Association between the *TP53* Arg72Pro polymorphism and clinical outcomes in acute myeloid leukemia

In acute myeloid leukemia (AML), alterations involving the tumor suppressor gene *TP53*, including mutations, deletions, or both, are frequently associated with older age and very poor prognosis.[1] In addition, they are closely associated with complex aberrant karyotype; of note, *TP53* alterations are rarely observed outside this group.[2] On the other hand, several polymorphisms in the *TP53* gene have been described[3] of which the non-synonymous *TP53* Arg72Pro (G215C) polymorphism appears to be a promising genetic modifier in human tumors, particularly because of its role as a modulator of the apoptotic activities of the encoded p53 protein.[4] In terms of the biological significance of the *TP53* Arg72Pro polymorphism, there are discernible functional differences between variants at this site. While the *TP53* variant that encodes proline (Pro72) results in 3- to 5-fold decreased apoptotic activity[5] and an increased risk of cancer,[6] the *TP53* arginine (Arg72) variant has an increased ability to induce apoptosis and to repress cellular transformation.[5]

To date, little is known about the frequency and prognostic impact of the *TP53* Arg72Pro polymorphism in hematologic malignancies, particularly in AML. The few available data suggest that the interaction between the *TP53* Arg72Pro polymorphism and the MDM2 SNP309 variant could modulate responses to genotoxic therapy and increase the risk of therapy-related AML.[7] Here, we assessed the frequency of the *TP53* Arg72Pro polymorphism in healthy volunteers and patients with AML, and evaluated its clinical impact on outcomes of non-selected patients with AML (excluding acute promyelocytic leukemia) at two university hospitals who were followed from June 2003 to January 2016.

Overall, 429 subjects were included. Two-hundred and five adult patients with *de novo* AML were retrospectively analyzed. One hundred and fifty-one (74%) patients were treated in Recife (northeast Brazil), while 54 (26%) patients were treated in Ribeirao Preto (southeast Brazil). Baseline characteristics were similar between centers. The treatment protocol has been described previously.[8] Briefly, in patients up to 60 years of age, the treatment protocol was adapted according to performance status and the presence of comorbidities (in particular, cardiac disorders). Conventional chemotherapy consisted of daunorubicin (60-90 mg/m^2/d for 3 days) and cytarabine (100-200 mg/m^2/d for 7 days) or TAD-9 as induction, followed by three or four cycles of consolidation therapy with high doses of cytarabine (>1 g/m^2/d). For patients who did not achieve complete remission (CR) after one course of chemotherapy, a second course was administered between days 28 and 35 after the end of the first course. CR was assessed by bone marrow examination on day 28 after each course of chemotherapy. For those who needed it, a post-remission therapy based on autologous or allogeneic transplantation was performed. Patients older than 60 years were treated with low-dose ARA-C, a combination of etoposide, thioguanine, and idarubicin, or best supportive care.

For the healthy control group, peripheral blood samples from 224 age- and sex-matched healthy volunteers (median age 51 years, range 21-83 years; 55% female) with no history of hematologic disease were obtained from the University Hospital, Federal University of Pernambuco, Recife, Brazil. Patients with therapy-related AML or with a previous history of myelodysplastic syndrome were not included. Informed consent was obtained from all patients and healthy volunteers in accordance with the Declaration of Helsinki and approval was obtained from the local research ethics board. The *TP53* Arg72Pro polymorphism was evaluated by polymerase chain reaction-restriction fragment length polymorphism.[9] Details of the statistical analysis and clinical end points have been described previously.[10]

The *TP53* Arg72Pro polymorphism was successfully genotyped in 413 (96%) subjects, including 189 of 205 patients with AML (92%) and 224 of 224 healthy volunteers (100%). No deviation from Hardy-Weinberg equilibrium was detected in the patient (P>0.05) or control (P>0.05) groups. In addition, there were no differences in the baseline characteristics or outcomes of the patients

Table 1. Distribution of allele and genotype frequencies of the TP53 Arg72Pro polymorphism.

TP53 Arg72Pro	Acute myeloid leukemia, n (%)	Healthy volunteers, n (%)	Odds ratio (95% CI)	*P*
Allele				
Arg (G allele)	203 (54)	285 (64)	Reference	
Pro (C allele)	175 (46)	163 (36)	1.51 (1.14-1.99)	0.004
Codominant				
Arg/Arg	57 (30)	89 (40)	Reference	0.011
Arg/Pro	89 (47)	107 (48)	1.30 (0.84-2.01)	0.286
Pro/Pro	43 (23)	28 (12)	2.40 (1.34-4.29)	0.004
Dominant				
Arg/Arg	57 (30)	89 (40)	Reference	
Arg/Pro + Pro/Pro	132 (60)	135 (60)	1.53 (1.01-2.30)	0.054
Recessive				
Arg/Arg + Arg/Pro	146 (77)	196 (88)	Reference	
Pro/Pro	43 (23)	28 (12)	2.06 (1.22-3.48)	0.008
Over dominant				
Arg/Arg + Pro/Pro	100 (53)	117 (52)	Reference	
Arg/Pro	89 (47)	107 (48)	1.03 (0.70-1.51)	0.969

included in this study *versus* patients who were not included because of poor quality genomic DNA or unavailable DNA samples (*data not shown*). To determine whether the *TP53* Arg72Pro polymorphism is associated with risk of AML, we compared the frequency of this polymorphism in patients with AML and healthy subjects. The allelic (P=0.004) and genotypic (P=0.008) frequencies of the *TP53* Pro72 variant were higher in patients with AML (Table 1). Next, we restricted our analysis to patients with AML and analyzed the association of the frequency of the *TP53* Arg72Pro polymorphism with clinical and laboratory features. We also evaluated its clinical impact on induction and post-induction outcomes. The recessive model (i.e. Pro/Pro *vs.* Arg/Arg+Arg/Pro, hereafter called non-Pro/Pro) was used because it had the best fit to our data (Table 1). There were no significant differences between patients with the Pro/Pro genotype (43 patients, 23%) *versus* the non-Pro/Pro genotype (146 patients, 77%) with respect to clinical and laboratory features (Table 2).

Of the 189 enrolled patients, 2 (1%) patients who started the induction treatment were lost to follow up without being assessed for CR. In addition, 68 (36%) patients did not receive conventional chemotherapy (main reasons for treatment failure have been previously described[8]) and were considered ineligible for the induction and post-induction therapy analyses. CR was achieved in 68 of 119 (57%) patients, of whom 18 of 27 (67%) and 50 of 92 (54%) patients were assigned to the Pro/Pro and non-Pro/Pro groups, respectively. The *TP53* Arg72Pro polymorphism had no impact on CR (P=0.278). Of the 51 patients who failed to reach CR, 16 (33%) experienced early mortality, mainly due to bacterial and fungal infections. Although the early mortality rate was proportionally higher in patients with the non-Pro/Pro genotype (44% *vs.* 30%) there was no difference between groups (P=0.449).

The median follow up for the entire cohort was 135 days [95% confidence interval (CI): 69-200 days] with an estimated 5-year overall survival (OS) rate of 22% (95%CI: 17%-29%). Patients with the Pro/Pro genotype had significantly longer survival (median 264 days, 95%CI: 201-657 days) than patients with the non-Pro/Pro genotype (median 114 days, 95%CI: 70-158 days). Univariate analysis showed that patients with the Pro/Pro genotype had a higher 5-year OS rate (42%) than patients with non-Pro/Pro genotype (12%) (P=0.031) (Figure 1A), although this difference was no longer significant after adjustment for age, cytogenetic risk stratification, and leukocyte counts at diagnosis [hazard ratio (HR): 0.66, 95%CI: 0.37-1.17; P=0.163]. We also analyzed the clinical impact of the *TP53* Arg72Pro polymorphism in each cytogenetic risk group. The *TP53* Arg72Pro polymorphism had no impact on the clinical outcome of patients assigned to the favorable (24 patients; P=0.6) and adverse (16 patients; P=0.561) groups. In contrast, the TP53 Pro/Pro genotype was significantly associated with longer survival (P=0.035) for patients assigned to the intermediate cytogenetic risk group (57 patients) (Figure 1B), even though these results were not consistent with the multivariate analysis (HR: 0.44, 95%CI: 0.14-1.37; P=0.164). The *TP53* Arg72Pro polymorphism had no impact on disease-free survival (P=0.77).

Regarding the prognostic relevance of age in AML, we decided to evaluate the prognostic impact of the *TP53* Arg72Pro polymorphism separately in younger (<60 years of age; n=125) and older (>60 years; n=64) patients. For patients up to 60 years of age, the *TP53* Arg72Pro polymorphism had no impact on CR (P=0.648), and DFS

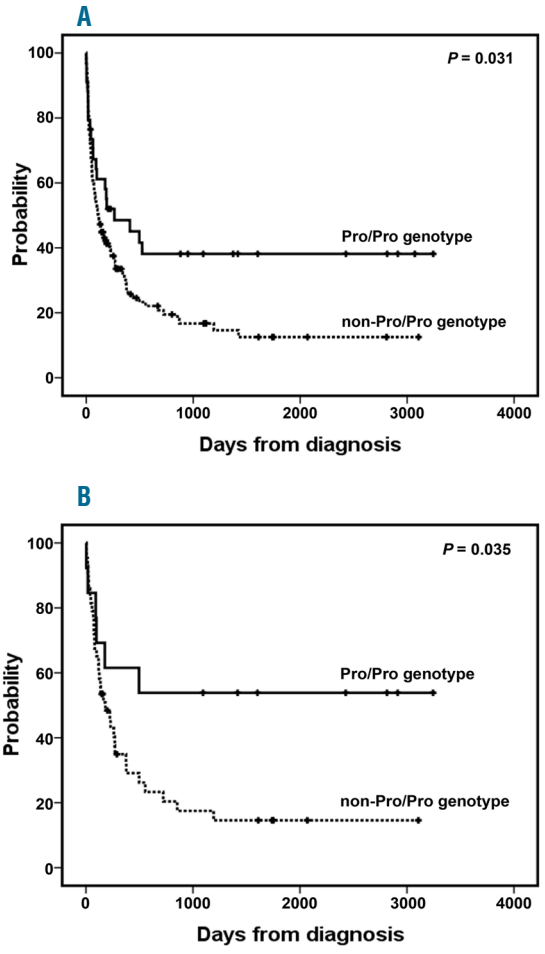

Figure 1. Patients' survival. The probability of overall survival (OS) (A) in patients with acute myeloid leukemia (AML) according to the presence of the *TP53* Arg72Pro polymorphism (entire cohort). OS (B) in patients with intermediate-risk karyotype according to the presence of the *TP53* Arg72Pro polymorphism. Survival curves were estimated using the Kaplan-Meier method and the log-rank test was used for comparison.

(P=0.856), but there was a trend towards a higher OS rate for patients with Pro/Pro genotype (17% *vs.* 46%; P=0.059). *TP53* Arg72Pro polymorphism was not associated with treatment outcomes in patients over 60 years of age (CR: P=0.154, DFS: P=0.201, OS: P=0.643).

Although most studies have described the *TP53* Pro72 allele as a poor prognostic factor in several types of cancer,[6] evidence suggests that the Pro72 variant is more efficient in both activating DNA-repair target genes[11] and inducing cell-cycle arrest.[12] Particularly in myeloid neoplasms, the *TP53* Pro72 variant exerts a protective effect against therapy-related AML in individuals with lower levels of the MDM2 protein.[7] Finally, Pro72 allele carriers show significantly lower frequency of *TP53* mutations in specific types of human non-hematologic tumors.[13] Here, we demonstrated that the *TP53* Pro/Pro genotype was associated with higher risk of leukemia and favorable outcome in AML patients treated with conventional therapy, particularly those assigned to the intermediate cytogenetic risk group. Therefore, it seems that the *TP53* Arg72Pro polymorphism may have different tissue- and context-specific functions, and the prognostic importance

Table 2. Baseline characteristics.

	All		TP53 Arg72Pro polymorphism (recessive model) Pro/Pro		non-Pro/Pro		P[1]
	N.	%	N.	%	N.	%	
Age (years)							0.62
18-40	66	34.9	17	39.5	49	33.6	
40-60	59	31.2	14	32.6	45	30.8	
>60	64	33.9	12	27.9	52	35.6	
Age, median (range)	47 (18-93)		41 (18-80)		49 (18-93)		0.203
Sex							0.862
Female	102	54	24	55.8	78	53.4	
Male	87	46	19	44.2	68	46.6	
FAB subtype							0.364
M0	16	9.1	6	16.7	10	7.2	
M1	27	15.4	7	19.4	20	14.4	
M2	59	22.7	8	22.2	51	36.7	
M4	52	29.7	11	30.6	41	29.5	
M5	16	9.1	4	11.1	12	8.6	
M6	3	1.7	–	–	3	2.2	
M7	2	1.1	–	–	2	1.4	
Missing data	14	–	7	–	7	–	
Cytogenetic abnormalities							0.215
Normal karyotype	47	42	10	40	37	42.5	
t(8;21)(q22;q22)	14	12.5	–	–	14	16.1	
inv(16)(p13;q22)/t(16;16)(p13;q22)	10	5.3	4	16	6	6.9	
Monosomy 7	4	3.6	1	4	3	3.4	
Trisomy 8	2	1.8	1	4	1	1.1	
Complex karyotype	7	6.3	2	8	5	5.7	
11q23 abnormalities	1	0.9	1	4	–	–	
Others	12	10.7	2	8	10	11.5	
Missing data[2]	77	–	18	–	59	–	
Cytogenetic risk stratification[3]							0.778
Favorable	24	24.7	4	19	20	26.3	
Intermediate	57	58.8	13	62	44	57.9	
Adverse	16	16.5	4	19	12	15.8	
Missing data	92	–	22	–	70	–	
FLT3-ITD							0.832
Mutated	38	20.3	8	18.6	30	20.8	
Non-mutated	149	79.7	35	81.4	114	79.2	
Missing data	2	–	–	–	2	–	
NPM1 mutations							0.687
Mutated	46	24.6	9	20.9	37	25.7	
Non-mutated	141	75.4	34	79.1	107	74.3	
Missing data	2	–	–	–	2	–	
Molecular risk group[4]							0.815
Low-risk group	31	16.6	6	14	25	17.4	
High-risk group	156	83.4	37	86	119	82.6	
Missing data	2	–	–	–	2	–	
WBC ($\times 10^9$/L), median	46.5		45.5		47.5		0.695
Range	0.5-312		2-285		0.5-312		
Hemoglobin (g/dL), median	7.8		8.3		7.7		0.393
Range	3-14		5.5-11		3-14		
PLT ($\times 10^9$/L), median	47		48		47		0.614
Range	6-652		6-143		8-625		

FAB: French-American-British classification; WBC: white blood cells; PLT: platelets; FLT3-ITD: internal tandem duplication of the *FLT3* gene; *NPM1*: nucleophosmin. [1]Missing values were excluded for calculation of *P*-values. [2]Material not available or no metaphases detected. [3]The cytogenetic risk groups were defined according to Medical Research Council (MRC) criteria.[16] [4]The high-risk molecular group was defined as NPM1non-mutated/*FLT3*-ITD^{negative}, *NPM1*$^{non\text{-}mutated}$/*FLT3*-ITD^{positive}, or *NPM1*^{mutated}/*FLT3*-ITD^{positive}. The low-risk molecular group was defined by the presence of any *NPM1* mutations and the absence of FLT3-ITD mutations.

of each allele may depend on the type of cancer and on the particular treatment. Importantly, screening for the *TP53* mutations was not performed in the present study. Nevertheless, we should point out that, in our cohort, only 6% of patients had complex karyotype, and abnormalities involving chromosome 17 [17p-, monosomy 17, or i(17q)] were not observed. One may argue that *TP53* alterations could be responsible for inferior outcomes in our cohort; however, considering the rarity of these mutations in non-complex karyotype AML, this seems unlikely.

Several published data have investigated the relationship between the *TP53* Arg72Pro polymorphism and risk of leukemia, but the impact of this polymorphism on predisposition to leukemia remains controversial. Most recently, in a meta-analysis involving seven AML cohorts with 1054 patients and 4337 healthy subjects, Tian *et al.* reported an absence of association between the *TP53* Arg72Pro polymorphism and increased risk of AML.[14] Nevertheless, it is important to note that the Authors conducted their analyses based on studies from Asian (eight studies) and Caucasian (five studies) populations. These may not reflect the Brazilian genetic background, which is well-known for its mixed genetic population.

Although we have demonstrated that the *TP53* Arg72Pro polymorphism could be prognostically relevant in AML, these results must be treated with caution. First, a sizable number of patients were excluded from induction and post-induction analyses because either they did not receive conventional chemotherapy (36%) or because they experienced early mortality (33%), which could bias our analyses. In addition, because of the relatively small sample size and the current lack of validation in independent cohorts, it is probably premature to use *TP53* Arg72Pro genotype information in treatment decisions. It would be reasonable to suppose that our findings will be confirmed by other groups with larger sample sizes and well-designed studies. Despite its limitations, the germline genetic profile should not be overlooked, taking into account the genomic landscape of AML and its role in the clonal evolution of the disease.[15]

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Funding: we gratefully acknowledge financial support from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; grant #444656/2014–2016). MFB received a fellowship from CNPq (Grant #486359/2013-1).

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doi:10.3324/haematol.2016.155069

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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