	Alive
405	*NUP98-KDM5A*	M	non-CR	2.2	M7	46,XY,del(3)(q13.2),add(6)(p25),ins(11;?)(q13;?),ins(12;?)(q13;?),del(13)(q12q14)[10]/49,idem,+2,+9,+del(13)(q12q14),-17,+21[1]/46,XY[9]	-	+	+	-	Alive
98	*NUP98-NSD1*	M	HR	16.8	M4	47,XY, +8[12]/46, XY[8]	+	+	-	+	Dead
222	*NUP98-NSD1*	F	non-CR	11.2	M4	46,XX[20]	+	+	-	-	Dead
225	*NUP98-NSD1*	M	non-CR	17.3	M5a	47,XY,+6[19]/48,XY,+6,+17[1]	+	+	-	+	Dead
111	*NUP98-NSD1*	M	IR	5.6	M1	46,XY,del(9)(q?)[20]	-	+	-	+	Dead
117	*NUP98-NSD1*	M	non-CR	13.1	M5a	46,XY[20]	-	+	-	+	Dead
37	*NUP98-NSD1*	M	HR	13.8	M0	47,XY,+8[20]	+	+	-	-	Alive
395	*NUP98-NSD1*	M	non-CR	14.8	M5a	47,XY,+8[2]/47,idem,add(10)(q11.2),add(10)(q22),add(12)(q24.1)[1]/46,XY[16]	+	+	-	+	Dead
285	*NUP98-NSD1*	F	non-CR	12.3	M5b	46, XX[20]	-	+	-	-	Alive
128	*NUP98-NSD1*	M	HR	10.8	M2	46,XY[20]	+	+	-	+	Dead
385	*NUP98-NSD1*	M	non-CR	6	M5a	46,XY[20]	+	+	-	+	Dead
397	*NUP98-NSD1*	M	non-CR	13.4	M5a	47,XY,+8[17]/46,XY[3]	+	+	-	+	Dead
81	*CBFA2T3-GLIS2*	M	non-CR	0.8	M7	47, XY, +21[9]/46, XY[11]	-	-	-	+	Dead
116	*CBFA2T3-GLIS2*	M	HR	1.3	M7	46,XY[20]	+	-	-	+	Dead
119	*CBFA2T3-GLIS2*	F	HR	1.9	M7	47,XX,+3[11]/46,XX[9]	+	-	-	+	Alive
144	*CBFA2T3-GLIS2*	M	IR	1.2	M7	46,XY,t(15;16)(q24;q24)[1]/47,XY,+Y[1]/46,XY[18]	-	-		+	Dead
159	*CBFA2T3-GLIS2*	M	IR	0.3	M7	46,XY,[20]	-	-	-	+	Dead
192	*CBFA2T3-GLIS2*	F	IR	0.8	M7	48,XX,+3,+21[9]/46,XX[11]	-	-	-	+	Alive
282	*CBFA2T3-GLIS2*	M	IR	0.8	M7	49,XY,+Y,+12,+21[2]/50,sl,+Y,+8,-12[18]	-	-	-	+	Dead
315	*CBFA2T3-GLIS2*	M	IR	1.2	M7	48, XY,+14,+21[20]	-	-	-	-	Alive
352	*CBFA2T3-GLIS2*	M	non-CR	0.9	M7	46,XY[20]	-	-	-	+	Alive
429	*CBFA2T3-GLIS2*	F	IR	0.8	M7	48, XX, t(3;21)(q27;q22),+21,+21[11]/48,idem,der(19)(t(1;19)(q21;p13)[3]/90,idem×2,-4,-7,-9,-15,-18,-21[3]/46,XX[3]	-	-	-	+	Dead

Table S4. The clinical features of 29 patients with de novo AML without any gene alterations other than FAB M7.

RNA No.	Sex	Age (y)	Risk	FAB	Chromosome	Gene mutations	Relapse	Event	Outcome
248	M	4.8	non-CR	M4	46,XY,add(7)(q32),del(9)(q?),ins(12;?)(q13;?)[20]	*KIT*	-	+	Dead
256	M	14.1	IR	M1	46,XY,t(1;14)(p36.1;q32)[1]/46,XY[19]	*KIT*	-	+	Dead
265	M	15.8	non-CR	M2	46,XY,t(11;20)(p11.2;p11.2)[17]/46,XY[3]	-	+	+	Dead
294	F	13.8	HR	M2	45,X,-X,add(2)(q31),add(5)(q22),add(7)(p11.2),del(9)(q?),add(12)(p11.2),add(12)(p11.2),del(16)(p?)[1]/45,sl,del(13)(q?)[15]/45,sdl1, -add(7),+add(7)(p11.2)[2]/46,XX[1]	-	+	+	Dead
16	M	7.8	HR	RAEB-T	46,XY[20]	-	+	+	Dead
130	F	11	IR	M5a	46, XX, t(11;11)(p15;q22)[4]/46,idem,add(17)(p11.2)[2]/46, XX[14]	-	+	+	Dead
251	F	2.3	IR	RAEB-T	46,XX,add(1)(p13),add(3)(q21),del(3)(q21),del(13)(q?)[1]/46,XX[19]	-	+	+	Dead
415	F	12.3	IR	M6a	45,XX,ins(1;?)(q21;?), add(4)(q12), add(7)(q36),der(17;18)(q10;q10)[20]	-	+	+	Dead
105	M	10.8	IR	M4	46,XY,+Y,add(1)(p11),del(2)(q?),del(5)(q?),add(8)(p11.2),-9,-9,-11,-17,add(18)(q21),-19,add(22)(q11.2),+del(?)t(?;11)(?;q13),+mar1,+mar2,,+mar3[2]/88,sl,×2, -3,-del(5)×2,-6,+9,-20,-20,-21,-mar1,-mar3×2,+5mar[1]/47,XY,+Y[9]	-	+	+	Dead
314	M	1.3	IR	M4	46,XY,add(6)(q11),add(7)(p11.2),add(9)(p13),del(12)(p?)[16]/46,sl,del(5)(q?),-add(7),+der(7)add(7)t(5;7)(q13;q22)[4]	-	+	+	Dead
228	F	0.3	non-CR	M5a	46,XX,add(1)(q42),del(1)(q23q25),add(3)(p25),del(7)(q11.2)[7]/47,sl,+19[3]/47,sdl1,der(3)add(3)(p13)del(3)(q24q25),add(5)(q13)[10]	-	-	+	Dead
279	F	12.1	IR	M1	46, XX[20]	-	-	+	Dead
412	F	11.8	IR	M2	46,XX,inv(9)(p12q13)[20]	*WT1*	-	-	Alive
396	F	7.6	IR	M1	46,XX[20]	*WT1*	-	-	Alive
180	F	0.5	HR	M2	48,XX,t(7;12)(q36;p13),+8,+19[8]/47,sl,-20,add(22)(p11.2)[12]	*KIT*	+	+	Alive
430	F	11	IR	M1	46,XX[20]	*KIT*	+	+	Alive
359	F	13.9	IR	M2	46,XX[20]	*KIT*	-	-	Alive
135	M	11.3	IR	M4	48,XY,+8,+8[20]	*KIT*	-	-	Alive
127	F	0.4	HR	M5a	47,XX,t(7;12)(q36;p13),+19[20]	-	+	+	Alive
393	F	13.3	IR	M1	46,XX,?t(5;6)(p15;q24)[6]/47,idem,+mar,inc[10]	-	+	+	Alive
277	M	15.1	IR	M5a	46,XY[20]	-	+	+	Alive
278	M	5.8	IR	M5a	46,XY,t(1;3)(p32;p25),t(12;12)(p13;q13)[18]/46,XY[2]	-	+	+	Alive
379	M	1.6	non-CR	M6	46,XY,-7[20]	-	-	+	Alive
21	F	14.9	HR	M6a	46,XX[20]	-	-	-	Alive
118	F	14.6	IR	M2	46,XX[20]	-	-	-	Alive
41	F	4.1	IR	M2	47,XX,+21[4]/48,XX,+4,+21[2]/46,XX,i(21)(q10)[1]/46,XX[13]	-	-	-	Alive
94	M	1.1	IR	M5a	47,Y,add(X)(q22),add(1)(q21),add(5)(q11),der(7)add(7)(p11)add(7)(q22),add(13)(q32),+19[4]/46,XY[3]	-	-	-	Alive
46	M	1.1	IR	M2	47,XY,der(18)t(1;18)(q25;p11.2),+i(21)(q10)[19]/46,XY[1]	-	-	-	Alive
367	M	7	IR	M0	47,XY,+11[18]/54,idem,+x,+10,+11,+13,+14,+20,+21[1]/46,XY[1]	-	-	-	Alive

Table S5. The clinical features of de novo pediatric AML patients with *KMT2A* fusions.

RNA No.		sex	Risks	Age (y)	FAB	Chromosome	*PRDM16 high*	*MECOM high*	relapse	Dead	Cause of death
157	*KMT2A-ELL*	F	Non-CR	0.6	M5a	46,XX[20]	+	-	-	Dead	ARDS
86	*KMT2A-ELL*	M	Non-CR	0.6	M4	47,XY,+8,i(10)(q10),t(11;19)(q23;p13.1)[18]/46, XY[2]	-	-	-	Dead	ARDS
122	*KMT2A-ELL*	F	Non-CR	0.6	M5a	48,XX,+8,+18,-19,+20[19]	-	-	-	Dead	Primary disease
417	*KMT2A-ELL*	M	IR	5.6	M1	46,XY,t(11;19)(q23;p13.1)[17]/47,idem, +8[1]/46,XY[2]	-	+	+	Alive	-
113	*KMT2A-ELL*	F	IR	10.3	M2	46,XX,add(12)(p11)[12]/46,XX[8]	-	+	+	Alive	-
204	*KMT2A-ELL*	M	IR	11.7	M1	47,XY,+22[1]/45,XYadd(2)(q31),-5,-7,-9,-14,-15,-19,+5mar[1]/46,XY[18]	-	-	-	Alive	-
304	*KMT2A-ELL*	M	IR	0.8	ND	46, XY, t(11;19)(q23;p13.1)[19]/46,XY[1]	-	-	-	Alive	-
150	*KMT2A-MLLT1*	M	IR	13.7	M5a	48,XY,+8,+8,t(11;19)(q23;p13.3)[12]/46,XY	-	-	+	Alive	-
10	*KMT2A-MLLT1*	F	IR	14.5	M0	46,XX,t(11;19)(q23;p13.3)[19]/46.sl,dup(1)(q21q32)[1]	-	+	-	Alive	-
70	*KMT2A-MLLT1*	M	IR	4.9	M5a	46, XY,t(11;19)(q23;p13.3)[13]/46,sl,t(1;6)(p22;q13)[4]/46, XY[2]	+	-	+	Dead	-
439	*KMT2A-MLLT1*	F	IR	8.9	M4	46, XX,t(11;19)(q23;p13.1)[20]	-	+	+	Alive	-
374	*KMT2A-MLLT1*	F	IR	1.8	M5a	45,XX,add(8)(p11.2),t(11;19)(q23;p13.3),der(12;19)(q10;p10)[19]/46,XX[1]	-	-	-	Alive	-
188	*KMT2A-MLLT10*	F	IR	5.8	M5a	46,XX[20]	+	+	+	Dead	Primary disease
155	*KMT2A-MLLT10*	M	IR	0.8	RAEB-T	48,XY,add(11)(q13), +21,+21[4]/46,XY[16]	-	+	+	Dead	Primary disease
259	*KMT2A-MLLT10*	M	IR	10.6	M5a	46,XY[20]	-	-	+	Dead	Primary disease
202	*KMT2A-MLLT10*	F	IR	1	M5a	46,XX,der(10)t(10;11)(p12;q14)del(11)(q23),der(11)t(10;11)[16]/46,XX[4]	-	-	+	Dead	ARDS, GVHD
201	*KMT2A-MLLT10*	F	IR	1.8	M5b	46,XX,t(8;10)(q13;p15),add(11)(q23)[17]/46, XX[3]	+	+	+	Alive	-
356	*KMT2A-MLLT10*	F	IR	13.4	M4	45, XX,der(1;19)(q10;p10),-9,+19[18]/46,XX[2]	+	+	-	Alive	-
411	*KMT2A-MLLT10*	F	IR	5.6	M5a	46,XX,ins(10;11)(p12;q13q23)[20]	+	-	-	Alive	-
214	*KMT2A-MLLT10*	F	IR	4	M5a	46,XX,add(10)(p11.2)[16]/46,XX[4]	-	-	-	Alive	-
219	*KMT2A-MLLT10*	F	IR	1.1	M5a	46,XX,add(7)(q22),der(10;11)(q10;q10)add(11)(q23),+mar1[17]/46,XX[3]	-	-	-	Alive	-
298	*KMT2A-PICALM*	F	IR	1.8	M4	47,XX,+8,del(11)(q21q23)[18]/46,XX[2]	-	-	-	Alive	-
441	*KMT2A-LASP1*	F	IR	0.9	M4	46, XX,t(11;17)(q23;q21)[15]/47,idem,+8[1]/46,XX[4]	-	-	-	Alive	-
347	*KMT2A-SEPT6*	F	IR	2.3	M4	46, XX[20]	-	-	-	Alive	-
437	*KMT2A-R*$^{\#}$	F	IR	0.6	M5b	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]	-	-	-	Alive	-
51	*KMT2A-R*$^{\#}$	F	IR	0	M5a	46,XX,-10,add(11)(q23),+mar1[16]/46,XX[4]	-	-	-	Alive	-
376	*KMT2A-R*$^{\#}$	F	Non-CR	7.8	M4	46,X,add(X)(q22),del(11)(q23),add(14)(q11.2)[7]/45,idem,-X[13]	-	-	-	Alive	-

**KMT2A-MLLT3* and *KMT2A -AFDN* were excluded from this table. *KMT2A* -R$^{\#}$ were identified by FISH analysis. ARDS, acute respiratory distress syndrome; GVHD, graft versus host disease.

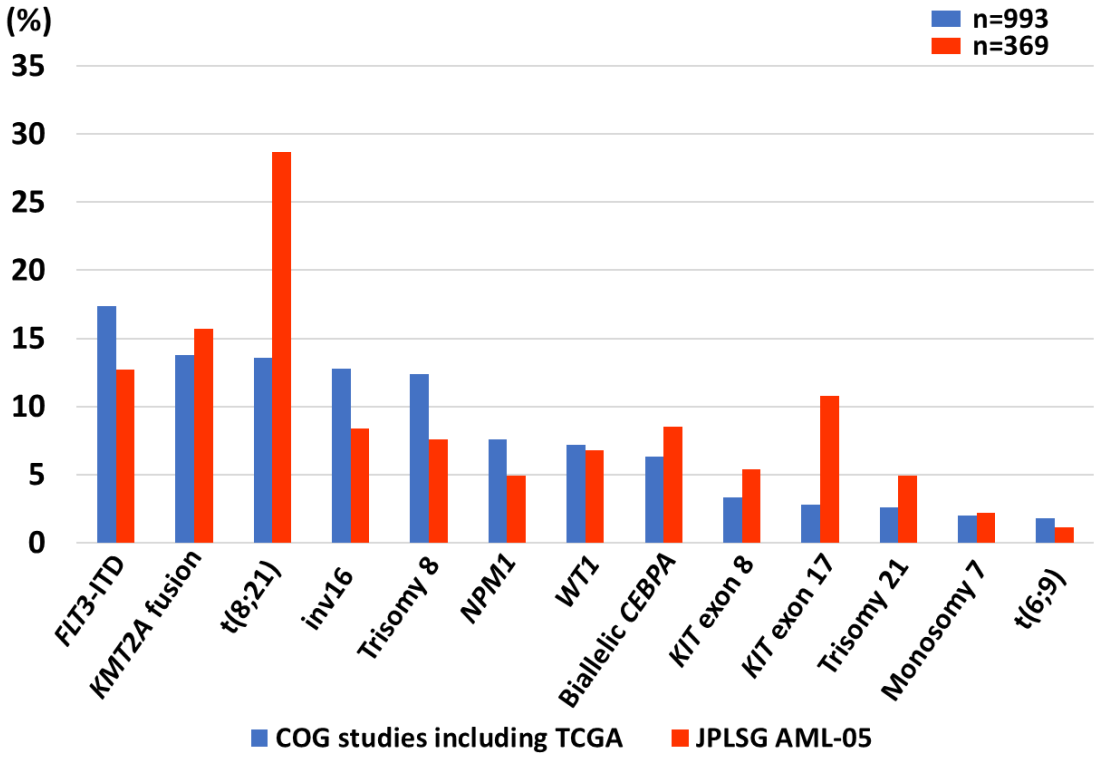

Figure S1. **Comparison of cytogenetic alterations between Children's Oncology Group (COG) and Japan Pediatric Leukemia/Lymphoma Study Group (JPLSG).** Frequencies of the clinically established molecular aberrations of COG data included TCGA AML patients (n=993 patients) have been reported previously reported.*

*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. *Nat Med.* 2018;24(1):103–112.

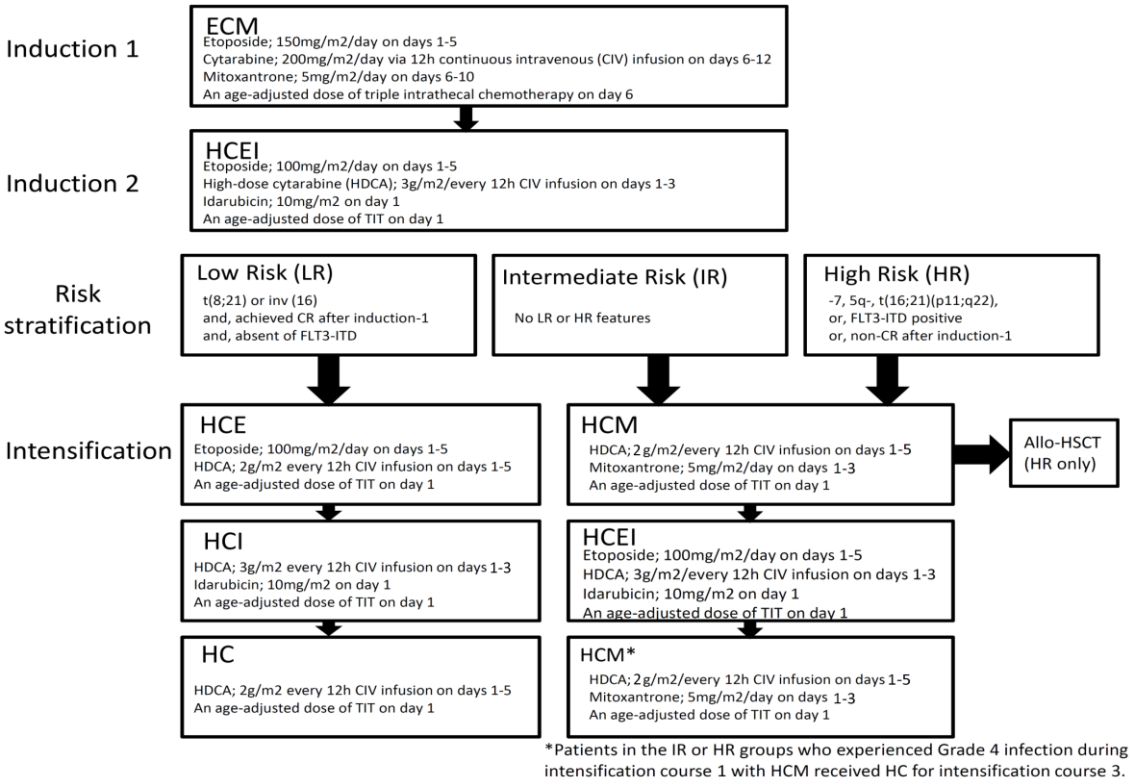

Figure S2. Treatment scheme in the AML-05 clinical trial. Patients who failed to achieve hematological complete remission (CR) after the second course were identified as non-CR and disengaged from the trial, and they had to select any other treatment regimens themselves but were eligible for this survey. Allogeneic hematopoietic stem cell transplantation (HSCT) was indicated in all high-risk (HR) patients after three or more treatment courses. All patients with the *FLT3*-ITD alteration underwent HSCT irrespective of the *FLT3*-ITD allelic ratio (AR). Asterisks indicate patients in the intermediate-risk or HR groups who experienced Grade 4 infection during intensification course 1 with HCM and received HC for intensification course 3. Ind-1, induction course 1; Ind-2, induction course 2; Allo-HSCT, allogeneic hematopoietic stem cell transplantation.

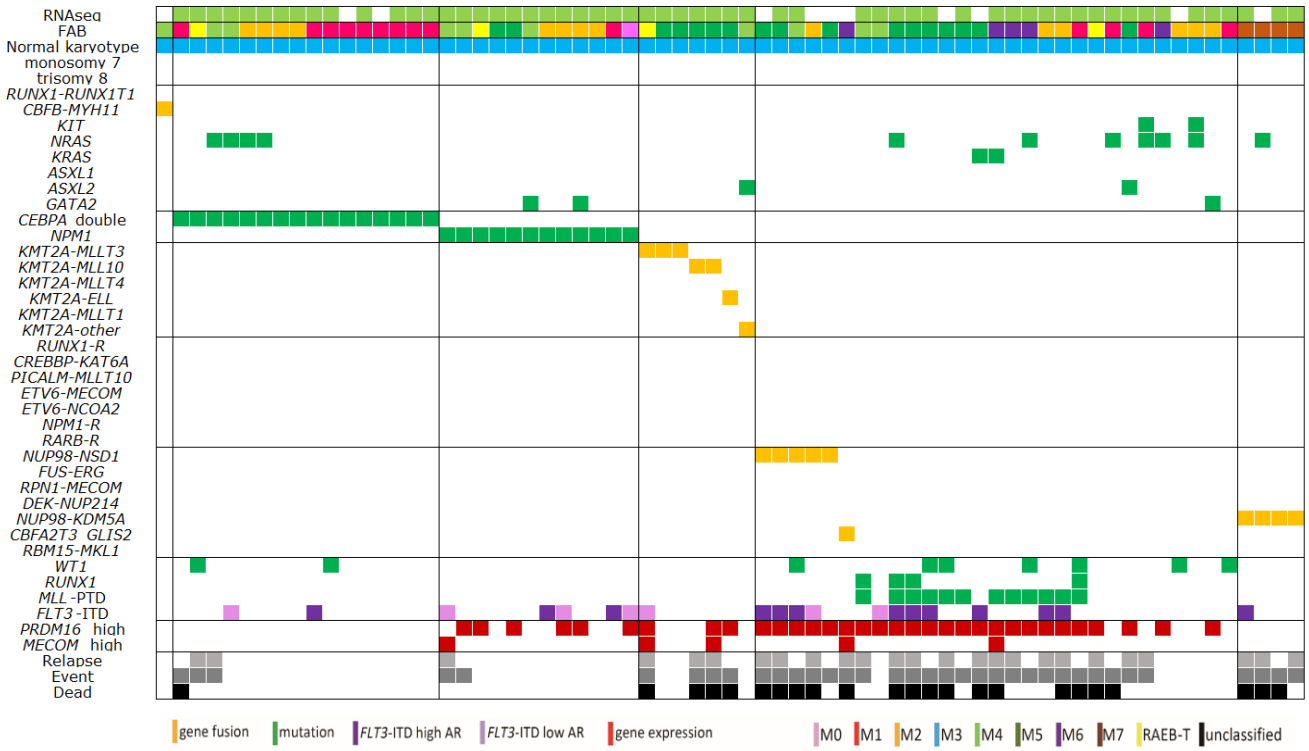

Figure S3: Clinical and genetic profiles of 70 patients with AML with a normal karyotype. Each column indicates one patient.

Figure S4: Clinical and genetic profiles of 84 patients with AML with high *PRDM16* gene expression. Each column indicates one patient.