MicroRNA expression-based outcome prediction in acute myeloid leukemia: novel insights through cross-platform integrative analyses

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Supplemetary Materials and methods

Datasets selection

The training dataset including clinical information and processed and filtered miRNA expression profiles of 91 patients previously analyzed at Ulm University, Germany (hereafter referred as the Ulm dataset; samples were derived from patients entered within the German AML Study Group HD-98A trial, NCT00146120) [1].

To select other datasets to be used for validation we searched public repositories for microarray experiments with available clinical information on survival. The data portal of The Cancer Genome Atlas (https://tcga-data.nci.nih.gov/tcga/tcgaHome2.jsp) provided data on a total of 200 AML cases. We were able to extract clinical data and RNA-Sequencing data for 177 patients. This dataset served as a validation set for our study (hereafter referred as the USA dataset). The number of features in the Ulm and the USA datasets were different. We matched them and selected a total of 168 microRNAs that were common between the two datasets. The expression matrices with only these 168 microRNAs were used in the study.

Ulm dataset detailed description

Adult AML patient samples [56 peripheral blood (PB) and 35 bone marrow (BM) specimens from 91 AML cases] were provided by the German and Austrian AML Study Group (AMLSG) with patient informed consent and institutional review board approval from all participating centers. All patients had been entered between February 1998 and October 2002 into the AMLSG treatment protocol AML HD98A for younger adults (age $\leq$ 60 years). Patients received intensive induction and consolidation therapy (for protocol details see Schlenk et al. [2] 2004 and Schaich et al. [3]). Patient age at the time of diagnosis ranged from 19 to 60 years (median age 45). Clinical characteristics at the time of diagnosis were available for almost all cases as detailed in Table S1 below.

Conventional cytogenetic (chromosome banding), and molecular genetic analyses (screening for *FLT3* internal tandem-duplications (ITD) and tyrosine kinase domain (TKD) mutations, CEPBA and *NPM1* mutations) were performed as previously described at the central reference laboratory of the German and Austrian AMLSG at Ulm University [4-7].

To screen miRNA expression in AML a miRNA microarray platform with a commercially available oligonucleotide probe set was used (Ambion mirVana™ miRNA Probe Set, Ambion

Inc.), which has already been successfully used to investigate miRNA profiles in tumors and for which the performance has been validated by both Northern Blot analysis and quantitative RT-PCR [8-10].

In brief, this probe set representing 328 human miRNAs was spotted onto GAPS coated glass slides (Corning Inc.). Then, total RNA was isolated from tumor samples and different tumor cell lines, which served as common reference using the mirVana™ miRNA Isolation Kit (Ambion Inc.). Of each total RNA sample 20µg was fractionated by polyacrylamide gel electrophoresis (PAGE) using Ambion's flashPAGE Fractionator System (Ambion Inc.), and the miRNA fractions for each sample were recovered. With the aid of the mirVana miRNA Labeling Kit (Ambion Inc.) miRNA samples were prepared for microarray analysis using an end-labeling strategy. In the first step, E. coli Poly(A) Polymerase and a mixture of unmodified and amine-modified nucleotides were used to add a 20–50 nucleotide tail to the 3' end of each miRNA sample. The amine-modified miRNAs were then purified and coupled to amine-reactive labeled NHS-ester CyDye™ fluorochromes (Amersham Biosciences). Following purification the Cy5-labeled AML and Cy3-labeled common reference samples were mixed, cohybridized for 14 hours onto the miRNA microarrays and washed according to the manufacturer's protocol (Ambion Inc.). Finally, microarrays were scanned using an Axon GenePix 4000B laser scanner (Axon Instruments) and extracted fluorescence ratios (tumor/common reference) after subtracting the background using the GenePix Pro 6.0 software (Axon Instruments) were obtained. To identify differential miRNA expression between samples, the fluorescent ratios were log2 transformed, normalized and filtered as previously described [11].

Model Building

In order to build a model based on the expression of survival-associated microRNAs we used the Robust Likelihood-Based Survival Modeling with Microarray Data method [12], which was implemented to the *rbsurv* package for the R statistical environment. This technique utilizes the partial likelihood of the Cox model and functions through the generation of multiple gene (microRNAs in this case) models. It also divides the input dataset into training and validation sets and performs multiple cross-validations of a series of gene models so that it finally provides the optimal model based on the Akaike Information Criterion (AIC). Cox regression coefficients for the microRNAs included in the model for both datasets were obtained using the *survival* package for R. A total continuous score was calculated for each patient sample using the Cox regression coefficients obtained for the Ulm dataset according to the formula:

$si = \sum wj * xij$ where xij is the log-transformed expression value for the microRNA j in patient i, and wj is the weight assigned to probe set j (here wj was the Cox regression coefficient from the univariate analysis in the training set). The total score was calculated for each patient sample in the training and the validation dataset. To build a binary score classifying the sample to either a high or low score group it was obviously not possible to select a cutoff identical for both datasets because the microRNA expression profiling platforms were different. For that reason we defined a cut-off value specific for each total score. This was performed using an on-line R based program for finding the optimal cut-off values of Receiver Operating Characteristics (ROC) curves (http://molpath.charite.de/cutoff/) [13]. Univariate and multivariate analyses for correlation of the continuous and discrete scores with overall survival were performed for each of the datasets using the *survival* package. In the subgroups analyses intermediate risk patients were analyzed together because of the relatively small number in that subgroup.

Gene expression analysis

Raw mRNA expression data (.cel) files were downloaded from TCGA website. The expression set was built with the *affy* package and preprocessed with the RMA procedure from the same. Gene filtering was performed based on the IQR and CV values. Unsupervised clustering was performed using the built-in R functions hclust and heatmap. Supervised clustering and identification of the differentially expressed probes/genes was performed using the *limma* package. P-values of the differentially expressed genes were corrected with the Bonferoni-Hocheger procedure. Probe level data were collapsed to gene level using the WGCNA package and annotated with the *annotate* and *hgu133plus2.db* packages.

DNA methylation analysis

Level 3 (processed) data including beta values from Infinium II platform for 41 patients with CN-AML aged less than 61 years was retrieved from TCGA data portal. Differentially methylated CpG sites were identified using the *limma* package and plotted as heatmaps using the R low level heatmap function. CpG level data were collapsed to gene level after averaging the beta values using the WGCNA package and annotated with the *annotate* and *IlluminaHumanMethylation450k.db* packages.

Gene ontology analysis

Gene ontology analysis of differentially expressed genes was perfomed using the on-line tools from the PANTHER database (http://www.pantherdb.org/). Statistical overrepresentation

testing was performed as described before [14]. KEGG pathways overrepresentation analysis was performed using the Web Gestal server (http://bioinfo.vanderbilt.edu/webgestalt/) [15].

Gene set enrichment analysis

Gene set enrichment analysis (GSEA) was performed using the conventional on-line tool developed at the Broad Institute (http://www.broadinstitute.org/gsea/index.jsp) [16]. Gene level expression profiles for patients from the validation set were preprocessed and collapsed as described above and loaded onto the server as .txt files. Phenotype containing files were generated with Texmaker and loaded onto the server as .plc files. The collections of oncogenic and gene ontology signatures from the Molecular Signatures Database (MSigDB) were tested for enrichment.

Regulatory network building and analysis

MicroRNA-mRNA regulatory network was built using the CyTargetLinker [17] plug-in for the Java-based program Cytoscape v. 3.0.2 [18]. Gene ontology visualization of the enriched molecular functions in the built microRNA-mRNA network was performed with the BiNGO plug-in for the same platform [19].

Differential exon usage (DEU) analysis

Level 3 (processed) RNA-Seq data for expressed exons for 40 CN-AML patients under 61 years-of-age were downloaded from TCGA web-site. The tag raw count level data were compiled into a single data frame using conventional R functions. This expression set was filtered for tags with low expression, i.e. tags with lower than 5 reads per million total reads (cpm) in less than 10 samples were removed. Differentially expressed exons were identified through a binomial test based procedure implemented by the *edgeR* package for R/Bioconductor.

Common statistical procedures

All statistical procedures were performed using the R v. 3.0.1 environment for statistical computing. Chi-squared test was used for assessment of the independence in the distribution of categorical variables. Two sided t-test for independent samples was used to compare the means of normally distributed continuous variables. Wilcoxon-Mann-Whitney test was used to compare the medians of continuous variables without normal distribution. Shapiro-Wilk test was used to assess the normality of distribution of continuous variables. For all statistical test an alpha level of 0.05 was considered statistically significant.

Supplementary Figures

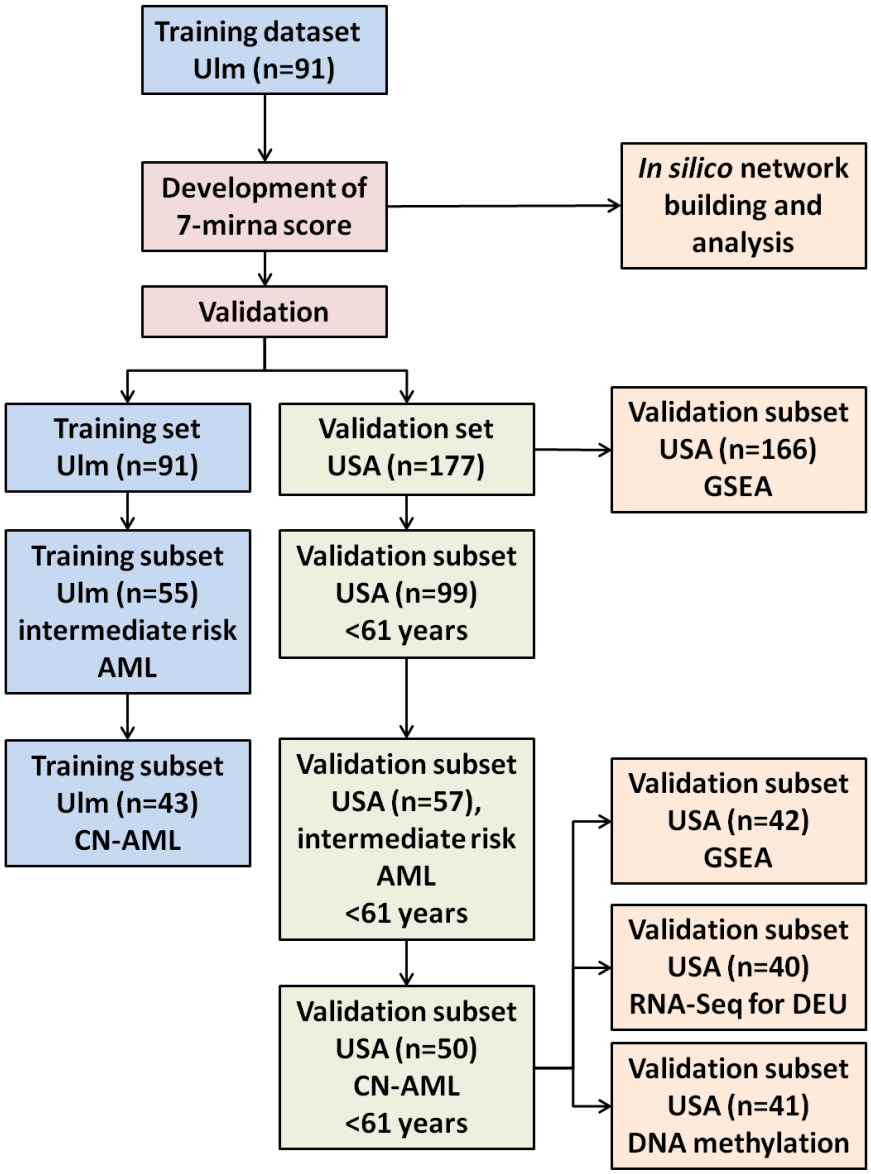


Figure S1. Flow chart of the performed analysis. The Ulm dataset was used to develop a 7-microRNA prognostic score for OS in adult AML. The score was further validated in the Ulm and the USA dataset, as well is subsets including younger patients (<61 years) and patients with intermediate risk AML or CN-AML. The 7 microRNAs were used to build a regulatory network including their validated and predicted target mRNAs. Gene set enrichement analysis (GSEA) was performed on the GEP data for patients from the entire USA dataset and from the USA subset of patients with CN-AML, aged less than 61 years. Differential exon usage (DEU) analysis and DNA methylation analysis were also performed only for USA subset of patients with CN-AML and aged less than 61 years.

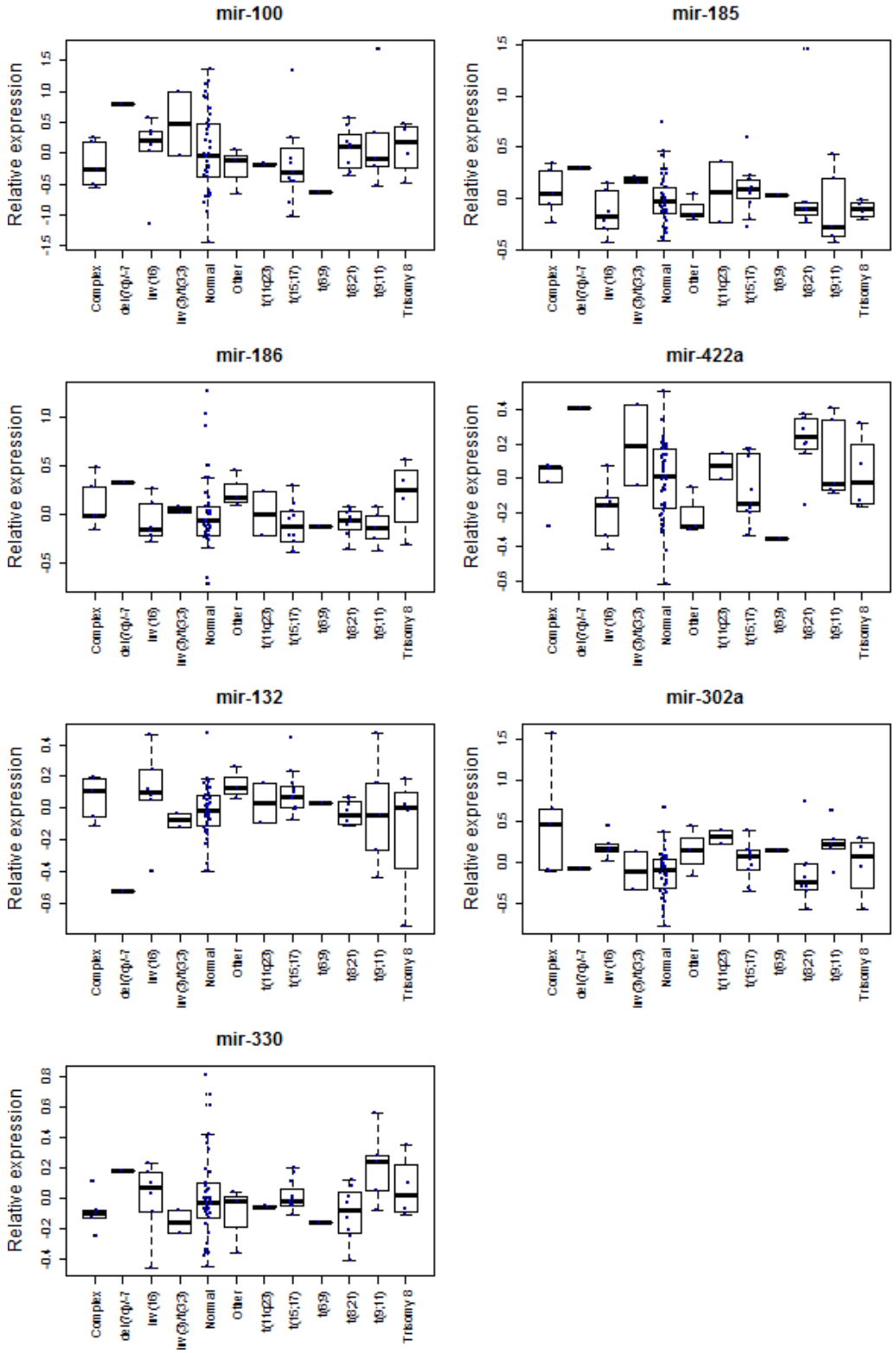


Figure S2. Expression of the microRNAs included in the prognostic score per cytogenetic group in the Ulm dataset.

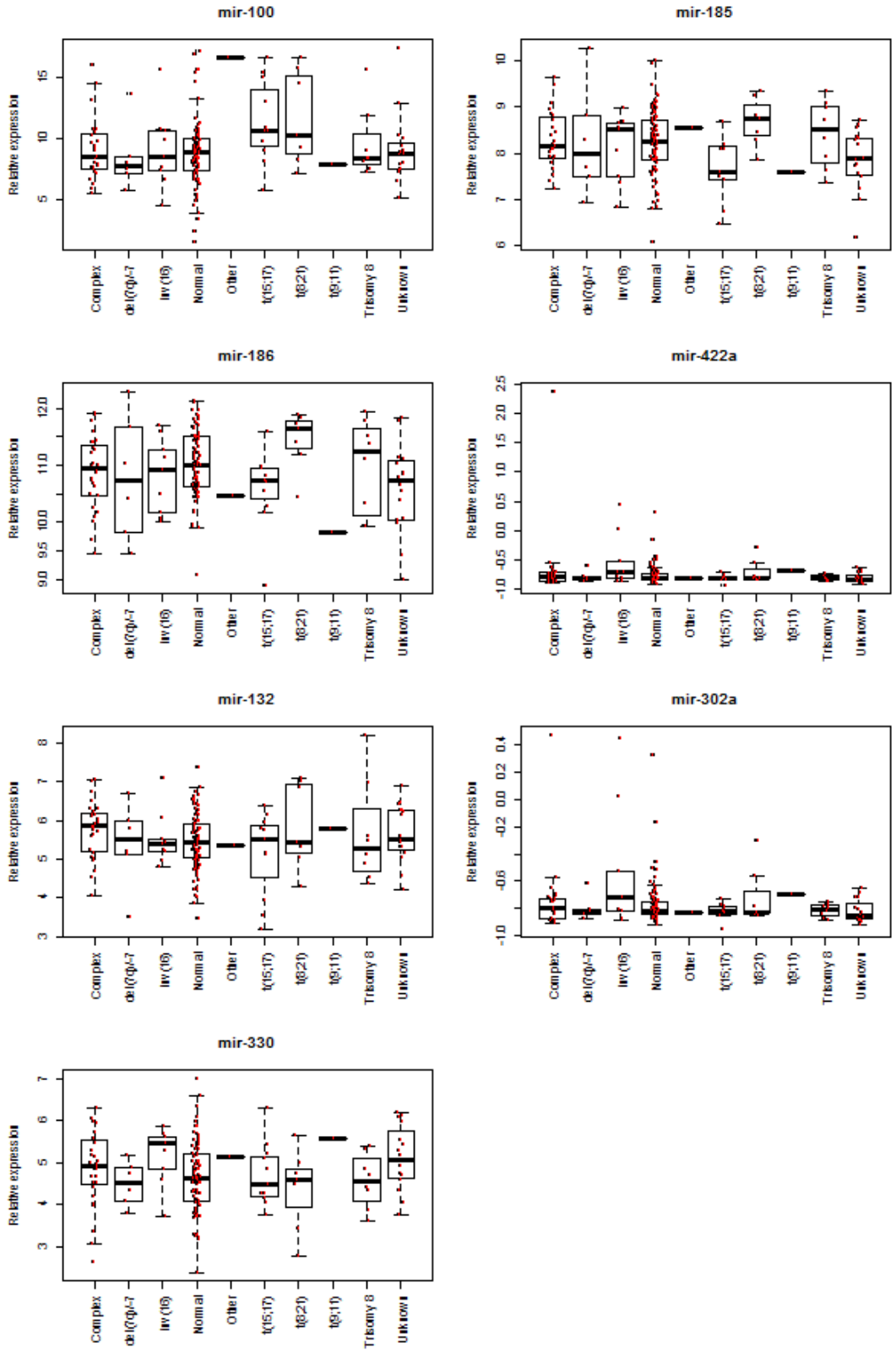
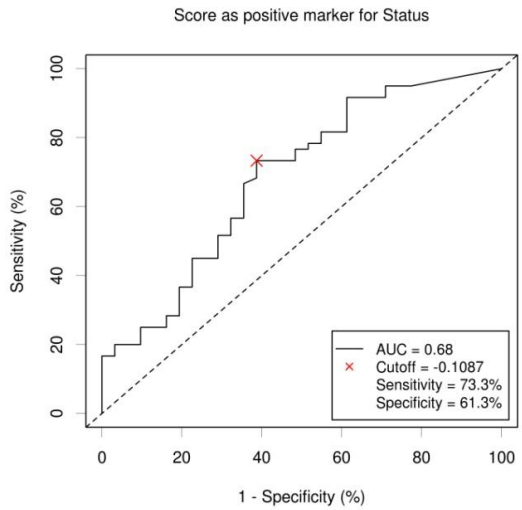


Figure S3. Expression of the microRNAs included in the prognostic score per cytogenetic group in the TCGA dataset.



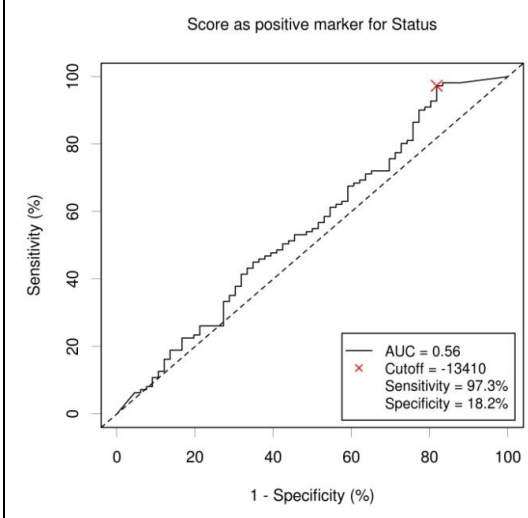
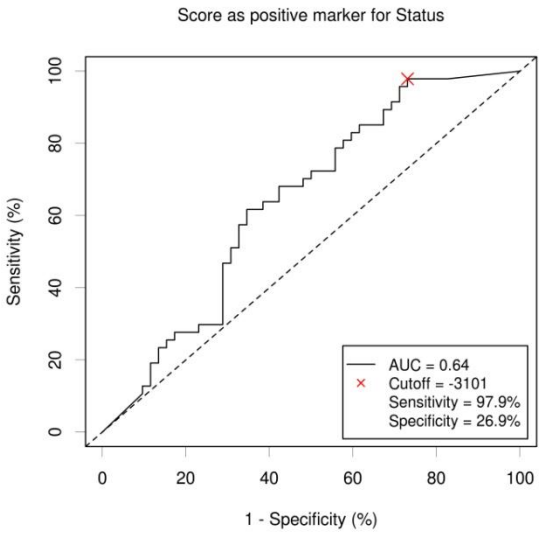


Figure S4. Optimal cut-off for the total score in the entire training and validations sets. **Left panel:** ROC curve with the position of the cut-off point selected based on the lowest p-value on the Log-rank test for the training dataset. **Right panel:** ROC curve with the position of the cut-off point selected based on the lowest p-value on the Log-rank test for the validation dataset.



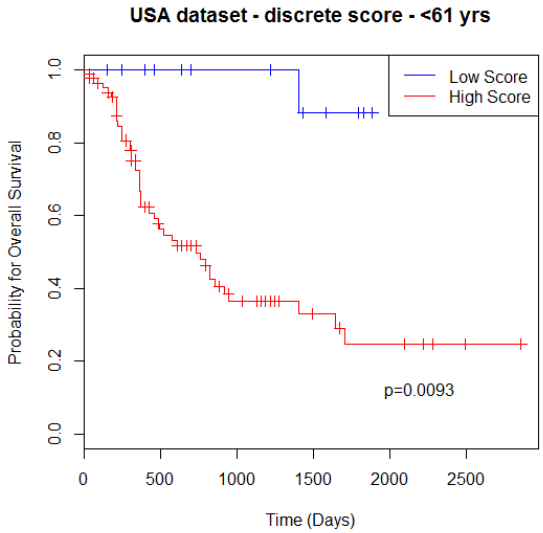


Figure S5. Univariate analysis of the discrete score in the validation set including only the patients under 61 years of age. **Left panel:** ROC curve for the continuous score with the optimal cut-off point position. **Right panel:** Effect of the newly defined discrete score on overall survival in the same subset of patients (Cox regression analysis).

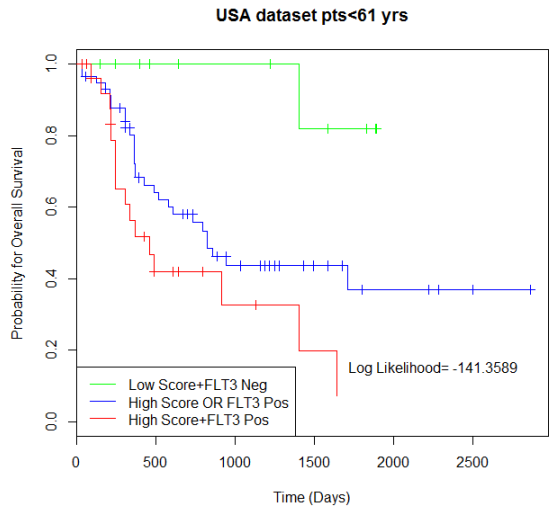
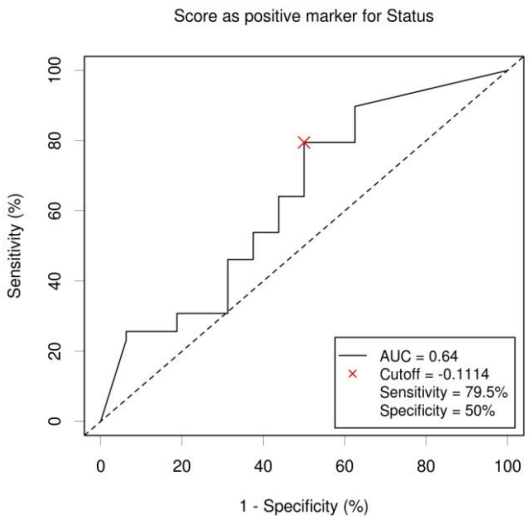


Figure S6. Survival curves based on the univariate analysis (Cox regression) of the combined effect of discrete score and *FLT3*-ITD mutational status on the OS in the validation set with patients under 61 years. Patients were stratified in 3 subgroups based on the High score or *FLT3*-ITD mutations.



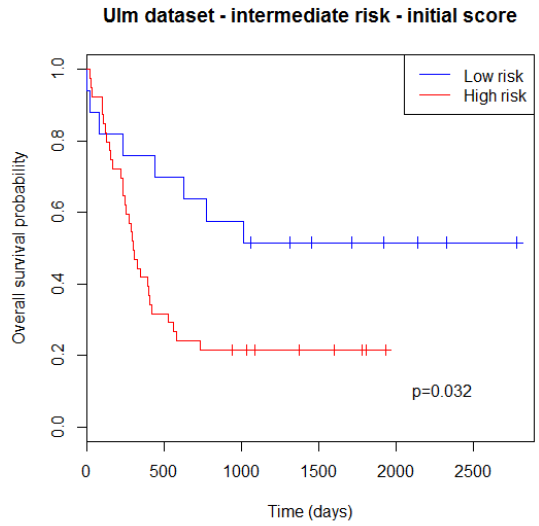


Figure S7. Univariate analysis of the discrete score in the training set including only the patients with intermediate cytogenetic risk under 61 years of age. **Left panel:** ROC for the continuous score with the optimal cut-off point position. **Right panel:** Effect of the newly defined discrete score on overall survival in the same subset of patients (Cox regression analysis).

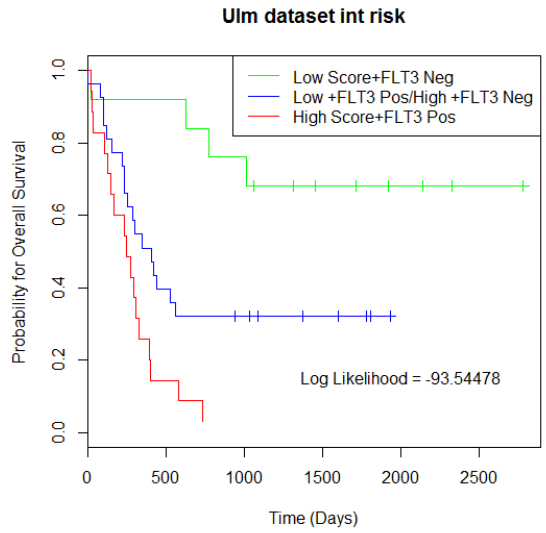
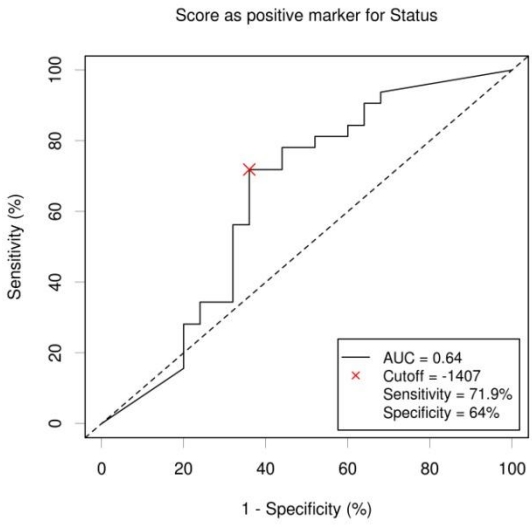


Figure S8. Survival curves based on the univariate analysis (Cox regression) of the combined effect of discrete score and *FLT3*-ITD mutational status on the OS in the training set including patients with intermediate cytogenetic risk under 61 years. Patients were stratified in 3 subgroups based on High score or *FLT3*-ITD mutations.



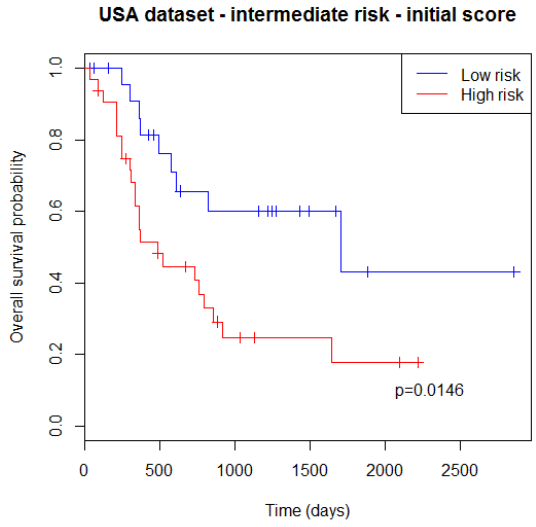


Figure S9. Univariate analysis of the discrete score in the validation set including only the patients with intermediate cytogenetic risk under 61 years of age. **Left panel:** ROC for the continuous score with the optimal cut-off point position. **Right panel:** Effect of the newly defined discrete score on overall survival in the same subset of patients (Cox regression analysis).

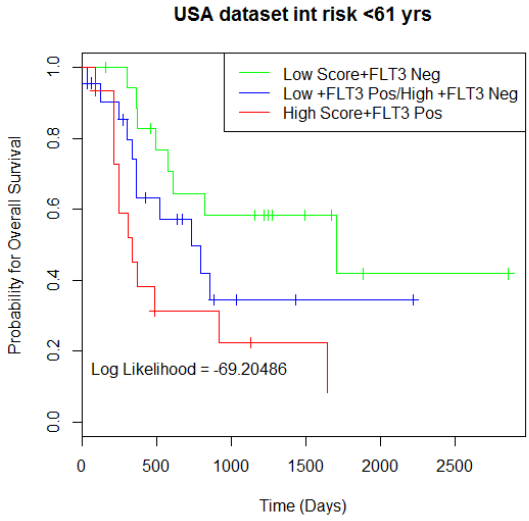
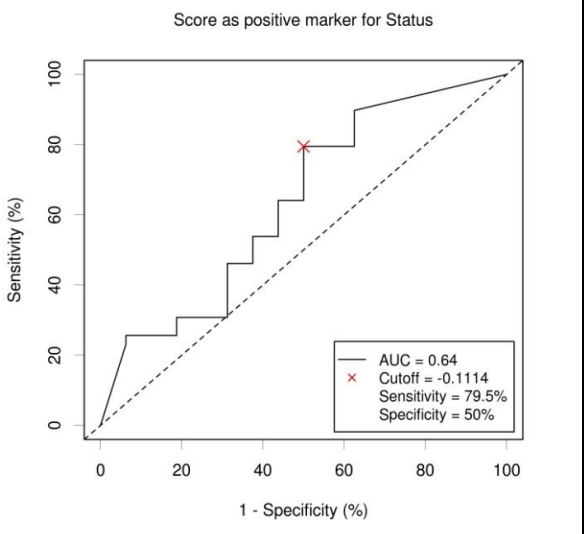


Figure S10. Survival curves based on the univariate analysis (Cox regression) of the combined effect of discrete score and *FLT3*-ITD mutational status on the OS in the validation set including patients with intermediate cytogenetic risk under 61 years. Patients were stratified in 3 subgroups based on the based on High score or *FLT3*-ITD mutations.



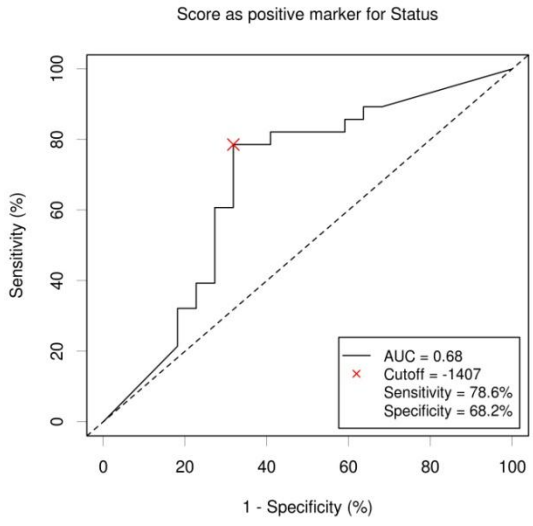


Figure S11. Univariate analysis of the discrete score in the training and validation datasets including only the patients with CN-AML under 61 years of age. **Left panel:** ROC for the continuous score with the optimal cut-off point position for the training dataset. **Right panel:** ROC for the continuous score with the optimal cut-off point position for the validation dataset.

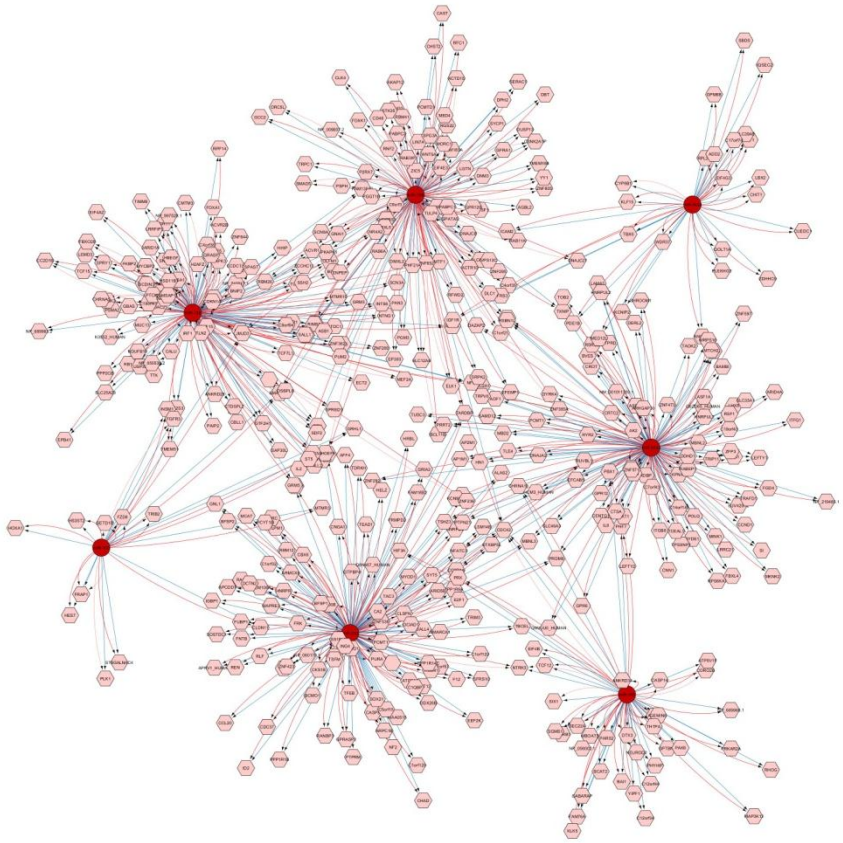


Figure S12. Computational network built between microRNAs and their targets. The dark purple nodes represent each of the microRNAs included in the score. The pink nodes represent an mRNA target present in at least 2 of the used databases. Each arrow represents the presence of the microRNA-mRNA interaction in a database.

Figure S13. Relationship of the overrepresented molecular functions in the microRNA-mRNA network. The size of each circle is proportional to the relative enrichment of the respective gene ontology term.

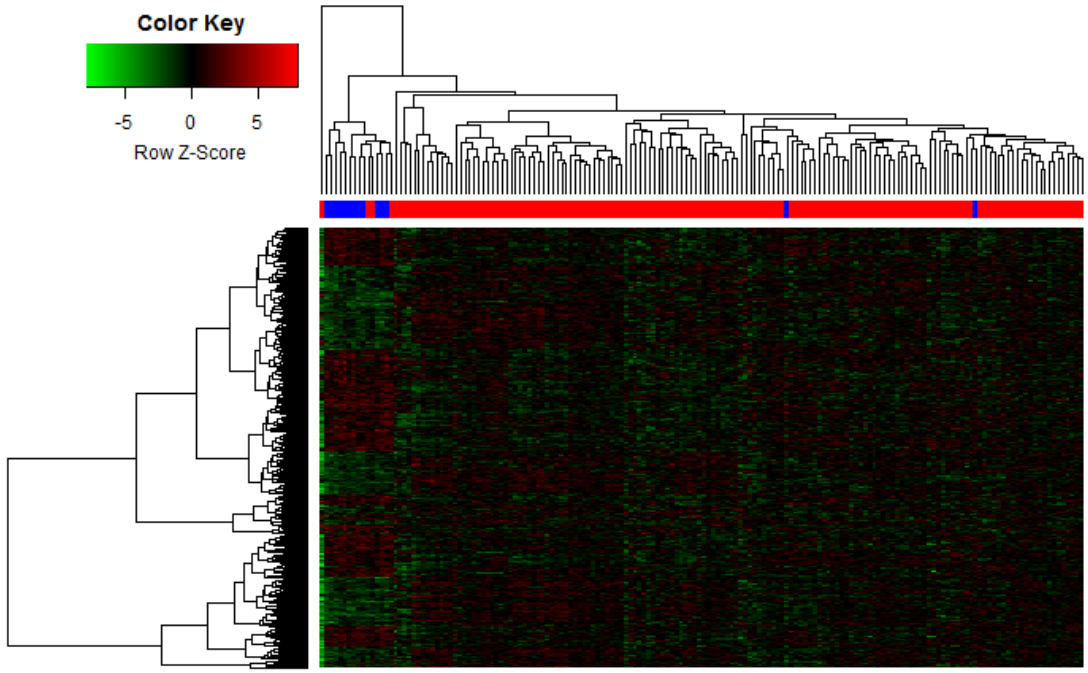
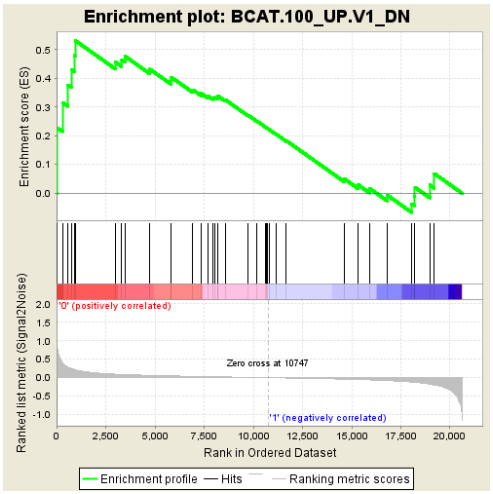


Figure S14. Hierarchical clustering of differentially expressed genes between patients from the entire validation dataset. Samples' annotation– Low Score patients, red color – High Score patients.



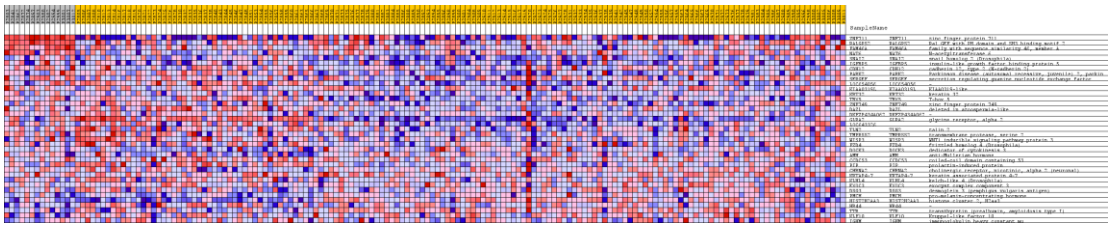


Figure S15. Snapshot of the GSEA output. BCAT.100_UP.V1_DN signature is enriched in the Low score vs. High score patients in the validation dataset. Only patients with CN-AML <61 years of age were included. Gene ontology signatures were analyzed. **Upper panel:** Enrichment plot. **Lower panel:** Heatmap of expressions of the genes from the signatures.

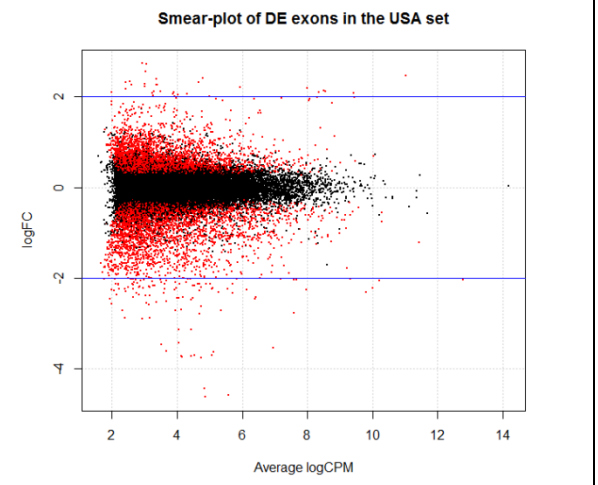


Figure S16. Results of the analysis of the differential exon usage (DEU) between Low vs. High Score subgroup based on RNA-Seq data of 40 patients with CN-AML less than 61 years of age. **Left panel:** Smear plot representing log-fold change (logFC) (i.e. the log of the ratio of expression levels for each tag between two experimental groups) against the log-concentration (i.e. the overall average expression level for each tag across the two groups expressed as the log count per million reads (logCPM)). The red dots represent tags with differential expression at level of significance of 0.05. The blue lines represent the 2-fold logFC change. **Right panel:** Multidimensional Scaling Plot (MDS) plot representing the similarity between the two subgroups of patients.

Supplementary Tables

Table S1. Summary of patients'characteristics in the training and validation datasets.

Parameter	Ulm	USA	p-values
Number	91	177	
Age			1.29E-09**
Median	45.03	58	
range	(19.15÷60.39)	(18÷88)	
Gender			0.6206*
Male(%)	53 (58.24)	96 (54.24)	
Female(%)	38 (41.76)	81 (45.76)	
FAB subtype			0.3313*
M0	4	16	
M1	18	39	
M2	19	38	
M3	12	16	
M4	26	40	
M5	10	21	
M6	4	2	
M7	0	3	
Unclassified	0	2	
Cytogenetics			9.28E-05*
Normal	43	90	
t(15;17)	11	11	
t(8;21)	8	7	
inv(16)	6	9	
inv(3)/t(3;3)	2	0	
t(6;9)	1	0	
t(9;11)	5	1	
Trisomy 8	4	8	
del(7q)/-7	1	6	
t(11q23)	2	0	
Complex	5	28	
Other	3	1	
Unknown	0	16	
Risk group			0.1198*
Favourable	25	34	
Intermediate	55	103	
Poor	11	38	
Unknown	0	2	
FLT3-ITD mutation			0.1252*
Positive	31	51	
Negative	60	119	
Unknown	0	7	
NPM1 mutation			2.20E-16*
Positive	22	44	
Negative	20	130	
Unknown	49	3	

*Chi-squared test; **Wilcoxon test

Table S2. Output from the *rbsurv* package – training (Ulm) dataset. nloglik – negative log-likelihood, AIC – Akaike Information Criterion.

Order gene	nloglik	AIC	Selected
10	241.15	484.3	*
60	240.23	484.46	*
61	239.08	484.17	*
157	238.64	485.28	*
26	236.92	483.84	*
111	236.07	484.14	*
126	234.68	483.37	*

Table S3. Cox regression coefficients from univariate analysis of the correlation of the selected 7 microRNAs with the overall survival (OS). The coefficients for the Ulm dataset were used for the calculation of the total score in each dataset.

MicroRNA	Ulm dataset		USA dataset	
	ln(HR)	p-value	ln(HR)	p-value
miR-100	-0.449	0.034	-1.69E-05	0.02
miR-185	0.253	0.56	0.00157	0.012
miR-186	-0.698	0.13	0.000132	0.27
miR-422a	-0.972	0.086	-10.9	0.99
miR-132	-1.1	0.081	0.00178	0.56
miR-302a	0.286	0.43	0	NaN
miR-330	-0.145	0.77	0.00118	0.85

Table S4. Univariate and multivariate analyses of the affect on overall survival (OS) of the 7-microRNAs based score. Data for the training dataset. Two types of multivariate models were test – extended and short for both the continuous and the discrete score.

Univariate analysis Ulm dataset all patients						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	2.392	1.426	4.013	0.872	0.264	0.000955
Score discrete optimal cut-off point	2.6293	1.473	4.693	0.9667	0.2956	0.00107
Age continuous	1.03821	1.013	1.064	0.0375	0.01275	0.00327
Gender	1.4729	0.8844	2.453	0.3872	0.2603	0.137
FLT3 mut status	2.5571	1.525	4.287	0.9389	0.2637	0.00037
NPM1 mut status	1.3817	0.6797	2.809	0.3233	0.362	0.372
Risk group low	0.1654	0.06864	0.3985	-1.7994	0.4487	6.06E-05
Risk group intermediate	0.4183	0.21136	0.8279	-0.8716	0.3483	0.0123

Multivariate analysis Ulm dataset all patients						
Extended model with continuous score						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	1.48841	0.88461	2.5043	0.39771	0.26547	0.1341
Age continuous	1.0268	0.99952	1.0548	0.02644	0.01373	0.05419
Gender	1.4225	0.83517	2.4229	0.35242	0.27171	0.19462
FLT3 mut status	3.4592	1.88804	6.3379	1.24104	0.30894	5.89E-05
NPM1 mut status	1.10315	0.47041	2.587	0.09817	0.43486	0.82139
Risk group low	0.16175	0.05608	0.4666	-1.8217	0.54049	0.00075
Risk group intermediate	0.3942	0.15165	1.0249	-0.93078	0.48745	0.0562
Short model with continuous score						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	1.5632	0.9235	2.6462	0.4468	0.2686	0.09619
FLT3 mut status	3.3849	1.92767	5.9436	1.2193	0.2873	2.19E-05
Risk group low	0.1253	0.04594	0.3415	-2.0773	0.5118	4.92E-05
Risk group intermediate	0.3175	0.15501	0.6501	-1.1474	0.3657	0.00171
Extended model with discrete score optimal cut-off point						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score discrete	1.7726	0.93508	3.3603	0.57244	0.32632	0.079387
Age continuous	1.02487	0.99721	1.0533	0.02457	0.01396	7.85E-02
Gender	1.40268	0.8242	2.3872	0.33839	0.2713	0.212292
FLT3 mut status	3.4749	1.89677	6.3662	1.24558	0.30889	5.52E-05
NPM1 mut status	1.1151	0.4737	2.6252	0.10898	0.01396	0.078453
Risk group low	0.1555	0.05594	0.4321	-1.8612	0.52154	0.000359
Risk group intermediate	0.3657	0.14203	0.9415	-1.00598	0.48251	0.037081
Short model with discrete score optimal cut-off point						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score discrete optimal cut	1.9279	1.03508	3.5909	0.6564	0.3173	0.03858
FLT3 mut status	3.3916	1.93922	5.9317	1.2213	0.2852	1.85E-05
Risk group low	0.1223	0.04653	0.3215	-2.1013	0.4931	2.03E-05
Risk group intermediate	0.2896	0.14229	0.5893	-1.2393	0.3625	0.00063

Table S5. Univariate and multivariate analyses of the effect on overall survival (OS) of the 7-microRNAs based score. Data for the validation dataset. Two types of multivariate models were tested – extended and short for both the continuous and the discrete score.

Univariate analysis USA dataset all patients						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	1	1	1	1	1.40E-05	0.0225

Score discrete optimal cut	5.9459	1.879	18.81	1.7827	0.5877	0.00242
Age continuous	1.043356	1.028	1.059	0.042442	0.007364	8.24E-09
Gender-male	0.9706	0.6681	1.41	-0.02979	0.19057	0.876
FLT3 mut status	1.06886	0.7009	1.63	0.06659	0.21527	0.757
NPM1 mut status	1.2246	0.8075	1.857	0.2026	0.2125	0.34
Risk group favorable	0.06842	0.01479	0.3166	-2.68206	0.78169	6.01E-04
Risk group intermediate	0.17979	0.04278	0.7557	-1.71595	0.73258	1.92E-02
Risk group poor	0.25097	0.05822	1.0818	-1.38241	0.74544	0.063671
Multivariate analysis USA dataset all patients						
Extended model with continuous score						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	1	0.99999	1	1.58E-05	1.46E-05	0.27884
Age continuous	1.0448	1.02932	1.0605	4.38E-02	7.60E-03	8.35E-09
Gender-male	0.9869	0.66719	1.4599	-1.32E-02	2.00E-01	0.9475
FLT3 mut status	1.5174	0.93161	2.4714	4.17E-01	2.49E-01	0.09387
NPM1 mut status	0.7067	0.42063	1.1874	-3.47E-01	2.65E-01	0.18979
Risk group favorable	0.0666	0.01261	0.3517	-2.71E+00	8.49E-01	0.00142
Risk group intermediate	0.1664	0.0375	0.7387	-1.79E+00	7.60E-01	0.01836
Risk group poor	0.1583	0.03309	0.7573	-1.84E+00	7.99E-01	0.021
Short model with continuous score						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	1.00E+00	0.99999	1	1.56E-05	1.51E-05	0.30246
FLT3 mut status	1.15E+00	0.74706	1.7618	1.37E-01	2.19E-01	0.53025
Risk group favorable	8.55E-02	0.01791	0.4077	-2.46E+00	7.97E-01	0.00203
Risk group intermediate	2.04E-01	0.0481	0.8632	-1.59E+00	7.37E-01	0.0308
Risk group poor	2.65E-01	0.06073	1.1587	-1.33E+00	7.52E-01	0.07771
Extended model with discrete score optimal cut						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score discrete optimal cut	3.57339	1.01769	12.5472	1.273515	0.640818	0.04689
Age continuous	1.0432	1.02779	1.0588	0.042293	0.007592	2.53E-08
Gender	1.010292	0.68304	1.4943	0.010239	0.199721	0.95911
FLT3 mut status	1.611723	0.986	2.6345	0.477304	0.250721	0.05695
NPM1 mut status	0.672344	0.3982	1.1352	-0.39699	0.267258	0.13744
Risk group favorable	0.075226	0.01441	0.3928	-2.58726	0.843341	0.00216
Risk group intermediate	0.163755	0.0369	0.7268	-1.80938	0.760341	0.01733
Risk group poor	0.153652	0.03209	0.7357	-1.87307	0.799067	0.01907
Short model with discrete score optimal cut						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score discrete optimal cut	4.1125	1.19758	14.1227	1.414	0.6295	0.02468
FLT3 mut status	1.2254	0.797	1.8841	0.2033	0.2195	0.35435
Risk group favorable	0.1119	0.02367	0.529	-2.1902	0.7925	0.00572
Risk group intermediate	0.2108	0.04976	0.8928	-1.557	0.7365	0.03452
Risk group poor	0.2764	0.0633	1.2067	-1.286	0.752	0.08725

Table S6. Univariate and multivariate analyses of the affect on overall survival (OS) of the 7-microRNAs based score. Data for the validation dataset for patients under 61 years of age. Two types of multivariate models were tested – extended and short for both the continuous and the discrete score.

Univariate USA dataset patients <61 years						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	1.00E+00	1	1	7.23E-05	3.40E-05	0.0332
Score discrete optimal cut	13.941	1.915	101.5	2.635	1.013	0.00928
Age continuous	1.007978	0.9835	1.033	0.007946	0.012566	5.27E-01
Gender-male	0.8517	0.4797	1.512	-0.1605	0.2929	0.584
FLT3 mut status	1.469	0.7987	2.702	0.3846	0.3109	0.216
NPM1 mut status	1.6043	0.8739	2.945	0.4727	0.3099	0.127
Risk group favorable	0.01355	0.001288	0.1427	-4.301	1.20097	3.42E-04
Risk group intermediate	0.04858	0.005362	0.4401	-3.02456	1.12447	7.15E-03
Risk group poor	0.07677	0.008028	0.7342	-2.56691	1.152	0.025866
Multivariate USA dataset patients <61 years						
Extended model with continuous score						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	1.00E+00	0.999984	1.0001	5.44E-05	3.59E-05	0.12923
Age continuous	1.00E+00	0.975908	1.0266	9.18E-04	1.29E-02	0.94334
Gender-male	9.82E-01	0.540862	1.7821	-1.84E-02	3.04E-01	0.95176
FLT3 mut status	1.82E+00	0.908587	3.6404	5.98E-01	3.54E-01	0.09118
NPM1 mut status	8.45E-01	0.384785	1.8533	-1.69E-01	4.01E-01	0.67334
Risk group favorable	2.35E-02	0.001904	0.2907	-3.75E+00	1.28E+00	0.00346
Risk group intermediate	6.82E-02	0.006991	0.6644	-2.69E+00	1.16E+00	0.02078
Risk group poor	1.10E-01	0.00961	1.2649	-2.21E+00	1.25E+00	0.07653
Short model with continuous score						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	1.00E+00	0.999984	1.0001	5.25E-05	3.52E-05	0.13516
FLT3 mut status	1.73E+00	0.907291	3.2834	5.46E-01	3.28E-01	0.09622
Risk group favorable	2.69E-02	0.002453	0.2939	-3.62E+00	1.22E+00	0.00305
Risk group intermediate	7.15E-02	0.007687	0.665	-2.64E+00	1.14E+00	0.02043
Risk group poor	1.26E-01	0.012385	1.2708	-2.08E+00	1.18E+00	0.0789
Extended model with discrete score – optimal cut						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score discrete optimal cut	10.763019	1.417533	81.7213	2.376116	1.034304	0.0216
Age continuous	0.995353	0.970035	1.0213	-0.00466	0.013146	0.72309
Gender	0.896894	0.491162	1.6378	-0.10882	0.307232	0.7232
FLT3 mut status	1.805553	0.892781	3.6515	0.590867	0.359334	0.10011
NPM1 mut status	0.933087	0.418977	2.078	-0.06926	0.408519	0.86538

Risk group favorable	0.031455	0.002578	0.3838	-3.45919	1.276363	0.00672
Risk group intermediate	0.07895	0.008125	0.7672	-2.53893	1.160187	0.02864
Risk group poor	0.130794	0.011419	1.4982	-2.03414	1.244088	0.10204
Short model with discrete score – optimal cut						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score discrete optimal cut	10.1781	1.354744	76.4674	2.32024	1.02891	0.02413
FLT3 mut status	1.73021	0.909048	3.2931	0.54824	0.32837	0.095
Risk group favorable	0.031	0.002868	0.335	-3.47387	1.21447	0.00423
Risk group intermediate	0.07497	0.008071	0.6965	-2.59061	1.13721	0.02272
Risk group poor	0.12495	0.012328	1.2664	-2.07987	1.18167	0.07839

Table S7. Comparison of the distribution of Low and High Score patients per cytogenetic abnormality in the two sets. P-values from Chi-squared test.

Dataset	Ulm		USA		
Discrete Score	Low	High	Low	High	p-value
Normal	13	30	3	87	2.97E-05
t(15;17)	8	3	8	3	1
t(8;21)	3	5	0	7	0.2442
inv(16)	4	2	0	9	0.02354
inv(3)/t(3;3)	1	1	0	0	-
t(6;9)	0	1	0	0	-
t(9;11)	1	4	0	1	1
Trisomy 8	1	3	0	8	0.7119
del(7q)/-7	0	1	1	5	1
t(11q23)	0	2	0	0	-
Complex	3	2	3	25	0.04522
Other	1	2	0	1	1
Unknown	0	0	0	16	-
Total	35	56	15	162	6.55E-09

Table S8. Univariate and multivariate analyses of the affect on overall survival (OS) of the 7-microRNAs based score. Data for the training dataset for patients with intermediate risk cytogenetics and under 61 years of age. Two types of multivariate models were tested – extended and short for both the continuous and the discrete score.

Univariate Ulm intermediate risk patients						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	1.996	1.056	3.774	0.6911	0.325	0.0334
Score discrete optimal cut for intermediate risk	2.374	1.079	5.226	0.8648	0.4025	0.0317
Age continuous	1.01937	0.9894	1.05	0.01918	0.01522	0.208

Gender	1.3624	0.7257	2.558	0.3093	0.3213	0.336
FLT3 mut status	4.5278	2.282	8.983	1.5102	0.3496	1.56E-05
NPM1 mut status	1.3817	0.6797	2.809	0.3233	0.362	0.372
Multivariate Ulm intermediate risk patients						
Extended model with continuous score						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	1.99993	0.8974	4.457	0.69311	0.40888	0.090044
Age continuous	1.05387	1.0134	1.096	0.05247	0.01997	0.008588
Gender-female	3.53504	1.5771	7.924	1.26272	0.4118	0.002167
FLT3 mut status	8.29041	2.7066	25.394	2.1151	0.57114	0.000213
NPM1 mut status	0.39092	0.1507	1.014	-0.93925	0.48634	0.053451
Short model with continuous score						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	1.6407	0.8498	3.168	0.4951	0.3357	0.14
FLT3 mut status	4.0613	2.0332	8.112	1.4015	0.353	7.18E-05
Extended model discreet score – optimal cut						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score discrete optimal cut for intermediate risk	2.16295	0.8145	5.7441	0.77147	0.49832	0.121588
Age continuous	1.05741	1.0158	1.1007	0.05583	0.02047	0.006376
Gender	3.44067	1.5384	7.6951	1.23567	0.41068	0.002622
FLT3 mut status	8.42975	2.7784	25.5764	2.13177	0.56629	0.000167
NPM1 mut status	0.33293	0.1241	0.8934	-1.09981	0.50362	0.028976
Short model with discrete score – optimal cut						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score discrete optimal cut	1.7088	0.753	3.878	0.5358	0.4181	0.200045
FLT3 mut status	3.9762	1.969	8.029	1.3803	0.3585	1.18E-04

Table S9. Univariate and multivariate analyses of the affect on overall survival (OS) of the 7-microRNAs based score. Data for the validation dataset for patients with intermediate risk cytogenetics and under 61 years of age. Two types of multivariate models were tested – extended and short for both the continuous and the discrete score.

Univariate USA intermediate risk pts <61 years						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	1.000593	1	1.001	0.000593	0.000288	0.0397
Score discrete optimal cut for intermediate risk	2.6234	1.21	5.688	0.9645	0.3949	0.0146
Age continuous	1.006797	0.9788	1.036	0.006774	0.014371	0.637
Gender-Male	0.94274	0.4697	1.892	-0.05896	0.35545	0.868
FLT3 mut status	2.0713	0.9987	4.296	0.7282	0.3722	5.04E-02
NPM1 mut status	1.2383	0.6113	2.508	0.2137	0.3602	0.553

Multivariate USA intermediate risk patients <61 years						
Extended model with continuous score						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	1.000523	0.9999	1.001	0.000523	0.000301	0.0822
Age continuous	0.993784	0.964	1.025	-0.00624	0.015532	0.6881
Gender-male	0.892001	0.4273	1.862	-0.11429	0.375479	0.7608
FLT3 mut status	2.263223	0.947	5.409	0.81679	0.444512	0.0661
NPM1 mut status	0.697945	0.2958	1.647	-0.35962	0.438048	0.4117
Short model with continuous score						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	1.000468	0.9999	1.001	0.000467	0.000286	0.102
FLT3 mut status	1.847827	0.8543	3.997	0.61401	0.393628	1.19E-01
Extended model with discrete score optimal cut-off						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score discrete optimal cut for intermediate risk	2.775351	1.1963	6.439	1.020777	0.429373	0.0174
Age continuous	0.995008	0.9635	1.028	-0.005	0.016418	0.7605
Gender	0.97774	0.4641	2.06	-0.02251	0.380225	0.9528
FLT3 mut status	1.754128	0.7715	3.988	0.561972	0.419083	0.1799
NPM1 mut status	0.782724	0.3381	1.812	-0.24498	0.428224	0.5673
Short model with discrete score optimal cut-off						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score discrete optimal cut	2.586	1.145	5.839	0.95	0.4156	0.0223
FLT3 mut status	1.559	0.731	3.325	0.4441	0.3864	2.51E-01

Table S10. Univariate and multivariate analyses of the affect on overall survival (OS) of the 7-microRNAs based score. Data for the training dataset for patients with CN-AML and under 61 years of age. Two types of multivariate models were tested – extended and short for both the continuous and the discrete score.

Univariate analysis Ulm CN-AML patients						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	2.012	0.9743	4.157	0.6994	0.3701	0.0588
Score discrete optimal cut	2.3387	0.9947	5.499	0.8496	0.4362	0.0514
Age continuous	1.03875	1.001	1.078	0.03802	0.01893	0.0446
Gender	1.629	0.801	3.312	0.4878	0.3621	0.178
FLT3 mut status	4.4572	1.982	10.03	1.4945	0.4136	0.000302
NPM1 mut status	1.3817	0.6797	2.809	0.3233	0.362	0.372
Multivariate analysis Ulm CN-AML patients						
Extended model continuous score						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	1.99993	0.8974	4.457	0.69311	0.40888	0.090044

Age continuous	1.05387	1.0134	1.096	0.05247	0.01997	0.008588
Gender	3.53504	1.5771	7.924	1.26272	0.4118	0.002167
FLT3 mut status	8.29041	2.7066	25.394	2.1151	0.57114	0.000213
NPM1 mut status	0.39092	0.1507	1.014	-0.93925	0.48634	0.053451
Short model continuous score						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	3.9597	1.736	9.033	1.3762	0.4208	0.00107
FLT3 mut status	1.5619	0.728	3.351	0.4459	0.3895	2.52E-01
Extended model discrete score optimal cut-off						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score discrete optimal cut	2.16295	0.8145	5.7441	0.77147	0.49832	0.121588
Age continuous	1.05741	1.0158	1.1007	0.05583	0.02047	0.006376
Gender	3.44067	1.5384	7.6951	1.23567	0.41068	0.002622
FLT3 mut status	8.42975	2.7784	25.5764	2.13177	0.56629	0.000167
NPM1 mut status	0.33293	0.1241	0.8934	-1.09981	0.50362	0.028976
Short model discrete score optimal cut-off						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score discrete optimal cut	1.7242	0.6737	4.412	0.5448	0.4794	0.255845
FLT3 mut status	5.0587	1.9768	12.946	1.6211	0.4794	0.000721
NPM1 mut status	0.548	0.2259	1.329	-0.6015	0.4521	0.183376

Table S11. Univariate and multivariate analyses of the affect on overall survival (OS) of the 7-microRNAs based score. Data for the validation dataset for patients with CN-AML and under 61 years of age. Two types of multivariate models were tested – extended and short for both the continuous and the discrete score.

Univariate USA CN-AML patients <61 years						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	1.00074	1	1.001	0.00074	0.000324	0.0224
Score discrete optimal cut	4.183	1.675	10.44	1.431	0.4669	0.00218
Age continuous	1.01	0.9815	1.04	0.01039	0.01484	0.484
Gender MALE	0.8907	0.4237	1.873	-0.1157	0.3791	0.76
FLT3 mut status	2.0971	0.9627	4.568	0.7406	0.3972	0.0623
NPM1 mut status	1.319	0.6159	2.824	0.2767	0.3885	0.476
Multivariate USA CN-AML patients <61 years						
Extended model continuous score						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score continuous	1.000714	1	1.001	0.000714	0.000372	0.0551
Age continuous	0.996167	0.9652	1.028	-0.00384	0.016125	0.8118
Gender	0.861151	0.3861	1.921	-0.14949	0.409231	0.7149
FLT3 mut status	1.838843	0.7349	4.601	0.609136	0.467973	0.193
NPM1 mut status	0.662707	0.265	1.657	-0.41142	0.46761	0.3789
Short model continuous score						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p

Score continuous	1.000617	0.9999	1.001	0.000617	0.000341	0.0706
FLT3 mut status	1.576361	0.6678	3.721	0.455119	0.438182	2.99E-01
Extended model discrete score optimal cut-off						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score discrete optimal cut	6.645227	2.1373	20.661	1.893899	0.578762	0.00107
Age continuous	0.991339	0.9578	1.026	-0.0087	0.017535	0.61983
Gender	0.991991	0.4331	2.272	-0.00804	0.422817	0.98483
FLT3 mut status	1.246165	0.5297	2.932	0.220071	0.436486	0.61413
NPM1 mut status	0.626944	0.2538	1.549	-0.4669	0.461337	0.31151
Short model discrete score optimal cut-off						
Parameter	HR	95% CI lower	95% CI upper	ln(HR)	se(HR)	p
Score discrete optimal cut	6.1157	2.0838	17.949	1.8109	0.5493	0.000979
FLT3 mut status	1.2621	0.5322	2.993	0.2328	0.4406	0.597225
NPM1 mut status	0.595	0.2524	1.403	-0.5192	0.4375	0.235372

Table S12. List of common genes in the netweork analysis.

Number	Gene Symbol
1	PHYHIP
2	HELZ
3	RPL23A
4	SERAC1
5	IL8
6	FRAP1
7	PAIP2
8	DCTN2
9	TFEB
10	H2AFZ
11	SPRY1
12	C9orf3
13	ZNF423
14	SETD1B
15	ENOSF1
16	RBM26
17	HRBL
18	PCYT1B
19	Q9NUU0_HUMAN
20	PABPC1
21	MYCBP2
22	PTPN21
23	CDC42
24	GRM3
25	SLC33A1
26	EPB41

27	FAM76A
28	SPG3A
29	PCMT1
30	SCN3A
31	FIGF
32	YLPM1
33	KPNA2
34	ICAM2
35	PTPRD
36	PARP8
37	NFATC3
38	ASF1A
39	FBXW4
40	CHRNA10
41	C9orf94
42	LBX2
43	GTPBP4
44	GRM5
45	RFWD2
46	MTF1
47	AP1M1
48	C1orf122
49	APRV1_HUMAN
50	DMXL2
51	BCAT2
52	PRDM8
53	GRHL1
54	HBEGF
55	FGD4
56	CCL20
57	TRIP11
58	AFF4
59	BCAN
60	CHST2
61	POLQ
62	KLF15
63	SNRPD3
64	PCMT1
65	Q9HCM3_HUMAN
66	MED12L
67	KHDRBS3
68	AK2
69	AGBL2
70	LAMA3
71	TCF12

72	TTK
73	CUEDC1
74	PRX
75	RBM12
76	CASP14
77	BAI1
78	C14orf145
79	RSRC2
80	SPATA5
81	C4orf35
82	GNL1
83	ANGPT4
84	EFCAB5
85	ACTR10
86	ZFP3
87	BBS1
88	FOXA1
89	PRRT2
90	RHOG
91	SPAST
92	C5orf15
93	CLDN11
94	GTF2H1
95	DDX26B
96	SYCP1
97	AOF1
98	SMARCA5
99	PURA
100	RGS22
101	CAST
102	ARPC1B
103	DDHD1
104	SDF2
105	HNRPUL2
106	GABARAP
107	PBX1
108	PCMTD1
109	RPP14
110	LIN7A
111	GFRA1
112	PABPC3
113	KIAA0515
114	MTCH2
115	MLL3
116	SUV420H2

117	MBNL3
118	GAPVD1
119	RSBN1L
120	TLE4
121	FRK
122	KCNMA1
123	EIF4A2
124	PARS2
125	HSD11B1
126	HIF3A
127	ASF1B
128	LSM14B
129	GPM6B
130	PUM2
131	PPP1R1B
132	SLC2A1
133	PLK1
134	GRASP
135	CASP8
136	TCEAL1
137	ACVR1
138	CNN1
139	OLFM1
140	SHROOM1
141	MORC1
142	SOX21
143	ZNF292
144	RAPH1
145	ASB1
146	C1QBP
147	ENPEP
148	TCF7L1
149	RAI2
150	CENTG1
151	MED4
152	ORC5L
153	CC2D1A
154	FOXP2
155	TAOK2
156	TBX6
157	TRIM3
158	TDRKH
159	NTRK3
160	MAPRE3
161	PHF21A

162	LRRFIP1
163	INSM1
164	SLC39A9
165	BFSP2
166	TUSC3
167	ZNF571
168	ING4
169	SLC25A28
170	C1orf128
171	ZDHHC9
172	SI
173	APBA1
174	SYDE1
175	PGGT1B
176	PAK6
177	ARID5B
178	SPTBN4
179	PHF7
180	SNX7
181	NTNG1
182	TEAD1
183	SBDS
184	TAC3
185	ARID4A
186	SLC46A3
187	APCDD1
188	SALL4
189	ARHGEF9
190	ZNF289
191	DUSP13
192	RIT1
193	MYOD1
194	MCAT
195	ZNF473
196	TRIM41
197	F12
198	IGF1R
199	CTDSPL2
200	HS3ST2
201	PLEKHG2
202	REN
203	SRPK2
204	HHIP
205	MBOAT5
206	PPP1R14C

207	GPRASP2
208	CXorf36
209	FUBP1
210	FAM18B2
211	MTMR3
212	GEMIN8
213	EPB41L3
214	BVES
215	FGFR3
216	ST5
217	FBXL4
218	KCNIP2
219	SMARCA1
220	STXBP5L
221	CHIT1
222	CLSPN
223	Q8N467_HUMAN
224	RABEP1
225	PTCH1
226	YY1
227	ACVR2B
228	JARID1A
229	RUVBL1
230	ECT2
231	IL2
232	TUSC2
233	PPP1R9A
234	TRPV6
235	SLC12A8
236	FXR1
237	K0552_HUMAN
238	ZNF238
239	FNTB
240	ALAS2
241	ZNF597
242	IGBP1
243	TIMM9
244	MBD2
245	ITGB8
246	GOLT1A
247	VPS13C
248	RASA1
249	MTMR10
250	RLF
251	NP_997528.1

252	DNAJA2
253	TMEM51
254	EIF4B
255	DYRK4
256	RORA
257	MAPK3
258	FRMPD2
259	SOSTDC1
260	PGM3
261	CRTC2
262	RELA
263	C11orf57
264	PPP2CB
265	NM_001011700.1
266	LRRC21
267	INTS6
268	NP_060170.1
269	C1orf32
270	MINK1
271	RYR2
272	KCNJ12
273	SIRT1
274	AMOT
275	C17orf74
276	NRAS
277	ZNF362
278	C19orf47
279	CROT
280	TP53INP1
281	NP_689963.2
282	CLK4
283	ARMCX6
284	TCF15
285	ATG9A
286	SSH2
287	FOXP2
288	IQSEC2
289	HN1
290	TMEM106A
291	LEMD3
292	NFIB
293	YIPF1
294	USP38
295	ITFG1
296	HES7

297	HNRPR
298	CKS1B
299	NP_742017.1
300	FOXO3A
301	FAM130A2
302	RFC1
303	ZNF536
304	ARX
305	CNGA1
306	DNM3
307	TSHZ3
308	DNAJC8
309	PTPRM
310	NP_689968.1
311	PEA15
312	DARC
313	DBT
314	NP_219485.1
315	BCMO1
316	JMJD3
317	TXNIP
318	WDR37
319	PRKAR2A
320	SIX1
321	CHAD
322	SGMS1
323	TLN2
324	GDF5
325	CBLL1
326	SFRS10
327	RAB3IP
328	RSF1
329	NDUFB10
330	RPS6KA1
331	EP300
332	CMTM3
333	IRF1
334	RDBP
335	GCC2
336	PSPH
337	TMEM16B
338	TBCEL
339	NP_009007.2
340	MAP3K13
341	AP2M1

342	TRIB2
343	ARHGAP30
344	SALL1
345	MUC13
346	ANKRD29
347	DYNLL2
348	C19orf43
349	KCND2
350	FKBP2
351	SEPT12
352	EIF4G3
353	NP_056002.1
354	FZD8
355	C12orf44
356	LGTN
357	MBNL2
358	VDAC2
359	RANBP3
360	ATP6V1F
361	OCIAD1
362	Q6ZU65_HUMAN
363	CCDC131
364	GPR128
365	HNRPUL2
366	CA2
367	ID2
368	SAMD12
369	MIA3
370	TRPC1
371	ADD2
372	S100B
373	MAPKAPK5
374	PDE1B
375	SCN9A
376	GPR6
377	EIF2S3
378	CC2D1B
379	EIF4E3
380	SYT5
381	BCDIN3
382	MKNK2
383	TJAP1
384	GBAS
385	PAN3
386	NEUROD2

387	SFRS3
388	CSNK2A1P
389	SEC22A
390	ZNF644
391	GTDC1
392	EEF2K
393	EFEMP1
394	LHX8
395	SLC6A15
396	GPR12
397	E2F5
398	ST6GALNAC4
399	ANKRD34
400	LEFTY1
401	TARDBP
402	SS18L2
403	BAMBI
404	DERL2
405	HOXA1
406	CYP8B1
407	ZCCHC11
408	NR4A2
409	DTX3
410	DPH2
411	KLK5
412	GRIA3
413	BFSP1
414	DTWD1
415	TAF15
416	MRPS16
417	CBX6
418	NF2
419	C12orf34
420	CRK
421	THTPA
422	RBM41
423	CDC37
424	NP_055530.2
425	TRAFD1
426	SAP30L
427	NRP1
428	TSFM
429	C7orf43
430	CTSA
431	PSMA2

432	RB1
433	MEF2A
434	OSBPL8
435	CHRNA5
436	BNIP2
437	ZNF652
438	ZNF236
439	NAP1L1
440	FBXO28
441	SPRED1
442	MAP3K3
443	CALU
444	CAMSAP2
445	LEFTY2
446	C1orf21
447	FOXK1
448	ZBTB21
449	SMAD5
450	CD46
451	RAB6A
452	TMEM183A
453	ZNF805
454	FOXN2
455	STK35
456	DLC1
457	RNF2
458	WNT5A
459	AKT1
460	FBXL5
461	CORO2B
462	RAB11A
463	ZNF385A
464	TULP4
465	GNAI1
466	BCL11B
467	RIMS3
468	DNAJC27
469	SCARB1
470	KCTD15
471	ZIC5
472	ELK1
473	AKAP12
474	TOB2
475	DAZAP2
476	CDKN1A

477	E2F1
478	P2RX7
479	CCND1

Table S13. Top 10 overrepresented KEGG pathways among the target genes in the network analysis (Table S12).

Gene Symbol	**Gene Name**
Pathways in cancer	
rawP=4.40e-14	**adjP=3.56e-12**
EP300	E1A binding protein p300
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog
AKT1	v-akt murine thymoma viral oncogene homolog 1
FZD8	frizzled family receptor 8
SLC2A1	solute carrier family 2 (facilitated glucose transporter), member 1
PTCH1	patched 1
FIGF	c-fos induced growth factor (vascular endothelial growth factor D)
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
HHIP	hedgehog interacting protein
MAPK3	mitogen-activated protein kinase 3
WNT5A	wingless-type MMTV integration site family, member 5A
CCND1	cyclin D1
FGFR3	fibroblast growth factor receptor 3
TCF7L1	transcription factor 7-like 1 (T-cell specific, HMG-box)
RB1	retinoblastoma 1
LAMA3	laminin, alpha 3
CDC42	cell division cycle 42 (GTP binding protein, 25kDa)
CRK	v-crk sarcoma virus CT10 oncogene homolog (avian)
IGF1R	insulin-like growth factor 1 receptor
IL8	interleukin 8
E2F1	E2F transcription factor 1
CKS1B	CDC28 protein kinase regulatory subunit 1B
CASP8	caspase 8, apoptosis-related cysteine peptidase
RELA	v-rel reticuloendotheliosis viral oncogene homolog A (avian)
Bladder cancer	
rawP=2.99e-10	**adjP=1.21e-08**
CCND1	cyclin D1
FGFR3	fibroblast growth factor receptor 3
RB1	retinoblastoma 1
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog
IL8	interleukin 8
E2F1	E2F transcription factor 1
FIGF	c-fos induced growth factor (vascular endothelial growth factor D)
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)

MAPK3	mitogen-activated protein kinase 3
TGF-beta signaling pathway	
rawP=8.24e-10	**adjP=2.22e-08**
EP300	E1A binding protein p300
LEFTY2	left-right determination factor 2
ACVR1	activin A receptor, type I
LEFTY1	left-right determination factor 1
E2F5	E2F transcription factor 5, p130-binding
PPP2CB	protein phosphatase 2, catalytic subunit, beta isozyme
ID2	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
SMAD5	SMAD family member 5
ACVR2B	activin A receptor, type IIB
GDF5	growth differentiation factor 5
MAPK3	mitogen-activated protein kinase 3
Prostate cancer	
rawP=1.55e-09	**adjP=3.14e-08**
CCND1	cyclin D1
EP300	E1A binding protein p300
RB1	retinoblastoma 1
TCF7L1	transcription factor 7-like 1 (T-cell specific, HMG-box)
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog
AKT1	v-akt murine thymoma viral oncogene homolog 1
IGF1R	insulin-like growth factor 1 receptor
E2F1	E2F transcription factor 1
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
RELA	v-rel reticuloendotheliosis viral oncogene homolog A (avian)
MAPK3	mitogen-activated protein kinase 3
Renal cell carcinoma	
rawP=3.40e-08	**adjP=5.51e-07**
EP300	E1A binding protein p300
PAK6	p21 protein (Cdc42/Rac)-activated kinase 6
FIGF	c-fos induced growth factor (vascular endothelial growth factor D)
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog
AKT1	v-akt murine thymoma viral oncogene homolog 1
CRK	v-crk sarcoma virus CT10 oncogene homolog (avian)
CDC42	cell division cycle 42 (GTP binding protein, 25kDa)
SLC2A1	solute carrier family 2 (facilitated glucose transporter), member 1
MAPK3	mitogen-activated protein kinase 3
Chronic myeloid leukemia	
rawP=4.95e-08	**adjP=6.68e-07**
CCND1	cyclin D1
RB1	retinoblastoma 1
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog

AKT1	v-akt murine thymoma viral oncogene homolog 1
CRK	v-crk sarcoma virus CT10 oncogene homolog (avian)
E2F1	E2F transcription factor 1
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
RELA	v-rel reticuloendotheliosis viral oncogene homolog A (avian)
MAPK3	mitogen-activated protein kinase 3
MAPK signaling pathway	
rawP=1.03e-07	**adjP=1.19e-06**
MAP3K3	mitogen-activated protein kinase kinase kinase 3
FGFR3	fibroblast growth factor receptor 3
TAOK2	TAO kinase 2
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog
AKT1	v-akt murine thymoma viral oncogene homolog 1
CRK	v-crk sarcoma virus CT10 oncogene homolog (avian)
CDC42	cell division cycle 42 (GTP binding protein, 25kDa)
MAP3K13	mitogen-activated protein kinase kinase kinase 13
RPS6KA1	ribosomal protein S6 kinase, 90kDa, polypeptide 1
MAPKAPK5	mitogen-activated protein kinase-activated protein kinase 5
MKNK2	MAP kinase interacting serine/threonine kinase 2
ELK1	ELK1, member of ETS oncogene family
RELA	v-rel reticuloendotheliosis viral oncogene homolog A (avian)
RASA1	RAS p21 protein activator (GTPase activating protein) 1
MAPK3	mitogen-activated protein kinase 3
Focal adhesion	
rawP=1.32e-07	**adjP=1.34e-06**
CCND1	cyclin D1
PAK6	p21 protein (Cdc42/Rac)-activated kinase 6
CHAD	chondroadherin
LAMA3	laminin, alpha 3
ITGB8	integrin, beta 8
AKT1	v-akt murine thymoma viral oncogene homolog 1
IGF1R	insulin-like growth factor 1 receptor
CRK	v-crk sarcoma virus CT10 oncogene homolog (avian)
CDC42	cell division cycle 42 (GTP binding protein, 25kDa)
FIGF	c-fos induced growth factor (vascular endothelial growth factor D)
TLN2	talin 2
ELK1	ELK1, member of ETS oncogene family
MAPK3	mitogen-activated protein kinase 3
Glioma	
rawP=2.81e-07	**adjP=2.53e-06**
CCND1	cyclin D1
RB1	retinoblastoma 1
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog
AKT1	v-akt murine thymoma viral oncogene homolog 1
IGF1R	insulin-like growth factor 1 receptor

E2F1	E2F transcription factor 1
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
MAPK3	mitogen-activated protein kinase 3
Pancreatic cancer	
rawP=5.03e-07	**adjP=4.07e-06**
CCND1	cyclin D1
RB1	retinoblastoma 1
AKT1	v-akt murine thymoma viral oncogene homolog 1
CDC42	cell division cycle 42 (GTP binding protein, 25kDa)
E2F1	E2F transcription factor 1
FIGF	c-fos induced growth factor (vascular endothelial growth factor D)
RELA	v-rel reticuloendotheliosis viral oncogene homolog A (avian)
MAPK3	mitogen-activated protein kinase 3

Table S14. List of genes common between the differentially expressed genes (High vs. Low score patients in the entire validation dataset) and the target genes in the network analysis (Table S11).

Number	Gene Symbol
1	ARHGAP30
2	ARPC1B
3	ASF1B
4	BNIP2
5	FOXN2
6	FXR1
7	GBAS
8	GRASP
9	IGBP1
10	IRF1
11	LEMD3
12	MAP3K3
13	MIA3
14	MINK1
15	PLEKHG2
16	PRKAR2A
17	RB1
18	SLC33A1
19	TCEAL1
20	TFEB

Table S15. Statistical overrepresentation testing for gene ontology terms using the PANTHER database. The tested gene list is the one of differentially expressed genes between Low and High risk patients' samples in the entire validation dataset. The p-values are unadjusted for multiple testing.

	Homo sapiens REFLIST (21804)	Diff gene list (148)	Diff gene list (expected)	Diff gene list (over/ under)	Diff gene list (P-value)
Pathway					
General transcription regulation	32	3	0.22	+	1.43E-03
Transcription regulation by bZIP transcription factor	48	3	0.33	+	4.45E-03
B cell activation	64	3	0.43	+	9.76E-03
Alpha adrenergic receptor signaling pathway	25	2	0.17	+	1.28E-02
PDGF signaling pathway	132	4	0.9	+	1.29E-02
Histamine H1 receptor mediated signaling pathway	29	2	0.2	+	1.69E-02
Parkinson disease	88	3	0.6	+	2.26E-02
Angiotensin II-stimulated signaling through G proteins and beta-arrestin	37	2	0.25	+	2.66E-02
Notch signaling pathway	40	2	0.27	+	3.07E-02
2-arachidonoylglycerol biosynthesis	6	1	0.04	+	3.99E-02
Biological Process					
protein folding	194	11	1.32	+	1.16E-07
metabolic process	8613	75	58.46	+	3.79E-03
transport	2564	28	17.4	+	7.52E-03
localization	2636	28	17.89	+	1.08E-02
proteolysis	899	1	6.1	-	1.45E-02
protein complex biogenesis	79	3	0.54	+	1.71E-02
protein complex assembly	79	3	0.54	+	1.71E-02
sulfur compound metabolic process	84	3	0.57	+	2.00E-02
polyphosphate catabolic process	3	1	0.02	+	2.02E-02
lipid metabolic process	902	12	6.12	+	2.05E-02
Unclassified	9422	52	63.95	-	2.78E-02
system development	1645	5	11.17	-	2.93E-02
nervous system development	1008	2	6.84	-	3.06E-02
fatty acid biosynthetic process	41	2	0.28	+	3.21E-02
multicellular organismal process	1798	6	12.2	-	3.50E-02
single-multicellular organism process	1798	6	12.2	-	3.50E-02
nucleobase-containing compound transport	110	3	0.75	+	3.96E-02
regulation of catalytic activity	1119	13	7.6	+	4.18E-02
cellular component biogenesis	114	3	0.77	+	4.33E-02
cellular calcium ion homeostasis	49	2	0.33	+	4.43E-02
regulation of molecular function	1140	13	7.74	+	4.73E-02
cellular defense response	387	6	2.63	+	4.94E-02

Molecular Function					
protein disulfide isomerase activity	16	5	0.11	+	1.08E-07
small GTPase regulator activity	400	11	2.72	+	1.01E-04
isomerase activity	169	6	1.15	+	1.12E-03
lyase activity	209	5	1.42	+	1.45E-02
peroxidase activity	27	2	0.18	+	1.48E-02
protein binding	2855	29	19.38	+	1.67E-02
antioxidant activity	30	2	0.2	+	1.80E-02
anion channel activity	35	2	0.24	+	2.40E-02
guanyl-nucleotide exchange factor activity	166	4	1.13	+	2.72E-02
transmembrane transporter activity	1076	13	7.3	+	3.21E-02
catalytic activity	5529	48	37.53	+	3.22E-02
enzyme regulator activity	1091	13	7.41	+	3.53E-02
peptidase activity	747	1	5.07	-	3.59E-02
pyrophosphatase activity	271	5	1.84	+	3.83E-02
transporter activity	1145	13	7.77	+	4.86E-02
PANTHER Protein Class					
G-protein modulator	487	12	3.31	+	1.36E-04
isomerase	168	6	1.14	+	1.09E-03
Hsp70 family chaperone	17	2	0.12	+	6.13E-03
DNA binding protein	861	1	5.84	-	1.82E-02
Unclassified	9728	53	66.03	-	1.85E-02
anion channel	35	2	0.24	+	2.40E-02
guanyl-nucleotide exchange factor	166	4	1.13	+	2.72E-02
transporter	1069	13	7.26	+	3.07E-02
lyase	183	4	1.24	+	3.69E-02

Table S16. Statistical overrepresentation testing for gene ontology terms using the PANTHER database. The tested gene list is the one of differentially expressed genes between Low and High risk patients' samples in the validation dataset including only CN-AML cases <61 years of age. The p-values are unadjusted for multiple testing.

	Homo sapiens REFLIST (20000)	Diff gene list (127)	Diff gene list (expected)	Diff gene list (over/ under)	Diff gene list (P-value)
PANTHER Protein Class					
chaperonin	28	3	0.18	+	8.04E-04
ribonucleoprotein	125	5	0.79	+	1.29E-03
mRNA polyadenylation factor	89	4	0.57	+	2.62E-03
RNA binding protein	1059	15	6.72	+	3.05E-03
receptor	1838	4	11.67	-	7.35E-03
mRNA splicing factor	278	6	1.77	+	8.96E-03
kinase	660	10	4.19	+	9.68E-03

chaperone	224	5	1.42	+	1.46E-02
Unclassified	6898	32	43.8	-	1.57E-02
ribosomal protein	235	5	1.49	+	1.75E-02
mRNA processing factor	325	6	2.06	+	1.80E-02
non-receptor tyrosine protein kinase	95	3	0.6	+	2.31E-02
kinase activator	97	3	0.62	+	2.44E-02
extracellular matrix protein	548	0	3.48	-	2.94E-02
HMG box transcription factor	42	2	0.27	+	2.97E-02
aminoacyl-tRNA synthetase	44	2	0.28	+	3.23E-02
amino acid transporter	110	3	0.7	+	3.35E-02
actin family cytoskeletal protein	485	7	3.08	+	3.57E-02
transferase	1547	16	9.82	+	3.65E-02
G-protein coupled receptor	504	0	3.2	-	3.91E-02
protein kinase	496	7	3.15	+	3.96E-02
Cellular Component					
ribonucleoprotein complex	125	5	0.79	+	1.29E-03
extracellular region	601	0	3.82	-	2.08E-02
extracellular matrix	558	0	3.54	-	2.75E-02
actin cytoskeleton	485	7	3.08	+	3.57E-02
Pathway					
Cell cycle	23	2	0.15	+	9.62E-03
Pyridoxal phosphate salvage pathway	2	1	0.01	+	1.26E-02
Vitamin B6 metabolism	3	1	0.02	+	1.89E-02
Parkinson disease	101	3	0.64	+	2.70E-02
Pentose phosphate pathway	8	1	0.05	+	4.95E-02
Molecular Function					
RNA binding	538	12	3.42	+	1.76E-04
poly(A) RNA binding	89	4	0.57	+	2.62E-03
receptor activity	1852	4	11.76	-	6.87E-03
RNA splicing factor activity, transesterification mechanism	278	6	1.77	+	8.96E-03
kinase activity	664	10	4.22	+	1.01E-02
nucleic acid binding	3715	34	23.59	+	1.46E-02
catalytic activity	5263	45	33.42	+	1.46E-02
structural constituent of ribosome	235	5	1.49	+	1.75E-02
mRNA binding	325	6	2.06	+	1.80E-02
non-membrane spanning protein tyrosine kinase activity	95	3	0.6	+	2.31E-02
kinase activator activity	97	3	0.62	+	2.44E-02
DNA replication origin binding	43	2	0.27	+	3.10E-02
aminoacyl-tRNA ligase activity	44	2	0.28	+	3.23E-02
amino acid transmembrane transporter activity	110	3	0.7	+	3.35E-02
G-protein coupled receptor activity	504	0	3.2	-	3.91E-02
Unclassified	7724	39	49.05	-	3.94E-02
protein kinase activity	500	7	3.18	+	4.10E-02

transferase activity	1601	16	10.17	+	4.73E-02
Biological Process					
cell cycle	1602	23	10.17	+	1.84E-04
primary metabolic process	7813	68	49.61	+	6.60E-04
metabolic process	8127	70	51.61	+	6.86E-04
RNA metabolic process	663	12	4.21	+	1.11E-03
rRNA metabolic process	123	5	0.78	+	1.20E-03
mRNA processing	456	9	2.9	+	2.65E-03
nuclear mRNA splicing, via spliceosome	371	8	2.36	+	2.67E-03
mRNA polyadenylation	93	4	0.59	+	3.07E-03
mRNA 3'-end processing	95	4	0.6	+	3.31E-03
nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	3782	37	24.02	+	3.49E-03
vitamin catabolic process	1	1	0.01	+	6.33E-03
DNA replication	262	6	1.66	+	6.81E-03
protein metabolic process	3178	31	20.18	+	8.47E-03
sensory perception	722	0	4.58	-	9.38E-03
Unclassified	6816	31	43.28	-	1.21E-02
fatty acid biosynthetic process	38	2	0.24	+	2.47E-02
MAPKKK cascade	454	7	2.88	+	2.64E-02
vesicle-mediated transport	1061	2	6.74	-	3.27E-02
tRNA aminoacylation for protein translation	48	2	0.3	+	3.78E-02
nitric oxide mediated signal transduction	49	2	0.31	+	3.93E-02
unsaturated fatty acid biosynthetic process	7	1	0.04	+	4.35E-02
DNA metabolic process	510	7	3.24	+	4.48E-02
endocytosis	480	0	3.05	-	4.57E-02

Table S17. Top 20 enriched gene lists identified by GSEA comparing Low vs. High Score patients in the validation dataset. Data from all patients in the validation dataset. Oncogenic pathways signatures were analyzed. ES-enrichment score; NES-normalized enrichment score; FDR-false discovery rate (q-value), FWER-familywise error rate.

NAME GENE SET	SIZE	ES	NES	p-val	FDR	FWER
BCAT.100 UP.V1 DN	37	0.531712	1.654186	0.009324	0.130983	0.204
ESC J1 UP EARLY.V1 UP	151	0.348619	1.314087	0.058691	1	0.858
BMI1 DN.V1 UP	142	0.36913	1.267653	0.064588	1	0.914
ESC V6.5 UP LATE.V1 UP	171	0.321308	1.191186	0.147541	1	0.958
SNF5 DN.V1 DN	146	0.338239	1.152179	0.185615	1	0.975
JAK2 DN.V1 DN	132	0.3549	1.150518	0.257143	1	0.975
P53 DN.V1 UP	178	0.372911	1.114061	0.256724	1	0.985
BCAT BILD ET AL DN	41	0.334562	1.093417	0.340344	1	0.988
ESC J1 UP LATE.V1 UP	172	0.324724	1.093247	0.300683	1	0.988
PDGF ERK DN.V1 UP	127	0.279767	1.092072	0.279441	0.919673	0.988
MTOR UP.V1 DN	164	0.289025	1.085196	0.263279	0.863668	0.989

PIGF UP.V1 DN	171	0.314093	1.081415	0.345982	0.805912	0.99
PRC1 BMI UP.V1 DN	162	0.301105	1.080786	0.283019	0.746304	0.99
BCAT GDS748 DN	40	0.362938	1.077304	0.354762	0.704737	0.991
P53 DN.V2 DN	135	0.300739	1.071589	0.274554	0.67526	0.991
CRX NRL DN.V1 DN	111	0.293225	1.068822	0.343049	0.642169	0.991
ESC V6.5 UP LATE.V1 DN	152	0.277008	1.05273	0.356132	0.654048	0.994
CAHOY OLIGODENDROCUTIC	89	0.293335	1.0467	0.357616	0.6344	0.994
PKCA DN.V1 DN	140	0.293089	1.044589	0.353081	0.606774	0.994
CRX DN.V1 UP	119	0.271124	1.028763	0.397849	0.61998	0.996

Table S18. Top 20 enriched gene lists identified by GSEA comparing High vs. Low Score patients in the validation dataset. Data from all patients in the validation dataset. Oncogenic pathways signatures were analyzed. ES-enrichment score; NES-normalized enrichment score; FDR-false discovery rate (q-value), FWER- familywise error rate.

NAME GENE SET	SIZE	ES	NES	p-val	FDR	FWER
GLI1 UP.V1 DN	22	-0.53873	-1.62392	0.017391	0.729481	0.261
MEL18 DN.V1 DN	134	-0.53199	-1.59184	0.00349	0.501767	0.321
ALK DN.V1 DN	131	-0.48925	-1.57607	0.01105	0.382616	0.352
MYC UP.V1 DN	139	-0.48839	-1.56473	0.001848	0.323381	0.381
HOXA9 DN.V1 DN	166	-0.52424	-1.55985	0.011472	0.270334	0.397
STK33 UP	250	-0.60137	-1.49928	0.020992	0.392742	0.547
STK33 SKM UP	249	-0.58368	-1.49667	0.019048	0.344834	0.554
PRC2 EDD UP.V1 UP	171	-0.39758	-1.49526	0.048872	0.306649	0.56
LTE2 UP.V1 DN	178	-0.48957	-1.48404	0.021825	0.303987	0.581
SRC UP.V1 DN	147	-0.41173	-1.46672	0.007722	0.318405	0.608
MEK UP.V1 DN	172	-0.49615	-1.4661	0.031809	0.29121	0.611
KRAS.KIDNEY UP.V1 DN	131	-0.45794	-1.4596	0.031423	0.281826	0.626
E2F1 UP.V1 UP	154	-0.41542	-1.44741	0.094567	0.288197	0.649
SIRNA EIF4GI DN	82	-0.41182	-1.44627	0.051485	0.269739	0.651
JNK DN.V1 UP	171	-0.40855	-1.44446	0.016791	0.254112	0.652
HOXA9 DN.V1 UP	169	-0.59047	-1.41711	0.078	0.305232	0.714
MTOR UP.V1 UP	145	-0.41327	-1.41693	0.043825	0.287756	0.714
SNF5 DN.V1 UP	163	-0.44226	-1.41015	0.051429	0.287675	0.721
TGFB UP.V1 DN	174	-0.41922	-1.40776	0.030142	0.27851	0.725
HINATA NFKB IMMU INF	17	-0.71183	-1.40639	0.079336	0.268742	0.732

Table S19. Top 20 enriched gene lists identified by GSEA comparing Low vs. High Score patients in the validation dataset. Data from all patients. All gene ontology signatures were used in the analysis. NES-normalized enrichment score; FDR-false discovery rate (q-value), FWER- familywise error rate.

NAME GENE SET	SIZE	ES	NES	p-val	FDR	FWER
EMBRYONIC MORPHOGENESIS	17	0.738631	1.933975	0	0.099862	0.097
METALLOPEPTIDASE ACTIVITY	44	0.558203	1.772094	0.004415	0.405964	0.49
ENDOSOME TRANSPORT	23	0.653164	1.737987	0.019531	0.41998	0.609
ACETYLCHOLINE BINDING	16	0.699724	1.719776	0	0.388881	0.679
EXOPEPTIDASE ACTIVITY	28	0.59943	1.717045	0.008316	0.320121	0.688
EXTRACELLULAR MATRIX STRUCTURAL CONSTITUENT	25	0.692305	1.701184	0	0.317686	0.743
INTRAMOLECULAR OXIDOREDUCTASE ACTIVITY	18	0.655936	1.678978	0.017429	0.342555	0.801
BASEMENT MEMBRANE	34	0.614939	1.671175	0.006711	0.323619	0.816
EXTRACELLULAR MATRIX PART	54	0.632017	1.665345	0.009412	0.305759	0.83
ATPASE ACTIVITY COUPLED TO TRANSMEMBRANE MOVEMENT OF IONS PHOSPHORYLATIVE MECHANISM	19	0.615005	1.662373	0.008403	0.285404	0.837
EARLY ENDOSOME	17	0.579626	1.654198	0.024621	0.282964	0.861
AMINE BINDING	22	0.621164	1.653911	0.008529	0.259998	0.861
CELLULAR MORPHOGENESIS DURING DIFFERENTIATION	49	0.50404	1.626503	0.002427	0.312428	0.905
INTERMEDIATE FILAMENT CYTOSKELETON	21	0.558707	1.616212	0.014862	0.320569	0.923
INTERMEDIATE FILAMENT	21	0.558707	1.616211	0.014862	0.299198	0.923
COLLAGEN	23	0.747389	1.595667	0.025943	0.338205	0.944
SERINE TYPE PEPTIDASE ACTIVITY	41	0.511643	1.589854	0.011111	0.335251	0.95
AXONOGENESIS	43	0.506797	1.575638	0.005	0.356644	0.962
SERINE HYDROLASE ACTIVITY	42	0.504956	1.574882	0.015521	0.339929	0.962
ATPASE ACTIVITY COUPLED TO TRANSMEMBRANE MOVEMENT OF IONS	22	0.531693	1.565894	0.02045	0.348379	0.966

Table S20. Top 20 enriched gene lists identified by GSEA comparing High vs. Low Score patients in the validation dataset. Data from all patients. All gene ontology signatures were used in the analysis. NES-normalized enrichment score; FDR-false discovery rate (q-value), FWER- familywise error rate.

NAME GENE SET	SIZE	ES	NES	p-val	FDR	FWER
VIRAL REPRODUCTIVE PROCESS	35	-0.57443	-1.89928	0	0.462649	0.15
VIRAL GENOME REPLICATION	20	-0.6563	-1.85839	0	0.4097	0.223

VIRAL INFECTIOUS CYCLE	31	-0.55503	-1.82549	0.001984	0.4117	0.301
REGULATION OF JNK ACTIVITY	20	-0.68939	-1.77204	0.003899	0.598964	0.448
TRANSLATION	154	-0.39181	-1.76046	0	0.546608	0.481
IMMUNE EFFECTOR PROCESS	37	-0.62989	-1.75297	0.001825	0.49277	0.498
DOUBLE STRANDED RNA BINDING	16	-0.68154	-1.74748	0.009634	0.446529	0.514
POSITIVE REGULATION OF JNK ACTIVITY	18	-0.70717	-1.73399	0.003953	0.446997	0.557
SENSORY PERCEPTION OF CHEMICAL STIMULUS	16	-0.68807	-1.72405	0.001859	0.439978	0.591
REGULATION OF RAS PROTEIN SIGNAL TRANSDUCTION	17	-0.72079	-1.71808	0.00381	0.419031	0.608
RESPONSE TO VIRUS	47	-0.62093	-1.71187	0.00365	0.403924	0.629
REGULATION OF DEFENSE RESPONSE	18	-0.73872	-1.70735	0.001969	0.388848	0.644
RESPONSE TO ORGANIC SUBSTANCE	28	-0.56564	-1.69952	0.013944	0.383828	0.662
RESPONSE TO WOUNDING	173	-0.58322	-1.69752	0.005495	0.364243	0.669
REGULATION OF SMALL GTPASE MEDIATED SIGNAL TRANSDUCTION	21	-0.667	-1.69528	0.003781	0.346916	0.674
CELL PROJECTION BIOGENESIS	23	-0.63204	-1.69433	0.00996	0.32762	0.675
REGULATION OF SIGNAL TRANSDUCTION	200	-0.46633	-1.68949	0.005758	0.322812	0.689
VIRAL REPRODUCTION	40	-0.47741	-1.68741	0.002049	0.31131	0.694
INFLAMMATORY RESPONSE	115	-0.61775	-1.6747	0.005629	0.333534	0.735
MICROTUBULE BASED MOVEMENT	16	-0.55829	-1.6734	0.017647	0.320388	0.74

Table S21. Top 20 enriched gene lists identified by GSEA comparing Low vs. High Score patients in the validation dataset. Data from CN-AML patients under 61 years in the validation dataset. Oncogenic pathways signatures were analyzed. ES-enrichment score; NES-normalized enrichment score; FDR-false discovery rate (q-value), FWER- familywise error rate.

NAME	SIZE	ES	NES	NOM	FDR	FWER
GCNP SHH UP LATE.V1 UP	88	0.389021	1.395961	0.074	1	0.919
GCNP SHH UP EARLY.V1 UP	81	0.357089	1.389002	0.093069	1	0.928
ERB2 UP.V1 DN	74	0.485871	1.373032	0.138943	1	0.934
PIGF UP.V1 UP	96	0.400453	1.365455	0.18618	1	0.942
CAHOY OLIGODENDROCUTIC	19	0.43277	1.353207	0.087318	0.929165	0.946
TBK1.DN.48HRS DN	20	0.419822	1.309505	0.121704	0.98995	0.968
JAK2 DN.V1 DN	58	0.443678	1.293639	0.159309	0.924756	0.975
MEK UP.V1 DN	64	0.416323	1.267586	0.214844	0.926577	0.983
BCAT BILD ET AL DN	24	0.413627	1.205744	0.278846	1	0.99
DCA UP.V1 DN	47	0.425082	1.204118	0.22619	0.996961	0.99

MTOR UP.N4.V1 DN	78	0.354735	1.188789	0.245211	0.966499	0.992
RAF UP.V1 DN	37	0.37104	1.15733	0.284884	1	0.997
EGFR UP.V1 DN	54	0.377276	1.15531	0.273453	0.939211	0.997
EIF4E DN	49	0.335271	1.140868	0.310476	0.924653	0.999
TBK1.DF DN	157	0.29864	1.079449	0.361905	1	1
VEGF A UP.V1 DN	84	0.277932	1.078071	0.36773	1	1
CSR EARLY UP.V1 UP	66	0.266372	1.037359	0.388889	1	1
PRC2 EZH2 UP.V1 UP	55	0.304612	1.024429	0.415686	1	1
PRC2 EDD UP.V1 DN	43	0.324125	1.008824	0.455285	1	1
PDGF ERK DN.V1 UP	58	0.253623	0.998386	0.452191	1	1

Table S22. Top 20 enriched gene lists identified by GSEA comparing High vs. Low Score patients in the validation dataset. Data from CN-AML patients under 61 years in the validation dataset. Oncogenic pathways signatures were analyzed. ES-enrichment score; NES-normalized enrichment score; FDR-false discovery rate (q-value), FWER- familywise error rate.

NAME	SIZE	ES	NES	p-val	FDR	FWER
E2F1 UP.V1 DN	60	-0.49059	-1.69655	0.008163	0.696177	0.334
KRAS.BREAST UP.V1 DN	19	-0.66701	-1.6532	0.013462	0.531894	0.453
KRAS.KIDNEY UP.V1 DN	17	-0.65603	-1.62072	0.013359	0.488943	0.538
RAPA EARLY UP.V1 DN	50	-0.48164	-1.53196	0.024482	0.788412	0.749
MEK UP.V1 UP	44	-0.53099	-1.47915	0.053131	0.940313	0.826
ESC V6.5 UP EARLY.V1 DN	31	-0.52536	-1.47314	0.049213	0.819023	0.834
AKT UP.V1 UP	42	-0.45927	-1.45189	0.051527	0.80975	0.859
NOTCH DN.V1 UP	34	-0.48582	-1.45014	0.038745	0.716431	0.861
MEL18 DN.V1 UP	25	-0.58551	-1.43641	0.091082	0.695913	0.874
CYCLIN D1 UP.V1 UP	51	-0.46194	-1.41923	0.096899	0.703676	0.891
PTEN DN.V1 DN	17	-0.59107	-1.41215	0.107724	0.669542	0.899
ATM DN.V1 UP	23	-0.52689	-1.39295	0.1	0.691151	0.915
PIGF UP.V1 DN	34	-0.48923	-1.39264	0.099808	0.639206	0.916
KRAS.600 UP.V1 DN	30	-0.52728	-1.3878	0.111111	0.614895	0.924
CRX NRL DN.V1 UP	32	-0.45747	-1.37917	0.079696	0.60513	0.928
IL2 UP.V1 DN	24	-0.44046	-1.37059	0.086172	0.595495	0.931
P53 DN.V2 UP	18	-0.49527	-1.37019	0.098077	0.562112	0.931
PRC2 EZH2 UP.V1 DN	26	-0.50147	-1.35671	0.142857	0.573399	0.938
ESC J1 UP EARLY.V1 DN	64	-0.36384	-1.35456	0.067179	0.549327	0.939
ESC J1 UP LATE.V1 UP	19	-0.46942	-1.35214	0.093117	0.528183	0.94

Table S23. Top 20 enriched gene lists identified by GSEA comparing Low vs. High Score patients in the validation dataset. Gene ontology signatures were tested. Data only from CN-AML patients <61 years of age. ES-enrichment score; NES-normalized enrichment score; FDR-false discovery rate (q-value), FWER-familywise error rate.

NAME GENE SET	SIZE	ES	NES	p-val	FDR	FWER
RNA SPLICING	55	0.566	1.859	0.004	0.287	0.174
MRNA PROCESSING GO 0006397	46	0.579	1.809	0.002	0.267	0.288
RNA PROCESSING	103	0.498	1.799	0.002	0.197	0.309
MRNA METABOLIC PROCESS	53	0.561	1.796	0.000	0.152	0.314
PROTEIN RNA COMPLEX ASSEMBLY	39	0.538	1.775	0.010	0.157	0.369
RIBONUCLEOPROTEIN COMPLEX BIOGENESIS AND ASSEMBLY	50	0.513	1.743	0.014	0.185	0.453
RNA SPLICINGVIA TRANSESTERIFICATION REACTIONS	23	0.600	1.725	0.017	0.189	0.503
TRANSLATION REGULATOR ACTIVITY	19	0.559	1.687	0.013	0.236	0.591
TRANSLATIONAL INITIATION	19	0.550	1.670	0.035	0.246	0.636
RNA BINDING	123	0.438	1.655	0.010	0.256	0.677
TRANSLATION FACTOR ACTIVITY NUCLEIC ACID BINDING	18	0.553	1.635	0.025	0.276	0.709
HELICASE ACTIVITY	26	0.551	1.602	0.065	0.337	0.774
DNA DEPENDENT DNA REPLICATION	30	0.573	1.565	0.078	0.417	0.832
RNA METABOLIC PROCESS	415	0.352	1.538	0.008	0.473	0.866
SPLICEOSOME	29	0.471	1.537	0.070	0.446	0.868
DNA REPLICATION	57	0.504	1.521	0.091	0.467	0.890
MICROTUBULE BINDING	17	0.515	1.514	0.048	0.464	0.894
REGULATION OF TRANSLATIONAL INITIATION	15	0.524	1.492	0.097	0.509	0.916
RIBONUCLEOPROTEIN COMPLEX	78	0.410	1.485	0.083	0.502	0.920
ONE CARBON COMPOUND METABOLIC PROCESS	17	0.495	1.471	0.097	0.529	0.931

Table S24. Top 20 enriched gene lists identified by GSEA comparing High vs. Low Score patients in the validation dataset. Data only from CN-AML patients <61 years of age. Gene ontology signatures were tested. ES-enrichment score; NES-normalized enrichment score; FDR-false discovery rate (q-value), FWER-familywise error rate.

NAME GENE SET	SIZE	ES	NES	p-val	FDR	FWER
ACTIVE TRANSMEMBRANE TRANSPORTER ACTIVITY	33	-0.50482	-1.71966	0.006186	1	0.507
SUBSTRATE SPECIFIC TRANSPORTER ACTIVITY	82	-0.4715	-1.66382	0.012632	1	0.646
RECEPTOR SIGNALING PROTEIN ACTIVITY	31	-0.55097	-1.65845	0.02	0.976944	0.66
INTRINSIC TO PLASMA MEMBRANE	157	-0.43399	-1.60681	0	1	0.762
INTEGRAL TO PLASMA MEMBRANE	157	-0.43399	-1.60681	0	0.937743	0.762
PLASMA MEMBRANE PART	200	-0.42923	-1.60264	0.008493	0.813661	0.77
TRANSMEMBRANE	73	-0.44029	-1.60049	0.023061	0.71084	0.776

TRANSPORTER ACTIVITY						
MEMBRANE FRACTION	101	-0.41988	-1.59873	0.004348	0.629701	0.776
SUBSTRATE SPECIFIC TRANSMEMBRANE TRANSPORTER ACTIVITY	67	-0.448	-1.57839	0.033613	0.664343	0.811
INTEGRAL TO MEMBRANE	270	-0.3846	-1.56085	0.006303	0.688293	0.839
ION TRANSPORT	30	-0.49599	-1.55371	0.031579	0.662274	0.844
INTRINSIC TO MEMBRANE	272	-0.38062	-1.54793	0.012739	0.635059	0.854
PLASMA MEMBRANE	274	-0.40315	-1.54792	0.022495	0.586208	0.854
ENZYME ACTIVATOR ACTIVITY	51	-0.42612	-1.53705	0.024691	0.591628	0.869
MOLECULAR ADAPTOR ACTIVITY	17	-0.55516	-1.5281	0.051653	0.590938	0.881
INTEGRAL TO ORGANELLE MEMBRANE	23	-0.51485	-1.51789	0.051383	0.6002	0.893
TRANSFERASE ACTIVITY TRANSFERRING ACYL GROUPS	27	-0.46745	-1.49092	0.045635	0.687275	0.924
MEMBRANE	500	-0.33689	-1.48992	0.027027	0.653518	0.926
MEMBRANE PART	398	-0.33709	-1.48987	0.024742	0.619346	0.926
REGULATION OF HYDROLASE ACTIVITY	24	-0.4923	-1.48902	0.051125	0.59169	0.926

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