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Acute Myeloid Leukemia

Limited value of *FLT3* mRNA expression in the bone marrow for prognosis and monitoring of patients with acute myeloid leukemia

We studied wild-type *FLT3* mRNA expression at diagnosis in bone marrow samples from 85 patients with acute myeloid leukemia (AML), 23 of whom were in complete remission, and determined its utility as a marker for minimal residual disease (MRD). We conclude that *FLT3* expression is of limited value as a prognostic marker and for MRD monitoring.

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FLT3, expressed in the blast cells in a majority of patients with acute myeloid leukemia (AML),[1,2] is a novel therapeutic target[3] and might be a candidate for minimal residual disease (MRD) monitoring. Only a few studies are available regarding *FLT3* mRNA expression in leukemic cells in AML patients,[4-6] and to our knowledge this is the first report concerning the use of *FLT3* mRNA expression for MRD monitoring. Eighty-five non-consecutive previously untreated adult AML patients were studied in this retrospective analysis. Their clinical characteristics are given in Table 1. The diagnosis of AML was established according to FAB criteria. Induction therapy was daunorubicin 45 mg/m^2 days 1-3, etoposide 100 mg/m^2 days 1-5, and cytosine arabinoside 2×100 mg/m^2 days 1-7 (n=79) and ATRA-containing chemotherapy for M3 patients (n=6). Fifteen patients underwent allogeneic stem cell transplantation in first complete remission (CR). Real-time polymerase chain reaction (PCR) from cDNA from unsorted frozen bone marrow (BM) or peripheral blood mononuclear cells was performed with the ABI Prism 7000 Sequence Detector (Applied Biosystems (AB)) according to the manufacturer's instructions with *FLT3*-specific primers (AB, Assay ID: Hs00174690). β-actin (AB, pre-developed VIC™-labeled TaqMan® Assay) was used as an endogenous control. Delta values were calculated as the difference between CT (threshold cycle) values ($\Delta CT = CT_{\beta\text{-actin}} - CT_{FLT3}$). Material for *FLT3*-ITD detection was available for 77/85 patients; *FLT3*-ITD assessment was performed as previously described.[7] BM of 4 patients staged for lymphoma without BM involvement was used as a control. Mean *FLT3* mRNA expression in these samples was taken as having a value of 1. *FLT3* mRNA was overexpressed in 84 of 85 patients (range 0.6- to 214-fold, median 22.09-fold) in comparison to our controls.

We found that high *FLT3* expression correlated with a high percentage of BM blasts (p=0.002) and FAB classification (M5 was associated with low and M1 with high expression, p=0.002 and p=0.04, respectively). Regarding clinical parameters, we found no correlation with cytogenetic risk groups, white blood count and serum lactate dehydrogenase, but a skewed sex distribution with higher expression in males. Expression levels were higher in patients with FLT3-ITD, but the difference was not statis-

Table 1. Clinical characteristics and laboratory parameters of the 85 patients.

FLT3 mRNA expression (median)	22.09-fold
Sex	M=38 (45%)
Age (median)	57 years
WBC (median)	27.2×10^9/L
LDH (median)	385 U/L
BM blasts n=53	74%
FAB classification	n=80
M0	5 (6%)
M1	14 (18%)
M2	12 (15%)
M3	6 (8%)
M4Eo	6 (8%)
M4	17 (21%)
M5	16 (20%)
M6	4 (5%)
Cytogenetics	n=82
Good risk	17 (21%)
Intermediate risk	52 (63%)
Poor risk	13 (16%)
Median observation time	25.1 months
Median overall survival	26.6 months
Overall survival at 60 months	35.5%
CR rate	78 (92%)
FLT3-ITD: n=77	
FLT3-ITD+	16 (21%)
FLT3-ITD-	61 (79%)

p values are not significant unless indicated: p<0.05. Associations between FLT3, mRNA expression and clinical and laboratory parameters are described by Spearman's correlation. Data analysis was performed using WinStat® for Windows (1996 Version 3.3). For cytogenetic analysis,15-20 metaphases were analyzed. The definition of high, intermediate and low risk groups was that of the MRC AML 10 Trial.[10]

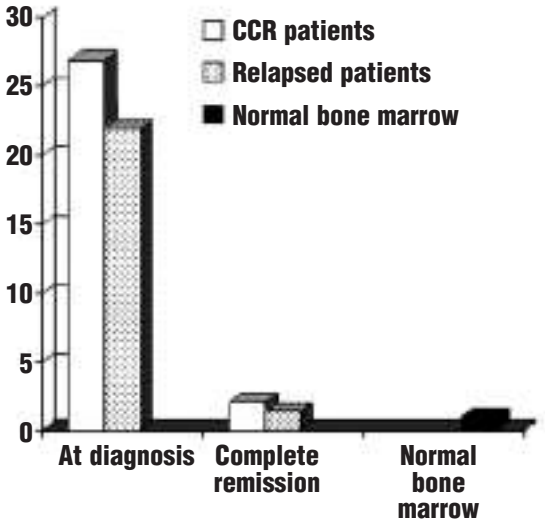

Figure 1. ***FLT3*** **expression in patients in CCR and relapsed patients at diagnosis and at time of remission. At diagnosis the patients showed a median 26.9-fold (CCR patients) or 21.9-fold (relapsed patients) FLT3 overexpression compared to normal bone marrow (normal bone marrow expression=1). In CR the expression decreased to a mean of 2.1-fold (CCR patients) or 1.4-fold (relapsed patients) higher than in normal bone marrow.**

tically significant (p=0.19). In univariate analysis we found no influence of *FLT3* expression on overall survival (OS) (p=0.54), even when M3 patients were excluded, which is in concordance with a report by Ozeki *et al.*, who could not find an association between overexpression of *FLT3* transcript and outcome in 119 AML patients.[4] In contrast, Bullinger *et al.* described an association of elevated *FLT3* mRNA expression levels with poor clinical outcome.[5] We found no association between OS and *FLT3* expression in patients lacking *FLT3*-ITD (n=61, p=0.13), in contrast to Ozeki who described that overexpression was associated with poor OS in this group.[4,8] However, our cut-off level is not comparable to that used by Ozeki.[4] We could not reproduce the data by Libura *et al.* concerning particularly high *FLT3* expression in M5 and low expression in M3 but, like us, this group found no association between *FLT3*-ITD and expression levels.[6] Differences in patient selection or PCR protocols might account for the differences observed.

CR samples were available for 23 patients, enabling us to measure the dynamics of *FLT3* mRNA expression during the course of disease. These patients were divided into a group with continuous CR (CCR >1 year, mean disease-free survival: 57 months) (n=9) and a group of patients who relapsed (n=14, mean disease-free survival: 15 months). In 7 cases we had samples from the time of relapse, in two cases samples in long-term CR at 15 or 60 months, respectively. The median expression level at diagnosis was 26.9 in the CCR group and 21.9 in the relapse group. In CR *FLT3* expression decreased to levels similar to those in healthy donors (median expression in the CCR group 2.1-fold, in the relapse group 1.4-fold). *FLT3* levels remained low in continuous CR and increased at the time of relapse. The drop in *FLT3* expression from diagnosis to CR was not significantly different between the CCR and the relapse group (Figure 1).

We show that *FLT3* mRNA is overexpressed in almost all patients with AML, but the degree of overexpression in diagnostic BM samples has no influence on OS. A good marker for MRD monitoring should decrease by more than 3 logs in CR, which was never the case in our group of patients, because the background expression in normal bone marrow was too high to allow for sensitive detection of MRD.[9] *FLT3* Therefore, we suggest that the usefulness of *FLT3* expression as a marker for MRD is limited.

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