E-26 Transformation-specific Related Gene Expression and Outcomes in Cytogenetically Normal Acute Myeloid Leukemia: A Meta-analysis

Jian-Fei Fang1,2, Hai-Ning Yuan1, Yong-Fei Song1, Pei-Bei Sun1, Xiao-Liang Zheng1, Xiao-Ju Wang1,2
1 Center for Molecular Medicine, Zhejiang Academy of Medical Sciences, Hangzhou, Zhejiang 310013, China
2 Institute of Lung Cancer, Zhejiang Academy of Medical Sciences, Hangzhou, Zhejiang 310013, China

Abstract

Background: The E-26 transformation-specific related gene (ERG) is frequently expressed in cytogenetically normal acute myeloid leukemia (CN-AML). Herein, we performed a meta-analysis to investigate the relationship between the prognostic significance of ERG expression and CN-AML.

Methods: A systematic review of PubMed database and other search engines were used to identify the studies between January 2005 and November 2016. A total of 667 CN-AML patients were collected from seven published studies. Of the 667 patients underwent intensive chemotherapy, 429 had low expression of ERG and 238 had high expression of ERG. Summary odds ratio (OR) and the 95% confidence interval (CI) for the ERG expression and CN-AML were calculated using fixed- or random-effects models. Heterogeneity was assessed using Chi-squared-based Q-statistic test and F statistics. All statistical analyses were performed using R 3.3.1 software packages (R Foundation for Statistical Computing, Vienna, Austria) and RevMan5.3 (Cochrane Collaboration, Copenhagen, Denmark).

Results: Overall, patients with high ERG expression had a worse relapse (OR = 2.5127, 95% CI: 1.5177–4.1601, P = 0.0003) and lower complete remission (OR = 0.3495, 95% CI: 0.2418–0.5051, P < 0.0001). With regard to the known molecular markers, both internal tandem duplications of the fms-related tyrosine kinase 3 gene (OR = 3.8634, 95% CI: 1.8285–8.1626, P = 0.004) and brain and acute leukemia, cytoplasmic (OR = 3.1538, 95% CI: 2.0537–4.8432, P < 0.0001) were associated with the ERG expression. In addition, the results showed a statistical significance between French-American-British (FAB) classification subtype (minimally differentiated AML and AML without maturation, OR = 4.7902, 95% CI: 2.7772–8.2624, P < 0.0001; acute monocytic leukemia, OR = 0.2324, 95% CI: 0.0899–0.6006, P = 0.0026) and ERG expression.

Conclusion: High ERG expression might be used as a strong adverse prognostic factor in CN-AML.

Key words: Leukemia, Myeloid, Acute; Meta-analysis; Prognosis; Recurrence

Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease with diverse genomic aberrations in the subtypes. While a great number of cytogenetic abnormalities have been identified in AML, approximately 45% of de novo adult AML patients and 20% of pediatric AML patients are diagnosed with cytogenetically normal AML (CN-AML). It is important to study the predictive molecular markers so that patients can get better treatment. The molecular aberrations that have been previously studied in CN-AML patients include internal tandem duplications of the fms-related tyrosine kinase 3 gene (FLT3-ITD), the nucleophosmin gene (NPM1) mutations, MLL partial tandem duplication (MLL-PTD), E-26 transformation-specific related gene (ERG) expression and outcomes in Cytogenetically Normal Acute Myeloid Leukemia: A Meta-analysis. Chin Med J 2017;130:1481-90.
The ERG, a member of the E-26 transformation-specific (ETS) family of transcription factors, plays a major role in multiple cancers, such as Ewing’s sarcoma,[12,13] prostate cancer,[14] and leukemia.[10,15] The ERG, which is located on chromosome band 21q22, was involved in cell proliferation, apoptosis, and differentiation.[16] Moreover, ERG over-expression was demonstrated with complex karyotypes in AML patients and found in patients with CN-AML.[17] In addition, Marcucci et al. [18] also was the first time to study and report the prognostic significance of ERG in CN-AML. In accordance with the prior report, Marcucci et al.[19] sought to validate and demonstrated that the ERG overexpression was associated with worse outcome. Therefore, ERG overexpression might be used as important markers for CN-AML patients.

Until now, however, a comprehensive analysis of all reported ERG-related CN-AML is lacking. Therefore, in the current study, we assess the prognostic values of the expression of ERG in the patients with de novo CN-AML by a meta-analysis on all published studies. The well-established genetic markers were also investigated.

**Methods**

**Search strategy**

We searched the PubMed database and other search engines for all articles on the association between ERG and leukemia (last search update Nov 3, 2016). The following terms were used in the search: “ERG or ETS-related” and leukemia and “CN-AML or cytogenetically normal acute myeloid leukemia”. All eligible studies on the topic were identified by a manual search for references of retrieved articles. Finally, 239 studies were identified.

**Selection criteria**

The association studies between ERG overexpression and CN-AML were included if all the following conditions were met:[19,17-20] ERG gene expression was analyzed and grouped into “high” and “low” in studies; the cancer type is CN-AML; the study provides the total number of ERG high and low patients; the study is published in English or Chinese; the publication year range from January 2005 to November 2016.

The major exclusion criteria were as follows: Duplicate data; abstract, comment, review or editorial; poor study quality; the incomplete data.

We have no contact with authors. Ethical approval and informed patient consent are not required as this study is a literature review and have no direct patient contact or influence on patient care.

**Data extraction and quality assessment**

Two investigators independently extracted data from each study by following the predefined selection criteria and discrepancies were resolved by consensus of all investigators. All study personnel was blinded throughout the meta-analysis.

The following information was recorded for each study: The surname of the first author; year of publish; cancer type; ethnicity; number of cases and controls; risk factors.

Two investigators conducted the risk of bias assessments independently using the Cochrane Collaboration tool.[21] To assess the quality of each eligible study, two investigators worked independently to determine the adequacy of the studies, and discrepancies were resolved by discussion with all investigators. All assessors were blinded throughout the meta-analysis.

**Statistical analysis**

The Chi-squared-based Q-statistic test and F statistics were used to assess the heterogeneity. When the result of the heterogeneity test was $P < 0.005$, the random-effects model was used.[22] Otherwise, the fixed-effects model was selected. Funnel plots were used to diagnose a potential publication bias.[23] For the possible publication bias, trim and fill method were used to evaluate the influence to the result.[24] Sensitivity analyses were performed to assess the stability of the results by excluding one study at a time. All analyses were performed using R.3.3.1 software package (R Foundation for Statistical Computing, Vienna, Austria). All the $P$ values were two-sided. The value of $P < 0.05$ was defined as statistical significance. The risk of bias assessment was performed in RevMan5.3 (Cochrane Collaboration, Copenhagen, Denmark).

**Results**

**Characteristics of studies**

Of the 239 studies identified initially, seven studies met criteria and were included in the analysis [Figure 1]. Overall, these studies contained a total of 667 patients with high/low expression of ERG. Characteristics of the included studies are summarized in Tables 1-4. The association analyses were performed between the ERG expression and the risk factor are indicated in Table 1.

**Association between the E-26 transformation-specific related gene expression level and gender risk**

Gender information was included in the seven studies [Table 2]. The result showed a statistical significance of heterogeneity between studies ($I^2 = 0.2732; F = 55.6%; P = 0.0354$; Figure 2); thus, random effects model was used for this analysis. Compared with the low expression ERG, there was no statistically significant difference among gender (odds ratio $[OR] = 0.9639$, 95% confidence interval $[CI]: 0.5640–1.6476$, $P = 0.8932$).

**Association between the E-26 transformation-specific related gene expression level and race risk**

It has been shown previously that the frequency of ERG overexpression varied significantly between white and nonwhite population.[15-19] Here, three studies were included to assess the effect of race [Table 2]. There was no evidence of heterogeneity between studies ($I^2 = 0; F = 0; P = 0.9193$;
Table 1: General information of the patients reported in the seven references

| References   | Groups     | Cases (n) | Age (years) | Risk factor                  |
|--------------|------------|-----------|-------------|------------------------------|
| Marcucci et al.[18] | Low ERG    | 63        | 18–59       | Sex; race; CR; relapse; FAB; M |
|              | High ERG   | 21        | 26–59       | Sex; race; CR; relapse; FAB; M |
| Marcucci et al.[19] | Low ERG    | 38        | 19–59       | Sex; race; CR; relapse; M     |
|              | High ERG   | 38        | 19–59       | Sex; race; CR; relapse; M     |
| Metzeler et al.[9] | Low ERG    | 157       | 17–83       | Sex; CR; relapse; M; FAB      |
|              | High ERG   | 53        | 18–78       | Sex; CR; relapse; M; FAB      |
| Eid et al.[20] | Low ERG    | 20        | 18–42       | Sex; CR                      |
|              | High ERG   | 10        | 16–40       | Sex; CR                      |
| Schwind et al.[17] | Low ERG    | 79        | 60–81       | Sex; CR; race; M             |
|              | High ERG   | 79        | 60–83       | Sex; CR; race; M             |
| Aref et al.[1] | Low ERG    | 25        | 3–14        | Sex; CR;                     |
|              | High ERG   | 22        | 2–15        | Sex; CR;                     |
| Zheng YC[25] | Low ERG    | 47        | 24–77       | Sex; CR; FAB                 |
|              | High ERG   | 15        | 20–76       | Sex; CR; FAB                 |

ERG: E-26 transformation-specific related gene; M: Relative gene mutations; CR: Complete remission; FAB: French-American-British.

Figure 3), thus fixed effects model was used for the analysis. Compared with the low expression ERG, there was no statistically significant difference among race (OR = 1.2012, 95% CI: 0.5645–2.5564, P = 0.6342).
### Table 2: ERG expression levels and clinic-pathologic report in the seven references

| References | Groups | Sex (female), n/N | Race (white), n/N | CR, n/N | Relapse, n/N |
|------------|--------|------------------|------------------|--------|-------------|
| Marcucci et al.[9] | Low ERG | 28/63 | 54/63 | 52/63 | 17/63 |
| High ERG | 11/21 | 19/21 | 16/21 | 13/21 |
| Marcucci et al.[9] | Low ERG | 23/38 | 35/38 | 37/38 | 19/38 |
| High ERG | 24/38 | 35/38 | 30/38 | 27/38 |
| Metzeler et al.[9] | Low ERG | 100/157 | – | 108/157 | 99/157 |
| High ERG | 22/53 | – | 25/53 | 41/53 |
| Eid et al.[9] | Low ERG | 7/20 | – | 19/20 | – |
| High ERG | 5/10 | – | 3/10 | – |
| Schwind et al.[17] | Low ERG | 41/79 | 70/79 | 60/79 | – |
| High ERG | 30/79 | 71/79 | 51/79 | – |
| Aref et al.[31] | Low ERG | 12/25 | – | 21/25 | – |
| High ERG | 11/22 | – | 9/22 | – |
| Zheng YC[25] | Low ERG | 21/47 | – | 31/42 | – |
| High ERG | 11/15 | – | 6/15 | – |

ERG: E-26 transformation-specific related gene; CR: Complete remission; –: No data.

### Table 3: ERG expression levels and other molecular markers in the six references

| References | Groups | FLT3-ITD (present), n/N | MLL-PTD (yes), n/N | BAALC (high), n/N | NPM1 (mutation), n/N |
|------------|--------|------------------------|-------------------|------------------|----------------------|
| Marcucci et al.[9] | Low ERG | 10/63 | 5/63 | 27/63 | – |
| High ERG | 4/21 | 3/21 | 15/21 | – |
| Marcucci et al.[9] | Low ERG | 10/38 | 4/38 | 18/38 | 25/38 |
| High ERG | 25/38 | 1/38 | 23/38 | 29/38 |
| Metzeler et al.[9] | Low ERG | 57/157 | 20/157 | – | 87/157 |
| High ERG | 30/53 | 6/53 | – | 27/53 |
| Eid et al.[9] | Low ERG | – | – | 13/20 | – |
| High ERG | – | – | 8/10 | – |
| Schwind et al.[17] | Low ERG | 12/79 | 4/79 | 28/79 | 45/79 |
| High ERG | 46/79 | 2/79 | 51/79 | 52/79 |
| Aref et al.[31] | Low ERG | 0/25 | – | 6/25 | 1/25 |
| High ERG | 6/22 | – | 17/22 | 4/22 |

ERG: E-26 transformation-specific related gene; MLL-PTD: Partial tandem duplication of the MLL gene; FLT3-ITD: Internal tandem duplication of the FLT3 gene; BAALC: Brain and acute leukemia, cytoplasmic; NPM1: Nucleophosmin gene; –: No data.

### Table 4: ERG expression levels and FAB classification reports in the three references

| References | Groups | M0/M1, n/N | M2, n/N | M4, n/N | M5, n/N | M6, n/N |
|------------|--------|------------|--------|--------|--------|--------|
| Marcucci et al.[9] | Low ERG | 9/63 | 21/63 | 17/63 | 11/63 | 1/63 |
| High ERG | 10/21 | 6/21 | 5/21 | 0/21 | 0/21 |
| Metzeler et al.[9] | Low ERG | 35/157 | 53/157 | 35/157 | 25/157 | 7/157 |
| High ERG | 28/53 | 12/53 | 11/53 | 0/53 | 21/53 |
| Zheng YC[25] | Low ERG | 0/47 | 14/47 | 10/47 | 19/47 | 4/47 |
| High ERG | 4/15 | 5/15 | 11/15 | 5/15 | 0/15 |

ERG: E-26 transformation-specific related gene; FAB: French-American-British; M0: Minimally differentiated acute myeloid leukemia; M1: Acute myeloid leukemia without maturation; M2: Acute myeloid leukemia with maturation; M4: Acute myelomonocytic leukemia; M5: Acute monocytic leukemia; M6: Erythroleukemia.

### Association between the E-26 transformation-specific related gene expression level and complete remission

Complete remission was reported in all seven studies,[1,9,17-20,25] and the results indicated that there was no heterogeneity between studies ($I^2 = 0.3021; F = 49.7\%; P = 0.0634$; Figure 4), thus fixed effects model was employed in the merging analysis. Compared with the low expression ERG, there was statistically significant difference among complete remission ($OR = 0.3495, 95\% CI: 0.2418-0.5051, P < 0.0001$).

### Association between the E-26 transformation-specific related gene expression level and relapse

The relapse information was reported in three studies.[9,18,19] Since there was no heterogeneity between studies ($I^2 = 0; F = 0; P = 0.4739$; Figure 5), the fixed effects model was used for this analysis. Compared with the low expression ERG, there was statistically significant difference in relapse ($OR = 2.5127, 95\% CI: 1.5177-4.1601, P = 0.0003$).
Association between the E-26 transformation-specific related gene expression level and internal tandem duplication of the fms-related tyrosine kinase 3 gene
A total of five studies reported data for FLT3-ITD. There was evidence of statistically significant heterogeneity between studies ($\tau^2 = 0.4047, I^2 = 62.0\%$; $P = 0.0325$; Figure 6) and a random effects model was used for merging analysis. Compared with the low expression ERG, there was statistically significant difference in FLT3-ITD ($OR = 3.8634, 95\% CI: 1.8285–8.1626, P = 0.004$).

Association between the E-26 transformation-specific related gene expression level and brain and acute leukemia, cytoplasmic
A total of five studies reported data for BAALC. There was no evidence of statistically significant heterogeneity between studies ($\tau^2 = 0.0777, I^2 = 21.8\%$; $P = 0.046$; Figure 7). Compared with the low expression ERG, there was no statistically significant difference in MLL-PTD ($OR = 0.7817, 95\% CI: 0.3915–0.4078, P = 0.4851$).

Association between the E-26 transformation-specific related gene expression level and the nucleophosmin gene mutations
A total of four studies reported data for NPM1 mutations. There was no evidence of statistically significant heterogeneity between studies ($\tau^2 = 0.046; F = 19.0\%$; $P = 0.2953$; Figure 9) and merging analysis is
using fixed effects model. Compared with low expression ERG, there was no statistically significant difference in NPM1 (OR = 1.2471, 95% CI: 0.8378–1.8563, \( P = 0.2766 \)).

**Association between the E-26 transformation-specific related gene expression level and French-American-British classification**

A total of three studies reported data for French-American-British subtype.\(^{[9,18,25]}\) Compared with low ERG expression, there was statistically significant difference in minimally differentiated AML (M0)/AML without maturation (M1) and acute monocytic leukemia (M5) [Table 5].

**Sensitivity analysis**

Sensitivity analyses were performed by sequential removal of each eligible study to assess the influence of each study on the pooled OR in each comparison in complete remission. The results showed the reliability of the prognostic impact [Figure 10]. Due to the limited studies included in this analysis, we did not carry out the sensitivity analysis for the relapse risk factor.

**Subgroup analysis**

Six studies\(^{[9,17-20,25]}\) conducted in adult-only population for complete remission were selected for subgroup analysis. Compared with low expression ERG, there was statistically significant difference among complete remission (OR = 0.38, 95% CI: 0.2586–0.5584, \( P < 0.0001 \)). There was no heterogeneity between studies (\( t^2 = 0.2663; I^2 = 47.8%; P = 0.0880 \); Figure 11); thus; fixed effects model was employed in the merging analysis.

Four studies\(^{[1,9,17,19]}\) with median as criteria for ERG high and low expression were selected for subgroup analysis. Compared with low expression ERG, there was statistically significant difference among complete remission (OR = 0.3779, 95% CI: 0.2472–0.5778,
We selected four studies \([1,9,17,19]\) and the results indicated that there was no heterogeneity \( ( \tau^2 = 0.1764; I^2 = 42.6\%; P = 0.1561; \text{Figure 12}) \); thus, fixed effects model was employed in the merging analysis.

**Table 5: Analyses of FAB classification reports in the three references**

| FAB classification | \( Q \) | \( I^2 \) (%) | \( P \) | OR     | 95% CI       | \( P \) |
|--------------------|-------|---------------|-------|--------|-------------|-------|
| M0/M1              | 2.23  | 10.2          | 0.3285| 4.7902 | 2.7772–8.2624 | <0.0001|
| M2                 | 1.02  | 0.0           | 0.6016| 0.7095 | 0.4143–1.2151 | 0.2112 |
| M4                 | 10.37 | 80.7          | 0.0056| 1.8455 | 0.4528–7.5214 | 0.3927 |
| M5                 | 4.90  | 59.2          | 0.0863| 0.2324 | 0.0890–0.6006 | 0.0026 |
| M6                 | 8.07  | 75.2          | 0.0177| 2.1898 | 0.1426–33.6154 | 0.5738 |

CI: Confidence interval; OR: Odds ratio; FAB: French-American-British; M0: Minimally differentiated acute myeloid leukemia; M1: Acute myeloid leukemia without maturation; M2: Acute myeloid leukemia with maturation; M4: Acute myelomonocytic leukemia; M5: Acute monocytic leukemia; M6: Erythroleukemia.

\( P < 0.0001 \). We selected four studies\([1,9,17,19]\) and the results indicated that there was no heterogeneity \( ( \tau^2 = 0.1764; I^2 = 42.6\%; P = 0.1561; \text{Figure 12}) \); thus, fixed effects model was employed in the merging analysis.
Risk of bias assessment
Assessments using the Cochrane Risk of Bias tool[21] are presented in Figure 13. Detailed are provided in Supplementary Information.

Publication bias
The funnel plot found the evidence for publication bias in complete remission. The trim and fill method showed that the funnel plot needed three studies to be symmetrical [Figure 14]. Since there was heterogeneity between studies ($\tau^2 = 0.6399$; $I^2 = 62.9$%; $P = 0.0039$), the merging analysis was performed using random effects model. Compared to the low expression $ERG$, there was statistically significant difference ($OR = 0.4527$, 95% CI: 0.2301–0.8905, $P = 0.0217$). We did not test the publication bias in relapse due to the limited number of relevant studies.

Discussion
$ERG$, located on chromosome 21q22,[26] is widely overexpressed in AML patients with complex karyotypes. The product of $ERG$ is involved in many important pathways, such as cell proliferation, differentiation, and apoptosis.[16,27,28] In this study, we performed a systematic study between $ERG$ and cancer risk based on seven studies. Although the results suggested that there was no association with race ($OR = 1.2012$, 95% CI: 0.5645–2.5564, $P = 0.6342$) or gender ($OR = 0.9639$, 95% CI: 0.5640–1.6476, $P = 0.8932$), the analysis showed high $ERG$ expression level was significantly associated with high relapse ($OR = 2.5127$, 95% CI: 1.5177–4.1601, $P = 0.0003$) and inferior complete remission ($OR = 0.3495$, 95% CI: 0.2418–0.5051, $P < 0.0001$). In accordance with previous studies, $ERG$ overexpression predicted the increased relapse risk and fewer complete remission.[18,19]

$FMS$-like tyrosine kinase-3 gene ($FLT3$), a receptor tyrosine kinase, is important for the development of the hematopoietic and immune systems. Activating mutations of $FLT3$ are now recognized as the most common molecular abnormality in AML. $FLT3/ITD$ occurs in 20–30% of young adults with AML and is associated with poor prognosis.[29-31] In the meta-analysis, we detected an association between $FLT3$-ITD positive and $ERG$ expression ($OR = 3.8634$, 95% CI: 1.8285–8.1626, $P = 0.004$), in line with the previous report by Marcucci et al.[9,19]

$BAALC$, located on chromosome 8q22.3, is widely expressed in CN-AML.[32] While no significant association was observed between the mutations in $NPM1$ and $ERG$ expression, we found a correlation between $ERG$ and $BAALC$ expression ($OR = 3.1538$, 95% CI: 2.0537–4.8432, $P < 0.0001$). The association between high $ERG$ expression and high $BAALC$ was consistent with the previous studies.[1,9,19,33]

Although the number of the study was small in this meta-analysis, there was no heterogeneity among studies in complete remission ($\tau^2 = 0.3021$; $I^2 = 49.7$%; $P = 0.0634$) and relapse ($\tau^2 = 0$; $I^2 = 0$; $P = 0.4739$), indicating the results were reliable. Publication bias was found in the complete remission, however, the trim and fill analysis proved that the combined effect was statistically significant ($OR = 0.4527$, 95% CI: 0.2301–0.8905, $P = 0.0217$) in random effect model. Moreover, sensitivity analysis results showed the results of
ERG expression is an independent prognostic factor and allows refined risk stratification in cytogenetically normal acute myeloid leukemia: A comprehensive analysis of ERG, MNI, and BAALC transcript levels using oligonucleotide microarrays. J Clin Oncol 2009;27:5031-8. doi: 10.1200/JCO.2008.20.5328.

10. Salek-Ardakani S, Somoa G, de Boer J, Sebire NJ, Morris M, Rainis L, et al. ERG is a megakaryocytic oncogene. Cancer Res 2009;69:4665-73. doi: 10.1158/0008-5472.CAN-09-0075.

11. Baldus CD, Tanner SM, Ruppert AS, Whitman SP, Archer KJ, Marcucci G, et al. BAALC expression predicts clinical outcome of de novo acute myeloid leukemia patients with normal cytogenetics: A Cancer and Leukemia Group B Study. Blood 2003;102:1613-8. doi: 10.1182/blood-2003-02-0359.

12. Sorensen PH, Lessnick SL, Lopez-Terrada D, Liu XF, Triche TJ, Denny CT. A second Ewing’s sarcoma translocation, t(21;22), fuses the EWS gene to another ETS-family transcription factor, ERG. Nat Genet 1994;6:146-51. doi: 10.1038/ng0294-146.

13. Erdogan KE, Deveci MA, Hakkomyaz ZR, Gönlüsen G. Therapy-induced neural differentiation in Ewing’s sarcoma: A case report and review of the literature. Turk Patoloji Derg 2017; [Epub ahead of print]. doi: 10.1182/tjpath.2017.01390.

14. Tomlins SA, Rhodes DR, Pernar S, Dhanasekaran SM, Mehra R, et al. A second Ewing’s sarcoma translocation, t(21;22), fuses the EWS gene to another ETS-family transcription factor, ERG. Nat Genet 1994;6:146-51. doi: 10.1038/ng0294-146.

15. Erdogan KE, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. Blood Rev 2004;18:115-36. doi: 10.1016/S0268-960X(03)00040-7.

16. Mead AJ, Linch DC, Hills RK, Mayakonda A, Takao S, Liu L, et al. Diagnosis and relapse: Cytogenetically normal acute myelogenous leukemia without FLT3-ITD or MLL-PTD. Leukemia 2017;31:762-6. doi: 10.1038/leu.2016.343.

17. Döhner K, Schlenk RF, Hoft, Scholl C, Scholl C, Rücker FG, Corbacioglu A, et al. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: Interaction with other gene mutations. Blood 2005;106:3740-6. doi: 10.1182/blood-2005-05-2164.

18. Döhner K, Tobis K, Ulrich R, Fröhling S, Benner A, Schlenk RF, et al. Prognostic significance of partial tandem duplications of the MLL gene in adult patients 16 to 60 years old with acute myeloid leukemia and normal cytogenetics: A study of the Acute Myeloid Leukemia Study Group Ulm. J Clin Oncol 2002;20:3254-61. doi: 10.1200/JCO.2002.09.088.

19. Metzeler KH, Dufour A, Bentshaus T, Himmel M, Sauerland MC, Heinzeke A, et al. ERG expression is an independent prognostic factor and allows refined risk stratification in cytogenetically normal acute myeloid leukemia: A comprehensive analysis of ERG, MNI, and BAALC transcript levels using oligonucleotide microarrays. J Clin Oncol 2009;27:5031-8. doi: 10.1200/JCO.2008.20.5328.

20. Mrőzek K, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. Blood Rev 2004;18:115-36. doi: 10.1016/S0268-960X(03)00040-7.
Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 2005;310:644-8. doi: 10.1126/science.1117679.

15. Loughran SJ, Kruse EA, Hacking DF, de Graaf CA, Hyland CD, Willson TA, et al. The transcription factor Erg is essential for definitive hematopoiesis and the function of adult hematopoietic stem cells. Nat Immunol 2008;9:810-9. doi: 10.1038/ni.1617.

16. Oikawa T. ETS transcription factors: Possible targets for cancer therapy. Cancer Sci 2004;95:626-33. doi: 10.1111/j.1349-7006.2004.tb03200.x.

17. Schwind S, Marcucci G, Maharry K, Radmacher MD, Mrózek K, Holland KB, et al. BAALC and ERG expression levels are associated with outcome and distinct gene and microRNA expression profiles in older patients with de novo cytogenetically normal acute myeloid leukemia: A Cancer and Leukemia Group B study. Blood 2010;116:5660-9. doi: 10.1182/blood-2010-06-290536.

18. Marcucci G, Baldus CD, Ruppert AS, Radmacher MD, Mrózek K, Whitman SP, et al. Overexpression of the ETS-related gene, ERG, predicts a worse outcome in acute myeloid leukemia with normal karyotype: A Cancer and Leukemia Group B study. J Clin Oncol 2005;23:9234-42. doi: 10.1200/JCO.2005.03.6137.

19. Marcucci G, Maharry K, Whitman SP, Vukosavljevic T, Paschka P, Langer C, et al. High expression levels of the ETS-related gene, ERG, predict adverse outcome and improve molecular risk-based classification of cytogenetically normal acute myeloid leukemia: A Cancer and Leukemia Group B Study. J Clin Oncol 2007;25:3337-43. doi: 10.1200/JCO.2007.10.8720.

20. Eid MA, Attia M, Abdou S, El-Shazly SF, Elahwal L, Farrag W, et al. BAALC and ERG expression in acute myeloid leukemia with normal karyotype: Impact on prognosis. Int J Lab Hematol 2010;32:197-205. doi: 10.1111/j.1751-553X.2009.01168.x.

21. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The Cochrane collaboration’s tool for assessing risk of bias in randomised trials. BMJ 2011;343:d5928. doi: 10.1136/bmj.d5928.

22. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177-88. doi: 10.1016/0197-2456(86)90046-2.

23. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629-34. doi: 10.1136/bmj.315.7109.629.

24. Duval S, Tweedie R. Trim and fill: A simple funnel-plot-based method of adjusting for publication bias in meta-analysis. Biometrics 2000;56:455-63. doi: 10.1111/j.0006-3 41X.2000.00455.x.

25. Zheng YC. Expression and Significance of ERG and BAALC in Acute Myeloid Leukemia with Normal Cytogenetics [In Chinese, Dissertation]. Sheng Yang, CN: China Medical University; 2010.

26. Baldus CD, Liyanarachchi S, Mrózek K, Auer H, Tanner SM, Guimond M, et al. Acute myeloid leukemia with complex karyotypes and abnormal chromosome 21: Amplification discloses overexpression of APP, ETS2, and ERG genes. Proc Natl Acad Sci U S A 2004;101:3915-20. doi: 10.1073/pnas.0400272101.

27. Oikawa T, Yamada T. Molecular biology of the Ets family of transcription factors. Gene 2003;303:11-34. doi: 10.1016/ S0378-1119(02)01156-3.

28. McLaughlin F, Ludbrook VJ, Cox J, von Carlowitz I, Brown S, Randi AM. Combined genomic and antisense analysis reveals that the transcription factor Erg is implicated in endothelial cell differentiation. Blood 2001;98:3332-9. doi: 10.1182/blood.V98.12.3332.

29. Cancer Genome Atlas Research Network, Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med 2013;368:2059-74. doi: 10.1056/NEJMoa1301689.

30. Tse KF, Mukherjee G, Small D. Constitutive activation of FLT3 stimulates multiple intracellular signal transducers and results in transformation. Leukemia 2000;14:1766-76. doi: 10.1038/sj.leu.2401905.

31. Fleischmann M, Schnetzke U, Schrenk KG, Schmidt V, Sayer HG, Hilgendorf I, et al. Outcome of FLT3-ITD-positive acute myeloid leukemia: Impact of allogeneic stem cell transplantation and tyrosine kinase inhibitor treatment. J Cancer Res Clin Oncol 2017;143:337-45. doi: 10.1007/s00432-016-2290-5.

32. Baldus CD, Thiede C, Soucek S, Bloomfield CD, Thiel E, Ehnunger G. BAALC expression and FLT3 internal tandem duplication mutations in acute myeloid leukemia patients with normal cytogenetics: Prognostic implications. J Clin Oncol 2006;24:790-7. doi: 10.1200/JCO.2005.01.6253.

33. Langer C, Radmacher MD, Ruppert AS, Whitman SP, Paschka P, Mrózek K, et al. High BAALC expression associates with other molecular prognostic markers, poor outcome, and a distinct gene-expression signature in cytogenetically normal patients younger than 60 years with acute myeloid leukemia: A Cancer and Leukemia Group B (CALGB) study. Blood 2008;111:5371-9. doi: 10.1182/blood-2007-11-124958.
### Table for Risk of Bias

| Items                                      | Zheng YC[25]                          | Aref et al.[1]                        | Schwind et al.[17]                     | Eid et al.[20]                         | Metzeler et al.[9]                    | Marcucci et al.[18]                   | Marcucci et al.[19]                   |
|--------------------------------------------|---------------------------------------|--------------------------------------|---------------------------------------|---------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| Random sequence generation (selection bias) | Unclear/no information on sequence generation was reported | Unclear/no information on sequence generation was reported | Unclear/no information on sequence generation was reported | Unclear/no information on sequence generation was reported | Unclear/no information on sequence generation was reported | Unclear/no information on sequence generation was reported | Unclear/no information on sequence generation was reported |
| Allocation concealment (selection bias)    | Unclear/this information was not reported | Unclear/this information was not reported | Unclear/this information was not reported | Unclear/this information was not reported | Unclear/this information was not reported | Unclear/this information was not reported | Unclear/this information was not reported |
| Blinding of participants and personnel (performance bias) | High risk/the patients were treated | High risk/the patients were treated | High risk/the patients were treated | High risk/the patients were treated | High risk/the patients were treated | High risk/the patients were treated | High risk/the patients were treated |
| Blinding of outcome assessment (detection bias) | Unclear/this information was not provided | Unclear/this information was not provided | Unclear/this information was not provided | Unclear/this information was not provided | Unclear/this information was not provided | Unclear/this information was not provided | Unclear/this information was not provided |
| Incomplete outcome data (attrition bias)    | Low risk/all participants completed the trial | Low risk/all participants completed the trial | Low risk/all participants completed the trial | Low risk/all participants completed the trial | Low risk/all participants completed the trial | Low risk/all participants completed the trial | Low risk/all participants completed the trial |
| Selective reporting (reporting bias)        | Low risk/the outcome measures were reported | Low risk/the outcome measures were reported | Low risk/the outcome measures were reported | Low risk/the outcome measures were reported | Low risk/the outcome measures were reported | Low risk/the outcome measures were reported | Low risk/the outcome measures were reported |
| Other bias                                  | Low risk/there was no exist inappropriate influence | Low risk/there was no exist inappropriate influence | Low risk/there was no exist inappropriate influence | Low risk/there was no exist inappropriate influence | Low risk/there was no exist inappropriate influence | Low risk/there was no exist inappropriate influence | Low risk/there was no exist inappropriate influence |