Clinical impact of *BAALC* expression in high-risk acute promyelocytic leukemia

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Key Points

- *BAALC* expression is significantly lower in APL compared with other subsets of AML and healthy volunteers.

- *BAALC* overexpression can independently predict shorter DFS in patients with high-risk disease.

Although overexpression of the brain and acute leukemia, cytoplasmic (*BAALC*) gene is associated with primary resistant disease and shorter relapse-free, disease-free, and overall survival in different subsets of acute myeloid leukemia (AML), little is known about its clinical impact in acute promyelocytic leukemia (APL). Using real-time reverse transcriptase polymerase chain reaction, we showed that *BAALC* expression is significantly lower in APL compared with other subsets of AML ($P < .001$). We also demonstrated that *BAALC* overexpression was associated with shorter disease-free survival (DFS) (hazard ratio [HR], 4.43; 95% confidence interval [CI], 1.29-15.2; $P = .018$) in 221 consecutive patients (median age, 35 years; range, 18-82 years) with newly diagnosed APL homogeneously treated with all-*trans* retinoic acid and anthracycline-based chemotherapy. Cox proportional hazard modeling showed that *BAALC* overexpression was independently associated with shorter DFS in the total cohort (HR, 5.26; 95% CI, 1.52-18.2; $P = .009$) and in patients with high-risk disease (ie, those with initial leukocyte counts $>10 \times 10^9$/L) (HR, 5.3; 95% CI, 1.14-24.5; $P = .033$). We conclude that *BAALC* expression could be useful for refining risk stratification in APL, although this needs to be confirmed in independent cohorts.

Introduction

Although clinical trials evaluating outcomes of patients with acute promyelocytic leukemia (APL) treated with all-*trans* retinoic acid (ATRA) combined with anthracyclines have reported overall remission rates of up to 95% and long-term overall survival (OS) rates higher than 80%,[1-6] other studies have demonstrated that the outcomes are less favorable for patients treated outside well-controlled studies or in low- and middle-income countries.[7-9] Excluding the well-known differences between low- and middle-income vs high-income countries, outcomes in APL are variable among studies,[1,5,7,10] and, in our

experience, patients with newly diagnosed APL may present a specific genomic profile important for predicting prognosis.[11-14]

In line with these findings, overexpression of the brain and acute leukemia, cytoplasmic (BAALC) gene, either alone or in association with the aberrant expression of other genes, is frequently associated with poor prognosis in different subsets of acute myeloid leukemia (AML),[15-18] although its functional role in leukemogenesis has not been investigated to the same extent. In contrast, very few studies have investigated the clinical impact of BAALC expression in APL. To date, only 1 German study demonstrated the isolated overexpression of the BAALC gene in APL as an independent prognostic marker for OS in 86 patients treated according to the German AML Cooperative Group (AMLCG) trial, although this independent association was not found for relapse-free survival.[19] Corroborating these findings in a cohort treated according to the International Consortium on Acute Promyelocytic Leukemia (IC-APL) study,[11] we provide evidence that BAALC overexpression is associated with poor clinical outcomes in APL and may independently predict shorter disease-free survival (DFS) in patients with high-risk disease.

Patients and methods

Patients and treatment protocol

A total of 221 consecutive patients with newly diagnosed APL who were enrolled in the IC-APL study were included. Details about the diagnosis, eligibility criteria, and classification of patients are published elsewhere.[11] The treatment protocol was identical to that of the LPA2005 trial reported by the Programa Español de Tratamiento en Hematologia/Dutch-Belgian Hemato-Oncology Cooperative Group (PETHEMA/HOVON) except for the replacement of idarubicin by daunorubicin due to its greater availability and lower cost for the participating centers. Following the tenets of the Declaration of Helsinki, informed written consent was obtained from all patients. The local research ethics board of each participating center approved the study. For comparison purposes, the analysis included total bone marrow (BM) mononuclear cells (BMMCs) from 27 healthy volunteers, along with 53 BM samples from patients with de novo core-binding factor leukemia (CBF leukemia), 51 from patients with cytogenetically normal AML (CN-AML), and 98 from patients with AML not otherwise specified (AML-NOS).

Gene expression profile of BAALC

All samples used for the gene expression analyses were obtained at diagnosis from BM aspirates and were processed using standard techniques. Following total RNA extraction, real-time quantitative polymerase chain reaction (PCR) assays were performed in duplicate using sample-derived complementary DNA (cDNA) on MicroAmp optical 96-well plates using a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA) with the ABL FusionQuant Standard kit as the endogenous control. BAALC gene expression was determined by real-time reverse transcriptase PCR using the TaqMan Gene Expression Assay (Hs00227249_m1; Applied Biosystems) following the manufacturer's instructions. The gene expression of BAALC was calculated relative to a reference cDNA (NB4 cell line, a human APL cell line positive for the PML/RARA fusion gene). Importantly, the same reference cDNA was used as an internal control in all experiments to ensure that the results of different experiments could be comparable. The relative gene expression values for BAALC were quantified using the Δ cycle threshold (Ct)
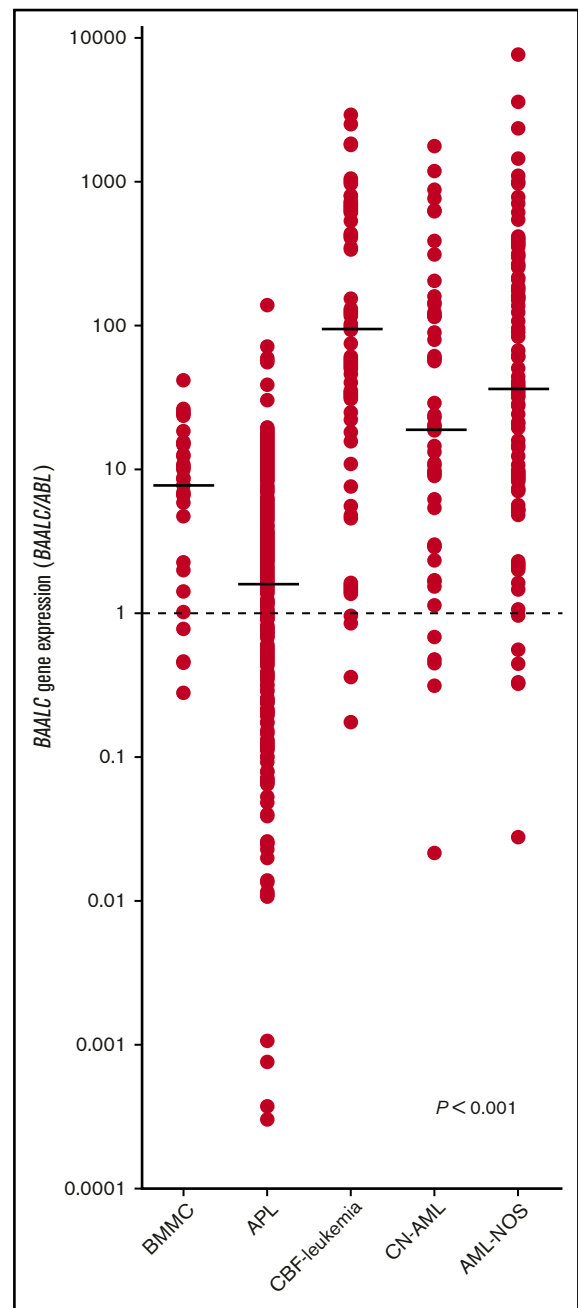

Figure 1. Differential gene expression of *BAALC* in BMMCs, APL, and in different subtypes of AML. The expression of the *BAALC* gene was analyzed by real-time quantitative PCR using the ABL FusionQuant Standard kit as the endogenous control. The horizontal bars represent the median value of *BAALC* expression relative to expression of the *ABL* gene. *BAALC* expression was lower in APL samples as determined by the Kruskal-Wallis test followed by the Dunn posttest.

method, and the results were expressed using $2^{-\Delta\Delta Ct}$, in which $\Delta\Delta Ct = \Delta Ct_{patients} - \Delta Ct_{NB4\ cell\ line}$.

Statistical analysis

The patients' baseline characteristics are reported descriptively. The Fisher exact test or the χ^2 test, as appropriate, was used to compare categorical variables, and the Kruskal-Wallis test was

Table 1. Baseline characteristics

| | All patients | | BAALC expression | | | | |
| | | | Low expression | | High expression | | |
Characteristic	No.	%	No.	%	No.	%	P
Sex							.686
Female	119	53.8	61	55	58	52.3	
Male	102	46.2	49	45	53	47.7	
Age, median (range)	35.3 (18.9, 82.5)		35.2 (19, 82.5)		36.1 (18.9, 81.9)		
ECOG performance status							.88
0	109	55.6	52	55.3	57	55.9	
1	46	23.5	23	24.5	23	22.5	
2	17	8.7	7	7.4	10	9.8	
≥3	24	12.2	12	12.8	12	11.8	
Unknown	25	–	16	–	11	–	
Relapse risk group							.979
Low risk	39	19.4	19	17.3	20	18	
Intermediate risk	101	44.4	50	45.5	51	45.9	
High risk	81	36.3	41	37.3	40	36	
Morphologic subtype							.066
Hypergranular (FAB M3)	203	92.7	98	89.1	105	96.3	
Microgranular (FAB M3 variant)	16	7.3	12	10.9	4	3.7	
Unknown	2	–	–	–	2	–	
PML breakpoint							.379
BCR1	104	58.1	48	54.5	56	61.5	
BCR2	6	3.4	2	2.3	4	4.4	
BCR3	69	38.5	38	43.2	31	34.1	
Unknown	42	–	22	–	20	–	
WBC counts, median (range), $\times 10^9$/L	4.4 (0.8, 134)		5 (1, 134)		3.4 (0.8, 128.5)		.907
Platelet counts, median (range), $\times 10^9$/L	24 (7, 230)		24 (7, 230)		25.5 (8, 110)		.87
Hemoglobin, median (range), g/dL	8.7 (3, 14.9)		8.6 (4.4, 14.9)		8.75 (3.2, 14.1)		.464
Creatinine, median (range), mg/dL	0.8 (0.3, 4.3)		0.8 (0.34, 1.8)		0.8 (0.3, 4.3)		.1
Uric acid, median (range), mg/dL	3.8 (1.1, 10.3)		3.7 (1.7, 9.2)		3.8 (1.1, 10.3)		.545
Fibrinogen, median (range), mg/dL	163 (10, 898)		165.5 (10, 460)		161 (0.5, 898)		.933
Albumin (range), g/dL	4 (2.2, 5.42)		4 (2.2, 5.2)		4 (2.2, 5.4)		.59

Patients were dichotomized into 2 groups according to the median value of *BAALC* expression (low expression, <1.54; high expression, ≥1.54). Details can be found in "Statistical analysis."
–, not taken into account to calculate the frequency; ECOG, Eastern Cooperative Oncology Group; WBC, white blood cell.

used to compare continuous variables. Using survival receiver operating characteristic curve analysis[20] and the C index,[21] we dichotomized patients into 2 groups according to the median value of *BAALC* expression (low expression, <1.54; high expression, ≥1.54). The clinical end points were described previously.[14]

Univariable and multivariable proportional hazards regression analysis was performed for potential prognostic factors for OS and DFS. The potential prognostic factors that were examined in multivariable regression analysis were as follows: age at diagnosis, sex, white blood cell (WBC) counts, platelet counts, hemoglobin levels, coagulopathy, French–American–British (FAB) classification, *PML* breakpoint, creatinine, albumin, uric acid, and fibrinogen. The proportional hazards assumption was tested for each variable of interest. The linearity assumption for all continuous variables was examined using restricted cubic spline estimates of the relationship between the continuous variable and the log relative hazard/risk. All P values were 2-sided with a significance level of .05. All calculations were performed using Stata statistical data analysis software version 14.1 (StataCorp, College Station, TX), Statistical Package for the Social Sciences (SPSS) 19.0, and R 3.3.2 (The CRAN project, www.r-project.org) software.

Results

Figure 1 shows the relative quantification of *BAALC* expression in BMMCs from healthy volunteers and in samples from patients with newly diagnosed APL, de novo CBF leukemia, CN-AML, and AML-NOS. *BAALC* expression was significantly lower in APL in comparison with BMMCs from healthy volunteers and to other AML groups (P < .001). Supplemental Table 1 summarizes the central tendency and dispersion measurements of the relative

Table 2. Summary of outcomes of patients with APL according to *BAALC* expression

	No. (%)	CR %	OS		DFS		CIR	
			No.	5-y % (95% CI)	No.	5-y % (95% CI)	No.	5-y % (95% CI)
All patients	221 (100)							
Low *BAALC* expression		89	110	82 (70, 90)	89	95 (86, 98)	87	5 (1, 10)
High *BAALC* expression		79	111	73 (63, 81)	96	78 (66, 86)	91	21 (11, 30)
P		.067		.077		.009*		.01*
Low-risk patients	39 (17)							
Low *BAALC* expression		87	19	93 (63, 99)	14	100	13	0
High *BAALC* expression		82	20	77 (54, 90)	19	91 (54, 99)	18	7 (0, 21)
P		1.00		.225		.48		.5
Intermediate-risk patients	101 (46)							
Low *BAALC* expression		95	51	97 (85, 99)	44	96 (79, 99)	43	3 (0, 9)
High *BAALC* expression		84	50	90 (79, 96)	46	87 (71, 94)	45	11 (2, 23)
P		.062		.143		.158		.15
High-risk patients	81 (37)							
Low *BAALC* expression		81	40	59 (35, 76)	31	90 (66, 97)	31	9 (0, 20)
High *BAALC* expression		72	41	47 (25, 66)	31	53 (28, 72)	28	41 (11, 62)
P		.432		.237		.019*		.019*

Summary of outcomes of patients with APL according to *BAALC* expression (in total cohort, and according to PETHEMA/GIMEMA criteria for relapse).[22]
CI, confidence interval; CIR, cumulative incidence of relapse; CR, complete remission.
*Statistically significant differences ($P < .05$).

quantification of *BAALC* expression in all of the enrolled subjects. Next, we restricted our analysis to patients with APL. The baseline features were similar between patients with low and high *BAALC* expression levels (Table 1).

Details for patient outcomes are summarized in Table 2. With a median follow-up of 32 months (range, 1-101 months), the estimated 5-year OS rate was 79% (95% confidence interval [CI], 72-84). Overall, 185 of 221 patients (84%) achieved complete hematological remission (CR). Of 36 patients (16%) who failed to achieve CR, 26 (72%) experienced early mortality (death within 30 days after diagnosis). The main causes of death during induction were hemorrhage (14 patients; 54%), followed by infection (10 patients; 38%), central nervous system thrombosis (1 patient; 4%), and differentiation syndrome (1 patient; 4%). *BAALC* expression had no impact on CR achievement ($P = .067$). In contrast, the early mortality rate was significantly higher in patients with high *BAALC* expression (17% vs 8%; $P = .046$). Although patients with high *BAALC* expression had a lower 5-year OS rate (73%; 95% CI, 63-81) than patients with low *BAALC* expression (82%; 95% CI, 70-90), this difference did not reach statistical significance ($P = .077$; Figure 2A).

Of the 185 patients who achieved CR, 19 patients (10%) relapsed at a median time of 40 days (range, 1-389 days). The estimated 5-year DFS rate was 87% (95% CI, 80-92), and the cumulative incidence of relapse (CIR) rate was 14% (95% CI, 8-20). Patients with high *BAALC* expression had a lower 5-year DFS rate (78%; 95% CI, 66-86) than those with low *BAALC* expression (95%; 95% CI, 86-98) ($P = .009$) (Figure 2B). Cox proportional hazards modeling showed that high expression of *BAALC* was independently associated with shorter DFS (hazard ratio [HR], 5.26; 95% CI, 1.52-18.2) ($P = .009$) (Table 3). In addition, the CIR rate was

higher in patients with high *BAALC* expression (21%; 95% CI, 11-30) than in patients with low *BAALC* expression (5%; 95% CI, 0-10) ($P = .01$) (Figure 2C).

Considering the importance of the PETHEMA/Gruppo Italiano Malattie EMatologiche dell'Adulto (GIMEMA) criteria for predicting relapse,[22] we opted to evaluate the impact of *BAALC* expression separately in patients with low-, intermediate-, and high-risk disease. For low-risk patients (39 patients; 17%), *BAALC* expression had no impact on CR ($P = 1.00$), OS ($P = .225$), DFS ($P = .48$), or CIR ($P = .5$). Similar results were observed for intermediate-risk patients (101 patients; 46%; CR, $P = .062$; OS, $P = .143$; DFS, $P = .158$; CIR, $P = .15$). In contrast, high-risk patients (81 patients; 37%) with high *BAALC* expression had a lower 5-year DFS rate (53%; 95% CI, 28-72) than those with low *BAALC* expression (90%; 95% CI, 66-97) ($P = .019$; Figure 2D). *BAALC* expression retained its significant prognostic value in multivariable analysis (HR, 5.3; 95% CI, 1.14-24.5; $P = .033$) (Table 3). Also, patients with high *BAALC* expression had a higher CIR rate (41%; 95% CI, 11-62) than patients with low *BAALC* expression (9%; 95% CI, 0-20) ($P = .019$). The CR ($P = .432$) and OS ($P = .237$) rates were similar in patients with low vs high *BAALC* expression (Table 2).

Discussion

Although the overexpression of *BAALC* is widely accepted as a predictor of inferior outcomes in AML,[15,17,18] and, more recently, has been used as a marker to detect minimal residual disease in CN-AML,[23] the biological mechanisms underlying these findings await clarification. Eisfeld et al showed that *BAALC* overexpression is closely related to a heritable polymorphism in the promoter region of the gene.[24] This polymorphism, rs62527607, is thought to create a high-affinity binding site for the activating RUNX1 transcription
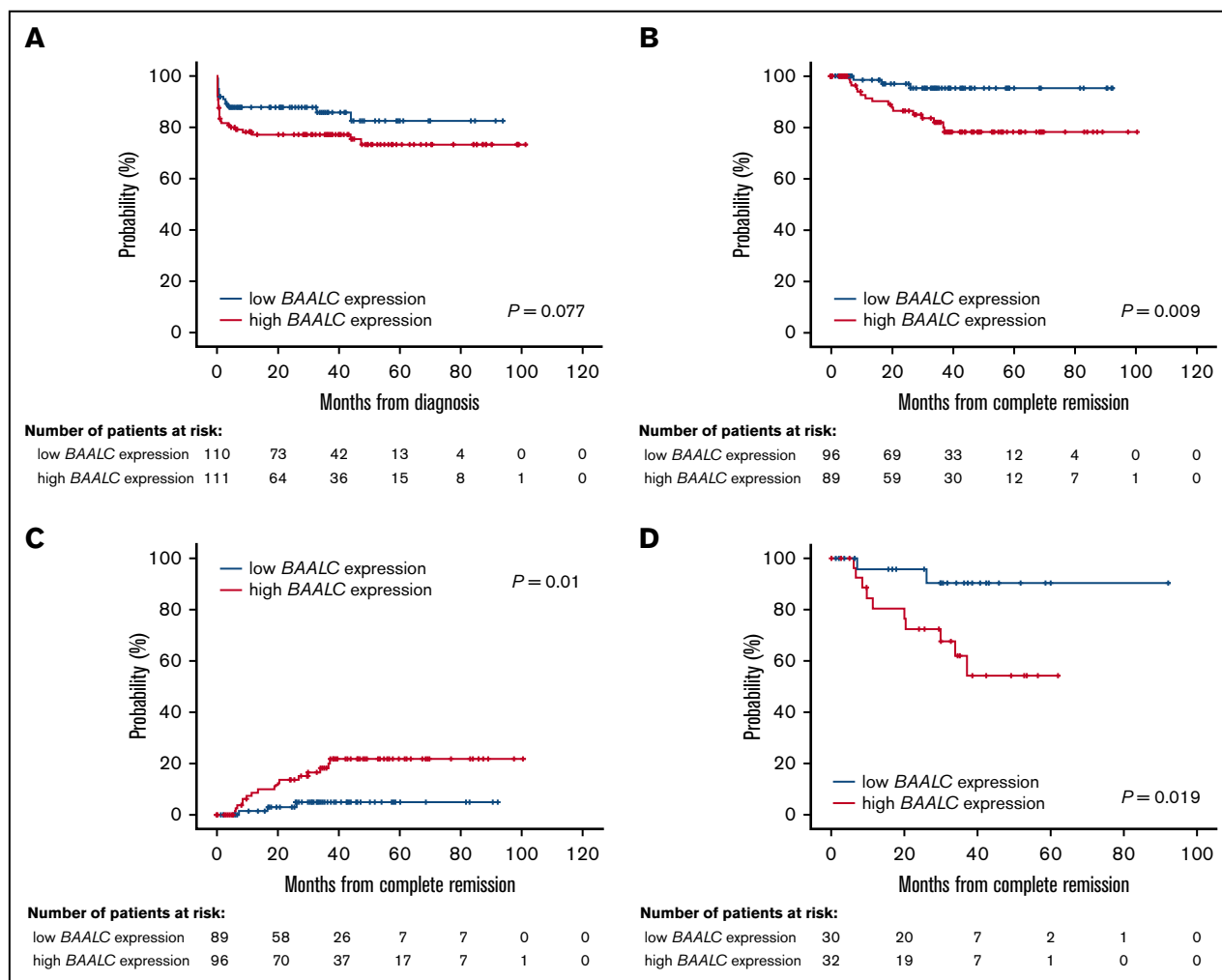
Figure 2. Patient survival. The probability of OS (A), DFS (B), and cumulative incidence of relapse (C) in patients with APL according to *BAALC* expression (entire cohort). DFS (D) in patients with a high risk of relapse according to *BAALC* expression.

factor, which in turn leads to high constitutive *BAALC* expression.[24] Of note, the methylation status of the promoter regions of the *BAALC* gene in primary APL blasts at diagnosis and relapse seems to be unrelated to its overexpression (V.M.d.D.W., unpublished data). In terms of its biological significance, *BAALC* expression may reflect functional differences in normal hematopoiesis and leukemogenesis. Although constitutive activation of *BAALC* does not appear to alter the proliferation/survival rates of healthy hematopoietic stem or progenitor cells,[25] its induced expression promotes cell-cycle progression of leukemia cell lines by sustaining extracellular signal-regulated kinase activity.[26] This is mediated by an interaction with the scaffold protein MEK kinase-1, which inhibits the interaction between extracellular signal-regulated kinase and MAPK phosphatase 3 (MKP3/DUSP6). In addition, there is evidence that *BAALC* can block myeloid differentiation and thereby promote leukemogenesis when combined with the self-renewal promoting oncogene *HOXA9*.[25] The functional role of *BAALC* in the induction of an APL-leukemic phenotype remains unknown.

Here, we demonstrated that *BAALC* expression is significantly lower in APL than in other subsets of AML. We also described the prognostic importance of *BAALC* expression in a cohort of patients

homogeneously treated with ATRA and anthracycline-based chemotherapy and addressed these findings in a large (at least in countries of Latin ancestry[7]) and clinically heterogeneous subset of APL, such as patients with high-risk disease. Most importantly, *BAALC* overexpression could be used to identify patients with poor prognosis regardless of leukocyte count and regardless of the treatment regimens we and others[19] have conducted. Although our results are in accordance with those reported by Nolte et al,[19] in our cohort, *BAALC* overexpression was independently associated with lower DFS in high-risk APL, which was not found by Nolte et al. It is important to emphasize that the studies have some methodological differences. Specifically, the Nolte et al study had longer follow-up, a lower frequency of patients with high-risk disease, a higher median patient age, and a higher frequency of the microgranular subtype. However, the most important difference between the AMLCG trial and IC-APL studies was the adopted treatment protocol. The AMLCG treatment protocol included a more intensive chemotherapy regimen with higher doses of cytarabine compared with the IC-APL study; this may lead to differences in prognostic markers.

Several findings over the last 2 decades show that highly effective treatment strategies can overcome characteristics that were

Table 3. Multivariable Cox model for OS and DFS

	OS				DFS			
	HR	95% CI		*P*	HR	95% CI		*P*
All patients								
Age: continuous variable	1.01	0.99	1.03	.187	0.98	0.95	1.02	.578
Sex: male vs female	0.93	0.5	1.73	.824	1.07	0.4	2.5	.987
WBC, ×10⁹/L: continuous variable	2.3	1.7	5.03	<.001	3.44	1.54	7.66	.002
BAALC expression: high vs low	1.69	0.88	3.24	.11	5.26	1.52	18.2	.009
Low-risk patients								
Age: continuous variable	0.95	0.88	1.03	.236	—*	—*		—*
Sex: male vs female	1.08	0.19	6.05	.928	—*	—*		—*
BAALC expression: high vs low	3.51	0.4	30.7	.256	—*	—*		—*
Intermediate-risk patients								
Age: continuous variable	0.99	0.93	1.05	.845	0.96	0.9	1.02	.248
Sex: male vs female	1.29	0.26	6.42	.752	2.24	0.41	12.2	.35
BAALC expression: high vs low	4.29	0.49	36.8	.184	4.38	0.51	37.6	.177
High-risk patients								
Age: continuous variable	1.03	1.01	1.05	.025	1.09	0.96	1.05	.684
Sex: male vs female	1.06	0.51	2.2	.87	0.87	0.26	2.83	.819
BAALC expression: high vs low	1.26	0.6	2.64	.53	5.3	1.14	24.5	.033

*Multivariable Cox modeling was hampered by the small number of events (relapses) in the low-risk group; therefore, no statistical analysis was performed.

previously associated with an adverse outcome, thereby making those prognostic markers less relevant.[27-29] This may also be true in patients with APL who are treated with the state-of-the-art combination of ATRA and arsenic trioxide (ATO). Recent clinical trials show that the ATRA-ATO combination is less toxic and more effective than ATRA plus chemotherapy. Consequently, patients with low- or intermediate-risk APL[30,31] and with high-risk APL show higher long-term and leukemia-free survival with ATRA-ATO.[32] In addition, the advantages of ATRA-ATO over ATRA chemotherapy increase over time, showing significantly greater and more sustained antileukemic efficacy.[30,32] It remains unknown whether prognostic markers that were previously related to inferior outcomes in APL will retain their clinical relevance in the ATRA-ATO era or whether they will be needed considering the encouraging results with this regimen. For example, Cicconi et al demonstrated that *fms*-related tyrosine kinase 3–internal tandem duplication mutations had no impact on either event-free survival or CIR in low-risk and intermediate-risk patients receiving ATRA and ATO regimens as first-line therapy. These results suggested that ATRA-ATO treatment abrogates the negative prognostic impact of *fms*-related tyrosine kinase 3–internal tandem duplication mutations.[33] In agreement, Powell et al showed that the addition of ATO on consolidation therapy significantly improved the prognosis (event-free, DFS. and OS) of adults with newly diagnosed APL and negated all previously identified prognostic factors, including initial leukocyte count.[34] Although further studies are needed to confirm all of these findings, it is possible that the prognostic influence of *BAALC* expression and other important markers for predicting outcomes in APL[35] may be different or even irrelevant for patients treated with ATRA-ATO. It is important to note that even in light of these findings, most of the patients in countries in Latin America still rely on the publicly funded health care system and arsenic treatment is still not available in most of them. In particular, in Brazil, the use of arsenic for APL treatment is not authorized by health surveillance agencies, most likely because of its cost. Apparently, there is still a long way to go before the ATRA-ATO combination will be implemented as the therapy of choice for APL in these countries.

Although initial leukocyte and platelet counts are currently used as markers to predict relapse in APL,[22] these parameters can change significantly in a short period of time. Based on our experience[12-14] and on the experiences of others,[36-38] the use of genetic data could improve risk stratification in APL. This could help clinicians make decisions about consolidation strategies and also provide the possibility of early intervention if molecular relapse occurs. However, the findings of all of these studies should be interpreted with caution because most of them, including our own, lack validation in independent cohorts and are limited by their sample sizes. Furthermore, we cannot rule out the possibility that methodological differences between studies could lead to different results and conclusions. More importantly, it is very likely that all of the molecular markers described so far will not remain clinically valid if the ATRA-ATO regimen becomes the most used protocol worldwide. Meanwhile, alternative strategies for improving prognostication in APL should be tested, particularly in countries in which the clinical use of arsenic has not yet been authorized.

Acknowledgments

Authorship

Contribution: A.R.L.-A. conceived and designed the study, performed experiments, analyzed and interpreted data, performed the statistical analyses, and drafted the article; D.A.P.-M. and L.C.K. updated the clinical data, performed experiments, and collected data; P.L.F.-N., V.M.d.D.W., and J.L.C.-S. performed experiments and collected data; R.A.M.M., R.B., K.P., R.P., C.S.C., E.M.F., and M.d.L.C. provided samples and updated the clinical data; S.L.S., M.S.T., R.C.R., D.G., A.G., B.L., F.L.-C., M.A.S., N.B., and E.M.R. designed the treatment protocol; M.A.S. performed and reviewed the statistical analyses; E.M.R. approved the final version of the manuscript; and all authors reviewed and approved the manuscript.

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