Concomitant *WT1* mutations predict poor prognosis in acute myeloid leukemia patients with double mutant *CEBPA*

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Supplementary Table 1. Clinical characteristics of AML patients according to the status of *CEBPA* mutations

Variables	*CEBPA*wt N=654	*CEBPA*^{sm} N=33	*CEBPA*^{dm} N=69	P value[c]	P value[d]
Sex				0.685	0.909
Male	372	20	41		
Female	282	13	28		
Age, years[a]	49 (23-82)	52 (18-85)	40 (17-90)	<0.0001	0.038
Lab[a]					
WBC (k/μL)	6.39 (0.3-212.7)	37.3 (0.98-271.5)	44.2 (2.41-387.4)	<0.0001	0.739
Hb (g/dL)	8.1 (4.2-13.9)	8.2 (4.5-10.9)	8.8 (3-13.6)	0.006	0.045
Platelet (k/μL)	28 (5-122)	28 (10-712)	41 (5-204)	0.077	0.707
PB Blast (k/μL)	0.77 (0-134.0)	20.9 (0.21-260.6)	29.2 (0.72-371.9)	0.006	0.634
FAB[b]					
M0	18 (2.8)	0 (0)	0 (0)	0.403	-
M1	114 (17.6)	11 (33.3)	39 (56.5)	<0.0001	0.028
M2	213 (32.4)	17 (51.5)	27 (39.1)	0.259	0.237
M3	63 (9.6)	0 (0)	0 (0)	0.007	-
M4	180 (27.5)	3 (9.1)	3 (4.3)	<0.0001	0.386
M5	30 (4.6)	1 (3.0)	0 (0)	0.099	0.324
M6	26 (4.0)	1 (3.0)	0 (0)	0.162	0.324
Undetermined	10 (1.5)	0 (0)	0 (0)	0.610	-
Cytogenetics[b]					
Favorable	146 (23.1)	0 (0)	0 (0)	<0.0001	-
Intermediate	392 (61.9)	30 (96.8)	67 (98.5)	<0.0001	0.325
Unfavorable	95 (15.0)	1 (3.2)	1 (1.5)	0.002	0.545
2016 WHO classification					
Recurrent geneic abnormalities					
t(8;21)	57	0	0	0.013	-
inv(16)	27	0	0	0.162	-
APL	63	0	0	0.009	-
t(9;11)	9	0	0	>0.999	-
t(6;9)	3	0	0	>0.999	-
Inv(3)	3	0	0	>0.999	-
t(1;22)	0	0	0	-	-
CEBPA^{dm}	0	0	69	<0.0001	<0.0001
BCR-ABL	1	0	0	>0.999	-

NPM1	132	8	0	<0.0001	<0.0001
RUNX1	76	0	0	0.004	-
Myelodysplasia-related change	92	1	0	0.001	0.324
NOS	191	24	0	<0.0001	<0.0001
2017 ELN classification					
Favorable					
t(8;21)	57	0	0	0.011	-
inv(16)	27	0	0	0.099	-
NPM1+/*FLT3*-ITD-	78	4	0	0.002	0.01
CEBPA^{dm}	0	0	69	<0.0001	<0.0001
Intermediate					
NPM1+/*FLT3*-ITD+	53	4	0	0.014	0.01
t(9;11)	9	0	0	>0.999	-
Other cytogenetics	107	17	0	<0.0001	<0.0001
Unfavorable					
t(6;9)	3	0	0	>0.999	-
t(v;11q23)	11	1	0	0.612	0.324
t(9;22)	1	0	0	>0.999	-
inv(3)	3	0	0	>0.999	-
-5/-7	19	0	0	0.384	-
complex	72	1	0	0.004	0.324
NPM1-/*FLT3*-ITD+	50	1	0	0.01	0.324
RUNX1	60	0	0	0.009	-
ASXL1	35	4	0	0.04	0.01
TP53	10	1	0	0.610	0.324

Abbreviations: APL, acute promyelocytic leukemia; *CEBPA*^{sm}, *CEBPA* single mutation; *CEBPA*^{dm}, *CEBPA* double mutation; NOS, not otherwise specified; PB, peripheral blood;

[a] Median (range)

[b] Number of patients (%)

[c] *CEBPA*^{dm} patients vs *CEBPA* wild-type patients

[d] *CEBPA*^{dm} patients vs *CEBPA*^{sm} patients

Supplementary Table 2. Characterization of the mutations in the *CEBPA* coding region

			N terminal		C terminal	
UPN	**Age/Sex**	**Karyotype**	**DNA change**	**Protein change**	**DNA change**	**Protein change**
1	75M	CN	c.324C>G	p.Y108X	c.992T>G	p.L331R
2	48M	CN	c.196_197insGT	p.A66GfsX95	c.925_926insTGG	p.V308_E309insV
3	32F	CN	c.264_303delins20	p.Q88HfsX75	c.920_921ins9	p.R306_N307insKQR
4	41F	CN	c.243_244insGTCG	p.F82LfsX27	c.930_931insACG	p.T310_Q311insT
5	90M	CN	c.68_69insC	p.H24AfsX84	c.772_787del	p.A258SfsX55
6	20F	CN	c.322_326del	p.Y108GfsX60	c.939_940insAAG	p.K313_V314insK
7	80F	CN	c.273_274insC	p.K92QfsX16	c.898C>G	p.R300G
8	37M	CN	c.161_180del20	p.G54VfsX47	c.964_965ins36	p.N321_D322ins12
9	36M	CN	c.209_210insC	p.A71GfsX37	c.961_962ins33	p.D320_N321ins11
10	24M	CN	c.68_69insC	p.H24AfsX84	c.951_952insCTG	p.L317_T318insL
11	66M	CN	c.259_260insC	p.Q87PfsX21	c.920_921ins9	p.R306_N307insKQR
12	39M	CN	c.196_197insT	p.A66VfsX42	c.922_924dup	p.N307_V308insG
13	68F	CN	c.229_235del	p.F77PfsX81	c.916_917insAGC	p.Q305_R306insQ
14	31F	CN	c.50_56del	p.H18RfsX140	c.947_948ins36	p.L315_E316ins12
15	69F	CN	c.247del	p.Q83SfsX77	c.942_943insGTG	p.V314_L315insV
16	64M	CN	c.68_69insCCCG	p.H24PfsX85	c.930_931ins45	p.T310_Q311ins15
17	71F	CN	c.201C>A	p.Y67X	c.815_816dup	p.K273AfsX46
18	49F	CN	c.196_197del	p.A66LfsX41	c.943_944ins3	p.L315_E316insL
19	40M	CN	c.178_182del	p.T60HfsX46	c.895_942dup	p.Q311_Q312ins16
20	55M	CN	c.103del	p.R35GfsX125	c.912_914dup	p.K304-Q305insK
21	31F	CN	c.321_322insT	p.Y108LfsX62	c.931_939dup	p. K313_V314insQQK
22	32F	CN	c.180_189del	p.I62AfsX95	c.925_942del	p.E309_V314del
23	48M	CN	c.283_284insG	p.V95GfsX13	c.934_935insAGA	p.Q312_K313insK
24	55M	CN	c.134del	p.P45HfsX115	c.916_917insAGC	p.Q305_R306 insQ
25	55M	CN	c.247del	p.Q83SfsX77	c.927_928insGAG	p.E309_T310insE
26	57M	CN	c.201_202del	p.I68RfsX39	c.939_940insAAG	p.K313_V314insK
27	69M	NM	c.247del	p.Q83SfsX77	c.961A>TCAT	p.N321>SY
28	32F	sp:+10	c.324C>G	p.Y108X	c.940_941ins33	p.K313_V314ins11
29	53F	sp:+21, -x	c.332_339del	p.A111GfsX56	c.939_962dup	p.D320_N321insKVLELTSD
30	31F	del(9)(q22q34)	c.204_205insC	p.D69RfsX39	c.944_945insCAC	p.L315_E316ins T
31	51M	sp: +21	c.247del	p.Q83SfsX77	c.939_940insAAG	p.K313_L315insV

32	45F	complex	c.262C>T	p.Q88X	c.936_937insCAA	p.Q312_K313insQ
33	81F	CN	c.318del	p.F106LfsX54	c.921_977dup	p.307_325 dup
34	43M	CN	c.183_184insGTCC	p.I62VfsX47	c.917_934del18	p.R306_Q311del
35	50M	CN	c.68_69insC	p.H24AfsX84	c.933_934insTTC	p.Q311_Q312insF
36	17F	CN	c.326_327insC	p.A111RfsX59	c.909_944dup	p.L315_E316ins12
37	28F	CN	c.346del	p.G116AfsX44	c.939_940insAAG	p.K313_V314insK
38	59M	CN	c.183del	p.I62SfsX98	c.907_912dup	p.K304_Q305insAK
39	32M	CN	c.337del	p.A113RfsX47	c.939_940insAAG	p.K313_V314insK
40	29M	CN	c.64_65del	p.P23AfsX84	c.939_940insAAG	p.K313_L315insV
41	50M	CN	c.247del	p.Q83SfsX77	c.939_940insAAG	p.K313_L315insV
42	54M	CN	c.206_207AC>T	p.D69VfsX91	c.939_940insAAG	p.K313_L315insV
43	36M	CN	c.177_186del10	p.I62AfsX95	c.907_912dup	p.K304_Q305insAK
44	19F	CN	c.68_69insC	p.H24AfsX84	c.934_936dup	p.Q312_K313insQ
45	38F	CN	c.129del	p.A44PfsX116	c.929_930insTCT	p.T310_Q311insL
46	73F	CN	c.213_214insCACCG	p.A72HfsX90	c.914A>C	p.Q305P
47	34M	CN	c.201_202insCTAC	p.I68LfsX41	c.907_915del	p.A303_Q305del
48	27M	CN	c.203T>CG	p.I68TfsX40	c.939_940insAAG	p.K313_V314insK
49	43M	CN	c.126_127insTC	p.L43SfsX116	c.901_918dup	p.R306_N307insDKAKQR
50	59M	CN	c.178_179insCC	p.S61RfsX100	c.912_913insTTG	p.K304_Q305insL
51	37M	CN	c.247del	p.Q83SfsX77	c.904_906dup	p.K302_A303insK
52	36F	CN	c.177_186del10	p.I62AfsX95	c.921_936del	p.N307_Q312del
53	57M	CN	c.183del	p.I62SfsX98	c.940_942dup	p.V314_L315insV
54	40M	CN	c.247del	p.Q83SfsX77	c.937_941delinsCA	p.K313_V314delinsQ
55	29F	CN	c.287_311del25	p.G96AfsX56	c.934_939del	p.Q312_K313del
56	35M	CN	c.238_239insGTCG	p.D80GfsX29	c.932_933ins24	p.T310_Q311insHKQRNVET
57	35M	CN	c.318_319insT	p.D107X	c.912_913ins9	p.K304_Q305insKAK
58	56F	CN	c.68del	p.P23RfsX137	c.939_940insAAG	p.K313_V314insK
59	36M	CN	c.71_119del	p.H24RfsX120	c.949_950ins33	p.E316_L317ins11
60	18M	CN	c.134del	p.P45HfsX115	c.944_945insGGT	p.L315_E316insV
61	32M	CN	c.64del	p.P23RfsX137	c.939_940insAAG	p.K313_V314insK
62	28F	CN	c.247del	p.Q83SfsX77	c.939_940insAAG	p.K313_L315insV
63	34M	del(9)(q13q32)	c.284_285ins10	p.G96CfsX15	c.916_917insAGC	p.Q305_R306insQ
64	56F	sp: del (11)(q14q23)	c.247_248insC	p.Q83PfsX25	c.920_922dup	p.N307_V308insD
65	22F	sp:del(9q)	c.70_71insC	p.H24AfsX84	c.911_928del	p.K304_E309del
66	30F	sp:i(21q)	c.169del	p.E57SfsX103	c.951_952insCTG	p.L317_T318insL

67	49M	sp:+21	c.107_120delinsC	p.G36AfsX120	c.939_940insAAG	p.K313_V314insK
68	37M	sp: -Y	c.232del	p.L78WfsX82	c.962_963ins66	p.D320_N321ins22
69	48M	der(9)del(9)(p13p22) del(9)(q22q33)	c.157_194dup	p.S65RfsX10	c.911_912insGTT	p.L304_Q305insL

Abbreviations: CN, cytogenetically normal; NM, no mitosis; UPN, unique patient number

Supplementary Table 3. Additional mutations in the *CEBPA*^{dm} patients

UPN	Gene	Protein change	UPN	Gene	Protein change
4	***RUNX1***	R271K	36	***FLT3***	ITD
5	***ASXL1***	G646WfsX12		***FLT3***	TKD
	SRSF2	P95L	38	***GATA2***	A318V
	TET2	Y1628X/H1219N	39	***GATA2***	A318T
	TP53	G240E/R209Q		***KIT***	D816V
6	***ASXL1***	G646WfsX12	40	***FLT3***	ITD
	GATA2	R330Q	41	***GATA2***	G320V
7	***GATA2***	T354M	42	***GATA2***	A318G
	FLT3	ITD	43	***GATA2***	L321F
8	***TET2***	G1275E	44	***GATA2***	G320A
9	***TET2***	S1586X		***FLT3***	TKD
12	***FLT3***	ITD	46	***DNMT3A***	R882H
14	***FLT3***	ITD		***SF3B1***	K700E
15	***GATA2***	A318V		***TET2***	R1216X, W1198X
17	***NRAS***	G12D	48	***GATA2***	A318G
	TET2	R550X, W1847X	49	***GATA2***	R308P
	U2AF1	S34F		***NRAS***	G12D, G13D
19	***GATA2***	L321F	50	***WT1***	R369G
	NRAS	G13D	52	***GATA2***	R307Q
20	***GATA2***	A318T		***NRAS***	G13D
21	***GATA2***	K324E	53	***GATA2***	L321H, N317H
23	***DNMT3A***	P731L		***TET2***	S716YfsX35
	FLT3	ITD	54	***WT1***	K399fsX448
24	***WT1***	P355C	55	***GATA2***	A318V
25	***GATA2***	R330L		***NRAS***	G13D
	TET2	L668YfsX32		***FLT3***	ITD
26	***TET2***	R1359H	56	***FLT3***	TKD
27	***TET2***	R550X		***WT1***	R458X
	WT1	N381fsX450	57	***KRAS***	G13D
28	***GATA2***	T387_G392del		***NRAS***	G13D
	NRAS	G12D	59	***GATA2***	K324E
29	***WT1***	D377fsX384		***NRAS***	G12D
31	***GATA2***	A318V	60	***FLT3***	ITD

	RUNX1	K110Q	62	***WT1***	K399fsX400
32	***WT1***	Y402X	63	***FLT3***	ITD
34	***IDH1***	R132H	65	***GATA2***	A318T
	NRAS	Q61L	68	***GATA2***	G320V
35	***FLT3***	TKD	69	***NRAS***	Q61R

Supplementary Table 4. Prognostic factors for OS and DFS in total AML patients

	Overall survival		Disease-free survival	
	Multivariate P value	RR(CI)	Multivariate P value	RR(CI)
Age[a]	<0.0001	1.773 (1.368-2.296)	0.003	1.435 (1.131-1.820)
WBC[b]	<0.0001	1.932 (1.471-2.537)	<0.0001	1.757 (1.371-2.252)
Cytogenetics[c]	<0.0001	2.923 (2.014-4.242)	<0.0001	2.506 (1.763-3.563)
CEBPA[dm]	0.002	0.420 (0.246-0.718)	0.006	0.544 (0.351-0.842)
NPM1+/FLT3-ITD-[d]	<0.0001	0.389 (0.232- 0.652)	0.002	0.476 (0.300-0.753)
ASXL1	0.970	0.992 (0.653-1.507)	0.634	1.097 (0.749-1.608)
TP53	0.016	1.929 (1.129-3.295)	0.260	1.364 (0.795-2.339)
RUNX1	0.556	1.129 (0.753-1.694)	0.287	1.220 (0.846-1.758)
IDH2	0.015	0.577 (0.372-0.897)	0.058	0.691 (0.471-1.013)
DNMT3A	0.001	1.851 (1.307-2.621)	0.006	1.581 (1.144-2.185)
SF	<0.0001	2.127 (1.405-3.220)	<0.0001	2.159 (1.473-3.165)

Abbreviations: CI, confidence interval; RR, relative risk; SF, splicing factors, including *SF3B1*, *SRSF2*, *U2AF1*; WBC, white blood cell.

[a]Age≧50 relative to age <50 (the reference)

[b]WBC greater than 50 000/μl *vs*. less than 50 000/μl.

[c]Unfavorable cytogenetics *vs*. others.

[d]*NPM1*+/*FLT3*-ITD− *vs*. other subtypes.

Supplementary Table 5. Clinical characteristics of *CEBPA*^{dm} AML patients according to the status of *WT1* mutations[a]

Variables	*WT1* mutated N=8	*WT1* wild-type N=60	P value
Sex			>0.999
Male	5	35	
Female	3	25	
Age, years[b]	49 (28-69)	38.5 (17-90)	0.397
Lab[b]			
WBC (k/μL)	127.3 (3.3-387.4)	36.83 (2.41-380.2)	0.077
Hb (g/dL)	7.1 (3.0-13.6)	9.0 (5.7-12.7)	0.308
Platelet (k/μL)	43.5 (10-54)	40.5 (5-204)	0.761
PB Blast (k/μL)	74.0 (2.95-371.9)	17.3 (0.72-354.0)	0.094
LDH (U/L)	1369 (437-7747)	1037 (375-11329)	0.381
FAB[c]			
M0	0	0	-
M1	5 (62.5)	34 (56.7)	>0.999
M2	3 (37.5)	23 (38.3)	>0.999
M3	0	0	-
M4	0	3 (5.0)	>0.999
M5	0	0	-
M6	0	0	-
Induction Response[d]	7	54	
Complete Resonse	5 (71.4)	50 (92.5)	0.140
Relapse	4 (80.0)	17 (34.0)	0.047

Abbreviation: PB, peripheral blood

[a] *WT1* mutation data was available in 68 of 69 *CEBPA*^{dm} patients.

[b] Median (range)

[c] Number of patients (%)

[d] Only the 61 patients who received conventional intensive induction chemotherapy and then consolidation chemotherapy if CR was achieved, as mentioned in the text, were included in the analysis.

Supplementary Table 6. Sequential studies of *WT1* mutations in AML patients[a]

UPN	Date	Status	*WT1* mutation AA change	Mutation burden	Other mutations
24	2011/2/7	Initial	P355C	33.2	-
	2011/4/26	CR1	-	-	-
	2011/11/23	Relapse	P355C	5.6	
29	2005/5/20	Initial	D377fsX384	11.71	-
	2005/6/22	CR1	-	-	-
	2006/5/16	Relapse	D377fsX384	61.55	-
32	2007/7/9	Initial	Y402X	47.04	
	2007/9/11	CR1	-	-	
70	2000/7/27	Initial	-	-	*NPM1*
	2000/11/10	CR1	-	-	-
	2001/7/17	Relapse	D379fsX386	20.7	*NPM1*
71	2002/4/18	Initial	-	-	
	2002/5/13	CR1	-	-	
	2002/11/19	Relapse	R471T	38.3	
72	1995/6/16	Initial	-	-	*NRAS*, *FLT3*-ITD
	1996/6/3	CR1	-	-	*NRAS*, *FLT3*-ITD
	1996/11/15	Relapse	H465Y	41.6	*NRAS*, *FLT3*-ITD

[a]Only patients with *WT1* mutation at diagnosis, relapse or both are shown and those without the mutation at both diagnosis and relapse are not shown.

Supplementary Table 7. Prognostic impact of concomitant mutations in *CEBPA*^{dm} patients

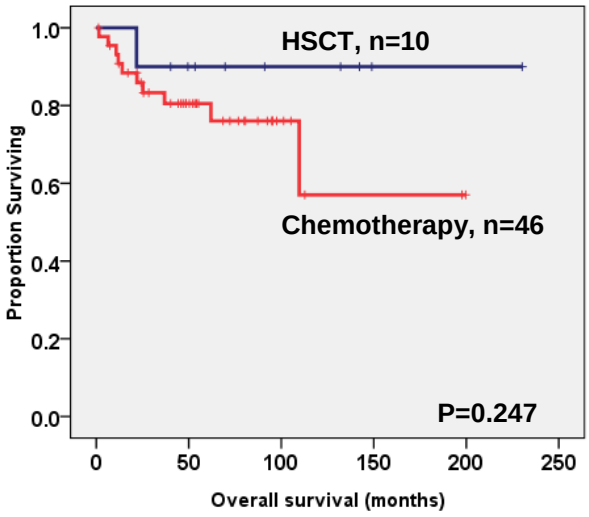
	No. of patients	CR1 (%)	Relapse (%)	Relapse in patients with C/T alone (%)	CR2 (%)
Total pts	62	56 (90.2)	21 (37.5)	21/46 (45.6)	14/21 (66.7)
GATA2	21	20 (95.2)	6 (30.0)	6/16 (37.5)	5/6 (83.3)
FLT3-ITD	8	6 (75.0)	1 (16.7)	1/3 (33.3)	1/1 (100)
NRAS	9	8 (88.9)	2 (25.0)	2/3 (67.7)	2/2 (100)
TET2	7	6 (85.7)	1 (16.7)	1/6 (16.7)	1/1 (100)
WT1	7	5 (71.4)	4 (80.0)*	4/4 (100)*	1/4 (25)

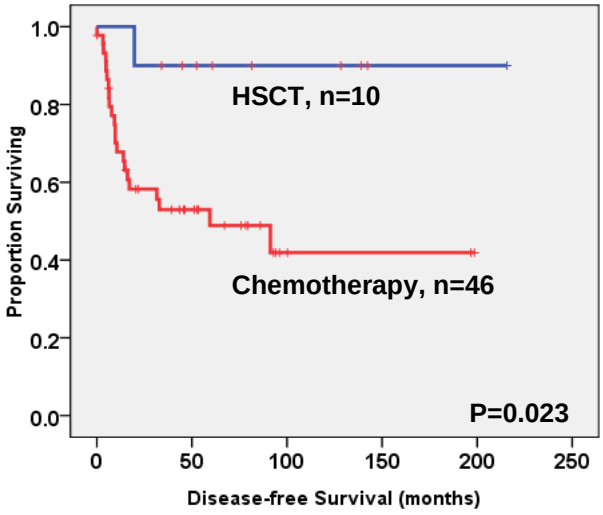
Abbreviations: C/T, chemotherapy; CR, complete remission

*denotes statistically significance compared to those without the mutation (P<0.05)

Supplementary Figure 1.
Kaplan-Meier plots for (A) OS and (B) DFS in *CEBPA*^{dm} patients according to transplantation or not in first CR

(A)

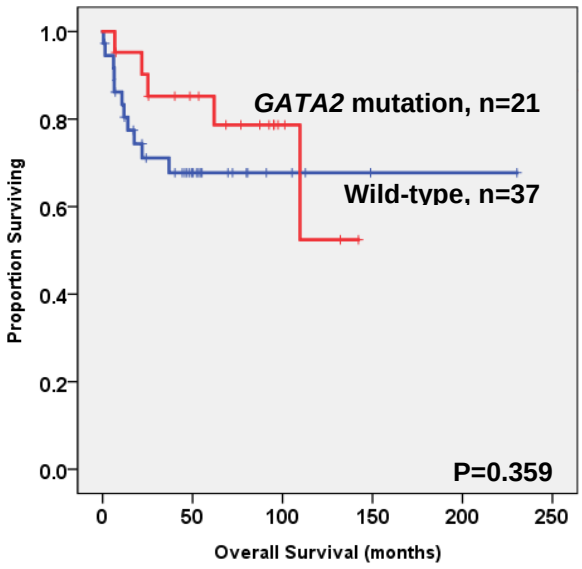


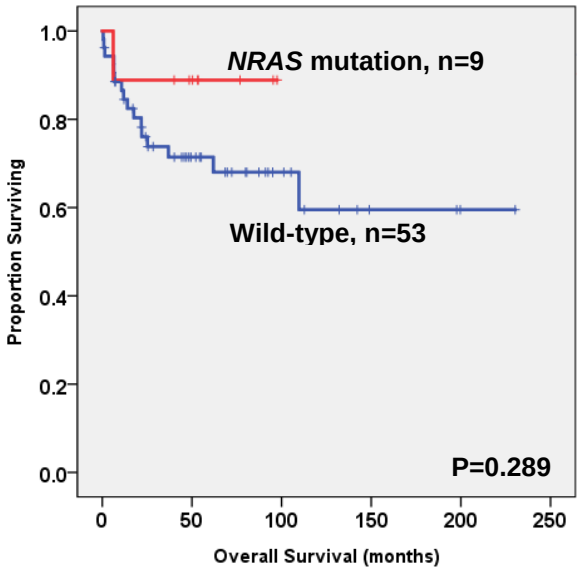


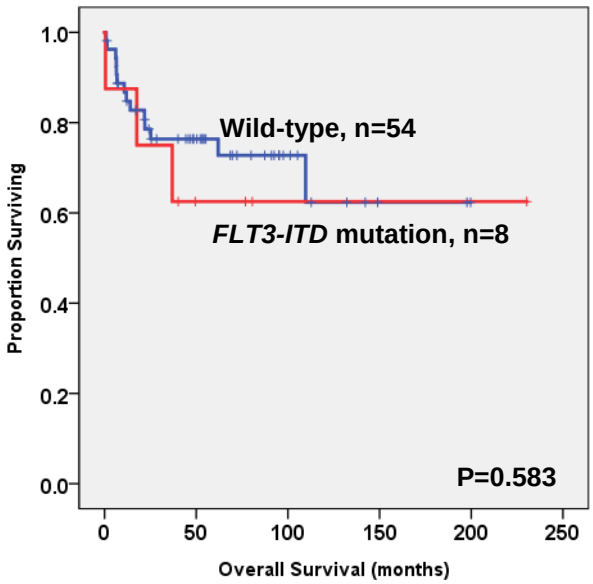
Supplementary Figure 2.

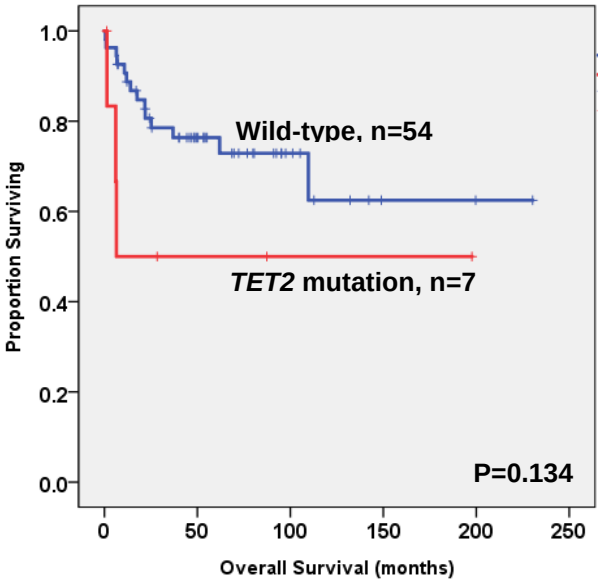
Kaplan-Meier plots for (A) OS and (B) DFS according to the status of different concomitant mutations

(A)

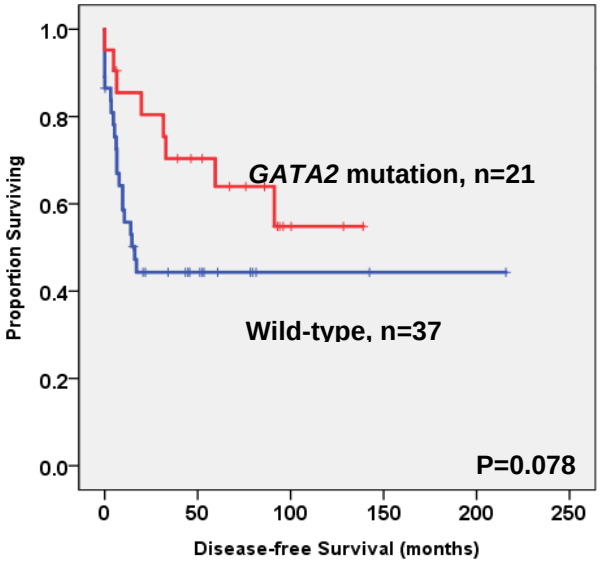


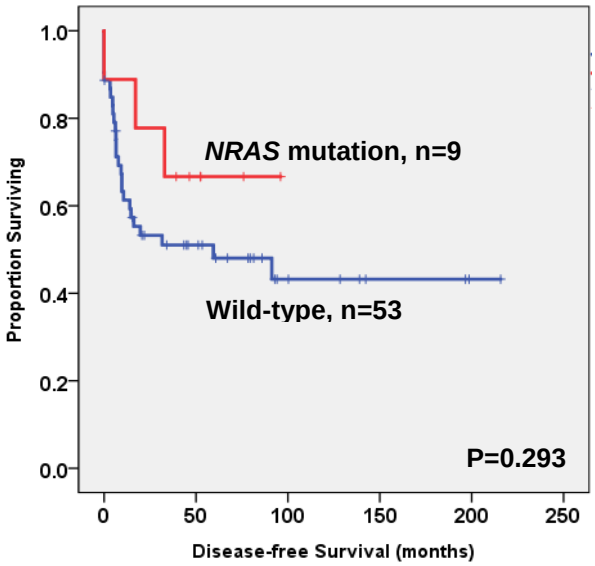


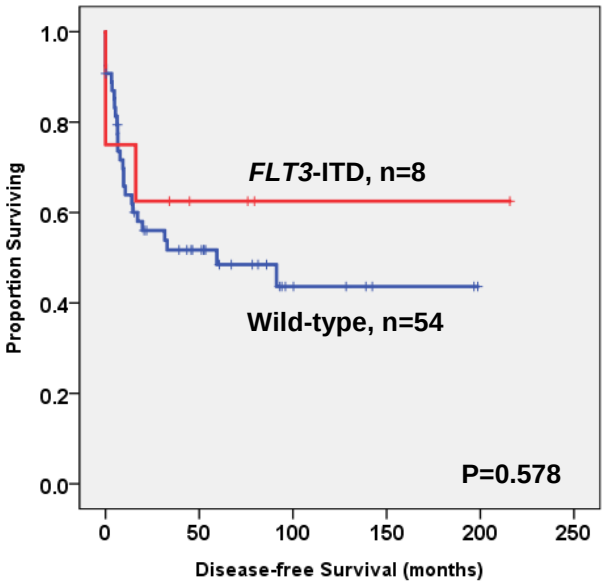


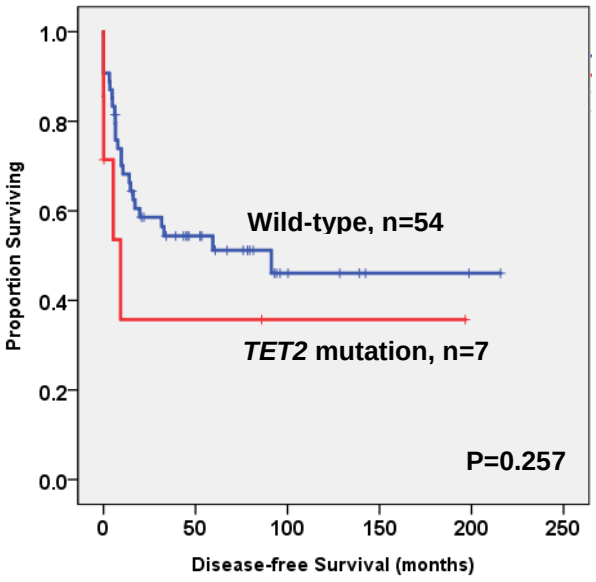


(B)


Proportion Surviving
1.0
0.8
0.6
0.4
0.2
0.0
GATA2 mutation, n=21
Wild-type, n=37
P=0.078
0
50
100
150
200
250
Disease-free Survival (months)


Proportion Surviving
1.0
0.8
0.6
0.4
0.2
0.0
NRAS mutation, n=9
Wild-type, n=53
P=0.293
0
50
100
150
200
250
Disease-free Survival (months)


Proportion Surviving
1.0
0.8
0.6
0.4
0.2
0.0
FLT3-ITD, n=8
Wild-type, n=54
P=0.578
0
50
100
150
200
250
Disease-free Survival (months)


Proportion Surviving
1.0
0.8
0.6
0.4
0.2
0.0
Wild-type, n=54
TET2 mutation, n=7
P=0.257
0
50
100
150
200
250
Disease-free Survival (months)