patients with azathioprine-related MDS. Our data suggest that long-term treatment with azathioprine increases the risk of developing secondary MDS about 100-fold over the risk of developing primary MDS in the general population. Patients in the hematology registry who did not receive long-term azathioprine treatment still had a considerably higher risk (>15-fold) than that of the general population. This may be attributable to mechanisms inherent to their hematological diseases or, more likely, to other DNA-damaging drugs such as cyclophosphamide.

Referring to an MDS registry, we may have missed cases of de novo AML developing after azathioprine treatment. Therefore, our data probably represent an underestimation of the true incidence of azathioprine-induced hematologic malignancies. Our analysis shows that sMDS following treatment with azathioprine is associated with abnormalities of chromosome 7. Frequent involvement of chromosome 7 is also seen in sAML/sMDS secondary to alkylating agents.

In our MDS registry, the median survival of patients with sMDS after azathioprine (9 months) was comparable to the median survival of 120 patients with MDS after chemotherapy (8 months). However, the survival among patients with azathioprine-related sMDS was significantly shorter than that in 2269 cases of primary MDS (9 vs 24 months) (p=0.00005). In conclusion, long-term treatment with azathioprine should be used with caution because it seems to be associated with an increased risk of developing MDS and subsequent leukemic transformation.

Saline Knipp,* Barbara Hildebrandt,° Jutta Richter, Rainer Haas,* Ulrich Gemming, Norbert Gattermann

*Department of Hematology, Oncology and Clinical Immunology;
°Institute of Human Genetics; Department of Rheumatology, Heinrich-Heine-University, Düsseldorf, Germany

Acknowledgments: we are grateful to Dr. G. Jacobs, Saarbrücken, Dr. A. Grüneisen (Berlin) and Dr. Giagounidis (Duisburg) for sending slides and comments on the course of diseases in two patients. We are also grateful to Dr. Tost, who performed FISH analyses.

Funding: this work was supported by Bundesministerium für Bildung und Forschung, Kompetenz-netz "Acute and chronic Leukämien".

Key words: azathioprine, hematologic malignancy, myelodysplastic syndrome, chromosome 7.

Correspondence: Saline Knipp, MD, Department of Hematology, Oncology and Clinical Immunology, Heinrich-Heine-University, Moorenstr. 5, 40225 Düsseldorf, Germany. Phone: international +49.211.8117780. Fax: international +49.211.8118853, E-mail: knipp@med.uni-duesseldorf.de

Acute Myeloid Leukemia

Microsatellite instability and p53 mutations are characteristic of subgroups of acute myeloid leukemia but independent events

Microsatellite instability (MSI) and p53 alterations which may represent major mechanisms of genetic instability, are rarely observed in de novo acute myeloid leukemia (AML) but may play a substantial role in subgroups characterized by either a myelodysplastic prephase (sAML), previous chemotherapy (tAML) or a complex aberrant karyotype.

We performed allelotyping and p53 mutation analysis in 75 patients with morphologically and cytogenetically classified AML.

Bone marrow samples were classified morphologically according to FAB-criteria and cytogenetically (GAG-band technique, fluorescent in situ hybridization of 5q, 7q, 11q, 17p). Clinically defined subgroups (sAML, tAML, de novo AML) were investigated for MSI and p53-tumor-suppressor gene mutations. AML with complex aberrant karyotype (cAML) was separately evaluated. MSI was investigated by a panel of 16 genomic loci including the consensus primers and regions prone to chromosomal rearrangements in AML (5q,7q,8q,11q,21q). Allelic imbalance in ≥2 loci was defined as MSI. Occurrence of shadow bands and artefacts (e.g. formamide slippage) was excluded by extensive control experiments. p53 mutation analysis was performed by polymerase chain reaction amplification with specific primers (exons 5-8) and subsequent sequence analysis (ABI PRISM™ 310 Genetic Analyzer). For statistical analyses, the χ² test (MSI) and Fisher’s exact test (p53 mutations) were applied. Seventy-five patients with a primary diagnosis of AML were studied (M2: 38.7%; M4: 22.7%; M5: 12%; M1: 9.3%; M6: 6.6%; M3: 2.6%; Table 1). Chromosomal aberrations were found in 42 patients (56.0%) including 10 cases (13.3%) with a complex aberrant karyotype. MSI (≥2 loci) was detected in 24 patients (32%). High frequency mutators were observed less frequently (≥3 loci: 13.3%; ≥4 loci: 6.6%).

References


haematologica/the hematology journal | 2005; 90(5) | 693 |
The highest rate of MSI was detected in loci frequently rearranged in AML (D7SS15 (14.7%), IRF 1 (13.3%)) whereas the consensus primers as defined in colorectal cancer were rarely involved in AML (0–6.7%).

MSI was significantly more frequent in cAML (60.0%, 6/10 patients) than in patients with a normal karyotype (21.1%, 7/26 patients, \(p=0.044\); Figure 1A) whereas the MSI rate in AML with cytogenetic aberrations was intermediate (34.4%; \(n=11/32\) patients). Similarly, MSI was significantly more frequent in patients with tAML/sAML (45%, 9/20 patients) than in patients with de novo AML and a non-complex karyotype (20.4%, 10/49 patients; \(p=0.038\), Figure 1B). p53 gene mutations (exon 5-8) were observed in 50% (4/8 patients) of cAML but in only 8.5% of AML with a non-complex karyotype (5/59 patients, \(p=0.009\)). p53 mutations were also more frequent in sAML/tAML (17.6%, 3/17 patients) than in de novo AML (2.4%; 1/42 patients) although this difference was not statistically significant because of the small number of p53 mutations (\(p=0.068\)). Surprisingly, the rate of p53 mutations was independent of the occurrence of MSI (13.3% vs. 13.6%).

Survival data from 37 patients (median follow-up 271 days) demonstrated the poor survival in patients with sAML/tAML or a complex aberrant karyotype (1/7 patients alive after a median follow-up of 159 days) and a remarkably poor clinical outcome in patients with p53-mutations (\(n=5\); all died after a median follow-up of 97 days) in contrast to patients with de novo AML (18/30 patients alive after median follow-up of 199 days; \(p=0.042\)).

MSI is a rare event in de novo AML, but the previously reported rates differ widely (0–43%). High MSI rates were found in small groups of patients with MDS or post-MDS AML (50%), \(1\) in patients with relapsed AML (50%) \(2\) or in genetically defined subgroups with alterations in cellular repair enzymes genes coding for (50-94%) (e.g. MSH2, \(p53\)). In accordance with previously published smaller studies we found statistically significant higher MSI rates in patients with complex aberrant karyotypes and sAML/tAML. With an observed rate of 13.4%, p53 mutations were only rarely detected in our study. These results are in line with previously obtained results (4.5%–16.7%). However, higher rates were detected in the subgroups of patients with complex aberrant karyotypes, sAML and tAML. Since the p53 gene directs cells to apoptosis or G1 cell cycle arrest after DNA damage, mutations in p53 were previously thought to be related to MSI. However, this hypothesis is not confirmed by our finding of similar MSI rates in patients with p53 mutations or wild type p53. Although p53 mutations and mismatch repair representing MSI are not specific for secondary AML with a myelodysplastic prephase, therapy-related AML and/or AML with complex aberrant karyotype, we conclude that in contrast to de novo AML, defects in mismatch repair and cell cycle regulation play significant but independent roles in malignant transformation of these subsets of AML. Additional work is needed to characterize more precisely these molecular mechanisms of DNA repair, which are involved in the malignant transformation of the hematopoietic cells.

Key words: leukemia, microsatellite, p53, mismatch repair.

Correspondence: Dr. Martin Dreyling, Dept. of Medicine III, University Hospital Grosshadern, LMU, Munich, Germany and GSF / Clinical Cooperative Group Leukemia, Munich; *Bundeswehr Institute of Radiobiology, Munich, Germany; †Dept. of Internal Medicine, Bundeswehr Hospital, Koblenz, Germany.

References


---

**Table 1. FAB subtypes, MSI rate (≥ 2 loci) and p53 mutations.**

<table>
<thead>
<tr>
<th>FAB subtype</th>
<th>all AML</th>
<th>sAML</th>
<th>tAML</th>
<th>chromosomal aberrations/complex karyotype</th>
<th>MSI</th>
<th>p53-mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML M 1</td>
<td>7</td>
<td>2</td>
<td>3/0</td>
<td>2 (28.6%)</td>
<td>0/6</td>
<td></td>
</tr>
<tr>
<td>AML M 2</td>
<td>29</td>
<td>9</td>
<td>19/5</td>
<td>10 (34.5%)</td>
<td>4/26 (15.4%)</td>
<td></td>
</tr>
<tr>
<td>AML M 3</td>
<td>2</td>
<td>1</td>
<td>2/0</td>
<td>0 (0%)</td>
<td>2/0</td>
<td></td>
</tr>
<tr>
<td>AML M 4</td>
<td>17</td>
<td>3</td>
<td>10/1</td>
<td>6 (35.2%)</td>
<td>2/16 (12.5%)</td>
<td></td>
</tr>
<tr>
<td>AML M 5</td>
<td>9</td>
<td>2</td>
<td>4/1</td>
<td>1 (11.1%)</td>
<td>0/7</td>
<td></td>
</tr>
<tr>
<td>AML M 6</td>
<td>5</td>
<td>0</td>
<td>3/2</td>
<td>3 (60.0%)</td>
<td>1/5 (20%)</td>
<td></td>
</tr>
<tr>
<td>Not classified</td>
<td>6</td>
<td>3</td>
<td>1/1</td>
<td>2 (33.3%)</td>
<td>2/5 (40%)</td>
<td></td>
</tr>
<tr>
<td>Total number</td>
<td>75</td>
<td>20</td>
<td>42/10</td>
<td>24 (32%)</td>
<td>9/67 (13.4%)</td>
<td></td>
</tr>
</tbody>
</table>

---

**Figure 1. Rate of MSI and p53-mutations (%) in cytogenetically (A) and clinically (B) defined AML. a. Complex aberrant karyotype; b. normal karyotype; c. sAML/tAML; d. de novo without complex karyotype.**

Gerhard Herzog,† Juxian Lu-Hesselmann,* Yvonne Zimmermann,* Torsten Hafelrach,* Wolfgang Hiddemann,* Martin Dreyling*

*Dept. of Internal Medicine III, University Hospital Grosshadern, LMU, Munich, Germany and GSF / Clinical Cooperative Group Leukemia, Munich; †Bundeswehr Institute of Radiobiology, Munich, Germany; †Dept. of Internal Medicine, Bundeswehr Hospital, Koblenz, Germany.

E-mail: martin.dreyling@med.uni-muenchen.de
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.

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

<table>
<thead>
<tr>
<th>FLT3 mRNA expression (median)</th>
<th>CR rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.09-fold</td>
<td>78 (92%)</td>
</tr>
<tr>
<td>FLT3-ITD: n=77</td>
<td>16 (21%)</td>
</tr>
<tr>
<td>FLT3-ITD*</td>
<td>61 (79%)</td>
</tr>
<tr>
<td>FLT3-ITD-</td>
<td>61 (79%)</td>
</tr>
</tbody>
</table>

*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. 9

FLT3, expressed in the blast cells of a majority of patients with acute myeloid leukemia (AML),12 is a novel therapeutic target7 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,13 and to our knowledge this is the first report concerning the use of FLT3 mRNA expression for MRD monitoring. Eighty-five non-competitive 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/m² days 1-3, etoposide 100 mg/m² days 1-5, and cytosine arabinoside 2×100 mg/m² 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 (ΔCT=CTFLT3−CTβ-actin). Material for FLT3-ITD detection was available for 77/85 patients; FLT3-ITD assessment was performed as previously described. 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-