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Association of specific cytogenetic aberrations with *mdr1* gene expression in adult myeloid leukemia and its implication for treatment outcome

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Background and Objectives. Cytogenetics and *mdr1* expression are established prognostic factors for treatment outcome in adult acute myeloid leukemia (AML). The association, however, between specific cytogenetic aberrations and mdr1 expression has not yet been examined in a large cohort of patients.

Design and Methods. We therefore looked for *mdr1* gene expression at diagnosis within specific cytogenetic aberrations in 331 previously untreated adult patients with *de novo* or secondary AML (not including t(15;17)) entered into the German SHG AML96 treatment trial.

Results. The proportion of *mdr1* positive blast probes was significantly higher in patients with aberrant karyotypes than in those with normal karyotypes (39% vs. 15%; $p<0.001$). Looking at specific cytogenetic aberrations significantly more *mdr1* positive AML patients were found within t(8;21), +8, +21, del(7q), del(5q), -7, abn(3q) and multiple aberrations. In contrast, no patient with inv(16) was positive for *mdr1*. Only 26% of mdr1 positive patients with aberrant karyotypes achieved complete remission (CR) whereas 54% of the *mdr1* negative counterparts did so ($p=0.002$). Furthermore, within abn(11q), +21, +22, -5 or abn(3q) no *mdr1* positive patient reached CR, whereas the *mdr1* negative counterparts had CR rates comparable to the CR rate of patients with a normal karyotype. This was most impressive in *mdr1* negative patients with multiple aberrations achieving a CR rate of 63% ($p=0.019$). In the multivariate analysis age, disease status and *mdr1* expression were the strongest independent predictors for induction treatment failure.

Interpretation and Conclusions. The correlation described here between *mdr1* gene expression and some cytogenetic aberrations might explain the prognostic divergence of such cytogenetic aberrations in different AML treatment trials due to the amount of mdr-drugs used within the protocols.

Key words: *mdr1* expression, cytogenetics, AML, adult, treatment outcome.

The outcome of adult patients with acute myeloid leukemia (AML) shows a great variability which might reflect the broad heterogeneity found in this type of disease. Therefore, prognostic factors are needed to identify high or low risk patients who might benefit from more or less intensified treatment strategies. It is well established that the major risk factor in AML is the patient's age. Patients older than 55 years have a significantly worse prognosis than do younger patients.[1]

Moreover, cytogenetic analysis has developed as a powerful prognostic tool. Bloomfield *et al.*[2] suggested that cytogenetic changes such as t(15;17), inv(16) or t(8;21) reflect abnormalities with a good prognosis. In contrast, chromosomal changes involving deletions of parts of the chromosome 5 [del(5q)], loss of chromosome 7 [-7], and complex cytogenetic aberrations are associated with a poorer response to anti-leukemic therapy. Furthermore, they defined a subgroup of patients with an intermediate outcome according to the appearance of a normal karyotype in the blast sample.[2] The MRC study group extended this cytogenetic intermediate risk group and found that patients carrying cytogenetic aberrations such as 11q23 abnormalities, trisomies 8, 21, and 22, and deletion of the chromosomal parts 9q and 7q also have an intermediate prognosis.[3] In contrast, the SWOG coded patients with 7q-, abn9q, 11q, 20q, 21q, 17p, t(6;9) and t(9;22) as unfavorable aberrations.[4] Thus, there are quite a few discrepancies concerning which kind of aberrations should be considered as high or intermediate risk.

Mdr1 expression is another well established risk factor for treatment failure in AML patients and has been shown to be associated with complete remission (CR)-failure after induction therapy.[5-7] There is growing evidence that *mdr1* expression might be mainly correlated with certain unfavorable karyotypes in AML.[8,9]

As the *mdr1* gene is located at 7q21.1,[10] especially aberrations involving chromosome 7 were correlated with enhanced *mdr1* expression in cell line models.[11-13] Recently, an upregulation of *mdr1* at the transcriptional level was found for AML patients with –7/7q-.[14] In addition one survey found a positive correlation between the favorable karyotype t(8;21) and *mdr1* expression in pediatric AML.[15]

Therefore, we looked at a large cohort of AML patients, investigating their *mdr1* expression within specific cytogenetic aberrations. We focused on the question of whether there are other distinct aberrations beside chromosome 7 and t(8;21) which are correlated with *mdr1* expression. Furthermore, we evaluated the correlation between *mdr1* expression and response to induction treatment within carriers of these aberrations.

Design and Methods

Patients

Three hundred and thirty-one adult patients with *de novo* or secondary AML were studied. All of them were included between February 1996 and February 2000 and treated within the German multi-center treatment trial of the SHG AML96 study group. The patients' characteristics are listed in Table 1. Patients diagnosed with AML of the subtype FAB-M3 were not included and were treated within other European trials.

The treatment schedule of the SHG AML96 trial has been previously published.[16]

Briefly, double induction therapy was stratified according to age. Patients ≤ 60 years old received one course of MAV (mitoxantrone 10 mg/m^2 days 4-8, cytosine arabinoside 100 mg/m^2 days 1-8, VP-16 100 mg/m^2 days 4-8) and a second course of MAMAC (cytosine arabinoside 2×1,000 mg/m^2 days 1-5, m-amsacrine 100 mg/m^2 days 1-5).

Patients over 60 years old were treated with two courses of DA (daunorubicin 45 mg/m^2 days 3-5, cytosine arabinoside 100 mg/m^2 days 1-7).

Complete remission (CR) was defined as the presence of < 5% of blast cells in a standardized bone marrow puncture after the second course of induction therapy. Only patients with a fully regenerated peripheral blood count were considered to be in CR.

Post-remission therapy for individuals ≤ 60 years old was priority-based and adapted according to cytogenetic risk.[16] The study was approved by the ethics committee of the University of Dresden. Each patient gave written informed consent.

The control group for drug resistance gene expression consisted of 12 healthy bone marrow donors.

Table 1. AML patient and disease characteristics by *mdr1* gene expression (n=331).

	All patients (n=331)	*Mdr1– patients (n=248)*	*Mdr1+ patients (n=83)*	*p value#*
Age [years]				
Median	56	54	58	–
Range	15-78	15-78	19-77	–
Disease status [n]				
De novo	276	212	64	–
Secondary	55	36	19	–
WBC [Gpt/l]				
Median	26.3	33.5	10.9	<0.001
Range	0.8–380.0	0.9–380.0	0.8–224.3	–
Blast count in BM [%]				
Median	66	70	59	0.004
Range	30 – 99	30-99	30-93	–
CD34 [%]				
Positive	47	41	87	<0.001
Negative	53	59	13	–
LDH [mmol/sL]				
Median	15.8	17.9	12.5	<0.001
Range	4.3–149.3	4.4-113.7	4.3-149.3	–
FAB class [n]				
M0	15	8	7	–
M1	74	52	22	–
M2	105	68	37	–
M4	58	50	8	0.004
M5a	49	47	2	0.001
M5b	20	19	1	0.026
M6	5	1	4	–
M7	5	3	2	–

#p values reflect significant differences between mdr1-positive and –negative patients. p values of non-parametric variables were obtained by two-tailed Mann-Whitney U-test, of parametric variables by two-tailed Fisher's exact test, and FAB subtypes were compared to FAB class M2 in a logistic regression analysis.

Sample handling

Bone marrow or peripheral blood samples were taken at diagnosis. Samples were divided for routine analysis (determination of FAB-type, blast count), cytogenetics, and immunocytochemical analysis. One part of the sample was frozen in liquid nitrogen. At the time of RNA extraction samples were thawed according to routine protocols. Probes containing fewer than 80% of myeloblasts were referred to CD-3 depletion. We performed depletion using CD-3-coated dynabeads (Dynal, Hamburg, Germany) according to the manufactur-

er's recommendations. CD-3 positive cells could be eliminated with a sensitivity of 98% (*data not shown*).

mRNA extraction and c-DNA synthesis

RNA extraction and cDNA synthesis were carried out as described previously.[17]

Reverse transcription polymerase chain reaction for mdr1 gene expression

Polymerase chain reaction (PCR) analysis was done as previously described.[16] Briefly, a final volume of 50 μL was used containing 1× reaction buffer (Perkin Elmer-Applied Biosystems, Weiterstadt, Germany), 2.0 μM MgCl2, 15 pmol of each primer, 200 μM each dNTP kit (Pharmacia, Freiburg, Germany), and 1.5 U AmpliTaqGold-Polymerase (Perkin Elmer-Applied Biosystems, Weiterstadt, Germany). GAPDH primers were obtained from Clontech (Heidelberg, Germany). *Mdr1* oligonucleotides were used as previously described.[18] The primer pair was tested in cycle kinetic analysis in order to assure amplification in the exponential range of PCR (*data not shown*). All PCR reactions were run at least twice.

GAPDH and *mdr1* PCR reaction products were ethanol-precipitated and subsequently loaded on a 5% polyacrylamide gel. After electrophoresis the gel was ethidium bromide stained and evaluated using the BioDoc II video documentation system (Biometra, Göttingen, Germany). Densitometric analysis was done with the ScanPack™ 3.0 software (Biometra, Göttingen, Germany). The area under the curve was evaluated for each amplified gene product. Relative amounts of resistance gene expression were determined by division with the observed value of GAPDH. The accuracy of PCR amplification was controlled using CCRF-VCR100 as the positive reference cell line.[19]

The mean value of the relative *mdr1* expression intensities of 12 bone marrow probes of healthy donors was approximately 0.019 compared to the corresponding GAPDH expression intensities. Thus the threshold of *mdr1* positivity for the patients' blast probes was set at 0.02 to rule out that contaminating *mdr1* positive normal bone marrow cells besides T-cells may lead to false positive samples. All AML blast probes displaying a relative *mdr1* expression of ≥ 0.02 of the corresponding GAPDH level were therefore regarded as positive. The probes of the control group were T-cell-depleted, were handled exactly as the probes of the study population, and consisted of previously taken bone marrow aspirates.

Flow cytometry

For the discrimination of $CD34^+$ cells, CD34 monoclonal antibody QBEnd10 (Coulter-Immunotech Diagnostics, Hamburg, Germany) was used according to previously published protocols.[20] CD34 positivity was defined as ≥ 20% CD34 positive blast cells within the examined blast samples.

Cytogenetics

Chromosome analyses were done in all 331 AML patients studied and were performed on metaphases from direct preparations, as well as from 24h and 48 h cultures of bone marrow and/or peripheral blood samples as described previously.[21] The cytogenetic preparation and G-banding were done following routine laboratory procedures.

Cytogenetic risk groups were defined as follows: *high risk*: -5/del(5q), -7/del(7q), hypodiploid karyotypes (besides 45,X,-Y or –X), inv(3q), abnl12p, abnl11q,+11, +13, +21, +22, t(6;9); t(9;22); t(9;11); t(3;3), multiple aberrations; *intermediate risk*: patients without a low risk or high risk constellation; *low risk*: t(8;21) and t(8;21) combined with other aberrations.

Statistical analysis

Basic statistical data such as mean values, standard deviations and frequencies were obtained using the SPSS software package. Differences in *mdr1* gene expression between the analyzed cytogenetic subgroups and univariate analyses of the correlation between experimental findings and response to induction therapy were evaluated by a two-tailed Fisher's exact test. Multivariate analyses of the correlation between experimental or clinical parameters and therapy response were done by stepwise logistic regression. Multivariate analyses of the correlation between experimental or clinical parameters and survival were done using Cox regression models.

Overall and disease-free survival analyses were performed using the Kaplan-Meier method and survival curves were compared using the log-rank test.

Results

Patients' characteristics and cytogenetics

All 331 AML patients with a median age of 56 years (range 18-78) had evaluable chromosome analyses. *De novo* AML was diagnosed in 276 patients and secondary AML in 55 others. The distribution of FAB subtypes, white blood cell counts (WBC), blast-counts and lactate dehydrogenase (LDH) levels are shown in Table 1.

Aberrant karyotypes [not including t(15;17)] were

found in 135 (41%) of the AML patients, whereas 196 (59%) patients had a normal karyotype.

Two hundred and twenty-seven patients had no numerical abnormalities. Aneuploidy was found in 104 samples with 39 displaying a hypodiploid karyotype, 39 a hyperdiploid karyotype and 26 others an aneuploid karyotypes.

The frequencies of the most common cytogenetic aberrations in the 331 AML patients at diagnosis are shown in Table 2. The distribution of most of the abnormalities was well comparable to the distribution found by Grimwade *et al.*[3] However, we discovered that 12% of patients had multiple aberrations (i.e. 3 or more detected aberrations) whereas 6% of patients within the MRC trial had multiple aberrations but in this latter trial the cut-off level was 5 or more detected aberrations.

Mdr1 gene expression

Correlations between *mdr1* gene expression and AML patients' disease characteristics are shown in Table 1.

Eighty-three of the 331 (25%) patients showed relative *mdr1* gene expression levels higher than or equal to the threshold level of 0.02 and were considered to be *mdr1* positive. Of these positive patients 26 had low (0.02-0.029), 15 moderate (0.03-0.039) and 42 high (≥0.04) levels of *mdr1* gene expression. Patients older than the median age of 56 years were more often *mdr1* positive than younger patients (31% vs. 19%; p=0.01).

Mdr1 positive patients had significantly lower peripheral WBCs (p<0.001) and blast counts in the bone marrow (p=0.004). Furthermore, LDH levels were significantly lower in these patients (p<0.001).

CD34 expression correlated strongly with *mdr1* gene expression: 87% of *mdr1*$^+$ AML patients expressed CD34 on their blasts whereas only 41% of their *mdr1*$^-$ counterparts expressed CD34 (p<0.001). No correlation was found between CD34 expression and age (p=0.88).

The frequency of *mdr1* expression was significantly lower in monocytic FAB subtypes M4 (p=0.004), M5a (p=0.001) and M5b (p=0.026) than in FAB subtype M2.

In the multivariate analysis CD34 expression (p<0.001) and low leukocyte count in the peripheral blood (p<0.005) were independently predictive of *mdr1* expression.

Mdr1-expression frequency within specific cytogenetic aberrations

We observed a higher incidence of *mdr1*$^+$ blast samples in patients with aberrant karyotypes compared to in patients with normal karyotypes (39% vs. 15%; p<0.001).

Table 2. Mdr1 gene expression by specific cytogenetic aberrations in AML patients.

Aberration	*All patients n[1](%)*	*Mdr1+ patients n (%)*	*p value[#]*
Overall	331 (100%)	83 (25%)	
Normal karyotype	196 (59%)	30 (15%)	
Aberrant karyotype	135 (41%)	53 (39%)	<0.001
t(8;21)	18 (5%)	9 (50%)	0.001
inv(16)	7 (2%)	0 (0%)	
+8	22 (7%)	9 (41%)	0.007
abn11q	15 (5%)	1 (7%)	
+21	8 (2%)	4 (50%)	0.028
+22	6 (2%)	2 (33%)	
abn12p	7 (2%)	2 (29%)	
del(7q)	14 (4%)	7(50%)	0.004
-5	9 (3%)	3 (33%)	
del(5q)	11 (3%)	6 (55%)	0.004
-7	22 (7%)	12 (55%)	<0.001
abn(3q)	3 (1%)	3 (100%)	0.004
Other monosomies[2]	19 (6%)	6 (32%)	
Multiple aberrations	39 (12%)	15 (39%)	0.002
Other aberrations	79 (24%)	25 (32%)	0.004

[1]All patients with a specific aberration are considered, irrespective of the presence of additional cytogenetic changes. Therefore numbers of the specific aberrations do not add up to 135; [#]p-values reflect significant differences in mdr1 gene expression between normal karyotype and the specific cytogenetic aberrations calculated by two-tailed Fisher's exact test; [2]other monosomies include other not mentioned hypodiploid karyotypes, except 45,X,-Y or -X.

Breaking this down to specific cytogenetic aberrations, *mdr1* expression was significantly more frequent in AML patients with t(8;21), +8, +21, del(7q), -7, del(5q), abn(3q) or multiple aberrations compared to in patients with a normal karyotype (Table 2). Interestingly, 4/9 *mdr1* positive blast probes with t(8;21) were CD56 positive compared to 1/9 *mdr1* negative probes with the same aberration.

Lower *mdr1* expression frequencies were seen in patients with inv(16) and abn(11q). Only one out of 15 patients with abn(11q), including three patients with t(9;11), and none out of 7 patients with inv(16) was positive for *mdr1* expression (Table 2).

Influence of mdr1 expression on treatment response of AML patients with specific cytogenetic aberrations

The total CR rate was 54% for patients with normal karyotypes. The CR rates were higher in patients displaying t(8;21) and inv(16), being 94% and 86%, respectively. Lower CR rates were found in patients with +8, +21, +22, abn(12p), del(7q), -5, del(5q), -7, abn(3q) and other monosomies.

Overall 56% of *mdr1*-negative patients com-

Table 3. CR rates of AML patients with specific aberrations by *mdr1* gene expression.

Aberration	*Total CR-rates %*	*Patients in CR of mdr1+ patients n/n [%]*	*Patients in CR of mdr1⁻ patients n/n [%]*	*p values#*
Overall	50	26/83 (31%)	137/248 (56%)	<0.001
Normal karyotype	54	12/30 (40%)	93/166 (56%)	
Aberrant karyotype	43	14/53 (26%)	44/82 (54%)	0.002
t(8;21)	94	8/9 (89%)	9/9 (100%)	
inv(16)	86	No mdr1 pos. patient	6/7 (86%)	
+8	27	1/9 (11%)	5/13 (39%)	
abn11q	67	0/1 (0%)	10/14 (71%)	
+21	13	0/4 (0%)	1/4(25%)	
+22	33	0/2 (0%)	2/4 (50%)	
abn12p	29	1 /2 (50%)	1 /5 (20%)	
del(7q)	36	1/7 (14%)	4/7 (57%)	
-5	33	0/3 (0%)	3/6 (50%)	
del(5q)	27	1/6 (17%)	2/5 (40%)	
-7	36	3/12 (25%)	5/10 (50%)	
abn(3q)	0	0/3 (0%)	No mdr1 neg. pat.	
Other monosomies[1]	37	0/6 (0%)	7/13 (54%)	0.044
Multiple aberrations	46	3/15 (20%)	15/24 (63%)	0.019
Other aberrations	47	7/25 (28%)	30/54 (56%)	0.030

#p values reflect significant differences between the CR rate of mdr1 negative and the CR rate of mdr1 positive patients within the specific cytogenetic aberrations calculated by two-tailed Fisher's exact test; [1]other monosomies include other not mentioned hypodiploid karyotypes, except 45,X,-Y or -X.

pared to 31% of *mdr1*-positive patients reached CR criteria ($p<0.001$) (Table 3). The influence of *mdr1* expression was more pronounced in AML patients with aberrant karyotypes with a CR rate of only 26% ($p=0.002$) in *mdr1*-positive patients whereas in patients with normal karyotypes *mdr1* expression showed no correlation with response to induction therapy. This was mainly due to resistant disease. Thirty percent of *mdr1*⁺ patients with aberrant karyotypes, compared to 14% of their *mdr1* negative counterparts, did not respond to the first induction therapy ($p<0.04$). Early death rate, however, was not significantly different between the two groups (9% vs. 17%; $p=0.18$).

Looking into specific cytogenetic aberrations, no *mdr1*-positive patient with abn(11q), abn(3q),+21, +22, -5, or other monosomies reached CR (Table 3).

Low CR rates in *mdr1*-positive patients were also found in patients with +8, del(7q), del(5q), -7, multiple aberrations or the summarized group of other aberrations (Table 3).

In contrast, within the patients with del(5q), del(7q), +22, -5, -7, other monosomies or multiple aberrations, those who were negative for *mdr1* gene expression had CR rates that were comparable to the CR rate in patients with normal karyotypes. This was most impressive for *mdr1*-negative patients with multiple aberrations who had a CR rate of 63% ($p=0.019$).

In the multivariate analysis the strongest predictors for induction treatment failure were age ($p<0.001$), mdr1 gene expression ($p<0.001$) and disease status ($p=0.001$) (Table 4). High-risk cytogenetics did not prove to be an independent predictor for induction treatment failure ($p=0.85$). This was also true when the high-risk cytogenetic group was coded according to the MRC trial[3] ($p=0.27$) (*data not shown*).

Low-risk cytogenetics, however, were an independent positive prognostic factor for achieving CR ($p=0.002$).

Independent prognostic factors for overall survival were age, disease status and low-risk cytogenetics, whereas high- and low-risk cytogenetics predicted disease-free survival in the multivariate analysis (Table 5).

As disease status is one of the strongest predictors of overall survival and secondary AML may have a different biological behavior anyway, survival was plotted for secondary and *de novo* AML separately. Overall survival was worse in *mdr1*-positive secondary AML patients compared to in their *mdr1*-negative counterparts ($p=0.03$), whereas there was no significant difference in overall survival between patients with *mdr1*-positive and negative *de novo* AML (Figure 1). No influence of

Table 4. Correlation with response to induction therapy (CR-rate) in all AML patients (n=331): multivariate analysis.

	Stepwise forward logistic regression		
	RP	*95% CI*	*p-value*
Age	0.30	0.18-0.51	<0.001
Disease status	0.29	0.14-0.60	0.001
Mdr1 expression	0.30	0.16-0.55	<0.001
Low risk cytogenetics	28.54	3.45-236.26	0.002
High risk cytogenetics	–	–	0.85

**cytogenetic risk groups were defined according to the protocol of the SHG AML96 study group with t(8;21) as low risk and -5/del(5q), -7/del(7q), hypodiploid karyotypes (excluded 45,X,-Y or –X), inv(3q), abnl12p, abnl11q,+11, +13, +21, +22, t(6;9); t(9;22); t(9;11); t(3;3), multiple aberrations as high risk cytogenetics. CR, complete remission; RP, relative probability of reaching CR; CI, confidence interval.*

mdr1 expression on disease-free survival could be found in either group of patients (Figure 2).

Discussion

Diagnostic cytogenetics is regarded as one of the most valuable prognostic factors for remission induction and survival in AML patients. However, there are still discrepancies about the value of certain cytogenetic abnormalities.[3,4] As other prognostic markers, such as *mdr1* expression, have been shown to influence remission induction and survival in AML,[7] the expression of resistance genes might alter the influence of cytogenetics on remission induction. Therefore, we looked for *mdr1* expression within specific cytogenetic aberrations of a large cohort of previously untreated adult patients with AML. Flow cytometry and functional tests are thought to be the most valid and practical methods to determine *mdr1* expression in AML blast samples, especially if they are used in

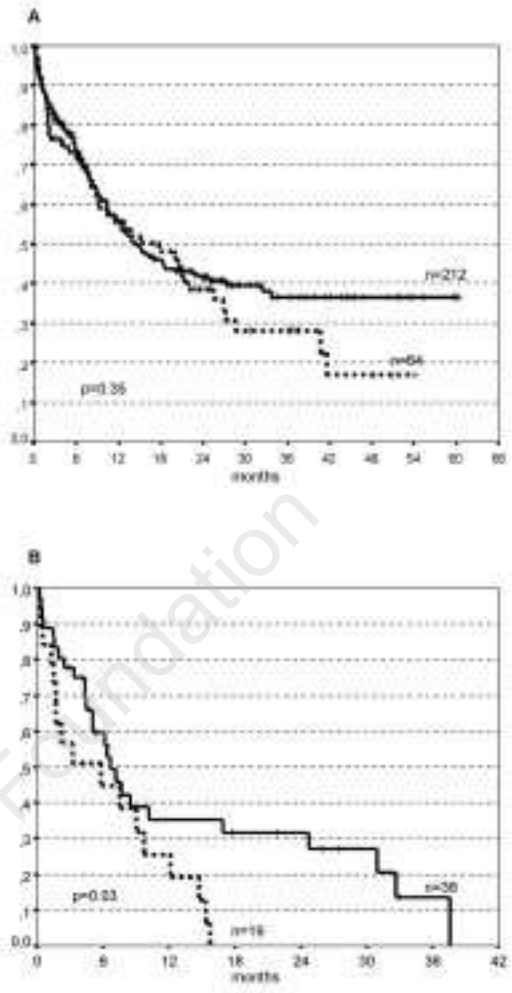

Figure 1. Overall survival of *de novo* (A) and secondary (B) AML patients by *mdr1* expression (*mdr1* positive patients: *dotted line*; *mdr1* negative patients: *solid line*). Patients with an allogeneic stem cell transplantation were censored at the time of transplantation.

Table 5. Prognostic factors for survival in all AML patients: multivariate analysis.

	Stepwise forward cox regression					
	Overall survival (n=331)			*Disease-free survival (n=163)*		
	RP	*95% CI*	*p-value*	*RP*	*95% CI*	*p-value*
Age	1.03	1.01-1.04	<0.001	–	–	0.15
Disease status	1.61	1.13-2.29	0.009	–	–	0.87
Mdr1 expression	–	–	0.07	–	–	0.24
Low risk cytogenetics	0.38	0.16-0.94	0.04	0.26	0.09-0.73	0.01
High risk cytogenetics	–	–	0.13	2.05	1.13-3.72	0.02

**cytogenetic risk groups were defined according to the protocol of the SHG AML96 study group with t(8;21) as low risk and -5/del(5q), -7/del(7q), hypodiploid karyotypes (excluded 45,X,-Y or –X), inv(3q), abnl12p, abnl11q,+11, +13, +21, +22, t(6;9); t(9;22); t(9;11); t(3;3), multiple aberrations as high risk cytogenetics; RP, relative probability of event; CI, confidence interval*

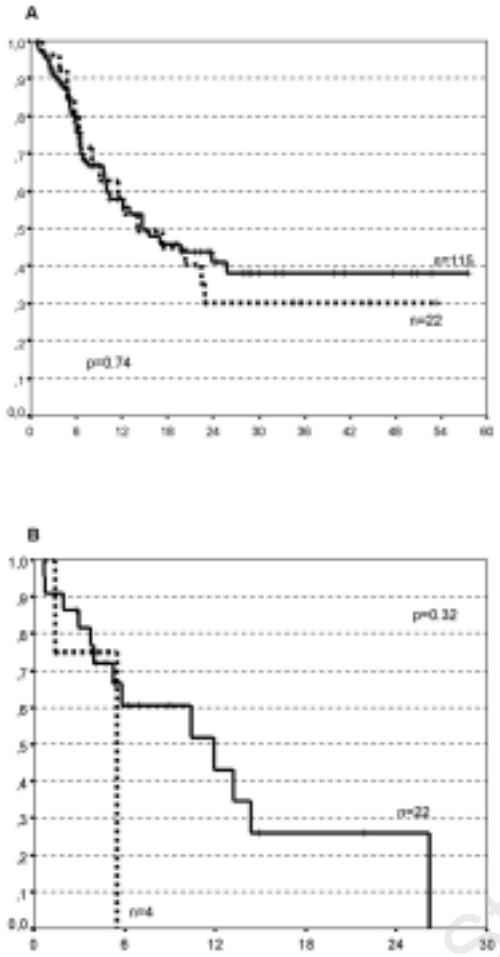

Figure 2. Disease-free survival of *de novo* (A) and secondary (B) AML patients by *mdr1* expression (*mdr1* positive patients: *dotted line*; *mdr1* negative patients: *solid line*). Patients with an allogeneic stem cell transplantation were censored at the time of transplantation.

combination.[22,23] However, in our hands[24] and in accordance with the literature[25,26] *mdr1* mRNA expression determined by RT-PCR is a sensitive method and well comparable to flow cytometry or functional tests. Furthermore, CD3 depletion of the samples to rule out T-cell contamination as the main source of normal *mdr1*-positive cells further increases the reliability of the method. Thus, as material access was restricted in the presented multi-center study, RT-PCR was a reliable and feasible method.

In our survey AML patients with aberrant karyotypes showed a greater extent of *mdr1* overexpression compared to patients with normal karyotype. This finding could be explained by a survey of Knutsen *et al.*[27] In cell lines and patients with drug-refractory ALL they showed that random chromosomal rearrangements could activate *mdr1* by fusing it to other genes. Especially within so-called unfavorable cytogenetic aberrations, such as abn(3q), del(5q), del(7q), –7 or multiple aberrations, we found a significantly higher proportion of *mdr1* positive patients. This is in accordance with the findings of Wüchter *et al.*,[23] who showed that AML patients with unfavorable cytogenetics were more often P-glycoprotein (P-gp)-positive than patients with intermediate/favorable cytogenetics. Samdani *et al.*[8] also found P-gp expression to be more frequent in AML patients with unfavorable cytogenetics. However, both studies examined small numbers of patients.

Leith *et al.*[7] reported a trend towards correlation between high-risk cytogenetics and *mdr1* expression in a survey of 352 AML patients aged 17 to 69 years using flow-cytometry (p=0.09). However, only 81 patients had evaluable cytogenetics, with 23 patients being in the unfavorable group. In our survey we looked for *mdr1* expression within specific cytogenetic aberrations and found that some of the aberrations coded as unfavorable by the SWOG, such as –5 or abn(11q), were not correlated with *mdr1* in our study. This might be another reason for lack of significance in the study by Leith *et al.*[7] The use of different methods and threshold levels may further influence correlation analyses between *mdr1* expression and cytogenetics. Furthermore, patient selection plays an important role, as in another paper the same group showed that the frequency of *mdr1* expression is much higher in older AML patients.[28]

As the *mdr1* gene is located on the long arm of chromosome 7 at position 7q21.1,[10] structural and numerical aberrations of chromosome 7 were of special interest for differential *mdr1* expression. Whereas duplications and additions to chromosome 7 were correlated to the *mdr1* phenotype[12,13] in cell lines, we found that del(7q) and –7 were also associated with *mdr1* gene expression in AML patients. Recently, an upregulation of *mdr1* mRNA was seen in 19 AML patients with –7 or del(7q).[14] The reason for this is still unclear. One can speculate that most patients with –7/del(7q) harbor additional aberrations activating *mdr1* gene expression.

Interestingly, +8 and +21 karyotypes correlated with *mdr1* gene expression in our survey, in which +8 is regarded as an intermediate-risk aberration and +21 as a high-risk one. The MRC coded both +8 and +21 as intermediate risk,[3] whereas the SWOG classified +21 as an aberration with unknown cytogenetic risk.[4] In contrast, others con-

sider +8 as unfavorable.[8] In fact, we found low remission rates for both +8 and +21, which might be due to the higher frequency of *mdr1* expression: no *mdr1*-positive patient with +21 and only one with +8 achieved CR criteria.

Furthermore, half of our examined eighteen AML patients with the favorable t(8;21) were positive for *mdr1*. In the literature *mdr1* correlated strongly with t(8;21) in pediatric AML, whereas this correlation was not found in 11 adult t(8;21) cases.[15] Recently, Lutterbach *et al.*[29] substantiated this latter finding as they demonstrated that the fusion-protein AML1/ETO inhibits *mdr1* expression via repression of the *mdr1* promoter. Nevertheless, harboring of additional aberrations might be the reason for *mdr1* positivity within our subgroup of AML patients with t(8;21). In fact most of the *mdr1* positive t(8;21) cases had additional aberrations, such as del(9q), del(7q) or del(6q) (*data not shown*). Thus, transcriptional repression might be counteracted by gene activation associated with genetic instability. However, *mdr1* expression does not influence remission induction in patients with t(8;21) as 94% of such patients reached CR.

In contrast, no patient with inv(16) was positive for *mdr1* expression, which was in accordance with the findings of Samdani *et al.*[8]

Mdr1 expression predicted treatment failure in most patients with intermediate-risk and unfavorable cytogenetic aberrations, whereas *mdr1* negative patients had remission rates comparable to those with a normal karyotype. For example, patients with multiple aberrations, who were generally considered at high-risk of treatment failure, had a 63% CR rate, if they were *mdr1* negative. This finding was confirmed by the multivariate analysis, in which unfavorable cytogenetic risk was not an independent prognostic factor for induction treatment failure, whereas *mdr1* expression was highly significant. This might be due to the unselected cohort of patients examined in this study, in which age and disease status were the strongest independent predictors of treatment failure. If one looks at selected patients younger than 60 years with *de novo* AML, high-risk cytogenetics proves to be an independent prognostic factor besides *mdr1* expression (*data not shown*). To rule out a major influence of our coding of the high-risk cytogenetic group, the same analysis was done using the MRC coding. Again high-risk cytogenetics lost significance. Similar findings were made by Hunault *et al.*[30] In their survey of 110 AML patients cytogenetic analysis maintained its prognostic value only in *mdr1*-negative patients. An additive negative prognostic effect for *mdr1* expression in AML patients with unfavorable cytogenetics has been described by others.[9,31]

In accordance with Leith *et al.*[7] *mdr1* expression seems not to be an independent prognostic factor for overall or disease-free survival in all examined AML patients in our survey as once again age, disease status or cytogenetics were much stronger determinants. However, within distinct subgroups, such as secondary AML, overall survival of *mdr1* positive patients is worse than that of *mdr1* negative patients. This is also true for *de novo* AML patients harboring specific cytogenetic aberrations, such as t(8;21), +8 or multiple aberrations (*data not shown*). As the numbers of patients in the latter subgroups are relatively low, these findings need further prospective evaluation within intergroup studies.

In conclusion, our data indicate that the expression of *mdr1* resistance gene is different between specific cytogenetic aberrations in AML. In some unfavorable aberrations *mdr1* expression might be the important determinant of induction treatment failure. In patients with aberrations, such as +8, +21 or del(7q), without a consensus on whether they should be coded as intermediate or unfavorable cytogenetic risk, a high *mdr1* expression rate was seen. This could be an explanation for the different classification of these patients, since induction treatment outcome might depend on the amount of mdr-drugs used within the different induction regimens.

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MS and TI were the principal authors. They were primarily responsible for the work, from conception to submitted manuscript. The remaining authors qualified for authorship according to the World Association of Medical Editors (WAME) criteria and have taken specific responsibility for the following parts of the content: EHB, substantial parts of the molecular studies; UP and BM, cytogenetic analyses; SS, statistical analyses; GE, substantial contributions to conception, design and interpretation of data. Order of authorship: authors are listed according to a criterion of decreasing individual contribution to the work, with one exception: the last author had a major role as a principal author with major responsibility for the whole work as outlined above.

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Disclosures

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What is already known on this topic

It is recognized that *MDR1 positivity* is associated with resistance to therapy in AML. This paper reports on the frequency of MDR1 expression according to karyotype, another highly important prognostic factor in AML.

What this study adds

Perhaps the most clinically interesting observation is that, within certain cytogenetic groups, MDR1 status adds prognostic information. Thus MDR status can be used to reduce the prognostic heterogeneity within these groups.

Potential implications for clinical practice

As a result, physicians can, and should, use patient's MDR status in deciding whether to offer standard or investigational therapy.

Elihu Estey, Associate Editor