Impact of FLT3 mutations and secondary cytogenetic changes on the outcome of patients with newly diagnosed acute promyelocytic leukemia treated with a single agent arsenic trioxide regimen

Ninety-eight newly diagnosed cases of PML-RARα positive APL were treated with a regimen of single agent ATO. FLT3 activating mutations were seen in 33% and an additional cytogenetic finding was noted in 23.2%. FLT3 activating mutations were significantly associated with a bcr3 PML-RARα isoform (p=0.012) and a delay in achieving a molecular remission (p=0.022). Neither FLT3 activating mutations nor secondary cytogenetic changes had an impact on clinical outcome.

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The efficacy of arsenic trioxide (ATO) in the management of relapsed and newly diagnosed cases of acute promyelocytic leukemia (APL) has been established.[1,2] Fms-like tyrosine kinase 3 (FLT3) mutations are commonly seen in patients with APL.[3] The common activating mutations of FLT3 include the FLT3 internal tandem duplication (FLT3-ITD) and a point mutation in the activation loop (D835V).[3] A retrospective analysis of the impact of FLT3 mutations in patients with APL, treated with conventional ATRA plus chemotherapy regimens, reported a higher incidence of induction deaths in one study[4] while another study reported a trend towards a shorter overall survival.[5] Secondary cytogenetic changes have been reported to have an adverse impact in some subsets of AML.[6] However, a similar adverse effect was not reported in patients with APL treated with conventional chemotherapy.[7,8] We undertook a retrospective analysis to study the impact of these factors on the outcome of 98 patients with newly diagnosed promyelocytic leukemia/retinoic acid receptor α positive (PML/RARα +) APL treated at our centre with single agent ATO.

Details of the treatment schedule with single agent ATO, the supportive care administered, and the indications for the administration of hydroxyurea or an anthracycline in induction was as reported earlier by our group.[2]

Cytogenetic analyses were carried out in the Cytogenetics Unit of the institution. Karyotypes were designated according to the International System for Human Cytogenetic Nomenclature.[9]

FLT3-ITD and D835V point mutation analyses were carried out on archival genomic DNA or cDNA samples obtained at the time of diagnosis. With the exception of minor variations in the primers for amplifying the cDNA (forward: 5'-CAATTTAGGTATGAAAGCCAG-3', reverse: 5'-CTTTCAGCA TTTTGACGGCAACC-3') the rest of the procedure was based on standard established methods.[10]

Of the 98 patients, archival DNA samples were not available for 4 patients. Of the remaining 94 patients, a FLT3 mutation was identified in 31 (33%). Of these, FLT3-ITD was seen in 20 (21.3%) patients and a point mutation of codon D835V was seen in 11 (11.7%). None of the patients had both the FLT3-ITD and the D835V mutation. Baseline characteristics of patients with and without a FLT3 mutation are summarized in Table 1. Patients with FLT3 activating mutations had a trend towards a higher white cell count at presentation (p=0.073) and were significantly more likely to have the bcr3 PML/RARα isoform (p=0.012). For further analysis of the impact of these mutations on outcomes, patients with either a FLT3-ITD mutation or a D835V mutation were combined and compared with the cohort that did not have a FLT3 mutation.

Table 1. Baseline characteristics of 94 patients evaluated for presence of FLT3 mutations.

Characteristic	*FLT3-ITD Mean±SD/n (%) (n=20)*	*Heterozygous D835V Mean±SD/n (%) (n=11)*	*Wild Type Mean±SD/n (%) (n=63)*	*p value*
Age (years)	28.4±11.9	31.9±15.1	29.9±15.8	0.734
Sex: Male	7 (35.0)	5 (45.5)	36 (57.1)	0.209
Duration of symptoms prior to diagnosis (weeks)	3.1±1.6	2.6±0.9	3.4±2.2	0.625
Hb (g/L)	73±28	89±26	81±29	0.316
WBC (x 10^9/L)	18.2±25.5	17.9±21.4	8.0±21.4	0.073
Platelet (x 10^9/L)	30.1±46.7	35.1±51.9	28.4±34.4	0.580
High risk[@]	5 (25.0)	3 (27.3)	18 (28.6)	0.952
PT – Prolonged	3 (15.0)	4 (36.4)	13 (20.6)	0.371
APTT – Prolonged	–	–	2 (3.2)	0.605
Abnormal liver function test	5 (25.0)	4 (36.4)	11 (17.5)	0.332
LDH (IU/L)	619.3±228.2	722.9±437.1	590.3±373.3	0.225
Exposure to Anthracycline	2 (10.0)	3 (30.0)	8 (12.7)	0.289
Exposure to Hydroxyurea	15 (75.0)	8 (80)	50 (79.4)	0.911
Cytogenetics + [#]	1 (8.3)	2 (28.6)	13 (26.5)	0.390
RT-PCR				
bcr 1	9 (45.0)	5 (45.5)	45 (71.4)	0.047
bcr 2	–	2 (18.2)	5 (7.9)	0.176
bcr 3	11 (55.0)	4 (36.4)	13 (20.6)	0.012

[@]risk stratification as previously defined by our group;[2] [#]defined by the presence of an additional cytogenetic anomaly.

Of the 98 patients evaluated, karyotyping was performed in 86 (87.8%). The karyotype failed in 5 (5.1%) and in 12 (12.2%) there were inadequate metaphases with a normal karyotype in the available metaphases. Of the remaining 69 patients, an isolated t(15;17) karyotype was seen in 53 (76.8%) while additional cytogenetic abnormalities were detected in 16 (23.2%). There were no significant differences in the baseline characteristics between these two groups (*data not shown*). Between the group that had FLT3 activating mutation (FLT3-ITD and D835V) and the group that did not, there was no significant difference in response to therapy. The complete hematologic response (CHR) rate was 87.1% and 90.5% The mean time to CHR was 43.7±13 and 45.25±10.25 days respectively for patients with and without a FLT3 mutation.

We had earlier reported that the time at which the majority of patients on this regimen achieved a molecular remission (MR) was prior to initiation of consolidation therapy.[2] Of the 72 patients who had an RT-PCR at this time point, 60.9% of patients with a FLT3 activating mutation achieved MR versus 85.7% of those without (p=0.022, RR=3.8; 95% CI 1.2 – 12.2). A univariate analysis showed that only a low hemoglobin at diagnosis was also associated with a significant delay in achieving an MR (p=0.036, RR1.2; 95% CI 1.0–1.5). A multivariate analysis adjusted for hemoglobin, WBC and platelet count at diagnosis, only the presence of a FLT3 activating mutation maintained its statistical significance (p=0.017, RR=4.868; 95% CI 1.3 – 17.8).

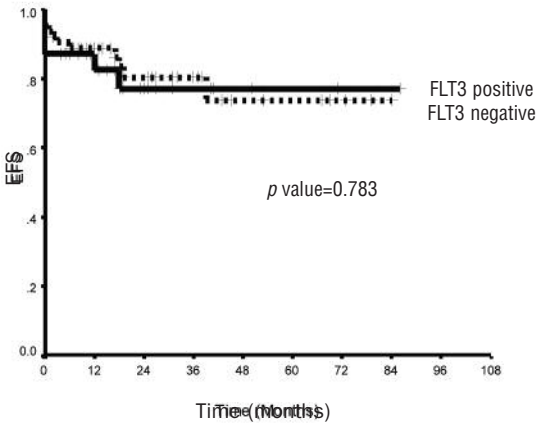

Figure 1. Kaplan-Meier product limit estimate of event free survival (EFS), based on FLT3 mutation status (n=94).

At a median follow up of 20 months (range: 4 – 97), the 3 year Kaplan-Meier estimate (± 1SE) of OS, EFS and DFS for patients with a FLT3 mutation was 82.51±7.24, 77.01±8.6, 88.82±7.48 percent, while for those without a FLT3 mutation it was 92.06±3.4, 80.45±5.86, 89.02±5.31 percent. Statistical analysis of these survival curves by a log rank test did not reveal any significant difference between the two groups. Figure 1 illustrates the impact of FLT3 activating mutation status on the OS and EFS in this study. The impact of FLT3-ITD and D835V mutations on OS, EFS and DFS were also analyzed independently and found not to be significantly different from each other and from the group without a FLT3 activating mutation (*data not shown*).

Of the 69 patients who were evaluated for the impact of cytogenetic findings, the presence of an additional cytogenetic finding in 16 (23.2%) did not alter the CHR, time to achieving CHR, time to MR, EFS, DFS or OS (*data not shown*).

This retrospective analysis suggests that neither the presence of a FLT3 activating mutation nor that of an additional cytogenetic finding appears to alter the clinical outcome of newly diagnosed patients with APL treated with single agent ATO in the short term.

In spite of the association of the FLT3 mutation with higher white cell counts, which is known to be the single most important predictor of outcome,[2] there was no difference in the EFS, DFS or OS in this study. Whether ATO is a specific or non-specific inhibitor of FLT3 tyrosine kinase activity remains to be demonstrated. Larger, preferably randomized controlled trials would be required to study if ATO has a beneficial effect in the subset of patients with newly diagnosed APL who have a FLT3 activating mutation.

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