Brief report

The \textit{FLT3}ITD mRNA level has a high prognostic impact in \textit{NPM1} mutated, but not in \textit{NPM1} unmutated, AML with a normal karyotype

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The impact of a \textit{FLT3}-internal tandem duplication (\textit{FLT3}ITD) on prognosis of patients with acute myeloid leukemia (AML) is dependent on the ratio of mutated to wild-type allele. In 648 normal karyotype (NK) AML patients, we found a significant independent effect of the quantitative \textit{FLT3}ITD mRNA level—measured as \textit{(FLT3}ITD/wt\textit{FLT3})/(\textit{FLT3}ITD/wt\textit{FLT3} + 1)—on outcome. Moreover, this effect was clearly seen in 329 patients with a mutated \textit{NPM1} gene (\textit{NPM1}+), but not in 319 patients without a \textit{NPM1} mutation (wt\textit{NPM1}). In a multivariate Cox regression model, the quantitative \textit{FLT3}ITD mRNA level showed an independent prognostic impact on overall survival (OS) and relapse-free survival (RFS) only in the \textit{NPM1}+ subgroup (OS: hazard ratio, 5.9; [95% confidence interval [CI]: 3.1-11.2]; RFS: hazard ratio, 7.5 [95% CI: 3.4-16.5]). The \textit{FLT3}ITD mRNA level contributes to relapse risk stratification and might help to guide postremission therapy in \textit{NPM1}-mutated AML. (\textit{Blood}. 2012;119(19):4383-4386)

Introduction

The prognosis of normal karyotype–acute myeloid leukemia (NK-AML) is influenced by the presence of gene mutations. \textit{NPM1} has been shown to be the most common single mutated gene in NK-AML occurring with a frequency of \textasciitilde 50%. Combinations of \textit{NPM1} mutations with \textit{FLT3}-internal tandem duplication (\textit{FLT3}ITD) have been described in \textasciitilde 20% of patients with NK-AML.1,2 The positive prognostic impact of the \textit{NPM1}+ on outcome is mainly evident in patients lacking a \textit{FLT3}ITD. Approximately 60% of patients carrying the \textit{NPM1}+/\textit{FLT3}–wild-type genotype survive > 10 years.3,4 The \textit{NPM1}+ NK-AML has been classified as an own entity of favorable prognosis in the revised World Health Organization and European LeukemiaNet classifications.5,6 Since 2001, there have been reports that not only the presence of a \textit{FLT3}ITD per se, but also the \textit{FLT3}ITD/\textit{FLT3}–wild-type (wt\textit{FLT3}) ratio is essential for prognosis.7,8 The aim of our work was to assess the influence of the \textit{FLT3}ITD mRNA level according to the mutation status of \textit{NPM1}.

Methods

Patients

Our analyses were based on patients with NK-AML treated within the AML Cooperative Group 99 study.9 Patients were randomly assigned for induction therapy with either TAD (thioguanine, conventional-dose AraC, daunorubicin) followed by HAM (high-dose AraC, mitoxantrone) or 2 courses of HAM. As consolidation therapy in first complete remission (CR), allogeneic transplantation from an unrelated donor was recommended for high-risk patients < 60 years whereas all other patients received treatment with TAD and maintenance therapy.9

End points

Overall survival (OS) was calculated from randomization to death from any cause or to the latest follow-up. Relapse-free survival (RFS) was determined from the first day of CR until relapse or death in CR.

Molecular analyses

Mutation analyses of \textit{NPM1}, \textit{FLT3}ITD, \textit{FLT3}–tyrosine kinase domain (\textit{FLT3}TKD), \textit{MLL}–partial tandem duplication (\textit{MLL}-PTD), and \textit{CEBPA} were performed according to standard protocols previously described.10-12 \textit{FLT3} mRNA RT-PCR and PCR were performed according to standard protocols.13 Labeled PCR products were electrophoresed on ABI 3100 (Applied Biosystems) according to protocol. The data were collected and analyzed with Genescan and Genotyper software (Applied Biosystems). The ratio of \textit{FLT3}ITD mRNA to wt\textit{FLT3} mRNA was calculated as previously published.8,14 The amount of \textit{FLT3}ITD mRNA in relation to the entire \textit{FLT3} transcript signal was defined as: quantitative “\textit{FLT3}ITD mRNA level” = \textit{(FLT3}ITD/wt\textit{FLT3})/(\textit{FLT3}ITD/wt\textit{FLT3} + 1).
Statistical analyses

Univariate Cox regression for OS was first performed in the complete cohort to evaluate the prognostic value of the quantitative FLT3 ITD mRNA level, independent of NPM1. For visualization of significant effects, we grouped patients according to the FLT3 ITD mRNA level using 5 potential threshold values. To reduce the potential bias of data-derived cutpoints, we fixed the biologically meaningful thresholds 0.00, to distinguish between FLT3 ITD and wt FLT3, 0.50, indicating a heterozygous mutation, and 1.00, indicating complete wild-type loss. In addition, we investigated the values 0.25 and 0.75 as potential thresholds. Very small patient groups (\(n = 11349\)) were combined to the next larger adjacent group.

Multiple Cox regression using the quantitative FLT3 ITD mRNA level, together with its interaction with NPM1 and clinical and molecular characteristics, was performed for OS and RFS. Kaplan-Meier estimation for OS and RFS and multiple Cox regression was also performed separately for NPM1-mutated and wtNPM1 patients. A significance level of 5% was used.

Results and discussion

Analyses were performed in 648 of 802 patients treated within the AMLCG99 trial (supplemental Figure 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article).

Patients (119 of 648) received allogeneic transplantation in first CR. Median follow-up for OS was 62.3 months. Median OS was 20.4 months with 414 events. In 427 of 648 (66%) patients in CR, median RFS was 18.0 months. In 173 of 648 FLT3 ITD-mutated patients, the median FLT3 ITD level was 0.42 (0.02-1.00). Patient characteristics are summarized in supplemental Tables 1 and 2.

Impact of FLT3 ITD mutation level on OS and definition of thresholds

Univariate Cox regression showed a significant impact of the FLT3 ITD mRNA level on OS (hazard ratio of 1.12 for a FLT3 ITD mutation level increased by 0.10, 95% confidence interval [CI], 1.08-1.17, \(P < .0001\)). Grouping patients using the prespecified threshold values, median OS for FLT3 ITD mRNA level 0.00 (\(n = 471\) of 648; 73%), 0.01-0.24 (\(n = 31\) of 648; 5%), 0.25-0.49 (\(n = 91\) of 648; 14%), 0.50-0.74 (\(n = 38\) of 648; 6%), and 0.75-1.00 (\(n = 17\) of 648; 3%) were 26, 24, 12, 8, and 8 months. The threshold level of 1.00 was excluded because only 7 patients had a complete wild-type loss. Because of the low patient number, FLT3 ITD-positive patients with a level below 0.25 were combined with those with a level between 0.25 and 0.50 into a low-level (0.01-0.49) FLT3 ITD group. Similarly,
patients with a positive FLT3 ITD mRNA level ≥ 0.50 were combined to one high-level (0.50-1.00) group. Finally, only the biologic meaningful cutoffs 0.00 and 0.50 were retained. Median OS in FLT3 ITD-negative (73%), low-level (19%), and high-level FLT3 ITD (8%) were 26.2, 15.6, and 7.8 months, respectively (P < .001).

Impact of FLT3 ITD mutation level on outcome according to NPM1 mutation status

In the NPM1-mutated cohort, median OS was 97.8 months in the FLT3 ITD-negative, 15.6 months in the low-level (0.01-0.49), and 8.2 months in the high-level FLT3 ITD (0.50-1.00) group (P < .001, Figure 1A). Significant differences between these risk groups were evident regarding RFS (P < .001; Figure 1B). Median OS in wtNPM1 patients without a FLT3 ITD, with a FLT3 ITD level < 0.50 and ≥ 0.50 were not statistically different (16.8 months, 12.8 months, and 6.0 months, respectively, P = .133, Figure 1A). FLT3 ITD mRNA level may not impact on survival in patients with wtNPM1, although this conclusion is limited by the low statistical power because of the relatively small number of patients with a high FLT3 ITD mRNA level (n = 9; 1%).

In the multivariate Cox regression model with all 648 patients, the independent prognostic impact of the quantitative FLT3 ITD mRNA level on outcome was detectable in NPM1+ patients (P < .001), but not in wtNPM1 (Table 1). This was true for both age subgroups (≤60 years, data not shown). In multiple regression in NPM1+ patients, the FLT3 ITD low-level group had an adjusted hazard ratio of 1.5 (95% CI, 0.96-2.3) for OS (P = .078), and the FLT3 ITD high-level group an adjusted hazard ratio of 3.1 (95% CI 1.9-5.2, P < .001) compared with wtFLT3 (supplemental Table 3). Within wtNPM1 patients, the FLT3 ITD mRNA level did not appear as an independent prognostic factor. Similar results were observed for RFS.

Whitman et al were the first to show that a complete loss of wtFLT3 was associated with worse outcome compared to patients without a FLT3 ITD (wtFLT3/wtFLT3) or a heterozygous FLT3 ITD (wtFLT3/FLT3 ITD) mutation. Thiede et al defined the FLT3 ITD/wt ratio as the relative proportion of the area under the curve (AUC) of mutant and wtFLT3 alleles (AUC-FLT3 ITD/AUC-wtFLT3) in Genescan analysis. A FLT3 ITD/wtFLT3 ratio above the median of the cohort was associated with an unfavorable prognosis. Median-defined risk groups have to be determined in large patient cohorts before a definite statement about individual prognosis can be made. In contrast, we defined the FLT3 ITD mRNA level as the relative amount of FLT3 ITD mRNA to the total FLT3 transcript, with a range from 0 (absence of mutation) to 1 (complete loss of wild type), facilitating the estimation of the FLT3 ITD mutational load. This has the advantage of direct estimation of individual prognosis according to a patient’s FLT3 ITD mutant level and better comparability in different clinical studies.

The focus of our analyses was the investigation of the impact of the FLT3 ITD mRNA level according to the NPM1 mutation status in NK-AML. Univariate and multivariate analyses demonstrated a distinct dose-dependent effect of the FLT3 ITD mutant level on OS and RFS only in NPM1+, but not in wtNPM1 patients. In NPM1-mutated patients, multivariate analyses revealed a FLT3 ITD level of 0.50 as cutoff between an intermediate group (26% long-term survivors) and a poor-risk group with 9% survivors in 7 years. In accordance with Whitman et al, these observations suggest different pathophysiology of heterozygous FLT3 ITD versus FLT3 ITD with a complete loss of the wild-type allele.

Our data suggest a significantly worse outcome with regard to OS and RFS for patients harboring an NPM1 mutation and higher FLT3 ITD mRNA expression compared to those NPM1-mutated patients with a low FLT3 ITD mRNA expression. Thus, the FLT3 ITD mRNA level might guide the decision for allogeneic
transplantation in NPM1\(^+\) AML. However, such a strategy should be prospectively evaluated.

**Authorship**

Contribution: F.S. performed statistical analysis and wrote the manuscript; E.H., M.U., A.H., and M.C.S. provided statistical support; S.S., A.D., T. Benthaus, G.M., E.Z., and P.M.K. performed molecular diagnostics; S.K.B., M.F.-B., C.B., J.B., and K.S. performed central diagnostics; W.E.B., T. Büchner, B.J.W., and W.H. were principal investigators of AMLCG99 study; and K.S. wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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**References**

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