High expression of transcription factor 4 (*TCF4*) is an independent adverse prognostic factor in acute myeloid leukemia that could guide treatment decisions

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Supplementary data

Supplementary Table 1. Patients characteristics. Characteristics for *TCF4*^{high} (n=131) and *TCF4*^{low} patients of the first cohort (n=394). Good risk (defined as patients with t(8;21), inv(16), t(16;16) or t(15;17); 2) Poor risk (defined as patients with ≥ 3 cytogenetic abnormalities (complex karyotype), monosomy of chromosome 5 or 7, deletion of 5q or 7q, abnormalities in 3q, t(6;9), t(9;22) or t(9;11); 3) Intermediate risk (defined as all other patients).

	TCF4^{low} (n = 394)	TCF4^{high} (n = 131)	***P* value**
Age			0.292
Under 60 years	331 (85.1%)	113 (87.6%)	
Above 60 years	58 (14.9%)	16 (12.4%)	
Sex			0.520
Male	194 (49.9%)	64 (49.6%)	
Female	195 (50.1%)	65 (50.4%)	
Cytogenetic risk classification			**<0.001**
Good	96 (24.9%)	8 (6.3%)	**<0.001**
Intermediate	240 (62.3%)	93 (72.7%)	**0.023**
Poor	49 (12.7%)	27 (21.1%)	**0.017**
ELN risk classification			**<0.001**
Favourable risk group	143 (37.1%)	13 (10.2%)	**<0.001**
Intermediate-I	85 (22.1%)	58 (45.3%)	**<0.001**
Intermediate-II	101 (26.5%)	29 (22.7%)	0.422
Adverse risk group	56 (14.5%)	28 (21.9%)	0.053
Flt3-ITD			**<0.001**
Negative	308 (78.2%)	74 (56.5%)	
Positive	86 (21.8%)	57 (43,5%)	
NPM1 mutation			0.338
Negative	273 (69.3%)	94 (71.8%)	
Positive	121 (30.7%)	37 (28.2%)	
CEBPA biallelic mutation			**0.005**
Negative	369 (93.7%)	130 (99.2%)	
Positive	25 (6.3%)	1 (0.8%)	
White Bloodcell Count (*10^{9})			0.133
Less than 100	326 (82.7%)	102 (77.9%)	
More than 100	68 (17.3%)	29 (22.1%)	
FAB classification			**<0.001**
M0	8 (2.1%)	10 (7.9%)	**0.005**
M1	55 (14.4%)	45 (35.7%)	**<0.001**
M2	102 (26.6%)	29 (23.0%)	0.230
M3	23 (6.0%)	0 (0.0%)	**0.001**
M4	77 (20.1%)	16 (12.7%)	**0.035**
M5	90 (23.5%)	26 (20.6%)	0.279
M6	7 (1.8%)	0 (0.0%)	0.132
Raeb-t	17 (4.4%)	0 (0.0%)	**0.007**
Raeb	4 (1.0%)	0 (0.0%)	0.316

FAB classification indicates French-American-British classification

Supplementary Table 2. Multivariate Cox Regression survival analysis A, Factors predicting OS and EFS in AML patients of the first cohort with available complete data of all molecular parameters (n=506), with *TCF4* as a continues variable per 100 AU. B, Factors predicting OS and EFS in AML patients of the first cohort with normal karyotype (n=214), with *TCF4* as a categorical variable per 100 AU. C, Factors predicting OS and EFS in AML patients of the first cohort with normal karyotype (n=214), with *TCF4* as a continuous variable per 100 AU.

A.

	OS, n = 506				**EFS, n = 506**			
Variable	χ^2 (Wald)	DF	*P*	HR (95% CI)	χ^2 (Wald)	DF	*P*	HR (95% CI)
Favorable ELN risk group, (reference)	44.83	3	<0.0001		38.52	3	<0.0001	
Intermediate-I ELN risk group	22.01	1	<0.0001	2.10 (1.54 - 2.86)	16.12	1	<0.0001	1.81 (1.35 - 2.41)
Intermediate-II ELN risk group	10.86	1	0.001	1.71 (1.24 - 2.36)	9.51	1	0.002	1.59 (1.19 - 2.14)
Adverse ELN risk group	42.74	1	<0.0001	3.17 (2.24 - 4.48)	37.53	1	<0.0001	2.78 (2.00 - 3.85)
Age (above 60 years)	15.61	1	<0.0001	1.80 (1.34 - 2.40)	8.56	1	0.003	1.52 (1.15 - 2.01)
WBC (>100 *10^9)	11.52	1	0.001	1.61 (1.22 - 2.12)	15.97	1	<0.0001	1.70 (1.31 - 2.20)
TCF4 expression (continuous per 100 AU)	5.09	1	0.024	1.04 (1.01-1.07)	9.20	1	0.002	1.05 (1.02-1.08)

ELN indicates European LeukemiaNet, DF indicates degrees of freedom, HR indicates Hazard ratio, CI indicates Confidence Interval

B.

	OS, n = 214				**EFS, n = 214**			
Variable	χ^2 (Wald)	DF	*P*	HR (95% CI)	χ^2 (Wald)	DF	*P*	HR (95% CI)
ELN risk group	6.76	1	0.009	1.68 (1.14 - 2.47)	6.57	1	0.010	1.61 (1.12 - 2.31)
Age (above 60 years)	5.19	1	0.023	1.72 (1.08 - 2.73)	2.17	1	0.141	1.40 (0.90 - 2.19)
WBC (>100 *10^9)	8.63	1	0.003	1.74 (1.20 - 2.51)	9.62	1	0.002	1.75 (1.23 - 2.49)
TCF4^{high} expression	8.56	1	0.003	1.75 (1.20 - 2.54)	7.83	1	0.005	1.66 (1.16 -2.37)

ELN indicates European LeukemiaNet, DF indicates degrees of freedom, HR indicates Hazard ratio, CI indicates Confidence Interval

C.

	OS, n = 214				**EFS, n = 214**			
Variable	χ^2 (Wald)	DF	*P*	HR (95% CI)	χ^2 (Wald)	DF	*P*	HR (95% CI)
ELN risk group	7.77	1	0.005	1.73 (1.18 - 2.54)	6.78	1	0.009	1.62 (1.13 - 2.33)
Age (above 60 years)	4.21	1	0.040	1.61 (1.02 - 2.54)	1.66	1	0.198	1.34 (0.86 - 2.08)
WBC (>100 *10^9)	9.27	1	0.002	1.79 (1.23 - 2.59)	10.32	1	0.001	1.80 (1.26 - 2.57)
TCF4 expression (continuous per 100 AU)	8.18	1	0.004	1.07 (1.02-1.13)	8.83	1	0.003	1.08 (1.03-1.13)

ELN indicates European LeukemiaNet, DF indicates degrees of freedom, HR indicates Hazard ratio, CI indicates Confidence Interval

Supplementary Table 3. Multivariate Cox Regression survival analysis including consolidation treatment. A, Factors predicting OS and EFS in *TCF4*^{high} patients of the first cohort (n=126). B, Factors predicting OS and EFS in *TCF4*^{low} patients of the first cohort (n=359).

A.

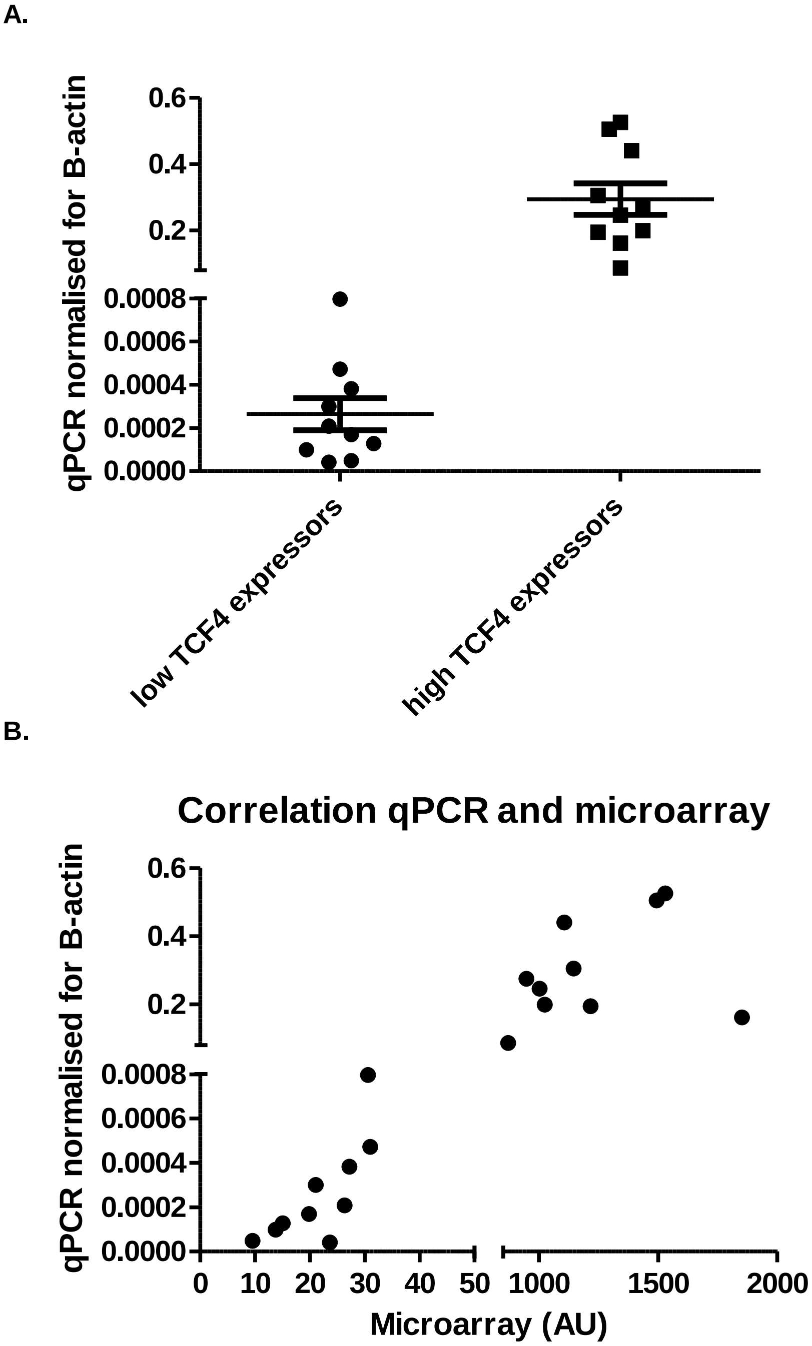
	OS, n = 126					EFS, n = 126			
Variable	χ^2 (Wald)	DF	*P*	HR (95% CI)		χ^2 (Wald)	DF	*P*	HR (95% CI)
Favorable ELN risk group, (reference)	8.73	3	0.033			10.89	3	0.012	
Intermediate-I ELN risk group	6.64	1	0.010	2.89 (1.29 - 6.47)		7.86	1	0.005	3.15 (1.41 - 7.02)
Intermediate-II ELN risk group	4.18	1	0.041	2.48 (1.04 - 5.94)		6.67	1	0.010	3.10 (1.31 - 7.29)
Adverse ELN risk group	8.30	1	0.004	3.55 (1.50 - 8.39)		10.84	1	0.001	4.25 (1.80 - 10.06)
Age (above 60 years)	5.31	1	0.021	2.10 (1.12 - 3.95)		2.75	1	0.097	1.68 (0.91 - 3.09)
WBC (>100 *10^9)	6.29	1	0.012	1.86 (1.15 - 3.02)		3.47	1	0.063	1.58 (0.98 - 2.57)
Additonal CT (reference)	16.78	2	<0.0001			12.15	2	0.002	
AutoHSCT	0.85	1	0.356	0.73 (0.37 - 1.43)		0.26	1	0.609	0.84 (0.43 - 1.63)
AlloHSCT	16.78	1	<0.0001	0.35 (0.21 - 0.58)		11.99	1	0.001	0.42 (0.26 - 0.69)

ELN indicates European LeukemiaNet, DF indicates degrees of freedom, HR indicates Hazard ratio, CI indicates Confidence Interval

B.

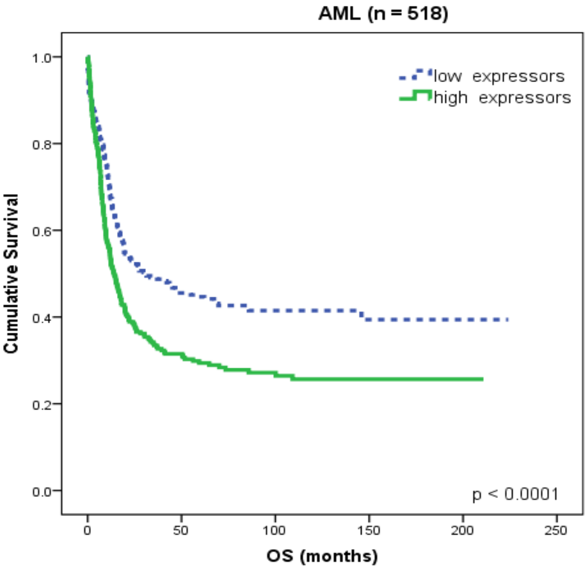
	OS, n = 359					EFS, n = 359			
Variable	χ^2 (Wald)	DF	*P*	HR (95% CI)		χ^2 (Wald)	DF	*P*	HR (95% CI)
Favorable ELN risk group, (reference)	36.02	3	<0.0001			31.49	3	<0.0001	
Intermediate-I ELN risk group	11.70	1	0.001	1.93 (1.32 - 2.81)		6.89	1	0.009	1.60 (1.13 - 2.28)
Intermediate-II ELN risk group	5.03	1	0.025	1.52 (1.05 - 2.18)		3.87	1	0.049	1.40 (1.00 - 1.96)
Adverse ELN risk group	34.36	1	<0.0001	3.40 (2.26 - 5.12)		31.05	1	<0.0001	3.09 (2.08 - 4.59)
Age (above 60 years)	4.82	1	0.028	1.51 (1.05 - 2.19)		2.47	1	0.116	1.33 (0.93 - 1.90)
WBC (>100 *10^9)	4.12	1	0.042	1.43 (1.01 - 2.02)		11.03	1	0.001	1.73 (1.25 - 2.38)
Additonal CT (reference)	11.42	2	0.003			5.24	2	0.073	
AutoHSCT	7.89	1	0.005	0.53 (0.34 - 0.82)		3.39	1	0.065	0.69 (0.47 - 1.02)
AlloHSCT	6.27	1	0.012	0.66 (0.48 - 0.92)		3.30	1	0.069	0.75 (0.56 - 1.02)

ELN indicates European LeukemiaNet, DF indicates degrees of freedom, HR indicates Hazard ratio, CI indicates Confidence Interval

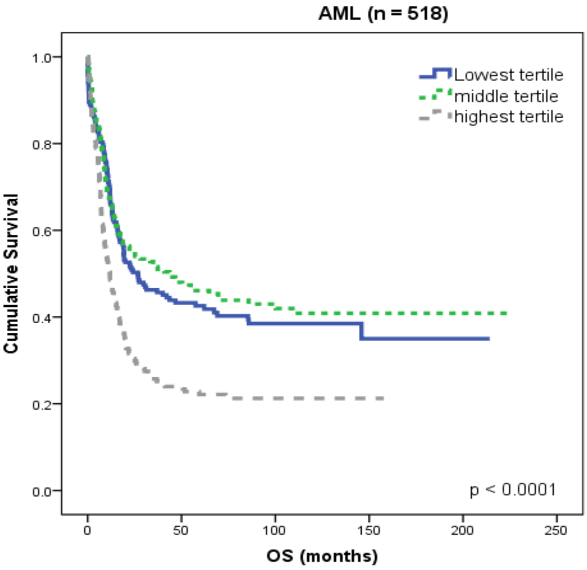


Supplementary Figure 1. To confirm the microarray expression data, qPCRs were performed on cDNA of 10/20 highest expressors (by microarray) and of 10/20 lowest expressors. The highest expressors by microarray had also the highest expression by qPCR (normalized for β-actin) (A); and the lowest expressors by microarray had also the lowest expression by qPCR (normalized for β-actin). The difference in expression level by qPCR by high expressors vs low expressors was about 1000 fold; and differed significantly ($P < 0.0001$; T test). The correlation between the microarray values and the qPCR values was high: Spearman's correlation coefficient 0.8887 (95% CI 0.73-0.96, $P < 0.0001$) (B). Of note: The following primers were used: TCCAGGTTTGCCATCTTCAGT (F) GCCTGGCGAGTCCCTATTG (R).

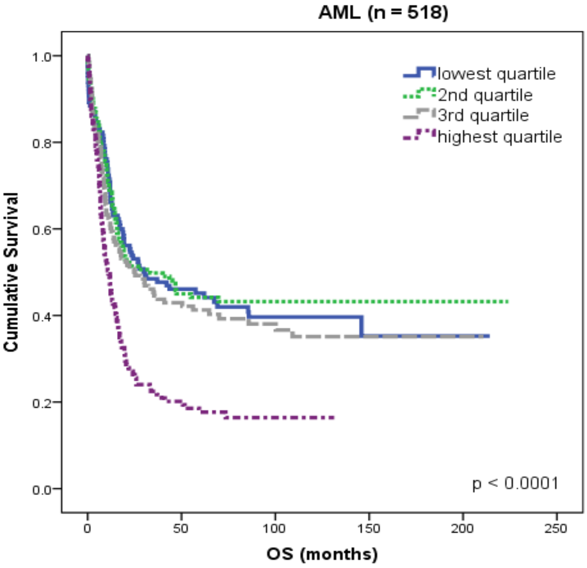
A.


AML (n = 518)
low expressors
high expressors
Cumulative Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
OS (months)
p < 0.0001

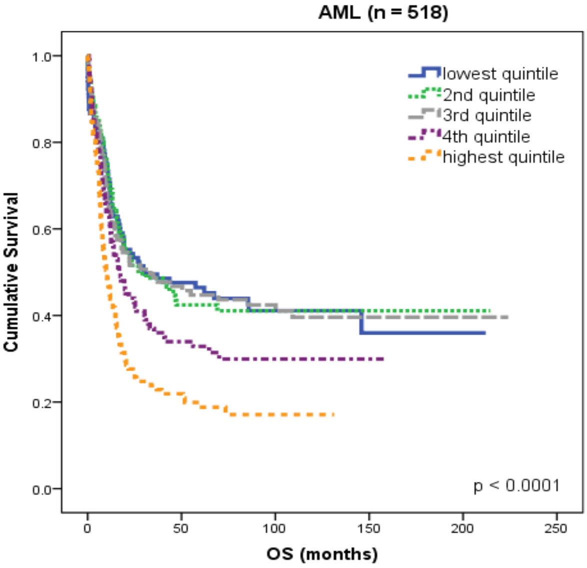
B.


AML (n = 518)
Lowest tertile
middle tertile
highest tertile
Cumulative Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
OS (months)
p < 0.0001

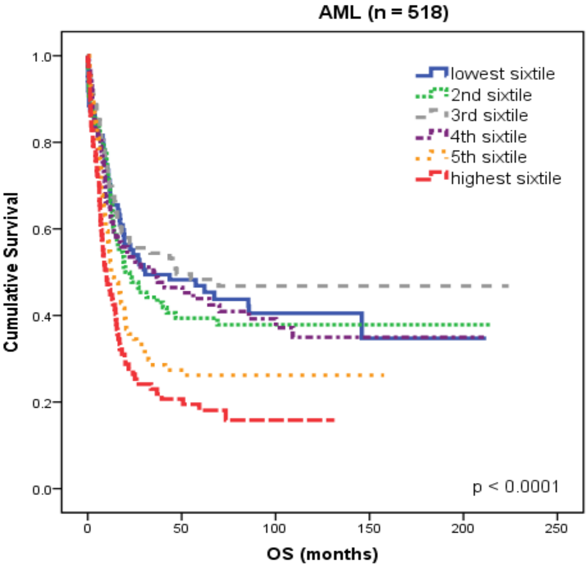
C.


AML (n = 518)
lowest quartile
2nd quartile
3rd quartile
highest quartile
Cumulative Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
OS (months)
p < 0.0001

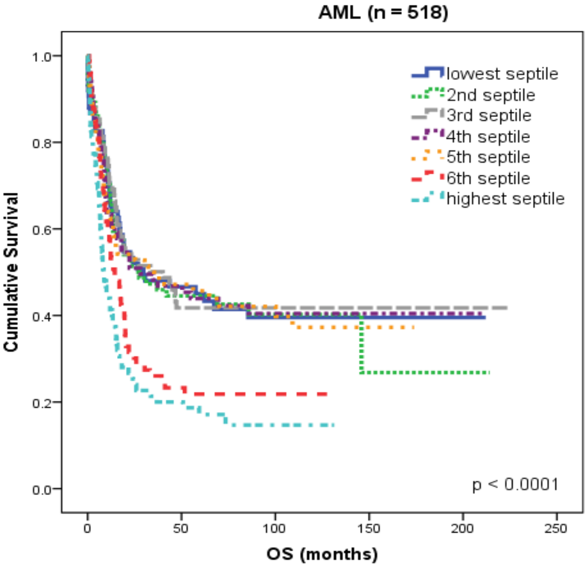
D.


AML (n = 518)
lowest quintile
2nd quintile
3rd quintile
4th quintile
highest quintile
Cumulative Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
OS (months)
p < 0.0001

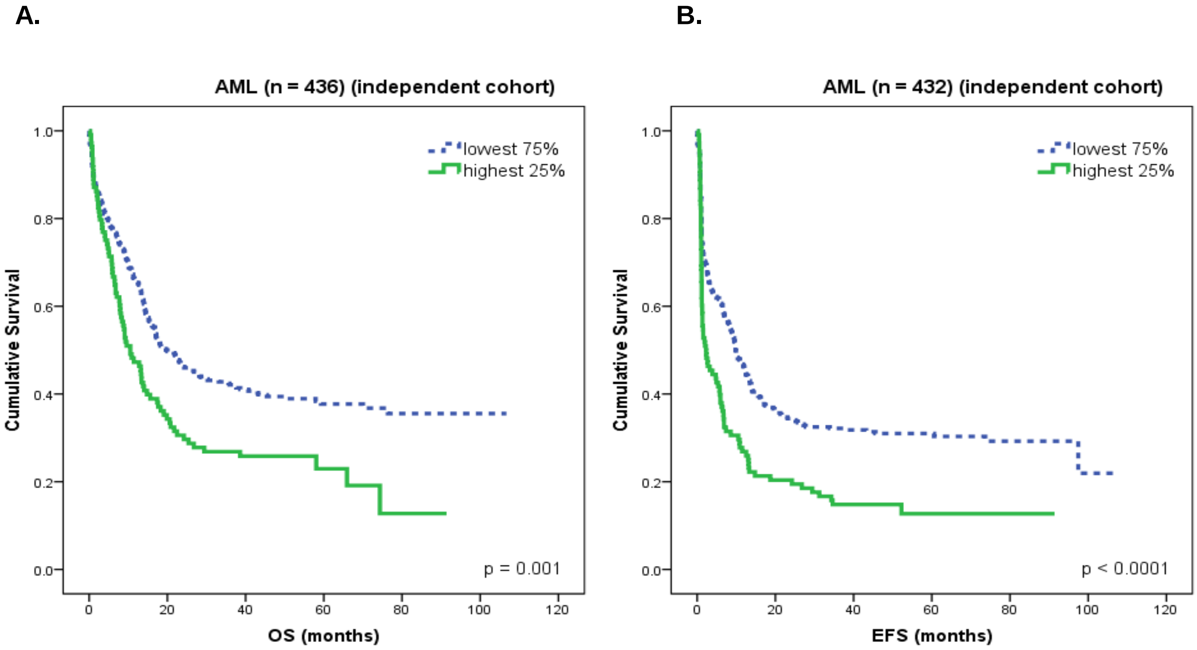
E.


AML (n = 518)
lowest sixtile
2nd sixtile
3rd sixtile
4th sixtile
5th sixtile
highest sixtile
Cumulative Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
OS (months)
p < 0.0001

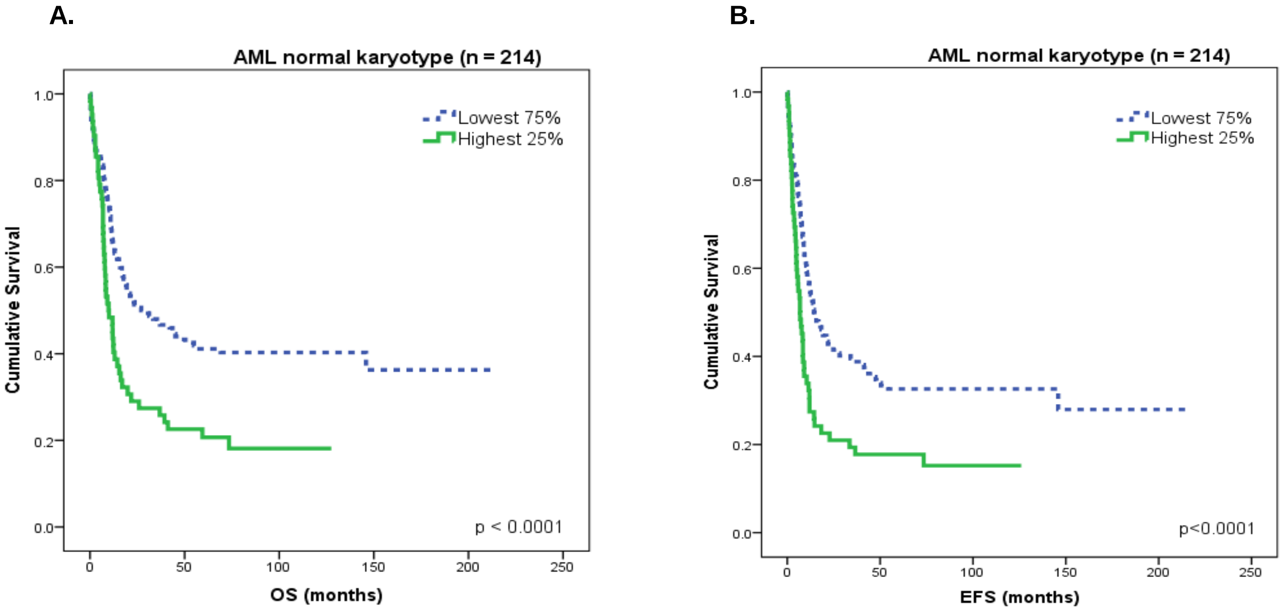
F


AML (n = 518)
lowest septile
2nd septile
3rd septile
4th septile
5th septile
6th septile
highest septile
Cumulative Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
OS (months)
p < 0.0001

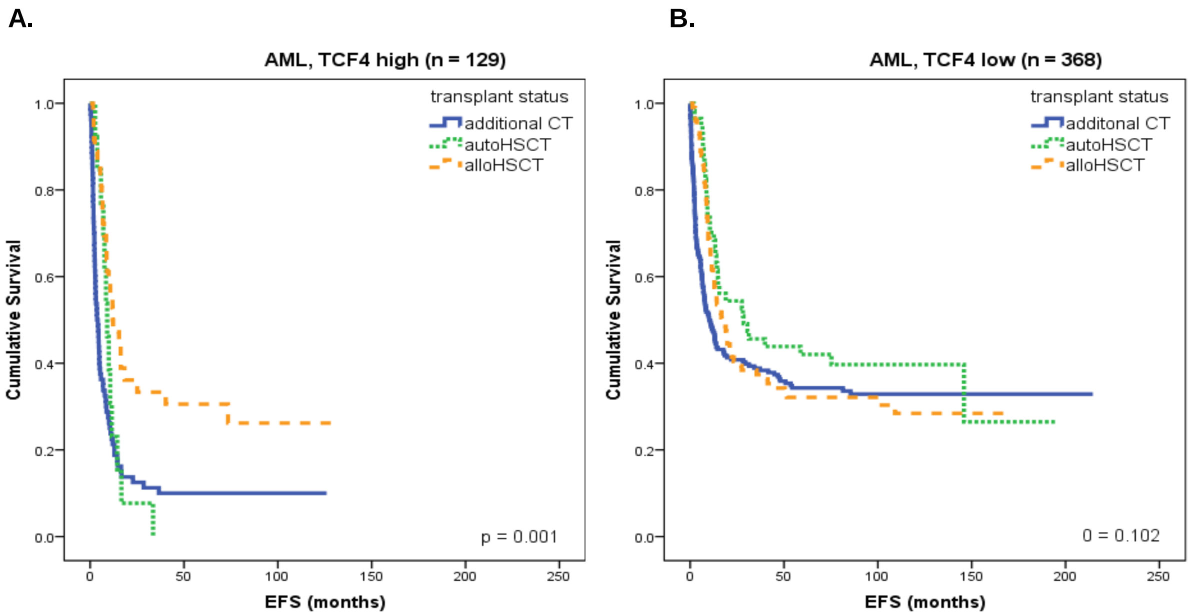
Supplementary Figure 2. OS curves for 518 AML patients of the first cohort. A, Patients with *TCF4* expression stratified by above median (n=257) and below median (n=261) *TCF4* expression. B, Patients with *TCF4* expression stratified by lowest tertile (n=174), middle tertile (n=173), highest tertile (n=171). C, Patients with *TCF4* expression stratified by quartiles, lowest quartile (n=131), 2nd quartile (n=130), 3rd quartile (n=128), highest quartile (n=129). D, Patients with *TCF4* expression stratified by quintiles, lowest quintile (n=105), 2nd quintile (n=104), 3rd quintile (n=104), 4th quintile (n=100), highest quintile (n=105). E, Patients with *TCF4* expression stratified by sixtiles, lowest sixtile (n=87), 2nd sixtile (n=87), 3rd sixtile (n=87), 4th sixtile (n=86), 5th sixtile (n=84), highest sixtile (n=87). F, Patients with *TCF4* expression stratified by septiles, lowest septile (n=75), 2nd septile (n=74), 3rd septile (n=75), 4th septile (n=74), 5th septile (n=72), 6th septile (n=73), highest septile (n=75).



Supplementary Figure 3. A, OS curves for 436 AML patients of the second cohort stratified by *TCF4*^{high} (n=109) and *TCF4*^{low} (n=327). B, EFS curves for 436 AML patients of the second cohort stratified by *TCF4*^{high} (n=108) and *TCF4*^{low} (n=324)



Supplementary Figure 4 A. OS curves for AML patients with a normal karyotype and available follow-up data (n=214) stratified by *TCF4*^{high} (n=62) and *TCF4*^{low} (n=152). B, idem for EFS.



Supplementary Figure 5.

EFS curves for *TCF4*^{high} AML patients with available follow up and consolidation treatment data (n=129) stratified for conditioning with alloHSCT (n=36), autoHSCT (n=13) or additional CT (n=80). F, EFS curves for *TCF4*^{low} AML patients with available follow up and consolidation treatment data (n=386) stratified for conditioning with alloHSCT (n=99), autoHSCT (n=57) or additional CT (n=212).