# Breast Cancer Resistance Protein and P-Glycoprotein in 149 Adult Acute Myeloid Leukemias

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## ABSTRACT

***Purpose:*** **Recently, a new ABC protein, breast cancer resistance protein (BCRP), was described. But its prognosis is not known in acute myeloid leukemia (AML). In addition, the prognosis of P-glycoprotein (Pgp) and BCRP in patients treated homogeneously by the same anthracycline (daunorubicin, idarubicin, or mitoxantrone) during all of the treatment with aracytine is not known. Therefore, we have evaluated the relationship between drug resistance phenotype, *in vitro* anthracene sensitivity, and the relation to treatment outcome.**

***Experimental Design:*** **We have analyzed 149 AML treated according to protocol of the European Organization for Research and Treatment of Cancer group. The prognostic value of BCRP and Pgp were analyzed in the whole population and according to intercalating agent.**

***Results:*** **BCRP was a prognostic factor, for achievement of complete remission (43% in positive patients and 69% in negative patients, $P = 0.005$), the 4-year disease-free survival (12% *versus* 33%, $P = 0.03$), and the 4-year overall survival (19% *versus* 38%, $P = 0.003$). When BCRP expression and Pgp function were categorized in three groups, +/+, +/− or −/+, and −/−, the achievement of complete remission was 45%, 66%, and 90% ($P = 0.0003$), the 4-year disease-free survival was 8%, 26%, and 40% ($P = 0.01$), and the 4-year overall survival was 16%, 37%, and 48% ($P = 0.001$), respectively. Pgp function was a prognostic factor in patients treated by daunorubicin and idarubicin but not by mitoxantrone. In contrast, BCRP expression was a prognostic factor in patients treated by daunorubicin and mitoxantrone but not by idarubicin.**

***Conclusions:*** **BCRP would be implicated in the resistance to chemotherapies in AML. But these are the patients expressing both BCRP and Pgp who have the poorest prognosis.**

## INTRODUCTION

Despite improvements accomplished in the last thirty years with the use of combination of cytarabine (Ara-C) and intercalating agents, the overall prognosis of adult acute myeloid leukemia (AML) remains poor (1). One of the best-characterized resistance mechanisms in AML is the drug extrusion mediated by ABCB1 [P-glycoprotein (Pgp)], the product of the multidrug resistance-1 (MDR1) gene, which has been shown to be associated with a poor outcome. In most studies, Pgp expression and function correlate with a poor prognosis (2–8). An approach that might improve therapy is to use intercalating agents, as idarubicin or mitoxantrone, that are less susceptible to Pgp-mediated *in vitro* resistance than the classic drug daunorubicin (9). But at this time, the results of the clinical studies comparing these anthracyclines are debated (10–12). A collaborative overview with individual patient data were done to compare idarubicin to daunorubicin or other anthracyclines, when used with Ara-C as induction chemotherapy for newly diagnosed AML (1,052 patients). In this study, the induction regimens based on idarubicin showed better remission rates and a better overall survival than those based on daunorubicin (10). But in the preliminary results of the European Organization for Research and Treatment of Cancer-Gruppo Italiano Malattie Ematologiche dell' Adulto AML-10 randomized trial (2,113 patients), the results of mitoxantrone and idarubicin are not superior to daunorubicin (12). The predictive role of these ABC proteins in the patients receiving only mitoxantrone, idarubicin, or daunorubicin is not clearly defined until now.

Recently, a new ABC protein ABCG2 [breast cancer resistance protein (BCRP)] was described in a MDR1-negative, ABCC1 (MRP1)-negative cell lines resistant to anthracycline and mitoxantrone (13–15). The *in vitro* drug-resistant profile of BCRP-positive cell lines was done by Litman *et al.* (16). BCRP-positive cells were cross-resistant to mitoxantrone, daunorubicin, doxorubicin, bisantrene, and topotecan but remained sensitive to vinblastine and paclitaxel. In recent clinical studies, in few patients, expression of BCRP was sufficiently frequent to warrant more investigations, to determine the relation of treatment outcome (17, 18), in contrast to other studies (19, 20). In one report, the tranduced clonal cell lines expressing various levels of BCRP expression conferred resistance to mitoxantrone but not to idarubicin. In another study, 59 childhood AML patients who expressed high levels of BCRP had a worse prognosis (21). Therefore, at this time, the prognosis of BCRP is not well known in AML.

Here, we report the analysis of Pgp activity and BCRP expression in a population of AML patients receiving the same intercalating agents during all of the treatment: daunorubicin, idarubicin, or mitoxantrone in combination with standard doses

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of aracytine. Both BCRP and Pgp were analyzed in whole population and according to the intercalating agent. We have evaluated the relationship between drug resistance phenotype, *in vitro* anthracene sensitivity, and the relation to treatment outcome.

## MATERIALS AND METHODS

### Patients

Between January 1995 and August 2000, 149 samples from *de novo* adult AML patients were successfully tested for Pgp function and BCRP expression. The diagnosis was based on French-American-British criteria (22). Immunophenotyping was done by using flow cytometry. Acute promyelocytic leukemia patients were excluded from the study (due to retinoic acid treatment). Patients with t(9;22) were also excluded from the study. For each patient, several clinical and biological characteristics were analyzed (age, WHO performance status, WBC at diagnosis, lactate dehydrogenase level, CD34 expression, cytogenetic, Pgp function, and BCRP expression). Unfavorable karyotypes were defined as follows: abnormalities of chromosomes 5 or 7, abnormalities of 11q2.3 band, or complex abnormalities. Inversion in chromosome 16 or t(8;21) indicated good prognosis, and the other karyotypes, including normal, indicated intermediate prognosis (23).

No patients had a history of prior therapies with anticancer drugs. All of the patients of this study were given a combination of Ara-C (100 mg/m$^2$/day) for 10 days, and either daunorubicin (45 mg/m$^2$/day; 59 patients) or idarubicin (10 mg/m$^2$/day; 30 patients) or mitoxantrone (7 mg/m$^2$/day; 60 patients) for 3 days, and etoposide (100 mg/m$^2$/day) for 5 days. Those patients who achieved complete remission (CR) after one or two cycles of therapy received one cycle of consolidation therapy (with the same intercalating agent). Patients achieving CR were subsequently scheduled to proceed to allogeneic bone marrow transplantation if a matched sibling donor was available (13 patients); patients >45 years old or lacking a suitable donor received an autograft. A CR was defined by cellular marrow with <5% blasts, no Auer rods, and peripheral granulocyte and platelet counts of at least $1 \times 10^9$/l and $100 \times 10^9$/l, respectively.

### Level of BCRP Expression by Reverse Transcription (RT)-PCR

**RNA Extraction and RT-PCR.** Total RNA was extracted from $5 \times 10^6$ AML blasts with a TRI Reagent kit (Molecular Research Center, Inc., Cinncinati, OH). One microgram of RNA was reverse-transcribed (M-MLV Reverse Transcriptase, Invitrogen, Carlsbad, CA) and supplemented with $H_2O$ up to a final volume of 40 μL.

**LightCycler System.** The LightCycler is a real-time PCR instrument that allows both rapid PCR cycling and continuous monitoring of product formation (24). SYBR Green I, an intercalating dye that fluoresces strongly when bound to double-stranded DNA, is included in the reactions so that when PCR products are formed, fluorescence increases (25).

**RT-PCR Protocol.** Real-time RT-PCR was done with the LightCycler FastStart DNA with Master SYBR Green I kit (Roche, Paris, France). For the amplification of a 446 pb of the BCRP gene, primers 5′-TTA-GGA-TTG-AAG-CCA-AAG-G-3′ (sense) and 5′-TAG-GCA-ATT-GTG-AGG-AAA-ATA-3′ (antisense) were used. A 0.6 μmol/L of each primer, 3 mmol/L $MgCl_2$, and 5 μL of cDNA (or water) were added. After initial denaturation step at 95°C for 8 minutes, PCR was run for 50 cycles (95°C for 10 seconds, 55°C for 10 seconds, and 72°C for 20 seconds) in a Trioblock thermal cycler. After a final cooling step for 20 seconds at 40°C, melting curve analysis was done. From the external standards, a calibration curve was automatically generated. Samples were quantified accordingly (LightCycler Software) and standardized for kit h-β2 Microglobulin housekeeping Gene Set (Roche).

Values shown are the ratio between the BCRP gene expression obtained in the AML patient and PC13 2-2 transfected cell line that expressed a high level of BCRP gene (PC13 is a lung large cell carcinoma cell line; ref. 26). If necessary, a threshold of $200 \times 10^{-6}$ was used to assess a positive expression of BCRP. This threshold was selected because the positive patients have a higher level of $LC_{50}$ of daunorubicin and etoposide than negative patients in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In addition, positive patients have a level of BCRP above the level of BCRP of sensitive cell lines ($120 \times 10^{-6}$). Four sensitive cell lines were tested, HL60, MCF7/S, K562, and PC13 V12.

All of the samples contain at least 90% of blast cells. In addition, normal mature hematopoietic cells do not express BCRP, except natural killer lymphocytes (27). In 40 new samples of AML, we find a good correlation between functional assay with mitoxantrone ± fumitremorgin C and RT-PCR ($r$ = 0.75; $P$ = 0.001; personal data), in contrast to Pgp highly expressed in normal hematopoietic cells (28). Therefore, RT-PCR may be used to evaluate BCRP in AML samples.

### Functional Assay of Pgp with JC-1

In previous study, we have shown that JC-1 seemed to be a more convenient and simple way to detect a functional Pgp in clinical AML samples than rhodamine 123. Pgp function was measured as described previously (8, 29). In brief, for staining, cells were washed twice, resuspended in PBS containing 0.1 μmol/L JC-1 monomer at a concentration of $5 \times 10^5$ cells/mL, and incubated at 37°C for 15′ without or with modulator [cyclosporine (2 μmol/L)] to assess Pgp. Cells were washed twice in cold PBS, and samples were analyzed. Cells fluorescence were recorded with a FACSort flow cytometer (Becton Dickinson, Meylan, France) equipped with a 488-nm argon laser and three fluorescence detectors [FL1 (530-nm band-pass filter), FL2 (585 nm band-pass filter), and FL3 (650 nm band-pass filter)]. JC-1 fluorescences were analyzed on the FL1 and FL2 channels for detection of the fluorescence of the dye monomer and liquid crystal form, respectively. The function of Pgp was established with blast cells selected by CD34 antibody (FL3 channel; HPCA 2 clone, Becton Dickinson, Le Pont de Claix, France) or with physical characteristics, only if blast cells did not express characteristic markers. Intensity of JC-1 fluorescence in presence or absence of CsA was compared with the Kolmorogov-Smirnov test. A higher D value (ranging from 0 to 1) indicates a wider difference between the two functions, thus a more resistant group of cells. For each sample, 4,000 events were collected. If necessary, and in accordance with our previous studies, a thresh-

old of positivity of 0.4 (D value) was used to assess a positive activity of Pgp.

### MTT Cytotoxicity Test

*In vitro* cytotoxicity was measured as described previously (30). We used the MTT assay to assess the *in vitro* resistance to drugs. *In vitro* sensitivity of cells to daunorubicin, Ara-C, and etoposide was determined by planting $2 \times 10^5$ cells in a 200 μL growth medium, without any specific growth factor, containing several dilutions of the drug in 96-well microtiter plates. Each concentration of drugs was repeated in six wells. After incubation for 3 days at 37°C with 5% $CO_2$, cell viability was determined with this assay. Briefly, 20 μL of MTT (5 mg/mL in PBS) were added to each well and incubated for 6 hours. The medium and MTT were then removed from the wells by centrifugation, and formazan crystals were dissolved in 200 μL of DMSO. The absorbance was recorded in a microplate reader (Model MR5000, Dynatech Laboratories, Grenoble, France) at the wavelength of 550 nm. The effect of drug on growth inhibition could be assessed as follows: % of growth inhibition = 1 − [(absorbance of drug treated cells/absorbance of untreated cells) × 100]. The $LC_{50}$ was determined as the drug concentration, which resulted in a 50% growth inhibition. Samples were considered evaluable if the drug-free control wells contained >80% of leukemic cells before and >70% of leukemic cells after 3 days of culture. The MTT assay gave reliable results under these conditions. Percentage of blast cells was determined by the May-Grünwald-Giemsa stain and by immunophenotyping, which was done by flow cytometry.

### Statistical Analysis

The association between variables was analyzed by the Fisher's exact test for categorical variables and by the Mann Whitney *U* test or Kruskal-Wallis test for continuous variables. Clinical and biological factors were investigated for their influence on remission rate by the Fisher's exact test for binary variables and by the Mann Whitney *U* or Krushkal-Wallis tests for continuous variables. The rates of disease-free survival was measured from establishment of CR until relapse or death from any cause, with observation censored for patients last known alive without report of relapse; and the rates of overall survival was measured from diagnosis until death from any cause, with observation censored for patients last known alive. They were estimated by the Kaplan-Meier method (31) and compared by the log-rank test. Analyses of prognostic factors for treatment outcomes were based on proportional hazards regression models for disease-free survival and overall survival (32). Significance was defined as a two-tailed *P* of 0.05. The Cox proportional model was used for the multivariate analyses on disease-free survival and overall survival (32). The median follow-up time for censored patients was 1,098 days. The timepoint used for the proportion of disease-free survival and overall survival was December 31, 2003.

## RESULTS

### All Patients

Ninety-eight of 149 patients achieved a CR (66%). The percentage of disease-free survival was 28% ± 5% at 5 years (median disease-free survival 352 days), and the percentage of overall survival was 25% ± 4% at 5 years (median overall survival 337 days).

**Pgp Function and BCRP Expression.** As previously reported, Pgp function was a prognostic factor in all of the patients for achievement of CR (in patients achieving CR the mean value of Pgp function was 0.3 *versus* 0.48 in other patients, $P = 0.04$), disease-free survival ($P = 0.04$; relative risk = 1.7), and overall survival ($P = 0.01$; relative risk = 1.7) when analyzed in continuous variable. BCRP expression was not correlated with the other patient characteristics (Table 1), age, lactate dehydrogenase, hemoglobin, leukocyte level, CD34 expression, cytogenetic, WHO performance status, and gender. A low BCRP expression was correlated with a low *in vitro* resistance to daunorubicin ($100 \pm 120 \times 10^{-8}$ mol/L in positive patients *versus* $10 \pm 20 \times 10^{-8}$ mol/L in negative patients, $P = 0.01$) and etoposide ($2300 \pm 1200 \times 10^{-8}$ in positive patients *versus* $900 \pm 420 \times 10^{-8}$ in negative patients, $P = 0.02$) but not to Ara-C (not significant).

BCRP was a prognostic factor for achievement of CR (43% in positive patients *versus* 69% in negative patients; $P = 0.005$), the disease-free survival (12% ± 5% *versus* 33% ± 7% at 4 years; median disease-free survival, 224 *versus* 574 days; $P = 0.03$; Fig. 1*A*), and the overall survival (19% ± 4% *versus* 38% ± 6% at 4 years; median overall survival, 270 *versus* 617 days; $P = 0.003$; Fig. 1*B*). When analyzed in continuous variable, BCRP was also a prognostic factor for achievement of CR ($P = 0.04$), disease-free survival ($P = 0.02$), and overall survival ($P = 0.008$). BCRP expression was not correlated with Pgp function ($r = 0.04$; $n = 65$; not significant; data not shown). However, when BCRP expression and Pgp function were categorized in three groups [−/− (31 patients), +/− or −/+ (78 patients), +/+ (40 patients)], the −/− group had a better prognosis than the +/+ group. In the same way, the +/− or −/+ group have an intermediate prognosis. The achievement of CR was 90%, 66%, and 45% ($P = 0.0003$), respectively. The 4-year disease-free survival was 40% ± 8% (median, 574 days), 26% ± 11% (median, 370 days), and 8% ± 5% (median, 190 days; $P = 0.01$; Fig. 2*A*), respectively. The 4-year overall

*Table 1* Comparisons of clinical and biological variables and achievement of CR in patients BCRP+ or BCRP−

| Characteristics | BCRP+ (78 patients) | BCRP− (71 patients) | *P* |
|---|---|---|---|
| Age (year) | 51 ± 19 | 53 ± 18 | NS |
| WHO status (0, 1/2, 3, 4) | 55/23 | 53/18 | NS |
| Leukocytes ($\times 10^9$/l) | 95 ± 114 | 73 ± 68 | NS |
| LDH (UI/l) | 2655 ± 3088 | 2179 ± 2225 | NS |
| Karyotype (G/I/P) | 7/58/13 | 12/53/6 | NS |
| CD34 (+ patients) | 48 | 43 | NS |
| Pgp function * | 0.44 ± 0.28 | 0.39 ± 0.27 | NS |
| $LC_{50}$ DNR ($10^{-8}$ mol/L) | 100 ± 10 | 10 ± 20 | $P = 0.01$ |
| $LC_{50}$ etoposide ($10^{-8}$ mol/L) | 2300 ± 1200 | 900 ± 420 | $P = 0.02$ |
| $LC_{50}$ Ara-C ($10^{-}$8 mol/L) | 1850 ± 1200 | 1400 ± 2100 | NS |
| Treatment outcome | | | |
| Achievement of CR (%) | 52 | 80 | $P = 0.0005$ |

Abbreviations: NS, not significant; LDH, lactate dehydrogenase; DNR, daunorubicin.

* D value.

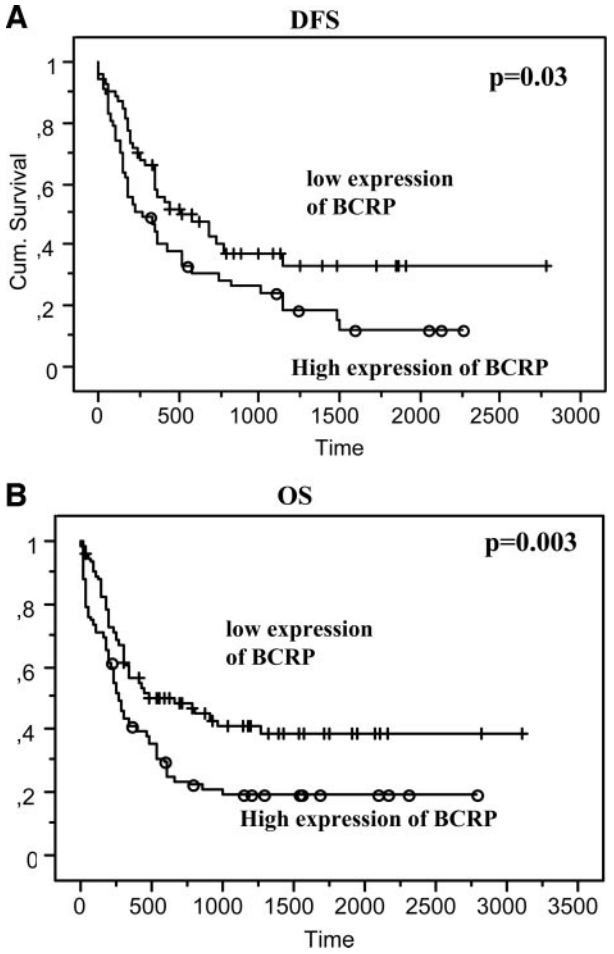


*Fig. 1* Disease-free survival (*A*) and overall survival (*B*) according to BCRP expression. (*DFS*, disease-free survival; *OS*, overall survival)

survival was 48% ± 6% (median, 784 days), 37% ± 10% (median, 320 days), and 16% ± 6% (median, 230 days; $P = 0.001$; Fig. 2*B*), respectively.

**Multivariate Analysis.** Table 2 shows other clinical and biological characteristics and treatment outcome in all of the patients in univariate analysis.

In multivariate analysis, cytogenetic ($P = 0.001$), BCRP expression ($P = 0.002$), and age ($P = 0.01$) were statistically significant for achievement of CR. For disease-free survival, cytogenetic ($P = 0.01$), age ($P = 0.02$), BCRP expression ($P = 0.02$), and Pgp function ($P = 0.02$) were statistically significant. For overall survival, achievement of CR ($P < 0.0001$), Pgp function ($P = 0.004$), BCRP expression ($P = 0.008$), and cytogenetic ($P = 0.02$) were statistically significant.

### BCRP and Pgp with Regard to Anthracycline Regimens

**Response Assessment with Regard to Anthracycline Regimen.** The three groups of patients (daunorubicin, idarubicin, and mitoxantrone) did not have different clinical and biological features at diagnosis and a different treatment outcome (Table 3).

**BCRP Expression and Pgp Function.** We have analyzed the prognostic value of Pgp function in regard to intercalating agents received (Table 4). Pgp function was a prognostic factor in patients receiving daunorubicin and idarubicin but not in mitoxantrone regimen for achievement of CR, disease-free survival ($P = 0.03$, $P = 0.04$, and $P = 0.64$, respectively), and overall survival ($P = 0.04$, $P = 0.03$, and $P = 0.96$, respectively). In contrast, BCRP expression was a prognostic factor in patients receiving daunorubicin and mitoxantrone but not in idarubicin regimen for achievement of CR, disease-free survival ($P = 0.02$, $P = 0.05$, and $P = 0.19$, respectively), and overall survival ($P = 0.03$, $P = 0.01$, and $P = 0.25$, respectively).

## DISCUSSION

After several years of discordant results, several large studies with robust methodology confirmed the importance of functional Pgp in clinical resistance in AML (2, 3, 5–8, 33, 34). But the results of randomized trials adding potent Pgp inhibitor, like cyclosporinA, quinine, or PSC833, showed contradictory results, raising the question of alternative mechanism of resist-

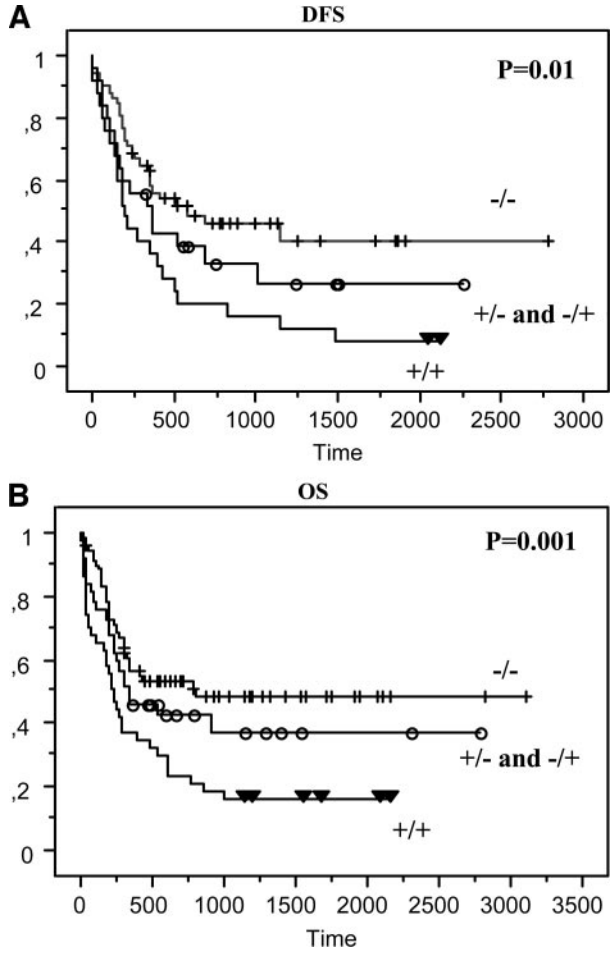


*Fig. 2* Disease-free survival (*A*) and overall survival (*B*) according to BCRP expression and Pgp function categorized in three groups [+/+ (▼), +/− or −/+ (○), and −/− (I)]. (*DFS*, disease-free survival; *OS*, overall survival)

ance (35–38). Recently, a new ABC protein BCRP was described simultaneous by different groups (13–15). In the first study in AML, high expression of BCRP mRNA was sufficiently frequent in AML (33% of patients were positive) to warrant more extensive investigations to determine the relation of disease and treatment outcome to BCRP expression and function (17). In contrast, in another study, only 7% of the 40 AML samples tested contained BCRP mRNA levels within the range of their drug-resistant clone, although another 78% were higher than normal blood and bone marrow (18). Steinbach *et al.* (19) studied 59 childhood AML and found that BCRP gene expression was >10 times higher in patients who did not achieve remission after the first phase of chemotherapy as compared with patients who did achieve remission at this stage. At this time, there is no other study that evaluates the relationship between BCRP expression and the treatment outcome in AML. In our study, with real-time RT-PCR, BCRP expression was an independent prognosis factor. We have previously shown that the coexpression of Pgp and MRP1 was of poorer prognostic factor than the expression of only one of these proteins (30). In the same way, we have shown in this study that patients who coexpressed both BCRP and Pgp had a poorer prognosis than patients who expressed only one or none of these two proteins. Therefore, our interesting results needs to be additionally confirmed with BCRP protein expression and function in a more extensive prospective study.

AML may be treated with one of these intercalating agents, daunorubicin, mitoxantrone, and idarubicin in combination with aracytine (12). However, at this time, the results of the studies comparing these anthracyclines are debated (10–12, 39), and the role of Pgp in these patients receiving one of these different intercalating agents is not clear. In our study, although the number of patients allocated to each of the anthracenes has significant imbalance with respect to patient number assigned to the specific anthracene, Pgp function was a prognostic factor only in patients receiving daunorubicin or idarubicin but not mitoxantrone. However, patients treated with mitoxantrone do

*Table 2* Univariate and multivariate analysis of achievement of CR, DFS, and OS in 149 AML patients

| Variables | CR achievement | DFS | OS |
|---|---|---|---|
| Achievement of CR | | | |
| Univariate | | | $P<0.0001$ |
| Multivariate | | | $P<0.0001$ |
| Age * | | | |
| Univariate | $P=0.001$ | $P=0.004$ | $P=0.001$ |
| Multivariate | $P=0.01$ | $P=0.02$ | NS |
| Cytogenetics | | | |
| Univariate | $P=0.03$ | $P=0.003$ | $P=0.02$ |
| Multivariate | $P=0.001$ | $P=0.01$ | $P=0.02$ |
| WHO (0–1 *versus* 2–4) | | | |
| Univariate | $P=0.03$ | NS | $P=0.02$ |
| Multivariate | NS | | NS |
| WBC count * | | | |
| Univariate | NS | NS | NS |
| Multivariate | | | |
| LDH level * | | | |
| Univariate | NS | NS | NS |
| Multivariate | | | |
| Pgp function * | | | |
| Univariate | $P=0.05$ | $P=0.04$ | $P=0.01$ |
| Multivariate | NS | $P=0.02$ | $P=0.004$ |
| BCRP expression * (mRNA) | | | |
| Univariate | $P=0.04$ | $P=0.03$ | $P=0.003$ |
| Multivariate | $P=0.002$ | $P=0.02$ | $P=0.008$ |

Abbreviations: DFS, disease-free survival; OS, overall survival; NS, not significant; LDH, lactate dehydrogenase.

* Analyzed in continuous variables.

*Table 4* Prognostic value of Pgp function and BCRP expression according to treatment arm (DNR+AraC, IDA+AraC, or MIT+AraC)

| | DNR | IDA | MIT |
|---|---|---|---|
| Pgp function | | | |
| CR | $P=0.02$ | $P=0.05$ | NS |
| DFS | $P=0.03$ | $P=0.04$ | NS |
| OS | $P=0.04$ | $P=0.03$ | NS |
| BCRP expression | | | |
| CR | $P=0.02$ | NS | $P=0.04$ |
| DFS | $P=0.02$ | NS | $P=0.05$ |
| OS | $P=0.03$ | NS | $P=0.01$ |

DNR, daunorubicin; IDA, idarubicin; MIT, mitoxantrone; NS, not significant; DFS, disease-free survival; OS, overall survival.

*Table 3* Comparison of clinical and biological variables and treatment outcome in patients receiving either DNR, IDA, or MIT + aracytine

| Characteristic | DNR (59 patients) | IDA (30 patients) | MIT (60 patients) | *P* |
|---|---|---|---|---|
| Age (year) | 51 ± 19 | 50 ± 9 | 58 ± 17 | NS |
| WHO status (0,1/2,3,4) (%) | 45/14 | 24/6 | 39/21 | NS |
| Leucocytes ($\times 10^9$/l) | 67 ± 89 | 52 ± 67 | 45 ± 80 | NS |
| LDH (UI/l) | 2277 ± 2753 | 2325 ± 2112 | 1365 ± 1782 | NS |
| Karyotype (G/I/P) | 7/45/7 | 4/21/5 | 8/45/6 | NS |
| CD34 (+ patients) | 36 | 18 | 37 | NS |
| Pgp function* (JC-1) | 0.34 ± 0.29 | 0.48 ± 0.3 | 0.45 ± 0.25 | NS |
| BCRP expression ($\times 10^{-6}$) (mRNA) | 302 ± 120 | 250 ± 150 | 279 ± 170 | NS |
| Treatment outcome | | | | |
| Achievement of CR (%) | 64 | 75 | 60 | NS |
| DFS (% at 5 years/median in days) | 25 ± 8/290 | 32 ± 7/360 | 28 ± 10/354 | NS |
| OS (% at 5 years/median in days) | 23 ± 9/240 | 30 ± 9/345 | 25 ± 7/340 | NS |

Abbreviations: DNR, daunorubicin; IDA, idarubicin; MIT, mitoxantrone; NS, not significant; LDH, lactate dehydrogenase; DFS, disease-free survival; OS, overall survival.

* D value.

not have a better prognosis than other patients. Therefore, other resistant mechanisms are involved in patients receiving this anthracycline. Recently, using a transduced clonal cell lines expressing various levels of BCRP, Abbott *et al.* (18) found that BCRP expression conferred resistance to mitoxantrone but not to idarubicin. In accordance with all of these *in vitro* data, we showed clinical correlations between BCRP expression and treatment failure in patients receiving daunorubicin or mitoxantrone regimens but not with idarubicin regimen. In a recent clinical study by Broxterman *et al.* (40), there was no correlation between Pgp expression and complete response rate, event-free survival, or overall survival of patients treated with idarubicin+AraC during induction therapy. However, the second and third cycles included amsacrine and mitoxantrone, respectively. Therefore, the absence of correlation between Pgp function and event-free survival and overall survival might be related to the use of mitoxantrone in consolidation therapy and not to idarubicin (40). In Borg's study (3), in patients <55 years old who received only daunorubicin, Pgp function was a prognostic factor. In contrast, in the elderly patients (19 patients received only mitoxantrone and 25 received only daunorubicin), Pgp was not a prognostic factor. Therefore, in this study also, the lack of correlation between Pgp function and treatment outcome might be related to the use of mitoxantrone in half of these patients (3). In van der Kolk's (6) study, Pgp function was a prognostic factor for CR achievement but not for overall survival. In induction treatment, patients received either daunorubicin or idarubicin, but they received mitoxantrone in consolidation regimen. The lack of significance between Pgp function and overall survival might also be related to use of mitoxantrone in this study. Therefore, putative beneficial effects of the inclusion of Pgp modulator in mitoxantrone-containing therapy might be related to alternative mechanisms than to inhibition of Pgp-mediated mitoxantrone efflux. In the same way, in AML, the studies that used a combination of Pgp modulators with mitoxantrone do not improve the outcome of patients (35, 37). In contrast, in a recent study, the addition of cyclosporine A to an induction and consolidation regimen containing infusional daunorubicin dramatically reduces resistance to daunorubicin, prolongs the duration of remission, and improves overall survival in patients with poor-risk AML (37).

In conclusion, the "new" described ABC protein BCRP would be implicated in the resistance to chemotherapies in AML. However, BCRP is implicated in resistance to daunorubicin and mitoxantrone, in contrast to Pgp implicated in resistance to idarubicin and daunorubicin. Drugs that inhibit the function of P-gp should only be introduced into the daunorubicin and idarubicin therapy of AML. Modulation of not only Pgp but also BCRP by fumitremorgin C or GG918, which inhibited these two proteins for the latter (28), could be essential, in the group of patients treated by daunorubicin, to improve the results of treatment. Therefore, the sequential administration of different intercalating agents would also circumvent the resistance of AML according to the expression of these two ABC proteins

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