**Supplemental File** **May 2016**

# A three-gene expression based risk score can refine the European LeukemiaNet AML classification

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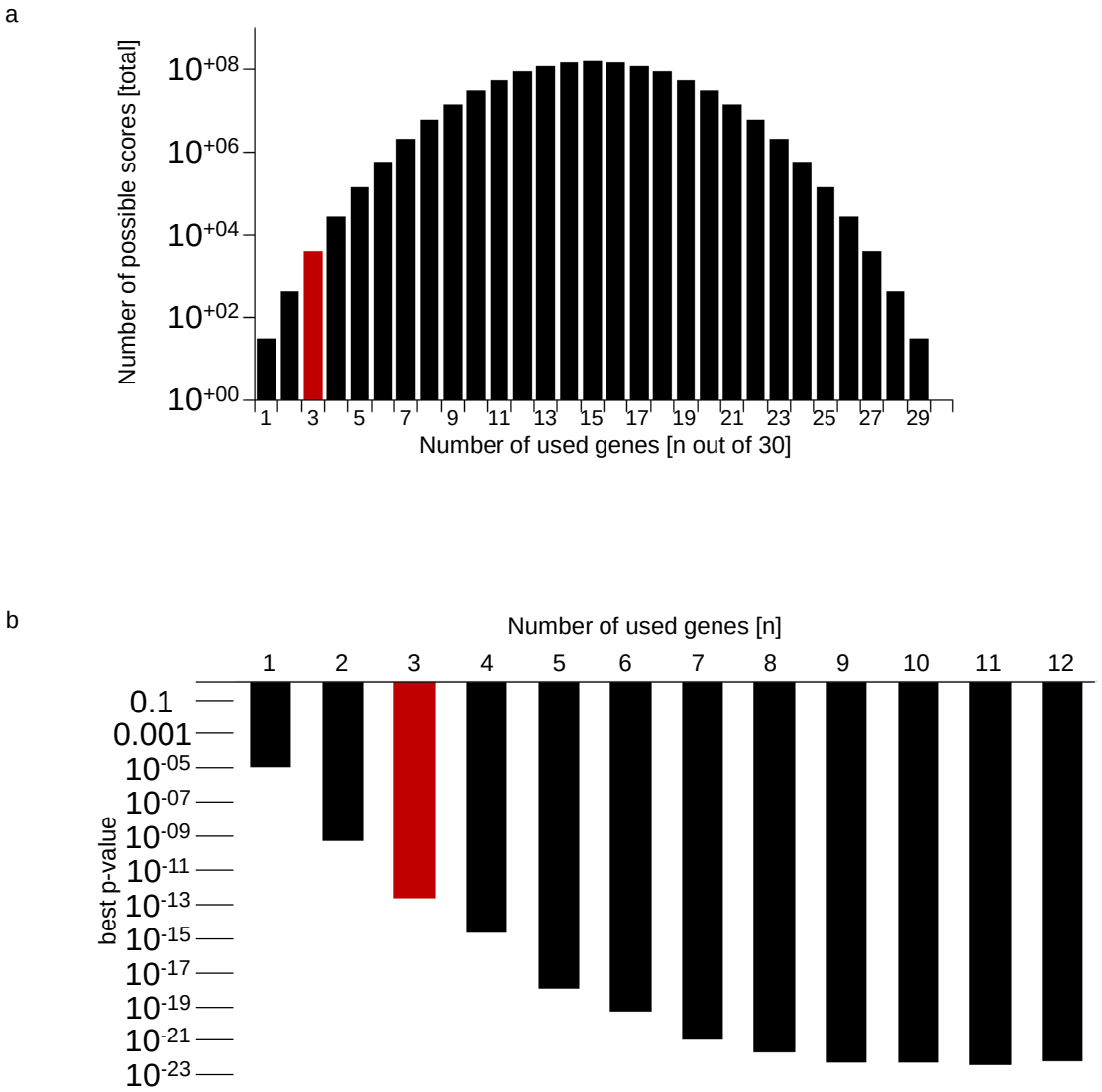
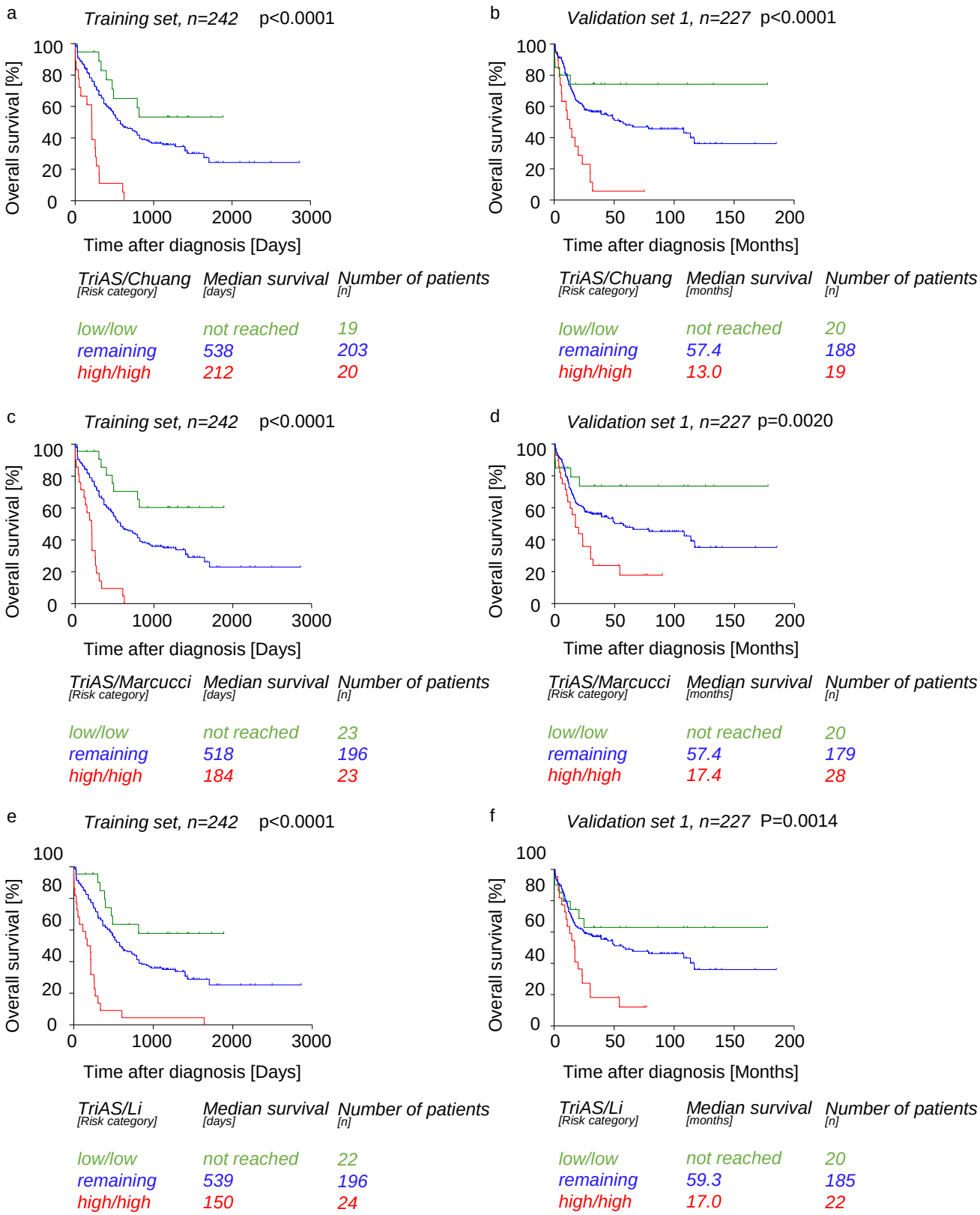
**Supplemental Figure 1: The significance level improves with incremental number of genes**




**Supplemental Figure 1:** The significance level improves with incremental number of genes

Each given number of candidate genes allows the creation of a large number of possible scores. The number of possible n-out-of-30 combinations **(a)** and the lowest multivariate p-value of the best n-out-of -30 score in a multivariate model including age and cytogenetic risk group in the training set **(b)** are shown.

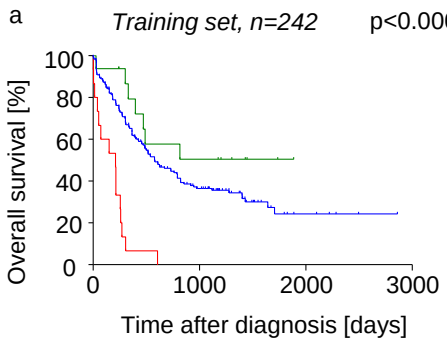
**Supplemental Figure 2:** Combination of risk scores improves the segregation of overall survival of AML patients



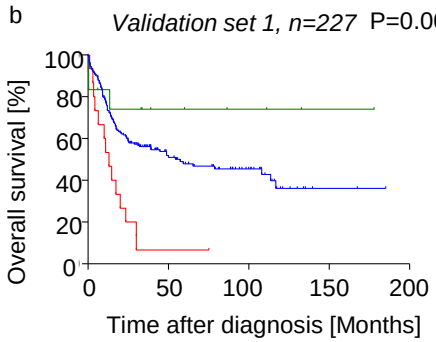

**Supplemental Figure 2:** Combination of risk scores improves the segregation of overall survival of AML patients

Expression based risk scores can be used synergistically. The OS according to the combination of either the TriAS/Chuang **(a, b)**, TriAS/Marcucci **(c, d)** as well as the TriAS/Li scores **(e, f)** for the training set **(a, c, e)** as well as for the NTUH validation set 1 **(b, d, f)** are shown.

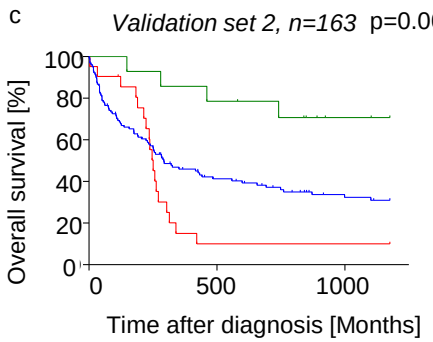
**Supplemental Figure 3:** Four risk scores can be used synergistically to segregate overall survival of AML patients




| TriAS/Chuang/Marcucci/Li [Risk category] | Median survival [days] | Number of patients [n] |
|---|---|---|
| low/low/low/low | Not reached | 16 |
| remaining | 539 | 209 |
| high/high/high/high | 150 | 17 |




| TriAS/Chuang/Marcucci/Li [Risk category] | Median survival [months] | Number of patients [n] |
|---|---|---|
| low/low/low/low | Not reached | 15 |
| remaining | 57.4 | 200 |
| high/high/high/high | 13 | 12 |




| TriAS/Chuang/Marcucci/Li [Risk category] | Median survival [dayss] | Number of patients [n] |
|---|---|---|
| low/low/low/low | Not reached | 14 |
| remaining | 293 | 128 |
| high/high/high/high | 251 | 21 |

**Supplemental Figure 3:** Four risk scores can be used synergistically to segregate overall survival of AML patients

Expression based risk scores can be used synergistically. The OS according to the combination of all four expression based scores (TriAS, Chuang, Marcucci, Li) for the training set **(a)** as well as for the validation set 1 **(b)** and set 2 **(c)** are shown.

**Supplemental Table 1:** Statistical analysis and hazard ratios of 30 genes using univariate as well as multivariate Cox regression analysis including age as a confounding factor in patients from the TCGA and enrolled in the AMLCG-1999 trial (GSE12417-GPL570) are shown.

| Gene name | HR uni [TCGA] | P-value uni [TCGA] | HR multi [TCGA] | P-value multi [TCGA] | HR uni [GSE570] | P-value uni [GSE570] | HR multi [GSE570] | P-value multi [GSE570] |
|---|---|---|---|---|---|---|---|---|
| APPL2 | 0.656 | 0.0326 | 0.646 | 0.0264 | 0.537 | 0.0367 | 0.528 | 0.032 |
| ATP9B | 0.582 | 0.0062 | 0.615 | 0.0146 | 0.429 | 0.0054 | 0.47 | 0.0137 |
| CAP1 | 1.976 | 0.0006 | 1.764 | 0.0049 | 2.081 | 0.014 | 2.324 | 0.0053 |
| CCDC137 | 1.712 | 0.0065 | 1.655 | 0.0114 | 2.116 | 0.0119 | 1.858 | 0.0412 |
| CD97 | 1.867 | 0.0017 | 1.672 | 0.0102 | 2.362 | 0.0041 | 2.251 | 0.0068 |
| CDYL | 0.668 | 0.0409 | 0.638 | 0.0233 | 0.532 | 0.0337 | 0.496 | 0.0195 |
| CPT1A | 1.511 | 0.0366 | 1.517 | 0.0348 | 2.031 | 0.0186 | 1.962 | 0.0251 |
| CXCR6 | 0.671 | 0.0423 | 0.465 | 0.0002 | 0.419 | 0.0042 | 0.445 | 0.0083 |
| DCBLD2 | 0.676 | 0.047 | 0.585 | 0.0074 | 0.546 | 0.0423 | 0.52 | 0.0292 |
| DNM1 | 0.572 | 0.0052 | 0.628 | 0.0212 | 0.424 | 0.0052 | 0.473 | 0.015 |
| EPAS1 | 0.592 | 0.0083 | 0.552 | 0.0029 | 0.532 | 0.033 | 0.558 | 0.0493 |
| FAM124B | 1.685 | 0.0084 | 1.531 | 0.0312 | 3.071 | 0.0003 | 2.871 | 0.0006 |
| FHL1 | 1.791 | 0.0034 | 1.488 | 0.0497 | 2.177 | 0.0097 | 2.098 | 0.0141 |
| FRYL | 0.656 | 0.0322 | 0.616 | 0.0148 | 0.381 | 0.0017 | 0.366 | 0.0011 |
| IGF2BP3 | 2.147 | 0.0002 | 1.669 | 0.0146 | 1.926 | 0.028 | 1.978 | 0.0232 |
| LEF1 | 0.67 | 0.0416 | 0.628 | 0.0189 | 0.37 | 0.0013 | 0.432 | 0.0079 |
| LRRN3 | 0.561 | 0.0033 | 0.631 | 0.0215 | 0.477 | 0.0141 | 0.516 | 0.0286 |
| LY9 | 0.666 | 0.0388 | 0.649 | 0.0286 | 0.436 | 0.0061 | 0.523 | 0.0379 |
| MYH11 | 0.561 | 0.0036 | 0.629 | 0.0212 | 0.494 | 0.019 | 0.528 | 0.0348 |
| OXCT1 | 1.475 | 0.0479 | 1.537 | 0.03 | 1.85 | 0.0388 | 1.892 | 0.0328 |
| PBX2 | 0.648 | 0.0281 | 0.586 | 0.0073 | 0.468 | 0.0125 | 0.514 | 0.0294 |
| PSMA7 | 1.964 | 0.0007 | 1.79 | 0.0037 | 1.883 | 0.032 | 2.084 | 0.0137 |
| RECK | 1.778 | 0.0038 | 1.582 | 0.0216 | 2.344 | 0.0046 | 2.38 | 0.004 |
| RPS6KA1 | 2.074 | 0.0003 | 2.217 | 0.000099 | 1.847 | 0.0381 | 1.793 | 0.0492 |
| SLC14A1 | 0.592 | 0.009 | 0.664 | 0.0412 | 0.529 | 0.0312 | 0.49 | 0.0164 |
| SORT1 | 1.794 | 0.0032 | 1.593 | 0.0195 | 1.875 | 0.0342 | 1.96 | 0.0242 |
| SPDYA | 0.614 | 0.0131 | 0.622 | 0.0163 | 0.517 | 0.0272 | 0.484 | 0.016 |
| TBL1XR1 | 0.649 | 0.0275 | 0.599 | 0.0101 | 0.474 | 0.0129 | 0.44 | 0.0065 |
| TRAT1 | 0.587 | 0.0073 | 0.565 | 0.004 | 0.363 | 0.001 | 0.317 | 0.0002 |
| ZBTB4 | 0.609 | 0.013 | 0.571 | 0.0053 | 0.519 | 0.0299 | 0.544 | 0.0442 |

**Supplemental Table 2:** Predictive AML genes are related to cellular stress and apopotosis pathways. Gene ontology classes of the significant genes using BiNGO plugin of the Cytoscape software are shown.

| GO ID | Gene ontology class description | p-value | Corrected p-value |
|---|---|---|---|
| 43620 | regulation of transcription in response to stress | 4.70E-05 | 2.29E-03 |
| 7169 | transmembrane receptor protein tyrosine kinase signaling pathway | 1.13E-04 | 3.71E-03 |
| 6950 | response to stress | 3.76E-04 | 6.95E-03 |
| 31325 | positive regulation of cellular metabolic process | 1.47E-03 | 1.96E-02 |
| 43154 | negative regulation of caspase activity | 1.66E-03 | 2.11E-02 |
| 10972 | negative regulation of G2/M transition of mitotic cell cycle | 2.20E-03 | 2.35E-02 |
| 6897 | endocytosis | 2.84E-03 | 2.80E-02 |
| 34142 | toll-like receptor 4 signaling pathway | 2.91E-03 | 2.80E-02 |
| 45893 | positive regulation of transcription. DNA-dependent | 3.87E-03 | 3.12E-02 |
| 60070 | canonical Wnt receptor signaling pathway | 4.17E-03 | 3.34E-02 |
| 45941 | positive regulation of transcription | 4.24E-03 | 3.37E-02 |
| 16573 | histone acetylation | 4.62E-03 | 3.42E-02 |
| 42981 | regulation of apoptosis | 6.19E-03 | 3.87E-02 |
| 43067 | regulation of programmed cell death | 6.35E-03 | 3.87E-02 |
| 82 | G1/S transition of mitotic cell cycle | 7.24E-03 | 3.87E-02 |
| 1817 | regulation of cytokine production | 7.29E-03 | 3.87E-02 |
| 10941 | regulation of cell death | 7.68E-03 | 4.00E-02 |
| 30099 | myeloid cell differentiation | 8.31E-03 | 4.12E-02 |
| 43555 | regulation of translation in response to stress | 1.10E-02 | 4.67E-02 |
| 43281 | regulation of caspase activity | 1.18E-02 | 4.93E-02 |

**Supplemental Table 3:** The clinical characteristics as available from the TCGA and NTUH data sets of the individual patient cohorts based on the categorized TriAS risk groups are shown.

| TCGA Training-Set [n=163] Parameter | Total | TriAS low | TriAS intermediate | TriAS high | p-value (ChiSq) |
|---|---|---|---|---|---|
| **Age** | | | | | 0.7283 |
| ≤ 65 years | 114 | 12 (75.00%) | 94 (70.15%) | 8 (61.54%) | |
| > 65 years | 49 | 4 (25.00%) | 40 (29.85%) | 5 (38.46%) | |
| **Gender** | | | | | 0.5466 |
| Male | 88 | 7 (43.75%) | 75 (55.97%) | 6 (46.15%) | |
| Female | 75 | 9 (56.25%) | 59 (44.03%) | 7 (53.85%) | |
| **Cytogenetic risk** | | | | | **0.0481** |
| Favorable | 32 | 7 (43.75%) | 25 (18.80%) | 0 (0.00%) | |
| Intermediate | 97 | 6 (37.50%) | 81 (60.90%) | 10 (83.33%) | |
| Adverse | 32 | 3 (18.75%) | 27 (20.30%) | 2 (16.67%) | |
| **Karyotype** | | | | | 0.0749 |
| Aberrant | 66 | 10 (62.50%) | 54 (44.63%) | 2 (18.18%) | |
| Normal | 82 | 6 (37.50%) | 67 (55.37%) | 9 (81.82%) | |
| Unknown | 15 | | | | |
| **FLT3-ITD** | | | | | **0.0250** |
| unmutated | 110 | 10 (66.67%) | 95 (74.22%) | 5 (38.46%) | |
| mutated | 46 | 5 (33.33%) | 33 (25.78%) | 8 (61.54%) | |
| Unknown | 7 | | | | |
| **NPM1** | | | | | **0.0342** |
| unmutated | 118 | 14 (87.50%) | 98 (74.81%) | 6 (46.15%) | |
| mutated | 42 | 2 (12.50%) | 33 (25.19%) | 7 (53.85%) | |
| Unknown | 3 | | | | |

| NTUH Validation-Set [n=227] Parameter | Total | TriAS low | TriASs intermediate | TriAS high | p-value |
|---|---|---|---|---|---|
| **Age** | | | | | 0.2108 |
| ≤ 65 years | 192 | 21 (75.00%) | 140 (84.85%) | 31 (91.18%) | |
| > 65 years | 35 | 7 (25.00%) | 25 (15.15%) | 3 (8.82%) | |
| **Gender** | | | | | 0.3922 |
| Male | 118 | 15 (53.57%) | 89 (53.94%) | 14 (41.18%) | |
| Female | 109 | 13 (46.43%) | 76 (46.06%) | 20 (58.82%) | |
| **FAB type** | | | | | **0.0015** |
| 0 | 2 | 0 (0.00%) | 2 (1.21%) | 0 (0.00%) | |
| 1 | 55 | 7 (25.00%) | 44 (26.67%) | 4 (11.76%) | |
| 2 | 73 | 4 (14.29%) | 52 (31.52%) | 17 (50.00%) | |
| 3 | 26 | 10 (35.71%) | 15 (9.09%) | 1 (2.94%) | |
| 4 | 55 | 4 (14.29%) | 41 (24.85%) | 10 (29.41%) | |
| 5 | 12 | 3 (10.71%) | 7 (4.24%) | 2 (5.88%) | |
| 6 | 4 | 0 (0.00%) | 4 (2.42%) | 0 (0.00) | |

| | | | | | |
|---|---|---|---|---|---|
| **Cytogenetic risk** | | | | | 0.0974 |
| Favorable | 52 | 10 (40.00%) | 34 (20.86%) | 8 (24.24%) | |
| Intermediate | 121 | 12 (48.00%) | 96 (58.90%) | 13 (39.39%) | |
| Adverse | 41 | 2 (8.00%) | 29 (17.79%) | 10 (30.30%) | |
| Unknown | 13 | | | | |
| **Karyotype** | | | | | 0.0671 |
| Normal | 110 | 14 (56.00%) | 74 (45.40%) | 22 (66.67%) | |
| Aberrant | 111 | 11 (44.00%) | 89 (54.60%) | 11 (33.33%) | |
| Unknown | 6 | | | | |
| **FLT3-ITD** | | | | | 0.1459 |
| Unmutated | 164 | 22 (78.57%) | 122 (73.94%) | 20 (58.82%) | |
| Mutated | 63 | 6 (21.43%) | 43 (26.06%) | 14 (41.18%) | |
| **C/EBPα** | | | | | 0.8575 |
| Unmutated | 205 | 26 (92.86%) | 148 (89.70%) | 31 (91.18%) | |
| Mutated | 22 | 2 (7.14%) | 17 (10.30%) | 3 (8.82%) | |
| **NPM1** | | | | | **0.0471** |
| Unmutated | 169 | 19 (67.86%) | 119 (72.12%) | 31 (91.18%) | |
| Mutated | 58 | 9 (32.14%) | 46 (27.88%) | 3 (8.82%) | |
| **Allogeneic HSCT** | | | | | 0.0548 |
| Untransplanted | 147 | 23 (82.14%) | 106 (64.24%) | 18 (52.94%) | |
| Allogeneic transplantation | 80 | 5 (17.86%) | 59 (35.76%) | 16 (47.06%) | |

**Supplemental Table 4:** TriAS independently predicts RFS of AML patients independently of other established risk factors. Multivariate Cox regression analysis for RFS of AML patients from the NTUH validation set 1 is shown. For other data sets, no relapse data was available.

| NTUH Validation-Set [n=177] | HR multivariate | P-value multivariate |
|---|---|---|
| **Cytogenetic risk group poor** | **1.929 (1.055-3.528)** | **0.0330** |
| **Cytogenetic risk group favorable** | **0.337 (0.174-0.655)** | **0.0013** |
| **C/EBPα mutated** | **0.365 (0.157-0.851)** | **0.0196** |
| FLT3 mutated | 1.396 (0.844-2.309) | 0.1940 |
| NPM1 mutated | 0.875 (0.498-1.538) | 0.6430 |
| Gender (female) | 0.840 (0.543-1.300) | 0.4344 |
| Age>65 | 1.209 (0.626-2.335) | 0.5715 |
| **TriAS** | **1.334 (1.027-1.732)** | **0.0306** |

**Supplemental Table 5:** Multivariate Cox regression analysis for OS of the CN-AML subcohort from the TCGA training and the NTUH validation set 1 comparing different expression based prediction scores separately

| **TCGA Training set CN-AML [n=82]** | **HR multivariate** | **P-value multivariate** |
|---|---|---|
| Gender (female) | 1.713 (0.988-2.973) | 0.0554 |
| **Age >65** | **3.832 (2.073-7.082)** | **<0.0001** |
| **TriAS** | **2.082 (1.464-2.961)** | **<0.0001** |

| **TCGA Training set CN-AML [n=82]** | **HR multivariate** | **P-value multivariate** |
|---|---|---|
| Gender (female) | 1.607 (0.928-2.784) | 0.0907 |
| **Age >65** | **2.334 (1.314-4.145)** | **0.0038** |
| Marcucci score | 1.331 (0.756-2.343) | 0.3211 |

| **TCGA Training set CN-AML [n=82]** | **HR multivariate** | **P-value multivariate** |
|---|---|---|
| Gender (female) | 1.537 (0.894-2.643) | 0.1198 |
| **Age >65** | **2.448 (1.349-4.443)** | **0.0032** |
| Chuang score | 1.004 (0.959-1.050) | 0.8757 |

| **TCGA Training set CN-AML [n=82]** | **HR multivariate** | **P-value multivariate** |
|---|---|---|
| Gender (female) | 1.669 (0.962-2.894) | 0.0683 |
| **Age >65** | **2.255 (1.278-3.977)** | **0.0050** |
| Li score | 1.693 (0.962-2.979) | 0.0677 |

| **NTUH Validation-Set CN-AML [n=111]** | **HR multivariate** | **P-value multivariate** |
|---|---|---|
| Gender (female) | 1.124 (0.657-1.925) | 0.6693 |
| **Age >65** | **3.099 (1.647 – 5.832)** | **0.0005** |
| **TriAS** | **1.560 (1.110 – 2.192)** | **0.0105** |

| **NTUH Validation-Set CN-AML [n=111]** | **HR multivariate** | **P-value multivariate** |
|---|---|---|
| Gender (female) | 1.123 (0.654 – 1.927) | 0.6749 |
| **Age >65** | **2.615 (1.392 – 4.913)** | **0.0028** |
| Marcucci score | 1.528 (0.894 – 2.612) | 0.1211 |

| NTUH Validation-Set CN-AML [n=111] Parameter | HR multivariate | P-value multivariate |
|---|---|---|
| Gender (female) | 0.887 (0.514 – 1.530) | 0.6669 |
| **Age >65** | **2.166 (1.156 – 4.057)** | **0.0158** |
| **Chuang score** | **1.104 (1.061 – 1.149)** | **<0.0001** |

| NTUH Validation-Set CN-AML [n=111] Parameter | HR multivariate | P-value multivariate |
|---|---|---|
| Gender (female) | 1.023 (0.593 – 1.763) | 0.9353 |
| **Age >65** | **2.543 (1.357 – 4.764)** | **0.0036** |
| Li score | 1.758 (0.998 – 3.095) | 0.0508 |

**Supplemental Table 6:** Multivariate Cox regression analysis for OS of the non-CN-AML subcohort from the TCGA training and the NTUH validation set 1 comparing different expression based prediction scores separately

| TCGA Training set non-CN-AML [n=66] | HR multivariate | P-value multivariate |
|---|---|---|
| Gender (female) | 0.887 (0.433-1.816) | 0.7434 |
| **Age >65** | **4.318 (1.960-9.514)** | **0.0003** |
| Cytogenetic risk group poor | 0.501 (0.159-1.579) | 0.2379 |
| **Cytogenetic risk group favorable** | **0.194 (0.058-0.651)** | **0.0079** |
| **TriAS** | **2.207 (1.382-3.524)** | **0.0009** |

| TCGA Training set non-CN-AML [n=66] | HR multivariate | P-value multivariate |
|---|---|---|
| Gender (female) | 0.723 (0.364-1.438) | 0.3553 |
| **Age >65** | **4.934 (2.242-10.858)** | **<0.0001** |
| Cytogenetic risk group poor | 0.551 (0.172-1.760) | 0.3143 |
| **Cytogenetic risk group favorable** | **0.230 (0.070-0.756)** | **0.0155** |
| Marcucci score | 1.263 (0.536-2.979) | 0.5930 |

| TCGA Training set non-CN-AML [n=66] | HR multivariate | P-value multivariate |
|---|---|---|
| Gender (female) | 0.701 (0.353-1.390) | 0.3088 |
| **Age >65** | **5.190 (2.386-11.289)** | **<0.0001** |
| Cytogenetic risk group poor | 0.602 (0.193-1.877) | 0.3820 |
| **Cytogenetic risk group favorable** | **0.235 (0.072-0.773)** | **0.0171** |
| Chuang score | 1.000 (0.934-1.071) | 0.9914 |

| TCGA Training set non-CN-AML [n=66] | HR multivariate | P-value multivariate |
|---|---|---|
| Gender (female) | 0.693 (0.350-1.374) | 0.2941 |
| **Age >65** | **5.299 (2.458-11.424)** | **<0.0001** |
| Cytogenetic risk group poor | 0.685 (0.211-2.229) | 0.5300 |
| **Cytogenetic risk group favorable** | **0.196 (0.054-0.712)** | **0.0133** |
| Li score | 0.698 (0.257-1.896) | 0.4803 |

| NTUH Validation-Set non-CN-AML [n=110] | HR multivariate | P-value multivariate |
|---|---|---|
| Gender (female) | 0.732 (0.424 – 1.263) | 0.2623 |
| Age >65 | 1.892 (0.831 – 4.305) | 0.1286 |
| Cytogenetic risk group poor | 2.013 (0.936 – 4.325) | 0.0732 |

| | | |
|---|---|---|
| Cytogenetic risk group favorable | 0.440 (0.187 – 1.037) | 0.0607 |
| **TriAS** | **1.389 (1.021 – 1.891)** | **0.0367** |

| **NTUH Validation-Set non-CN-AML [n=110]** | **HR multivariate** | **P-value multivariate** |
|---|---|---|
| Gender (female) | 0.816 (0.474 - 1.405) | 0.4637 |
| Age >65 | 2.019 (0.893 - 4.565) | 0.0912 |
| **Cytogenetic risk group poor** | **2.216 (1.037 - 4.734)** | **0.0400** |
| Cytogenetic risk group favorable | 0.445 (0.191 - 1.033) | 0.0595 |
| **Marcucci score** | **2.361 (1.340 - 4.159)** | **0.0029** |

| **NTUH Validation-Set non-CN-AML [n=110]** | **HR multivariate** | **P-value multivariate** |
|---|---|---|
| Gender (female) | 0.890 (0.513 - 1.543) | 0.6771 |
| Age >65 | 1.922 (0.846 - 4.364) | 0.1184 |
| **Cytogenetic risk group poor** | **2.559 (1.175 - 5.574)** | **0.0180** |
| Cytogenetic risk group favorable | 0.465 (0.197 - 1.094) | 0.0795 |
| **Chuang score** | **1.069 (1.016 - 1.124)** | **0.0105** |

| **NTUH Validation-Set non-CN-AML [n=110]** | **HR multivariate** | **P-value multivariate** |
|---|---|---|
| Gender (female) | 0.743 (0.431 - 1.282) | 0.2862 |
| Age >65 | 1.810 (0.799 - 4.102) | 0.1551 |
| **Cytogenetic risk group poor** | **2.237 (1.039 - 4.816)** | **0.0396** |
| Cytogenetic risk group favorable | 0.526 (0.219 - 1.264) | 0.1511 |
| **Li score** | **1.946 (1.099 - 3.446)** | **0.0224** |