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 rheumatology 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 rheumatological 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.[8] A Swedish group recently reported on seven patients with sAML or sMDS after treatment with azathioprine and cyclophosphamide, four of whom showed a complex karyotype including monosomy 7.[9]

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 sMDS after chemo/radiotherapy (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.

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

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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.

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Bone marrow samples were classified morphologically according to FAB-criteria and cytogenetically (GAG-banding 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-supressor 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).[1] 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 χ^2 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%).

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

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

The highest rate of MSI was detected in loci frequently rearranged in AML (D7S515 (14.7%), IRF 1 (13.3%)) whereas the consensus primers as defined in colorectal cancer were rarely involved in AML (0–6.7%).[1]

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 *s*AML, *t*AML 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%).[2-7] High MSI rates were found in small groups of patients with MDS or post- MDS AML (50%),[8] in patients with relapsed AML (35%)[6] 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 *s*AML/tAML.[3] 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%).[1,2,9,10] However, higher rates were detected in the subgroups of patients with complex aberrant karyotypes, *s*AML and *t*AML. 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.[3] However, this hypothesis is not confirmed by our finding of similar MSI rates in patients with *p53* mutations or wild

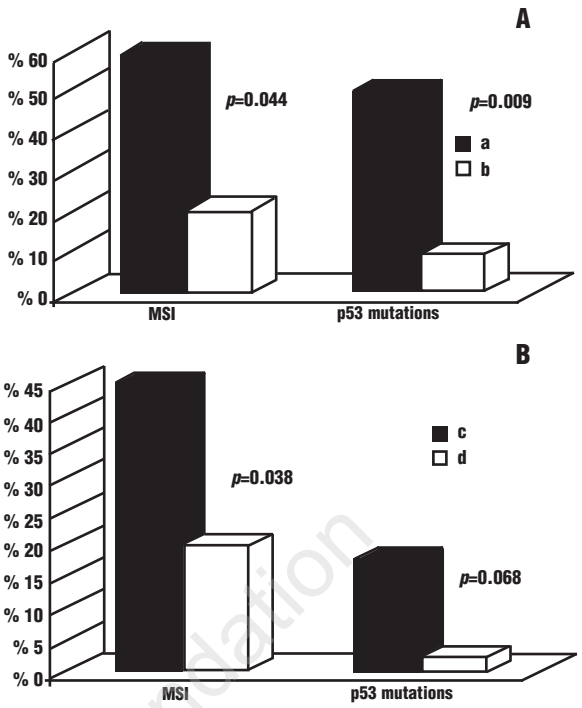

Figure 1. Rate of MSI and p53-mutations (%) in cytogenetically (A) and clinically (B) defined AML. a. Complex aberrant karyotype: b. normal karyotype; c. sAML//tAM: d. *de novo* without complex karyotype.

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.

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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]