P-glycoprotein and Multidrug Resistance Protein Activities in Relation to Treatment Outcome in Acute Myeloid Leukemia

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ABSTRACT

Despite treatment with intensive chemotherapy, a considerable number of patients with acute myeloid leukemia (AML) die from their disease due to the occurrence of resistance. Overexpression of the transporter proteins P-glycoprotein (P-gp) and multidrug resistance protein (MRP) 1 has been identified as a major cause of cross-resistance to functionally and structurally unrelated drugs. In the present study, the functional activity of P-gp and MRP was determined in 104 de novo AML patients with a flow cytometric assay using rhodamine 123 (Rh123) in combination with PSC833 and carboxyfluorescein (CF) in combination with MK-571. The results were compared with clinical outcome and with known prognostic factors. The functional activity of P-gp and MRP, expressed as Rh123 efflux blocking by PSC833 and CF efflux blocking by MK-571, demonstrated a great variability in the AML patients. A strong negative correlation was observed between Rh123 efflux blocking by PSC833 and Rh123 accumulation (rs = −0.69, P < 0.001) and between CF efflux blocking by MK-571 and CF accumulation (rs = −0.59, P < 0.001). A low Rh123 accumulation and a high Rh123 efflux blocking by PSC833 were associated with a low complete remission (CR) rate after the first cycle of chemotherapy (P = 0.008 and P = 0.01, respectively). Patients with both low Rh123 and CF accumulation (n = 11) had the lowest CR rate (6%), whereas patients with both high Rh123 and CF accumulation (n = 11) had a CR rate of 73%. AML patients with French-American-British classification M1 or M2 showed a lower Rh123 accumulation than patients with French-American-British classification M4 or M5 (P = 0.02). No association was observed between the multidrug resistance parameters and overall survival of the AML patients. Risk group was the only predictive parameter for overall survival (P = 0.003).

INTRODUCTION

Intensive chemotherapy consisting of Ara-C and anthracyclines results in a CR rate of 60–70% in patients with AML. Despite these intensive chemotherapy regimens, a considerable number of AML patients die from their disease predominantly because of a relapse of the disease. An important underlying mechanism is resistance to chemotherapeutic drugs at either the time of diagnosis or relapse. The overexpression of ATP-dependent membrane transporter proteins in AML cells has been identified as one of the major causes of resistance to structurally and functionally unrelated drugs, the so-called MDR. Overexpression of one of these proteins, P-gp (8, 9), which acts as an ATP-dependent membrane efflux pump and is encoded by the MDR1 gene, has been shown to be a poor prognostic factor in AML (10–12). Additional membrane transporters are MRP1 (12, 13) and its homologue, MRP2 (14). Expression of each of the P-gp, MR1, and MRP2 proteins results in a reduced cellular accumulation of cytostatic agents due to active efflux of these substrates (14–17). P-gp and MRP1 confer resistance to a similar but not identical spectrum of anticancer agents. The transport kinetics of anthracyclines by P-gp and MRP are very similar (18). However, the important difference between P-gp and MRP1 is that MRP1 transports cationic and neutral compounds only in the presence of GSH and that it also transports a wide range of multivalent organic anions, including GSH conjugates (19–21).

We have recently presented a functional assay for MRP activity in which the fluorescent MRP substrate CF was used in combination with the leukotriene D4 receptor antagonist and MRP inhibitor MK-571 in a flow cytometric assay (23).
MRP activity, expressed as CF efflux blocking by MK-571, correlated with the MRPI protein expression in AML blasts.

The data about the importance of the expression and functional role of MRPI in clinical drug resistance in AML are diverse. Some studies suggest that MRPI mRNA expression is a prognostic factor for the achievement of CR in AML (24, 25) and demonstrate increased expression of MRPI mRNA at relapse (25). Other studies describe no effect of MRPI mRNA and protein expression on the treatment outcome of de novo AML patients (26–28). An additional study by Legrand et al. (29) of 56 AML patients reported that MRP functional activity, as determined in a flow cytometric assay with calcine and the MRP inhibitor probenecid, but not MRPI protein expression, is an unfavorable prognostic factor for the achievement of CR. The same group recently presented a study in which a correlation is described between simultaneous activity of MRP and P-gp and in vivo resistance in AML (30).

In the present study, the clinical significance of the functional activity of MRP and P-gp and the relationship between both transporters in 104 de novo AML patients were studied. MRP and P-gp activities were determined in protocol-treated patients with a flow cytometric assay using CF with the MRP inhibitor MK-571 and Rh123 with the P-gp inhibitor PSC833. MRPI, MRPII, and MDR1 mRNA levels were determined by RT-PCR. GSH level was measured in a flow cytometric assay with the GSH substrate MCB. Univariate and multivariate analyses were performed, and the results were compared with known prognostic factors and with clinical outcome.

MATERIALS AND METHODS

Patients. Bone marrow or peripheral blood was collected for diagnostic evaluation from newly diagnosed patients with primary AML who were admitted during a 9-year period (1988–1997) in the participating centers. Patients were included in this functional study if a sufficient number of bone marrow or peripheral blood cells (≥20 x 10⁶) were collected. Several clinical characteristics were determined, such as biological characteristics (WBC count, FAB classification, and karyotype) and the response to treatment. Cytogenetic analysis was performed using standard binding techniques according to the International System for Human Cytogenetic Nomenclature ISCN 1995. The patients were treated according to the standard protocol of the Dutch-Belgian Hemato-Oncology Cooperative Hovon Group (31) for AML Hovon 4/4A or Hovon 29 (32). Protocol Hovon 4/4A consisted of daunorubicin (45 mg/m², i.v., days 1–3) plus Ara-C (200 mg/m², i.v., days 1–7), followed by a second induction cycle of amsacrine (120 mg/m², i.v., days 4–6) plus Ara-C (2 g/m², i.v., days 1–6). The AML Hovon 29 protocol consisted of the same regimen as the Hovon 4/4A protocol, except that idarubicin (12 mg/m², i.v., days 5–7) was used instead of daunorubicin in induction cycle 1. Hovon 4/4A patients were randomized to treatment with or without granulocyte macrophage colony-stimulating factor (5 µg/kg, s.c.) after each cycle of chemotherapy. Hovon 29 patients were randomized between treatment with or without granulocyte colony-stimulating factor (150 µg/m²/day, s.c.) during chemotherapy. Hovon 29 patients with FAB classification M3 received all-trans-retinoic acid. After the two induction cycles, patients were to receive a third cycle consisting of mitoxantrone (10 mg/m², i.v., days 1–5) plus etoposide (100 mg/m², i.v., days 1–5), an autograft, or a HLA-matched allograft, depending on the risk estimates according to protocol and the availability of a HLA-matched donor. CR status was determined after each cycle of chemotherapy and after transplantation and was defined as normocellular peripheral blood and <5% blasts in a bone marrow smear.

Cell Lines. The human small cell lung cancer cell line GLC4 (33) and the in vitro doxorubicin-selected MRPI-over-expressing sublines GLC4/ADR2x, GLC4/ADR25x, and GLC4/ADR150x (the numbers indicate the resistance factor for doxorubicin; Ref. 34) were cultured in RPMI 1640 (BioWhittaker, Brussels, Belgium) supplemented with 10% FCS (Hyclone, Logan, UT). The doxorubicin-selected cell lines were cultured in the presence of doxorubicin (2 µM; Pharmacia and UpJohn, Woerden, the Netherlands).

The MDRI-transfected cell line GLC4/P-gp (35, 36) was cultured in RPMI 1640 with 10% FCS in the presence of 50 nm vincristine (Teva Pharma BV, Mijdrecht, the Netherlands).

Sample Preparation. Mononuclear cells from bone marrow or peripheral blood were enriched by Ficoll-Isopaque (Nycomed, Oslo, Norway) density gradient centrifugation. The cells were cryopreserved in RPMI 1640 supplemented with 10% FCS and 10% DMSO (Merck, Amsterdam, the Netherlands) and stored at −196°C. On analysis, cells were thawed, centrifuged in normal calf serum (Life Technologies, Inc., Breda, the Netherlands), treated with DNase (Boehringer Mannheim, Mannheim, Germany), and washed with RPMI 1640. Subsequently, the AML cells were incubated for 1 h in RPMI 1640 supplemented with 10% FCS at 37°C in 5% CO₂. Viability of the cells was determined by trypan blue exclusion, and cases with a viability of <90% were excluded from this study.


Functional activity of the P-gp and MRP transporters was demonstrated as described previously (22). To determine MRP activity, the CFDA compound (Sigma Chemical Co., Bornem, Belgium) was used, which permeates the plasma membrane and is transformed into the fluorescent anion CF on cleavage of the ester bonds. CFDA was used in combination with the leukotriene D₄ receptor antagonist and MRP inhibitor MK-571 [provided by Dr. A.W. Ford-Hutchinson; Merck Sharp, Kirkland, Quebec, Canada (37)]. For the detection of P-gp activity, Rh123 (Sigma Chemical Co.) was used together with the P-gp inhibitor PSC833 (provided by Novartis Pharma Inc., Basel, Switzerland).

Cells (0.5 × 10⁶) were loaded for 20 min at 37°C in 5% CO₂ with 0.1 µM CFDA or 200 ng/ml Rh123 with or without inhibitor (20 µM MK-571 or 2 µg/ml PSC833) in RPMI 1640. Thereafter, cells were washed in ice-cold medium and incubated for 1 h in drug-free medium with or without inhibitor at 37°C in 5% CO₂ to allow efflux to occur. Efflux was stopped by pelleting the cells and adding ice-cold medium. Fluorescence of CF and Rh123 was analyzed with a FACStar flow cytometer (Becton Dickinson Immune Cytometry Systems, Mountain View, CA) equipped with an argon laser. The blast population was gated by forward- and side-scatter characteristics. CF and Rh123 fluorescence of 10,000 events was measured logarithmically through a 530 nm bandpass filter at an excitation wave-
length of 488 nm. Each time the assay was performed, a mixture of beads with several different, known numbers of molecules of FITC fluorescent dye (Flow Cytometry Standards Europe, Leiden, the Netherlands) was measured under the same conditions. The logarithmically acquired signals were converted geometrically into linear values and expressed as MESF units using the programs Quick Call for Winlist and Winlist 32 (Verity Software House, Inc., Topsham, ME). CF and Rh123 accumulation after 1 h of efflux, as a measure for MRP and P-gp activity, respectively, was expressed in MESF units. The efflux-blocking factors of the inhibitors were expressed as the median MESF value in inhibitor-blocked cells divided by the median MESF value in unblocked cells after 1 h of efflux. Each time the assay was performed, GLC4 cells served as a control.

Flow Cytometric Detection of GSH. GSH-bimane conjugates were detected in a flow cytometric assay as described by Lorico et al. (38), with some modifications. Cells (0.5 × 10⁶) were incubated with 10 μM MCB (Calbiochem-Behring, La Jolla, CA) for 1 h at 4°C. At this concentration and incubation time, a plateau of intracellular fluorescence was reached. The cellular fluorescence of GSH-bimane conjugates was measured with an Epics-Elite flow cytometer (Coulter Electronics, Hialeah, FL). Emission was collected with a 525 nm bandpass filter with an argon laser tuned at 350 nm. To standardize the assay, the fluorescence of MCB-treated GLC4 cells from a frozen stock was measured each time the assay was performed. GSH content of the patient samples was expressed as the median fluorescence percentage of the GLC4 cells, and a correction for cell size, as measured by forward-scatter characteristics, was applied.

RNA Extraction and RT-PCR Analysis for MRP1, MRP2, and MDR1. Total cellular RNA was isolated from 5–10 × 10⁶ AML blasts or cell line cells using Trizol reagent (Life Technologies, Inc.). RNA was extracted, precipitated, and was heated according to the manufacturer’s protocol. Five μg of RNA were reverse-transcribed and supplemented with H₂O up to a final volume of 50 μl. The PCR reactions were performed under the following conditions. For MRP1, 29 cycles of denaturation (95°C, 30 s), annealing (56°C, 30 s), and extension (72°C, 30 s) were performed. For the cell line samples, 27 PCR cycles were performed. For MRP2, 31 cycles (95°C, 58°C, and 72°C, 30 s) were performed; for MDR1, 31 cycles (95°C, 55°C, and 72°C, 30 s) were performed; and for β-2 microglobulin, 20 cycles (95°C, 55°C, and 72°C, 30 s) were performed. The amplified products contain 990 bp for MRP1, 1067 bp for MRP2, 161 bp for MDR1, and 268 bp for β-2 microglobulin. Reaction products were separated on a 1.5% agarose gel and visualized by ethidium bromide staining. The absorbance of the visualized bands was expressed as absorbance × mm² using the program Diversity One 1D (PDl, New York, NY). The mRNA expression the MDR genes was expressed as the absorbance × mm² of the MRP1, MRP2, or MDR1 PCR product divided by the absorbance × mm² of the β-2 microglobulin PCR product.

Statistical Analysis. The analysis presented here must be considered primarily exploratory. The implicit hypothesis or expectation at the initiation of the study was that low accumulation or high efflux blocking would go together with a low CR rate and a high overall death rate. However, because it was unknown whether such a relation would be more or less continuous or whether a cutpoint would exist depending on the parameter considered, the following explorative approach was followed. CR after cycle 1 was chosen as the primary end point for measuring response on treatment, whereas OS was used for measuring overall outcome. Logistic regression analysis was used to test for association between a factor and the probability of CR after cycle 1. Cox regression analysis was used to test for an association with death rate. Tests for trend in such an analysis with a continuous parameter were done by using a logarithmic transform of the parameter as a covariate in the analysis. Due to the lack of a priori cutpoints, the range of each continuous variable was arbitrarily subdivided into three subgroups of equal size to determine CR rates in these subgroups with low, medium, and high values. When a significant association was observed, isotonic regression (39) analysis was performed to search for an optimal cutpoint to subdivide low and high values. The method of Kaplan and Meier was used to calculate survival curves and probabilities (40). Tests for trend in regression analyses were performed with and without adjustment for risk group. Spearman rank correlation was used as a measure for the association between continuous variables. The Kruskal-Wallis test was used to test for differences between subgroups in the distribution of ordinal variables.

RESULTS
Clinical Characteristics and Treatment Outcome of AML Patients. In 116 patient cases with de novo AML, a sufficient number of bone marrow or peripheral blood cells were available. Twelve patients were excluded because follow-up data were lacking; thus, 104 patients were included in the present analysis. A bias for WBC count was observed because patients with high WBC counts (>50 × 10⁹) were overrepresented in the study. All patients were treated with at least one induction cycle of chemotherapy. Ninety-six patients received chemotherapy cycle 2; of these patients, 33 received chemotherapy cycle 3, and 23 patients were treated with an autograft or allograft transplantation. The clinical characteristics, FAB classifications, and WBC counts of these patients are listed in Table 1. Considering the FAB classifications and cytogenetics, the patients were subdivided into three groups, i.e., favorable karyotype [t(8;21); FAB classification M3 with t(15;17), inv(16), del(16)], very poor (−5q, −7q), or poor [other karyotypes (Table 1)]. Twenty-four patients (23%) achieved no CR at any moment, and 49 patients (47%) relapsed or died in CR; thus, 31 patients (30%) were in CR at the time of evaluation (Table 1). The median follow-up time of the patients still alive (n = 38) was 15 months (range, 2–109 months). The last patient was entered in March 1998, and the time of evaluation was November 1998.

P-gp and MRP Activity and GSH Level in AML Patients. The functional activity of P-gp and MRP demonstrated a great variability in efflux-blocking factors. Rh123 efflux-blocking factors of PSC833 varied between 0.7 and 37.8, and efflux-blocking factors of CF by MK-571 varied between 1.0–10⁹ MESF. Each time the assay was performed, a mixture of beads with several different, known numbers of molecules of FITC fluorescent dye (Flow Cytometry Standards Europe, Leiden, the Netherlands) was measured under the same conditions. The logarithmically acquired signals were converted geometrically into linear values and expressed as MESF units using the programs Quick Call for Winlist and Winlist 32 (Verity Software House, Inc., Topsham, ME). CF and Rh123 accumulation after 1 h of efflux, as a measure for MRP and P-gp activity, respectively, was expressed in MESF units. The efflux-blocking factors of the inhibitors were expressed as the median MESF value in inhibitor-blocked cells divided by the median MESF value in unblocked cells after 1 h of efflux. Each time the assay was performed, GLC4 cells served as a control.

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efflux-blocking factor PSC833 and Rh123 accumulation measured after 60 min of efflux ($r_s = 0.69; P < 0.001$) and between CF efflux-blocking factor of MK-571 and CF accumulation represent MRP function, and MCB content represents GSH levels of the AML patients as compared with the GSH level of GLC4 cells (100%).

GSH level, expressed as the percentage of MCB in AML cells compared with GLC4 cells, varied between 0% and 132% in the AML patients. A correlation was observed between GSH level and MRP function, as measured by CF efflux blocking by MK-571 ($r_s = 0.30; P = 0.005$).

No correlation was observed between the five MDR parameters (Rh123 efflux blocking by PSC833, Rh123 accumulation, CF efflux blocking by MK-571, CF accumulation, and GSH level) and age. A weak correlation was found between WBC count and Rh123 accumulation ($r_s = 0.25; P = 0.01$) and Rh123 efflux-blocking factor of PSC833 ($r_s = 0.23; P = 0.02$).

Considering the FAB classifications, AML patients with FAB classifications M1 or M2 showed a lower Rh123 accumulation than patients with FAB classifications of M4 or M5 ($P = 0.02$). However, Rh123 efflux-blocking factors of PSC833 did not differ between these groups ($P = 0.39$). With respect to CF accumulation or CF efflux blocking by MK-571 or GSH level, no differences were observed between patients with FAB classifications of M1 or M2 and patients with FAB classifications of M4 or M5.

MRP1, MRP2, and MDR1 mRNA Expression in Cell Lines and AML Patients. Total cellular RNA was obtained from 18 patient samples and from GLC4, GLC4/ADR2x, GLC4/ADR25x, GLC4/ADR150x, and GLC4/P-gp cell lines. mRNA expression of $\beta$-2 microglobulin and MRP1 of the cell lines is shown in Fig. 1A (MDR1 data not shown) and correlated well with the MRP and P-gp functions (expressed as efflux-blocking factors) of the cell lines (B).
cell line and its MDR1-transfected counterpart GLC4/P-gp demonstrated Rh123 efflux-blocking factors by PSC833 of 0.86 ± 0.3 (n = 3) and 3.0 ± 0.8 (n = 5), respectively (Fig. 1B).

A large variation in expression of MRP1, MRP2, and MDR1 mRNA was observed in the AML patient samples. Correlations were observed between MRP1 mRNA expression and CF efflux-blocking factors of MK-571 (r = 0.47; P < 0.05; n = 18; Fig. 2A) and between MDR1 mRNA expression and Rh123 efflux-blocking factors of PSC833 (r = 0.63; P < 0.01; n = 18; Fig. 2B). No correlation was observed between MRP2 mRNA expression and CF efflux-blocking factors of MK-571 in the AML patient samples (data not shown).

Relation between P-gp or MRP Activity or GSH Level and Treatment Outcome. Because all patients received induction cycle 1 with daunorubicin or idarubicin, which are substrates for P-gp and/or MRP, the achievement of CR after induction cycle 1 was chosen as an end point.

Eighty patients reached CR; of these patients, 49 achieved CR1. Table 3 shows the probabilities of reaching CR1 in subgroups of almost equal size defined by risk, age, and the MDR parameters considered. Risk group was the most important factor for reaching CR1; 100% of good risk patients versus 14% of very poor risk patients achieved CR1 (P < 0.001). No correlation was found between age and the achievement of CR. A low Rh123 accumulation and a high Rh123 efflux blocking of PSC833 were associated with a low CR1 rate (P = 0.008 and P = 0.01, respectively). It was observed that the CR1 rate in the subgroup with the lowest Rh123 accumulation was 25% versus 58% in the subgroup with the highest Rh123 accumulation. Isotonic regression analysis suggested 90,000 MESF units as an optimal cutpoint for a subdivision of low and high Rh123 accumulation. A low CF accumulation was also associated with a lower CR1 rate (P = 0.04); the subgroup with the lowest CF accumulation had a CR1 rate of 34% versus 48% in the group with the highest accumulation, but the differences were minor as

<table>
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<th>No. of patients</th>
<th>CR1 (%)</th>
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4 EBF, efflux-blocking factor.
5 Rh123 and CF accumulation, expressed as MESF units.
6 Low, Rh123 EBF of PSC833 ≤ 3.25 and CF EBF of MK-571 ≤ 2.3; high, Rh123 EBF of PSC833 > 3.25 and CF EBF of MK-571 > 2.3; medium, all other values.
7 Low, Rh123 accumulation ≤ 9,000 and CF accumulation ≤ 157,900 MESF units; high, Rh123 accumulation > 9,000 and CF accumulation > 350,000 MESF units, medium: all other values.

Fig. 2 Correlation between MRP1 mRNA expression and CF efflux-blocking factors of MK-571 (A) and between MDR1 mRNA expression and Rh123 efflux-blocking factors of PSC833 (B) in 18 AML patients. mRNA levels are expressed as absorbance × mm² of the visualized bands of MRP1 or MDR1 mRNA divided by absorbance × mm² of β-2 microglobulin mRNA.
compared with the Rh123 accumulation, and a test for trends based on the logarithmic transform showed no statistical significance. Of special interest is the fact that the subgroup of patients with high efflux-blocking factors of both Rh123 and CF had a CR1 rate of 0% ($n = 14$), and the subgroup of patients with both low efflux-blocking factors of Rh123 and CF showed a CR1 rate of 56% ($n = 57$; Table 3). The patients with low accumulation of both Rh123 (<90,000 MESF units) and CF (<157,900 MESF units) had the lowest CR1 rate (6%; $n = 16$), whereas the subgroup of patients with both high Rh123 (>90,000 MESF units) and CF (>350,000 MESF units) accumulation had a CR1 rate of 73% ($n = 11$; Table 3).
The GSH level showed a moderate positive association with the probability of CR1, but the trend was not significant. No significant association was observed between P-gp or MRP activity or GSH level and OS (Fig. 3; Table 3), although it is interesting to note that the lowest 5-year survival rates were observed for the patients with a low Rh123 and CF accumulation or a high Rh123 and CF efflux blocking (Table 3). Risk group was the only predictive parameter for OS ($P = 0.003$).

A subgroup of patients (33 of 104 patients), who did not receive an autograft or allograft transplantation, were treated with the MDR drug mitoxantrone in the third cycle of chemotherapy. In this group of patients, those with a high CF accumulation showed a better EFS after 24 months ($P = 0.02$). Also, patients with a low efflux blocking by MK-571 showed a better EFS rate after 24 months, but the difference was not statistically significant ($P = 0.16$). No association was found between EFS rate and Rh123 accumulation or efflux blocking by PSC833. However, the group of patients is relatively small (33 of 104 patients), and the differences observed between the low and high groups were not apparent in a test for trends based on the logarithmic transform.

**DISCUSSION**

In this study, P-gp and MRP activity was measured in a large number of patients to determine whether MRP activity, in addition to P-gp, might be of relevance for the clinical outcome in patients with AML. The measurement of fluorescence modulation of Rh123 by PSC833 and CF by MK-571 is a reliable approach to determine P-gp and MRP activity. Broxterman et al. (42) demonstrated that the modulation of Rh123 by PSC833 is the most sensitive assay to detect P-gp activity or expression. However, some studies have described discrepancies between P-gp protein or MDR1 mRNA expression and P-gp function (43, 44). With regard to MRP activity, we have observed previously (23) that a correlation exists between MRP activity expressed as the modulatory effect of probenecid on the fluorescence of calcein and MRP1 protein expression in AML patients. In the present study, a strong negative correlation was observed between Rh123 efflux blocking by PSC833 and Rh123 accumulation and between CF efflux blocking by MK-571 and CF accumulation.

![Fig. 3 D–E Continued.](image_url)
The measurement of Rh123 and CF accumulation appears to be a sensitive and easy method to determine P-gp and MRP activities; however the use of the inhibitors PSC833 and MK-571 remains necessary to control the specificity of the assay. Correlations were observed between MDR1 mRNA expression and P-gp function and between MRPI mRNA expression and MRP function, but not between MRP2 mRNA expression and MRP function, suggesting that MRP1 exerts a decisive role in the efflux of CF. Some discrepant AML cases were observed with high P-gp or MRP function in combination with a low mRNA expression or vice versa (Fig. 2). These discrepancies might reflect the presence of MRPI homologues, additional unknown efflux pumps, or the existence of a dysfunctional P-gp or MRPI protein. With regard to the GSH level, GSH content in AML cells correlated well with MRP function, as measured by CF efflux blocking of MK-571. In some other studies, chemotherapeutic drugs, such as vincristine and daunorubicin, are described as being transported by MRP in a GSH-dependent way (16, 21, 45). However, CF is transported by MRP in a GSH-independent manner. A coordinated induction of MRP and GSH-related enzymes is reported in drug-resistant human leukemia cells and colon cancer cells (46, 47), which might also be the case in the present study.

This study demonstrates that MRP activity is not an independent prognostic factor for the achievement of CR after chemotherapy cycle 1, although patients with both high P-gp and MRP activity show a lower CR rate after cycle 1 than patients with low P-gp and MRP activity. CR rate after cycle 1 was chosen as an end point for this study because all patients received the MDR drugs daunorubicin or idarubicin only in induction cycle 1, whereas only a subgroup of patients (33 of 104 patients) was treated with the MDR drug mitoxantrone in the third cycle of chemotherapy. Legrand et al. (30) recently reported that not only P-gp but also MRP activity was a prognostic factor for achieving CR in AML. In addition, their study described a correlation between the simultaneous activity of P-gp and MRP and the OS of the patients. The discrepancy between both studies with regard to OS is not clear because in both studies, MDR drugs were applied in the first course of chemotherapy.

When the subgroup of patients who received mitoxantrone in the third cycle of chemotherapy (33 of 104 patients) was analyzed separately, a trend was observed between MRP function and EFS after 24 months, but not between P-gp function and EFS, which suggests that mitoxantrone is effluxed by MRP and is not effluxed or is effluxed to a lesser extent by P-gp. Thus far, results of the studies on mitoxantrone as a substrate for P-gp or MRP are conflicting. Mitoxantrone has been described to be a substrate for P-gp (48, 49), whereas in certain P-gp-positive cell lines, no effect of a P-gp inhibitor on mitoxantrone sensitivity was observed (50). With regard to MRP, it has been reported that MRP does not play a role in effluxing mitoxantrone (51, 52), although a cross-resistance has been described for mitoxantrone in a MRPI-positive cell line selected for resistance to etoposide (53).

P-gp activity was higher in the very poor risk group as compared with the other risk groups, which was also observed in the study of Legrand et al. (30). However, when adjusted for risk group, P-gp activity was not an independent predictive factor for OS. The only predictive parameter for OS appeared to be the defined risk group.

Rh123 accumulation was lower in FAB classifications M1 and M2 than in FAB classifications M4 and M5, demonstrating a higher P-gp activity in myeloblastic cells than in monoblastic AML cells. Because the expression of the CD34 antigen is greater on myeloid cells than on monocytic cells, these findings are in agreement with the results of Te Boekhorst et al. (54), who reported a predominance of functional P-gp in CD34-positive AML cells.

In view of the results obtained, the possibility of measuring P-gp and MRP activities in newly diagnosed AML patients before the chemotherapy regimen should be considered to construct a risk analysis in which patients with high P-gp and MRP activities can be identified. This would allow us to analyze whether treatment with a limited number of MDR drugs or inhibitors of P-gp as well as MRP function might improve the results.

Thus far, the use of competitive inhibitors of P-gp such as cyclosporin A and PSC833 in Phase II/III clinical studies has shown diverse results. Some studies indicate that the use of inhibitors improves the uptake of drugs in cells of patients with AML and multiple myeloma (55–57), whereas other studies report an increased toxicity due to these inhibitors as a result of a change in the pharmacokinetics (58, 59). Furthermore, it is conceivable that in cases in which P-gp function is inhibited, additional MDR proteins such as MRPI or one of its homologues can take over the P-gp function, at least in part. Therefore, for a full understanding of the mechanism of MDR modulation, the evaluation of activity and expression of all known MDR proteins will be required in future studies.

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P-glycoprotein and Multidrug Resistance Protein Activities in Relation to Treatment Outcome in Acute Myeloid Leukemia

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