Transcription factor 4 (*TCF4*) expression predicts clinical outcome in *RUNX1* mutated and translocated acute myeloid leukemia

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Supplementary data

Methods

AML patients and TCF4 expression

TCF4 expression values were derived from a previously reported cohort of 436 AML patients (1;2). Gene expression profiling was performed as previously described using the Stanford cDNA microarray platform (2). Following Ficoll enrichment, all samples contained at least 80% leukemic cells. To determine the *TCF4* expression, an average of 7 probe sets (which bind at different locations of the gene) was used (IMAGE:854581, IMAGE:701710, IMAGE:701629, IMAGE:1603442, IMAGE:1592047, IMAGE:1597854, IMAGE:287722). In 330 patients the *RUNX1* mutational status was established as previously reported (3).

Statistics.

SPSS version 22.0 software (SPSS Inc, Chicago, IL) and Graphpad Prism 5.03 were used for statistical analysis. Differences in patient groups were calculated using the Mann-Whitney U test. The overall and event free survival (OS, EFS) as defined by the ELN 2017 guidelines (4) were calculated from the date of AML diagnosis to a relevant event date (death and first recurrence, respectively), or the last follow-up date. Survival curves were calculated by the Kaplan-Meier method and compared using the logrank test. Multivariate survival analysis was carried out using the Cox proportional hazards model, and covariates included were *RUNX1* mutation and white blood cell count (WBC > 100 x 10^9/L) with and without *TCF4* expression (lowest 75% vs highest 25%). For assessing the possible role of TCF4 as a mediator of the RUNX1 effect on prognosis, we first examined whether RUNX1 mutational status was related to survival. Subsequently, we checked whether *TCF4* expression was related to survival and whether TCF4 was related to *RUNX1* mutational status. If the examined relationships were significant, we examined the mediating role of TCF4 by adding *TCF4* expression levels to the model and estimate the reduction in HR for *RUNX1* mutational status. *P*-values equal or inferior to 0.05 were considered significant.

References

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Figure Legends

Supplementary Figure 1. Event free survival (EFS) curves for AML patients with available data stratified on **A.** *TCF4* expression, lowest 75% (n=324), highest 25% (n=108); **B.** *RUNX1* mutational status*, RUNX1* wild type (n=304), *RUNX1* mutation (n=26); **C.** Presence (n=31) or absence of t(8;21) (n=405); **D.** Presence (n=47) or absence of inv(16) (n=389).

Supplementary Figure 2. RUNX1 ChIP sequencing data of the *TCF4* promoter in *RUNX1* wild type, *RUNX1* mutated and AML-ETO Chip sequencing of the TCF4 promoter in AML-ETO positive primary AML cells.

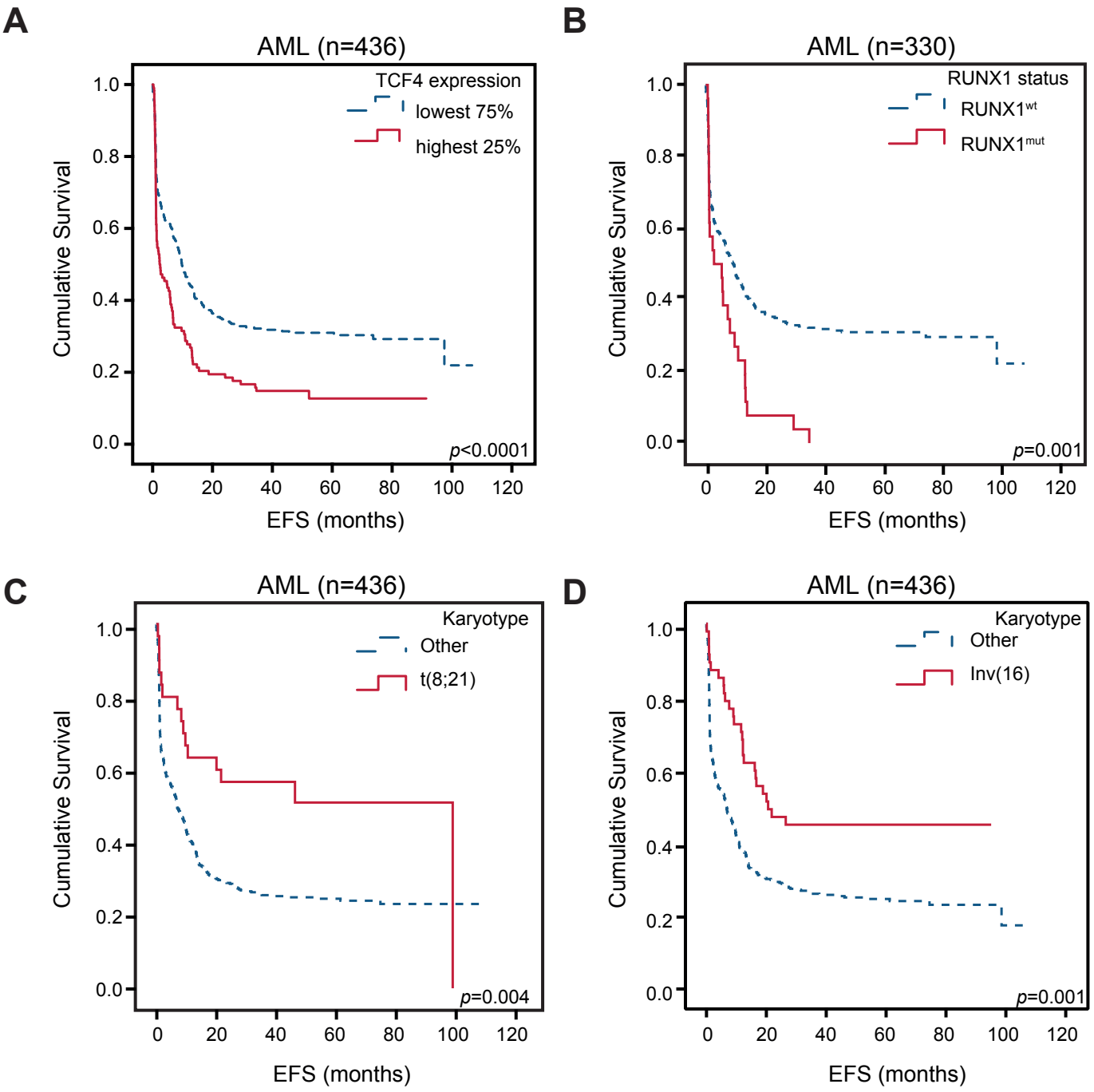
Supplementary Table 1. 5-Year overall and event free survival rates based on *TCF4* expression levels, *RUNX1* mutational status, presence of t(8;21) and presence of inv(16).

Supplementary Table 2. Multivariate Cox Regression analysis overall survival (OS); cytogenetics, CEPBA double mutation, FLT3-ITD, NPM1 status, WBC and age are included in the model, together with; **A.** RUNX1 status, ; **B.** TCF4 expression (highest quartile); **C.** TCF4 expression (highest quartile) and RUNX1 status; **D.** TCF4 expression (continues)

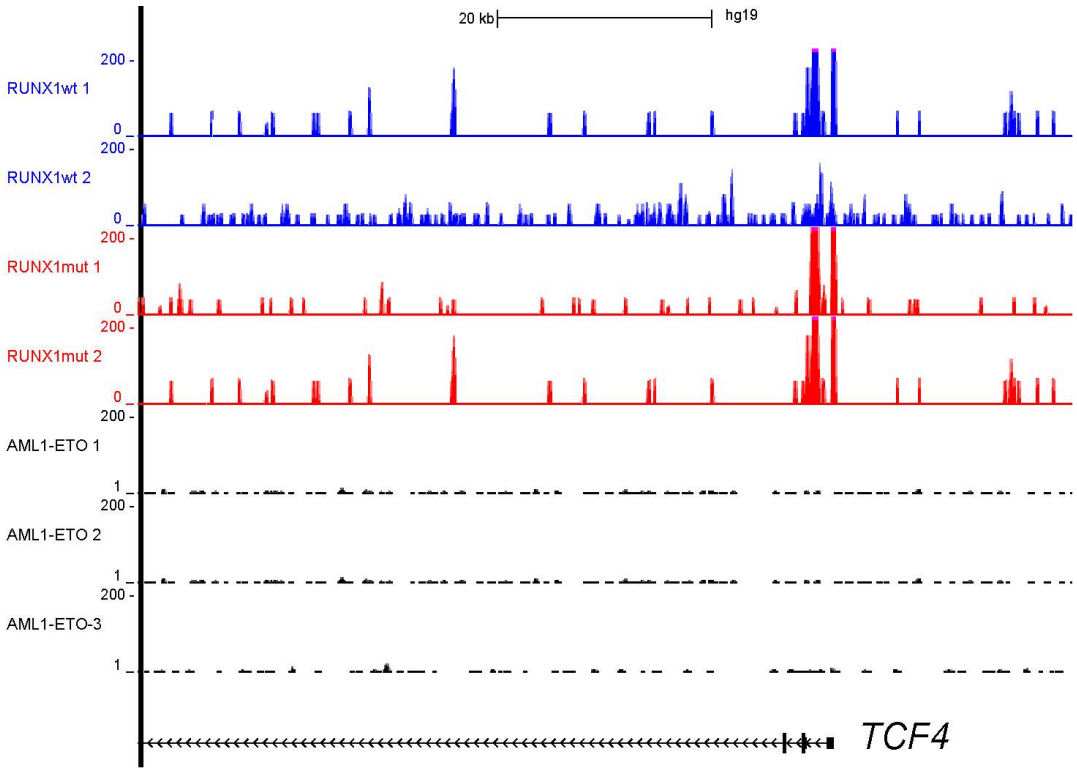
Supplementary Table 3. A. Cross tab with white blood cell count (WBC), age and cytogenetische risk group against *RUNX1* status, t(8;21), inv(16) and *TCF4* expression (n=436, for RUNX1 n=330). **B.** Multivariate Cox Regression analysis Event free survival (EFS); left *RUNX1* status and white WBC included in the model; and right *TCF4* expression, *RUNX1* status and WBC included in the model. **C.** Multivariate Cox Regression analysis EFS; left t(8;21) and WBC included in the model; and right *TCF4* expression, t(8;21) and WBC included in the model. **D.** Multivariate Cox Regression analysis EFS; left inv(16) and WBC included in the model; and right *TCF4* expression, inv(16) and WBC included in the model. Event free survival (EFS). DF= degrees of freedom, HR= Hazard Ratio, CI= Confidence interval.

Supplementary Table 4. A. Multivariate Cox Regression analysis overall survival (OS); left *TCF4* expression and white WBC included in the model; and right *TCF4* expression, *RUNX1* status and WBC included in the model. **B.** Multivariate Cox Regression analysis Event free survival (EFS); left *TCF4* expression and white WBC included in the model; and right *TCF4* expression, *RUNX1* status and WBC included in the model. **C.** Multivariate Cox Regression analysis OS; left *TCF4* expression and WBC included in the model; and right *TCF4* expression, t(8;21) and WBC included in the model. **D.** Multivariate Cox Regression analysis EFS; left *TCF4* expression and WBC included in the model; and right *TCF4* expression, t(8;21) and WBC included in the model **E.** Multivariate Cox Regression analysis OS; left *TCF4* expression, and WBC included in the model; and right *TCF4* expression, inv(16) and WBC included in the model. **F.** Multivariate Cox Regression analysis EFS; left *TCF4* expression, and WBC included in the model; and right *TCF4* expression, inv(16) and WBC included in the model. Event free survival (EFS). DF= degrees of freedom, HR= Hazard Ratio, CI= Confidence interval.

Supplemental Figure 1



Supplemental Figure 2



Supplementary Tables

Supplementary Table 1:

	5-year OS	*p*-value	5-year EFS	*p*-value
high TCF4 (highest 25%)	23.3%	0.001	12.7%	<0.0001
low TCF4 (lowest 75%)	37.7%		31.0%	
RUNX1 wt	38.8%	0.014	31.0%	0.001
RUNX1 mut	14.4%		0.0%	
t(8;21)	48.9%	0.035	51.0%	0.004
other cytogenetics	33.0%		24.6%	
inv(16)	63.0%	<0.0001	44.7%	0.001
other cytogenetics	30.8%		24.2%	

Supplementary Table 2:

A.

					HR 95.0% CI	
	Wald.	**df**	***p*-value**	**HR**	**Lower**	**Upper**
RUNX1 mutation	2.569	1	0.109	1.456	0.920	2.306
Cytogenetic low risk	32.546	2	0.000			
Cytogenetic intermediate risk	5.889	1	0.015	2.040	1.147	3.627
Cytogenetic poor risk	30.249	1	0.000	3.802	2.362	6.119
CEBPA double mutation	3.860	1	0.049	1.004	1.000	1.009
FLT3-ITD	1.689	1	0.194	0.997	0.993	1.001
NPM1 mutation	0.089	1	0.766	1.001	0.993	1.009
WBC (> 100 * 10^9)	11.009	1	0.001	1.928	1.308	2.842
Age (> 60 years)	5.474	1	0.019	1.552	1.074	2.243

B.

					HR 95.0% CI	
	Wald.	**df**	***p*-value**	**HR**	**Lower**	**Upper**
TCF4 highest quartile	5.931	1	0.015	1.387	1.066	1.806
Cytogenetic low risk	30.834	2	0.000			
Cytogenetic intermediate risk	5.908	1	0.015	1.847	1.126	3.030
Cytogenetic poor risk	28.932	1	0.000	2.983	2.003	4.442
CEBPA double mutation	3.003	1	0.083	1.003	1.000	1.007
FLT3-ITD	1.679	1	0.195	0.998	0.994	1.001
NPM1 mutation	0.006	1	0.938	1.000	0.994	1.006
WBC (> 100 * 10^9)	9.776	1	0.002	1.770	1.237	2.532
Age (> 60 years)	16.918	1	0.000	1.849	1.380	2.478

C.

					HR 95.0% CI	
	Wald.	**df**	***p*-value**	**HR**	**Lower**	**Upper**
TCF4 highest quartile	5.755	1	0.016	1.486	1.075	2.054
RUNX1 mutation	0.963	1	0.327	1.269	0.788	2.045
Cytogenetic low risk	28.814	2	0.000			
Cytogenetic intermediate risk	3.463	1	0.063	1.761	0.970	3.195
Cytogenetic poor risk	25.199	1	0.000	3.442	2.124	5.577
CEBPA double mutation	2.766	1	0.096	1.004	0.999	1.008
FLT3-ITD	1.616	1	0.204	0.997	0.993	1.001
NPM1 mutation	0.063	1	0.802	1.001	0.993	1.009
WBC (> 100 * 10^9)	10.228	1	0.001	1.886	1.279	2.783
Age (> 60 years)	7.202	1	0.007	1.666	1.148	2.420

D.

					HR 95.0% CI	
	Wald.	**df**	***p*-value**	**HR**	**Lower**	**Upper**
TCF4 continues variable	7.511	1	0.006	1.147	1.040	1.265
Cytogenetic low risk	30.529	2	0.000			
Cytogenetic intermediate risk	5.287	1	0.021	1.796	1.090	2.958
Cytogenetic poor risk	28.414	1	0.000	2.954	1.983	4.398
CEBPA double mutation	2.343	1	0.126	1.003	0.999	1.007
FLT3-ITD	1.187	1	0.276	0.998	0.994	1.002
NPM1 mutation	0.004	1	0.950	1.000	0.994	1.006
WBC (> 100 * 10^9)	10.941	1	0.001	1.833	1.280	2.624
Age (> 60 years)	18.844	1	0.000	1.928	1.433	2.593

Supplementary Table 3:

A.

		RUNX1 wt	RUNX1 mut	*p*-value	t(8;21)	non t(8;21)	*p*-value	inv(16)	non inv(16)	*p*-value	TCF4 low	TCF4 high	*p*-value
WBC	<100*10^9/L	267 (92%)	24 (8%)	0.752	29 (7%)	363 (93%)	0.757	349 (90%)	43 (91%)	1.000	294 (90%)	98 (90%)	1.000
	≥100*109/L	37 (95%)	2 (5%)		2 (5%)	42 (96%)		40 (10%)	11 (9%)		33 (10%)	11 (10%)	
Age	<60 years	249 (94%)	15 (6%)	0.008	27 (8%)	312 (92 %)	0.263	299 (77%)	40 (85%)	0.265	258 (79%)	81 (74%)	1.000
	≥60 years	55 (83%)	11 (17%)		4 (4%)	93 (96%)		90 (23%)	7 (15%)		69 (21%)	28 (26%)	
Cytogenetic	low	87 (29%)	0 (0%)	0.001	31 (100%)	81 (20%)	<0.001	65 (17%)	47 (100%)	<0.001	103 (32%)	9 (8%)	<0.001
risk group	intermediate	165 (54%)	24 (92%)		0 (0%)	245 (61%)		245 (63%)	0 (0%)		170 (52%)	75 (69%)	
	poor	52 (17%)	2 (8%)		0 (0%)	79 (19%)		79 (20%)	0 (0%)		54 (17%)	25 (23%)	

B.

		Excluding TCF4				Including TCF4		
EFS; Variable		**Wald.**	**HR (95% CI)**	***p*-value**	**df**	**Wald.**	**HR (95% CI)**	***p*-value**
TCF4	highest 25%				1	12.16	1.70 (1.26 - 2.29)	<0.001
RUNX1	mutation	11.24	2.02 (1.34 - 3.06)	0.001	1	3.88	1.55 (1.00 - 2.41)	0.049
WBC	>100*10^9/L	10.16	1.81 (1.26 - 2.61)	0.001	1	9.10	1.76 (1.22 - 2.53)	0.003

C.

EFS; Variable		Excluding TCF4				Including TCF4		
		Wald.	**HR (95% CI)**	***p*-value**	**df**	**Wald**	**HR (95% CI)**	***p*-value**
TCF4	highest 25%				1	13.70	1.59 (1.24 - 2.03)	<0.001
t(8;21)	present	7.82	0.48 (0.28 - 0.80)	0.005	1	5.66	0.53 (0.31 - 0.89)	0.017
WBC	>100*10^9/L	6.80	1.58 (1.12 - 2.23)	0.009	1	6.88	1.59 (1.12 - 2.24)	0.009

D.

EFS; Variable		Excluding TCF4				Including TCF4		
		Wald.	**HR (95% CI)**	***p*-value**	**df**	**Wald**	**HR (95% CI)**	***p*-value**
TCF4	highest 25%				1	15.30	1.63 (1.28 - 2.08)	<0.001
t(8;21)	present	10.53	0.51 (0.34 - 0.77)	0.001	1	9.32	0.53 (0.36 - 0.80)	0.002
WBC	>100*10^9/L	6.88	1.59 (1.12 - 2.24)	0.009	1	6.77	1.58 (1.12 - 2.23)	0.009

Supplementary Table 4:

A.

OS; Variable		Excluding RUNX1				Including RUNX1		
		Wald.	**HR (95% CI)**	***p*-value**	**df**	**Wald.**	**HR (95% CI)**	***p*-value**
TCF4	highest 25%	11.05	1.55 (1.20 – 2.00)	0.001	1	8.86	1.61 (1.18 - 2.21)	0.003
RUNX1	mutation				1	2.30	1.43 (0.90 - 2.28)	0.129
WBC	>100*10^9/L	11.03	1.81 (1.28 - 2.58)	0.001	1	12.10	1.96 (1.34 - 2.87)	0.001

B.

EFS; Variable		Excluding RUNX1				Including RUNX1		
		Wald.	**HR (95% CI)**	***p*-value**	**df**	**Wald.**	**HR (95% CI)**	***p*-value**
TCF4	highest 25%	17.10	1.67 (1.31 - 2.13)	<0.001	1	12.16	1.70 (1.26 - 2.29)	<0.001
RUNX1	mutation				1	3.88	1.55 (1.00 - 2.41)	0.049
WBC	>100*10^9/L	7.01	1.59 (1.13 - 2.25)	0.008	1	9.10	1.76 (1.22 - 2.53)	0.003

C.

OS; Variable		Excluding t(8;21)				Including t(8;21)		
		Wald.	**HR (95% CI)**	***p*-value**	**df**	**Wald.**	**HR (95% CI)**	***p*-value**
TCF4	highest 25%	11.05	1.55 (1.20 - 2.00)	0.001	1	9.09	1.49 (1.15 - 1.93)	0.003
t(8;21)	Present				1	2.75	0.63 (0.37 – 1.09)	0.098
WBC	>100*10^9/L	11.03	1.81 (1.28 - 2.58)	0.001	1	10.83	1.80 (1.27 - 2.56)	0.001

D.

EFS; Variable		Excluding t(8;21)				Including t(8;21)		
		Wald.	**HR (95% CI)**	***p*-value**	**df**	**Wald.**	**HR (95% CI)**	***p*-value**
TCF4	highest 25%	17.10	1.67 (1.31 - 2.13)	<0.001	1	13.70	1.59 (1.24 - 2.03)	<0.001
t(8;21)	Present				1	5.66	0.53 (0.31 - 0.89)	0.017
WBC	>100*10^9/L	7.01	1.59 (1.13 - 2.25)	0.008	1	6.88	1.59 (1.12 - 2.24)	0.009

E.

OS; Variable		Excluding inv(16)				Including inv(16)		
		Wald.	**HR (95% CI)**	***p*-value**	**df**	**Wald.**	**HR (95% CI)**	***p*-value**
TCF4	highest 25%	11.05	1.55 (1.20 - 2.00)	0.001	1	9.09	1.49 (1.15 - 1.93)	0.003
Inv(16)	Present				1	12.85	0.42 (0.26 - 0.67)	<0.001
WBC	$>100*10^9$/L	11.03	1.81 (1.28 - 2.58)	0.001	1	10.71	1.80 (1.27 - 2.55)	0.001

F.

EFS; Variable		Excluding inv(16)				Including inv(16)		
		Wald.	**HR (95% CI)**	***p*-value**	**df**	**Wald.**	**HR (95% CI)**	***p*-value**
TCF4	highest 25%	17.10	1.67 (1.31 - 2.13)	<0.001	1	15.30	1.63 (1.28 - 2.08)	<0.001
Inv(16)	Present				1	9.32	0.53 (0.36 - 0.80)	0.002
WBC	$>100*10^9$/L	7.01	1.59 (1.13 - 2.25)	0.008	1	6.77	1.58 (1.12 - 2.23)	0.009