# Estrogen Receptor–Positive, Progesterone Receptor–Negative Breast Cancer: Association With Growth Factor Receptor Expression and Tamoxifen Resistance

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***Background:*** **Clinical data indicate that estrogen receptor–positive/progesterone receptor–negative ($ER^+/PR^-$) breast cancers are less sensitive to tamoxifen than are $ER^+/PR^+$ tumors. It has also been reported that tamoxifen may be less effective in tumors that overexpress either HER-2 or HER-1 (epidermal growth factor receptor) and that signaling through these receptors reduces PR expression in experimental models. We hypothesized that $ER^+/PR^-$ breast tumors are more likely than $ER^+/PR^+$ breast tumors to have an aggressive phenotype, to express HER-1 and overexpress HER-2, and are less likely to benefit from tamoxifen adjuvant therapy.** ***Methods:*** **Clinical and biological features of 31 415 patients with $ER^+/PR^+$ tumors were compared with those of 13 404 patients with $ER^+/PR^-$ tumors. Association between disease-free survival (DFS) and HER-1 and HER-2 status was analyzed in a subset of 11 399 patients receiving adjuvant tamoxifen therapy. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using Cox regression or Kaplan–Meier analyses, and all statistical tests were two-sided.** ***Results:*** **$ER^+/PR^-$ tumors were more frequent in older patients, were larger in size, had a higher S-phase fraction, and were more likely to be aneuploid than $ER^+/PR^+$ tumors. Furthermore, three times as many $ER^+/PR^-$ tumors as $ER^+/PR^+$ tumors expressed HER-1 (25% versus 8%; $P<.001$) and 50% more overexpressed HER-2 (21% versus 14%; $P<.001$). Among all tamoxifen-treated women, recurrence was higher among women with HER-1–expressing tumors than with HER-1–negative tumors (HR = 1.9, 95% CI = 1.0 to 3.5; $P = .05$); a stronger association between worse DFS and HER-2 overexpression was observed (HR = 2.3, 95% CI = 1.2 to 4.3; $P = .006$). However, results varied by PR status. Among tamoxifen-treated women with $ER^+/PR^+$ tumors, HER-1 or HER-2 status was not associated with worse DFS. Among women with $ER^+/PR^-$ tumors, however, both HER-1 expression (HR = 2.4, 95% CI = 1.0 to 5.4; $P = .036$) and HER-2 overexpression (HR = 2.6, 95% CI = 1.1 to 6.0; $P = .022$) were associated with a higher likelihood of recurrence.** ***Conclusions:*** **$ER^+/PR^-$ tumors express higher levels of HER-1 and HER-2 and display more aggressive features than $ER^+/PR^+$ tumors. As in laboratory models, lack of PR expression in $ER^+$ tumors may be a surrogate marker of aberrant growth factor signaling that could contribute to the tamoxifen resistance observed in these tumors.** **[J Natl Cancer Inst 2005;97:1254–61]**

Nearly 30 years ago, it was recognized that transcription of the progesterone receptor (PR) gene was regulated by estrogen in breast and reproductive tissues and that estrogen receptor–positive ($ER^+$) breast tumors that lacked PR expression were less responsive to endocrine therapy than those that express PR. At that time, Horwitz and McGuire *(1)* hypothesized that PR loss was due to loss of ER activity, due to either low circulating estrogen in some older women or a nonfunctioning ER pathway *(2,3)*. This hypothesis, however, did not fully explain why some $ER^+/PR^-$ tumors respond to endocrine therapy, albeit at a lower frequency, than tumors that are both $ER^+$ and $PR^+$ ($ER^+/PR^+$). Later, it was recognized that ER and PR status are not always stable phenotypes and that they can in fact change over the natural history of the disease or as a consequence of endocrine treatment *(4)*. During tamoxifen therapy, levels of both PR and ER decrease but PR levels decrease more dramatically than ER levels, with up to half of the tumors completely losing PR expression as they develop tamoxifen resistance *(5)*. In patients with such tumors, the loss of PR translates into a more aggressive disease and worse overall survival, suggesting that other alterations in the molecular machinery driving tumor growth accompany the loss of PR receptor expression *(6)*.

Clinical data have confirmed in both the metastatic and adjuvant treatment settings that tamoxifen is less efficacious in $ER^+/PR^-$ tumors than in $ER^+/PR^+$ tumors *(7–13)*. Data from the large ATAC adjuvant trial, a worldwide trial comparing the efficacy of tamoxifen with that of the aromatase inhibitor anastrazole, showed overall that patients with $ER^+/PR^+$ tumors had a lower recurrence rate than those with $ER^+/PR^-$ tumors (7.6% versus 14.8%) *(14)*. This difference in overall recurrence was due largely to the lower efficacy of tamoxifen in the subgroup of patients with $ER^+/PR^-$ tumors; there was little difference in the recurrence rate of $PR^+$ versus $PR^-$ tumors in patients who were treated with anastrozole. The observation that patients with $ER^+/PR^-$ tumors respond nearly as well to anastrozole as those with $ER^+/PR^+$ tumors suggests that the ER signaling pathway is functional in many $ER^+/PR^-$ tumors and that these tumors are still

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dependent on estrogen for growth, despite having somewhat lower ER levels. Thus, the etiology of the $ER^+/PR^-$ phenotype, either de novo or acquired, cannot be attributed entirely to the nonfunctional ER hypothesis. Furthermore, ER activity has been observed in freshly prepared breast lysates of $ER^+/PR^-$ tumors *(15)*.

Several clinical reports have suggested that high growth factor signaling may be associated with decreased PR levels in breast cancer *(6,16–19)*. Indeed, a recent study showed that growth factors that activate the PI3K–Akt–mTOR pathway can decrease PR transcription *(20–22)*. Increased HER-1 or HER-2 activity may also lead to tamoxifen resistance in some patients, and this result has also been shown using several experimental preclinical models *(23–26)*. The cumulative data raise the possibility that PR loss is a surrogate marker for excessive growth factor receptor activation, which translates into reduced tamoxifen benefit.

The aim of this study was to determine whether $ER^+/PR^+$ and $ER^+/PR^-$ breast tumors represent distinct biologic and clinical entities and to investigate whether PR loss is associated with higher HER-1 (epidermal growth factor receptor [EGFR]) and HER-2 content in human breast cancer. Finally, we investigated whether PR expression is associated with the clinical outcome of patients treated with tamoxifen adjuvant therapy. Because of the limited sample size in some of the tamoxifen-treated HER-1, HER-2, and hormone receptor–defined subsets, this is a hypothesis-generating analysis.

## Subjects and Methods

### Study Population

The Breast Center at Baylor College of Medicine maintains databases of breast cancer patients whose biopsy or mastectomy specimens were sent to two central laboratories for steroid receptor assays. The reference laboratories were located at the University of Texas Health Science Center at San Antonio and at Nichols Institute in San Juan Capistrano, California, and they used identical assays and cooperated at regular intervals in quality control procedures. The patients were diagnosed and treated at more than 370 academic and community institutions throughout the United States. Follow-up information was obtained from tumor registries, by direct review of medical records performed by data managers, or by data collection forms completed by the office staff of the referring physicians. These databases contain information on receptor status and outcomes of 54 865 patients who were diagnosed between 1970 and 1998 with early breast cancer (stage I–IIIA, as defined by American Joint Committee on Cancer staging classification) *(27)*. The patient information contained in this report was obtained from two data repositories maintained by the Breast Center at Baylor College of Medicine. Each repository has been reviewed by institutional review boards at the University of Texas Health Science Center at San Antonio and at Baylor College of Medicine. Both institutional review boards determined that a waiver of written informed consent was appropriate for each of these repositories. No patient identifiers were provided to the authors.

### Prognostic Factors

ER levels were measured by the dextran-coated charcoal method, as previously described *(28)*. From 1970 to 1984, [$^3$H]estradiol was used as the labeled ligand. During the same period, PR levels were measured by sucrose density gradient *(29)*. In 1985, the standard multipoint dextran-coated charcoal assay was modified to incorporate [$^{125}$I]estradiol and [$^3$H]R5020 in a single assay, allowing the simultaneous determination of both ER and PR. Samples containing at least 3 fmol/mg protein were considered ER positive, and those containing at least 5 fmol/mg protein were considered PR positive, based on prior clinical studies *(7,10,30)*. DNA ploidy and S-phase fraction were evaluated by flow cytometry and the histograms were analyzed by Modfit (Verify Software House, Topsham, ME) using single-cut debris stripping *(31)*. Cutpoints were determined by calibrating S-phase fraction with clinical outcome in a group of more than 28 800 patients with breast cancer (low, <6%; intermediate, 6%–10%; high >10%) *(31)*. HER-2 status was determined by western blot analysis, using a rabbit polyclonal antibody directed against the C terminus of the HER-2 protein *(32)*. The cutoff value between low (negative) and high (positive) HER-2 expression was set at 1 U/μg protein based on prior studies in which protein level above this cutpoint was associated with several poor prognostic factors and a worse disease-free and overall survival *(32,33)*. HER-1 levels were measured by radioligand binding assay using a fixed concentration of radiolabeled EGF and various concentrations of unlabeled EGF. Levels of at least 10 fmol/mg were considered positive. This method for assessing HER-1 expression and cutoff value is similar to that used in previously published studies *(34,35)*.

### Statistical Methods

Descriptive statistics are reported as frequencies or as medians. The clinical and biologic characteristics of women with $ER^+/PR^+$ and $ER^+/PR^-$ tumors were compared using contingency tables, chi-square tests, and Fisher exact tests. ER and PR levels were compared between $ER^+/PR^+$ and $ER^+/PR^-$ tumors and by HER-1 and HER-2 positivity status using nonparametric Wilcoxon rank-sum tests.

Disease-free survival (DFS) was calculated from the date of the diagnostic biopsy, with first recurrences, local or distant, being scored as an event, and with censoring of other patients at the time of last follow-up or death. Overall survival (OS) was defined as the interval between the diagnostic biopsy and death from any cause, death being scored as an event. Patients who were still alive at the time of last follow-up were censored then. DFS and OS curves were estimated using the Kaplan–Meier product limit method and were compared by the log-rank test. A univariate Cox regression model was used to determine the association of HER-1 and HER-2 status with DFS and OS in tamoxifen-treated $ER^+/PR^+$ and $ER^+/PR^-$ patients. The assumption of proportionality of HER-1 and HER-2 on DFS and OS was verified by performing hypothesis tests of HER-1 and HER-2 status as time-dependant variables in the Cox model. Hazard ratios (HRs) are presented with their 95% confidence intervals (CIs). The simultaneous association of these growth factor receptors along with clinical and biological characteristics was assessed in a multivariable Cox regression model. The potential interaction between ER/PR status and HER-1 and HER-2 status was also tested in this multivariable model, which included the following variables and cutpoints were determined based on previous studies or conventional definitions: tumor size (≤2 cm versus >2 cm), axillary nodes (0, 1–3, ≥4), age (<50 years versus ≥50 years), ploidy (diploid versus aneuploid), S-phase fraction (low

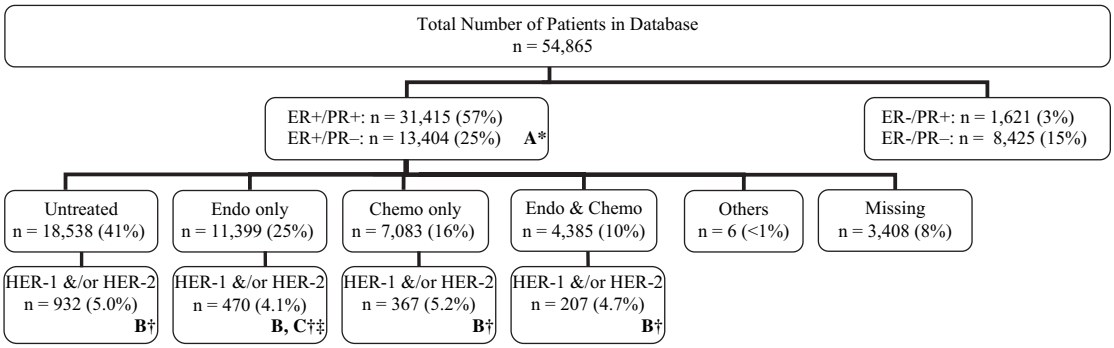


**Fig. 1.** Distribution of tumor types by ER, PR, HER-1, HER-2 status and treatment in the study population. ER = estrogen receptor; PR = progesterone receptor. *Used for Table 1. ER = estrogen receptor. PR = progesterone receptor. †Used for Tables 2 and 3. ‡Used for Table 4 and Figs. 2 and 3.

0%–<6%, intermediate 6%–10%, high >10%), HER-1 (negative <10, positive ≥10 fmol/mg), HER-2 (negative <1 U/μg, positive ≥1 U/μg), and ER and PR status ($ER^+/PR^+$ versus $ER^+/PR^-$). All analyses were performed using SAS 9.1 (SAS, Cary, NC). All statistical tests were two-sided, and comparisons made in which $P<.05$ were deemed statistically significant. Comparisons of the two growth factor receptors, HER-1 and HER-2, were performed in the overall population and by ER and PR subgroups ($ER^+/PR^+$ and $ER^+/PR^-$); therefore, $P$ values of .05 should be interpreted with caution. Median follow-up of all patients was 72 months (range = 0–120 months) at the time of last follow-up in 2002. Patients are no longer being actively followed up.

## Results

From a total of 54 865 patients with early breast cancer in the Baylor College of Medicine Breast Cancer databases, we identified 31 415 (57%) patients with $ER^+/PR^+$ tumors and 13 404 (25%) patients with $ER^+/PR^-$ tumors (Fig. 1). Forty-one percent (18 538) of the women with tumors of known ER and PR status did not receive systemic adjuvant therapy, and 11 399 (25%) received endocrine therapy as their only adjuvant treatment (Fig. 1), with 97% of the women who received endocrine therapy having received tamoxifen. The 11 399 patients who received endocrine therapy only are therefore referred to as the tamoxifen-treated group. Another 7083 (16%) of the patients received adjuvant chemotherapy only, 4385 (10%) received both endocrine therapy and chemotherapy, and for 3408 (8%) no information on adjuvant treatment was available. Tamoxifen therapy was used slightly more often in patients with $ER^+/PR^+$ tumors than in those with $ER^+/PR^-$ tumors (27% of $ER^+/PR^+$ patients versus 22% of $ER^+/PR^-$ patients) whereas chemotherapy was used more often in women with $ER^+/PR^-$ tumors (14% of $ER^+/PR^+$ patients versus 19% of $ER^+/PR^-$ patients).

### Clinical and Tumor Characteristics of $ER^+/PR^+$ and $ER^+/PR^-$ Tumors

The clinical and biologic tumor characteristics are summarized in Table 1. Overall, in women more than 50 years of age, $ER^+/PR^-$ tumors were found more frequently than $ER^+/PR^+$ tumors (82% versus 77% respectively; $P<.001$). However, $ER^+/PR^-$ tumors were larger (greater than 2 cm in diameter) than $ER^+/PR^+$ tumors (51% versus 45%, respectively; $P<.001$). In addition, 19% of patients whose tumors were $ER^+/PR^-$ had four or more axillary nodes involved with tumor compared with 16% of patients with $ER^+/PR^+$ tumors ($P<.001$).

### Biologic Characteristics of $ER^+/PR^+$ and $ER^+/PR^-$ Tumors

$ER^+/PR^-$ tumors displayed features of a more aggressive biologic phenotype than $ER^+/PR^+$ tumors (Table 1). For example, as has been previously shown in other studies *(10)*, the median level of ER in $ER^+/PR^-$ tumors was approximately half that in $ER^+/PR^+$ tumors (median = 47 fmol/mg, range = 3–2211, versus median = 103 fmol/mg, range = 3–3290, respectively; $P<.001$). Furthermore, $ER^+/PR^-$ tumors were more likely to be aneuploid than $ER^+/PR^+$ tumors (54% versus 48%, respectively; $P<.001$). In addition, $ER^+/PR^-$ tumors had higher proliferation rates than $ER^+/PR^+$ tumors, as evidenced by their higher S-phase fraction (33% for $ER^+/PR^-$ versus 19% for $ER^+/PR^+$; $P<.001$). Only 45% of $ER^+/PR^-$ tumors had low S-phase fractions, compared with 63% of the $ER^+/PR^+$ tumors.

**Table 1.** Clinical and biological characteristics in patients with $ER^+/PR^+$ and $ER^+/PR^-$ early breast cancer*

| Characteristic | $ER^+/PR^+$ | $ER^+/PR^-$ | $P$ |
|---|---|---|---|
| Age | | | |
| No. tested | 31 403 | 13 399 | |
| Median age (range) | 63 (22–104) | 64 (22–101) | |
| <50 years, % | 23 | 18 | <.001† |
| ≥50 years, % | 77 | 82 | |
| Tumor size | | | |
| No. tested | 30 258 | 12 850 | |
| ≤2 cm, % | 55 | 49 | <.001† |
| >2 cm, % | 45 | 51 | |
| Nodal status | | | |
| No. tested | 28 843 | 12 350 | |
| 0, % | 60 | 60 | |
| 1–3, % | 24 | 21 | <.001† |
| ≥4, % | 16 | 19 | |
| ER | | | |
| No. tested | 31 415 | 13 404 | |
| Median levels, fmol/mg (range) | 103 (3–3290) | 47 (3–2211) | <.001‡ |
| Tumor ploidy, % | | | |
| No. tested | 3813 | 1915 | |
| % Diploid | 52 | 46 | <.001† |
| % Aneuploid | 48 | 54 | |
| S phase§ | | | |
| No. tested | 3463 | 1719 | |
| Low, % | 63 | 45 | <.001† |
| Intermediate, % | 18 | 22 | |
| High, % | 19 | 33 | |

*ER = estrogen receptor; PR = progesterone receptor.

†$P$ values (two-sided) were calculated using the chi-square test.

‡$P$ value (two-sided) was calculated using Wilcoxon rank-sum test for ER level (fmol/mg).

§Cutpoint for S phase was low <6%, intermediate 6%–10%, high >10%.

**Table 2.** HER-1 and HER-2 status in patients with $ER^+/PR^+$ and $ER^+/PR^-$ early breast cancer*

| Characteristic | $ER^+/PR^+$ | $ER^+/PR^-$ | *P* |
|---|---|---|---|
| HER-1 status† | | | |
| No. tested | 1306 | 634 | |
| % Positive | 8 | 25 | <.001‡ |
| Median levels, fmol/mg | 24 (10–4071) | 40 (10–11 084) | .02§ |
| HER-2 status‖ | | | |
| No. tested | 1130 | 568 | |
| % Positive | 14 | 21 | <.001‡ |

*ER = estrogen receptor; PR = progesterone receptor.

†HER-1–positive tumors were defined as those with ≥10 fmol HER-1/mg of total protein.

‡*P* values (two-sided) were calculated using the chi-square test.

§*P* value (two-sided) was calculated using the Wilcoxon rank-sum test for HER-1 level.

‖HER-2–positive tumors were defined as ≥1 U HER-2/μg of total protein.

## HER-1 and HER-2 Status in $ER^+/PR^+$ and $ER^+/PR^-$ Tumors

HER-1 and HER-2 was performed on only a portion of patients because testing for these molecular markers was not the routine standard of care for all patients when this database was gathered. $ER^+/PR^-$ tumors were three times more likely than $ER^+/PR^+$ tumors to express HER-1 (25% versus 8%, respectively; *P*<.001), and the levels of HER-1 in $ER^+/PR^-$ tumors (median = 40 fmol/mg protein, range = 10–11 084) were nearly twice those in $ER^+/PR^+$ tumors (median = 24 fmol/mg of protein, range = 10–4071; *P* = .02) (Table 2). $ER^+/PR^-$ tumors were also statistically significantly more likely to overexpress HER-2 (21% for $ER^+/PR^-$ versus 14% for $ER^+/PR^+$; *P*<.001) (Table 2).

Both HER-1 and HER-2 are markers of tumor aggressiveness in breast and ovarian cancer cells *(36)*. Not surprisingly, therefore, tumors expressing either HER-2 or HER-1 were more likely to have a intermediate or high S-phase fractions than tumors negative for these two growth factors, regardless of PR status (Table 3). It is interesting, however, that the differential in S-phase fraction between $PR^+$ and $PR^-$ tumors was greater in tumors that expressed HER-1 than in those that did not (*P* = .01). A higher odds ratio for S-phase fraction was also observed for HER-2–positive tumors (for HER-2 positive, OR = 1.97, 95% CI = 1.2 to 3.3 versus OR = 1.67, 95% CI = 1.3 to 2.1 in HER-2–negative tumors), but the difference between these odds ratios was not statistically significant (*P* = .57).

Because HER-1 and HER-2 status was not available in all patients, we compared patients who had either HER-1 or HER-2, or both, performed versus those that had none performed (Supplementary Tables 1 and 2 available at http://jncicancerspectrum.oxfordjournals.org/jnci/content/vol97/issue17). There is no meaningful difference in age, tumor size, nodal status, ER level ploidy, or S phase between women in this group; thus, we could detect no bias using this comparison.

## Clinical Outcome of Tamoxifen-Treated Women With $ER^+/PR^+$ and $ER^+/PR^-$ Tumors According to HER-1 and HER-2 levels

In this database, 11 399 patients (8421 with $ER^+/PR^+$ breast cancer and 2978 with $ER^+/PR^-$ breast cancer) were treated with tamoxifen as the only systemic therapy (Supplementary Table 3 available at http://jncicancerspectrum.oxfordjournals.org/jnci/content/vol97/issue17). Among all 11 399 patients, information on HER-1 status was available for 465. Patients with HER-1–positive tumors had a higher likelihood of recurrence than patients with HER-1–negative tumors (HR = 1.9, 95% CI = 1.0 to 3.5; *P* = .05) (Fig. 2, A); no statistically significant difference was observed for OS (data not shown). Information on HER-2 status was available for a total of 392 of the 11 399 women (with $ER^+$ tumors treated with adjuvant tamoxifen). As with patients with HER-1–expressing tumors, patients with HER-2–overexpressing tumors had a higher recurrence risk than patients with HER-2–nonoverexpressing tumors (HR = 2.3, 95% CI = 1.24 to 4.3; *P* = .006) (Fig. 2, B). HER-2–positive patients also had a higher chance of death from any cause than HER-2–negative patients (HR = 1.6, 95% CI = 1.0, 2.75; *P* = .05).

**Table 3.** S-phase fraction in patients with $ER^+/PR^+$ versus $ER^+/PR^-$ early breast cancer according to HER-2 and HER-1 status*

| | S phase | | | | |
|---|---|---|---|---|---|
| | Low, n (%) | Intermediate/high, n (%) | OR† | 95% CI | *P*‡ |
| HER-1–positive tumors, n = 219 | | | | | |
| $ER^+/PR^-$ | 29 (23) | 98 (77) | 3.23 | (1.8 to 5.8) | <.001 |
| $ER^+/PR^+$ | 45 (49) | 47 (51) | | | |
| HER-1–negative tumors, n = 1494 | | | | | |
| $ER^+/PR^-$ | 226 (53) | 198 (47) | 1.46 | (1.2 to 1.8) | .001 |
| $ER^+/PR^+$ | 669 (63) | 401 (37) | | | |
| HER-2–positive tumors, n = 255 | | | | | |
| $ER^+/PR^-$ | 34 (31) | 76 (69) | 1.97 | (1.2 to 3.3) | .009 |
| $ER^+/PR^+$ | 68 (47) | 77 (53) | | | |
| HER-2–negative tumors, n = 1258 | | | | | |
| $ER^+/PR^-$ | 195 (51) | 188 (49) | 1.67 | (1.3 to 2.1) | <.001 |
| $ER^+/PR^+$ | 555 (63) | 320 (37) | | | |

*HER-1–positive tumors were defined as those with ≥10 fmol HER-1/mg of total protein; HER-2–positive tumors were defined as those with ≥1 U HER-2/μg of total protein; S phase: low <6%; intermediate/high ≥6%. ER = estrogen receptor; PR = progesterone receptor; CI = confidence intervals; OR = odds ratio.

†OR for high/intermediate S phase.

‡*P* values (two-sided) were calculated using the chi-square test.

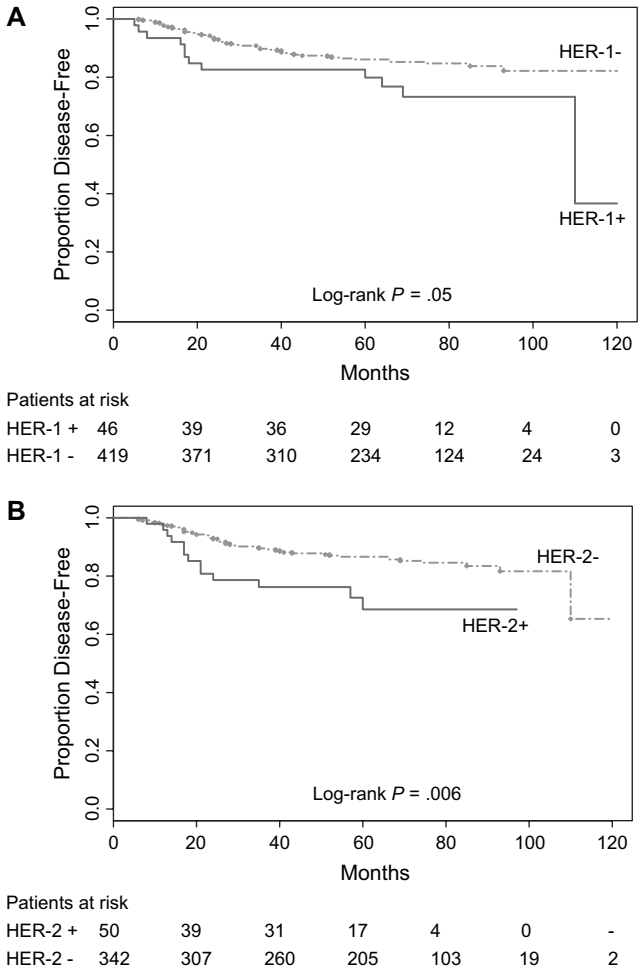


**Fig. 2.** Kaplan–Meier curves for disease free survival in tamoxifen-treated patients. **A**) Analysis by HER-1 in estrogen receptor-positive ($ER^+$) patients; proportion disease-free and 95% confidence interval at 20, 60, and 100 months, respectively: HER-1–negative = 0.95 (0.92 to 0.96), 0.86 (0.82 to 0.89), 0.82 (0.76 to 0.87), HER-1–positive = 0.85 (0.71 to 0.92), 0.80 (0.65 to 0.89), 0.73 (0.56 to 0.84) and (**B**) by HER-2 in $ER^+$ patients; proportion disease-free and 95% confidence interval at 20, 60, and 100 months, respectively: HER-2–negative = 0.94 (0.91 to 0.96), 0.87 (0.82 to 0.89), 0.82 (0.75 to 0.87), HER-2–positive = 0.85 (0.71 to 0.93), 0.68 (0.51 to 0.81), 0.68 (0.51 to 0.81). Patients with growth factor receptor–positive tumors (**solid line**) or growth factor receptor–negative tumors (**hatched line**) are shown. *P* values were two-sided.

It is important, however, that associations of recurrence and survival with HER status varied with the PR status of the tumors. In patients with $ER^+/PR^+$ tumors, neither the HER-1 expression nor the HER-2 overexpression was associated with DFS (Fig. 3, A and Fig. 3, C) or OS (data not shown). In contrast, among tamoxifen-treated patients with $ER^+/PR^-$ tumors, both HER-1 expression and HER-2 overexpression were associated with statistically significantly poorer DFS (for HER-1, HR of recurrence = 2.4, 95% CI = 1.0 to 5.4; $P = .036$; and for HER-2, HR = 2.6, 95% CI = 1.1 to 6.0; $P = .022$) (Fig. 3, B and Fig. 3, D). HER-2 overexpression was associated with a borderline statistically significantly worse OS in the $ER^+/PR^-$ group (HR = 2.2, 95% CI = 1.0 to 4.8; $P = .05$), but HER-1 expression showed no such association (HR = 1.7, 95% CI = 0.8 to 3.6; $P = .17$).

A multivariable Cox model that included all 11 399 patients treated with tamoxifen and included the following variables—tumor size, axillary nodes, age, ploidy, S-phase fraction, HER-1, HER-2, and ER and PR status ($ER^+/PR^+$ versus $ER^+/PR^-$)—was then used to determine their simultaneous association with DFS and OS. In this multivariable analysis, HER-2 but not HER-1 expression (HER-2 HR = 2.31, 95% CI = 1.18 to 4.51; $P = .014$) and nodal status (HR = 2.04, 95% CI = 1.48 to 2.82; $P<.001$) were statistically significantly associated with DFS, whereas only nodal status was associated with OS (HR = 1.91, 95% CI = 1.44 to 2.52; $P<.001$).

We next determined whether quantitative ER levels in the HER-1– or HER-2–positive groups alone were associated with outcome in tamoxifen-treated patients (Table 4). Overall, in both $ER^+/PR^+$ and in $ER^+/PR^-$ tumors, those with HER-1 expression had statistically significantly lower ER levels than those negative for HER-1. This difference was particularly evident in the $ER^+/PR^-$ cohort, with HER-1–negative tumors having an eightfold higher median ER level (median = 91 fmol, range = 3–1092) than HER-1–positive tumors (median = 11 fmol, range = 3–168; $P<.001$). However, further analysis of this association controlling for continuous levels of ER in a Cox model in the $ER^+/PR^-$ subgroup of patients still showed statistically significant worse DFS and OS in women with HER-1–positive tumors than in those with HER-1–negative tumors (data not shown), suggesting that the lower ER level in the HER-1–positive, $ER^+/PR^-$ tumors does not fully explain the worse outcome in these patients. In contrast, there was no statistically significant difference in the ER level among the HER-2–overexpressing versus HER-2–nonoverexpressing tumors in the $ER^+/PR^+$ patient subset. However, in patients with $ER^+/PR^-$ tumors, the median ER level was five times lower in the HER-2–overexpressing tumors (median = 18 fmol, range = 4–1092) than in the HER-2–nonoverexpressing tumors (median = 94 fmol, range = 3–777; $P = .004$). As with HER-1, after controlling for continuous levels of ER, HER-2 overexpression, compared with HER-2 nonoverexpression, remained statistically significantly associated with worse DFS ($P = .03$) and OS ($P = .04$) in $ER^+/PR^-$ tumors.

## Discussion

This study is, to our knowledge, the largest comprehensive evaluation of the biologic and clinical characteristics of invasive breast cancers that are $ER^+/PR^-$ compared with those that are $ER^+/PR^+$. The results, i.e., that $ER^+/PR^-$ tumors have worse clinical and biologic characteristics and that in women with PR-negative tumors, HER-1 expression and HER-2 overexpression are associated with worse outcome, suggest that $ER^+/PR^-$ tumors represent a distinct subset of breast cancer and that knowing the PR status of a breast cancer has important clinical relevance. The results presented here, derived from many patients with tumor receptor assays performed centrally by standardized techniques, provide clues to the origin of the distinct ER+PR phenotype. We find that ER+PR tumors have more aggressive features than $ER^+/PR^+$ tumors; they are larger, are more likely to be aneuploid, and proliferate more rapidly. Interestingly, $ER^+/PR^-$ tumors are also associated with a statistically significantly higher frequency of HER-2 overexpression and HER-1 expression than $ER^+/PR^+$ tumors. Finally, loss of PR in $ER^+$ tumors may be a surrogate marker of aberrant growth factor signaling that could contribute to the tamoxifen resistance found in the tumors in this study, i.e., poorer survival in tamoxifen-treated women.

Several recent clinical reports also indicate that high growth factor receptor activity may be associated with reduced PR levels in breast cancer *(6,16–18,37,38)*. Recent laboratory studies suggest a molecular basis for this observation. Growth factors in

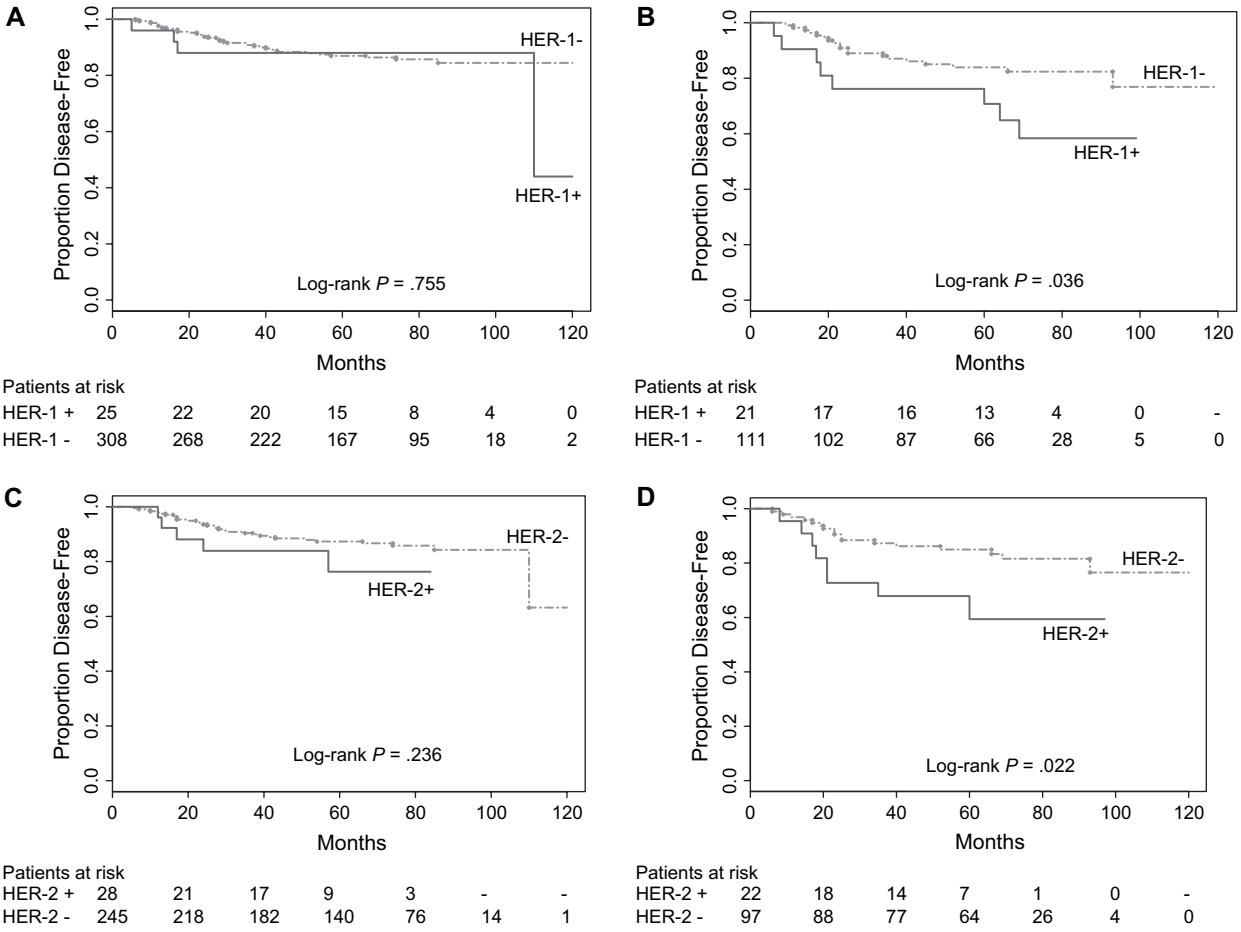


**Fig. 3.** Kaplan–Meier curves for disease-free survival in tamoxifen-treated patients. **A**) Analysis by HER-1 in $ER^+/PR^+$ patients; proportion disease-free and 95% confidence interval at 20, 60, and 110 months, respectively: HER-1–negative = 0.95 (0.92 to 0.97), 0.87 (0.82 to 0.90), 0.84 (0.79 to 0.89), HER-1–positive = 0.88 (0.67 to 0.96), 0.88 (0.67 to 0.96), 0.44 (0.01 to 0.86); (**B**) by HER-1 in $ER^+/PR^-$ patients; proportion disease-free and 95% confidence interval at 20, 60, and 100 months, respectively: HER-1–negative = 0.94 (0.87 to 0.97), 0.84 (0.75 to 0.90), 0.77 (0.61 to 0.87), HER-1–positive = 0.81 (0.57 to 0.92), 0.71 (0.46 to 0.86), 0.58 (0.33 to 0.77); (**C**) by HER-2 in $ER^+/PR^+$ patients; proportion disease-free and 95% confidence interval at 20, 60, and 100 months, respectively: HER-2–negative = 0.95 (0.91 to 0.97), 0.87 (0.82 to 0.91), 0.84 (0.78 to 0.89), HER-2–positive = 0.88 (0.67 to 0.96), 0.76 (0.50 to 0.90), 0.76 (0.50 to 0.90); and (**D**) by HER-2 in $ER^+/PR^-$ patients; proportion disease-free and 95% confidence interval at 20, 60, and 100 months, respectively: HER-2–negative = 0.93 (0.85 to 0.96), 0.85 (0.76 to 0.91), 0.76 (0.61 to 0.86), HER-2–positive = 0.82 (0.58 to 0.93), 0.59 (0.33 to 0.78), 0.59 (0.33 to 0.78). Patients with growth factor receptor–positive tumors (**solid line**) or growth factor receptor–negative tumors (**hatched line**) are shown. *P* values were two-sided.

the insulin-like growth factor (IGF) and epidermal growth factor (EGF) families that activate the P13K–Akt–mTor pathway can cause reduced expression of PR at the transcriptional level. This transcriptional suppression may be mediated by an AP-1 site in the PR gene promoter that blocks transcription *(22,39)*. Transcription factors in the AP-1 family are activated by several external cellular stimuli including stress, cytokines, and growth factor receptor signaling. These molecular data, in concert with our clinical findings, raise the possibility that loss of PR in some tumors is due to increased growth factor receptor activity rather than to a nonfunctional ER signaling pathway.

Although our data need to be confirmed by other studies, the results suggest the hypothesis that PR levels are a surrogate for the level of activity in the signaling cascade generated by HER-1 and/or HER-2 activation. First, $ER^+/PR^-$ tumors that were also positive for HER-1 or that overexpressed HER-2 had higher

**Table 4.** Median ER and PR levels according ER, PR, HER-1, and HER-2 status in tamoxifen-treated patients*

| Characteristic | HER-1 + | HER-1 − | *P*† | HER-2 + | HER-2 − | *P*† |
|---|---|---|---|---|---|---|
| $ER^+/PR^+$ | n = 25 | n = 308 | | n = 28 | n = 245 | |
| Median ER level, fmol/mg protein (range) | 64 (4–814) | 148 (4–890) | <.001 | 152 (12–747) | 127 (4–890) | .73 |
| Median PR level, fmol/mg protein (range) | 77 (11–1859) | 223 (10–2069) | <.001 | 184 (28–1104) | 213 (11–2069) | .68 |
| $ER^+/PR^-$ | n = 21 | n = 111 | | n = 22 | n = 97 | |
| Median ER level, fmol/mg protein (range) | 11 (3–168) | 91 (3–1092) | <.001 | 18 (4–1092) | 94 (3–777) | .004 |

*ER = estrogen receptor; PR = progesterone receptor. ER-positive ≥3 fmol/mg; PR-positive ≥5 fmol/mg.

†*P* values (two-sided) were calculated using the Wilcoxon rank-sum test.

proliferation rates (S-phase fraction). Second, tamoxifen-treated women whose tumors were $ER^+/PR^-$, and either HER-1 positive or overexpressed HER-2, had statistically significantly worse outcome than patients in the same subgroup whose tumors had no HER-1 or low HER-2. In contrast, the effect of HER-1 expression and HER-2 overexpression was not statistically significant in the subset of patients with $ER^+/PR^+$ tumors. One possible explanation for the difference between $ER^+/PR^+$ and $ER^+/PR^-$ tumors in terms of associations of outcome with HER-1 and HER-2 is that HER-1 and HER-2 levels were statistically significantly higher in the $ER^+/PR^-$ tumors than in the $ER^+/PR^+$ tumors (Table 2), and several clinical studies suggest that such high levels of expression of HER-1 and HER-2 are associated with tamoxifen resistance *(19,40–43)*. Consequently, HER-1/HER-2 signaling may be more active in $ER^+PR^-$ tumors, resulting in a smaller impact in response to tamoxifen.

Another possibility that would explain the tamoxifen resistance is that ER levels are considerably lower in $ER^+$ tumors that lack PR and also have abundant HER-1 or HER-2. Lower levels of ER are associated with less benefit from tamoxifen *(44)*. In this study, ER levels were approximately 60%–80% lower in HER-1–positive and HER-2–overexpressing tumors than in HER-1–negative and HER-2–nonoverexpressing tumors. However, multivariable analysis in this and other studies *(19,42)* that consider ER level as a continuous rather than dichotomous variable suggests that a low ER level is not the only factor explaining tamoxifen resistance in HER-1–expressing and HER-2–overexpressing tumors.

Laboratory studies provide a potential mechanism for the lower ER levels in HER-1–positive and HER-2–overexpressing tumors and suggest that cross-talk between ER and growth factor receptor signaling pathways in response to estrogen or tamoxifen can in part account for tamoxifen resistance. Tamoxifen, like estrogen, can directly activate the HER-1 and HER-2 tyrosine kinases and induce tumor growth when these membrane receptors are abundant *(45)*. Estrogen deprivation therapy, in contrast, remains highly effective in such tumors, because, unlike tamoxifen, estrogen deprivation therapy does not result in activation of the growth factor receptor pathways.

This study has several potential limitations. First, it is a retrospective study, and therapy or lack of therapy was not determined on a randomized basis. Second, there were few patients in the tamoxifen-treated subgroups whose HER-1 and HER-2 status was known; however, it remains the largest study to our knowledge to address the issue of HER-1 and HER-2 expression in hormone receptor phenotypes and its potential relationship to tamoxifen efficacy. Third, although the study focused primarily on HER-1 and HER-2 receptors as possible contributors to tamoxifen resistance, additional downstream factors could also contribute, and such factors were not analyzed. Fourth, HER-2 overexpression was analyzed by western blotting, which is not currently a standard method for assessing the status of this molecule in clinical practice. It is however, a standardized, reproducible methodology. Fifth, for a substantial portion of the patients reported in this database, follow-up was not obtained from primary tumor registries. Recurrences are probably somewhat underreported because tumor registries do not always capture this information; however, underreporting of recurrences would have varied by hospital and not by PR or HER-1 or HER-2 status. Primarily because of the retrospective nature of this study and the other issues raised above, these findings are for hypothesis generation only and need to be confirmed in prospective, large, randomized trials or retrospective analysis of a prospective study.

In summary, our findings support the hypothesis that loss of PR in $ER^+$ breast cancer is a surrogate marker for increased growth factor receptor tyrosine kinase activity that causes lower PR expression and tamoxifen resistance in some patients. The results raise the possibility that overexpression of only HER-1 and/or HER-2 affects tamoxifen response substantially only when PR is negative. If PR expression is maintained, perhaps signaling through the HER family pathways is low despite overexpression of the HER receptors themselves. Although response to trastuzumab has not been shown to vary by ER status, if the hypothesis that lack of PR expression is a reflection of active signaling in the HER family is correct, then the response to trastuzumab or other small-molecule HER-2 inhibitors might be different in the $PR^+$ and $PR^-$ subsets, an idea that could be explored in ongoing adjuvant clinical trials. Finally, if the link between PR negativity and high growth factor receptor signaling can be confirmed as a cause of tamoxifen resistance, then therapies targeting the growth factor pathways in combination with tamoxifen should be investigated in patients with $ER^+/PR^-$ tumors in future clinical trials.

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## Notes

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