Supplementary Table 1. Primer sequences and PCR conditions. The first four assays are for bisulfite-pyrosequencing. The next four are for mutation analysis.

Gene	Step	Annealing Temp. (°C)	Sequence 5' to 3'	5'-modified
SP140 (hg18, Chr2:230,798,573)	1	58	AGTTAAGGGAGGAGGAGTAGAGTT	
			CCTTAACAAAAACAAATAACCCTATC	
	2	60	AGTTAAGGGAGGAGGAGTAGAGTT	
			GGGACACCGCTGATCGTTTACCTTAACAAAAACAAATAACCCTATC	
			GGGACACCGCTGATCGTTTA	Biotin
	S		GGAGGAGGAGTAGAGTTAGT	
MCCC1 (hg18, Chr3:184,245,211)	1	56	GAATGATGGTTTGGTTTAGAATGT	
			TCAAATTCACTTCCCCCTAA	
	2	58	GAATGATGGTTTGGTTTAGAATGT	
			GGGACACCGCTGATCGTTTATCAAATTCACTTCCCCCTAA	
			GGGACACCGCTGATCGTTTA	Biotin
	S		AATTTTATTTGTTGGTTGTT	
EHMT1 (hg18, Chr9:	1	58	TGTAAGGGTAGGAGGGGTTGA	
			TTCCCTCCACTCTTAAAACTTTCT	

139,803,373)			TGTAAGGGTAGGAGGGGTTGA	
	2	60	GGGACACCGCTGATCGTTTATTCCCTCCACTCTTAAAACTTTCT	
			GGGACACCGCTGATCGTTTA	Biotin
	S		GTTGTTTTTAGATTTATAT	
MTSS1 (hg18, Chr8:125,683,079)	1	56	AAGTTTTAAATTGGTAGGGGTTTT	
			AATATACCCAACCTTACCCTACTC	
	2	58	AAGTTTTAAATTGGTAGGGGTTTT	
			GGGACACCGCTGATCGTTTAAATATACCCAACCTTACCCTACTC	
			GGGACACCGCTGATCGTTTA	Biotin
	S		GGGGTTTTTTTATTTTGA	
IDH1 (R132)	1	58	TGCCAACATGACTTACTTGATCC	
			AATATCCCCCGGCTTGTGA	Biotin
	S		TGATCCCCATAAGCAT	
IDH2 (R140)	1	58	TAGGCGTGGGATGTTTTTG	
			CAGAGTTCAAGCTGAAGAAGATGT	Biotin
	S		CCCCCCAGGATGTTC	
IDH2 (R172)	1	58	TGCCCAGGTCAGTGGATC	
			GGAGCCCATCATCTGCAAA	Biotin

	S		**TCGCCATGGGCGTGC**	
DNMT3A (R882)	**1**	**58**	**TGTGGTTAGACGGCTTCC** **GGGACACCGCTGATCGTTTAGAAGAGGTGGCGGATGA** **GGGACACCGCTGATCGTTTA**	**Biotin**
	S		**TGACGTCTCCAACATGA**	

Supplementary Table 2. Patient Characteristics for tet2-DMC-low and high defined by the clinically applicable thresholds*

Characteristics	tet2-DMC-Low	tet2-DMC-High	P value
Total number	**67**	**113**	
Age, years			
Mean	**51**	**49**	**0.66**
(Range)	**(19-68)**	**(17-73)**	
Gender:			
Male – no. (%)	**30 (45)**	**55 (49)**	**0.65**
Bone marrow blasts at diagnosis			**0.57**
Mean	**53**	**55**	
(Range)	**(7-94)**	**(16-99)**	
WBC at diagnosis (*10^3/uL)			
Mean	**21**	**26**	**0.96**
(Range)	**(1-129)**	**(1-228)**	
Cytogenetic risk group – no. (%)			**0.07**
Favorable	**3 (5)**	**0 (0)**	
Intermediate	**39 (59)**	**65 (59)**	
Poor	**24 (36)**	**45 (41)**	
Antecedent hematologic disorder–	**13 (19)**	**23 (20)**	**1**
Complete remission rate – no. (%)	**56 (84)**	**78 (69)**	**0.03**
Overall survival (months)			
Median	**79+**	**14**	**0.0006**
(Range)	**(0-79+)**	**(0-82+)**	
Mutations – no. (%)			
FLT3-ITD	**14 (22)**	**20 (18)**	**0.55**
FLT3-TKD	**3 (5)**	**10 (9)**	**0.38**
RAS	**6 (10)**	**16 (17)**	**0.25**
NPM1	**16 (28)**	**17 (18)**	**0.22**
IDH1	**2 (3)**	**6 (5)**	**0.71**
IDH2	**5 (8)**	**8 (7)**	**1**
IDH1/2	**7 (11)**	**13 (12)**	**1**

DNMT3A	**10 (16)**	**10 (9)**	**0.22**

***The P value were computed from Fisher's exact test for two-by-two contingency analyses, Mann-Whitney test to compare continuous variables (Age, Bone marrow blasts at diagnosis, and WBC at diagnosis), and the log-rank test for survival data. All p values were two-tailed and the threshold of statistical significance was P<0.05. tet2-DMCs = TET2-specific differentially methylated CpGs.**

Supplementary Table 3. Patient Characteristics for tet2-DMC-high with and without TET2/IDH mutations*

Characteristics	With mutations	Without mutations	P value
Age, years			
Mean	**61**	**60**	**0.52**
(Range)	**(27-77)**	**(18-88)**	
Gender:			
Male – no. (%)	**21 (51)**	**59 (56)**	**0.06**
Bone marrow blasts at diagnosis (%)			**0.32**
Mean	**48**	**29**	
(Range)	**(0-97)**	**(0-98)**	
WBC at diagnosis (*10^3/uL)			
Mean	**15**	**20**	**0.47**
(Range)	**(1-172)**	**(1-298)**	
Cytogenetic risk group – no. (%)			**0.36**
Favorable	**0 (0)**	**5 (5)**	
Intermediate	**29 (73)**	**70 (67)**	
Poor	**11 (27)**	**30 (28)**	
Mutations – no. (%)			
FLT3-TKD	**9 (24)**	**31 (31)**	**0.53**

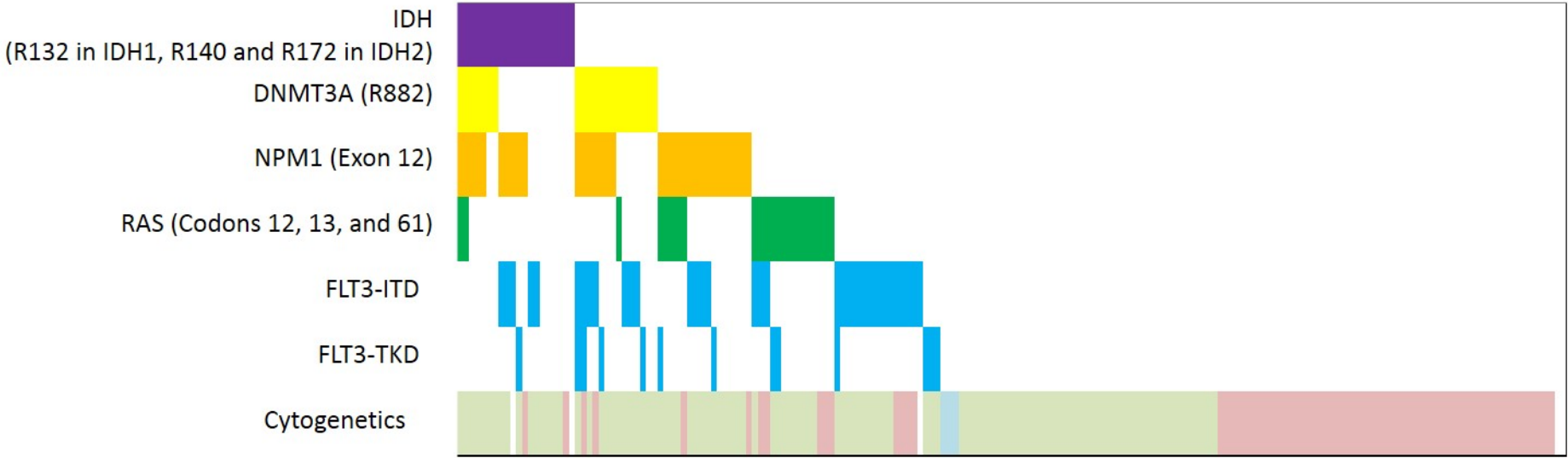
RAS	**2 (5)**	**7 (7)**	**1.0**
NPM1	**14 (36)**	**23 (22)**	**0.13**
ASXL1	**2 (9)**	**1 (3)**	**0.55**
DNMT3A	**6 (28)**	**11 (33)**	**0.77**
CEBPA	**2 (9)**	**4 (12)**	**1.0**

***The P value were computed from Fisher's exact test for two-by-two contingency analyses, Mann-Whitney test to compare continuous variables (Age, Bone marrow blasts at diagnosis, and WBC at diagnosis), and the log-rank test for survival data. All p values were two-tailed and the threshold of statistical significance was P<0.05.**

tet2-DMCs = TET2-specific differentially methylated CpGs.

Supplementary Figure 1. Mutation co-occurrence for 186 AML patients. Shown are somatic, nonsynonymous mutations in individual genes and sets of genes. Eighty-one (43%) had at least one mutation in one of the listed genes or sets. The cytogenetic risk for each patient is shown at the bottom of the chart. Blue, Favorable; Green, Intermediate; Red, Poor risk groups.

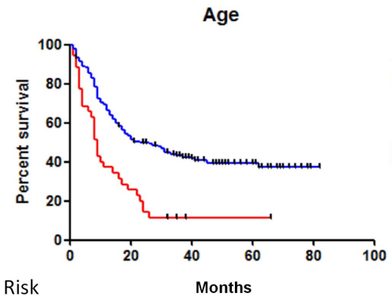
Supplementary Figure 1



Supplementary Figure 2. Analysis of OS for 186 AML patients. A) Univariate analyses of OS by clinical characteristics. Kaplan-Meier survival curves were drawn for each covariate. Age, cytogenetics, and AHD are associated with OS. P values are derived from the log-rank test. B) Univariate analyses of OS by genetic alterations. Kaplan-Meier survival curves were drawn for each gene. Only NPM1 mutations are associated with longer OS (P=0.01). P values are derived from the log-rank test.

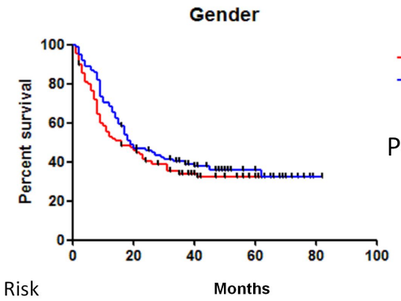
Supplementary Figure 2

A



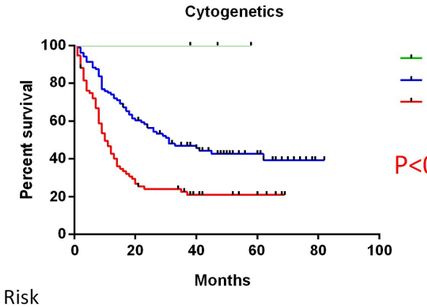
P<0.0001

No. at Risk				
>60	35	10	2	2
<60	153	80	54	49



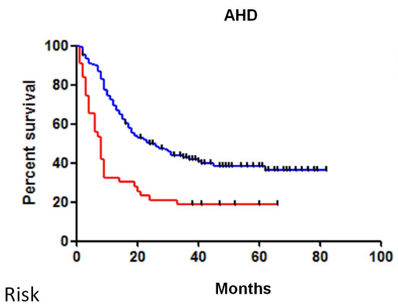
P=0.30

No. at Risk				
Male	90	41	22	10
Female	98	47	28	11



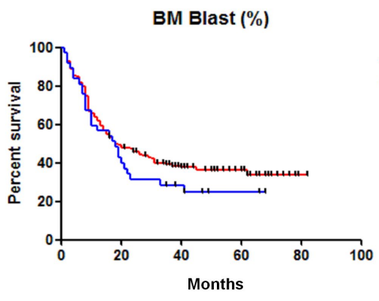
P<0.0001

No. at Risk				
Favorable	3	3	3	3
Intermediate	104	62	38	8
Poor	76	22	11	2



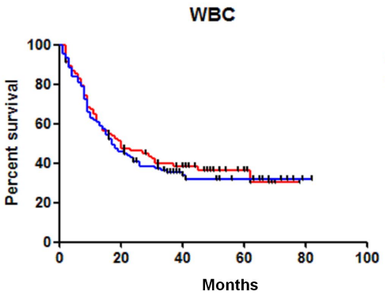
P<0.0001

No. at Risk				
Yes	43	12	8	3
No	145	76	43	18



P=0.28

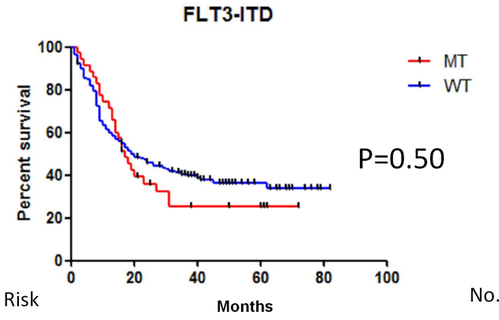
No. at Risk				
>30%	151	73	42	18
<30%	37	15	9	4



P=0.65

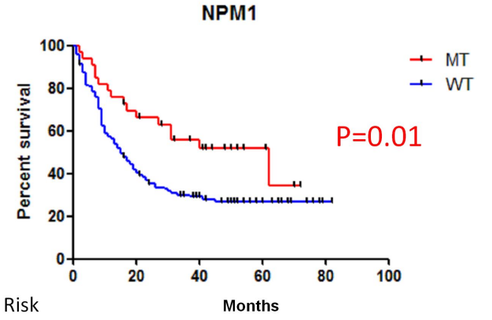
No. at Risk				
>10,000	83	41	27	9
<10,000	105	49	23	14

B



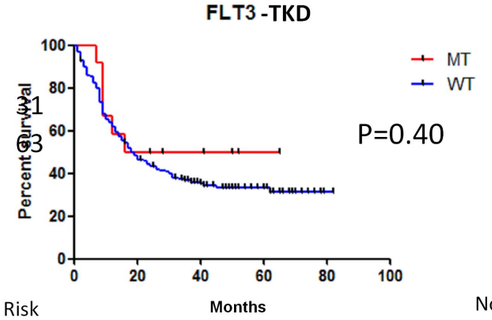
P=0.50

No. at Risk				
MT	35	14	7	5
WT	145	71	43	16



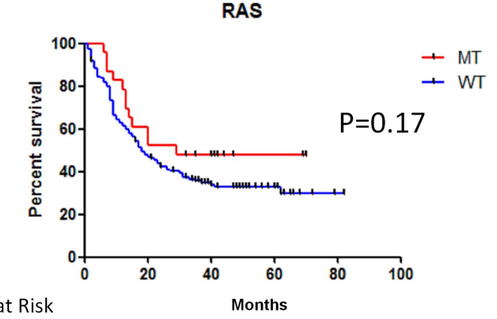
P=0.01

No. at Risk				
MT	33	22	13	5
WT	125	51	30	14



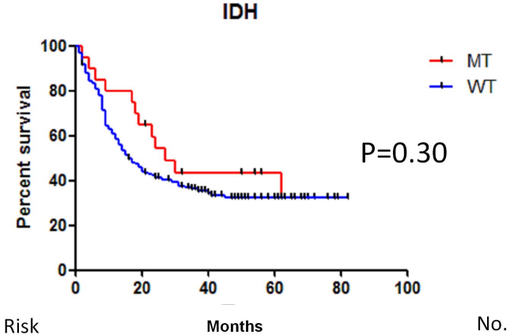
P=0.40

No. at Risk				
MT	12	7	5	2
WT	169	79	45	19



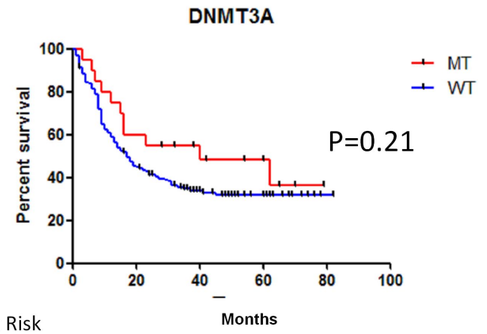
P=0.17

No. at Risk				
MT	23	14	9	2
WT	143	66	35	5



P=0.30

No. at Risk				
MT	20	14	8	5
WT	164	75	41	15

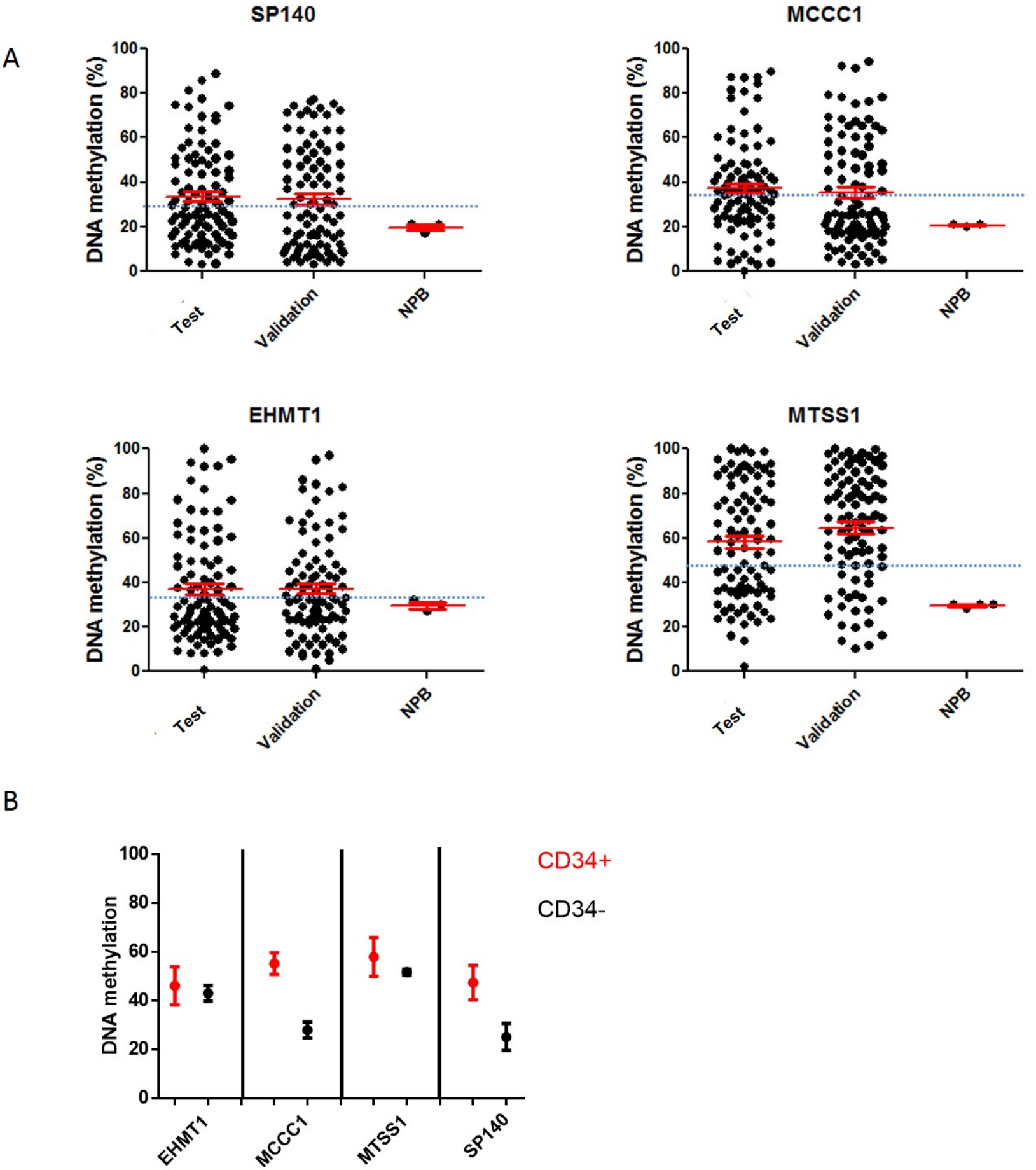


P=0.21

No. at Risk				
MT	20	14	8	5
WT	161	71	39	15

Supplementary Figure 3. DNA methylation status of 4 tet2-DMCs (a CpG site close (<50 bp) to the transcription start site of SP140 and CpG sites in gene-bodies of MCCC1, EHMT1, and MTSS1) in AML patients in the test and validation cohorts and normal peripheral blood (NPB) A) Mean ± SEM are shown. All 4 loci showed significant hypermethylation in AML compared to NPB. Note that there are some patients with tet2-DMC equal or lower than NPB. Horizontal blue lines represent the thresholds used for the clinically applicable tet2-DMC signature. B) DNA methylation status of 4 tet2-DMCs in CD34+ and 34- cells.

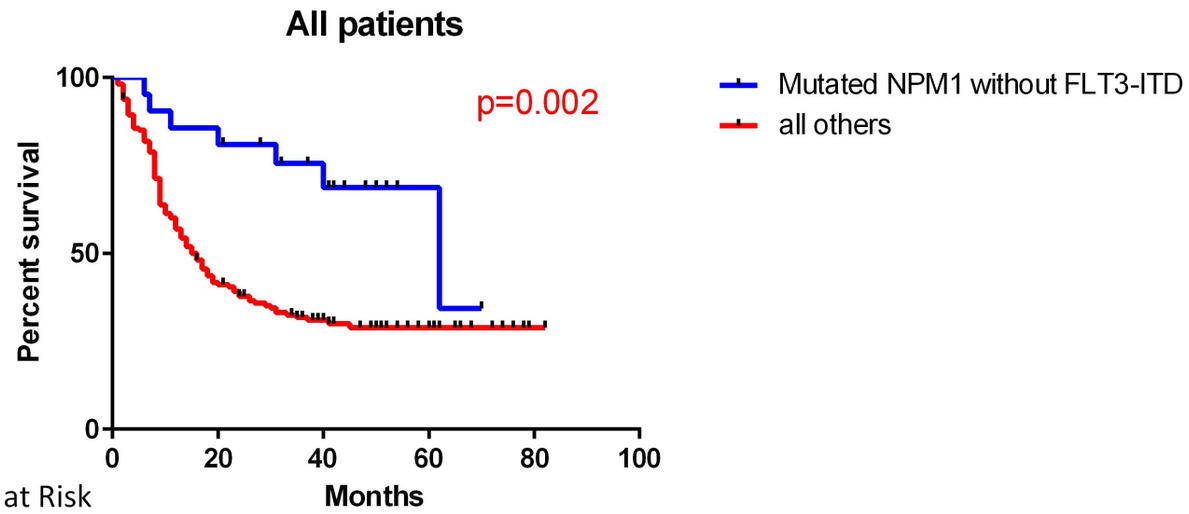
Supplementary Figure 3



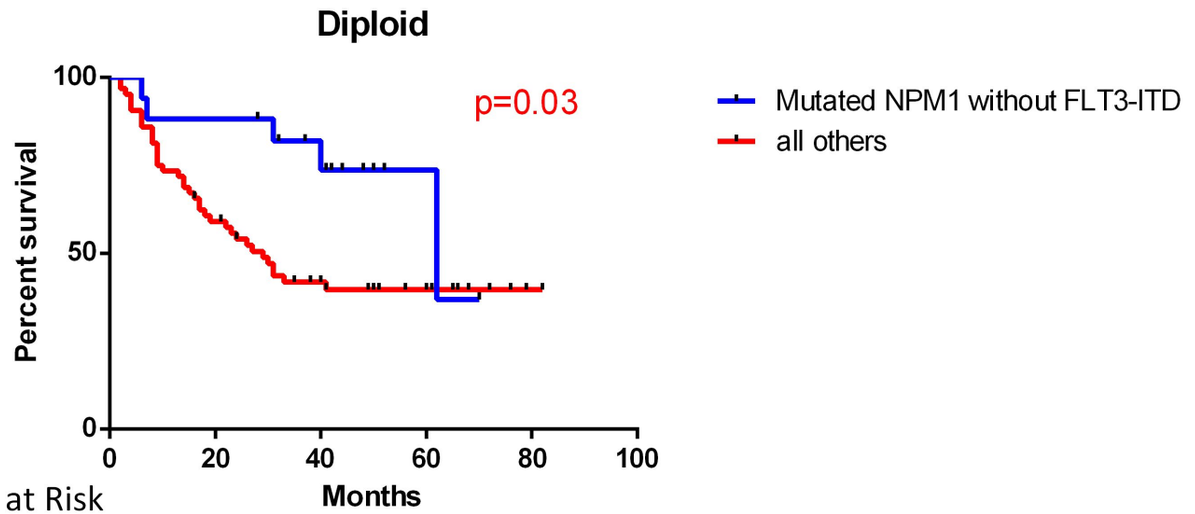
Supplementary Figure 4. Kaplan-Meier survival curves for patients with NPM1 mutation but no FLT3-ITD (N+F-) compared to other patients in the combined dataset A) and only among diploid patients B) in all patients (A). Kaplan-Meier survival curves for tet2-DMC-low (blue) and –high (red) patients in (N+F-) patients C) and all patients except for (N+F-) patients D) P values are derived from the log-rank test.

Supplementary Figure 4

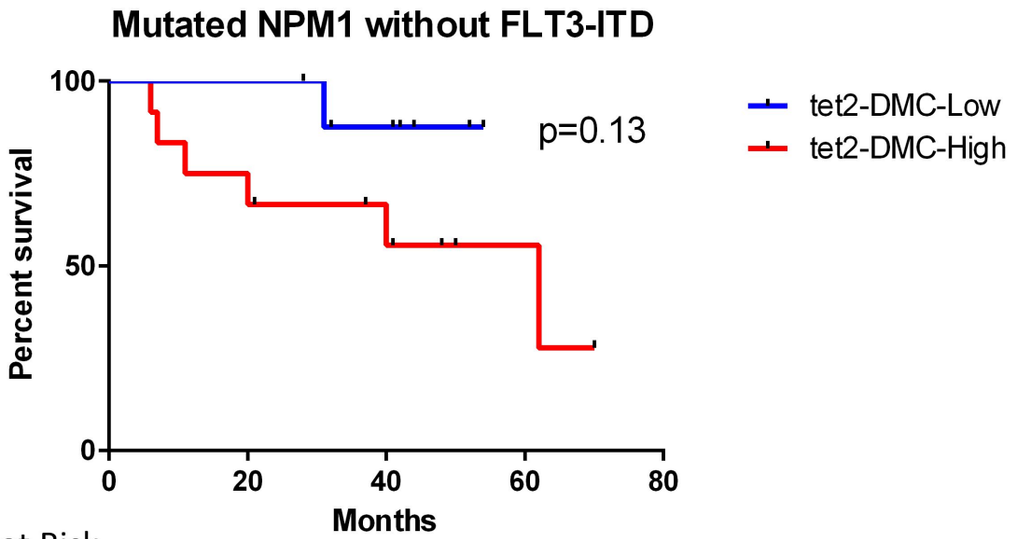
A



B



C



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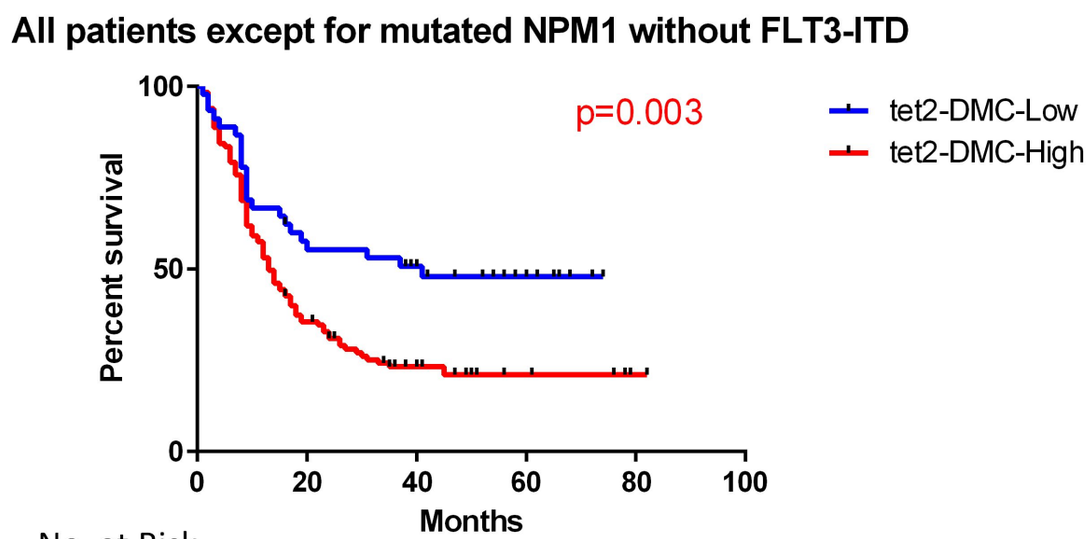
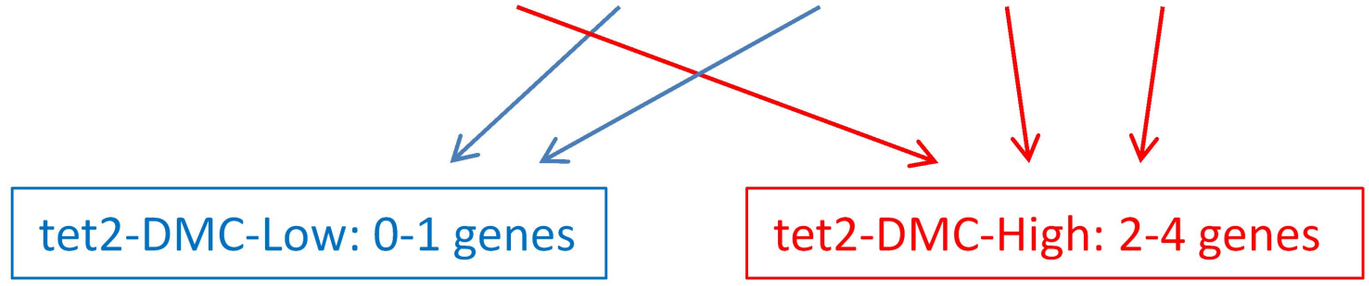
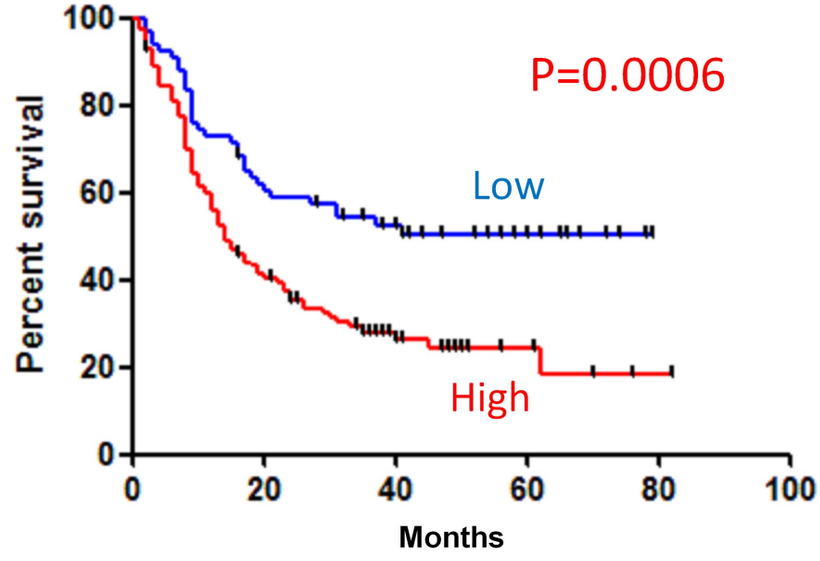


Supplementary Figure 5. A scheme for a clinically applicable tet2-DMC signature. Upper panel; methylation thresholds for each tet2-DMC to classify AML patients as being called `positive`. Examples of AML patients and the numbers of positives are shown. Lower panel; Kaplan-Meier survival curves for tet2-DMC-low (blue) and –high (red) patients defined by the method above. Tet2-DMC-low patients showed significantly longer OS compared to tet2-DMC-high in the analysis of all patients. P values are derived from the log-rank test.

Supplementary Figure 5

tet2-DMRs	Thresholds	AML 1	AML 2	AML3	AML 4	AML 5	.....
SP140	31.5%	70%	47%	8%	18%	61%	
MCCC1	38.0%	68%	25%	7%	26%	65%	
EHMT1	32.3%	8%	27%	26%	67%	71%	
MTSS1	42.4%	95%	25%	32%	94%	86%	
Number of Positive		3	1	0	2	4	





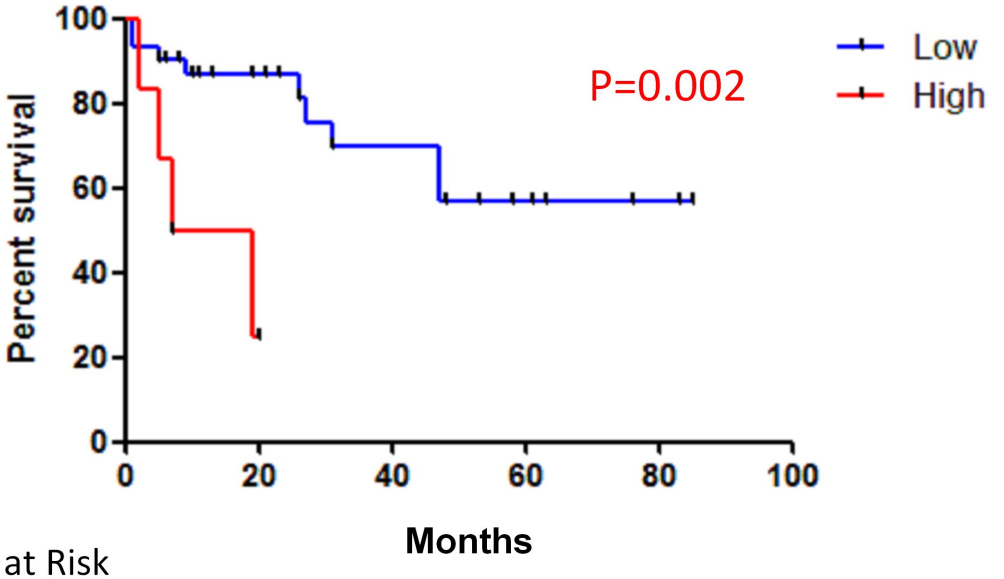
No. at Risk

Low	66	40	28	13	1
High	110	44	19	6	2

Supplementary Figure 6. Additive effects between tet2-DMC-low and M3/favorable-risk cytogenetic group. A) Kaplan-Meier survival curves in the TCGA dataset for M3/favorable-risk cytogenetic group. The patients are subdivided by tet2-DMC status. B) Kaplan-Meier survival curves in the TCGA dataset for tet2-DMC-low patients. The patients are subdivided by status of M3/favorable-risk cytogenetic group. C) Kaplan-Meier survival curves in the TCGA dataset for intermediate and poor cytogenetic group. The patients are subdivided by tet2-DMC status. P values are derived from the log-rank test.

Supplementary Figure 6

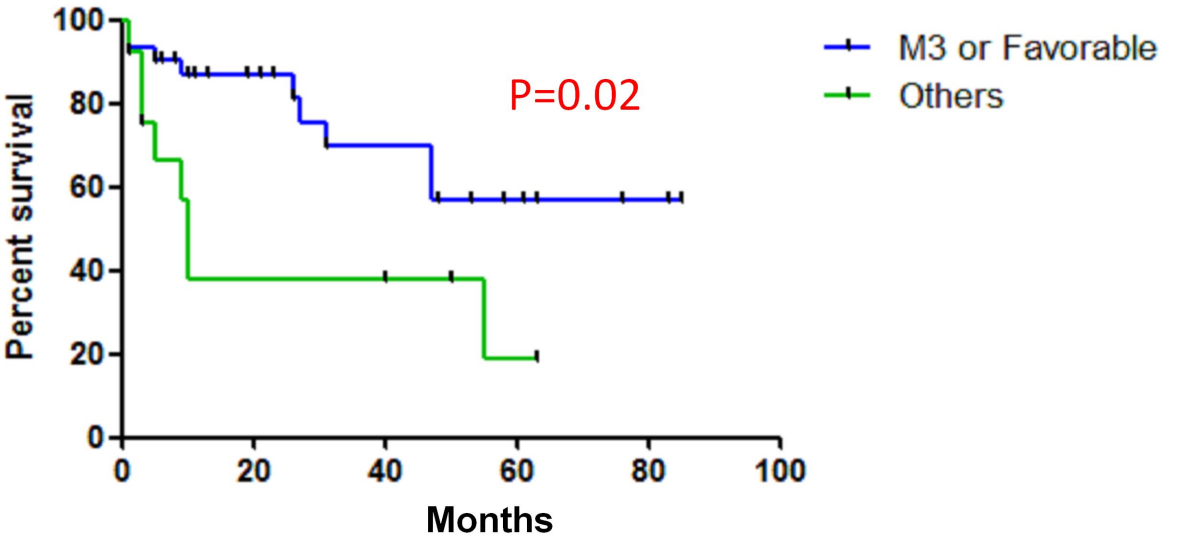
A



No. at Risk

Low	31	20	13	6
High	6	1	1	1

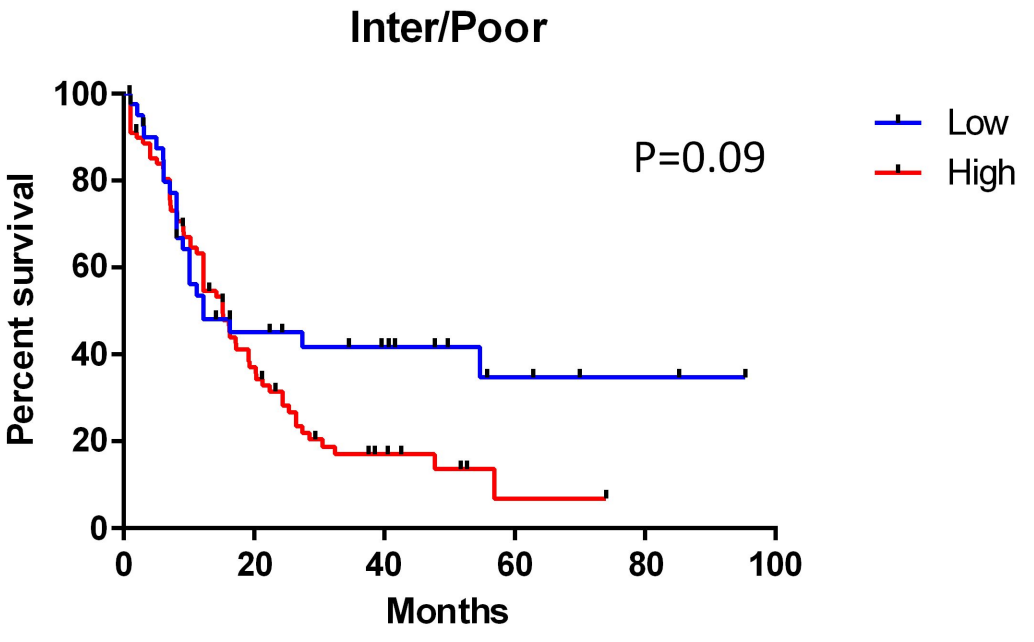
B



No. at Risk

	31	20	13	6
	13	6	4	2

C



No. at Risk

Low	42	16	11	5
High	90	26	9	2