Activating internal tandem duplication mutations of the fms-like tyrosine kinase-3 (*FLT3*-ITD) at complete response and relapse in patients with acute myeloid leukemia

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ABSTRACT

FMS-like tyrosine kinase 3 internal tandem duplication (*FLT3*-ITD) mutations are among the most frequent molecular aberrations in patients with acute myeloid leukemia. We retrospectively analyzed 324 patients with acute myeloid leukemia treated with front-line induction chemotherapy between October 2004 and March 2010. Fifty-six patients had *FLT3*-ITD mutation at diagnosis. Fifty-one (91%) patients with *FLT3*-ITD achieved complete remission. Thirteen patients had *FLT3* analysis at complete remission. None had *FLT3*-ITD. Twenty-five (49%) patients with *FLT3*-ITD relapsed. Of these, 13 (52%) had *FLT3*-ITD at relapse (3 negative and 9 not done). Among the 201 patients without *FLT3*-ITD at diagnosis who achieved complete remission, 77 (38%) relapsed among whom 8 (10%) patients acquired *FLT3*-ITD clone. We conclude that *FLT3*-ITD mutations are unstable at follow up and may occur for the first time at relapse. Therefore, *FLT3*-ITD is not a reliable marker for minimal residual disease in acute myeloid leukemia.

Key words: internal tandem duplication, acute myeloid leukemia, minimal residual disease, *FLT3*, mutations.

*Citation: Nazha A, Cortes J, Faderl S, Pierce S, Daver N, Kadia T, Borthakur G, Luthra R, Kantarjian H, and Ravandi F. Activating internal tandem duplication mutations of the fms-like tyrosine kinase-3 (*FLT3*-ITD) at complete response and relapse in patients with acute myeloid leukemia. Haematologica 2012;97(8):1242-1245. doi:10.3324/haematol.2012.062638*

Introduction

Survival of younger patients with acute myeloid leukemia (AML) has improved because of advances in supportive care measures as well as in the management of the favorable subgroup. Despite this, only approximately 40% of the patients will be cured and the majority of patients will relapse and die from their disease.[1] More recently, extensive effort has been directed at identifying molecular markers which could detect minimal residual disease (MRD) and predict early relapse, thereby allowing for early therapeutic interventions.

Recent studies have demonstrated a significant correlation between MRD and the outcome in patients with AML.[2,3] A number of molecular markers have been used to detect MRD in AML including specific products of fusion genes such as *AML1/ETO* arising from t(8;21), *CBFB/MYH11* from inv(16), and *PML/RARα* fusion transcript arising from the t(15;17).[4-6] Reappearance or persistence of some of these markers after induction chemotherapy or during follow up has been shown to have a significant impact on early relapse and prognosis. However, the use of these markers is limited to patients carrying the specific translocations, leading to the search for a more universally detected marker such as *WT1* and *NPM1*.[7,8]

FMS-like tyrosine kinase 3 (*FLT3*) mutations are among the most frequent molecular aberrations reported in patients with AML. Two main types have been identified: internal tandem duplication mutations (ITDs) detected in 20-25% of patients with AML, and tyrosine kinase domain (TKD) point mutations occurring in 5-10%.[9,10] Recent studies have suggested that higher allele burden of *FLT3*-ITD is associated with higher early relapse rate and inferior overall survival (OS) in patients with normal karyotype.[11,12] However, the prognostic impact of *FLT3*-TKD mutation remains controversial.[13]

Similarly, previous studies have shown conflicting results regarding the role of *FLT3*-ITD mutations as a marker for MRD monitoring in patients with AML.[14-17] In this study, we investigated the prognostic implication of *FLT3*-ITD mutation status at remission and relapse in patients with AML and explored its potential role as a marker for MRD.

Design and Methods

Patients

We conducted a retrospective, chart review analysis of patients with AML who were treated at the University of Texas - MD Anderson Cancer Center between October 2004 and March 2010. All patients were treated on front-line phase II induction protocols containing idarubicin and cytarabine (IA) with or without sorafenib (S), vorinostat

Manuscript received on January 19, 2012. Revised version arrived on March 13, 2012. Manuscript accepted on April 17, 2012.
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(V), or tipifarnib (T), and had bone marrow biopsy or aspiration, cytogenetic, and molecular studies at the time of diagnosis. Cytogenetic and molecular studies at complete remission (CR) and relapse were performed at the discretion of the treating physician. All patients gave their informed consent to participate in the study and the trials were conducted in accordance with the Declaration of Helsinki. All studies have been approved by the Institutional Review Board committee at the University of Texas - MD Anderson Cancer Center. CR and relapse were defined as described previously.[18] Bone marrow samples at diagnosis, CR and first relapse were reviewed for the presence of *FLT3*-ITD mutated clones.

Detection of FLT3 mutations

FLT3-ITD mutation status was determined in DNA from initial, post-induction and relapsed unsorted BM aspirate samples by a PCR-based method with an analytical sensitivity of 1-2% mutation-bearing cells. Genomic DNA from bone marrow samples was isolated using the Autopure extractor (QIAGEN/Gentra, Valencia, CA, USA). *FLT3*-ITD levels were determined by a semi-quantitative DNA-based polymerase chain reaction-capillary electrophoresis (PCR-CE) assay, as described previously.[19]

Statistical analysis

Differences among variables were evaluated by the χ^2 test and Mann-Whitney *U* test for categorical and continuous variables, respectively. All *P* values were two-sided and $P<0.05$ was significant.

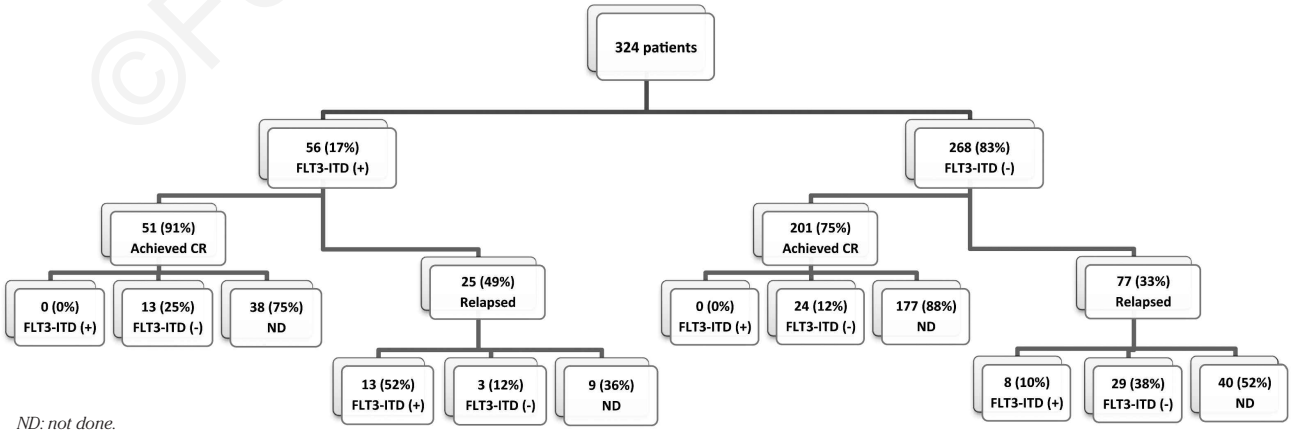
Results and Discussion

Three hundred and twenty-four patients were included in the final analysis. Fifty-six (17%) patients had *FLT3*-ITD mutations. Cytogenetic profile at diagnosis in *FLT3*-ITD mutated patients included normal karyotype in 37 (67%) patients, chromosome 5 and 7 abnormalities in 4 (7%), trisomy 8 in 2 (3%), complex karyotype in 2 (3%), insufficient metaphases in 2 (3%), and miscellaneous in 9 (17%). The clinical characteristics for the entire group as well as for the *FLT3*-ITD mutated patients are summarized in Table 1. Patients with *FLT3*-ITD mutation at presentation had higher WBC counts, lower platelet counts, lower hemoglobin and a higher percentage of blasts in the bone marrow and peripheral blood compared with those without *FLT3*-ITD mutation; this agrees with previous reports.[17] Fifty-one (91%) of the 56 patients with *FLT3*-ITD mutation achieved CR following induction chemotherapy. Thirteen (25%) patients had available samples at CR and all became negative for the *FLT3*-ITD (38 patients did not have samples tested at CR). Among the 51 patients achieving CR, 25 (49%) have relapsed with a median time to relapse of 31 weeks (range 4-86). Thirteen (52%) patients had *FLT3*-ITD at relapse and 3 (12%) patients were negative for the mutation (9 patients did not have samples tested at relapse). Among the 268 patients without *FLT3*-ITD at diagnosis, 201 (75%) achieved CR with none of the 24 patients testing positive for *FLT3*-ITD mutation. Interestingly, 77 (33%) patients relapsed with 8 (10%) patients acquiring a *FLT3*-ITD clone at relapse (Figure 1). Karyotype changes were identified in 6 patients whose *FLT3*-ITD status changed at relapse. Table 2 summarizes the cytogenetic changes associated with the changes in *FLT3* mutational status.

Monitoring MRD using sensitive molecular analysis is becoming an important aspect of treatment of patients with leukemia with the potential for early detection of impending relapse and the opportunity for prompt therapeutic

Table 1. Patients' characteristics in FLT3-ITD mutated and unmutated patients.

Parameter	FLT3-ITD (+)	FLT3-ITD (-)	*P*
Number (%)	56 (17)	268 (83)	
Median age, years (range)	51 (17-68)	53 (18-73)	0.18
Median WBC x 10^9/L (range)	16.7 (0.8-196.2)	4.1 (0.3-119)	<0.001
Median platelets x 10^9/L (range)	41.5 (6-189)	52 (2-676)	0.04
Median hemoglobin g/dL (range)	8.1 (5-10.8)	8.6 (2-13.9)	0.02
Median peripheral blood blast % (range)	56 (0-97)	11 (0-98)	<0.001
Median bone marrow blast % (range)	74 (16-98)	42 (5-96)	<0.001
ECOG performance status, n. (%)			0.08
0-2	54 (96)	267 (99)	
> 2	2 (4)	1 (1)	
AML history			0.09
de novo, n. (%)	53 (95)	232 (87)	
secondary, n. (%)	3 (5)	36 (13)	



ND: not done.

Figure 1. Schema describing the FLT3-ITD status at CR1 and first relapse.

Table 2. Karyotype analysis in patients with a change in FLT3-ITD status between diagnosis and relapse.

N. patients	Diagnosis	Relapse
5	Normal karyotype	Normal karyotype
1	-5q	Normal karyotype
2	Miscellaneous*	Normal karyotype
1	inv (9)	del (16)
1	Normal karyotype	Complex
1	+8	Normal karyotype

**One patient with del (16)(?,q22), and the other with +21*

interventions. The identification of *FLT3*-ITD mutation as one of the most frequent molecular aberrations in AML leads to its potential role as a marker for MRD in AML. The optimal marker for detecting MRD should be present at diagnosis as well as relapse. Previous studies have produced contradictory results regarding the role of *FLT3*-ITD mutation as a marker of MRD with several reports suggesting lack of consistent recurrence of the aberration at relapse.[14-17] Kottaridis *et al.* showed that *FLT3* mutations were unstable and recommended that they should be used only with great caution for MRD monitoring.[15] Among the 18 patients with positive *FLT3*-ITD mutation at diagnosis included in their study, 5 had no mutation at relapse.[15] These data are consistent with those previously reported by Shih *et al*. and Holvland *et al.*[14,16] as well as with our results reported above. On the contrary, Schnittger *et al.* suggested that *FLT3*-ITD mutations are reliable for monitoring MRD and early detection of relapse was possible in some cases in their series.[17,20] However, not all of the patients with positive *FLT3*-ITD mutation at presentation reported in their study had the mutation at relapse.[20] Among the 97 patients included in the study, 8 (8%) patients had discordant *FLT3*-ITD status between diagnosis and relapse.[20]

Interestingly, in our study, 8 of 77 (10%) of the patients with negative *FLT3*-ITD at presentation had a positive mutation at relapse suggesting that *FLT3* mutations may also be acquired and are not necessarily the primary leukemogenic event. Their acquisition may also be the driving force behind the worse than expected responses seen in some patients at relapse. Pratz *et al.* have shown that the inhibition of FLT3 autophosphorylation in *FLT3*-ITD positive cells does not by itself guarantee cell death, suggesting that some *FLT3*-ITD AML cells may not be addicted to the FLT3 signaling pathway. Furthermore, relapsed *FLT3*-ITD AML samples were more sensitive and responsive to FLT3 inhibitors compared to samples at diagnosis.[21] Additionally, a previous gene expression profiling study showed that not all *FLT3*-ITD cells harbor a respective ITD-specific gene expression pattern, suggesting that other alterations in *FLT3* or other genes such as JM domain point mutation or TKD (K663Q) mutation would have to account for the FLT3 pathway activation signature in these cases.[22]

Higher mutant/wild-type allelic ratio of *FLT3*-ITD mutation has been shown to correlate with more aggressive disease, higher early relapse rate in the first year and decreased overall survival.[21,23] Unfortunately, these data were not available for our patients and were not reported in this study; we could not, therefore, identify any potential variation in the MRD detection between patients with high and low allele burden. Another limitation of our study is the small sample size with only a small number of patients with molecular testing at CR and relapse.

In conclusion, *FLT3* mutations are likely secondary events occurring randomly in the previously transformed leukemogenic clone. *FLT3*-ITD mutations cannot be used as a reliable MRD marker since their presence at diagnosis and relapse is variable and unpredictable. Further studies to better understand the role of these mutations and their relationship to the primary leukemogenic events are warranted.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

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