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***BAALC* is an important predictor of refractoriness to chemotherapy and poor survival in intermediate-risk acute myeloid leukemia (AML)**

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Abstract We have analyzed brain and acute leukemia, cytoplasmic (*BAALC*) gene expression and other genetic markers (*ERG*, *EVII*, *MNI*, *PRAME*, *WT1*, *FLT3*, and *NPM1* mutations) in 127 intermediate-risk acute myeloid leukemia (AML) patients: 98 cytogenetically normal and 29 with intermediate-risk cytogenetic alterations. High versus low *BAALC* expressers showed a higher refractoriness to induction treatment (31% vs 10%; $p=.005$),

lower complete remission rate after salvage therapy (82% vs 97%; $p=.010$), and lower 3-year overall (23% vs 58%, $p<.001$) and relapse-free survival (26% vs 52%, $p=.006$). Similar results were found when cytogenetic subgroups were analyzed separately. Multivariate models confirmed the unfavorable prognosis of this marker. In conclusion, *BAALC* is a relevant prognostic marker in intermediate-risk AML.

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Introduction

The *BAALC* (brain and acute leukemia, cytoplasmic) gene, located at chromosome 8q22.3, is expressed primarily in neuroectoderm-derived tissues and hematopoietic precursors but not in mature marrow or blood mononuclear cells and encodes a protein with unknown function [1, 2]. Additionally, it has been detected in a subset of patients with acute myeloid leukemia (AML), acute lymphoblastic leukemia, and chronic myelogenous leukemia in blast crisis [2]. High *BAALC* RNA expression levels are associated with adverse prognosis in newly diagnosed AML patients with normal cytogenetics (CN-AML) [2–5]. This prognostic impact is particularly relevant in those CN-AML patients lacking *FLT3*-ITD and *CEBPA* mutations [6].

Several studies from the same research group have investigated the prognostic value of *BAALC* expression together with other prognostic molecular markers (*ERG* and *MNI* expression, as well as *CEBPA*, *FLT3*, *NPM1*, or *WT1* mutations) [5, 7–9], but all of them are restricted to CN-AML patients, and there are no confirmatory studies from other independent investigational groups. Thus, no reports have evaluated the prognostic value of *BAALC* in AML harboring chromosomal alterations with unclear prognostic impact (i.e., trisomy 8 or t(9;11) [1]) without either complex or monosomal karyotype [10]. Very recently, our group has published a molecular score based on *ERG*, *PRAME*, and *EVII* gene expression that allows to stratify CN-AML patients in four risk categories [11]. For this reason, we aimed to evaluate the clinical relevance of *BAALC* together with other relevant markers in AML (*FLT3*-ITD, *ERG*, *EVII*, *MNI*, *NPM1* mutations, *PRAME*, and *WT1*) in a series of de novo AML patients with intermediate-risk cytogenetics. Therefore, the present study represents an external validation of *BAALC* adverse prognosis in patients enrolled into PETHEMA/CETLAM Spanish clinical trials. In addition, it is the first description about the clinical relevance of *BAALC* gene expression levels in AML patients harboring chromosomal abnormalities without a well-defined prognostic value.

Materials and methods

One hundred and twenty-seven adult patients with untreated primary AML and intermediate-risk cytogenetics were included in this study. We considered as intermediate-risk cytogenetics those patients with a normal karyotype ($n=98$) and patients harboring one or two abnormalities without

a well-defined impact on clinical outcome [1, 10] and CN-AML with an altered fluorescence in situ hybridization analysis or an *MLL* transcript fusion detected by real-time polymerase chain reaction (PCR; $n=29$; Supplementary Table 1). We included five patients harboring an *MLL-MLLT3* (alias *MLL-AF9*) since de novo AML with t(9;11)(p22;q23) show a better clinical outcome than traditionally adverse AML group with other 11q23 rearrangements [12, 13], and they are now considered as an independent AML subtype according to the 2008 World Health Organization classification of myeloid neoplasias. All patients were treated according to the Spanish PETHEMA LAM-99 [14] or ICE-CETLAM [15] protocols. Twelve patients (9.4%) died during induction treatment, 92 (72.4%) achieved complete remission (CR) with induction therapy, and 23 (18.1%) were refractory to the standard induction treatment. Eleven patients from this latter group achieved CR after salvage therapy. Finally, 52 of the 103 (50.5%) patients who achieved CR eventually relapsed during the evaluation period. The median follow-up for censored patients was 29 months (range 6–107). Additionally, ten bone marrow (BM) samples from healthy donors were evaluated as controls for gene expression analysis. Informed consent to use biological samples and clinical data was obtained in all cases.

Total RNA from diagnostic BM was obtained using phenol-chloroform extraction, and subsequent reverse transcription was performed using high capacity commercial kit (Applied Biosystems, Foster City, CA, USA). All samples were analyzed for *FLT3*-ITD [16], mutations in *NPM1* [17], and relative expression of the following genes: *ABL1* (as control gene), *BAALC*, *ERG*, *EVII*, *MNI*, *PRAME*, and *WT1*, using the TaqMan® gene expression assays (Applied Biosystems). Relative quantification was calculated using the equation $2^{-\Delta\Delta C_t}$, as previously described [18]. Additionally, we tested *DEK-NUP214* [t(6;9)(p23;q34)], *MLL-MLLT2* [t(4;11)(q21;q23)], *MLL-MLLT3* [t(9;11)(p22;q23)], *MLL-MLLT4* [t(6;11)(q26;q23)], *MLL-MLLT10* [t(10;11)(p12;q23)], *RUNX1/RUNX1T1* [t(8;21)(q22;q22)], *PML-RAR α* [t(15;17)(q22;q12-21)], and *CBF β /MYH11* [inv(16)(p13q22)] fusion transcripts by real-time PCR [19].

All tests were carried out by using the Statistical Package for the Social Sciences (SPSS) 15.0 program (SPSS, Chicago, IL, USA). For univariate analyses, the χ^2 and t student test was performed to evaluate the relationship between refractoriness to treatment and gene expression. The relapse-free survival (RFS) and overall survival (OS) were calculated using the Kaplan–Meier method. The impact of multiple predictor variables on refractoriness to treatment was estimated based on logistic regression analysis. Similarly, multivariate analysis on RFS and OS was assessed using the Cox regression model. Logistic regression and multivariate analyses were built including

Table 1 Clinical and biological characteristics of acute myeloid leukemia patients at diagnosis according to *BAALC* gene expression levels

Parameter	Total (n=127)	Low <i>BAALC</i> (n=63)	High <i>BAALC</i> (n=64)	<i>p</i>
Age, years, median (range)	54 (15–77)	56 (20–77)	53 (15–75)	.35
Sex, male, n (%)	64 (50.4)	32 (50.8)	32 (50.0)	.54
WBC, $\times 10^9/L$, median (range)	14.0 (0.2–337)	9.2 (0.2–186)	27.8 (0.6–337)	.057
Hemoglobin, g/L, median (range)	94 (40–150)	88 (44–150)	98 (40–127)	.59
Platelets, $\times 10^9/L$ median (range)	55 (5–930)	62 (7–930)	48 (5–226)	.10
BM blasts,% median (range)	68 (20–98)	70 (20–98)	67 (20–98)	.39
FLT3-ITD, n (%)	34 (26.8)	13 (20.6)	21 (32.8)	.088
<i>NPM1</i> mutated, n (%)	55 (43.3)	43 (68.3)	12 (18.8)	<.001
<i>ERG</i> gene expression, median (range)	0.5 (0.0–6.2)	0.4 (0.0–4.3)	0.8 (0.1–6.2)	.011
<i>EVII</i> gene expression, median (range)	0.05 (0.0–253)	0.04 (0.0–230)	0.06 (0.0–253)	.081
<i>MNI</i> gene expression, median (range)	53.2 (0–18,342)	39.8 (0–3,628)	201.7 (0–18,342)	.006
<i>PRAME</i> gene expression, median (range)	6.1 (0–18,163)	6.1 (0–11,391)	6.0 (0–18,163)	.23
<i>WT1</i> gene expression, median (range)	164.6 (0–3,987)	187.0 (0–856)	122.4 (0–3,987)	.21
Response to induction therapy, n (%)				
Complete remission (CR)	92 (80.0)	54 (90.0)	38 (69.1)	.005
Refractoriness to treatment	23 (20.0)	6 (10.0)	17 (30.9)	
Death before CR	12	3	9	
CR after induction/salvage therapy, n (%) ^a				
Yes	103 (89.6)	58 (96.7)	45 (81.8)	.010
No	12 (10.4)	2 (3.3)	10 (18.2)	
Relapse, n (%)				
No	51 (49.5)	34 (58.6)	17 (37.8)	.028
Yes	52 (50.5)	24 (41.4)	28 (62.2)	
Exitus, n (%)				
No	60 (47.2)	40 (63.5)	20 (31.3)	<.001
Yes	67 (52.8)	23 (36.5)	44 (68.7)	

^a A total of 103 patients achieved complete remission, including 92 with standard induction therapy and 11 with salvage treatment

Table 2 Multivariate analysis for refractoriness to induction therapy, overall and relapse-free survival

Parameter	Refractoriness			OS			RFS		
	OR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
FLT3-ITD	4.6	1.5–13.7	.006						
High <i>BAALC</i> (median)	3.3	1.0–10.9	.036	3.0	1.7–5.2	<.001	2.4	1.1–3.1	.005
High <i>ERG</i> (median)	2.9	1.0–9.4	.053						
High <i>PRAME</i> (75th percentile)	–	–	.076						
Age >65 years				3.2	1.7–5.8	<.001	2.7	1.4–5.2	.003
WBC >50 $\times 10^9/L$				1.7	1.1–2.8	.026	–	–	.12
Molecular score ^a				1.4	1.0–1.9	.041	1.4	1.0–2.0	.049

Variables considered for refractoriness model (*p* value in Pearson chi-square analysis for categorical variables or Mann–Whitney *U* test for continuous variables): *FLT3*-ITD (.001), *BAALC* gene expression, median (.003), *ERG* gene expression (.023), *PRAME* gene expression (.037), *NPM1* mutations (.040), and *MNI* gene expression, median (.081). *BAALC*, *ERG*, and *MNI* gene expression levels were dichotomized by median value and *PRAME* expression were dichotomized by 75th percentile value to perform the logistic regression analysis

Variables considered for OS model (*p* value in Kaplan–Meier [log-rank test] analysis): high *BAALC* (<.001), age >65 years (.006), high molecular score (.007), WBC > 50 $\times 10^9/L$ (.010), high *ERG* (.013), *NPM1*mut+*FLT3*wt (.017), low *PRAME* (.039), high *EVII* (.071), and high *MNI* (.097)

Variables considered for RFS model (*p* value in Kaplan–Meier [log-rank test] analysis): high *BAALC* (.006), age >65 years (.013), high molecular score (.024), high *ERG* (.038), low *PRAME* (.060), and WBC > 50 $\times 10^9/L$ (.090)

^a Based on *ERG*, *EVII*, and *PRAME* gene expression levels. From Santamaria et al. [11]

only those variables reaching a p value <0.1 in the respective univariate analysis.

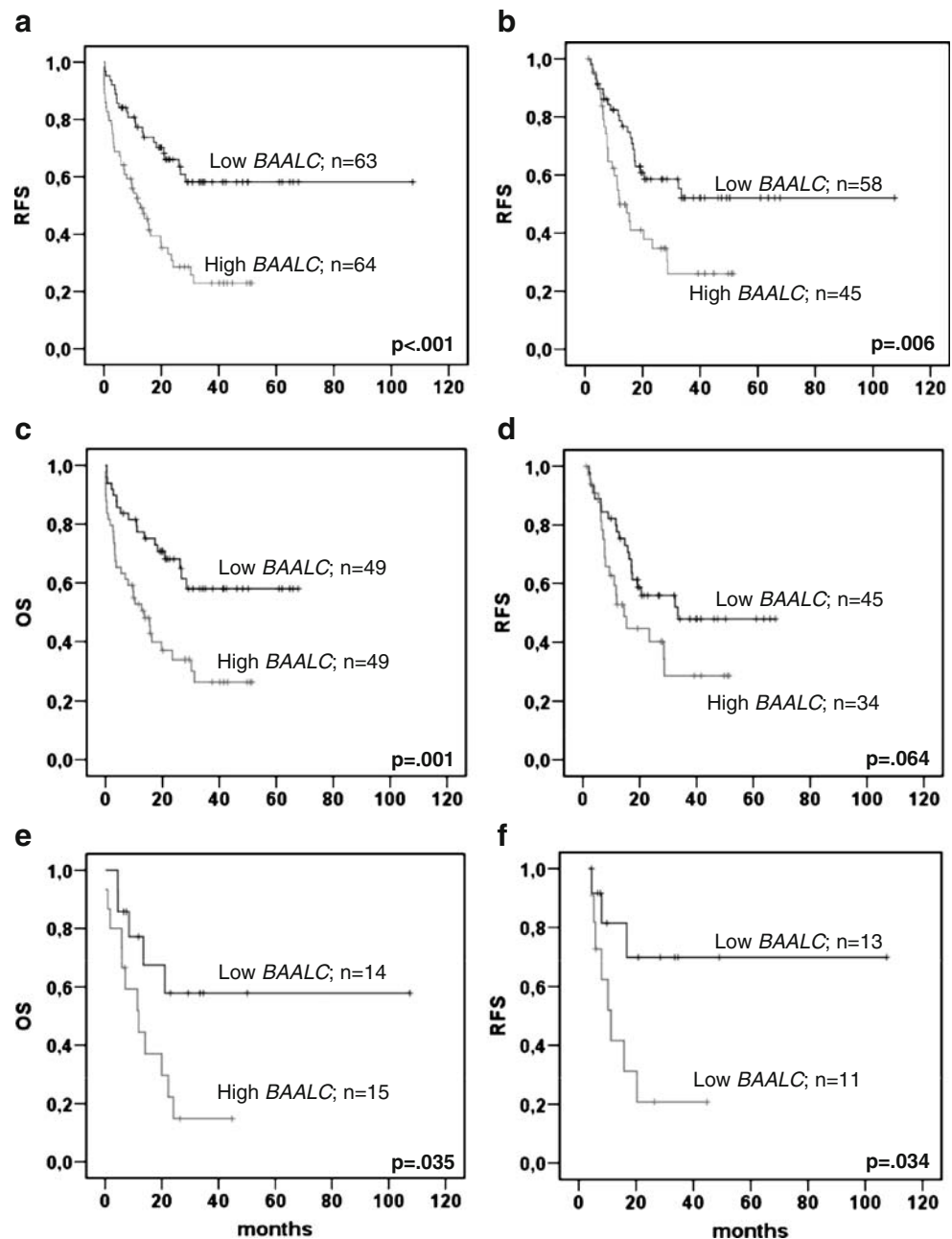
Results and discussion

In order to test if all patients could be considered as a uniform series, we compared survival, clinical features, and gene expression profile of CN-AML ($n=98$) and AML with intermediate-risk karyotype ($n=29$). No differences in either overall (OS; $p=.52$) or RFS ($p=.96$) were observed between these subgroups. In addition, no differences in age,

white blood cells (WBC), platelets or BM blasts counts, as well as *ERG*, *EVII*, *MNI*, or *PRAME* gene expression were observed. Only *WTI* was overexpressed in AML patients with altered karyotype ($p=.021$), especially in those patients harboring trisomy 11. In addition, there was a trend toward higher *BAALC* ($p=.062$) in patients with trisomy 8 as compared to CN-AML patients.

Clinical and biological features of the 127 intermediate-risk AML patients are summarized in Table 1. At diagnosis, patients with high *BAALC* gene expression (values above the median value) showed several adverse prognostic parameters such as lower incidence of *NPM1* mutation

Fig. 1 Overall survival (OS) and relapse-free survival (RFS) of intermediate-risk acute myeloid leukemia (AML) patients based on *BAALC* gene expression levels. **a, b** OS and RFS for the 127 intermediate-risk AML patients, respectively. **c, d** Survival analyses for 98 AML patients with normal cytogenetics. High *BAALC* expressers displayed a shorter 3-year OS (26% vs 58%, $p=.001$) and a trend toward a poorer RFS (29% vs 48%, $p=.064$) than low *BAALC* expressers. **e, f** Survival analyses for 29 AML patients with intermediate-risk chromosomal/molecular abnormalities. High *BAALC* expressers showed a shorter 3-year OS (15% vs 58%, $p=.035$) and RFS (21% vs 70%, $p=.034$) than low *BAALC* expressers



(18.8% vs 68.3%; $p < .001$), higher *MNI* and *ERG* gene expression levels (median 201.7 vs 39.8; $p = .006$, and median 0.8 vs 0.4; $p = .011$, respectively), and a trend towards higher WBC counts (27.8 vs $9.2 \times 10^9/L$; $p = .057$), higher *EVII* gene expression levels (median 0.06 vs 0.04; $p = .081$) and higher incidence of *FLT3*-ITD (32.8% vs 20.6%; $p = .088$) in comparison to low *BAALC* expressers. Furthermore, high *BAALC* RNA levels were associated with high mortality rate (69% vs 36%; $p < .001$), refractoriness to standard induction treatment (31% vs 10%; $p = .005$), low CR rate after salvage therapy (82% vs 97%; $p = .010$), and high relapse rates (62.2% vs 41.4%; $p = .028$). In fact, chemotherapy-resistant patients displayed significantly higher incidence of *FLT3*-ITD (52% vs 17%; $p = .001$), higher *BAALC* (mean 16.4 ± 40.2 vs 6.7 ± 17.4 , $p = .003$) and *ERG* (mean 1.0 ± 0.8 vs 0.7 ± 0.8 , $p = .023$) gene expression levels, lower *PRAME* RNA levels (mean 30 ± 52 vs $742 \pm 2,797$, $p = .037$), and lower incidence of *NPM1* mutation (26% vs 49%; $p = .040$) than non-refractory patients at diagnosis. In a multivariate model, *FLT3*-ITD mutation (odds ratio, 4.6; $p = .006$), high *BAALC* (odds ratio, 3.3; $p = .036$), and high *ERG* RNA levels (odds ratio, 2.9; $p = .053$) were associated with refractoriness to induction therapy, confirming previous findings in CN-AML [4, 11] (Table 2). In this regard, a recent study has described a singular gene expression signature in CN-AML with high *BAALC* gene expression, identifying the multidrug resistance gene *ABCBI* (MDR1) as one of the most highly upregulated genes in these patients, which could explain the refractoriness to treatment observed in high *BAALC* expressers [5].

Patients expressing high *BAALC* RNA levels had a shorter 3-year OS (23% vs 58%, $p < .001$) and 3-year RFS (26% vs 52%, $p = .006$) in comparison to low *BAALC* expressers (Fig. 1a, b). Similar results were obtained when patients were sub-divided in CN-AML (Fig. 1c, d) and those with other molecular or cytogenetic intermediate-risk abnormalities (Fig. 1e, f). These results confirm the adverse prognostic impact of *BAALC* in CN-AML described previously by the Cancer and Leukemia Group B research group [2–4] and add novel information about prognostic relevance of *BAALC* gene expression levels in de novo AML, by including also those patients with intermediate-risk cytogenetic abnormalities. Multivariate analysis confirmed the prognostic value of *BAALC* in the whole series; the features selected as having an independent prognostic value for either a shorter OS or RFS were age > 65 years ($p < .001$ and $p = .003$), high *BAALC* gene expression ($p < .001$ and $p = .005$), WBC counts $> 50 \times 10^9/L$ ($p = .026$ and $p = .12$), and our recently proposed molecular score [11] ($p = .041$ and $p = .049$, respectively; Table 2). Taken together, these results confirm the prognostic value of *BAALC* gene expression levels in CN-AML. Moreover, we

have also analyzed patients with other chromosomal abnormalities that do not qualify neither for high or low risk categories, describing for the first time that *BAALC* RNA levels show a clinical impact in the whole intermediate-risk cytogenetic AML sub-group.

In summary, we demonstrated that high *BAALC* RNA levels are associated with low CR rate and refractoriness to chemotherapy in intermediate-risk AML patients, resulting in a short survival. This suggests that *BAALC* is a relevant marker in intermediate-risk AML.

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Author's contributions

Santamaría C. and Chillón M. C. carried out all molecular studies and prepared the database for the final analysis. Santamaría C. performed the statistical analysis and prepared the initial version of the paper.

García-Sanz R. helped in the design of the work, reviewed the database, and contributed towards the statistical analysis. He provided the pre-approval of the final version.

Pérez C., Caballero D., Mateos M. V., Ramos F., García de Coca A., Alonso J. M., Giraldo P., Bernal T., Queizán J. A., Rodríguez J. N., and Díaz-Mediavilla J. were the clinicians responsible for the patients and those who ensured the application of protocol, sampling, and the collection of clinical data.

Puig N., Balanzategui A., Sarasquete M. E., and Alcoceba M. participated in the generation of the molecular results.

San Miguel J. F. and González M. initially promoted the study and were responsible for securing financial support. Both were responsible for the group and were the persons responsible for the most important revision of the draft. González M. was the person who approved the final version to be sent to the editor.

References

- Mrozek K, Bloomfield CD (2006) Chromosome aberrations, gene mutations and expression changes, and prognosis in adult acute myeloid leukemia. *Hematology* 2006:169–177
- Tanner SM, Austin JL, Leone G, Rush LJ, Plass C, Heinonen K, Mrozek K, Sill H, Knuutila S, Kolitz JE, Archer KJ, Caligiuri MA, Bloomfield CD, de la Chapelle A (2001) *BAALC*, the human member of a novel mammalian neuroectoderm gene lineage, is implicated in hematopoiesis and acute leukemia. *Proc Natl Acad Sci USA* 98:13901–13906
- Baldus CD, Tanner SM, Ruppert AS, Whitman SP, Archer KJ, Marcucci G, Caligiuri MA, Carroll AJ, Vardiman JW, Powell BL, Allen SL, Moore JO, Larson RA, Kolitz JE, de la Chapelle A, Bloomfield CD (2003) *BAALC* expression predicts clinical outcome of de novo acute myeloid leukemia patients with normal cytogenetics: a Cancer and Leukemia Group B Study. *Blood* 102:1613–1618
- Baldus CD, Thiede C, Soucek S, Bloomfield CD, Thiel E, Ehninger G (2006) *BAALC* expression and *FLT3* internal tandem duplication mutations in acute myeloid leukemia patients with

- normal cytogenetics: prognostic implications. *J Clin Oncol* 24:790–797
5. Langer C, Radmacher MD, Ruppert AS, Whitman SP, Paschka P, Mrozek K, Baldus CD, Vukosavljevic T, Liu CG, Ross ME, Powell BL, de la Chapelle A, Kolitz JE, Larson RA, Marcucci G, Bloomfield CD (2008) 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* 111:5371–5379
 6. Bienz M, Ludwig M, Leibundgut EO, Mueller BU, Ratschiller D, Solenthaler M, Fey MF, Pabst T (2005) Risk assessment in patients with acute myeloid leukemia and a normal karyotype. *Clin Cancer Res* 11:1416–1424
 7. Langer C, Marcucci G, Holland KB, Radmacher MD, Maharry K, Paschka P, Whitman SP, Mrozek K, Baldus CD, Vij R, Powell BL, Carroll AJ, Kolitz JE, Caligiuri MA, Larson RA, Bloomfield CD (2009) Prognostic importance of MN1 transcript levels, and biologic insights from MN1-associated gene and microRNA expression signatures in cytogenetically normal acute myeloid leukemia: a cancer and leukemia group B study. *J Clin Oncol* 27:3198–3204
 8. Marcucci G, Maharry K, Whitman SP, Vukosavljevic T, Paschka P, Langer C, Mrozek K, Baldus CD, Carroll AJ, Powell BL, Kolitz JE, Larson RA, Bloomfield CD (2007) 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* 25:3337–3343
 9. Paschka P, Marcucci G, Ruppert AS, Whitman SP, Mrozek K, Maharry K, Langer C, Baldus CD, Zhao W, Powell BL, Baer MR, Carroll AJ, Caligiuri MA, Kolitz JE, Larson RA, Bloomfield CD (2008) Wilms tumor 1 gene mutations independently predict poor outcome in adults with cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B Study. *J Clin Oncol* 26:4595–4602
 10. Breems DA, van Putten WL, de Greef GE, Van Zelderren-Bhola SL, Gerssen-Schoorl KB, Mellink CH, Nieuwint A, Jotterand M, Hagemeyer A, Beverloo HB, Lowenberg B (2008) Monosomal karyotype in acute myeloid leukemia: a better indicator of poor prognosis than a complex karyotype. *J Clin Oncol* 26:4791–4797
 11. Santamaria CM, Chillon MC, Garcia-Sanz R, Perez C, Caballero MD, Ramos F, de Coca AG, Alonso JM, Giraldo P, Bernal T, Queizan JA, Rodriguez JN, Fernandez-Abellan P, Barez A, Penarrubia MJ, Balanzategui A, Vidriales MB, Sarasquete ME, Alcoceba M, az-Mediavilla J, San Miguel JF, Gonzalez M (2009) Molecular stratification model for prognosis in cytogenetically normal acute myeloid leukemia. *Blood* 114:148–152
 12. Krauter J, Wagner K, Schafer I, Marschalek R, Meyer C, Heil G, Schaich M, Ehninger G, Niederwieser D, Krahl R, Buchner T, Sauerland C, Schlegelberger B, Dohner K, Dohner H, Schlenk RF, Ganser A (2009) Prognostic factors in adult patients up to 60 years old with acute myeloid leukemia and translocations of chromosome band 11q23: individual patient data-based meta-analysis of the German Acute Myeloid Leukemia Intergroup. *J Clin Oncol* 27:3000–3006
 13. Mrozek K, Heinonen K, Lawrence D, Carroll AJ, Koduru PR, Rao KW, Strout MP, Hutchison RE, Moore JO, Mayer RJ, Schiffer CA, Bloomfield CD (1997) Adult patients with de novo acute myeloid leukemia and t(9; 11)(p22; q23) have a superior outcome to patients with other translocations involving band 11q23: a Cancer and Leukemia Group B study. *Blood* 90:4532–4538
 14. Suarez L, Vidriales MB, Moreno MJ, Lopez A, Garcia-Larana J, Perez-Lopez C, Tormo M, Lavilla E, Lopez-Berges MC, de Santiago M, San Miguel JF, Orfao A (2005) Differences in anti-apoptotic and multidrug resistance phenotypes in elderly and young acute myeloid leukemia patients are related to the maturation of blast cells. *Haematologica* 90:54–59
 15. Oriol A, Ribera JM, Esteve J, Guardia R, Brunet S, Bueno J, Pedro C, Llorente A, Tormo M, Besalduch J, Sanchez JM, Batlle M, Vivancos P, Carreras E, Vila JM, Julia A, Sierra J, Montserrat E, Feliu E (2004) Feasibility and results of autologous stem cell transplantation in de novo acute myeloid leukemia in patients over 60 years old. Results of the CETLAM AML-99 protocol. *Haematologica* 89:791–800
 16. Chillon MC, Fernandez C, Garcia-Sanz R, Balanzategui A, Ramos F, Fernandez-Calvo J, Gonzalez M, Miguel JF (2004) FLT3-activating mutations are associated with poor prognostic features in AML at diagnosis but they are not an independent prognostic factor. *Hematol J* 5:239–246
 17. Schnittger S, Schoch C, Kern W, Mecucci C, Tschulik C, Martelli MF, Haferlach T, Hiddemann W, Falini B (2005) Nucleophosmin gene mutations are predictors of favorable prognosis in acute myelogenous leukemia with a normal karyotype. *Blood* 106:3733–3739
 18. Santamaria C, Chillon MC, Garcia-Sanz R, Balanzategui A, Sarasquete ME, Alcoceba M, Ramos F, Bernal T, Queizan JA, Penarrubia MJ, Giraldo P, San Miguel JF, Gonzalez M (2008) The relevance of preferentially expressed antigen of melanoma (PRAME) as a marker of disease activity and prognosis in acute promyelocytic leukemia. *Haematologica* 93:1797–1805
 19. Gabert J, Beillard E, van der Velden V, Bi W, Grimwade D, Pallisgaard N, Barbany G, Cazzaniga G, Cayuela JM, Cave H, Pane F, Aerts JL, De Micheli D, Thirion X, Pradel V, Gonzalez M, Viehmann S, Malec M, Saglio G, van Dongen JJ (2003) Standardization and quality control studies of ‘real-time’ quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia—a Europe Against Cancer program. *Leukemia* 17:2318–2357