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ORIGINAL REPORT

ERG Expression Is an Independent Prognostic Factor and Allows Refined Risk Stratification in Cytogenetically Normal Acute Myeloid Leukemia: A Comprehensive Analysis of *ERG*, *MN1*, and *BAALC* Transcript Levels Using Oligonucleotide Microarrays

Klaus H. Metzeler, Annika Dufour, Tobias Benthaus, Manuela Hummel, Maria-Cristina Sauerland, Achim Heinecke, Wolfgang E. Berdel, Thomas Büchner, Bernhard Wörmann, Ulrich Mansmann, Jan Braess, Karsten Spiekermann, Wolfgang Hiddemann, Christian Buske, and Stefan K. Bohlander

From the Department of Internal Medicine III, the Institute for Medical Informatics, Biometry, and Epidemiology, University Hospital Großhadern, Ludwig-Maximilians-Universität; the Clinical Cooperative Group Leukemia; and Helmholtz Zentrum München, National Research Centre for Environment and Health, Munich; the Department of Medical Informatics and Biomathematics; and the Department of Medicine A, Hematology and Oncology, University of Münster, Münster; and the Department of Hematology and Oncology, Municipal Hospital, Braunschweig, Germany.

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C.B. and S.K.B. contributed equally to this study.

Corresponding author: Stefan K. Bohlander, MD, or Christian Buske, MD, Department of Internal Medicine III, University Hospital Großhadern, Ludwig-Maximilians Universität, Campus Großhadern, Marchioninistr 15, 81377 München, Germany; e-mail: stefan.bohlander@med.uni-muenchen.de.

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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ABSTRACT

Purpose
Recently, several novel molecular prognostic markers were identified in cytogenetically normal acute myeloid leukemia (CN-AML). In addition to the well-known influence of *FLT3, NPM1,* and *CEBPA* mutations, high transcript levels of the *ERG, BAALC,* and *MN1* genes have been associated with inferior outcomes, but the relative importance of these risk markers remains to be defined.

Patients and Methods
We analyzed *ERG*, *BAALC,* and *MN1* expression levels in a cohort of 210 patients with CN-AML who received intensive chemotherapy. Expression levels of *ERG*, *BAALC,* and *MN1* were determined in bone marrow samples by using oligonucleotide microarrays.

Results
High transcript levels of *ERG*, *BAALC*, and *MN1* were predictors for inferior overall survival (OS) and a lower rate of complete remissions (CRs). There were significant positive correlations between the expression levels of all three genes. *ERG* expression levels predicted OS in elderly patients (ie, age 60 years or older) with CN-AML ($P = .006$) as well as in younger patients ($P = .013$). In multivariate analyses, high *ERG* expression was independently associated with a lower CR rate ($P = .013$), shorter event-free survival ($P = .008$), and shorter OS ($P = .005$). Patients who had low *ERG* levels and absent *FLT3* internal tandem duplication (ITD) had a 5-year OS of 44%, and patients who had high *ERG* expression and *FLT3* ITD had a 5-year OS of only 5%.

Conclusion
We analyzed a comprehensive set of molecular risk factors in a large, homogeneous CN-AML patient cohort. In this study, high *ERG* expression levels emerged as a strong negative prognostic factor and provided prognostic information in addition to established molecular markers.

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INTRODUCTION

Approximately 45% of patients with acute myeloid leukemia (AML) have a normal karyotype without chromosomal aberrations on standard cytogenetic analysis,[1] and this subgroup is heterogeneous with regard to molecular genetic alterations and therapeutic outcomes.[2] Submicroscopic genetic lesions not only are relevant for the pathogenesis of the disease but also influence response to therapy and survival.[2,3] The most frequent molecular abnormalities in cytogenetically normal AML (CN-AML) are mutations in the nucleophosmin 1 (*NPM1*) gene and internal tandem duplications (ITDs) of the fms-like tyrosine kinase 3 gene (*FLT3*). Mutations in the tyrosine kinase domain (TKD) of *FLT3* occur more rarely, and there are conflicting data on their prognostic relevance.[4,5] Other molecular aberrations found in smaller proportions of patients with CN-AML include mutations in the *CEBPA* gene, which convey a favorable prognosis, and partial tandem duplications (PTDs) of the *MLL* gene.[2,6-8]

More recently, quantitative differences in the expression levels of several genes (*BAALC*,[9-12] *ERG*,[13,14] *MN1*,[15,16] *WT1*,[17,18] and *EVI1*[19]),

measured by quantitative real-time polymerase chain reaction (qPCR), have been shown to carry prognostic information in patients with CN-AML. However, information on the interactions and relative importance of these risk factors is sparse. In this study, we used data from oligonucleotide microarrays to study the transcript levels of prognostically relevant genes in a well-characterized population of patients with CN-AML. Our approach allowed us to investigate correlations between various molecular markers and to identify those that carry independent prognostic information.

PATIENTS AND METHODS

Patients and Treatment

We studied a cohort of 210 patients with previously untreated CN-AML who received intensive induction and consolidation chemotherapy between 1999 and 2004. Two hundred (95.2%) of these patients were enrolled on the AMLCG-1999 multicenter trial of the German AML Cooperative Group,[20] and 10 patients were enrolled on the preceding AMLCG-1992 trial[21] (details in Data Supplement, online only). The study protocols were approved by the ethics committees of the participating centers, and all patients provided written informed consent. All patients had normal karyotypes on conventional cytogenetic examination of at least 20 metaphases. Patients were characterized at the molecular level with regard to *FLT3* ITD, *MLL* PTD, *NPM1*, *CEBPA*, and *FLT3* TKD (D835) mutations, as described previously.[22-24]

Microarray Analyses

Pretreatment bone marrow samples were analyzed by using Affymetrix HG-U133A (n = 154) or HG-U133 Plus 2.0 (n = 56) oligonucleotide microarrays (Affymetrix, Santa Clara, CA). Details regarding sample preparation, hybridization, and image acquisition have been described previously.[25,26] For combining individual oligonucleotide probes to probe sets and for the annotation to genes, we used custom chip definition files that were based on the GeneAnnot database.[27] Thereby, we ensured that each gene was represented by a single set of oligonucleotide probes and that only one single expression value was obtained per gene (details in Data Supplement, online only).

Statistical Analyses

We studied genes for which an association between transcript levels and prognosis of patients with CN-AML had been reported in the literature (*BAALC*, *ERG*, and *MN1*), and tested their associations with OS and event-free survival (EFS) in univariate Cox models. To delineate distinct patient subgroups on the basis of *BAALC* and *MN1* expression, patients with expression values greater than the median of all samples were classified as having high *BAALC* or *MN1* expression.[9-11,15,16] *ERG* overexpression was defined as a transcript level greater than the 75th percentile of all measurements.[13]

Survival curves were generated by the Kaplan-Meier method, and *P* values were calculated by the log-rank test.[28] Multivariate Cox proportional hazard regression models were constructed for OS, EFS, and relapse-free survival (RFS), and factors that predicted the chance to reach complete remission (CR) were analyzed in a logistic regression model (details in Data Supplement, online only). All analyses were performed by using the R 2.7.2 software package (available at www.r-project.org).[29]

RESULTS

Patient Characteristics

Baseline clinical and molecular characteristics of the 210 patients included on this study are detailed in Table 1. Overall, 188 patients (89.5%) were fully characterized for all five mutations considered in this analysis (*FLT3* ITD, *NPM1*, *CEBPA*, *MLL* PTD, and *FLT3* TKD). The frequencies and combinations of genetic aberrations among these 188 patients are displayed in Appendix Figure A1 (online only). The median OS for the total cohort was 12.7 months, and the median follow-up for survivors was 46 months (range, 2 to 92 months).

Measurement of BAALC, ERG, *and* MN1 *Expression Levels by Oligonucleotide Microarrays*

In an initial step, we tested the association of *ERG*, *BAALC*, and *MN1* transcript levels, determined by oligonucleotide microarray measurements and treated as continuous variables, with patient survival. By using univariate Cox proportional hazards models, we found that higher transcript levels of *ERG*, *BAALC*, and *MN1* were significantly associated with shorter EFS, and high *BAALC* and *ERG* levels also correlated with shorter OS (Appendix Table A1, online only). Importantly, we also found that the transcript levels of *ERG*, *BAALC*, and *MN1* showed significant positive pairwise correlations. This correlation was particularly strong between *BAALC* and *MN1* (Spearman $\rho = 0.77$; $P < .0001$), although it was less marked between *BAALC* and *ERG* ($\rho = 0.36$; $P < .0001$) and between *ERG* and *MN1* ($\rho = 0.27$; $P = .0001$; Appendix Fig A2, online only). Consequently, the dichotomized variables that indicated high versus low *BAALC*, *ERG*, and *MN1* expression also showed strong pairwise correlations (Appendix Table A2, online only).

Correlation of ERG, BAALC, *and* MN1 *Expression Levels With Other Molecular Alterations, Clinical Characteristics, and Treatment Outcomes*

On the basis of the microarray-measured transcript levels of *BAALC*, *ERG*, and *MN1*, we defined patient subgroups with high and low expression of each gene. For *BAALC* and *MN1*, this dichotomization was performed at the median of all expression values, the threshold used in most previous reports.[9-11,15,16] High *ERG* expression was defined as a transcript level greater than the 75th percentile. Different cut points for *ERG* have been used in earlier studies,[13,14] but the 75th percentile was chosen after inspection of survival curves within quantiles of *ERG* expression (Appendix Fig A3, online only).

Table 1 lists the clinical characteristics of the entire cohort and of patient subgroups distinguished by high versus low expression of *BAALC*, *ERG*, and *MN1*. Patients with high transcript levels of *ERG*, *BAALC*, or *MN1* frequently had AML with immature (ie, French-American-British classification [FAB] FAB M0 or M1) cytomorphology, whereas those with low expression of these genes more often showed monocytic differentiation (ie, FAB M5). High *ERG* expression was also associated with higher leukocyte and bone marrow blast counts, and high expression of all three genes was more frequent among men. High expression of *BAALC* was strongly correlated with wild-type *NPM1* and the presence of *MLL* PTD. Interestingly, 16 (89%) of 18 patients with *CEBPA* mutations had high *BAALC* levels. High *MN1* expression was also associated with wild-type *NPM1* and mutated *CEBPA*, whereas high *ERG* expression correlated with the presence of an *FLT3* ITD.

The patient subgroups with high expression of *ERG*, *BAALC*, or *MN1* had lower CR rates and shorter EFS and OS than patients with low expression of these genes (Table 2; Fig 1). Furthermore, high transcript levels of *ERG* and *MN1* were associated with shorter RFS among patients who reached CR (Appendix Fig A4, online only). High *ERG* expression was the marker with the strongest

Table 1. Associations of *BAALC, ERG,* and *MN1* Expression Levels With Pretreatment Clinical Characteristics and Other Molecular Markers

Variable	Patient Data in Total Cohort (N = 210)	Patient Data by Expression Level								
		BAALC Expression			*ERG* Expression			*MN1* Expression		
		Low (n = 105)	High (n = 105)	*P*	Low (n = 157)	High (n = 53)	*P*	Low (n = 105)	High (n = 105)	*P*
Female sex				.07			.006			.017
No.	122	71	51		100	22		70	52	
%	58	65	51		64	42		66	50	
Age, years										
Median	59	57	61	.77	57	62	.37	56	61	.05
Range	17-83	17-80	18-83		17-83	18-78		17-78	18-83	
WHO performance status, No.*				.72			.58			.82
0	46	21	25		37	9		23	23	
1	89	47	42		69	20		46	43	
2, 3, or 4	64	32	32		46	18		31	33	
AML type, No.				.86			.68			.50
De novo AML	200	101	99		150	50		100	100	
sAML	8	3	5		6	2		3	5	
tAML	2	1	1		1	1		2	0	
FAB classification, No.				< .001			< .001			.001
M0	6	0	6		2	4		0	6	
M1	57	22	35		33	24		18	39	
M2	65	27	38		53	12		35	30	
M4	46	28	18		35	11		27	19	
M5	25	21	4		25	0		18	7	
M6	9	6	3		7	2		6	3	
MDS-RAEB	2	1	1		2	0		1	1	
Leukocyte count, $\times 10^9$/L				.17			.039			.12
Median	31.8	33.7	26.0		26.3	41.9		33.7	24.0	
Range	0.85-486	0.85-486	1.0-440		0.90-486	0.85-287		0.75-486	1.0-289	
Hemoglobin level, g/L				.30			.80			.63
Median	92	90	93		92	91		92	93	
Range	40-147	40-142	42-147		40-147	42-131		40-142	49-147	
Platelet count, $\times 10^9$/L				.20			.14			.12
Median	59	60	51		60	48		61	51	
Range	6-471	9-280	6-471		9-471	6-367		10-471	6-367	
Bone marrow blasts, %										
Median	85	82	85	.67	80	86	.006	80	90	.01
Range	11-100	17-100	11-100		11-100	20-98		17-100	11-100	
FLT3 ITD status										
FLT3 ITD negative				.58			.015			.58
No.	123	64	59		100	23		64	59	
%	59									
FLT3 ITD positive										
No.	87	41	46		57	30		41	46	
%	41									
NPM1 status										
NPM1 wild type				< .001			.63			< .001
No.	96	23	73		70	26		31	65	
%	46									
NPM1 mutated										
No.	114	82	32		87	27		74	40	
%	54									
CEBPA status†										
CEBPA wild type				.001			.58			.005
No.	175	94	81		129	46		91	84	
%	91									
CEBPA mutated		2	16		12	6		3	15	
No.	18									
%	9									

(continued on following page)

Table 1. Associations of *BAALC, ERG,* and *MN1* Expression Levels With Pretreatment Clinical Characteristics and Other Molecular Markers (continued)

Variable	Patient Data in Total Cohort (N = 210)	Patient Data by Expression Level								
		BAALC Expression			*ERG* Expression			*MN1* Expression		
		Low (n = 105)	High (n = 105)	*P*	Low (n = 157)	High (n = 53)	*P*	Low (n = 105)	High (n = 105)	*P*
MLL PTD status‡										
MLL PTD negative				.011			.81			.40
No.	178	97	81		131	47		92	86	
%	87									
MLL PTD positive										
No.	26	7	19		20	6		11	15	
%	13									
FLT3 D835 status§										
FLT3 D835 wild type				.21			.25			.13
No.	192	93	99		141	51		93	99	
%	92									
FTL3 D835 mutated										
No.	17	11	6		15	2		12	5	
%	8									

NOTE. All 210 patients were characterized with regard to *FLT3* ITD and *NPM1* mutations.
Abbreviations: AML, acute myeloid leukemia; sAML, secondary AML after preceding myelodysplastic syndrome; tAML, therapy-related AML after previous chemotherapy or radiotherapy; FAB, French-American-British classification; MDS-RAEB, myelodysplastic syndrome–refractory anemia with excess of blasts; ITD, internal tandem duplication; PTD, partial tandem duplication.
*Information on performance status was missing for 11 patients.
†The *CEBPA* status was known for 193 patients.
‡The *MLL* PTD status was known for 204 patients.
§Information on *FLT3* tyrosine kinase domain (D835) status was available for 209 patients. *P* values were calculated by using Fisher's exact test and the Mann-Whitney *U* test, as appropriate.

impact on survival. In the entire cohort of patients, with a median age of 59 years, patients with high and low *ERG* transcript levels had median OS times of 7.7 and 18.4 months, respectively ($P < .001$) and estimated 5-year OS rates of 17% and 36%, respectively. The effect of high *ERG* levels was similar in patients age 60 years or older (5-year OS, 11% *v* 28%; $P = .006$) and in younger patients (5-year OS, 27% *v* 43%; $P = .013$; Fig 2). Similar results were obtained if surviving patients who had been observed for less than 24 months were excluded (Data Supplement, online only).

Prognostic Value of ERG, BAALC, *and* MN1 *Expression Levels in the Context of Other Risk Factors*

Given the correlations we observed between various molecular risk markers, we performed multivariate analyses to identify those factors that independently predicted prognosis in CN-AML (Table 3). High expression levels of *ERG* predicted a lower likelihood of reaching CR ($P = .013$) after analysis adjustment for *NPM1* status. Among patients who reached CR, the presence of an *NPM1* mutation without

Table 2. Clinical Outcomes in Patient Subgroups Groups Defined According to Expression Levels of *BAALC, ERG,* and *MN1*

Outcome	*BAALC* Expression			*ERG* Expression			*MN1* Expression		
	Low (n = 105)	High (n = 105)	*P*	Low (n = 157)	High (n = 53)	*P*	Low (n = 105)	High (n = 105)	*P*
CR									
No. of patients	75	57	.022	108	25	.008	75	58	.022
Rate, %	71	55		69	47		71	55	
RFS									
Median, months	16.8	10.8	.08	14.6	6.1	.022	14.6	8.8	.038
Rate at 5 years, %	42	24		37	23		42	23	
95% CI, %	31 to 55	15 to 38		28 to 47	11 to 48		32 to 55	14 to 38	
EFS									
Median, months	8.4	4.1	.007	9.0	2.3	< .001	9.5	3.9	.002
Rate at 5 years, %	28	13		24	11		29	13	
95% CI, %	21 to 39	8 to 22		18 to 32	5.1 to 24		21 to 39	8 to 22	
OS									
Median, months	15.5	9.5	.049	18.4	7.7	< .001	17.7	9.5	.047
Rate at 5 years, %	39	24		36	17		39	24	
95% CI, %	30 to 50	16 to 35		29 to 45	9.5 to 33		30 to 50	17 to 35	

Abbreviations: CR, complete remission; RFS, relapse-free survival; EFS, event-free survival; OS, overall survival.

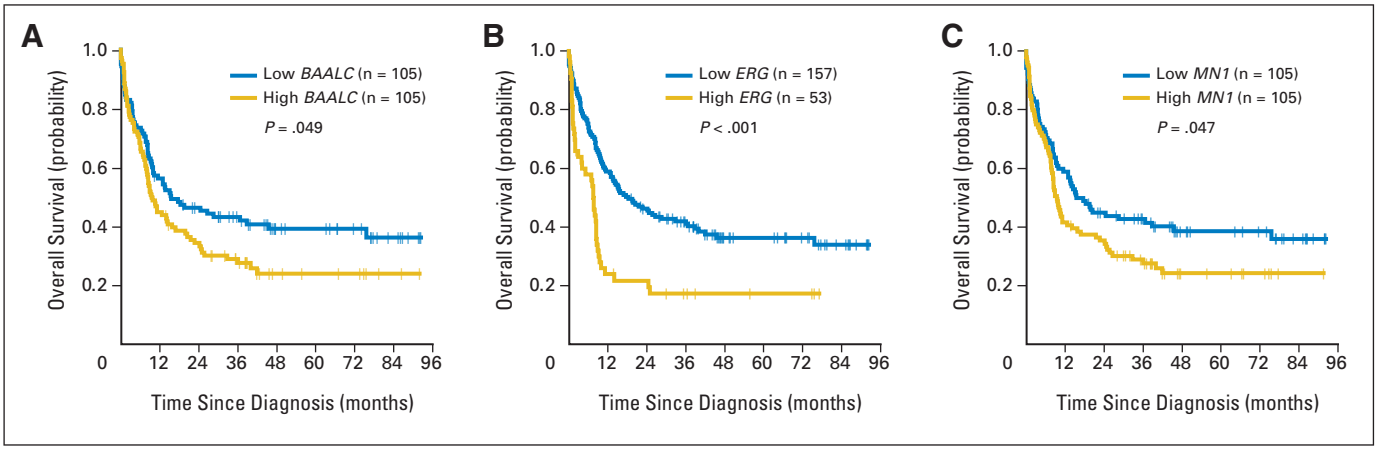

Fig 1. High expression of *BAALC, ERG,* or *MN1* predicts shorter overall survival (OS) in cytogenetically normal acute myeloid leukemia. Kaplan-Meier plots show the OS of patient subgroups with high versus low transcript levels of (A) *BAALC,* (B) *ERG,* and (C) *MN1.*

concurrent *FLT3* ITD (*NPM1*-positive/*FLT3* ITD-negative genotype) was the only independent predictor of RFS. In a multivariate model for EFS, high *ERG* expression was a significant prognostic factor ($P = .008$) together with age and the *NPM1*-positive/*FLT3* ITD-negative genotype. Patients with high *ERG* expression also had shorter OS ($P = .005$) than those with low expression after analysis adjustment for age, *FLT3* ITD, and platelet count at diagnosis. Neither *BAALC* nor *MN1* expression levels exhibited independent prognostic value in any of these multivariate analyses.

High *ERG* expression and *FLT3* ITD were both independent predictors of inferior OS, and we observed no statistically significant interaction between the effects of both factors on survival. Thus, we investigated patient stratification by using a combination of *ERG* transcript level and *FLT3* ITD status (Fig 3). Patients with low *ERG* expression in the absence of a *FLT3* ITD constituted a favorable subset of patients with CN-AML who had a median OS of 36 months, whereas the median OS for the three other groups combined was 8.5 months ($P < .001$). For patients with low *ERG* levels and absent *FLT3* ITD, the estimated 5-year OS was 44% and the RFS at 5 years was 43%. In contrast, the outcomes of patients with both high *ERG* expression and *FLT3* ITD were dismal, as the 5-year OS and RFS rates were only 5% and 9%, respectively. These differences were observed both in patients age younger than 60 years and those who were age 60 years or older (Appendix Fig A5, online only).

Patients with isolated *NPM1* mutations in the absence of *FLT3* ITDs seem to form a distinct, prognostically favorable subgroup of patients with AML; therefore, several study groups classify patients with CN-AML into low molecular risk (*NPM1*-mutant/*FLT3* ITD-negative) and high molecular risk (*NPM1* wild-type or *FLT3* ITD) categories.[7,30] We investigated whether *ERG* expression levels are useful to refine the risk stratification for patients with CN-AML who were already classified according to *NPM1* and *FLT3* ITD status. Patients in the high molecular risk category who also had high *ERG* expression had significantly worse OS than those with low *ERG* levels (estimated 5-year OS, 13% *v* 27%; $P = .003$; Appendix Fig A6B, online only). The majority (48 of 57) of patients in the low molecular risk category also had low *ERG* levels. However, among the nine patients with high *ERG* expression, there were five deaths within the first 8 months (Appendix Fig A6A; $P = .10$).

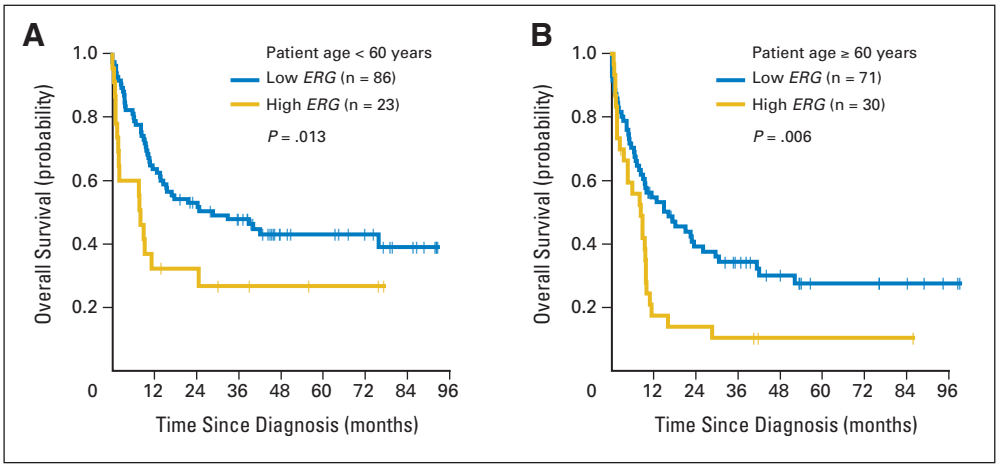

Fig 2. Prognostic value of *ERG* expression levels in younger and elderly patients with cytogenetically normal acute myeloid leukemia. High *ERG* expression levels predicted shorter overall survival in patients (A) younger than 60 years and (B) 60 years and older.

Table 3. Multivariate Analyses of Clinical Outcomes in 188 Patients With CN-AML

Variable by Clinical Outcome	Analysis			
	HR	OR	95% CI	*P*
CR				
ERG expression, highest quartile		0.43	0.22 to 0.84	.013
NPM1 mutation		2.16	1.17 to 3.96	.013
RFS				
NPM1-positive/*FLT3* ITD-negative genotype	0.30		0.17 to 0.54	< .001
EFS				
ERG expression, highest quartile	1.65		1.14 to 2.39	.008
NPM1-positive/*FLT3* ITD-negative genotype	0.41		0.27 to 0.62	< .001
Age per 10-year increase	1.23		1.08 to 1.39	.002
OS				
ERG expression (highest quartile)	1.82		1.20 to 2.75	.005
FLT3 ITD*	2.16		1.48 to 3.21	< .001
Age per 10-year increase	1.26		1.10 to 1.45	.001
Thrombocytes per increase of 50,000/μL	0.86		0.75 to 0.99	.034

NOTE. Only the 188 patients with complete information on *FLT3* ITD and tyrosine kinase domain mutation, *MLL* partial tandem duplication, and *NPM1* and *CEBPA* mutational status were considered in these analyses. The following variables had a $P < .2$ in univariate models and were included in the initial multivariate analyses: For overall survival, age, sex, performance status, de novo *v* secondary AML, pretreatment leukocyte and thrombocyte counts, *FLT3* ITD, *NPM1*, *FLT3* tyrosine kinase domain, *CEBPA* mutations, and *BAALC* and *ERG* expression levels; for EFS, age, sex, performance status, pretreatment leukocyte and thrombocyte counts, the *FLT3* ITD-negative/*NPM1*-positive genotype, *MLL* partial tandem duplication, and *BAALC*, *ERG*, and *MN1* expression levels; for RFS, age, performance status, pretreatment hemoglobin level and leukocyte count, the *FLT3* ITD-negative/*NPM1*-positive genotype, and *BAALC*, *ERG*, and *MN1* expression levels; for CR, age, sex, performance status, de novo *v* secondary AML, pretreatment thrombocyte count, *FLT3* ITD, *MLL* partial tandem duplication, *NPM1* mutations, and *BAALC*, *ERG*, and *MN1* expression levels. A stepwise backward variable selection technique was used, so that variables remaining in the final models were significant at $\alpha < .05$.

Abbreviations: CN-AML, cytogenetically normal acute myeloid leukemia; HR, hazard ratio; OR, odds ratio; CR, complete remission; RFS, relapse-free survival; ITD, internal tandem duplication; EFS, event-free survival; OS, overall survival.

*In the model for overall survival, *FLT3* ITD violated the proportional hazards assumption. Therefore, *FLT3* ITD was included in the model together with a time-dependent covariate. The hazard ratio for *FLT3* ITD is given for the time point 12 months after diagnosis, and the associated *P* value refers to a Wald test with two degrees of freedom for *FLT3* ITD and the time-dependent covariate.

DISCUSSION

Currently, the prediction of treatment outcomes and selection of treatment strategies for patients with CN-AML are frequently based on *FLT3* ITD and *NPM1* status, the most extensively characterized molecular prognostic markers. In recent years, however, the situation has become more complicated because of the description of several novel prognostic factors, including quantitative measurements of *ERG, BAALC,* and *MN1* transcript levels. Although the individual prognostic relevance of each of these risk factors has been well documented, it is unclear how molecular markers are best combined to allow for an improved prognostic stratification of patients with CN-AML. The aim of this study was to jointly evaluate a comprehensive set of risk markers in a relatively large and well-characterized patient cohort to delineate which markers are most informative for predicting a patient's risk for treatment failure, relapse, and death.

In our cohort of 210 patients, we found that transcript levels of the *ERG*, *BAALC,* and *MN1* genes correlated with treatment outcomes in univariate analyses. Our findings extend the results of earlier studies[9-16] in several important aspects: This report, to our knowledge, is the largest study so far that simultaneously evaluated expression levels of all three genes. Therefore, we had the possibility to explore correlations between the expression levels of these genes as well as with other molecular risk factors. We found that the expression levels of *MN1, BAALC*, and (to a lesser degree) *ERG* showed significant positive correlations with each other. Consistent with these results, *MN1* was among the most strongly upregulated genes in patients with high *BAALC* expression in a previous microarray-based analysis,[11] and an association between high *BAALC* and high *ERG* expression was described in two earlier studies.[11,14] Taken together, these data indicate that the prognostic information conveyed by *BAALC, MN1,* and *ERG* transcript levels may be partially overlapping and redundant. This underlines that multivariate analyses that include all three genes are necessary to determine their relative importance as prognostic markers.

Most of the patients on this study were fully characterized with regard to *FLT3* ITD, *FLT3* TKD, *MLL* PTD, *NPM1,* and *CEBPA* mutations, which thus allowed consideration of a comprehensive panel of molecular and clinical prognostic factors. In multivariate analyses, *ERG* expression levels emerged as one of the strongest predictors for treatment outcomes in CN-AML. *ERG* is a transcription factor expressed in hematopoetic stem cells and is involved in early lymphocyte differentiation[31] and angiogenesis.[32] Of note, the *ERG* gene is also involved in a recurring chromosomal translocation found in approximately 50% to 60% of all prostate adenocarcinomas,[33] which leads to an aberrantly high expression of *ERG*. High *ERG* transcript levels have been associated with worse outcomes, not only in AML but also in prostate cancer and T-cell acute lymphoblastic leukemia.[34,35] In this study, patients with CN-AML who had high *ERG* expression were less likely to respond to induction chemotherapy, as indicated by the lower CR rate, but their early death rate (within 28 days) was similar to patients with low *ERG* expression (Data Supplement, online only). High *ERG* levels, thus, primarily seem to predict resistance to induction chemotherapy and early treatment failure. The lower CR rate possibly explains the association of high *ERG* transcript levels with shorter EFS and OS. When the analysis was adjusted for other risk factors, no influence of high *ERG* transcript levels on RFS was observed. These results are in accordance with previous studies in younger patients.[13,14] Additional research is needed to identify the mechanisms that determine *ERG* expression levels in CN-AML and the signaling pathways that are responsible for the prognostic differences associated with varying *ERG* levels.

Both *ERG* expression and *FLT3* ITD were independent predictors of OS in this study. Therefore, we investigated whether improved risk stratification in CN-AML can be achieved by combining these two markers. Indeed, the combination of wild-type *FLT3* and low *ERG* expression identified a subset of patients who had relatively good outcomes. At the other end of the spectrum, patients with both an *FLT3* ITD and high *ERG* transcript levels had extremely poor prognoses, comparable to those with a complex aberrant karyotype. Although detailed information on salvage treatments was not available, the median OS of 7.5 months and a 5-year OS rate of only 5% suggest that most diseases with these traits are refractory to current therapies. Patients with this combination of risk markers apparently do not

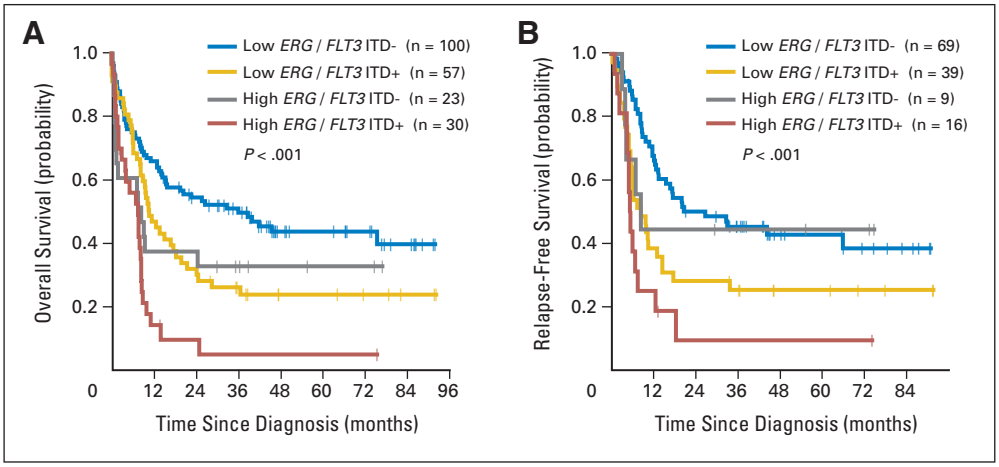

Fig 3. Risk stratification of patients with cytogenetically normal acute myeloid leukemia by a combination of *FLT3* internal tandem duplication (ITD) and *ERG* expression. (A) Overall survival and (B) relapse-free survival are shown for the four subgroups defined by *FLT3* ITD and *ERG* status. *P* values are given for the overall comparison across all four groups.

profit from intensive chemotherapy, with the possible exception of those who are candidates for allogeneic transplantation.

Almost all previous studies on the prognostic relevance of *ERG, BAALC,* and *MN1* expression levels exclusively focused on patients with CN-AML who were younger than age 60 years. Only one study that evaluated the prognostic impact of *BAALC* expression levels[12] also included elderly patients. In our cohort, the median age was considerably higher than in the previous study by Bienz et al,[12] and nearly half of all patients were age 60 years or older. We did not observe a significant interaction between age and the prognostic impact of *ERG, BAALC,* or *MN1* expression levels, which indicated that these genes are useful prognostic markers in older patients. For example, for patients who were age 60 years or older and who fell into the *FLT3* ITD-negative group with low *ERG* levels, the 5-year OS rate was 34%, which indicated that some of these elderly patients might benefit from intensive chemotherapy.

ERG expression levels also provided additional prognostic information for patients who were already stratified according to their *NPM1* and *FLT3* status. On the basis of findings in this study and results from other recent studies,[7,14] we propose a refined algorithm for risk stratification in CN-AML (Appendix Fig A7A, online only) that could be evaluated in future clinical trials. According to our results, patients with an *FLT3* ITD and high *ERG* levels should be classified as high risk. Because their outcomes resemble those of patients with a complex aberrant karyotype, they may be candidates for allogeneic transplantation or experimental therapies (Appendix Fig A7B). In contrast, patients with *NPM1* mutations, low *ERG* levels, and absent *FLT3* ITD, as well as those with mutant *CEBPA* (Appendix Fig A8, online only), can be considered as low risk.[7,8,14] In this cohort, the majority of these low-risk patients were cured with intensive chemotherapy. All remaining patients fell into an intermediate-risk category, and the optimum treatment strategy for these patients still needs to be defined.

All previous studies on the prognostic impact of *BAALC, ERG,* and *MN1* expression were performed by using qPCR,[9-15] whereas we used expression measurements from oligonucleotide microarrays. Although microarray measurements tend to underestimate differences in gene expression compared with qPCR, there is generally a good correlation between expression ratios measured by qPCR and by those obtained from oligonucleotide microarrays.[11,36,37] Of note, our analysis relied on relative expression differences between samples and not on absolute expression levels, and our results were remarkably consistent with those of previous, PCR-based studies. Nonetheless, before quantitative measures of *ERG* expression can be used for clinical decision making, additional standardization of the methods used to determine *ERG* expression levels in combination with prospective trials is necessary.

In summary, this work is, to our knowledge, the largest study so far that simultaneously analyzed *ERG, BAALC,* and *MN1* expression levels in a uniformly treated patient population. Several important conclusions can be drawn from this study: We found that the expression levels of *ERG, BAALC,* and *MN1* are strongly correlated, which suggests that their prognostic significance may be overlapping; this necessitates a comprehensive analysis of molecular risk factors in CN-AML. Our patient cohort was well characterized for the most relevant molecular aberrations and gene expression changes. In multivariate models, high *ERG* expression emerged as a strong negative prognostic marker that independently predicted a poor response to induction chemotherapy and shorter survival. Unlike most previous studies, our analysis also included patients who were older than 60 years of age, and this study is the first to show that high *ERG* expression is a negative prognostic factor in elderly patients with CN-AML. We demonstrated that combining novel risk markers, such as high *ERG* expression, with established prognostic factors results in refined prognostic subclassification of CN-AML.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Klaus H. Metzeler, Christian Buske, Stefan K. Bohlander
Financial support: Ulrich Mansmann, Wolfgang Hiddemann, Christian Buske, Stefan K. Bohlander
Administrative support: Maria-Cristina Sauerland, Achim Heinecke
Provision of study materials or patients: Annika Dufour, Tobias Benthaus, Wolfgang E. Berdel, Thomas Büchner, Bernhard Wörmann, Jan Braess, Karsten Spiekermann, Wolfgang Hiddemann
Collection and assembly of data: Klaus H. Metzeler, Annika Dufour, Tobias Benthaus, Manuela Hummel, Maria-Cristina Sauerland, Achim Heinecke, Ulrich Mansmann

Data analysis and interpretation: Klaus H. Metzeler, Manuela Hummel, Ulrich Mansmann, Jan Braess, Karsten Spiekermann, Christian Buske, Stefan K. Bohlander

Manuscript writing: Klaus H. Metzeler, Christian Buske, Stefan K. Bohlander

Final approval of manuscript: Klaus H. Metzeler, Annika Dufour, Tobias Benthaus, Manuela Hummel, Maria-Cristina Sauerland, Achim Heinecke, Wolfgang E. Berdel, Thomas Büchner, Bernhard Wörmann, Ulrich Mansmann, Jan Braess, Karsten Spiekermann, Wolfgang Hiddemann, Christian Buske, Stefan K. Bohlander

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