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MYELOID NEOPLASIA

Combining gene mutation with gene expression analysis improves outcomes prediction in acute promyelocytic leukemia

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ABSTRACT

Combining the analysis of mutations with aberrant expression of genes previously related to poorer prognosis in both acute promyelocytic leukemia (APL) and acute myeloid leukemia, we proposed an integrative score in APL (ISAPL) and demonstrated its relationship with clinical outcomes of patients with APL treated with all-trans retinoic acid (ATRA) in combination with anthracycline-based chemotherapy. Based on *FLT3*-ITD mutational status, ΔNp73/TAp73 expression ratio, *ID1 BAALC ERG* and *KMT2E* gene expression levels, ISAPL was fully modeled in 159 patients (median ISAPL score: 3, range: 0-10). Early mortality (P<0.001), complete remission (P=0.004), overall survival (P<0.001), cumulative incidence of relapse (P=0.028), disease-free survival (P=0.03), and event-free survival (P<0.001) rates were significantly different between patients assigned to the low- and high-risk groups. In summary, ISAPL modeling identified two distinct groups of patients, with significant differences in remission achievement, relapse, and survival, therefore, may improve consolidation treatment stratification in APL patients treated with ATRA and anthracycline-based chemotherapy.

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INTRODUCTION

In the context of all-trans retinoic acid (ATRA) and anthracycline-based chemotherapy, heterogeneity of clinical outcomes of patients with acute promyelocytic leukemia (APL) may be higher than expected, mainly outside well-controlled clinical trials.[1–3] Although well-succeed initiatives, such as the International Consortium on Acute Promyelocytic Leukemia (IC-APL) study, has significantly improved the treatment outcomes of patients who live in low- and middle-income countries (LMIC),[4] these results are still inferior to those reported by high-income countries (HIC),[5] reinforcing the idea that the prognosis of APL is not as favorable as is frequently stated.

Based on the results from clinical trials using arsenic trioxide associated with ATRA and minimal chemotherapy for those patients with high risk disease, it is conceivable that many factors associated with unfavorable prognosis as well as differences between the results of treatment in LMIC and HIC would be reduced.[6–8] However, most patients who live in LMIC does not benefit from these recent improvements, mainly because this compound is still not available in public healthcare programs. Therefore, at least for a near future, alternative strategies for predicting outcomes in patients treated with ATRA and chemotherapy should be tested. Here, we combined recurrent mutations with aberrant expression of genes previously associated with poor prognosis in both APL[9–16] and acute myeloid leukemia (AML),[17–21] and proposed an integrative score in APL (ISAPL) for outcomes prediction.

DESIGN and METHODS

Patients

Between October 2006 and June 2015, diagnostic bone marrow samples from 183 adult patients with APL who were enrolled in the IC-APL study were analyzed. Details about the diagnosis, eligibility criteria and treatment protocol are published elsewhere.[4] Written informed consent was obtained from all patients, following the Declaration of Helsinki recommendations. The local Research Ethics Board of each participating center approved the study.

DNA extraction and screening for FLT3-ITD mutations

Genomic DNA was extracted using the Puregene kit (Gentra System) according to the manufacturer's protocol. Screening for the *FLT3*-ITD mutations was performed by PCR according to the method of Kiyoi *et al*,[22] followed by electrophoresis on 3% agarose gel stained with ethidium bromide. Internal and external validations for *FLT3*-ITD mutations screening were described elsewhere.[11]

Gene expression profile

Real-time quantitative polymerase chain reactions assays using patient-derived cDNA were accomplished in duplicate on MicroAmp optical 96-well plates using a 7500 Real-Time PCR System (Applied BioSystems). Transcript levels of *TP73* isoforms (TAp73, Assay ID: Hs00232088_m1; and ΔNp73 Assay ID: Hs01065727_m1, Applied BioSystems), *KMT2E* (Assay ID: Hs00218773_m1, Applied BioSystems) and *BAALC* genes (Assay ID: Hs00227249_m1, Applied BioSystems) were determined as previously described.[9,10,15] Expression levels of *ERG* (Assay ID: Hs01554635_m1, Applied BioSystems), *ID1* (Assay ID: Hs00357821_g1, Applied BioSystems), *PIM2* (Assay ID: Hs00179139_m1, Applied BioSystems), *PRAME* (Assay ID: Hs01022301_m1, Applied BioSystems), and *WT1*

genes (Assay ID: Hs01103751_m1, Applied BioSystems) were determined using the TaqMan Gene Expression, following the manufacturer's instructions. The comparative cycle threshold (Ct) method was used to determine the relative expression levels of *ERG*, *ID1*, *PIM2, PRAME*, and *WT1* genes by using the *ABL* FusionQuant Standard Kit as endogenous control (Ipsogen). The gene expression profile was calculated relative to a reference cDNA (NB4 cell line) and results were expressed as $2^{-\Delta\Delta Ct}$. Details can be found elsewhere.[9]

Dichotomization strategy and samples categorization

Patients presenting with *FLT3*-ITD mutations were designated mutated, while patients without *FLT3*-ITD mutations were defined non-mutated. For continuous variable, we used two different strategies to define optimal cutoffs: first, we divided the total cohort into quartiles (Q) according to expression levels of each gene. Based on the survival curves (using overall survival as a primary parameter), quartiles with similar event probabilities were grouped. Next, we confirmed these findings using survival receiver operating characteristic (ROC) curve analysis[23] and the C index.[24] Whether no cutoff could be evidenced by these two strategies, patients with expression values higher than the median were classified as having high expression. According to these criteria, patients were dichotomized at the median value of *PIM2*, and *PRAME* expression, lowest 25% expression of *ERG* and *WT1* genes (i.e., those assigned to the first quartile, Q1) and highest 75% expression of *ID1* gene (i.e., those assigned to the fourth quartile, Q4). The gene expression profile and dichotomization strategies for ΔNp73/TAp73 expression ratio, *KMT2E,* and *BAALC* gene were previously described.[9,10,15]

Statistical analysis and clinical endpoints

Fisher's exact test or Chi-square test, as appropriate, was used to compare categorical variables. Kruskal-Wallis test was used to compare continuous variables. Overall survival (OS), disease-free survival (DFS) and event-free survival (EFS) were estimated using the Kaplan–Meier method. OS was defined as the time from diagnosis to death from any cause; those alive or lost to follow-up were censored at the date last known alive. Early mortality was defined as death occurring within 30 days from diagnosis. For patients who achieved CR, DFS was defined as the time from CR achievement to the first adverse event: relapse, development of secondary malignancy, or death from any cause, whichever occurred first. EFS was defined as the time from the initiation of induction therapy to disease relapse, development of secondary malignancy, or death from any cause, whichever occurred first. Patients who were alive without disease relapse or secondary malignancy were censored at the time they were last seen alive and disease-free. The log-rank test was used for comparisons of Kaplan–Meier curves. Cumulative incidence curves for non-relapse death and relapse with or without death were constructed to reflect time to relapse and time to non-relapse death as competing risks. Time to relapse and time to non-relapse death were measured from the date of CR.

Univariable and multivariable logistic regression analyses were performed in order to identify prognostic factors for CR. Univariable and multivariable proportional hazards regression analysis was performed for potential prognostic factors for OS, DFS, and EFS. Potential prognostic factors examined and included in multivariable regression analysis were age at diagnosis (analyzed as a continuous variable), and initial leukocyte counts (analyzed as a continuous variable).

Proportional hazards assumption for each continuous variable of interest was tested. Linearity assumption for all continuous variables was examined in logistic and proportional hazards models using restricted cubic spline estimates of the relationship between the continuous variable and log relative hazard/risk. All *P*-values were two-sided with a significance level of 0.05. All calculations were performed using Stata Statistic/Data Analysis version 12 (Stata Corporation), and R 3.3.2 (The CRAN project, www.r-project.org) software.

Assignment of weights to prognostic markers

Integer weights for the risk score were derived from Cox proportional hazard model, identical to that reported by Damm et al.,[25] using OS as endpoint and including *P*-values lower than 0.05 in the model. Variables considered for the model inclusion were the following: *FLT3*-ITD status, gene expression profile of ΔNp73/TAp73 ratio and transcript levels of *KMT2E*, *BAALC*, *ID1,* and *ERG* genes. Other candidates, such as *PIM2, WT1*, *PRAME,* and *IDH1*, were not associated with lower OS and not included in the score. Hazard ratios (HR) for OS were calculated for each variable separately. The HR was converted to integer weights according to the following: variables with HR ≤ 1 were excluded from analyses; variables with HR > 1 and ≤ 1.5 were assigned a weight of 1; variables with HR > 1.5 and ≤ 2.5 were assigned a weight of 2; variables with HR > 2.5 were assigned a weight of 3. The final score was the sum of these integer weights.

RESULTS

ISAPL modeling in APL

Complete data for ISAPL modeling was available for 159 of 183 (87%) patients. The remaining 24 patients (13%) were not included in the score because biological material (DNA and/or RNA) was not available at diagnosis or due to lack of one or more genetic markers needed to compose the ISAPL in full. To test whether patients not included in the ISAPL model were missing at random, the OS was evaluated for patients with and without ISAPL data. Estimated 5-year OS rate did not differ between patients included (82%, 95% confidence interval, CI: 72-88%) and not included (90%, 95% CI: 78-95%) in the score (*P*=0.509). Univariable Cox proportional hazard analysis revealed that *FLT3*-ITD mutational status (hazard ratio, HR: 2.72, 95% confidence interval, CI: 1.2-6.22; *P*=0.018), high ΔNp73/TAp73 expression ratio (HR: 4.43, 95% CI: 1.83-10.7; *P*=0.001), high expression of *ID1* (HR: 3.41, 95% CI: 1.42-8.22; *P*=0.006), and *BAALC* genes (HR: 2.68, 95% CI: 1.04-6.92; *P*=0.041), and low expression of *ERG* (HR: 2.65, 95% CI: 1.15-6.42; *P*=0.03) and *KMT2E* genes (HR: 3.26, 95% CI: 1.18-8.99; *P*=0.022) were associated with lower OS, and, therefore, were used to generate the ISAPL (Table 1).

Clinical and laboratory features

According to the median value of ISAPL modeling (median value: 3, range: 0-10), we dichotomized patients into two groups (i.e., low-risk, < 3; high-risk, ≥ 3). Descriptive analyses were performed for patient baseline features (Table 2). The median age was 35 years (range: 18-82 years) with 82 males (44%). According to PETHEMA/GIMEMA criteria for predicting relapse,[26] 31% and 50% of patients assigned to the low- and high-risk groups were deemed high-risk patients, respectively (*P*=0.037).

Induction outcome

Patient follow-up was last updated in September 2018. Of the 183 subjects included in the study, 24 patients were lost to follow-up prior to the assessment of remission status and thus were not counted in the induction outcome analysis. Overall, 131/159 (82%) patients achieved complete hematological remission (CR). Of 28 patients (18%) who failed to achieve CR, 21 (75%) experienced early mortality (i.e., death within 30 days after diagnosis). The main causes of death during induction were hemorrhage (11 patients, 52%), followed by infection (nine patients, 43%) and central nervous system thrombosis (one patient, 5%). Early mortality was significantly higher in patients with high-risk (23%) than patients with low-risk (4%) (P<0.001). CR rates according to the ISAPL modeling were 91% and 73% for low- and high-risk, respectively (P=0.004). In univariate logistic regression analysis, ISAPL modeling was significantly associated with CR (odds ratio, OR: 0.26, 95% CI: 0.17-0.66; P=0.005). These results were consistent with multivariable analysis (OR: 0.23, 95% CI: 0.1-0.63; P=0.004), considering age, and leukocyte counts as confounders (Table 3).

With a median follow-up time among survival of 32 months (range: 1-101 months), the estimated 5-year OS rate was 78% (95% CI: 71–82%). Patients assigned to the high-risk group exhibited significantly lower 5-year OS rate (55%, 95% CI: 40-68%) than patients assigned to the low-risk group (91%, 95% CI: 81-95%) (P<0.001; Figure 1A). Cox proportional hazards modeling showed that ISAPL modeling was independently associated with poor OS (HR: 5.77, 95% CI: 2.33-8.27; P<0.001) (Table 3).

Post-remission outcomes

Out of the 82 patients who achieved CR, nine patients (11%) relapsed at a median time of 43 days (range: 23-389 days). Considering non-relapse death as a competing cause of failure, the 5-year cumulative incidence of relapse (CIR) rate was 12% (95% CI: 7-17%). CIR rates for patients with low- and high-risk were 9% (95% CI: 2-17%) and 25% (95% CI: 11-39%) respectively (*P*=0.028; Figure 1B). The estimated 5-year DFS and EFS rates were 87% (95% CI: 80–92%), and 70% (95% CI: 63-76%), respectively. Patients with high ISAPL score had a significantly lower DFS rate (71%, 95% CI: 53-84%) in comparison to patients with low score (90%, 95% CI: 80-95%) (*P*=0.03; Figure 1C). This result was consistent with the multivariable proportional hazards analysis (HR: 2.25, 95% CI: 1.8-6.29; *P*=0.012) (Table 3). In agreement, EFS rate was significantly lower in high-risk patients (45%, 95% CI: 31-58%) than those with low-risk (83%, 95% CI: 72-90%) (*P*<0.001; Figure 1D). Accordingly, ISAPL modeling was associated with shorter EFS in an independent manner (HR: 3.97, 95% CI: 1.98-7.97; *P*<0.001) (Table 3).

DISCUSSION

We and others have previously demonstrated that a relatively large set of genes are aberrantly expressed or frequently mutated in APL. Most important, these genetic findings may be prognostically relevant in a clinical setting in which ATRA and anthracycline-based chemotherapy constitute the basis for induction trearapy.[9–11,15,27–30] Here, we hypothesized that if these molecular prognostic markers were pooled together into a single prognostic risk score, the resulting information could be more accurate than focus on a single molecular marker at a time. Such approach has been already demonstrated in both AML non-APL[25,31–33]

and myelodysplastic syndromes,[34] although score systems for prognosis prediction in APL have not been explored in the same extent. To the best of our knowledge, the first study to propose a molecular risk score in APL was reported by Hecht et al.. Although the sample size was limited (79 patients), the authors demonstrated that *BAALC*, *ERG* and *WT1* expression levels integrated into a score could be a promising approach to guide monitoring of patients with APL treated with ATRA and high doses of cytarabine.[35,36] Here, we and extended this panel and demonstrated that gene mutations and aberrant gene expression combined could be a useful tool to robustly improve outcomes prediction in patients with APL, at least for those treated with ATRA in combination with chemotherapy. Our ISAPL modeling has resulted in the separation of two distinct groups of patients, with significant differences for remission achievement, relapse and survival.

Despite the promising results, other issues should be taken into consideration for molecular monitoring purposes in APL, including validation data in independent cohorts, the establishment of universal controls, and cut-off values that uniformly defined in studies. Furthermore, considering time from diagnosis to treatment initiation as one of the most important steps in attaining success in induction therapy in acute leukemias, one may argue if the ISAPL modeling or others schemes for APL risk stratification[35] could be available to the medical team soon after diagnosis. In our experience, with proper infrastructure and skilled labor, a reference laboratory would be able to conclude all genetic markers for ISPAL modeling within 24-48 hours. Moreover, the progressive decreasing in sequencing costs and the relative facility to obtain high quality of gene quantification in a high number of samples suggest that, in a near future, such strategy will become more accessible and could be incorporated into the clinical

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practice, even in countries with socioeconomic heterogeneity and limited resources, as Brazil.

Here, we included nine genetic markers previously associated with prognosis in both APL and AML to compose the ISAPL modeling. Part of these candidates was functionally evaluated by us[37] and others[38–40] in order to understand the biological significance described in the clinical setting. Previously, we demonstrated that ΔNp73 exerted an important role in cell survival, providing resistance to drug-induced apoptosis.[41] Following the same experimental strategy, several studies have demonstrated that *FLT3*-ITD mutations, overexpression of *BAALC*, *PIM2* in the cooperation in the induction of a leukemic phenotype. In an APL context, unpublished data from our group strongly suggest that the overexpression of *KMT2E*, *BAALC*, and *ΔNp73* in both *in vitro* (using NB4/NB4-R2 cell lines as target cells) and *in vivo* (through murine bone marrow cells transplantation model from hCG-PML/RARA transgenic mice) are able to modulate granulocytic differentiation pathways and directly act in the responsiveness of APL leukemic cells to ATRA therapy. Moreover, standardized assays are already available for prognostication of patients with intermediate cytogenetic risk acute myeloid leukemia are current using some of these genetic markers in the clinical practice.[42]

We acknowledge that our modeling or any other prognostic risk score in APL could become clinically irrelevant if the frontline ATO-ATRA combination is as effective as recent clinical trials have been demonstrating.[6–8] If this efficacy is proven, ATO-ATRA treatment may overcome diagnostic characteristics previously associated with adverse outcomes in APL,[43] with great benefits for patients. Nevertheless, as we mentioned in previous studies, the most important reasons for

ATRA-ATO combination does not constitute the therapy of choice for patients with APL in Latin America are due to its low availability in most reference centers and higher cost for the public healthcare system.[15] Since ATRA plus chemotherapy still constitutes the basis for APL treatment in most LMIC and, apparently, this scenario may endure for some years to come, we believe that our scheme for risk stratification represents a viable alternative for identifying patients who need a closer follow-up.

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AUTHOR CONTRIBUTIONS

A.R.L.A. conceived and designed the study, performed experiments, analyzed and interpreted data, performed the statistical analyses, and drafted the article. L.C.K. updated the clinical data, performed experiments, collected data, and reviewed the paper. P.L.F-N, V.M.D-W, J.L.C-S. performed experiments, collected data, and reviewed the paper. R.A.M., R.B., K.P., R.P., C.S.C., E.M.F., and M.L.C., provided the samples, updated the clinical data, and reviewed the paper. S.L.S., M.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 and reviewed the paper. M.A.S. performed and reviewed the statistical analyses. E.M.R. gave final approval of the submitted version.

DISCLOSURE of CONFLICTS of INTEREST

The authors have no competing financial interests.

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Table 1. Variables used for the determination of the integrated score in APL (ISAPL).

Variables analyzed	Dichotomization strategy	Learning set	
		Cox proportional hazard modeling	Integer weight[1]
		HR (95% CI); *P*-value	
FLT3-ITD status: mutated *vs* non-mutated	Not applicable	2.72 (1.2 to 6.22); 0.018	3
KMT2E gene expression: low *vs* high	Q1-Q2 *vs* Q3-Q4	3.26(1.18 to 8.99); 0.022	2
BAALC gene expression: high *vs* low	Q3-Q4 *vs* Q1-Q2	2.68 (1.04 to 6.92); 0.041	2
ΔNp73/TAp73 ratio: high *vs* low	Q4 *vs* Q1-Q3	4.43 (1.83 to 10.7); 0.001	3
ID1 gene expression: high *vs* low	Q4 *vs* Q1-Q3	3.41 (1.42 to 8.22); 0.006	2
ERG gene expression: low *vs* high	Q1 *vs* Q2-Q4	2.65 (1.15 to 6.42); 0.03	3
WT1 gene expression: low *vs* high	Q1 vs Q2-Q4	6.78 (0.9 to 50.7); 0.061	-
PRAME gene expression: high *vs* low	Q3-Q4 *vs* Q1-Q2	1.02 (0.42 to 2.45); 0.96	-
PIM2 gene expression: high *vs* low	Q3-Q4 *vs* Q1-Q2	1.23 (0.5 to 2.96); 0.644	-

1: Integer weights for the risk score were derived from Cox proportional hazard model, using overall survival as endpoint.

Table 2. Baseline characteristics.

Characteristics	All patients			ISPAL modeling						*P*-value[1]
				Low-risk			High-risk			
	No.	%	Median (range)	No.	%	Median (range)	No.	%	Median (range)	
Gender										0.008*
Female	101	55.2		61	64.9		40	44.9		
Male	82	44.8		33	35.1		49	55.1		
Age, years			35.6 (18.3, 82.5)			37.7 (18.3, 82.5)			34.9 (18.4, 66.5)	0.517
18-40	89	56.7		44	55.7		45	57.7		
41-60	56	35.7		26	32.9		30	38.5		
≥60	13	7.6		9	11.4		3	3.8		
Unknown	25	-		15	-		11	-		
ECOG performance status										0.483
0	78	54.5		40	55.6		38	53.5		
1	33	23.1		16	22.2		17	23.9		
2	14	9.8		9	12.5		5	7		
≥3	18	12.6		7	9.7		11	15.5		
Unknown	40	-		22	-		18			
Leukocyte counts, $\times 10^9$/L			5.37 (0.8, 128.5)			3..4 (0.22, 102.7)			9.9 (0.8, 128.5)	0.027*
<5	77	48.7		45	56.3		32	41		
5-10	17	10.8		10	12.5		7	9		
10-50	47	29.7		20	25		27	34.6		
≥50	17	10.8		5	6.3		12	15.4		
Unknown	25	-		14	-		11	-		
Platelet counts, $\times 10^9$/L			25.5 (7, 230)			26 (4, 230)			25 (7, 157)	0.314
<40	123	77.8		59	73.8		64	82.1		
≥40	35	22.2		21	26.3		14	17.9		
Unknown	25	-		14	-		11	-		
Relapse-risk group										0.037*
Low risk	24	15.2		16	20		8	10.3		

Intermediate risk	70	44.3		39	48.8		31	39.7	
High risk	64	40.5		25	31.2		39	50	
Unknown	25	-		14	-		11	-	
Hemoglobin, g/dL			8.7 (3.2, 21.8)			8.6 (3.4, 21.8)			8.7 (3.2, 14.9)	0.939
<10	118	74.7		57	71.3		61	78.2	
≥10	40	25.3		23	28.7		17	21.8	
Missing	25	-		14	-		11	-	
Creatinine, mg/dL			0.8 (0.8, 4.3)			0.8 (0.4, 2.8)			0.81 (0.4, 4.3)	0.48
<1.4	146	94.8		78	98.7		68	90.7	
≥1.4	8	5.2		1	9.6		7	9.3	
Unknown	29	-		15	-		14	-	
Uric acid, mg/dL			3.9 (1.1, 10.3)			3.8 (1.1, 8.1)			4.1 (2, 10.3)	0.227
<7	132	89.8		66	90.4		66	89.2	
≥7	15	10.2		7	9.6		8	10.8	
Unknown	36	-		21	-		15	-	
Fibrinogen (mg/dL)			160 (10, 898)			163 (10, 898)			159 (0.5, 549)	0.86
<170	80	53.7		40	52.6		40	54.8	
≥170	69	46.3		36	47.4		33	45.2	
Unknown	34	-		18	-		16	-	
Albumin (g/dL)			3.9 (2.2, 5.42)			3.9 (2.2, 5.42)			3.9 (2,4, 5)	0.754
<3.5	27	22.3		12	20		15	24.6	
≥3.5	94	77.7		48	80		46	75.4	
Unknown	62			34	-		28	-	
Morphologic subtype										0.328
Hypergranular	148	93.7		76	96.2		72	91.1	
Microgranular	10	6.3		3	3.8		7	8.9	
Unknown	25	-		15	-		10	-	
PML breakpoint										0.383
BCR1	76	62.3		40	62.5		36	62.1	
BCR2	2	1.6		2	3.1		22	37.9	

BCR3	44	36.1	22	34.4	58	
Unknown	61	-	30	-	31	-

NOTE: * Indicate differences statistically significant.

1: Missing values were excluded in the calculation of *P*-values.

2: Classification according to PETHEMA-GIMEMA criteria. (PMID: 10942364)

Table 3. Univariable and multivariable.

End point	Model Variables	Univariable Analysis					Multivariable Analysis				
		HR	OR	95% CI		*P* -value	HR	OR	95% CI		*P*-value
CR	ISAPL modeling: low *vs* high		0.26	0.17	0.66	0.002		0.23	0.1	0.63	0.004
	Leukocyte counts (×10^9/L): continuous variable		0.57	0.34	0.96	0.036		0.9	0.47	1.73	0.771
	Age at diagnosis: continuous variable		0.4	0.2	0.79	0.008		0.7	0.42	1.15	0.164
OS	ISAPL modeling: low *vs* high	5.1		2.22	11.6	<0.001	5.77		2.33	8.27	<0.001
	Leukocyte counts (×10^9/L): continuous variable	2.86		1.75	4.66	<0.001	1.92		1.1	3.44	0.027
	Age at diagnosis: continuous variable	1.5		1.05	2.26	0.047	2.34		1.35	4.08	0.002
DFS	ISAPL modeling: low *vs* high	2.86		1.1	7.88	0.04	2.25		1.8	6.29	0.012
	Leukocyte counts (×10^9/L): continuous variable	3.25		1.51	7	0.003	2.87		1.14	7.24	0.025
	Age at diagnosis: continuous variable	1.14		0.57	2.28	0.704	1.11		0.44	2.77	0.816
EFS	ISAPL modeling: low *vs* high	3.96		2.05	7.65	<0.001	3.97		1.98	7.97	<0.001
	Leukocyte counts (×10^9/L): continuous variable	2.69		1.77	4.1	<0.001	1.94		1.17	3.19	0.009
	Age at diagnosis: continuous variable	1.34		0.93	1.93	0.105	1.78		1.1	2.88	0.019

NOTE. Hazard ratios (HRs) or odds ratios (ORs) > 1 or < 1 indicate an increased or decreased risk, respectively, of an event for the first category listed.

Abbreviations: CR, complete remission; OS, overall survival; DFS, disease-free survival; EFS, event-free survival; HR, hazard ratio; OR, odds ratio.

FIGURE LEGENDS

Figure 1. Patient survival. The probability of overall survival (A), cumulative incidence of relapse (B), disease-free survival (C) and event-free survival (D) in patients with acute promyelocytic leukemia (APL) according to ISAPL modeling.