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ORIGINAL REPORT

Clinical Role of Multidrug Resistance Protein 1 Expression in Chemotherapy Resistance in Early-Stage Breast Cancer: The Austrian Breast and Colorectal Cancer Study Group

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Authors' disclosures of potential conflicts of interest are found at the end of this article.

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ABSTRACT

Purpose
The multidrug resistance protein 1 (MRP1) is expressed in human breast cancer cells and may contribute to the clinical drug resistance of breast cancer patients. Therefore, we determined the impact of MRP1 expression on the clinical outcome of adjuvant therapy in patients with early-stage breast cancer.

Patients and Methods
Immunostaining for MRP1 was performed on tissue sections from 516 premenopausal, hormone receptor–positive breast cancer patients with stage I and II disease. Statistical analyses were performed to assess the effect of MRP1 expression on survival and to test for interaction between MRP1 expression and treatment.

Results
MRP1 expression independently predicted shorter relapse-free survival (RFS) and overall survival (OS) in patients treated with cyclophosphamide, methotrexate, and fluorouracil (CMF; RFS: hazard ratio [HR] = 1.48; 95% CI, 1.16 to 1.88; $P = .002$; OS: HR = 1.82; 95% CI, 1.10 to 3.01; $P = .02$), but it did not predict shorter RFS and OS in patients who received tamoxifen plus goserelin (RFS: HR = 0.99; 95% CI, 0.74 to 1.31; $P = .9$; OS: HR = 0.68; 95% CI, 0.40 to 1.15; $P = .1$). Tests for interaction between MRP1 expression and treatment were statistically significant for both RFS ($P = .04$) and OS ($P = .006$).

Conclusion
Our data suggest that MRP1 expression plays an important role in the clinical resistance to adjuvant CMF chemotherapy but does not seem to affect response to adjuvant endocrine treatment with tamoxifen plus goserelin. Thus, MRP1 may be a useful marker for the selection of patients with early-stage breast cancer for the appropriate adjuvant therapy after prospective confirmatory evaluation.

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INTRODUCTION

Chemotherapy plays an important role in the management of breast cancer, but the efficacy of this treatment is limited by the presence and/or development of drug resistance. Various cellular pathways may be involved in tumor-cell drug resistance, and one of the mechanisms that may be clinically active in breast cancer patients is the prevention of the intracellular accumulation of anticancer drugs by the expression of transport proteins that pump drugs out of cells.[1] Several of these proteins belong to the adenosine triphosphate–binding cassette (ABC) proteins, a large superfamily of transmembrane proteins that use the energy of adenosine triphosphate hydrolysis to translocate

their substrates across biologic membranes.[1,2] The *MDR1* gene product P-glycoprotein, also called ABCB1, is one of the most thoroughly studied ABC proteins. In breast cancer, MDR1/P-glycoprotein expression occurs with various frequencies and, according to a meta-analysis of 31 reports from 1989 to 1996, both increased after chemotherapy and was associated with treatment failure.[3] This suggests a role of MDR1/P-glycoprotein in clinical drug resistance of breast cancers, but additional mechanisms are most likely active in this disease.

The multidrug resistance protein 1 (MRP1), also called ABCC1, is another member of the ABC transporter family.[4] Overexpression of this protein in tumor cells confers resistance to various anticancer drugs, such as anthracyclines or methotrexate,[4-7] which are drugs that are part of the chemotherapy regimens currently used in the treatment of breast cancer.[8] Prior studies suggest an association between MRP1 protein expression and shorter disease-free survival and overall survival (OS) of breast cancer patients.[9] However, the heterogeneity of these studies as a result of differences in the study populations, the number of patients examined, the length of follow-up, the use of different types of adjuvant endocrine treatment and/or chemotherapy regimens, and the detection methods used does not allow a definitive conclusion with regard to the impact of MRP1 expression on clinical outcome.

To further evaluate the clinical role of MRP1 in breast carcinomas, we studied the relationship between MRP1 expression of the primary tumors and survival of the patients who were enrolled onto the Austrian Breast and Colorectal Cancer Study Group (ABCSG) Trial 5, a prospective randomized trial comparing the efficacy of cyclophosphamide, methotrexate, and fluorouracil (CMF) chemotherapy with a combination endocrine treatment.

PATIENTS AND METHODS

Patients

The study population represents a subset of patients enrolled onto ABCSG Trial 5. The objective of ABCSG Trial 5 was to compare the efficacy of a combination endocrine treatment with standard CMF chemotherapy. The results of this study have previously been published.[10] From December 1990 to October 1999, a total of 1,099 patients were entered, of whom 1,034 patients were assessable for the final analysis. Patients were stratified by tumor size, number of involved lymph nodes, type of curative surgery, tumor grade, and hormone receptor status and were randomly assigned to receive either six cycles of CMF or 5 years of tamoxifen (Nolvadex; AstraZeneca Pharmaceuticals, Wilmington, DE) plus 3 years of goserelin (Zoladex; AstraZeneca Pharmaceuticals). CMF was administered intravenously for six cycles days 1 and 8 and recycled on day 28 at the following doses: cyclophosphamide 600 mg/m^2, methotrexate 40 mg/m^2, and fluorouracil 600 mg/m^2. Goserelin was administered subcutaneously at 3.6 mg per injection every 28 days for 3 years (a total of 39 injections). Tamoxifen was administered orally at 20 mg once a day for 5 years. None of the trial participants received tamoxifen after CMF treatment.

Tumor Specimens

All tumor specimens were obtained at the time of surgery before adjuvant therapy. Formalin-fixed, paraffin-embedded tumor blocks containing primary breast cancer tissue from patients who participated in ABCSG Trial 5 were retrospectively collected from the major participating centers. Details with regard to the collection of the samples and the preparation of slides have been published as part of a study describing the role of p27^{Kip1} expression in this population.[11]

Immunohistochemistry

A hematoxylin and eosin–stained slide was prepared from each block and used for pathologic confirmation of present invasive breast cancer by an experienced breast pathologist. Immunohistochemical analysis reported in this study was carried out in a single laboratory and performed as described previously.[12] Briefly, tissue sections of 4-μm thickness were prepared, mounted on poly-L-lysine–coated slides, deparaffinized, and rehydrated with distilled water. Endogenous peroxidase activity was blocked by incubation in 0.06% hydrogen peroxide for 10 minutes at room temperature. After washing in phosphate-buffered saline (pH 7.4), the tissues were preincubated for 20 minutes in normal serum (dilution 1:20; Dako, Glostrup, Denmark) before a 90-minute incubation with the anti-MRP1 monoclonal antibody MRPr1 (dilution 1:50; Alexis, Läufelfingen, Switzerland). Antibody binding was detected by the avidin-biotin-peroxidase method. Bound peroxidase was developed with 3,3′-diaminobenzidine (Dako). The slides were counterstained with Mayer's Hämalaun and mounted with Aquatex (Merck, Darmstadt, Germany). All of the washes were performed in phosphate-buffered saline (pH 7.4).

Normal human kidney tissue served as positive control for MRP1 expression. In addition, we used breast cancer specimens known to be positive or negative for MRP1 as positive and negative controls, respectively. Negative controls without the primary antibody were performed as described earlier. Staining of tumor cells was examined by an experienced breast pathologist who was blinded to the clinical outcome of the patients. All tumor cells on each slide were evaluated, and interpretation of the results was limited to the invasive portion of the tumor. MRP1 immunostaining was classified as previously described by us,[12] and was as follows: negative, 0% of tumor cells showing staining reactivity; low, less than 10% of tumor cells showing staining reactivity; intermediate, 10% to 30% of tumor cells showing staining reactivity; and high, more than 30% of tumor cells showing staining reactivity. To assess interobserver reproducibility, 100 randomly selected patients were analyzed by a second investigator, and κ statistics were used to assess interobserver reliability. The observed κ was 0.72, and therefore, interobserver reliability was considered satisfactory (data not shown). The rare discrepant cases were reassessed together by both investigators using a double-headed microscope, and a consensus was reached.

Statistical Analysis

Associations of MRP1 expression with age, tumor size, lymph node status, tumor grade, estrogen receptor (ER) expression, and progesterone receptor (PgR) expression were assessed by the Spearman rank correlation. Survival probabilities were estimated with the product limit method according to Kaplan and Meier.[13] Survival time was defined as the period between the time of randomization and death (OS) or the period between the time

of randomization and documented relapse (relapse-free survival [RFS]). Relapse was defined as the first reappearance of breast cancer at any local, distant, or contralateral site. Patients who died because of reasons other than breast cancer were considered as censored with death. Survival times of patients still alive were censored with the date of the last follow-up. Differences between survival curves were analyzed using the log-rank test. To describe the unadjusted effects of covariates on RFS and OS, univariate Cox proportional hazards regression models were used. Multiple Cox proportional hazards regression models were used to assess the independent effects of MRP1 expression on RFS and OS.[14] In addition, multiple Cox proportional hazards regression models with backward elimination of nonsignificant variables were applied. We used a backward elimination algorithm with a 0.1 significance level for removing explanatory variables. Variables were coded as follows: age, less than 35 years or ≥ 35 years; tumor size, pT1, pT2, or pT3; lymph node status, 0, 1 to 3, 4 to 10, or more than 10 axillary lymph node metastases; tumor grade, grade 1, grade 2, or grade 3; ER, negative, positive, or strongly positive; PgR, negative, positive, or strongly positive; treatment, combination endocrine therapy or chemotherapy; $p27^{Kip1}$, less than 50% or ≥ 50%; MRP1, negative, low, intermediate, or high; and an interaction term, the product of MRP1 and treatment (MRP1 × treatment). Tumor size, lymph node status, tumor grade, ER status, PgR status, and MRP1 expression were included as ordered factors in the statistical analyses on the assumption that the relative risk between two successive categories would remain the same. The Cox proportional hazards regression model was also applied to assess interactions between treatment and the other covariates. All *P* values are results of two-sided tests. SPSS 11.5.1 statistical software (SPSS Inc, Chicago, IL) was used for calculations.

Table 1. Comparison of MRP1 Study and ABCSG Trial 5

Variable	% of Patients	
	MRP1 Study (n = 516)	ABCSG Trial 5 (n = 1,034)
Age		
< 35 years	6	7
≥ 35 years	94	93
Pathologic tumor size		
pT1	59	57
pT2	37	39
pT3	4	4
Lymph node status		
Negative	52	51
Positive, 1-3 lymph nodes	34	34
Positive, 4-10 lymph nodes	12	12
Positive, > 10 lymph nodes	2	3
Tumor grade		
Grades 1 and 2	69	72
Grade 3	31	28
ER status		
ER negative	7	7
ER positive	66	68
Strongly ER positive	27	25
PgR status		
PgR negative	11	10
PgR positive	49	51
Strongly PgR positive	40	39
Adjuvant treatment		
Endocrine therapy	49	49
Chemotherapy	51	51
Relapses	20	19
Deaths	7	9

Abbreviations: MRP1, multidrug resistance protein 1; ABCSG, Austrian Breast and Colorectal Cancer Study Group; ER, estrogen receptor; PgR, progesterone receptor.

RESULTS

Tumor blocks were available from 516 of the 1,034 patients who participated in ABCSG Trial 5. These 516 patients were similar in age at surgery, tumor size, lymph node involvement, tumor grade, hormone receptor status, adjuvant treatment, relapses, and deaths to the 1,034 patients enrolled onto ABCSG Trial 5 (Table 1). Likewise, the patients included in the present study and those included in the parental clinical trial did not differ in RFS and OS. Moreover, the treatment effects observed in ABCSG Trial 5 were reproducible, if the same variables as in the clinical study were included into a Cox model (data not shown). Thus, the 516 patients were representative of the total study population. Furthermore, the percentage of patients was not significantly different between the two treatment arms with regard to age, tumor size, lymph node involvement, tumor grade, hormone receptor status, and MRP1 expression (Table 2).

MRP1 immunostaining was both membranous and cytoplasmic and ranged from 0% to 100% (median, 10%) of the breast cancer cells. For comparisons with clinical parameters, MRP1 expression was categorized as negative, low, intermediate, and high. MRP1 expression was negative in 148 patients (29%), low in 90 patients (17%), intermediate in 130 patients (25%), and high in 148 patients (29%).

When we examined the correlations between MRP1 expression and clinical parameters, MRP1 expression showed a weak positive correlation with tumor size ($r = 0.115$; $P = .009$) and tumor grade ($r = 0.096$; $P = .03$; Table 3). In contrast, MRP1 expression was not significantly correlated with age, lymph node status, hormone receptor status, treatment, and $p27^{Kip1}$ expression (Table 3). Similar results were obtained when MRP1 expression was analyzed as a continuous variable (data not shown).

At a median follow-up of 5.6 years, 105 patients (20%) had relapsed (60 CMF patients and 45 endocrine therapy patients), and 38 patients (7%) had died from cancer (22 CMF patients and 16 endocrine therapy patients). The 5-year RFS and OS rates were 80% and 93%, respectively. Univariate analyses demonstrated that younger age, larger tumor size, higher number of positive lymph nodes, lower levels of PgR expression, low $p27^{Kip1}$ expression, and increasing levels of MRP1 expression were significantly

Table 2. Patient and Tumor Characteristics

Variable	All Patients (N = 516) No.	%	CMF (n = 263) No.	%	Tamoxifen + Goserelin (n = 253) No.	%	P
Age, years							
< 35	33	6	16	6	17	7	.8
≥ 35	483	94	247	94	236	93	
Pathologic tumor size							
pT1	306	59	160	61	146	58	.7
pT2	190	37	94	36	96	38	
pT3	20	4	9	3	11	4	
Lymph node status							
Negative	270	52	128	49	142	56	.2
Positive, 1-3 lymph nodes	174	34	97	37	77	30	
Positive, 4-10 lymph nodes	64	12	32	12	32	13	
Positive, > 10 lymph nodes	8	2	6	2	2	1	
Tumor grade							
Grade 1	59	11	28	11	31	12	.7
Grade 2	296	58	155	59	141	56	
Grade 3	161	31	80	30	81	32	
Hormone receptor status							
ER status							
ER negative	37	7	17	7	20	8	.6
ER positive	340	66	179	68	161	64	
Strongly ER positive	139	27	67	25	72	28	
PgR status							
PgR negative	55	11	31	12	24	9	.06
PgR positive	255	49	140	53	115	46	
Strongly PgR positive	206	40	92	35	114	45	
MRP1 expression							
Negative	148	29	81	31	67	26	.1
Low	90	17	36	14	54	21	
Intermediate	130	25	70	26	60	24	
High	148	29	76	29	72	29	

Abbreviations: CMF, cyclophosphamide, methotrexate, and fluorouracil; ER, estrogen receptor; PgR, progesterone receptor; MRP1, multidrug resistance protein 1.

Table 3. Spearman Rank Correlations of MRP1 Expression With Clinical Variables (N = 516)

Variable	MRP1 Correlation Coefficient (r)	P
Age	−0.014	.8
Tumor size	0.115	.009
Lymph node status	0.082	.06
Tumor grade	0.096	.03
ER	−0.014	.7
PgR	−0.072	.1
Treatment	−0.003	.9
$p27^{Kip1}$	−0.03	.5

NOTE. Variables were coded as described in Patients and Methods.
Abbreviations: MRP1, multidrug resistance protein 1; ER, estrogen receptor; PgR, progesterone receptor.

associated with shorter RFS (Table 4). Larger tumor size, higher number of involved lymph nodes, higher tumor grade, low $p27^{Kip1}$ expression, and higher MRP1 expression were also significantly associated with shorter OS (Table 4).

The independent effects of MRP1 expression on RFS and OS were assessed by multiple Cox proportional hazards regression models. All variables listed in Table 4 were included in the models. Because previous studies suggested that MRP1 expression may be associated with resistance to CMF chemotherapy, we also tested for the existence of interaction between MRP1 expression and treatment by incorporating an interaction term, the product of MRP1 expression and treatment (MRP1 × treatment), into the Cox model. Age, tumor size, lymph node status, PgR expression, $p27^{Kip1}$ expression, and the interaction term (MRP1 × treatment) were identified as statistically significant risk factors for RFS (Table 4). Lymph node status, treatment, $p27^{Kip1}$ expression, and the interaction term (MRP1 × treatment) were independently associated with OS (Table 4). Interestingly, the interaction term was statistically significant for both RFS ($P = .04$) and OS ($P = .006$) in these analyses (Table 4). Similar results were obtained when multiple Cox proportional hazards regression models with backward elimination of nonsignificant variables were applied. In particular, the interaction term remained significant after elimination of the nonsignificant variables ($P = .04$ and $P = .004$ for RFS and OS, respectively; data not shown).

Because the interaction term was statistically significant for both RFS and OS, we determined the association of MRP1 expression with survival in subgroups of patients treated with either chemotherapy or endocrine therapy. To assess whether the effect of MRP1 expression by treatment is independent of other variables, hazard ratios were estimated before and after adjusting for age, tumor size, lymph node status, tumor grade, ER status, PgR status, and $p27^{Kip1}$ expression. The results of these analyses are listed in Table 5 and shown in Figure 1. In the cohort of CMF-treated patients, higher MRP1 expression was associated with shorter RFS and OS of the patients. The adjusted hazard ratios for relapse and death were 1.48 (95% CI, 1.16 to 1.88; $P = .002$) and 1.82 (95% CI, 1.10 to 3.01; $P = .02$), respectively (Table 5). In contrast, in patients who received combination endocrine therapy, MRP1 expression did not predict RFS and OS. The adjusted hazard ratios for relapse and death were 0.99 (95% CI, 0.74 to 1.31; $P = .9$) and 0.68 (95% CI, 0.40 to 1.15; $P = .1$), respectively (Table 5). These data were also shown in Figure 1. At 5 years, the RFS rates for CMF-treated patients were 92%, 84%, 76%, and 56% for patients with negative, low, intermediate, and high MRP1 expression, respectively (Fig 1A); the corresponding 5-year OS rates were 98%, 97%, 91%, and 79%, respectively (Fig 1C). In contrast, the 5-year RFS rates for patients assigned to endocrine therapy were 83%, 81%, 82%, and 84% for patients with negative, low, intermediate, and high MRP1 expression, respectively

Table 4. Cox Proportional Hazards Regression Analyses for RFS and OS in All 516 Patients

Variable	Univariate			Multivariate		
	HR	95% CI	*P*	HR	95% CI	*P*
RFS						
Age	0.25	0.15 to 0.41	< .001	0.25	0.15 to 0.44	< .001
Tumor size	2.42	1.77 to 3.30	< .001	1.79	1.31 to 2.45	< .001
Lymph node status	1.97	1.59 to 2.44	< .001	1.78	1.40 to 2.25	< .001
Tumor grade	1.29	0.94 to 1.77	.1	1.09	0.79 to 1.50	.6
ER	1.01	0.72 to 1.41	.97	0.93	0.64 to 1.36	.7
PgR	0.74	0.55 to 0.98	.04	0.75	0.56 to 0.99	.05
Treatment	1.39	0.94 to 2.04	.1	0.72	0.34 to 1.52	.4
$p27^{Kip1}$	0.57	0.37 to 0.88	.01	0.54	0.35 to 0.84	.006
MRP1	1.31	1.11 to 1.55	.002	1.00	0.77 to 1.31	.98
MRP1 × treatment	1.76	1.25 to 2.50	.001	1.45	1.01 to 2.07	.04
OS						
Age	0.51	0.20 to 1.33	.2	0.48	0.17 to 1.32	.2
Tumor size	2.44	1.51 to 3.95	< .001	1.58	0.97 to 2.59	.07
Lymph node status	2.40	1.69 to 3.41	< .001	1.88	1.24 to 2.83	.003
Tumor grade	1.73	1.01 to 2.97	.046	1.41	0.78 to 2.53	.3
ER	0.94	0.54 to 1.65	.8	1.12	0.59 to 2.10	.7
PgR	0.66	0.41 to 1.05	.08	0.77	0.48 to 1.23	.3
Treatment	1.51	0.78 to 2.91	.2	0.22	0.05 to 0.98	.05
$p27^{Kip1}$	0.33	0.17 to 0.63	.001	0.29	0.15 to 0.58	< .001
MRP1	1.38	1.04 to 1.83	.03	0.76	0.48 to 1.19	.2
MRP1 × Treatment	3.41	1.77 to 6.60	< .001	2.59	1.31 to 5.12	.006

NOTE. Variables were coded as described in Patients and Methods.
Abbreviations: RFS, relapse-free survival; OS, overall survival; HR, hazard ratio; ER, estrogen receptor; PgR, progesterone receptor; MRP1, multidrug resistance protein 1.

(Fig 1B); the corresponding 5-year OS rates for patients who received endocrine therapy were 95%, 88%, 98%, and 100%, respectively (Fig 1D). Similar results were obtained when we performed an analysis on patients with T1 and T2 tumors only (data not shown).

These results indicate that MRP1 expression independently predicted shorter RFS and OS in CMF-treated patients and suggest that MRP1 expression is associated with treatment failure in those patients. In contrast, no such association was found for MRP1 and outcome in patients receiving endocrine therapy with tamoxifen plus goserelin.

DISCUSSION

The development of molecular staging of breast cancer may have important implications for treatment. The definition of accurate predictive factors could help to select the appropriate adjuvant therapy for patients with early-stage breast cancer. Up to now, however, only a few molecular markers have been evaluated as predictors of response to specific treatments, and most of the currently available data are controversial and/or inconclusive.[15] The ABC transporter MRP1 is one of the most interesting molecular markers associated with resistance to various anticancer drugs.[1,2]

In the current study, we have examined MRP1 expression in a large, homogenous, and well-defined patient population consisting of premenopausal, hormone receptor-positive breast cancer patients with stage I and II disease who were enrolled onto a prospective randomized trial. Our results indicate that MRP1 expression is a prognostic factor for shorter RFS and OS in the total study population. These findings are consistent with previous reports from both our group[12,16] and other investigators.[17-19] More importantly, however, we found an interaction between MRP1 expression and adjuvant treatment. Patients who were treated with CMF chemotherapy and whose tumors were MRP1 negative experienced a significant reduction in relapse rate and a decrease in mortality compared with patients who had highly expressed MRP1. In contrast, in patients who received tamoxifen plus goserelin, MRP1 expression had no impact on the relapse rate or mortality. These results suggest that MRP1 expression is associated with clinical resistance to CMF chemotherapy but not with tamoxifen resistance and confirm the hypothesis derived from preclinical studies suggesting that methotrexate but not tamoxifen is transported by MRP1.

Several studies have investigated the expression of MRP1 in clinical breast cancer samples.[12,16-27] MRP1 mRNA was detected by reverse transcriptase polymerase chain reaction (RT-PCR) in 488 (98%) of 496 breast cancer specimens,[19-25] and MRP1 protein expression determined by immunohistochemistry was detectable in 291 (53%) of 544 samples.[12,16-18,21,26,27] The higher frequency of MRP1

Table 5. Multivariate Cox Proportional Hazards Regression Analyses for RFS and OS by Treatment

Variable	CMF			Tamoxifen + Goserelin		
	HR	95% CI	*P*	HR	95% CI	*P*
RFS						
Age	0.33	0.15 to 0.74	.007	0.23	0.11 to 0.51	< .001
Tumor size	1.63	1.06 to 2.49	.03	1.76	1.07 to 2.89	.03
Lymph node status	2.15	1.56 to 2.96	< .001	1.50	1.00 to 2.26	.05
Tumor grade	0.87	0.57 to 1.35	.5	1.50	0.92 to 2.46	.1
ER	0.83	0.48 to 1.43	.5	1.05	0.60 to 1.82	.9
PgR	0.81	0.55 to 1.20	.3	0.79	0.51 to 1.21	.3
$p27^{Kip1}$	0.86	0.45 to 1.64	.6	0.32	0.16 to 0.64	.001
MRP1	1.48	1.16 to 1.88	.002	0.99	0.74 to 1.31	.9
OS						
Age	0.82	0.18 to 3.77	.8	0.48	0.12 to 1.90	.3
Tumor size	1.67	0.80 to 3.47	.2	1.74	0.80 to 3.79	.2
Lymph node status	2.51	1.47 to 4.29	.001	1.16	0.52 to 2.55	.7
Tumor grade	1.04	0.48 to 2.28	.9	2.89	1.08 to 7.76	.04
ER	0.56	0.21 to 1.49	.2	1.93	0.78 to 4.85	.2
PgR	1.27	0.64 to 2.52	.5	0.49	0.24 to 0.97	.04
$p27^{Kip1}$	0.47	0.18 to 1.20	.1	0.20	0.06 to 0.68	.01
MRP1	1.82	1.10 to 3.01	.02	0.68	0.40 to 1.15	.1

NOTE. Variables were coded as described in Patients and Methods.
Abbreviations: RFS, relapse-free survival; OS, overall survival; CMF, cyclophosphamide, methotrexate, and fluorouracil; HR, hazard ratio; ER, estrogen receptor; PgR, progesterone receptor; MRP1, multidrug resistance protein 1.

expression obtained with RT-PCR is not surprising because MRP1 is ubiquitously expressed at low levels. Thus, by using RT-PCR, MRP1 mRNA can be detected in most breast cancer samples.

Only a few previous studies have correlated MRP1 expression with patients' clinical outcome. One study evaluated the impact of MRP1 expression on the prognosis and response to chemotherapy of recurrent breast cancer.[17] In 23 previously untreated patients who received first-line chemotherapy for recurrence, the presence of MRP1 protein was associated with a lower response rate (13% of MRP1-positive patients had an objective response compared with 40% of MRP1-negative patients) and with a significantly shorter progression-free survival, independent of the type of chemotherapy. No association between MRP1 expression and response rate or progression-free survival was observed in patients who received chemotherapy after treatment with endocrine therapy. The results of another study suggested that MRP1 may be associated with an increased relapse rate in subgroups of patients with a more favorable prognosis (eg, patients with T1 tumors or node-negative patients) and in node-positive patients who received adjuvant CMF chemotherapy.[18] MRP1 expression was also associated with an increased risk of death in patients with T1 tumors and in node-positive patients who were treated with adjuvant CMF chemotherapy. In our previous report, MRP1 expression in primary breast tumors correlated with shorter disease-free survival and OS in a heterogeneously treated cohort of patients.[16] In patients treated with preoperative chemotherapy, patients with MRP1 expression had a significantly shorter progression-free survival than patients without MRP1 expression, independent of the type of chemotherapy. In a multivariate Cox regression analysis, prechemotherapy MRP1 expression was identified as an independent prognostic factor for shorter progression-free survival.[12] A recently published study has shown MRP1 expression to be related to clinical outcome only in the subgroup of patients treated with anthracycline-based chemotherapy and not in patients who received CMF chemotherapy.[19] These data suggest that the clinical role of MRP1 expression in chemotherapy resistance of breast cancer may be restricted to anthracycline resistance. However, the results are based on low numbers of patients, particularly in treatment subgroups, and, therefore, have to be viewed with caution.

The results of these previous studies suffer from various shortcomings and are difficult to compare because of differences in the study populations, the number of patients examined, the length of follow-up, the use of different types of adjuvant endocrine treatment and/or chemotherapy regimens, and the detection methods used. However, the results suggest that MRP1 expression is of clinical relevance in breast cancer and support the results obtained in our present study.

The treatment protocol underlying the present study consists of reasonable treatment options with regard to the management of premenopausal women with early-stage hormone receptor-positive breast cancer. Various randomized trials have shown that ovarian ablation with or without

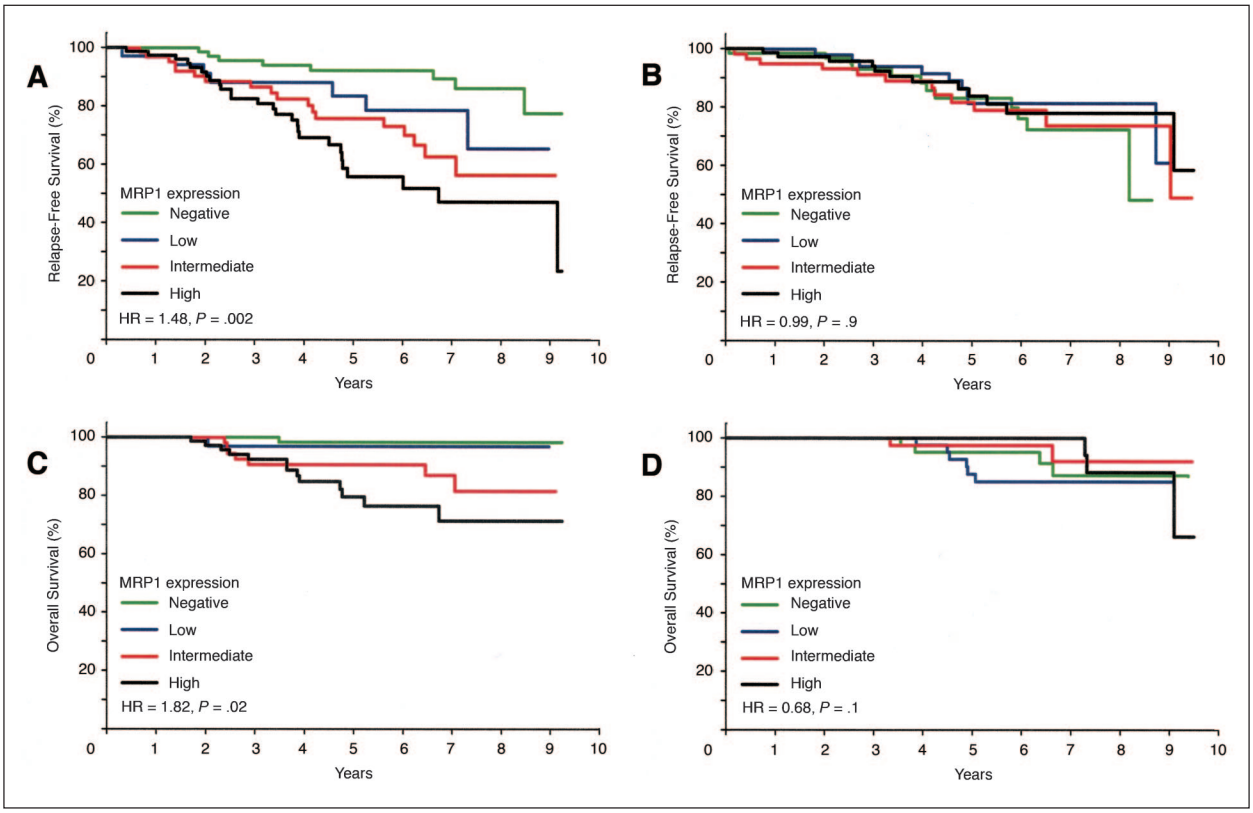

Fig 1. Kaplan-Meier plots for (A and B) relapse-free survival and (C and D) overall survival are shown with respect to multidrug resistance protein 1 (MRP1) expression. Hazard ratios (HR) of failure and *P* values shown on each plot are adjusted for age at surgery, tumor size, lymph node status, tumor grade, estrogen receptor, progesterone receptor, and $p27^{Kip1}$ expression. CMF, cyclophosphamide, methotrexate, and fluorouracil.

tamoxifen and standard chemotherapy regimens like CMF have similar benefits for premenopausal women with early-stage, receptor-positive breast cancer.[8] However, because adjuvant polychemotherapy improves survival, the National Institutes of Health Consensus Development Panel concluded that polychemotherapy should be recommended to the majority of women with localized breast cancer.[28] The inclusion of anthracyclines in adjuvant chemotherapy regimens results in a small but statistically significant improvement in survival over nonanthracycline-containing regimens. Four cycles of doxorubicin plus cyclophosphamide have been shown to be equivalent to six cycles of classical CMF, and only six cycles of anthracycline-containing regimens yield superior results but at the cost of greater toxicity.[8] Therefore, our present results may have important implications in the treatment of the majority of women with localized breast cancer by the more precise identification of those patients who benefit from adjuvant chemotherapy. Previously, we concluded that, "Overall, our data suggest that the goserelin-tamoxifen combination is significantly more effective than CMF in the adjuvant treatment of premenopausal patients with stage I and II breast cancer."[10] In light of the strong predictive effect of MRP1 among patients who received CMF chemotherapy, the results of the current study suggest that patients with intermediate or high levels of MRP1 expression should not be treated with CMF chemotherapy but may benefit from treatment with endocrine therapy, whereas MRP1-negative patients may be considered for treatment with CMF chemotherapy or anthracycline-based chemotherapy. However, this proposed treatment strategy has to be confirmed in well-designed clinical trials.

The current data are encouraging but require validation in a prospective trial before being implemented in clinical routine. Our results confirm the hypotheses derived from preclinical studies that suggest that higher MRP1 expression levels may be associated with clinical drug resistance in breast cancer. Thus, MRP1 may be a useful marker for the selection of patients with early-stage, hormone receptor-positive breast cancer for appropriate adjuvant therapy.

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Appendix

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Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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