FLT3-ITD confers poor prognosis in patients with acute promyelocytic leukemia treated with AIDA protocols: long-term follow-up analysis

Internal tandem duplication (ITD) of the fms-like tyrosine kinase 3 gene (*FLT3*/ITD) occurs in 20-30% of young adults with acute myeloid leukemia (AML) and is associated with poor prognosis.[1-4] *FLT3*/ITD mutation is detected in 35-40% of acute promyelocytic leukemia (APL) patients and is associated with a high WBC count on presentation, hypogranular variant (M3v) morphology and short (bcr3) PML-RARA isoform. However, the prognostic significance of this alteration remains controversial[5] whereas few data have been reported on the activity of arsenic trioxide in patients with *FLT3*/ITD mutation.[5] We previously investigated the clinical-biological correlations of *FLT3*/ITD in 90 APL patients receiving the AIDA protocol.[6] While we confirmed the association between *FLT3*/ITD and the above-mentioned base-line features, for *FLT3*/ITD$^+$ patients we found no difference in response to induction and only a trend towards an inferior outcome. In the present study, we report the *FLT3*/ITD prognostic impact on an expanded series of 147 APL patients with a considerably longer follow up.

During the period April 1993-October 2010, 147 patients with newly diagnosed APL were observed and treated with the AIDA0493[7] and AIDA2000[8] protocols at the Sapienza University of Rome. The diagnosis was initially established morphologically and confirmed by RT-PCR identification of PML/RARA fusion gene as reported.[7,8] Seventy-three patients received AIDA 0493 regimen,[7] while 74 patients diagnosed after May 2000 were treated according to the risk-adapted regimen AIDA-2000,[8] with distinct post-induction approaches based on the initial risk stratification (ATRA included in each consolidation course and reduced intensity chemotherapy for low and intermediate risk).[9] All patients received maintenance according to the protocols.[7,8] The following clinical characteristics at diagnosis were analyzed according to the *FLT3* status: age, gender, FAB classification, peripheral WBC count, platelet count, hemoglobin level, karyotype, PML/RARA isoform and relapse risk category. Molecular tests were performed after the third consolidation and thereafter every three months for two years and every six months after the end of maintenance. Molecular relapse was defined as positive RT-PCR test detected in two successive marrow samples collected at any time after consolidation and in the absence of morphologically detectable blasts in both the marrow and peripheral blood. Differentiation syndrome

Table 1. Differences between FLT3-positive and negative patients.

Features	FLT3$^+$ (33 pts)	FLT3 – (114 pts)	*P*
Gender (M/F)	19/14	46/68	0.02
Age (median) years	42	35	ns
M3/M3v	24/9	88/16	0.03
Relapse risk			
Low	10	67	0.03
Intermediate	11	37	
High	12	10	
WBC (median x10^9/L)	32	3.6	0.001
Type of transcript			
bcr1	12	77	0.002
bcr3	21	37	
Differentiation syndrome (%)	24%	14%	0.02
Relapse (%)	42%	20%	0.03
OS	39%	96%	0.0001
RFS	30%	90%	0.017
CIR	60%	4%	0.0001

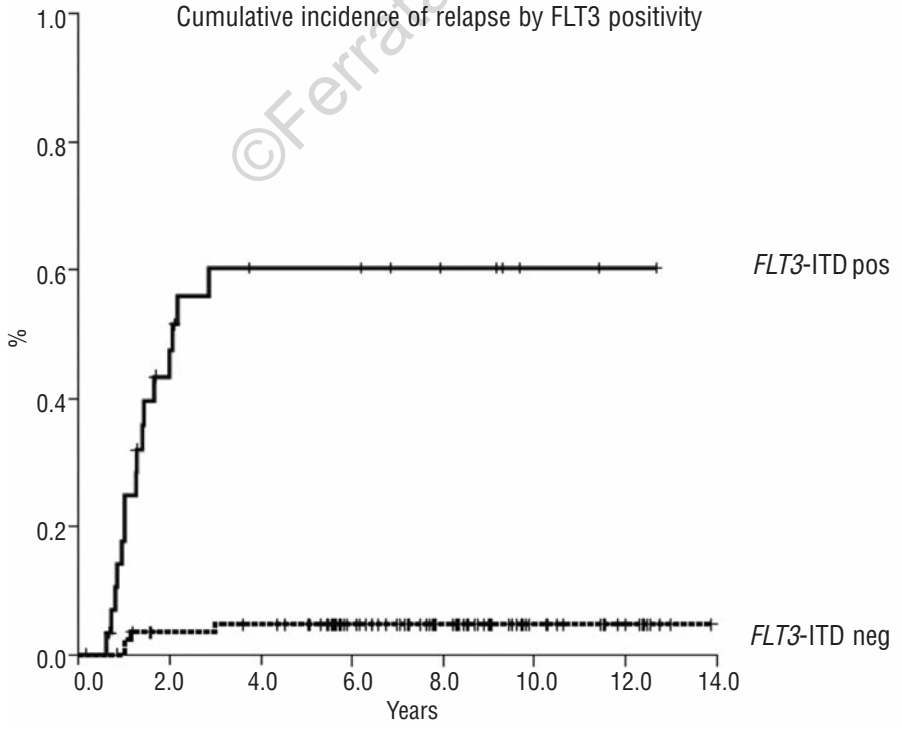

Figure 1. Cumulative incidence of relapse (CIR) according to *FLT3* status: continuous line represents CIR of *FLT3-ITD* positive patients; split curve represents *FLT3-ITD* negative patients.

(DS) was defined as "definitely present" when at least three major signs were recorded according to the criteria of Frankel *et al.*[10] Screening for *FLT3*-ITD mutations was performed as previously reported.[6] D835 mutations were also investigated, but not considered in this study due to low prevalence. Wilcoxon-Mann-Whitney test was performed for comparison of non-parametric series and Fisher's exact test was used to compare categories. Multivariable Cox proportional hazard regression models were performed and expressed as hazard ratios with 95% confidence intervals (CI). $P<0.05$ was considered statistically significant. Overall survival (OS) was estimated using the Kaplan-Meier method. Relapse-free survival (RFS) was estimated counting either relapse or death in CR as events. Cumulative incidence of relapse (CIR) was defined as the time from end of induction to date of hematologic or molecular relapse, whichever occurred first, considering death in remission as a competitive event.

Thirty-three of the 147 patients were *FLT3*-ITD$^+$, whereas FLT3-D835 mutation was detected in 9.5% of patients. Of the *FLT3*-ITD$^+$ patients, 19 were males (57%) and 14 females; 27% were diagnosed as having a M3v and 36% as high relapse risk. In the negative cohort of 114 patients, 46 (40%) were males, 12% were diagnosed as having a M3v and 15.7% as high relapse risk. Twenty-one *FLT3*-ITD$^+$ patients (63%) had the bcr3 transcript type as compared to 37 of 114 (32%) patients with germline *FLT3* (P=0.002). Eight *FLT3*-ITD$^+$ patients (24%) experienced a documented DS compared to 14 (12%) in the *FLT3*-ITD$^-$ cohort (P=0.02). After a median follow up of nine years (range 5-19), a significant difference was seen in terms of OS: 96% in the *FLT3*-ITD$^-$ cohort compared to 39% in the *FLT3*-ITD$^+$ cohort (P=0.0001). RFS was 90% and 30% in the *FLT3*-ITD-negative and -positive cohorts, respectively (P=0.017) (Table 1). The CIR at nine years was 4% (95%CI: 1,279-6,238) for the *FLT3*-negative and 60% (95%CI: 22,632-81,236) for the *FLT3*$^+$ subset (Figure 1; P=0.0001). Cox multivariable analysis was performed for hazard of relapse and OS: of all previously mentioned factors tested, only *FLT3*-ITD had an independent prognostic value (hazard ratio = 2.4; 95%CI: 1-4.5; P=0.01) and bcr3 that carried a poorer prognosis compared to bcr1 (hazard ratio of 2.2; 95%CI: 1-4.2; P=0.02). Following our initial study reported in 2002,[6] we prospectively analyzed *FLT3* for the presence of ITD in all newly diagnosed patients. In this final analysis, we found a weak correlation of *FLT3*-ITD mutation with male gender and we confirm a strong correlation with WBC count, M3v and bcr3 at baseline. Although we confirmed no difference in response to induction, we observed in a prolonged follow up a considerably more unfavorable outcome for *FLT3*-ITD$^+$ patients in terms of RFS, DFS and OS. The strong association of mutation with specific clinical features and the poor outcome demonstrated in long-term analysis, allows an aggressive subset of APL at diagnosis to be identified, which probably deserves an intensification of treatment in order to prevent relapse.

Conflicting results have so far been reported with respect to the correlation between *FLT3* status and OS. While Gale *et al.* reported no association with outcome, even though *FLT3*-ITD$^+$ patients had a higher rate of induction deaths,[11] several groups reported a less favorable outcome for *FLT3*-ITD$^+$ patients. A Spanish group described a shorter 5-year RFS in patients with ITD mutant/wild-type ratio or longer ITD size.[12] A German study found that patients with *FLT3* mutation/wild-type ratio of 0.5 or over had better 2-year OS and EFS rates compared to patients with a ratio less than than 0.5.[13] In the PETHEMA/HOVON experience,[14] in univariate analysis *FLT3*-ITD mutations were associated to higher relapse and lower OS not retained in multivariate analysis, probably due to a shorter median follow up. A Korean group also showed a higher relapse rate in *FLT3*-ITD$^+$ patients with respect to those *FLT3*-negative.[15] Given its distinct mechanism of action and non-crossresistance with chemotherapy and ATRA, it will be interesting to explore the efficacy of arsenic trioxide in APL patients harboring *FLT3*-ITD.

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