MDR1 gene–related clonal selection and P-glycoprotein function and expression in relapsed or refractory acute myeloid leukemia

Marry M. van den Heuvel-Eibrink, Erik A. C. Wiemer, Marjan J. de Boevere, Bronno van der Holt, Paula J. M. Vossebeld, Rob Pieters, and Pieter Sonneveld

The expression of P-glycoprotein (P-gp), encoded by the MDR1 gene, is an independent adverse prognostic factor for response and survival in de novo acute myeloid leukemia (AML). Little is known about MDR1 expression during the development of disease. The present study investigated whether MDR1 gene–related clonal selection occurs in the development from diagnosis to relapsed AML, using a genetic polymorphism of the MDR1 gene at position 2677. Expression and function of P-gp were studied using monoclonal antibodies MRK16 and UIC2 and the Rhodamine 123 retention assay with or without PSC 833. No difference was found in the levels of P-gp function and expression between diagnosis and relapse in purified paired blast samples from 30 patients with AML. Thirteen patients were homozygous for the genetic polymorphism of MDR1 (n = 7 for guanine, n = 6 for thymidine), whereas 17 patients were heterozygous (GT). In the heterozygous patients, no selective loss of one allele was observed at relapse. Homozygosity for the MDR1 gene (GG or TT) was associated with shorter relapse-free intervals (P = .002) and poor survival rates (P = .02), compared with heterozygous patients. No difference was found in P-gp expression or function in patients with AML with either of the allelic variants of the MDR1 gene. It was concluded that P-gp function or expression is not up-regulated at relapse/refractory disease and expression of one of the allelic variants is not associated with altered P-gp expression or function in AML, consistent with the fact that MDR1 gene–related clonal selection does not occur when AML evolves to recurrent disease. (Blood. 2001;97:3605-3611)

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Patients and methods

Patients
Bone marrow samples from 30 patients with AML (9 children, 21 adults) were obtained from the iliac crest at diagnosis and at time of first relapse (n = 27) or refractory disease (n = 3) (Table 1). Written informed consent was obtained from each patient and/or their parents to perform these studies. AML classification, according to the French-American-British (FAB) criteria was M1 (n = 8), M2 (n = 11), M4 (n = 2), M5 (n = 7), and M6 (n = 2). Cytogenetic analysis was carried out by standard techniques, and the findings were described according to the international nomenclature. Patients with a deletion or loss of chromosome 7 were not included in the study, because of the (possible) loss of one MDR1 gene which is located on 7q21.1, which complicates the analysis of polymorphism in these patients. All patients were treated according to the Helsinki agreement and were included in treatment protocols of the Dutch-Belgian Hemato-Oncology Collaborative Group (protocol HOVON 4/4a resp. HOVON 29) for young adults (n = 17), European Organisation for Research and Treatment of Cancer (EORTC) protocol LAM 9) (n = 1) for patients 60 years or older, and the Dutch Childhood Leukemia Study Group (DCLSG: protocol ANLL 87 and 94) (n = 9) for children (younger than 18 years old). After relapse or in case of refractory disease after induction therapy, adults were treated according to the HOVON 30 protocol. The pediatric patients received treatment according to institutional protocols (Table 2). For some patients, individual therapy choices were made (Table 1). CR

Introduction

Classic multidrug resistance (MDR) encoded by the MDR1 gene is characterized by expression of P-glycoprotein (P-gp), which acts as a drug efflux pump in the plasma membrane. Expression of MDR1 has been identified as an independent adverse prognostic factor for complete remission (CR) and survival in patients with acute myeloid leukemia (AML), especially in adults. Little is known about possible changes in MDR1 expression during the development to relapse or refractory disease. The present study investigated whether MDR1 gene–related clonal selection occurs in the development from diagnosis to relapsed AML, using a genetic polymorphism of the MDR1 gene at position 2677. Expression and function of P-gp were studied using monoclonal antibodies MRK16 and UIC2 and the Rhodamine 123 retention assay with or without PSC 833. No difference was found in the levels of P-gp function and expression between diagnosis and relapse in purified paired blast samples from 30 patients with AML. Thirteen patients were homozygous for the genetic polymorphism of MDR1 (n = 7 for guanine, n = 6 for thymidine), whereas 17 patients were heterozygous (GT). In the heterozygous patients, no selective loss of one allele was observed at relapse. Homozygosity for the MDR1 gene (GG or TT) was associated with shorter relapse-free intervals (P = .002) and poor survival rates (P = .02), compared with heterozygous patients. No difference was found in P-gp expression or function in patients with AML with either of the allelic variants of the MDR1 gene. It was concluded that P-gp function or expression is not up-regulated at relapse/refractory disease and expression of one of the allelic variants is not associated with altered P-gp expression or function in AML, consistent with the fact that MDR1 gene–related clonal selection does not occur when AML evolves to recurrent disease. (Blood. 2001;97:3605-3611)

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status was defined as normocellular marrow with <5% blasts in a bone marrow (BM) smear and normal peripheral blood cell counts.

**Methods**

**Patient samples.** Bone marrow aspirates were obtained in heparinized tubes. Mononuclear bone marrow cells (MNCs) were collected by Ficoll Hypaque density gradient centrifugation (density 1.077g/m³) (Pharmacia, Uppsala, Sweden). To obtain purified samples with more than 85% blasts, T-cell depletion and adherence depletion were performed.\(^1\)\(^6\) Cells were cryopreserved in Iscoves modified Dulbecco medium (IMDM; Gibco, Paisley, United Kingdom) supplemented with 10% dimethyl sulfoxide (DMSO; Merck, Darmstadt, Germany) and 20% fetal calf serum (FCS; Gibco) and stored in liquid nitrogen. On the day of the experiments bone marrow cells were thawed. For flowcytometry experiments, cells were washed and resuspended in IMDM supplemented with 10% FCS and gentamycin at a concentration of \(4\times10^6\) cells/mL. Total RNA was isolated using Trisolv extraction (Biotecx, Houston, TX).

**Oligonucleotide hybridization and dotblot analysis.** Both DNA and RNA were used as templates in the polymerase chain reaction (PCR). One microgram of genomic DNA was used as a template in the PCR for 40 cycles to investigate the genetic polymorphism at the DNA level. One microgram of total RNA was reverse transcribed and the cDNA template was subjected to 40 cycles of PCR. The following primers were used as described by Mickley\(^1\)\(^2\)\(^3\)\(^4\)\(^5\): 5\'-TCCTTGGGACAGGATTGC-3'-T and 5\'-CTCCTCCTCCCTGTAATC-3'.

**Table 1. Clinical characteristics of 30 patients with acute myeloid leukemia**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Expression of MDR1 gene polymorphism (G/T variant)</th>
<th>Diagnosis</th>
<th>Age (years)</th>
<th>FAB classification</th>
<th>Karyotype</th>
<th>Relapse free after 1st CR (months)</th>
<th>Treatment at time of relapse/refractory disease</th>
<th>Response to reinduction</th>
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<tbody>
<tr>
<td>1</td>
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<td>CR</td>
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<tr>
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<td>1</td>
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<td>2cdA/Ara-C/Idarubicin</td>
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<td>CR</td>
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<td>CR</td>
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<td>CR</td>
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<td>CR</td>
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<td>NT</td>
<td>CR</td>
</tr>
<tr>
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<td>25</td>
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<td>34</td>
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<td>No CR**</td>
</tr>
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<td>M2</td>
<td>Neutral</td>
<td>5</td>
<td>HOVON30</td>
<td>No CR</td>
</tr>
</tbody>
</table>

CR indicates complete remission after 1 or 2 courses of reinduction chemotherapy; No CR, refractory disease at time of relapse. No CR** indicates never CR after diagnosis; NT, not treated for relapse. For karyotypes, unfavorable indicates t(9;22), 11q23 with MLL rearrangements, complex karyotype, 5q-; favorable indicates inv(16), t(15;17) and t(8;21); and neutral indicates normal and other karyotypes.

**Table 2. Cumulative drug doses in the treatment protocols for acute myeloid leukemia**

<table>
<thead>
<tr>
<th>Drug</th>
<th>HOVON 4/4A N and N</th>
<th>DNR</th>
<th>Adria</th>
<th>Amsa</th>
<th>IdaVP16</th>
<th>Mitox</th>
<th>Pred</th>
<th>6TG</th>
<th>VCR</th>
<th>CP</th>
<th>CsA</th>
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<tr>
<td>Induction</td>
<td></td>
<td></td>
<td>1360</td>
<td>135</td>
<td>360</td>
<td>500</td>
<td>50</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>HOVON 29</td>
<td></td>
<td></td>
<td>1360</td>
<td></td>
<td>360</td>
<td>36</td>
<td>500</td>
<td>50</td>
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<td></td>
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<tr>
<td>DCLSG ANLL87*</td>
<td></td>
<td></td>
<td>22400</td>
<td>180</td>
<td>120</td>
<td>1050</td>
<td>1120</td>
<td>2580</td>
<td>6</td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>DCLSG ANLL94*</td>
<td></td>
<td></td>
<td>33400</td>
<td>120</td>
<td>36</td>
<td>950</td>
<td>20</td>
<td>1120</td>
<td>2520</td>
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</tr>
<tr>
<td>Reinduction</td>
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<td></td>
<td>90</td>
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<td></td>
<td></td>
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<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

(Total cumulative dose in mg/m².) Ara-C indicates cytosine arabinoside; Adria, Adriamycin; DNR, daunorubicin; Amsa, amsacrine; Ida, idarubicin; VP16, etoposide; Mitox, mitoxantrone; Pred, prednisolone; 6TG, 6-thioguanine; VCR, vincristine; CP, cyclophosphamide; and CsA, cyclosporin A.

*T Indicates +5 intrathecal Ara-C.

\(^{1}\)\(^{2}\)\(^{3}\)\(^{4}\)\(^{5}\) \(\text{GCAAATCTTGGGACAGGAAT}^{3606}\) van den HEUVEL-EIBRINK et al. BLOOD, 1 JUNE 2001 - VOLUME 97, NUMBER 11

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3′ RNA, 2′7′-CTCTTTCCGTGTTGAAAC; 3′, DNA, 2′6′-CTCTC2′6′
wCAGGTTGGTATT.

Reverse transcriptase treatment preceded amplification in order to
evaluate RNA expression. All PCR experiments included controls without
DNA or RNA. After amplification of 1 μg template, 30% of the PCR
product was loaded in each of 2 adjacent wells of a slot-blot apparatus. The
Zeta Probe nylon filter (Biorad, Hercules, CA) was cut out into 2 halves and
each half was hybridized with a different oligonucleotide. Two 19-bp
allele-specific oligonucleotide probes (HMO7 and HMO8) were 5′-
phosphorylated with [γ-32P]-ATP and T4 polynucleotide kinase. HMO7 and
HMO8 cover residues 2667 to 2685 and were used for hybridizations.
HMO7 possesses a G at position 2677 and HMO8 contains a T at the same
position. Internal controls for hybridization and specificity were included in
all experiments. For this purpose, two 30-bp oligonucleotides, designated
HMC3 and HMC4, were used. These oligonucleotides cover residues 2656
to 2685 of the MDR1 gene with HMC3 possessing a G at position 2677 and
HMC4 a T at the same position. Equal amounts of each control were spotted
on both sides of the filter. Because the hybridizations were performed under
identical conditions, with probes labeled to similar specific activities, the
signals from the control oligonucleotides were similar. For quantification of
the hybridization spots, the blots were exposed to a Phosphor Imager screen
(Molecular Dynamics, Sunnyvale, CA).

Expression of P-glycoprotein. For measurement of the expression of
P-gp, cells were incubated at room temperature with the monoclonal
anti-P-gp antibodies MRK16 and MRK16* (Kamiya Biomedical, Tukwila, WA) at
a concentration of 10 μg/mL and also, in separate tubes, with UC218
(Innogenetics, Gent, Belgium) at a concentration of 12.5 μg/mL or with an
isotype-matched mIgG2a control antibody (Sigma, St Louis, MO) at a
concentration of 10 μg/mL. Cell-bound antibodies were detected by
fluorescein isothiocyanate (FITC)–labeled rabbit antimouse immunoglobu-
lin antibodies (Dako, Glostrup, Denmark). Results are given as the ratio of
the mean fluorescence of cells incubated with the anti–P-gp antibody
divided by the mean fluorescence of cells incubated with the control
mIgG2a antibody. To measure the expression of P-gp in CD34-positive
cells, 5×10^5 cells were labeled with phycoerythrin-Cy5–labeled CD34 antibody
or a phycoerythrin-Cy5–labeled matched mIgG1 antibody (Immunotech).

Function of P-glycoprotein. For measurement of the function of P-gp,
the fluorescent molecule Rhodamine 123 (Rho 123) (Sigma) was used as a
P-gp substrate.31,32 Cells were incubated for 1 hour at 37°C at 5% CO2 in the
absence or presence of 2 μM of the P-gp modulator PSC 833 (Novartis,
Basel, Switzerland). After this incubation, 200 ng/mL Rho 123 was added
to the cells. A sample was taken at t = 0 minutes to correct for background
fluorescence and at t = 75 minutes to measure intracellular Rho 123
retention. Results were calculated as the PSC/Rho 123 retention ratio of the
mean intracellular Rho 123 fluorescence of cells exposed to PSC 833
divided by the mean intracellular Rho 123 fluorescence of cells not exposed
to PSC 833. As controls, the drug-sensitive human myeloma cell line 8226
S and the drug-resistant P-gp expressing variant 8226 D6 cells21 were
included in each experiment. Taking all experiments together, the mean
ratio of P-gp function of the negative control cell line 8226 S was
0.91 ± 0.07 (mean ± SD). The mean ratio of P-gp function of the positive
control cell line 8226 D6 was 7.03 ± 4.69 (mean ± SD).

For analysis of the function of P-gp in CD34-positive cells, cells were
labeled with phycoerythrin-Cy5–labeled CD34 antibody or a control
phycoerythrin-Cy5–labeled mIgG1 antibody (Immunotech). Fluorescence
was measured using a FACScalibur flowcytometer (Becton Dickinson, San
Jose, CA). Cells were incubated with 0.1 μM TO-PRO-3 (Molecular
Probes, Eugene, OR) to exclude nonviable cells in the functional
and expression studies.

Statistical analysis. Expression and functional levels of P-gp, either at
diagnosis or at relapse or refractory disease, were compared between
subgroups using the Mann-Whitney test in case of 2 subgroups, and the
Kruskal-Wallis test in case of 3 subgroups. Moreover, MDR1 expression at
relapse or refractory disease was compared with that at diagnosis using the
Wilcoxon matched-pairs signed-ranks test, which was restricted to patients
with data available both at diagnosis and at relapse or refractory disease. All
P values are 2-sided and a significance level of α = .05 was used.

Results

Thirty patients with AML were studied at diagnosis and during the
course of their disease. Twenty-seven patients developed a relapse
after reaching CR with induction chemotherapy. Three patients
were primary refractory to induction chemotherapy (Table 1).

Oligonucleotide hybridization and dotblot analysis

Oligonucleotide hybridization studies of position 2677 of the
MDR1 gene revealed 7 patients with a G variant, 6 patients with a T
variant, and 17 patients with a GT variant. The 17 patients with
heterozygous expression at diagnosis also showed GT expression
at relapse. In these patients, no up-regulation of either allele was
noticed during the development of disease at RNA level. Conse-
quently, in this group of patients no evidence of a MDR1
gene–associated selection of a resistant clone was found.

P-glycoprotein expression and function

MRK16 expression (n = 27) and UIC2 expression (n = 25) re-
vealed no differences in P-gp expression at relapse or refractory
disease as compared with diagnosis (P = .14 and P = .22, respec-
tively) (Table 3). No difference of MRK16 expression in the
CD34-positive subpopulation was found (n = 11) (P = 1.0) in the
paired analysis. The analysis of UIC2/CD34 in matched pairs
showed a trend to a lower expression level (P = .07) in relapsed/
refractory disease as compared with diagnosis, although the
number of patients that could be analyzed for UIC2/CD34 was

| Table 3. Paired analysis of P-glycoprotein expression and function in patients with acute myeloid leukemia at diagnosis and relapse/refractory disease |
|-------------|------------------|------------------|-----------------|-------------|
|            | At diagnosis      | At relapse and/or refractory disease | P value |
| MDR1       |                   |                   |                 |
| MRK16      | Median            | 2.16              | 1.83            | .14         |
|            | Range             | 1.22-7.65         | 1.02-5.55       |             |
|            | N                 | 27                |                 |             |
| MRK16/CD34 | Median            | 2.77              | 2.28            | 1.00        |
|            | Range             | 1.52-9.27         | 1.46-9.6        |             |
|            | N                 | 11                |                 |             |
| UIC2       | Median            | 2.37              | 1.74            | .22         |
|            | Range             | 1.47-12.3         | 0.99-6.39       |             |
|            | N                 | 25                |                 |             |
| UIC2/CD34  | Median            | 3.5               | 2.42            | .07         |
|            | Range             | 1.51-26.6         | 0.86-7.34       |             |
|            | N                 | 8                 |                 |             |
| PSC/Rho    | Median            | 1.13              | 1.10            | .26         |
|            | Range             | 0.87-2.11         | 0.81-2.19       |             |
|            | N                 | 27                |                 |             |
| PSC/Rho/CD34 | Median       | 1.43              | 1.22            | .39         |
|            | Range             | 0.98-2.7          | 0.82-3.26       |             |
|            | N                 | 12                |                 |             |

For P-gp expression, the monoclonal antibodies MRK16 and UIC2 were used; for P-gp function, the PSC/Rho 123 retention ratio was used. In samples with greater than 10% CD34 expression, the variables were also evaluated in the CD34+ subfraction of the blasts. The P values indicate the differences between diagnosis and relapse/refractory disease.

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small (n = 8) (Table 3; Figure 1C). The CD34 expression was not different at relapse as compared to diagnosis (P = .31). The PSC/Rho 123 retention ratio (n = 27) was not significantly different between diagnosis and relapsed/refractory AML (P = .26). When analyzed in the CD34-positive subpopulation of blasts (n = 12), comparable results were found (P = .39) (Table 3; Figure 1A). No difference was found in P-gp expression (P = .67 for MRK16 expression, P = .82 for UIC2 expression) or PSC/Rho ratio (P = .09) at diagnosis nor at relapse/refractory disease (P values of .42, .67, and .11, respectively) between adults and children.

P-glycoprotein versus MDR1 allelic expression

As the functional meaning of the genetic polymorphism of the MDR1 gene has not been established as yet, we analyzed P-gp in patients with expression of the G, T, and GT variants. The median MRK16 expression ratio was not significantly different in the various allelic variants (P = .72 at diagnosis and P = .34 at relapse). Also, no difference was found with monoclonal antibody UIC2 (P = .81 at diagnosis and P = .25 at relapse) and the PSC/Rho 123 retention ratio (P = .26 at diagnosis, P = .11 at relapse). No difference was found in P-gp expression or function when homozgyous patients were compared with heterozygous patients (Table 4). Similarly, in the CD34-positive fraction we did not find differences in P-gp expression and function between the different MDR1 allelic variants at diagnosis nor at relapse and/or refractory disease. The results show that there is no difference in P-gp expression and function in AML blast cells between the different specific allelic variants of the MDR1 gene. The therapeutic outcome of patients with the different allelic variants showed a significant difference, that is, homozygosity was associated with a

Table 4. Analysis of P-glycoprotein expression and function in the homozgyous vs the heterozygous allelic variants of the MDR1 gene at time of relapse/refractory disease

<table>
<thead>
<tr>
<th>Allelic Variants</th>
<th>GG/TT</th>
<th>GT</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRK16 Median</td>
<td>1.54</td>
<td>2.14</td>
<td>.15</td>
</tr>
<tr>
<td>MRK16 Range</td>
<td>1.02-5.45</td>
<td>1.18-5.55</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>13</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>UIC2 Median</td>
<td>1.56</td>
<td>2.28</td>
<td>.22</td>
</tr>
<tr>
<td>UIC2 Range</td>
<td>1.12-6.39</td>
<td>0.99-5.24</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>13</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>PSC/Rho Median</td>
<td>1.67</td>
<td>2.72</td>
<td>.10</td>
</tr>
<tr>
<td>PSC/Rho Range</td>
<td>0.86-7.34</td>
<td>1.69-5.15</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>7</td>
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<tr>
<td>PSC/Rho/CD34 Median</td>
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<td>1.42</td>
<td>.40</td>
</tr>
<tr>
<td>PSC/Rho/CD34 Range</td>
<td>1.07-1.72</td>
<td>0.82-3.26</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

For P-gp expression, the monoclonal antibodies MRK16 and UIC2 were used; for P-gp function, the PSC/Rho 123 retention ratio was used. In samples with more than 10% CD34 expression, the variables were also evaluated in the CD34− subfraction of the blasts. The P values indicate the differences between the homozygous and heterozygous patients.

Figure 1. P-glycoprotein expression and function in the CD34-positive population of the paired AML patients. (A) The UIC2 and (B) MRK16 ratios represent the expression of P-gp, and (C) the UIC2 ratio represents the function of P-gp. Dx indicates diagnosis; Rel/RD, relapsed/refractory AML. The dotted lines indicate the median values.

Figure 2. Survival of the patients with AML. Distinguishing patients that are homozygous (GG and TT) from patients that are heterozygous (GT) for the genetic polymorphism of position 2677 of the MDR1 gene. (A) Time from diagnosis until relapse/refractory disease. (B) Overall survival from relapse/refractory disease. N indicates number of patients investigated; O, observed events.

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shorter time from diagnosis to relapse ($P = .002$) and a shorter overall survival from relapse ($P = .02$) (Figure 2A,B).

**Discussion**

Clinical resistance to chemotherapy is a major problem in relapsed and/or refractory AML. *MDR1* expression in de novo AML is an adverse prognostic factor for CR and survival. It is conceivable that up-regulation of the *MDR1* gene is involved in the development of relapse and/or refractory disease, although this has not been investigated in paired analyses of respectable numbers of clinical samples of patients with AML. In the present study we analyzed whether clonal selection associated with the *MDR1* gene is involved in the development of relapsed AML. This is the first study that examined the allelic expression of *MDR1* in AML, using the genetic polymorphism of the *MDR1* gene. Our data show that there is no evidence of a *MDR1* gene–related clonal selection in the evolution of AML to relapse or refractory disease.

This is consistent with our observation that P-gp expression and function did not increase from diagnosis to relapsed/refractory state. Several studies have reported a higher *MDR1* expression at time of relapse as compared to diagnosis. However, most studies compared patients who were not matched and studies in paired patient samples are scarce and generally they were performed in small numbers of patients. Most studies suggest an identical expression or even lower level of *MDR1* in relapsed/refractory AML. Only the sequential analysis by Wood, who used immunocytochemistry techniques, showed a higher percentage of P-gp–positive samples in 14 relapsed patients with AML as compared with diagnosis. In pediatric patients, only 3 case reports are available. Therefore, although many studies have suggested that *MDR1* is up-regulated in relapsed and/or refractory AML, sequential studies do not support this theory (Table 5 and Table 6). The present analysis, which is the largest paired study in AML thus far, is an attempt to quantify MDR1 expression at genomic and protein level during the development toward resistant disease. In the 9 children and 21 adults studied, we did not find evidence that *MDR1*, although being a strong prognostic factor at the time of diagnosis, is up-regulated at time of relapse and/or refractory disease in AML. We suggest that similar sequential studies of other mechanisms of drug resistance should be performed in patients with AML during the course of their disease in order to determine which drug resistance proteins are associated with clonal selection at relapse. In these studies it will be important to analyze children and younger adults separately from elderly patients with AML, since different mechanisms might be important in different age groups. Until now, the only study that analyzed P-gp expression in a large group of children with AML showed that in contrast to adult AML, *MDR1* expression was not of prognostic significance. In the present study no difference was found in P-gp expression and function between adults and children.

Our study emphasizes that it is important to study *MDR1* expression in clinical samples from patients with AML. In many cell lines, including even AML cell lines, MDR expression may be up-regulated as a direct response of cells to antineoplastic drugs.
However, it seems apparent that this does not occur in patients with AML.\(^{1-3}\) This is the first analysis of the functional significance of the genetic polymorphism of \(MDR1\) in highly purified samples of AML. P-gp function and expression were similar in any one of the specific allelic variants (G, T, and GT). These findings suggest that the genetic polymorphism of the \(MDR1\) gene (at position 2677) lacks functional importance in AML. However, we found that patients with homozygous expression of the \(MDR1\) gene (GG or TT) had a shorter time to relapse and overall survival from relapse/refractory disease than heterozygous patients. This finding warrants further studies on the role of genetic polymorphisms of \(MDR1\) in AML.

\(MDR1\) expression at diagnosis is a strong adverse prognostic factor in AML. However, our sequential analysis reveals that there is no higher function or expression of P-gp at relapse or refractory disease, and that specific allelic expression is not related to increased P-gp expression or function. Since no loss of a specific \(MDR1\) allele has been observed in these patients with AML, \(MDR1\) gene–related clonal selection plays no role in the development of resistant disease. These data suggest that mechanisms other than \(MDR1\) might be responsible for the development of clinical resistance in these patients.

### Acknowledgment

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### References


MDR1 gene–related clonal selection and P-glycoprotein function and expression in relapsed or refractory acute myeloid leukemia

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