Annals of Surgical Oncology, 3(1):8–14
Published by Lippincott–Raven Publishers

Best Clinical Research Paper

p-Glycoprotein Expression as a Predictor of Breast Cancer Recurrence

Sharon Gregorcyk, MD, Yuan Kang, MD, PhD, Dörthe Brandt, MD, Paul Kolm, PhD, Gerry Singer, MD, and Roger R. Perry, MD

Background: Many new prognostic factors for breast cancer have been described, and yet the ability to predict patient outcomes remains poor. Overexpression of p-glycoprotein (p-gp), the multidrug resistance efflux pump, confers a worse prognosis to patients with certain leukemias and other tumors. The purpose of this study was to analyze the potential usefulness of p-gp expression as a prognostic factor in patients with breast cancer.

Methods: Paraffin blocks were obtained from 55 previously untreated patients who underwent surgery between 1987 and 1988. To determine p-gp expression, tumor cell suspensions were incubated with the p-gp–specific C219 monoclonal antibody and analyzed using an indirect immunofluorescent flow cytometric assay.

Results: Twenty-four (44%) of the tumors were p-gp positive and 31 (56%) were p-gp negative. Among the p-gp positive patients, 65% had recurrence of their disease, whereas only 13% of the p-gp negative patients experienced recurrence ($p = 0.0001$). The 5-year disease-free rate for p-gp positive patients was 39% compared with 83% for p-gp negative patients ($p = 0.0001$). In univariate analysis examining 10 different variables, significant predictors of recurrence were p-gp, stage, and tumor size. Multivariate analysis using Cox Proportional Hazards regression showed that only p-gp and stage were significant independent predictors of recurrence ($p = 0.0002$).

Conclusions: p-gp is frequently expressed in patients with untreated breast cancer, with p-gp–positive patients being at significantly greater risk for disease recurrence. p-gp appears to be a useful prognostic factor in breast cancer and could potentially help guide management.

Key Words: Breast cancer—p-glycoprotein—Prognostic factor—Recurrence—Multidrug resistance.

It is estimated that there will be 183,400 new breast cancer cases and 46,240 deaths due to this cancer in 1995 (1). The biologic behavior of breast cancer and the ultimate clinical outcome are often unpredictable. For instance, although patients with lymph node–negative tumors have a favorable prognosis, ~20–25% of these patients will die within 10 years (2). Despite the poor prognosis associated with "locally advanced" (stage III) breast cancer, ~30% of these patients will survive 10 years when treated with multimodality therapy (3). Therefore, methods of identifying those patients at increased risk of failure are essential in order to direct therapy to those who would most benefit. It is equally important to identify patients who do not require adjuvant therapy in order to avoid the toxicity and cost associated with these treatments.

A great deal of effort has been focused on improving the ability to predict the outcome of pa-

Received March 25, 1995; accepted July 17, 1995.

From the Division of Surgical Oncology, Department of General Surgery (S.G., Y.K., D.B., R.R.P.), and Office of Biostatistics (P.K.), Eastern Virginia Medical School, and Department of Pathology, DePaul Medical Center (G.S.), Norfolk, Virginia, USA.

Address correspondence and reprint requests to Dr. Roger R. Perry, Eastern Virginia Medical School, 825 Fairfax Avenue, Norfolk, VA 23507-1912, USA.

Presented at The 48th Cancer Symposium of the Society of Surgical Oncology, Boston, Massachusetts, March 23–26, 1995. Received SSO Annual Resident/Fellow Award for Best Clinical Research Paper.

tients with breast cancer. In addition to standard pathologic staging criteria, a variety of other prognostic factors have been examined, including histologic grade, estrogen (ER) and progesterone (PR) receptor levels, epidermal growth factor receptor, DNA ploidy and S-phase fraction, HER-2/neu expression, and cathepsin D levels (4). These factors can help identify a patient's risk of recurrence and appear most useful in patients with lymph node–negative disease (4). Unfortunately, the ability of these factors to predict patient outcomes is limited, and the most important prognostic factor remains the presence or absence of lymph node metastases (5). Thus, there is a major need to identify better prognostic factors (6).

p-Glycoprotein (p-gp), also known as P-170, is a 170-kDa membrane-bound protein that functions as an energy-dependent drug efflux pump and has been shown to mediate the multidrug resistance (MDR) phenotype (7). MDR cells are resistant to doxorubicin and to many other structurally and functionally dissimilar chemotherapeutic agents (8). Elevated expression of p-gp has been detected in a wide variety of solid malignancies, including gastric carcinoma (9), renal carcinoma (10), neuroblastoma (11), and pediatric sarcomas (12), as well as hematologic malignancies, including myeloma (13), lymphoma (14), and leukemia (15–17). Studies examining the relationship between p-gp expression and clinical outcome have shown variable results, possibly due to differences in the techniques used to measure p-gp (18). However, it appears that patients with certain leukemias (15–17), neuroblastoma (11), or renal cell cancer (10) whose tumors express p-gp have a worse prognosis.

Thus far, there are limited and contradictory data in the literature on the frequency of p-gp expression in human breast cancer and its clinical utility (19). In this study, archival breast cancer specimens were analyzed for p-gp expression, and the results were then compared with clinical data. The ability of p-gp to predict patient outcomes was compared with that of other currently accepted prognostic factors.

MATERIALS AND METHODS

Patients and Tumor Samples

Paraffin-embedded blocks containing surgical breast tumor specimens from the years 1987 to 1988 were obtained from a single institution (DePaul Medical Center). All blocks were reviewed by a single pathologist (G.S.) to ensure that they were representative of the patient's breast cancer. There were 55 specimens with sufficient tumor tissue available for study. The corresponding patient records were reviewed to obtain clinical data, standard pathologic information, ER and PR levels, flow cytometry data, treatment rendered, and follow-up data. Each patient was assigned a code number such that the investigators who conducted the p-gp assays (S.G., Y.K.) were blinded as to the clinical results.

Flow Cytometry Determination of p-gp

To determine p-gp by flow cytometry, the samples were processed using mechanical and enzymatic disaggregation to yield single cell suspensions. Briefly, the paraffin blocks were sliced into 10- to 20-μm sections using a microtome, and the slices were processed immediately to help prevent antigen loss. The specimens were deparaffinized with multiple xylene washes, each wash lasting >1 h. Rehydration of the samples was performed with multiple washes using decreasing concentrations of ethanol (100%, 95%, 85%, 75%, 65%, and 55%). The samples were transferred to glass containers with 20 ml of trypsin and stirred for 1 h in a water bath at 37°C. The samples were filtered through mesh to obtain single cell suspensions. The cells were centrifuged and then fixed in 2 ml methanol at −20°C for 15 min. They were then washed with ice-cold phosphate-buffered saline with 0.1% Tween 20 and incubated with 2.5 μg/ml of the p-gp–specific C219 mouse monoclonal antibody (Centocor Inc., Malvern, PA) or with 2.5 μg/ml of an appropriate isotype control (mouse myeloma IgG2a; Vector Laboratories, Burlingame, CA) for 30 min at 4°C as previously described (20). After another wash, the cells were incubated with FITC-labeled goat anti-mouse IgG (3.3 μg/ml; Vector) for 30 min at 4°C. The cells were immediately washed and analyzed on a Coulter Epics V flow cytometer equipped with an MDADS graphic display (Coulter Electronics, Hialeah, FL). Single histograms of FITC fluorescence were collected for 10,000 cells. Samples stained with the specific antibody were compared with the isotype controls, and electronic gates were set to exclude 99% of isotype-positive cells. Cells with fluorescence higher than that of the gated population were counted as being p-gp positive. The percentage of cells expressing p-gp was calculated by using an immunoanalysis program provided by the manufacturer (Coulter). ChR C5 and AuX B1

Chinese hamster cells were used as p-gp–positive and –negative controls, respectively (20). Preliminary experiments in 5-year-old breast cancer tissue blocks demonstrated that prolonged paraffin fixation followed by this tissue processing method does not harm membrane protein expression as judged by positive antibody staining for the constitutive membrane protein CD44. These experiments included samples that were p-gp positive and negative. Other work in our laboratory has shown a good correlation between results obtained using flow cytometry and immunohistochemistry, including a CD44-positive staining control (unpublished observations).

Statistical Analysis

Clinical characteristics are expressed as the mean ± standard deviation for continuous variables and as percentages for categorical variables. Categorical covariates were compared with results of the χ^2 test, and continuous covariates were compared using the *t* test. Univariate analysis of the effect of the categorical covariates on recurrence-free survival or overall survival was performed using the log-rank test. For continuous covariates, Cox regression analysis was used. Multivariate analysis was performed using the Cox Proportional Hazards regression.

RESULTS

All 55 patients were female and none of the patients received preoperative chemotherapy or radiotherapy. Flow cytometric analysis of the tumors for expression of p-gp showed that 24 (44%) were p-gp positive and 31 (56%) were p-gp negative (Fig. 1). The p-gp–positive and –negative groups had similar clinical characteristics, as shown in Table 1. No statistical difference was found between the two groups in patient age, stage, tumor size, nodal status, ER and PR status, ploidy, DNA index, or S-phase. There also was no significant difference between the two groups in the number of patients receiving postoperative treatment or the type of therapy they received (p = 0.26). Overall, 87% of the p-gp–positive patients and 74% of the p-gp–negative patients received chemotherapy and/or tamoxifen. The histologic distribution was similar in both groups, with infiltrating ductal carcinoma in 85% and various invasive carcinomas in the remaining 15%.

Recurrence of disease was defined as the development of local recurrence, metastatic disease, or progression of disease not present originally at the time of surgery. No follow-up data were available for one patient; thus, the survival data are based on the remaining 54 patients. The length of follow-up for surviving patients ranged from 47 to 81 months, with an average follow-up period of 60 months. Fifteen of 23 (65%) p-gp–positive patients had recurrence of their disease compared with four of 31 (13%) p-gp–negative patients (p = 0.0001). The 5-year disease-free rate was 39% for p-gp–positive patients, with a median time to recurrence of 44 months, compared with 83% for p-gp–negative patients, with a median time to recurrence of >81

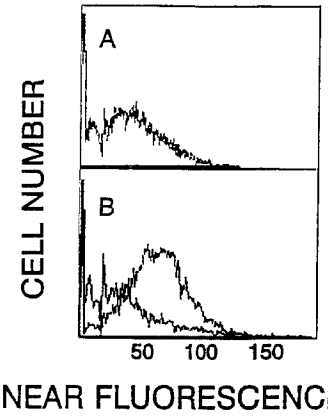

FIG. 1. Archival tumor samples were deparaffinized and processed into single cell suspensions. The samples were incubated with the p-gp–specific C219 monoclonal antibody or an appropriate isotype control and analyzed using an indirect immunofluorescent flow cytometric assay as described in Materials and Methods. Shown are representative histograms from a p-gp–negative tumor (A) and a p-gp–positive tumor (B).

TABLE 1. *Comparison of P-glycoprotein (+) and (−) groups*

	P-Glycoprotein		
	+	−	p[a]
Age (years)	57.2 ± 15.4	61.3 ± 12.8	0.28
Tumor size (cm)	2.8 ± 1.4	2.4 ± 1.3	0.27
Node (+)	54%	55%	0.96
Stage			
I	25%	32%	
II	62%	55%	
III	4%	6%	
IV	8%	6%	0.65
ER (+)	79%	72%	0.59
PR (+)	48%	55%	0.46
Aneuploid	71%	64%	0.66
DNA index	1.47 ± 0.36	1.32 ± 0.38	0.25
S-phase (%)	12.2 ± 8.9	9.9 ± 9.1	0.50

[a] Categorical covariates analyzed via χ^2. Continuous covariates analyzed via *t* tests.

months (Fig. 2). The results of univariate analysis of the different variables is shown in Table 2. p-gp, stage, and tumor size were the only variables that significantly influenced recurrence. When the parameters were analyzed using multivariate analysis, only p-gp and stage were significant predictors of recurrence ($p = 0.0002$). Stage and tumor size were highly correlated; therefore, size was no longer significant when adjusted for stage.

The effects of p-gp expression and tumor stage on recurrence are illustrated by the predicted recurrence-free curves generated from the Cox Proportional Hazards regression as shown in Fig. 3. The 5-year recurrence-free rates for p-gp–positive patients in stages I–IV here were 58%, 34%, 12%, and 2%, respectively, whereas the corresponding p-gp–negative patients had significantly higher recurrence-free rates of 93%, 86%, 75% and 57%, respectively. Of note, stage IV p-gp–negative patients had a recurrence-free survival (57%) comparable with that of stage I p-gp–positive patients (58%).

The influence of different types of postoperative treatment on the observed recurrence rates was examined using Cox Proportional Hazards regression. The overall analysis showed that patients of similar stage and treatment who were p-gp positive had significantly higher recurrence rates compared with patients who were p-gp negative ($p = 0.0003$). As an example, the recurrence-free survival curves for stage II patients generated from the Cox Proportional Hazards regression are shown in Fig. 4. Similar results were seen for patients in the other stages (data not shown). Thus, differences in postoperative treatment cannot explain the apparent influence of p-gp expression on recurrence-free survival.

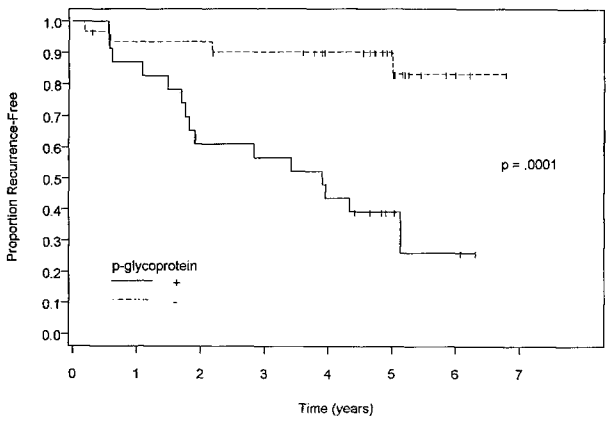

FIG. 2. Actuarial curves of recurrence-free survival stratified by p-gp expression.

TABLE 2. *Univariate analysis of factors affecting recurrence*

Covariate	p[a]
P-gp	0.0001
Age	0.55
Tumor size	0.014
Nodal status	0.81
Stage	0.04
ER status	0.69
PR status	0.63
Ploidy	0.44
DNA index	0.75
S-phase	0.92

[a] Categorical covariates analyzed via the log-rank test. Continuous covariates analyzed via Cox regression.

Among the 23 p-gp–positive patients, eight (35%) died from their breast carcinoma. Six of the 31 (19%) p-gp–negative patients likewise succumbed to their disease (Fig. 5). The difference in overall survival between the two groups was not statistically significant.

DISCUSSION

This study in previously untreated patients with breast cancer showed that 44% of the tumors were p-gp positive. It thus appears that many breast tumors possess intrinsic drug resistance that may severely limit the effectiveness of chemotherapeutic agents. When multiple factors were evaluated for their effect on prognosis, only p-gp and stage were independent predictors of breast cancer recurrence (Fig. 3). Overall, 65% of p-gp–positive patients experienced recurrence compared with only 13% of the p-gp–negative group. It is also interesting that p-gp was useful in predicting prognosis even in patients who did not receive postoperative chemotherapy. This suggests that p-gp, in addition to its association with MDR, may be a marker for cells that possess a more malignant phenotype.

The clinical characteristics of the p-gp–positive and p-gp–negative groups were similar, as shown in Table 1. The distribution of treatment modalities between the two groups was also similar. When comparing patients of similar stage treated with the same postoperative modalities, the p-gp–positive patients had a higher recurrence rate (Fig. 4). Thus, the findings in our study do not appear to be due to differences in clinical characteristics or the type of treatment administered.

Although treatment with chemotherapy can in-

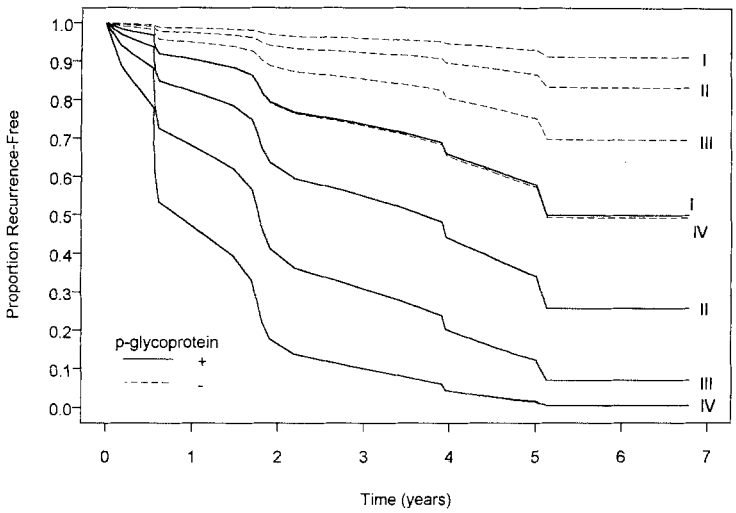

FIG. 3. Predicted recurrence-free survival curves stratified by p-gp expression and tumor stage, determined using the Cox Proportional Hazards Model. Roman numerals indicate tumor stage.

duce p-gp, tumors that have not been exposed to chemotherapy also can express p-gp, as demonstrated by our study and the work of others (21). It has been suggested that drug resistance may be a natural consequence of tumor progression (22–24). However, the mechanisms that regulate the expression of p-gp are poorly understood. Investigators have shown that wild-type p53 protein represses (25) and mutant p53 stimulates the MDR-1 promotor (26,27), even though the promotor lacks a p53 binding sequence. Others have found no correlation between p-gp expression and p53 status, but instead have shown a relationship between p-gp and HER-2/neu (28). It seems likely that the regulation of p-gp is complex and may occur via multiple mechanisms, including gene amplification, increased messenger RNA content, or increased protein content (29).

The frequency of p-gp expression in human solid tumors varies depending on the tumor type, the type of assay performed, and the treatment status of the patient. Studies on the frequency of p-gp expression in breast cancer have shown conflicting results. In the largest study to date, Southern, Northern, and Western blotting failed to detect any increase in p-gp gene copy number or expression in 248 patients with untreated primary and relapsing breast carcinoma (30). This failure to detect p-gp–positive cells may have been due to a large background of structural, inflammatory, and p-gp–negative breast cancer cells. A review of subsequent studies using immunohistochemical assay in smaller numbers of both treated and untreated patients with breast cancer suggests that p-gp is expressed in ~40–50% of patients (19). There contin-

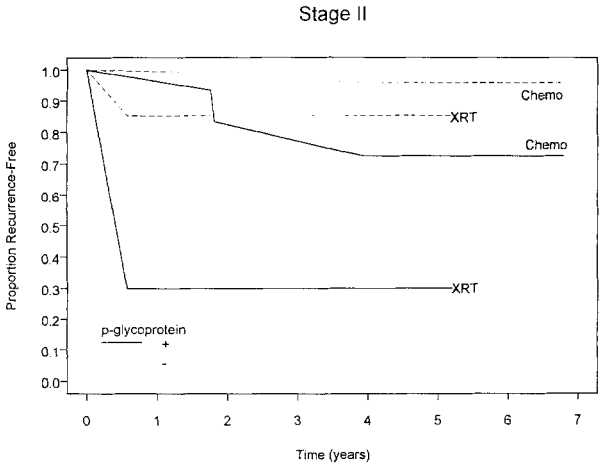

FIG. 4. Predicted recurrence-free survival curves stratified by p-gp expression, tumor stage, and type of postoperative treatment, determined using the Cox Proportional Hazards model. Shown is a representative series of curves for patients with stage II disease. Similar results were seen in patients in other stages.

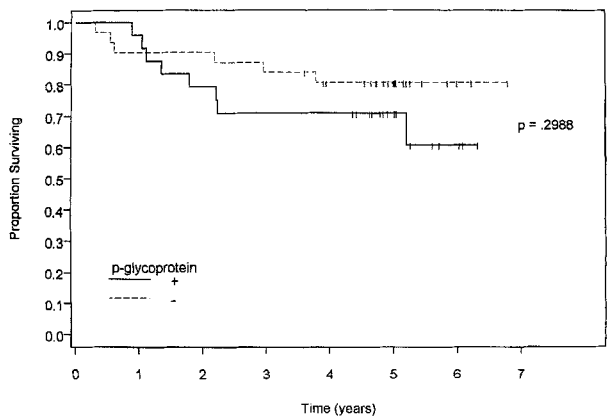

FIG. 5. Actuarial curves of overall survival stratified by p-gp expression.

ues to be uncertainty regarding the frequency of p-gp expression in previously untreated patients with breast cancer, with values ranging from 0% (31) to 85% (21). We found in our study of untreated patients that 44% of the tumors were p-gp positive using a flow cytometry technique. Other work in our laboratory has shown a good correlation between the results obtained using flow cytometry and immunohistochemistry (unpublished observations). Thus, it appears that immunohistochemistry and flow cytometry are preferable to blotting techniques to assess p-gp expression in breast cancer. However, controversy remains regarding the optimal technique, the antibody to be used, and the criteria for a positive result (18,32,33). Such issues will need to be resolved if assay of p-gp expression is to become more widely used.

Although increased expression of p-gp has been associated with a poor prognosis in several malignancies, including certain forms of leukemia (15–17), in general few data are available on its usefulness as a prognostic factor in solid tumors such as breast cancer. In a series of 20 untreated patients with breast cancer, p-gp expression correlated with a lack of response to chemotherapy and a shorter progression-free survival (21). In another study of 48 patients with locally advanced breast cancer treated with neoadjuvant chemotherapy and radiotherapy, 50% of the tumors were p-gp positive after treatment, and these patients had a poorer response to chemotherapy (34). Our study in untreated patients with breast cancer showed that p-gp expression was associated with a higher recurrence rate and a shorter time to recurrence, regardless of the type(s) of treatment that the patient received.

Although in our study 35% of the p-gp–positive group died compared with only 19% of the p-gp–negative group, the difference in overall survival was not statistically significant. The preliminary results of a recent study in 50 patients showed that p-gp expression was a significant independent predictor of postoperative survival (35). In that study, all patients had positive axillary lymph nodes and were treated with postoperative chemotherapy. Thus, both studies showed similar trends, even though the two study populations were somewhat different.

In summary, it appears that p-gp may be useful in predicting recurrence-free survival in breast cancer. Further study with a larger patient population and longer follow-up are needed to verify these findings and determine the usefulness of p-gp in predicting overall survival. The techniques used to measure p-gp and the criteria that constitute a positive result also need to be standardized so that data from different studies may be more easily compared. If the results of this study are confirmed, measurement of p-gp might help identify patients requiring more aggressive therapy with non-MDR drugs.

REFERENCES

1. The American Cancer Society. *Cancer facts and figures, 1995*. Atlanta, GA: The American Cancer Society.
2. Fisher B, Redmond C, Dimitrov NV, et al. A randomized clinical trial evaluating sequential methotrexate and fluorouracil in the treatment of patients with node-negative breast cancer who have estrogen-receptor–negative tumors. *N Engl J Med* 1989;320:473–8.
3. Piccart MJ, deValeriola D, Parideans R, et al. Six-year results of a multimodality treatment strategy for locally advanced breast cancer. *Cancer* 1988;62:2501–6.
4. Winchester DP. Adjuvant therapy for node-negative breast cancer: the use of prognostic factors in selecting patients. *Cancer* 1991;67(suppl):1741–3.
5. Clark GM, McGuire WL. Steroid receptors and other prognostic factors in primary breast cancer. *Semin Oncol* 1988; 15(suppl):20–5.
6. Osborne CK. Prognostic factors for breast cancer: have they met their promise? *J Clin Oncol* 1992;10:679–82.
7. Juliano RL, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochem Biophys Acta* 1976;455:152–62.
8. Beck WT. The cell biology of multiple drug resistance. *Biochem Pharmacol* 1987;36:2879–87.
9. Wallner J, Depisch D, Gsur A, Gotzl M, Haider K, Pirker R. MDR1 gene expression and its clinical relevance in primary gastric carcinomas. *Cancer* 1993;71:667–71.
10. Mickisch G, Bier H, Bergler W, Bak M, Tsschada R, Alken P. P-170 glycoprotein, glutathione and associated enzymes in relation to chemoresistance of primary human renal cell carcinomas. *Urol Int* 1990;45:170–6.
11. Chan HSL, Haddad G, Thorner PS, DeBoer G, Lin YP, Ondrusek N, Yeger H, Ling V. P-glycoprotein expression as a predictor of the outcome of therapy for neuroblastoma. *N Engl J Med* 1991;325:1608–14.
12. Chan HSL, Thorner PS, Haddad G, Ling V. Immunohistochemical detection of p-glycoprotein: prognostic correlation in soft tissue sarcoma of childhood. *J Clin Oncol* 1990;8:689–704.
13. Dalton WS, Grogan TM, Rybski JA, et al. Immunohistochemical detection and quantitation of p-glycoprotein in multiple drug-resistant human myeloma cells: association with level of drug resistance and drug accumulation. *Blood* 1989;73:747–52.
14. Miller TP, Grogan TM, Dalton WS, Spier CM, Scheper RJ, Salmon SE. P-glycoprotein expression in malignant lymphoma and reversal of clinical drug resistance with chemotherapy plus high-dose verapamil. *J Clin Oncol* 1991;9:17–24.
15. Goasguen JE, Dossot J, Fardel O, et al. Expression of the multidrug resistance-associated P-glycoprotein (P-170) in 59 cases of de novo acute lymphoblastic leukemia: prognostic implications. *Blood* 1993;81:2394–8.
16. Campos L, Guyotat D, Archimbaud E, et al. Clinical significance of multidrug resistance P-glycoprotein expression on acute nonlymphoblastic leukemia cells at diagnosis. *Blood* 1992;79:473–6.

17. Michieli M, Raspadori D, Damiani D, et al. The expression of multidrug resistance-associated glycoprotein in B-cell chronic lymphocytic leukaemia. *Br J Haematol* 1991;77: 460–5.
18. Benchimol S, Ling V. P-glycoprotein and tumor progression. *J Natl Cancer Inst* 1994;86:814–6.
19. Weinstein RS, Hansen KK, McBeath RB, Dalton WS. Expression of the MDR1 gene (p-glycoprotein) in breast cancer. *Recent Results Cancer Res* 1993;127:49–54.
20. Kang Y, Perry RR. Modulatory effects of tamoxifen and recombinant human α-interferon on doxorubicin resistance. *Cancer Res* 1993;53:3040–5.
21. Verrelle P, Meissonnier F, Fonck Y, et al. Clinical relevance of immunohistochemical detection of multidrug resistance P-glycoprotein in breast carcinoma. *J Natl Cancer Inst* 1991; 83:111–6.
22. Hill RP. Tumor progression: potential role of unstable genomic changes. *Cancer Metastasis Rev* 1990;9:137–47.
23. Weinstein RS, Jakate SM, Dominguez JM, et al. Relationship of the expression of the multidrug resistance gene product (P-glycoprotein) in human colon carcinoma to local tumor aggressiveness and lymph node metastasis. *Cancer Res* 1991;51:2720–6.
24. Rofstad EK. Radiation sensitivity in vitro of primary tumors and metastatic lesions of malignant melanoma. *Cancer Res* 1992;52:4453–7.
25. Ullrich SJ, Anderson CW, Mercer WE, Appelle E. The p53 tumor suppressor protein, a modulator of cell proliferation. *J Biol Chem* 1992;267:15259–62.
26. Chin K-V, Ueda K, Pastan I, Gottesman MM. Modulation of activity of the promoter of the human MDR1 gene by Ras and p53. *Science* 1992;255:459–61.
27. Zastawny RL, Salvino R, Chen J, Benchimol S, Ling V. The core promoter region of the p-glycoprotein gene is sufficient to confer differential responsiveness to wild-type and mutant p53. *Oncogene* 1993;8:1529–35.
28. Schneider J, Rubio M-P, Barbazan M-J, Rodriguez-Escudero FJ, Seizinger BR, Castresana JS. P-glycoprotein, HER-2/neu, and mutant p53 expression in human gynecologic tumors. *J Natl Cancer Inst* 1994;86:850–5.
29. Bradley G, Naik M, Ling V. P-glycoprotein expression in multidrug-resistant human ovarian carcinoma cell lines. *Cancer Res* 1989;49:2790–6.
30. Merkel DE, Fuqua SA, Tandon AK, Hill SM, Buzdar AU, McGuire WL. Electrophoretic analysis of 248 clinical breast cancer specimens for P-glycoprotein overexpression or gene amplification. *J Clin Oncol* 1989;7:1129–36.
31. Schneider J, Bak M, Efferth T, Kaufmann M, Mattern J, Volm M. P-glycoprotein expression in treated and untreated human breast cancer. *Br J Cancer* 1989;60:815–8.
32. Krishan A, Sauerteig A, Stein SH. Comparison of three commercially available antibodies for flow cytometric monitoring of P-glycoprotein expression in tumor cells. *Cytometry* 1991;12:731–42.
33. Charpin C, Vielh P, Duffaud F, et al. Quantitative immunocytochemical assays of p-glycoprotein in breast carcinomas: correlation to messenger RNA expression and to immunohistochemical prognostic indicators. *J Natl Cancer Inst* 1994;86:1539–45.
34. Ro J, Sahin A, Ro JY, Fritsche H, Hortobagyi G, Blick M. Immunohistochemical analysis of P-glycoprotein expression correlated with chemotherapy resistance in locally advanced breast cancer. *Hum Pathol* 1990;21:787–91.
35. He LR, Huang XF, Hao CY, Lin BY, Wang Y, Gao F. Evaluation of p-glycoprotein expression as a prognostic factor in breast cancer [Abstract 78]. *Breast Cancer Res Treat* 1994;32(suppl):48.