Clonal leukemic evolution in myelodysplastic syndromes with *TET2* and *IDH1/2* mutations

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Supplementary Data

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Figure S1. Chromatograms of patient T-016 at MDS and sAML phases and his anal mucosal biopsy. The point mutation, M638V, was located outside the conserved region of *TET2* gene, but was not present in the DNA derived from anal mucosa. This indicated that M638V was an acquired mutation. The left two panels showed that this mutation was present both at MDS and sAML phases and the mutant levels were comparable.

Figure S2. Chromatograms of patient T-018 at MDS and sAML phases and her skin biopsy. The point mutation, R814C, was located outside the conserved region of *TET2* gene, but was not present in the DNA derived from skin. This indicated that R814C was an acquired mutation. The upper left two and the lower panels showed that two mutations in patient T-018 were present both at MDS and sAML phases and the mutant levels were comparable.

Supplementary Table S1. Sequences of primer pairs, sequencing primers, and sequences for pyrosequencing

Patient No.	Gene	Mutation	Primer name	Primer sequence (5'-3')	Sequencing primer (5'-3')	Sequence to analyze
T-012	*TET2*	Q916X	M12A-F M12A-R-bio	AAATGGGACTGGAGGAAGTACAGA biotin-GGTCAGGCACAGGAAAAACATT	GCTGCGCAACTTGCT	C/TAGCAAAG GTACTTGATA CATAACCA
T-012	*TET2*	S231fs	M12B-F M12B-R-bio	GGTGCTACAGTTTCTGCCTCTTCC biotin-TCTGCACCGCAATGGAAA	ACTCCTGGAAAAAACACT	[T]GTCTCAA TATTATCCAG ATTGTGTT
T-024	*TET2*	A1512fs	M24-F M24-R-bio	AAGACTTGCCGACAAAGGAAACTA biotin-TACAAGTTGATGGGGGCAAAA	AAGCCAGGCTAAACAGT	TGG[C]AGGT AAATTTAATG TAAAGCATTT G
T-099	*TET2*	Q635X	M99-F M99-R-bio	GGCTCCCAAGGCAAGCTTA biotin-GGAGATGTTGGTCCACTGTACCT	AGCACAAGTCACAAATGTA	CC/TAAGTTG AAATGAATCA AGGGCAGTC
T-027	*IDH1*	R132H	IDH1-R132-F-bio IDH1-R132-gR	biotin-CGGTCTTCAGAGAAGCCATT TTGCCAACATGACTTACTTGATCC	TGATCCCCATAAGCAT	GAC/TGACCTATGATGATAGG
T-027	*JAK2*	V617F	JAK2-200F JAK2-200R-bio	GCAGAGAGAATTTTCTGAACTAT biotin-CTCTGAGAAAGGCATTAGAAAG	GGTTTTAAATTATGGAGTATGT	G/TTCTGTGG
T-099	*RUNX1*	A224fs	RUNX1-pyro668-F RUNX1-pyro668-R-bio	CTTGTCCTTTTCCGAGCG biotin-AGTGGGCTCCATCTGGTAC	CACGCCCAACCCTCG	[GG]TGCCTC CCTGAACCAC TCCACTGCC
T-015	*DNMT3A*	R771*	DNMT3A-pyroE19-F2 DNMT3A-pyroEX19-R2-bio	GAGCCACACCACTGTCCTATGC Biotin-CTGGCCAAACCAAGGTTGCT	GTGACAAGAGGGACATC	TCGC/TGATT TCTCGAGGTA TAGCCAGCA
T-027	*DNMT3A*	L647Vfs*2	DNMT3A-pyroE17-F DNMT3A-pyroE17-R-bio	CAGGCTCACCTGCCGAGA Biotin-TCCAGGTGCTGAGTGTGC	GGACCGCTACATTGC	CTCGG/TAGGTGTG TGAGGACTCC
T-035	*DNMT3A*	S663Rfs*42	DNMT3A-pyroE17-F DNMT3A-pyroEX17-R-bio	CAGGCTCACCTGCCGAGA Bio-TCCAGGTGCTGAGTGTGC	GGACCGCTACATTGC	C[T]CGGAGG TGTGTGAGGA CTCC
T-002	*ASXL1*	S847Ifs*4	AXSL1-pyroE12-2-F-bio AXSL1-pyroE12-2a-R	biotin-GCCAAGCTCTTGACAGTCATCC GTTTGGGCTGTTTCACTACCTCA	TTCAAGTTTTCTTGTCTAGT	[T]ATCACTT TCCCTCATAG GAGGG
T-022	*ASXL1*	E635Rfs*15	AXSL1-pyroE12-1-F-bio AXSL1-pyroE12-1-R	biotin-CCTAGGTCAGATCACCCAGTCAGTTA CCACAGGCCTCACCACCAT	ACCCCCCCCTCCGAT	GGCAGTGGTG A/GC

Supplementary Table S2. Characteristics of patients with myelodysplastic syndrome according to the presence or absence of *TET2* mutation

Features	*TET2* mutation		
	Mutated (N=31)	Not mutated (N=137)	*P*
Age (year)*	71.4 (67.6-75.1)	62.9 (60.3-65.5)	< 0.001
Sex (M/F)[†], No. (%)	22 (71)/9 (29)	91 (66)/46 (34)	0.678
Hb (g/dL)*	8.2 (7.1-9.2)	7.9 (7.5-8.4)	0.677
Platelet (x 10^9/L)*	83 (51- 115)	138 (106-169)	0.016
WBC (x 10^9/L)*	5.1 (3.5-6.7)	3.9 (3.3-4.5)	0.082
BM blasts (%)*	7.8 (5.9-9.7)	8.0 (7.0-9.0)	0.865
PB blasts (%)*	1.3 (0.5-2.1)	1.4 (0.8-2.0)	0.889
WHO subtypes[†]			0.972
RAEB-1, No. (%)	11 (36)	43 (31)	
RAEB-2, No. (%)	11 (36)	54 (39)	
RCMD, No. (%)	7 (23)	31 (23)	
Others, No. (%)	2 (7)	9 (7)	
IPSS [†] (No.)			0.220
Int-2/High, No. (%)	12 (40)	65 (54)	
Low/Int-1, No. (%)	18 (60)	55 (46)	
IPSS-R[†]			0.783
VL/L, No. (%)	4 (15)	18 (19)	
I/H, No. (%)	15 (58)	48 (50)	
VH	7 (27)	30 (31)	
sAML evolution (+/-)[†], No. (%)	18 (58)/ 13 (42)	60 (44)/77 (56)	0.167

Abbreviations: BM: bone marrow; Hb: hemoglobin; I/H: intermediate and high IPSS-R risk groups; IPSS-R: Revised International Prognostic Scoring System; PB: peripheral blood; sAML: secondary acute myeloid leukemia; VH: very high IPSS-R risk groups; VL/L: very low and low IPSS-R risk groups; WBC: white blood cell

* t-test and values representing mean (95% confidence interval)

[†] Fisher's exact or Chi-square tests

Supplementary Table S3. Characteristics of patients with myelodysplastic syndrome according to the presence or absence of *IDH1/2* mutations

Features	*IDH1/2* mutation				
	Mutated (N=17)		Not mutated (N=151)		*P*
Age (year)*	71.2	(67.8-74.7)	63.6	(61.1-66.2)	0.001
Sex (M/F)†, No. (%)	14 (82.4)/3 (17.6)		99 (65.6)/52 (34.4)		0.186
Hb (g/dL)*	8.9	(7.6-10.1)	7.9	(7.5-8.3)	0.095
Platelet (x 10^9/L)*	185	(47-323)	119	(94-144)	0.329
WBC (x 10^9/L)*	5.2	(2.3-8.2)	4.0	(3.5-4.5)	0.408
BM blasts (%)*	9.9	(7.4-12.5)	7.7	(6.8-8.6)	0.107
PB blasts (%)*	2.2	(0.5-3.9)	1.3	(0.8-1.8)	0.217
WHO subtypes†					0.351
RAEB-1, No. (%)	6 (35.3)		48 (31.8)		
RAEB-2, No. (%)	9 (52.9)		56 (37.1)		
RCMD, No. (%)	2 (11.8)		36 (23.8)		
Others, No. (%)	0 (0)		11 (7.3)		
IPSS-R†					0.170
VL/L, No. (%)	2 (17)		20 (18)		
I/H, No. (%)	9 (75)		54 (49)		
VH, No. (%)	1 (8)		36 (33)		
sAML evolution (+/-)†, No. (%)	10 (58.8)/7 (41.2)		68 (45.0)/83 (55.0)		0.314

Abbreviations: BM: bone marrow; Hb: hemoglobin; I/H: intermediate and high IPSS-R risk groups; IPSS-R: Revised International Prognostic Scoring System; PB: peripheral blood; sAML: secondary acute myeloid leukemia; VH: very high IPSS-R risk groups; VL/L: very low and low IPSS-R risk groups; WBC: white blood cell

* t-test and values representing mean (95% confidence interval)

† Fisher's exact or Chi-square tests

Supplementary Table S4. Characteristics of patients with myelodysplastic syndrome according to the presence of *TET2* or *IDH1/2* mutations

Features	*TET2* mutated (N=31)		*IDH1/2* mutated (N=17)		*P*
Age (year)*	71.4	(67.6-75.1)	71.2	(67.8-74.7)	0.962
Sex (M/F)[†], No. (%)	22 (71)/ 9 (29)		14 (82.4)/ 3 (17.6)		0.497
Hb (g/dL)*	8.2	(7.1-9.2)	8.9	(7.6-10.1)	0.392
Platelet (x 10^9/L)*	83	(51- 115)	185	(47-323)	0.142
WBC (x 10^9/L)*	5.1	(3.5-6.7)	5.2	(2.3-8.2)	0.951
BM blasts (%)*	7.8	(5.9-9.7)	9.9	(7.4-12.5)	0.174
PB blasts (%)*	1.3	(0.5-2.1)	2.2	(0.5-3.9)	0.263
WHO subtypes[†]					0.460
RAEB-1, No. (%)	11 (36)		6 (35.3)		
RAEB-2, No. (%)	11 (36)		9 (52.9)		
RCMD, No. (%)	7 (23)		2 (11.8)		
Others, No. (%)	2 (7)		0 (0)		
IPSS-R[†]					0.418
VL/L, No. (%)	4 (15)		2 (17)		
I/H, No. (%)	15 (58)		9 (75)		
VH	7 (27)		1 (8)		
sAML evolution (+/-)[†], No. (%)	18 (58)/ 13 (42)		10 (58.8)/ 7 (41.2)		1.000

Abbreviations: BM: bone marrow; Hb: hemoglobin; I/H: intermediate and high IPSS-R risk groups; IPSS-R: Revised International Prognostic Scoring System; PB: peripheral blood; sAML: secondary acute myeloid leukemia; VH: very high IPSS-R risk groups; VL/L: very low and low IPSS-R risk groups; WBC: white blood cell

* t-test and values representing mean (95% confidence interval)

[†] Fisher's exact or Chi-square tests

Supplementary Table S5. Univariate and multivariate analysis of time to sAML and overall survival in RAEB-2 and IPSS-R very high risk patients with myelodysplastic syndromes

RAEB-2

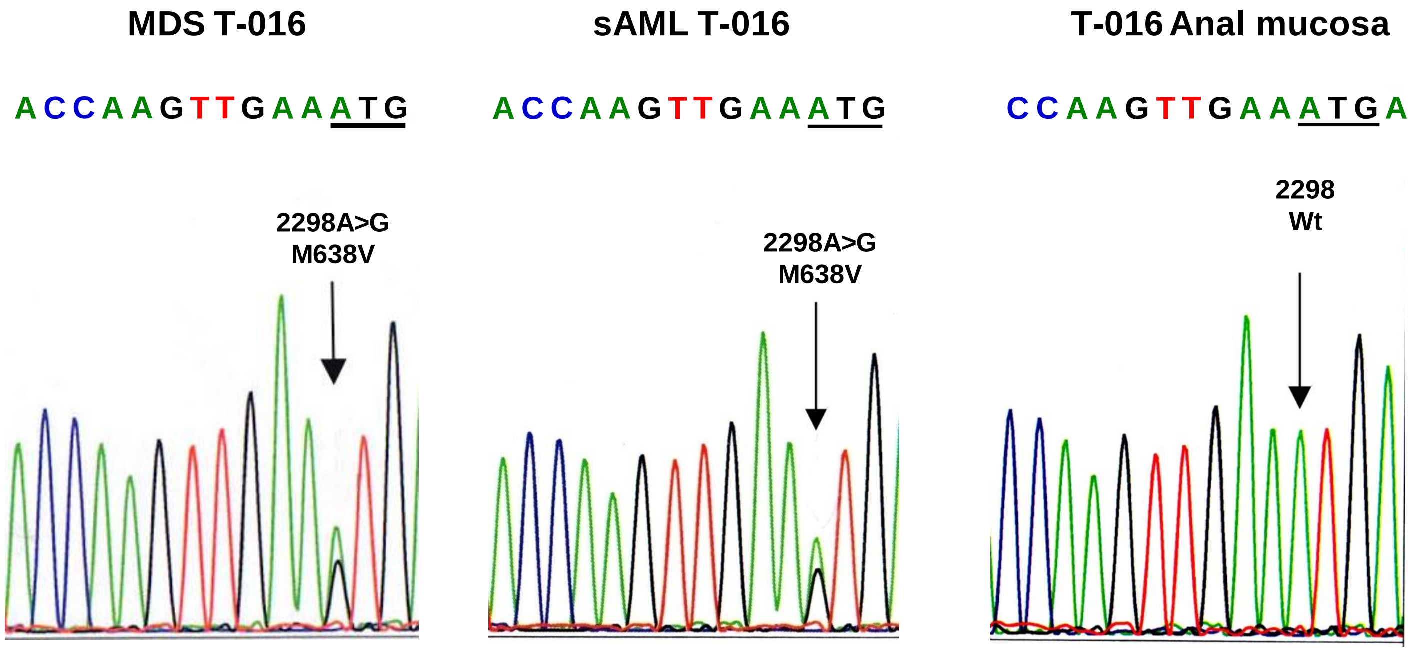
Genes	Time to sAML		Overall Survival			
	Univariate		Univariate		Multivariate	
	P	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)
TET2	0.001	4.52 (1.66 – 12.30)	0.026	2.24 (1.08 – 4.68)	0.044	9.02 (1.06 – 76.66)
TP53	0.236	2.83 (0.46 – 17.47)	0.004	3.78 (1.43 – 9.99)	0.312	2.53 (0.42 – 15.27)

IPSS-R very high risk group

Genes	Time to sAML		Overall Survival	
	Univariate		Univariate	
	P	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)
TET2	<0.001	4.97 (1.89 – 13.09)	0.082	1.90 (0.91 – 3.99)
TP53	0.390	0.47 (0.08 – 2.69)	0.592	1.34 (0.46 – 3.90)

Abbreviations: sAML: secondary acute myeloid leukemia; RAEB-2: refractory anemia with excess blasts-2; IPSS-R: revised International Prognostic Scoring System; CI: confidence interval

Supplementary Figure S1



Supplementary Figure S2