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LETTER TO THE EDITOR

Results of *FLT3* mutation screening and correlations with immunophenotyping in 169 Brazilian patients with acute myeloid leukemia

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Dear Editor,

Despite the fact that several studies have reported that 25–45% of patients with acute myeloid leukemia (AML) in developed countries harbor mutations of the *FLT3* gene, little is known about the incidence and clinical relevance of *FLT3* mutations in AML patients from developing countries. *FLT3* mutations are of two major types: internal tandem duplications (ITD) in exons 14 and 15, that map to the juxtamembrane domain, and missense point mutations in exon 20 within the activation loop of the tyrosine kinase domain (TKD), involving mainly D835/I836. The former group of mutations is the most frequent, being detected in 17–25% of adult and 5–22% of childhood AML cases, whereas point mutations of TKD are detected in 8–12% of cases. These mutations lead to the overexpression or constitutive activation of the tyrosine kinase receptor and are associated with shorter disease-free survival and overall survival (OS) [1].

FLT3 mutations may occur in any AML subtype but are most common in AML with t(6;9)(p23;q34), acute promyelocytic leukemia (APL), and cytogenetically normal acute myeloid leukemia (CN-AML). Geographic differences in the frequency of AML-associated recurrent genetic abnormalities as well as in mutations in receptor tyrosine kinase family members have been reported [2, 3]. In the present study, we used polymerase chain reaction single-stranded conformational polymorphism (PCR-SSCP) and conventional PCR analyses to evaluate the mutational status of *FLT3* in 169 de novo Brazilian AML patients and correlated this status with some clinical and laboratory features. Screening for *FLT3-ITD* mutations was performed by PCR according to Kiyoi et al. [4] and PCR-SSCP was used for the analyses of exons 12 to 20.

Chromosome analyses were performed using the trypsin-Giemsa banding technique and were successful in 120 cases (71.0%). The distribution of recurrent chromosomal abnormalities is shown in Fig. 1a. Screening for *NPM1* mutations was performed by PCR followed by sequencing in CN-AML patients and were detected in 43.7% of these cases. Despite the small number of cases, it must be pointed out that the detected higher frequency of patients with APL and lower of those with normal karyotype compared with US and European AML patients is in agreement with previous studies [5, 6]. On the other hand, the frequency of *NPM1* is approximately the same as those described in developed countries [7].

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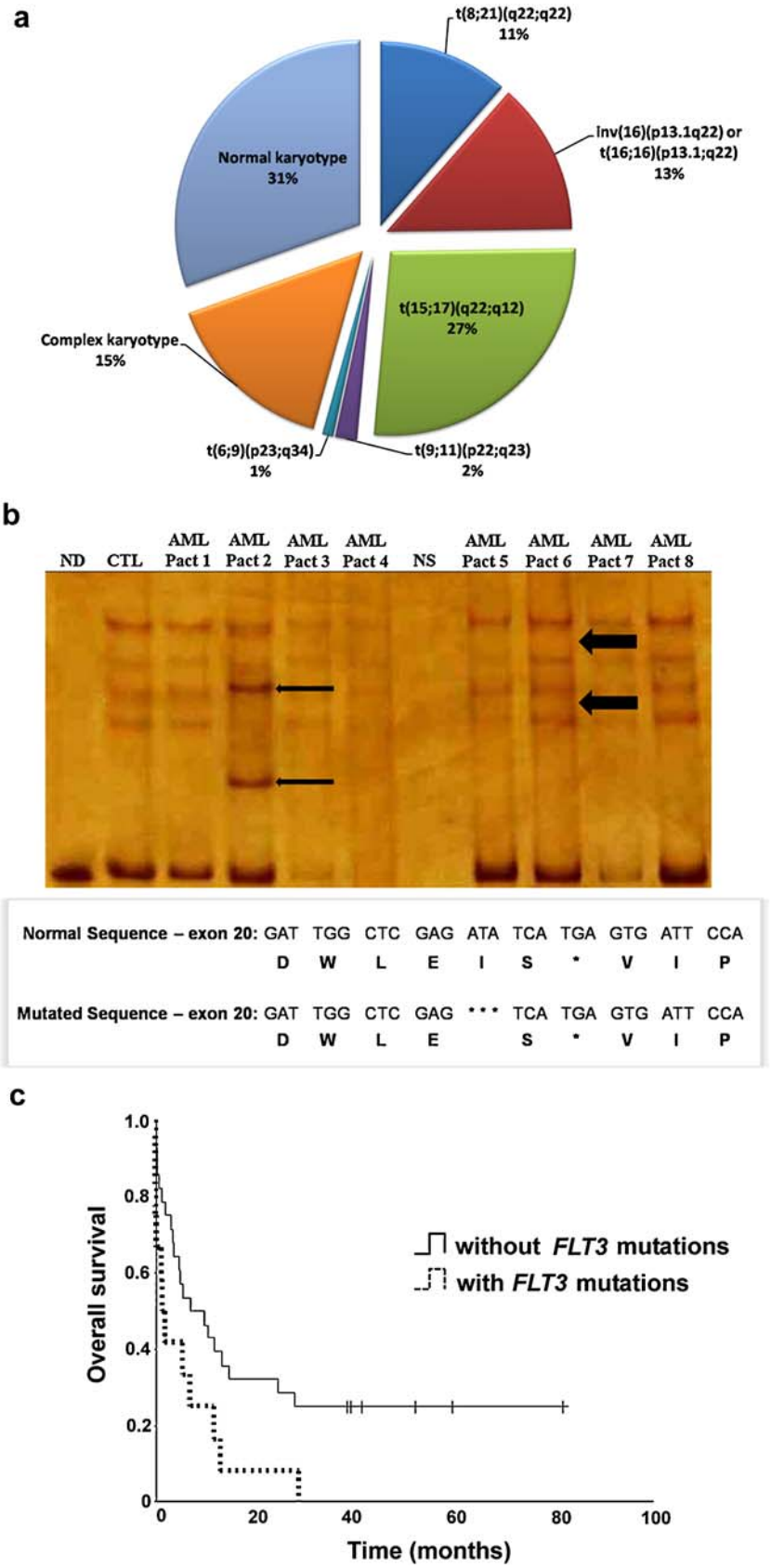

Fig. 1 **a** Distribution of AML patients according to recurrent chromosomal abnormalities. **b** Polyacrylamide gel electrophoresis analysis of exon 20. The *large arrows* represent the normal migration standard (*lanes CTL, 1*, and *3–8*). The *thin arrows* (*lane 2*) illustrate the altered migration standard. *ND* normal sample nondenatured, *CTL* normal sample denatured, *NS* no sample. Below the polyacrylamide gel is the sequencing result of this alteration (I836del). c Kaplan-Meier analysis of the overall survival of 49 AML patients (nonPML-RARa) according to the presence or absence of *FLT3* mutations. The *p* value is derived from the log-rank test (Mantel-Cox)

FLT3-ITD were detected in 40/169 patients (23.6%). There was complete agreement between the PCR-SSCP and conventional PCR results for the detection of *FLT3-ITD*. The mean leukocyte counts at diagnosis were roughly three times higher in patients with *FLT-ITD* mutations, but this difference was not significant. On the other hand, the hemoglobin level was higher in AML patients with *FLT3-ITD* (Table 1). Among the 31 patients with APL, the frequency of *FLT3-ITD* was 32%. Patients were further classified according to the cytogenetics findings in: favorable (n=54), intermediate (n=50), and unfavorable (n=16) risk group [8]. No association was observed between the presence of *FLT3-ITD* and cytogenetic subgroup.

One patient with *FLT3-ITD* also had altered results on polyacrylamide gel electrophoresis (Fig. 1b), which, after sequencing, was found to be associated with the deletion of isoleucine 836 (I836del). The I836del has been previously described [9] and it leads to constitutive activation of the FLT3 receptor. In addition, two previously not described synonymous mutations were detected, one in exon 12 (T526T) and the other in exon 17 (G697G; data not shown). Both patients also harbored *FLT3-ITD*. Mutations in exons 13, 16, 18, and 19 of *FLT3* were not detected in the AML patients. The detected frequency of *FLT3-ITD* was similar to European and American populations [1]. In contrast, only one case was found with a mutation within the TKD activation loop. Based on the existing data, the expected frequency of mutations in the TKD of *FLT3* would be between 5% and 22%, and the value detected in the present study (0.6%) suggests that these mutations are rare in our population. Accordingly, Chauffaille et al. [10] reported a frequency of 2.8% for D835 mutations in Brazilian APL patients and Emereciano et al. [11], studying 159 infant leukemia cases, detected six (3.8%) cases with *FLT3-ITD* and six (3.8%) cases with mutations involving D835.

We also evaluated if there was any association between immunophenotypic markers and the presence of *FLT3* mutations. There was a significant association between CD56 expression and the presence of *FLT3-ITD*. Compared with patients without *FLT3* mutations, those harboring *FLT3-ITD* presented a lower frequency of CD56 positivity (5% versus 27.2%, p=0.04). This difference remained significant when APL cases were excluded. Vidriales et al. associated CD56 expression with a tendency for a better outcome in AML patients [12]. In accordance with the latter study, our results showed that blasts presenting *FLT3-ITD* have lower CD56 expression.

For the analysis of the response to treatment, we restricted the analysis to 40 patients with de novo AML, excluding APL, who ranged in age from 15 to 65 years and were treated with a single protocol. The OS was defined as the time from diagnosis to death or last follow-up,

Table 1 Demographic and laboratory features of AML patients according to the presence or absence of *FLT3-ITD*

Patients characteristics	With FLT3-ITD (n=40)	Without FLT3-ITD (n=129)	TOTAL (n=169)	p value
Gender (M/F)	16:24	61:68	77:92	0.41[a]
Age[b] (years)	46.6 (17–91)	46.5 (2–88)	46.6 (2–91)	0.16[c]
Hb at diagnosis[b] (g/dl)	8.6 (7.3–9.9)	7.2 (5.6–8.9)	7.8 (6.4–9.3)	0.01[c,d]
WBC[b] × 10^3 (/mm^3)	54 (13.5–82.3)	19.5 (7.6–55.6)	59 (8.9–67.5)	0.18[c]
Platelets[b] × 10^3 (/mm^3)	38 (16–97.5)	33 (20–69)	35.5 (17.2–54)	0.93[c]
Cytogenetic analysis, n (%)				
Favorable risk[e]	12 (7.1)	42 (24.8)	54 (31.9)	0.97[a]
Intermediate risk[f]	13 (7.7)	37 (21.9)	50 (29.6)	
Unfavorable risk[g]	6 (3.5)	10 (5.9)	16 (9.4)	
No cytogenetic data available[h]	9 (5.3)	40 (23.6)	50 (29.6)	–
CN-AML, n (%)	11 (6.5)	22 (13)	33 (19.52)	

M male, *F* female, *Hb* hemoglobin, *WBC* white blood cell, *CN-AML* cytogenetically normal acute myeloid leukemia

[a] Calculated using the Chi-square test

[b] Values represent mean (range)

[c] Calculated using Student's t test

[d] Significant (p<0.05)

[e] Favorable risk: (15;17)(q22;q12-21), t(8;21)(q22;q22), inv(16)(p13q22)/t(16;16)(p13;q22)

[f] Intermediate risk: normal karyotype, t(9;11)(p22;q23), del(7q), del(9q), del(11q), del(20q), –Y, +8, +11, +13, +21

[g] Unfavorable risk: complex karyotype, inv(3)(q21q26)/t(3;3)(q21;q26), t(6;9)(p23;q34), t(6;11)(q27;q23), t(11;19)(q23;p13.1), del(5q), –5, –7

[h] No sample available or no metaphases detected

censoring patients alive at the last follow-up. All patients received two courses of induction therapy with cytarabine 100 mg/m^2/day administered as a continuous 7-day infusion plus daunorubicin 60 mg/m^2/day administered as a 1-hour infusion on each of the first 3 days of treatment, as widely used. For the patients who achieved a complete remission (CR) after induction, postremission therapy consisted of two to three courses of consolidation with mitoxantrone 10 mg/m^2/day, days 1–5, and cytarabine 1,000 mg/m^2 every 12 h, days 1–3. Patients with an HLA-identical sibling were scheduled to proceed to allogeneic stem cell transplantation, and were excluded from analysis. The remainders, without an HLA-matched sibling donor, were submitted to two to three cycles of consolidation treatment as described above. The patients who were not in CR after induction were submitted to alternatively salvage treatment including high doses of cytarabine. Figure 1c shows the OS curve for patients with (n=12) and without *FLT3-ITD* (n=28). The groups had a similar age distribution and frequency of cases with *RUNX1-RUNX1T1* and *CBFb-MYH11* rearrangements. The mean follow-up time was 64.7 months (ranging from 28.6–88.8 months). The mean OS of the *FLT3-ITD* group was 5.8 months (95% CI, 0.98–10.7 months), whereas it was 25.8 months (95% CI, 13.5–38 months) for the group without *FLT3-ITD* (p=0.004). It should be pointed out that all 12 patients with *FLT3-ITD* died within 1 year of diagnosis. In general, the estimated OS of patients with AML was inferior to that reported in developed countries, but it is in agreement with reports of Brazilian patients [13]. These results may be due to the high early mortality, the delay in diagnosis, and/or socioeconomic aspects, as recently demonstrated for APL [5].

In conclusion, this study reinforces the relevance of geographic differences in AML, by demonstrating that *FLT3-TKD* was rarely detected in Brazilian AML patients. In addition, our results suggest that there may be an inverse correlation between CD56 expression and the presence of *FLT3-ITD*.

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