Brief report

NPM1 but not FLT3-ITD mutations predict early blast cell clearance and CR rate in patients with normal karyotype AML (NK-AML) or high-risk myelodysplastic syndrome (MDS)

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Mutations in the NPM1 gene represent the most frequent genetic alterations in patients with acute myeloid leukemia (AML) and are associated with a favorable outcome. In 690 normal karyotype (NK) AML patients the complete remission rates (CRs) and the percentage of patients with adequate in vivo blast cell reduction 1 week after the end of the first induction cycle were significantly higher in NPM1+ (75% and 80%, respectively) than in NPM1– (57% and 57%, respectively) patients, but were unaffected by the FLT3-ITD status. Multivariate analyses revealed the presence of a NPM1 mutation as an independent positive prognostic factor for the achievement of an adequate day-16 blast clearance and a CR. In conclusion, NPM1+ blast cells show a high in vivo sensitivity toward induction chemotherapy irrespective of the FLT3-ITD mutation status. These findings provide insight into the pathophysiology and help understand the favorable clinical outcome of patients with NPM1+ AML. (Blood. 2009;113:5250-5253)

Introduction

In patients with a normal karyotype acute myeloid leukemia (NK-AML) submicroscopic genetic markers are essential for risk stratification according to the AMLCG 1999 study protocol. The details of the study have been published previously.11 Cytomorphology, cytogentic, and molecular analyses of bone marrow (BM) aspirates were performed according to standard protocols.1,12,13

All pretreatment clinical (age, sex, performance status [ECOG score], AML de novo, white blood cell count [WBC], hemoglobin level, platelet count, lactase dehydrogenase [LDH], BM blasts) and molecular markers (NPM1, FLT3-ITD, FLT3-TKD, CEBPA, and MLL-PTD) were included in univariate and multivariate analyses. Outcome parameters, CR rate, and early blast clearance were dichotomous. For univariate analyses, we performed cross tables and 2-sided exact fisher test for categoric characteristics and univariate logistic regression for categoric and continuous parameters. In multiple logistic regression, independent prognostic factors were identified by backward elimination using the Wald statistic14,15 with significance level α = .05. Since with regard to overall survival an interaction effect of NPM1 and FLT3-ITD is known,13 we analyzed a potential interaction effect on CR rate or early blast cell clearance by including the interaction term together with the main parameters NPM1 and FLT3-ITD in multiple logistic regression.

Results and discussion

We investigated the influence of pretherapeutic markers on early therapeutic response parameters in patients with NK-AML enrolled in the AMLCG 1999 trial.11 Patient characteristics are summarized in Table S1 (available on the Blood website; see the Supplemental Materials link at the top of the online article).


The online version of this article contains a data supplement.

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In 690 patients the FLT3-ITD mutation status and the NPM1 mutation status could be analyzed. The NPM1 mutation was present in 51% (n = 352/690) of patients. The FLT3-ITD mutation was found in 29% (n = 200/690) of patients.

Patients with known NPM1 and FLT3-ITD mutation status were divided into 4 groups: NPM1+/FLT3-ITD- (n = 211/690, 31%), NPM1+/FLT3-ITD+ (n = 141/690, 20%), NPM1-/FLT3-ITD- (n = 279/690, 40%), and NPM1-/FLT3-ITD+ (n = 59/690, 9%). In 589 patients with known NPM1/FLT3-ITD mutation status information about the early blast cell clearance was available.

NPM1 but not FLT3-ITD mutations predict early response parameters

Of 690 patients, 66% achieved a CR, 10% had persistent leukemia (PL), 15% died of early death (ED), and 9% remained hypoplastic. Seventy-five percent of patients carrying the NPM1 mutation and 57% of NPM1-negative patients achieved a CR (P < .001). CR rates did not differ significantly between the FLT3-ITD+ (68%) and the FLT3-ITD- (65%) group (P = .480). Response to induction therapy differed according to the combination of the NPM1 and FLT3-ITD mutations. In NPM1 mutated AML, the CR rates were 77% (NPM1+/FLT3-ITD-) and 71% (NPM1+/FLT3-ITD+). Significantly lower CR rates of 56% (NPM1-/FLT3-ITD-) and 58% (NPM1-/FLT3-ITD+) were found in NPM1 unmuted AML (P < .001).

The amount of residual leukemic blasts in the BM measured 1 week after the end of induction therapy (ie, on day 12 in the HAM regimen; on day 16 in the TAD regimen) can be used as an early independent prognostic parameter of response to therapy9 and is referred to as early blast cell clearance. In our data set the early blast clearance did not differ significantly between patients treated with either TAD or HAM as first induction course (P = .661). Four-hundred nine (68%) of 598 patients showed less than 10% of BM blasts 1 week after the end of induction chemotherapy.

Eighty percent of NPM1+ patients (80%: NPM1+/FLT3-ITD-; 79%: NPM1+/FLT3-ITD+) but only 57% of patients without the NPM1 mutation (58%: NPM1-/FLT3-ITD-; 52%: NPM1-/FLT3-ITD+) showed a residual blast cell percentage of less than 10%. Thus, the NPM1 mutation was associated with a significantly higher blast clearance (P < .001) compared with the NPM1 wild-type (WT) genotype.

Seventy-one percent of FLT3-ITD–positive and 67% of FLT3-ITD–negative patients showed a blast reduction to less than 10%. Thus the presence of the FLT3-ITD had no influence (P = .498) on the blast cell clearance (Table 1).

Similar results were seen in younger (< 60 years) and older (≥ 60 years) patients (Table S2).

The NPM1 mutation has independent prognostic impact on the CR rate and early blast cell clearance in univariate and multivariate analyses

The CR rate was analyzed in a model of the 9 clinical and 5 molecular independent prognostic factors mentioned in “Methods.” Parameters
with prognostic impact on the CR rate in univariate analyses included the presence of the NPM1 mutation (OR: 2.31; 95% CI: 1.67-3.19) and de novo AML (OR: 2.14; 1.44-3.17), as well as the presence of the MLL-PTD mutation (OR: 0.46; 0.26-0.82), age (OR: 0.78; 0.69-0.88), and WBC (OR: 0.75; 0.60-0.95). In multivariate analysis, the parameter with independent favorable impact on CR rate was the presence of the NPM1 mutation (OR: 2.81; 1.84-4.29), whereas a high WBC (OR: 0.53; 0.39-0.72) and higher age (OR: 0.81; 0.69-0.94) were significantly associated with a lower CR rate.

Regarding the effect on early blast clearance in univariate analyses, the presence of the NPM1 mutation (OR: 2.92; 2.03-4.20) and a high LDH level (OR: 1.97; 1.11-3.50) were strong positive indicators, whereas the MLL-PTD mutation (OR: 0.46; 0.24-0.89) negatively influenced blast clearance. Parameters not showing a significant effect included the hemoglobin level, WBC, platelets, BM blasts, age, sex, performance status, AML de novo, and mutations of FLT3 and CEBPA. In multivariate analysis, independent predictive variables were the NPM1 status (OR: 3.12; 2.01-4.83) and BM blasts at diagnosis (OR: 0.90; 0.82-0.98; Table 2).

Our data show that the presence of a NPM1 mutation is associated with the initial response to chemotherapy and thus represents a marker of sensitivity toward induction chemotherapy in vivo.

Pathophysiologically, this hypothesis is supported by in vitro experiments in which leukemic blasts carrying the NPM1 mutation showed significantly higher rates of apoptosis after treatment with chemotherapeutic agents compared with the NPM1 WT leukemic cells. Abnormal activation of the transcription factor NF-κB has been described in leukemic blasts resistant to chemotherapy. Cilloni et al. showed that NPM1 plays a central role in enhancing apoptotic cell death in AML by interacting with NF-κB. Interestingly, NF-κB activity did not differ in NPM1+/FLT3-ITD+ and NPM1+/FLT3-ITD− blasts, supporting our in vivo data that the presence of the NPM1 mutation is associated with a higher sensitivity toward induction chemotherapy regardless of the FLT3-ITD mutation status.

These data allow further insight into the biology of AML and provide a rationale for the better clinical outcome of this genetic AML subgroup.

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Authorship
Contribution: F.S. performed statistical analysis and wrote the paper; E.H., M.U., S.F., A.H. and M.C.S. performed statistical analysis; S.S., A.D., T. Benthaus, G.M., and E.Z. performed molecular diagnostics; W.E.B. and B.J.W. coordinated the inclusion of patients into the AMLCG99 study and acquired patient data in cooperating hospitals; S.K.B., M.F.-B., C.B., J.B., and K.S. performed central diagnostics; K.S. wrote the paper; and W.E.B., T. Buechner, B.J.W., and W.H. were principal investigators of AMLCG99 study.

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

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References

Table 2. Impact of clinical and molecular parameters on early response parameters (multivariate analysis)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Comparison</th>
<th>P</th>
<th>OR</th>
<th>Lower CI</th>
<th>Upper CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial BM blasts, %</td>
<td>Pos vs neg</td>
<td>&lt;.001</td>
<td>3.12</td>
<td>2.01</td>
<td>4.83</td>
</tr>
<tr>
<td>Age, y</td>
<td>+ 10</td>
<td>.019</td>
<td>0.90</td>
<td>0.82</td>
<td>0.98</td>
</tr>
<tr>
<td>WBC, x 10^9/L</td>
<td>10-fold</td>
<td>&lt;.001</td>
<td>2.81</td>
<td>1.84</td>
<td>4.29</td>
</tr>
</tbody>
</table>
| NPM1, FLT3-ITD, MLL-PTD, CEBPA, and the clinical parameters age, sex, ECOG performance status, AML de novo, platelet count, hemoglobin level, LDH, and amount of BM blasts. The analyses were performed using 514 complete cases with regard to CR rate and 448 complete cases with regard to day-16 blast clearance for the candidate prognostic factors. The multivariate prognostic factors were identified using backward Wald logistic regression model with a significance level of 5%. OR indicates odds ratio for achieving a CR/early blast cell clearance; lower CI, lower limit of the 95% confidence interval; and upper CI, upper limit of the 95% confidence interval.

The candidate prognostic factors included were mutations of the molecular markers NPM1, FLT3-ITD, FLT3-ITD, MLL-PTD, CEBPA, and the clinical parameters age, sex, ECOG performance status, AML de novo, WBC, platelet count, hemoglobin level, LDH, and amount of BM blasts. The analyses were performed using 514 complete cases with regard to CR rate and 448 complete cases with regard to day-16 blast clearance for the candidate prognostic factors. The multivariate prognostic factors were identified using backward Wald logistic regression model with a significance level of 5%. OR indicates odds ratio for achieving a CR/early blast cell clearance; lower CI, lower limit of the 95% confidence interval; and upper CI, upper limit of the 95% confidence interval.


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