

Changes in Estrogen Receptor, Progesterone Receptor, and pS2 Expression in Tamoxifen-resistant Human Breast Cancer¹

S. R. D. Johnston,² G. Saccani-Jotti, I. E. Smith, J. Salter, J. Newby, M. Coppen, S. R. Ebbs, and M. Dowsett

Academic Department of Biochemistry [S. R. D. J., J. S., J. N., M. D.] and the Breast Unit [S. R. D. J., I. E. S., J. N.], The Royal Marsden Hospital, Fulham Road, London, SW3 6JJ, England; Istituto di Anatomia ed Istologia Patologica, University of Parma, Parma, Italy [G. S.-J.]; and Departments of Pathology [M. C.] and Surgery [S. R. E.], Mayday University Hospital, Croydon, Surrey, CR 7 7YE, England

ABSTRACT

Changes in estrogen receptor (ER) expression and function may explain the development of tamoxifen resistance in breast cancer. ER expression was measured by an immunohistochemical assay, validated for use in tamoxifen-treated tumors against a biochemical enzyme immunoassay, in 72 paired biopsies taken before treatment and at progression or relapse on tamoxifen. Progesterone receptor (PgR) and pS2 gene expression were also measured immunohistochemically as an indicator of ER function.

Overall the frequency of ER expression was reduced from 37 of 72 (51%) pretamoxifen to 21 of 72 (29%) at progression or relapse, with a significant reduction in the quantitative level of ER ($P < 0.0001$; Wilcoxon signed rank sum test). Tumors treated with primary tamoxifen that responded but then developed acquired resistance frequently remained ER positive (ER+) at relapse: 16 of 18 (89%) were ER+ pretamoxifen (75% of these expressed either PgR or pS2) and 11 of 18 (61%) were ER+ at relapse (82% continued to express PgR or pS2). In contrast, only 3 of 20 (15%) tumors that progressed on primary tamoxifen with *de novo* resistance were ER+ pretamoxifen, and all tumors were ER- at progression. At progression, 6 of 20 (30%) of these tumors expressed high levels of PgR (mean H-score, 98) and/or pS2 (mean, 50% cells positive), despite being ER-. In tumors that recurred during adjuvant tamoxifen therapy, including locoregional and metastatic lesions, ER expression was significantly reduced from 18 of 34 (53%) in the original primary tumor to 10 of 34 (29%) at relapse ($P = 0.002$). PgR expression was likewise significantly reduced in this group ($P = 0.001$).

This study confirms that expression of a functional ER in breast cancer is a strong predictor for primary response to tamoxifen. Although ER was reduced in tamoxifen-resistant tumors overall, the development of acquired resistance was associated with maintained ER expression and function in many tumors, whereas *de novo* resistance remained related to lack of ER expression. Recurrence during adjuvant tamoxifen was associated with development of an ER/PgR-negative phenotype in some tumors. These data imply that separate mechanisms of resistance may occur in these different clinical subgroups.

INTRODUCTION

The response to the antiestrogen tamoxifen in human breast cancer occurs more frequently in tumors that contain significant quantities of ER³ (1). Many tumors that do not respond and thereby demonstrate primary *de novo* resistance to tamoxifen lack detectable ER protein, and this generally renders them resistant to other endocrine therapies including progestins and aromatase inhibitors. Most of the tumors that do respond initially to tamoxifen eventually progress with acquired resistance, although clinical evidence suggests that many remain sensitive to further endocrine therapies. These observations suggest

that a basic biological difference exists between tumors with acquired tamoxifen resistance and those with intrinsic resistance to the drug.

Tamoxifen may modulate the expression of ER in hormone-dependent breast cancer. Although the mechanism of action of the drug is to compete with estrogen for the binding site of ER and to inhibit estrogen-induced growth, there is evidence *in vitro* that ER expression itself may become up-regulated after tamoxifen (2). However, immunohistochemical studies have demonstrated that ER expression within breast cancers is heterogeneous, and theoretically through selective pressure tamoxifen could permit the survival of clones of ER- cells while inhibiting the growth of ER+ clones. Ultimately, this could allow the emergence of an ER- hormone-independent tumor that was no longer sensitive to tamoxifen.

There have been previous studies that have examined the expression of ER in tumor biopsies from patients during tamoxifen therapy (3-5). In general, these studies found tamoxifen-treated tumors to be ER-, supporting the hypothesis of clonal selection. However, the most commonly used technique to measure ER in these samples was the ligand-binding assay, and tamoxifen may have given false negative results due to competition with estrogen for the binding site of ER. In addition, this assay requires relatively large quantities of fresh tissue, which limits any retrospective comparisons of relapsed with primary tumors. The more recent development of IHAs with the use of mAbs has allowed ER to be measured in paraffin-embedded material. These assays have the advantages of detecting tamoxifen-bound receptor, requiring very small amounts of tissue, and permitting study of the heterogeneity of ER expression within tumors. Several groups, including our own, have now validated these assays against conventional biochemical techniques (6-8).

The functional activity of ER may be as important as its level of expression, particularly in determining whether endocrine therapy is of value. The expression of several proteins is known to be estrogen-regulated, including the PgR and the product of the pS2 gene (9, 10). ER+ tumors that express PgR have been shown to be more likely to benefit from endocrine therapy in the adjuvant setting (11). Likewise, pS2 expression in ER+ tumors has been found to improve the likelihood of response to endocrine therapy in advanced breast cancer (12). IHAs are now available to measure both of these proteins in paraffin-embedded tissue. This allows, therefore, a more complete characterization of the ER-related phenotype to be made, which may give an indication of the function of ER, in addition to its level of expression.

In this study, we have analyzed ER expression and function, as measured by PgR and pS2 expression, in 72 patients with documented resistance to tamoxifen. In all patients, a biopsy from the primary tumor had been taken before tamoxifen was started, allowing direct within-patient comparison of the change in expression of ER, PgR, and pS2 in relation to the development of tamoxifen resistance *in vivo*.

MATERIALS AND METHODS

Patients and Tissue Samples. Seventy-two women with breast cancer who progressed during tamoxifen therapy (20 mg daily) were studied. All patients attended either the Royal Marsden Hospital or the Mayday University

Received 2/9/95; accepted 5/23/95.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This study was supported by the Cancer Research Campaign. S. R. D. J. is a CRC Clinical Research Training Fellow. G. S.-J. was supported in part by the following grants: CNR ACRO Project, CNR Bilateral 94.02482.CT04, and MURST 40% (Italy).

² To whom requests for reprints should be addressed.

³ The abbreviations used are: ER, estrogen receptor; ER+, ER positive; ER-, ER negative; PgR, progesterone receptor; EIA, enzyme immunoassay; IHA, immunohistochemical assay.

Hospital. Thirty-eight patients had been treated with tamoxifen as primary medical therapy when they first presented with breast cancer. In general, these were postmenopausal women who were treated with tamoxifen rather than surgery because of age, tumor size, or advanced local disease. Of these patients, 18 responded to tamoxifen but subsequently relapsed with acquired resistance, and 20 progressed during initial tamoxifen therapy with primary *de novo* resistance. Response was defined clinically according to standard UICC criteria (13) in terms of change in bidimensional tumor measurements (caliper), with a partial response representing a greater than 50% reduction in the product of the two measurements, and a complete response when the tumor was no longer palpable. Progression during treatment included tumors in which there was a greater than 25% increase in size and those where there was no change in tumor measurement (<50% reduction or <25% increase in size). Three of the 20 tumors that progressed on primary tamoxifen had no change in tumor measurements for more than 6 months before progression. Some authors consider these to represent clinical "responses," but for the purpose of this study objective response to primary tamoxifen only included those with documented partial of complete response. In all these cases the tumor at relapse or progression was compared with a trucut biopsy taken from the same tumor before tamoxifen was started.

In an additional 34 patients, tamoxifen had been given as adjuvant therapy after initial surgical management, and in these cases the tamoxifen-relapsed tumor was compared with the original excised primary tumor. In 15 cases the tumor which developed during adjuvant therapy represented a local recurrence in the breast, whereas in 19 cases the recurrence was at a different site (11 as lymph node metastases and 8 as skin nodules). The demographic data for these three groups of patients are shown in Table 1.

At relapse, mastectomy or excision biopsy specimens were delivered fresh to histopathology after resection and were processed immediately. A portion of tumor, approximately 200 mg in size, was snap frozen in liquid nitrogen immediately after dissection from the breast, and stored at -80°C for analysis of ER by EIA. The remainder of the tumor was fixed for approximately 24 h in 10% buffered formalin. The tissues were embedded in paraffin wax after a routine processing procedure that did not exceed 60°C . Sequential adjacent sections (3 μm) were cut from the paraffin-embedded tumors onto slides coated with either 3-aminopropyltriethoxysilane (Sigma Chemical Co.) for the ER and PgR assays or poly-L-lysine for the pS2 assay. The sections were air dried overnight in an oven at 37°C , and one section was stained with hematoxylin and eosin for light microscope assessment.

ER IHA. We have previously described this IHA, which has been validated against the conventional biochemical EIA (8). In brief, sections were predigested in 10 mM citrate buffer (pH 6.0) by microwaving (750 W, full power) for two 5-min intervals. After blocking endogenous peroxidase activity, sections were incubated with monoclonal anti-human ER antibody 1D5 (Dako) for 2 h (1:100 dilution), rinsed in PBS, and incubated in biotinylated rabbit anti-mouse immunoglobulin (Dako) for 45 min (1:100 dilution). After incubation with horseradish peroxidase-conjugated streptavidin complex (dilution 1:200) for 1 h, a solution of 0.05% 3,3'-diaminobenzidine (Sigma) dissolved in dimethyl formamide plus 100 μl of 30 volumes hydrogen peroxide-100 ml PBS was used to develop the peroxidase activity. Previously identified strongly ER+ tumors were used as positive controls, with negative controls being derived by omission of the primary antibody.

Ten fields (minimum 500 cells) were chosen at random at $\times 400$ magnification for scoring of nuclear staining. Staining intensity was assessed as negative, weak, intermediate, or strong (index, 0 to 3), and the percentage of cells at each intensity estimated to give an overall "H-score," ranging from

0–300 (14). We have previously validated our scoring system for this assay against the biochemical EIA (ER-EIA) (8). Stroma, normal, and benign epithelial tissue were excluded, and a tumor was designated ER+ if the H-score was >20 .

PgR and pS2 IHAs. For the PgR assay, no predigestion or microwave enhancement was required. The methods were similar to those described above, although sections were incubated overnight with a 1:2 dilution of monoclonal anti-human PgR antibody (0.1 mg/ml) from the Abbott immunohistochemical kit, followed by a biotinylated rabbit anti-rat antibody at a dilution of 1:100 for 45 min. The detection method and scoring system were similar to those used for ER.

The pS2 assay used a mouse anti-pS2 mAb BC6 (gift from Professor P. Chambon, Paris, France), which we have described previously and validated against an immunoradiometric biochemical assay (15). Scoring was assessed by counting the number of malignant cells with cytoplasmic staining for pS2 and expressing this as a percentage of the total number of malignant cells with the use of a positive cutoff of 10%.

Comparison of ER IHA with ER EIA in Tamoxifen-treated Tumors. We have already demonstrated the close relationship between ER measured by the IHA method described above and by EIA (Abbott) in a separate series of 119 primary breast cancers (8). It has been shown previously that tamoxifen does not lead to false negative results in the EIA (16). To ensure that tamoxifen did not interfere with the IHA results in the resistant samples, a separate cohort of 33 primary breast cancers from postmenopausal women who had been treated with tamoxifen for 2–3 weeks before surgery was studied. In these tumors, we measured ER by IHA in paraffin-embedded sections and by EIA in frozen tumor samples. A similar comparison between ER IHA and ER EIA was also made in a total of 98 tamoxifen-resistant tumors. This cohort comprised 40 of the 72 resistant tumors described above, where a frozen sample of tumor was also available for EIA. In addition, 58 tamoxifen-resistant tumors were available from patients for whom no matched pretreatment biopsy existed for the currently reported paired immunohistochemical study, but in whom for validation purposes at relapse a frozen sample for EIA could be compared with a paraffin-embedded sample for IHA.

For the EIA method, the frozen tumor sample was pulverized in a micro-dismembrator (Braun Medical, Ltd.) for 1 min after cooling in liquid nitrogen. The powdered tumor was reconstituted 1:8 (w/v) in iced tris/molybdate buffer [5 mM sodium molybdate, 10 mM monothio glycerol, 1 mM dipotassium chloride EDTA, 3 mM sodium azide, and 10 mM TRIS (pH 7.4)], and the homogenate was centrifuged at 4°C for 20 min at $2000 \times g$, after which the cytosol fraction was removed and diluted 1:5 in tris/molybdate buffer for protein assay with the use of the Bio-Rad method with a bovine γ globulin standard. An aliquot of the cytosol was diluted to give a protein concentration of 1–2 mg/ml. The ER levels in the diluted cytosols were determined with the use of the ER EIA kit from Abbott Diagnostics, according to the manufacturer's instructions, and values >10 fmol/mg protein were regarded as positive.

Statistics. Comparisons between the semiquantitative scores for the IHA and EIA assays were made by linear regression analysis. The mean H-scores (ER and PgR) and the mean percentage positive cells (pS2) were calculated for all the pretamoxifen and tamoxifen relapse-positive tumors, and within each of the three clinical subgroups. In view of the wide range in values and absence of a normal distribution of quantitative data for ER, PgR, and pS2 in each group, nonparametric paired analysis of the change in absolute value for each parameter was performed with the use of the Wilcoxon signed rank sum test.

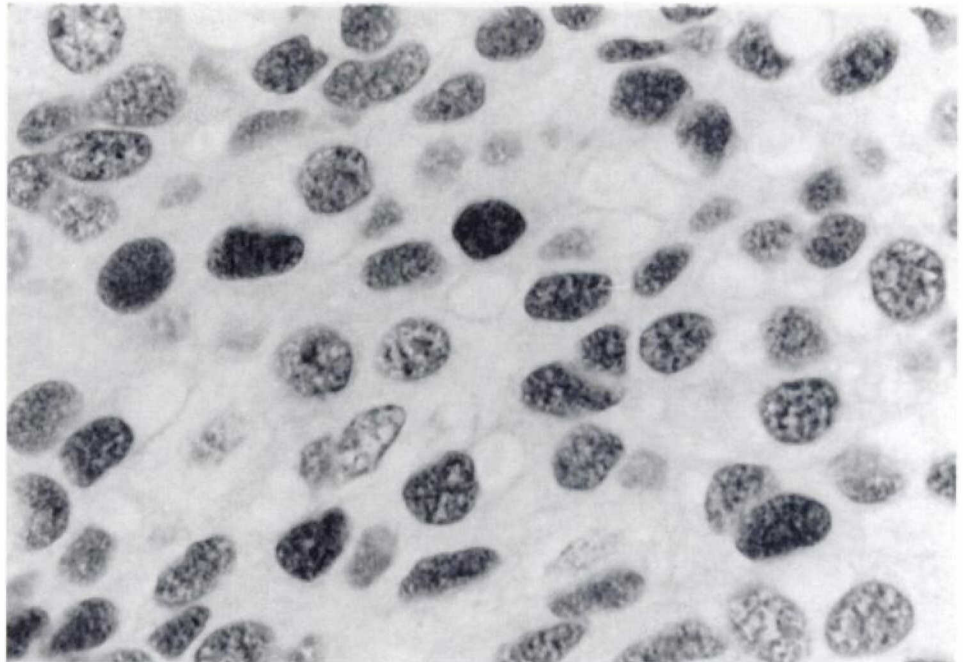
RESULTS

Comparison of ER IHA with ER EIA in Tamoxifen-treated Tumors. The immunohistochemical staining obtained with the 1D5 antibody on paraffin-embedded sections produced clear nuclear staining in invasive carcinoma cells (Fig. 1). In our previous comparison of the H-score system for ER with the biochemical EIA in 119 untreated primary breast cancers, we found a concordance rate of 86%, with a positive correlation between the scores ($r = 0.605$). In the 33 tamoxifen-treated primary breast cancers, the concordance rate was 96%; only 1 tumor was EIA+ and IHA–, and the IHA score was borderline negative (H-score 16). A strong positive correlation ($r = 0.934$) was shown between the IHA H-score for ER and the EIA value in fmol/mg

Table 1 Characteristics of patients with breast cancer who developed tamoxifen resistance

	No. of patients	Median age (yr)	Median time to relapse (mo.)
Primary tamoxifen			
Responders: acquired resistance	18	74	24
Nonresponders: <i>de novo</i> resistance	20	72	4
Adjuvant tamoxifen			
Locoregional recurrence	26	60	27
Metastatic recurrence	8	55	14

Fig. 1. Paraffin-embedded section of an invasive ductal carcinoma of the breast stained with 1D5 mAb against ER. Uniform nuclear staining is observed in tumor cells. $\times 1000$.



protein (Fig. 2). From these data it does not appear that tamoxifen prevents the detection of ER by the 1D5 antibody in paraffin-embedded sections.

In the 98 tamoxifen-resistant tumors, both the ER EIA and ER IHA scores for ER+ tumors were lower than scores for the primary ER+ tumors treated with short-term tamoxifen (Fig. 2). The concordance rate between the two methods was lower at 66%, which appeared to be largely explained by 30 tumors that were EIA+ but IHA- (Table 2). However, 25 of these 30 tumors had borderline scores on either or both assays; 15 tumors were borderline EIA+ (10–35 fmol/mg protein) and IHA-, 6 tumors were borderline IHA- (H-score 5–19) and EIA+, and 2 tumors were both borderline EIA+ and borderline IHA-. In addition 2 EIA+ tumors were IHA- within the invasive tumor component but contained benign epithelial cells that were strongly positive. The remaining 5 tumors, which were completely negative by IHA, had EIA scores which ranged between 46 and 112 fmol/mg protein. There were 3 tumors that were IHA+ but EIA-, but in all 3 cases the EIA score was borderline negative (5–9 fmol/mg protein). Overall, there was weaker positive correlation between the two methodologies for the tamoxifen-resistant tumors ($r = 0.561$) compared with either the short-term tamoxifen-treated cohort or our previous data on primary untreated tumors.

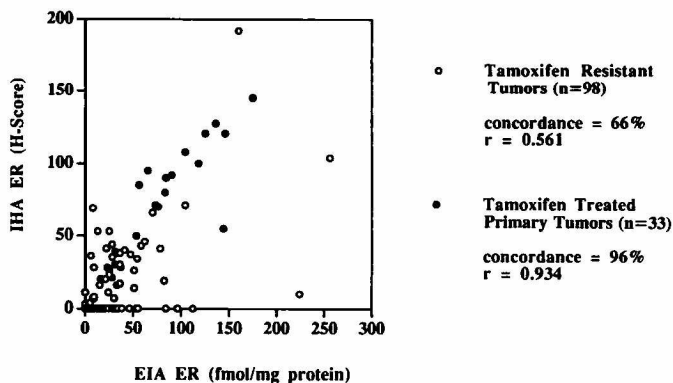


Fig. 2. Scattergram of EIA versus IHA for ER in 33 tamoxifen-treated primary tumors and 98 tamoxifen-resistant tumors.

Table 2 Concordance between ER EIA and ER IHA in tamoxifen-treated and tamoxifen-resistant human breast cancer

	Primary tamoxifen	Tamoxifen resistant
<i>n</i>	33	98
Concordance	96%	66%
EIA+/IHA+	22	26
EIA+/IHA-	1	30
EIA-/IHA+	0	3
EIA-/IHA-	10	39

Change in ER IHA in Tamoxifen-resistant Breast Cancer.

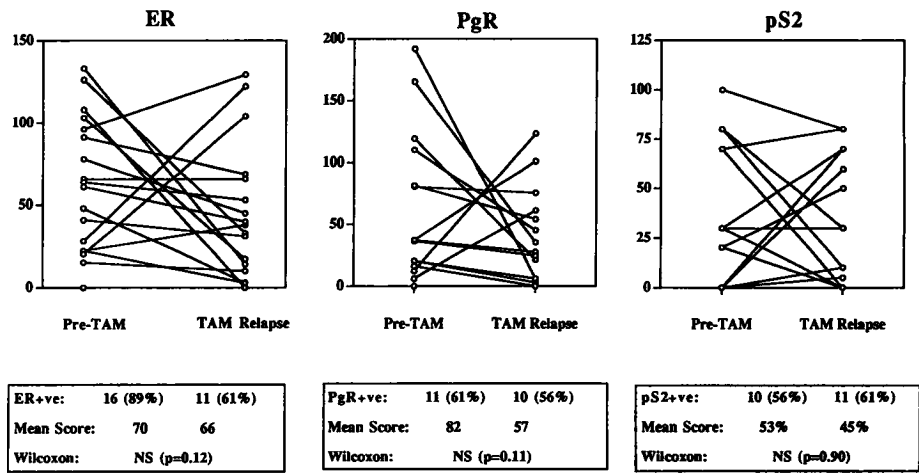
Before tamoxifen, 37 of 72 (51%) tumors were ER+ by the IHA assay, with a mean H-score for ER+ tumors of 90 ± 7 (SEM). At relapse, only 21 of 72 (29%) tumors were ER+ (mean H-score for ER+ tumors, 61 ± 9). A direct comparison of the changes in H-score between the 72 pairs of samples showed that, overall, there was a significant fall in ER expression at relapse (Z value, -4.52; $P < 0.0001$, Wilcoxon signed rank sum test).

Analysis of the three clinical subgroups of tamoxifen resistance revealed different patterns of ER expression. In tumors treated with primary tamoxifen, ER expression within the same tumor was compared between the pretreatment biopsy and the tumor at relapse or progression. Of those that responded to primary tamoxifen, 16 of 18 (89%) were ER+ at presentation (mean H-score for ER+ tumors, 70). At subsequent relapse in these patients analysis of the same tumor showed that 11 (61%) were still ER+ (mean H-score of 66), and in 4 of these cases the ER score had increased. In total, 5 ER+ tumors had become ER-, and 2 originally ER- tumors remained ER- at relapse. Paired comparison between the 18 presentation and relapse samples revealed no significant difference in ER score (Fig. 3).

Of the tumors treated with primary tamoxifen that progressed on treatment (*de novo* resistance), the majority (17 of 20) was ER- at presentation. At progression in the repeat biopsy from the same tumor, all 20 samples were ER-. On paired analysis of the ER scores, this represented a significant reduction (Z value, -2.67; $P = 0.008$; Fig. 4).

In the adjuvant group, comparison of ER expression was made between the original primary tumor and the recurrent tumor at relapse

Fig. 3. Change in ER, PgR, and pS2 status in 18 paired samples from patients with acquired tamoxifen (TAM) resistance. NS, not significant.



on tamoxifen. Of the original primary tumors, 18 of 34 (53%) were ER+ (mean H-score of ER+ tumors, 103). At progression on tamoxifen only 10 of 34 (29%) were ER+, and in these tumors the mean H-score was reduced to 57. Paired comparison showed a highly significant reduction in ER expression between the primary and relapsed tumor in this group (Z value, -3.77; P = 0.0002). Of the 18 ER+ tumors, 9 became ER-, and in 6 the ER score was reduced by more than 50% (Fig. 5). Of these original 18 ER+ primary tumors, 12 recurred on tamoxifen with locoregional tumor and 6 with metastatic skin nodules. In these patients, a greater number of locoregional recurrences than metastatic tumors remained ER+ (8 of 12 versus 2 of 6; Table 4).

Change in PgR and pS2 in Tamoxifen-resistant Breast Cancer. Overall, there was no significant difference in the frequency of PgR+ or pS2+ tumors between the pretreatment and tamoxifen-resistant groups (Table 3). The mean scores for the PgR+ or pS2+ tumors were similar, although paired comparison of the change in score for all 72 cases showed a significant reduction in PgR (P = 0.01) but not pS2 expression (P = 0.49).

Of the 18 primary treated tumors that developed acquired resistance, 11 (61%) were PgR+ and 10 (56%) were pS2+ at presentation. All of these tumors that expressed PgR or pS2 were also ER+. At relapse, 10 of 18 (56%) tumors were PgR+. Whereas the mean H-score in these tumors was lower at relapse compared with pretamoxifen (57 versus 82), paired comparison showed no significant difference (Fig. 3). Two tumors that were originally PgR- became

PgR+ at relapse, one of which was strongly PgR+ (H-score = 61) despite being completely ER- both at presentation and at relapse. pS2 expression was likewise unchanged in these 18 tumors, with 11 (61%) tumors pS2+ at relapse (Fig. 3).

Of the 20 tumors that progressed on primary tamoxifen, 3 (15%) were PgR+ and 1 (5%) was pS2+ before therapy (all but one of these tumors was ER+). Whereas all tumors were ER- at progression, 6 (30%) were PgR+, of which 3 were also pS2+ (Fig. 4). Four of these tumors had zero scores for ER, PgR, and pS2 in the pretamoxifen biopsy. The quantitative level of both PgR and pS2 expression in these ER- tumors at progression was relatively high (mean PgR H-score, 91; mean pS2 score, 50% positive cells). In sequential adjacent sections from one of these tumors (Fig. 6), populations of invasive carcinoma cells that were negative for ER stained positive for PgR and pS2.

In the adjuvant group, 13 (38%) of the original primary tumors were PgR+, and 12 (35%) tumors pS2+. There was a reduction in the expression of PgR in the relapsed tumor (38 to 12%), which was highly significant on paired analysis (Z value, -3.29; P = 0.001). In contrast, the frequency of pS2 expression did not change (Fig. 5). Ten of the 12 pS2+ tumors at relapse were local or nodal recurrence, whereas only 2 cases were metastatic tumors (both ER+). In addition, 4 of the 12 recurrent pS2+ tumors were ER-, including one that also expressed PgR. However, all the ER+ recurrences during adjuvant tamoxifen (5 local, 3 regional, and 2 metastatic) expressed either PgR (n = 3) or pS2 (n = 8; Table 4).

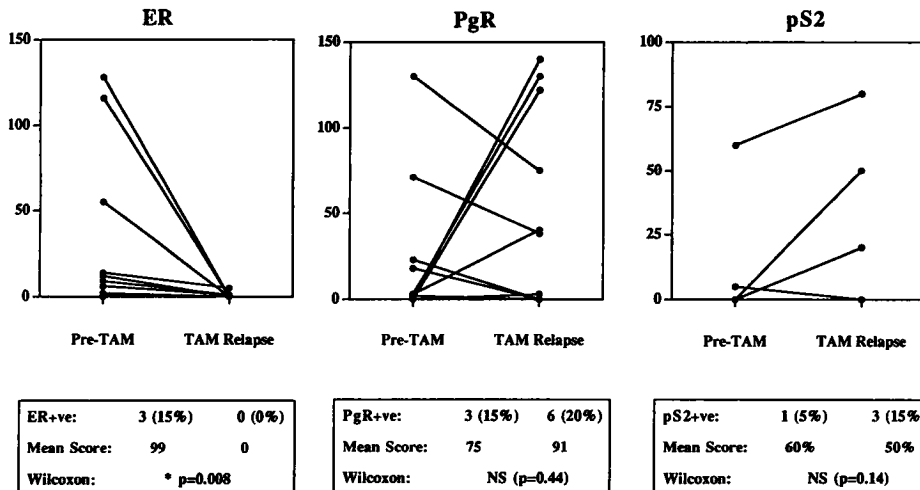


Fig. 4. Change in ER, PgR, and pS2 status in 20 paired samples from patients with de novo tamoxifen (TAM) resistance. NS, not significant.

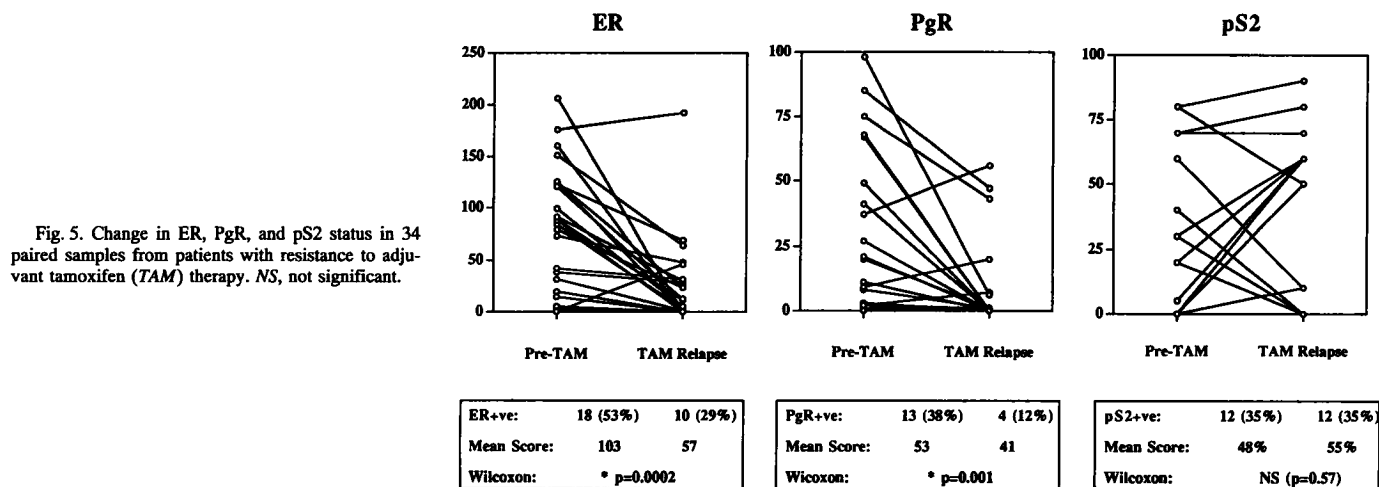


Fig. 5. Change in ER, PgR, and pS2 status in 34 paired samples from patients with resistance to adjuvant tamoxifen (TAM) therapy. NS, not significant.

DISCUSSION

The expression of ER within the majority of human breast cancers is heterogeneous (17). Immunohistochemical studies with mAbs to ER have identified mixed populations of ER+ and ER- cells in human breast carcinomas (18). A potential consequence of prolonged endocrine therapy could be the clonal selection of ER-, presumably hormone-insensitive cells from within an originally heterogeneous ER+ tumor (19). One mechanism for relapse after successful endocrine therapy, therefore, might be the emergence under selective pressure of tamoxifen of ER- hormone-resistant tumors.

Several groups have studied ER content in sequential tumor biopsies after intervening endocrine therapy. Allegra *et al.* (3) were among the first to report that whereas the ER content was similar between either multiple metastatic sites or over time without intervening therapy, a significant fall in ER content followed endocrine therapy. Taylor *et al.* (4) showed in 26 patients with advanced breast cancer that the ER content of metastatic skin deposits fell in both responding and nonresponding patients after 2-3 months of endocrine therapy. Hull *et al.* (5) demonstrated a significant decrease in tumor ER levels after tamoxifen, but not in patients in whom the second biopsy was taken more than 2 months after discontinuing the drug. However, in all of these studies a ligand-binding assay was used to measure ER, and it is probable that at the time of the second biopsy, receptor occupancy by tamoxifen resulted in a false negative ER assay for many tumors.

Another confounding variable is sequential comparison between different metastatic deposits of tumor. In a more recent study, ER was measured in the same tumor before and after systemic therapy in 63 patients with large operable primary breast cancer (20). No significant change in ER concentration was seen in those treated with surgical

oophorectomy, aromatase inhibitor, or chemotherapy, whereas a significant fall in ER was observed in those treated with tamoxifen. However, again it was concluded that this was due to interference by tamoxifen or its metabolites in the ligand-binding assay. The impact of such interference was recently demonstrated in a study where ER was measured by both ligand-binding assay and IHA in tumors from 34 patients on tamoxifen (21). ER was detected more frequently by immunohistochemical compared with ligand-binding assay, again implying that receptor occupancy by tamoxifen may interfere with the ligand-binding assay.

From our study it appears that tamoxifen does not interfere with our IHA for ER. In the subset of tamoxifen-treated primary tumors, comparison with the biochemical EIA (unaffected by ligand interaction) showed a 96% concordance rate and strong positive correlation for the immunohistochemical H-score for ER. These data suggest that tamoxifen does not reduce or inhibit ER detection by 1D5 antibody, which is targeted against the NH₂-terminal end of the receptor, away from the ligand-binding region. In the 98 tamoxifen-resistant tumors that were studied by both EIA and IHA, lower scores were observed by both assays (Fig. 2). The lower concordance rate between the two assays may largely be explained by borderline scores on either or both assays (Table 2). The technical difference between measuring ER in a tumor homogenate and on a histological section means that such discrepancies at the detection threshold for each assay are to be expected. However, 5 tumors were completely IHA- but clearly EIA+. In these tumors, for example, mutations or conformational changes within certain ER domains could explain why antibodies directed toward the NH₂-terminal epitopes (1D5) may not bind, whereas antibodies directed towards the COOH-terminal epitopes (H222) bind strongly.

Overall, the frequency and quantitative expression of ER appears to be reduced in tamoxifen-resistant tumors. The paired comparison between the primary and resistant tumor suggest that ER expression and function may change in association with certain types of tamoxifen resistance. In the patients treated with primary tamoxifen, the clinical response was strongly correlated with ER status; 89% of responders compared to only 15% of nonresponders were ER+ at presentation. In the biopsy from the same tumor taken at relapse or progression on tamoxifen, 61% of the responding group who had developed acquired resistance remained ER+, whereas all of the nonresponders who progressed on treatment were ER-. In those with acquired resistance, paired comparison of the quantitative scores showed no significant change (Fig. 3). Furthermore 82% of these tamoxifen-resistant tumors that were ER+ at relapse still expressed

Table 3 Overall change in frequency of ER, PgR, and pS2 expression and mean score in 72 paired biopsies taken before and at relapse on tamoxifen^a

	Pretamoxifen	Tamoxifen relapse
ER+		
No. (%)	37 (51)	21 (29)
Mean H-score ± SEM	90 ± 7	61 ± 9
PgR+		
No. (%)	27 (38)	20 (28)
Mean H-score ± SEM	66 ± 9	64 ± 9
pS2+		
No. (%)	23 (32)	26 (36)
Mean percentage positive ± SEM	52 ± 5	51 ± 5

^a ER and PgR scores are the mean H-scores for those positive cases; pS2 scores are the mean percentage positive cells in positive cases.

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.