Can Molecular Markers Predict When to Implement Treatment with Aromatase Inhibitors in Invasive Breast Cancer?

Sian Tovey,1 Barbara Dunne,2 Caroline J. Witton,1 Amanda Forsyth,1 Timothy G. Cooke,1 and John M. S. Bartlett1

Abstract

Purpose: Resistance to tamoxifen is linked to overexpression of HER2, and aromatase inhibitors show particular benefit in progesterone receptor (PR)–negative patients. We previously reported reduced survival in patients overexpressing HER1, HER2, and HER3. We now show that both HER1-3 and PR status predicts for early relapse in estrogen receptor (ER)–positive tamoxifen-treated breast cancer patients.

Experimental Design: Tissue microarray technology was used to analyze 402 ER-positive tamoxifen-treated patients. Immunohistochemistry using epidermal growth factor receptor, HER2, HER3, HER4, and PR antibodies was done. Kaplan-Meier life table and Cox Regression analysis (log-rank testing of differences in breast cancer–related relapse on tamoxifen) was done.

Results: HER1-3 (but not HER4) overexpression predicted for early relapse on tamoxifen ($P = 0.0060$). PR-negative cases were also significantly more likely to relapse while on tamoxifen ($P = 0.017$). HER1-3-positive and/or PR-negative patients combined as a “high-risk” group were significantly more likely to relapse on tamoxifen in univariate ($P < 0.0001$) and Cox’s multivariate analysis ($P = 0.0069$). However, this applied to early relapse on tamoxifen only, as any disease relapse after 3 years of tamoxifen was unrelated to PR/HER status.

Conclusions: We show that HER1-3 and PR status can identify time-dependent de novo tamoxifen resistance with risk declining markedly after 3 years of tamoxifen treatment. These results parallel data from the ATAC and Intergroup Exemastane Study trials which suggest that whereas PR-negative patients derive greater benefit from initial aromatase inhibitor treatment, PR status has no effect on response when given as delayed treatment to those disease free on tamoxifen after 3 years.

Tamoxifen is a potent anticancer agent and remains the standard for adjuvant endocrine therapy in breast cancer in the United Kingdom (SIGN guidelines 1998). Its chief mechanism of action is by a competitive inhibition of estrogen binding to estrogen receptors (ER). The subsequent reduction in expression of estrogen-related genes such as growth factors and angiogenic factors results in reduced cell proliferation, enhanced apoptosis, and reduced cell growth (1). Analysis of a large number of randomized trials (2) shows a 50% proportional reduction in the recurrence rate and a 28% proportional mortality reduction for ER-positive patients taking adjuvant tamoxifen for 5 years. Despite this success, resistance to tamoxifen is a significant clinical problem; almost all patients with metastatic disease experience progression and up to 20% of early breast cancer patients relapse while on adjuvant treatment (2). Tamoxifen resistance may be either de novo (present before tamoxifen treatment) or “acquired” during the course of treatment.

Identifying biological mechanisms behind tamoxifen resistance is important particularly as increasing clinical trials evidence implies superiority of aromatase inhibitors over tamoxifen (3). Whereas emerging data on the efficacy of novel aromatase inhibitors in the adjuvant setting does not yet identify a group of patients for whom additional benefit is derived in terms of overall survival (4, 5), both the ATAC and Intergroup Exemestane Study (IES) studies show a clear benefit in terms of disease-free survival. It is not currently clear whether this superiority is the case for all ER-positive patients, or whether, as is widely predicted, there is a particular group of patients resistant to tamoxifen whose tumors may be sensitive to aromatase inhibitors. There is however growing evidence that specific tumor markers may be used to identify tumors that exhibit resistance to tamoxifen as well as being linked to enhanced responsiveness to aromatase inhibitors (3, 6, 7). These markers include both conventional markers of endocrine responsiveness (ER and progesterone receptor, PR) and receptor tyrosine kinases such as the HER family of receptors.

The HER family of tyrosine kinase receptors HER1 (ErbB1/epidermal growth factor receptor), HER2 (ErbB2/neu), HER3

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Received 1/27/05; revised 3/23/05; accepted 4/19/05.

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(ErB3), and HER4 (ErB4) initiate a complex signal transduction cascade modulating cell proliferation, survival, adhesion, migration, and differentiation. Growth factor–induced HER signaling is essential for normal cellular processes and plays a key role in the aberrant development and growth of tumor cells (8). Each of the HER ligands promotes different homodimerization and hetrodimerization patterns within the HER family. HER2 enhances and stabilizes dimerization but has no ligand (9), whereas HER3 has no inherent kinase activity and requires dimerization with another HER family member to promote downstream signaling (10).

The role of the HER family in tamoxifen resistance was initially shown by in vitro studies, where ER-positive, hormonally sensitive MCF-7 cells transfected with very high levels of HER2 become resistant to tamoxifen (11). Tamoxifen-resistant MCF-7 cells, derived in vitro, have increased levels of both HER1 and HER2 (12, 13). Restoration of tamoxifen sensitivity can be achieved using HER1 inhibitors (Iressa) and HER2 blockade (Herceptin; refs. 14, 15). There have been many clinical studies reporting on the proposed influence of HER2 expression on hormonal resistance, but results are varied and confusing. Some studies have shown a clear link with resistance (16–20), whereas others have not shown any association (21–24). The picture for HER1 is equally unclear (24–26). There is little in vivo or in vitro evidence currently to link HER3 and HER4 to tamoxifen resistance (27, 28), although similar signaling pathways are activated by HER1-4. The most convincing clinical evidence for the role of HER1 and HER2 in tamoxifen resistance has come from neoadjuvant trials where HER1/2-positive patients have significantly greater response to aromatase inhibitors than to tamoxifen (29). Further evidence on the relative importance of other growth factor receptors, including HER1, HER3, and HER4, are required before these findings alter clinical practice particularly in the adjuvant setting.

We have previously reported the adverse association HER1-3 overexpression has on survival as well as evidence that HER4 is linked with a good prognostic outlook (30). In addition, data from the ATAC (3) and IES trials (31) suggest that differences exist in the molecular profile of patients who respond to early or to delayed treatment with aromatase inhibitors. In the ATAC trial, PR-negative patients derived greater benefit from initial aromatase inhibitor treatment compared with tamoxifen. In the ATAC from the ATAC (3) and IES trials (31) suggest that differences linked with a good prognostic outlook (30). In addition, data clinical practice particularly in the adjuvant setting.

Materials and Methods

Patients. The local ethics committee granted ethical approval for the study. Six hundred and eighty-six ER-positive or ER unknown patients, treated with adjuvant tamoxifen, were selected from a database of sequentially diagnosed patients presenting with operable breast cancer between 1980 and 1999. These patients had standard adjuvant treatment according to protocols at the time of diagnosis. Patients were subsequently excluded if lost to follow-up was not complete (n = 52, 8%), or if tissue blocks were not available or had insufficient tissue as judged by the pathologist (B.D.; n = 177, 26%). There was no significant difference between the final data set of 456 (66%) and the original group in terms of size, nodal status, grade, or age of patient (Pearson χ2).

Tissue microarray construction. Three 0.6-mm² cores of breast cancer tissue were removed from representative tumor areas on each block identified by a pathologist (B.D.). These cores were used to construct recipient array blocks in triplicate (80-120 cores per block). Cores of normal skin, smooth muscle, testes, lymph node, placenta, and tonsil were also included in the tissue microarray as controls. If one or more core was uninformative because of loss or lack of tumor in core then the scoring results were taken from the remaining core(s).

Immunohistochemistry. Herceptest and PR staining was done as part of diagnostic runs. Immunohistochemical staining for ER, HER1, HER3, and HER4 was done as described below. Sections were dewaxed and rehydrated then endogenous peroxidase was blocked with hydrogen peroxide. Antigen retrieval for HER1 was done by incubating the slides in 0.1% trypsin (Sigma, Poole, Dorset, United Kingdom; dissolved in 0.1% calcium chloride (Sigma) solution preheated in a 37°C water bath) for 10 minutes. Antigen retrieval for ER was done using pressure microwave treatment in Tris-EDTA buffer (0.07 g sodium EDTA (BDH Laboratory Supplies, Poole, Dorset, United Kingdom) and 0.55 g Tris base (Sigma) made up to 1 liter with distilled water). There was no antigen retrieval required for HER3 or HER4. Sections were incubated in 15 µl/mL horse serum (HER1 and ER protocols) or in serum-free block (DAKO). Ely, Cambridgeshire, United Kingdom; HER3 and HER4) to block nonspecific background staining. For HER3 and HER4 protocols, endogenous biotin was blocked with an Avidin/Biotin blocking agent (Vector Laboratories, Peterborough, Cambridgeshire, United Kingdom). Incubation with the primary antibodies diluted in Tris-HCl buffer (DAKO) was done at room temperature. The HER1 antibody (clone 31G7, Zymed, Invitrogen Paisley, Strathclyde, United Kingdom) was used at 1:50 dilution for 1 hour. The ER antibody (DAKO, clone 1D5) was used at 1:50 dilution for 30 minutes. Both the HER4 (H4.77.16, Neomarkers, Lab Vision, Newmarket, Suffolk, United Kingdom) and the HER3 antibodies (H3.105.5, Neomarkers) were used at a concentration of 50 µg/mL for 2 hours. The DAKO LSAB+ kit was used for signal amplification. Washes, between all steps, were done with TBS solution (Tris saline buffer, pH 7.6). Detection was then completed with incubation with a 3,3-diaminobenzidine solution (Vector Laboratories) diluted in distilled water for 10 minutes. Finally, the sections were counterstained, dehydrated, and mounted. A control slide was incubated in each run with an isotype-matched control antibody to insure no false-positive staining.

Scoring. One scorer scored all cases having previously double scored a series of tissue microarray slides (including the Herceptest and ER slides) with an experienced diagnostic scorer achieving an intraclass correlation coefficient of 0.94 (n = 890) for membrane staining and 0.84 (n = 827) for nuclear staining. Membrane staining for HER1-4 was done using the Herceptest scoring method. Cores with over 10% of strong membrane staining were assigned 3+. Cores with over 10% moderate staining were assigned 2+. Cores with over 10% weak staining were assigned 1+. When there was any discrepancy between cores, then the mean percentages stained at each intensity level were calculated. Thus, an average at least 10% of cells with strong membranous staining over analyzed cores would be required for the combined score to reach 3+. Nuclear scoring for PR and ER was done using the Histoscore method. This involves giving a weighted score for percentages of staining seen where the percentage of cells stained (0-100%) is multiplied by the staining intensity (1, 2, or 3) to give a maximum histoscore of 300. The histoscores for each core were then averaged. Patients with an average of
between HER3 and HER4 ($P = 0.2159$).

No significant difference in survival on tamoxifen (Fig. 1D; $P = 0.0278$, respectively). However, HER4-positive patients showed more likely to relapse on tamoxifen (Fig. 1B-C; $P = 0.4739$). HER2- and HER3-positive patients were significantly different ($P = 0.001$, Fishers exact test), between HER2 and HER3 ($P = 0.030$, $\chi^2$) and between HER3 and HER4 ($P < 0.001$, $\chi^2$).

### Results

**Clinical and pathologic characteristics.** ER expression was confirmed in 422 cases (92.5%) with 20 (4.4%) of these cases shown as ER negative. For 34 cases, there was insufficient material for ER analysis, either due to core loss or insufficient tumor material in cores (7.5%). These patients and the ER-negative cases were excluded from further analysis. For the remaining 402 patients, core availability for each antibody ranged from 84.8% to 98.9%.

Clinical and pathologic characteristics, for the ER-positive patients, including grade, nodal status, histology, size, Nottingham Prognostic Index, and age are shown in Table 1. In addition to tamoxifen, 99 of 399 (24.8%) patients had chemotherapy (three unknown) and 110 of 399 (27.57%) had radiotherapy (three unknown). The median duration of tamoxifen therapy was 5 years. The mean follow-up duration is 6.91 years (SD, 3.34 years) and median 6.45 years (range, 0.64-18.42 years). There were 74 breast cancer–specific deaths. There were 100 breast cancer relapses, 78 of which were while on tamoxifen.

**Immunohistochemistry: HER1-4 expression.** Cases with any membranous HER1 staining were classed as being positive for HER1 overexpression (6 of 393, 1.5%). Patients were considered positive for HER2 if they had at least 2+ staining intensity (i.e., at least 10% tumor cells were scored as being moderately positive); 51 of 397 (12.8%) cases were positive for HER2. The same 2+ cutoff was also used for HER3 and HER4 resulting in 56 of 353 (15.9%) cases considered positive for HER3 and 46 of 341 (13.5%) for HER4. There were significant correlations between expression of epidermal growth factor receptor and HER4 ($P = 0.019$, Fishers exact test), between HER2 and HER3 ($P = 0.030$, $\chi^2$) and between HER3 and HER4 ($P < 0.001$, $\chi^2$).

**Progesterone receptor expression.** There were 241 of 388 (62.1%) PR-positive patients and 147 of 388 (37.9%) PR-negative cases. There was no significant correlation between PR and ER Histoscore values ($P = 0.309$, Kendall's tau-b).

**Disease-free survival on tamoxifen: HER1-4.** Despite a marked separation of the relapse-free survival curves for HER1-positive patients relapsing on tamoxifen, statistical significance in this small group was not reached (Fig. 1A; $P = 0.4739$). HER2- and HER3-positive patients were significantly more likely to relapse on tamoxifen (Fig. 1B-C; $P = 0.0280$, 0.0278, respectively). However, HER4-positive patients showed no significant difference in survival on tamoxifen (Fig. 1D; $P = 0.2159$).

### Table 1. Patient clinical and pathologic variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number/total</th>
<th>Valid %</th>
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<tbody>
<tr>
<td>Grade</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>99/391</td>
<td>25.32</td>
</tr>
<tr>
<td>2</td>
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<tr>
<td>0</td>
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<tr>
<td>Lobular</td>
<td>45/397</td>
<td>11.3</td>
</tr>
<tr>
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<td>7.6</td>
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</tr>
<tr>
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</tr>
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</tr>
<tr>
<td>T2 (20-50)</td>
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<td>53.68</td>
</tr>
<tr>
<td>T3 (&gt;50)</td>
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</tr>
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<td>31.98</td>
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<tr>
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<tr>
<td>&lt;50</td>
<td>73/402</td>
<td>36.5</td>
</tr>
<tr>
<td>&gt;50</td>
<td>328/402</td>
<td>63.5</td>
</tr>
</tbody>
</table>

**Note:** Grade = Bloom Richardson grade. Nodal status = number of positive nodes. Pathologic type: ductal, invasive ductal carcinoma; lobular, invasive lobular carcinoma; other includes mucinous, mucoid, etc. Abbreviation: NPI, Nottingham Prognostic Index (grade + nodal status + 0.02 x size in mm).

Ninety-eight of 350 patients were positive for either one of HER1, HER2, or HER3 (HER1-3), and HER1-3 positivity predicted for early relapse on tamoxifen ($P = 0.0060$; Fig 2A). Interestingly, the trend for relapse on tamoxifen seems to increase if patients are positive for more than one HER family member ($P = 0.0093$; Fig 2B).

**Progesterone receptor: relationship with relapse on tamoxifen and correlation with HER1-3 status.** Patients negative for PR were significantly more likely to relapse while on tamoxifen (Fig. 3A; $P = 0.0017$). Table 2 shows the relationship between PR and each HER1-3 member. HER3 shows a significant inverse relationship with PR ($P = 0.001$, $\chi^2$). There is no significant relationship between PR and either HER1 or HER2, although when the group is combined as HER1-3 the significant inverse relationship persists ($P = 0.001$, $\chi^2$).

Figure 3B shows the significantly increased rate of relapse on tamoxifen ($P < 0.0001$) when HER1-3-positive and/or PR-negative patients are combined as a poor prognostic group. This “high risk” HER1-3-positive/PR-negative group also predicted...
for increased relapse in Cox's multivariate analysis ($P = 0.0069$) when analyzed alongside known prognostic variables such as size, grade, and nodal status.

Analysis of molecular markers as time-dependent variables. Kaplan-Meier survival analysis was subsequently done following exclusion of those patients who relapsed during the first 3 years of treatment, with the aim of focusing on those patients who had later relapse. Using this method of analysis the PR-negative/HER1-3-positive group no longer showed any significant difference in recurrence rates on tamoxifen ($P = 0.0858$).

Table 3 shows the number (and percentages) of recurrences on tamoxifen in years 1 to 3 compared with years 4 to 5 in the high/low-risk groups as identified by the relevant molecular markers. (Note that only patients remaining disease free at 3 years are included in the year 4-5 group). It also shows the hazard ratios (95% confidence interval) from Cox's multiple regression with the relevant factors analyzed alongside size, nodal status, and grade. The $P$s from this analysis show a significant difference between recurrences in the high-risk versus low-risk groups when analyzed for the years 1 to 3, whereas PR and HER1-4 status has no significant effect on recurrences in years 4 to 5.

Fig. 2. A, Kaplan-Meier survival curves showing cumulative disease-free survival differences between patients positive or negative for HER1-3 (positive for either one of HER1, HER2, or HER3). B, Kaplan-Meier survival curves showing cumulative disease-free survival differences between patients positive for more than one HER1-3 family member, patients positive for only one type of HER receptor, and patients negative for all HER family members. Cutoffs for positivity for variables are defined in the text. $P$s represent log-rank testing of the difference in cumulative disease-free survival.
Discussion

Data presented here shows the significant role played by the type I receptor tyrosine kinases HER1, HER2, and HER3 in promoting tamoxifen resistance in hormone-responsive breast cancers and underlines the different role of HER4 in this context. Furthermore, this data shows that PR-negative tumors have reduced responsiveness to tamoxifen, which is only weakly related to expression of HER1-3. Therefore, a phenotype of either HER1-3 positivity or PR negativity in ER-positive breast cancers seems linked to tamoxifen resistance, as defined by elevated risk of disease relapse despite endocrine therapy. Strikingly, however, it seems that this elevated risk applies in the first 3 years of tamoxifen therapy only. Most interestingly, this data seems to explain recently reported discrepancies relating to PR status and aromatase inhibitors in clinical trials (3, 31).

In the current study, the expression of the type I receptor tyrosine kinases, HER1-4, was analyzed in a large retrospective group of tamoxifen-treated, ER-positive cases. Patients whose tumors were positive for HER1-3 were at significantly greater risk of relapse while on adjuvant tamoxifen, supporting a role for the HER1-3 receptors in tamoxifen resistance. Conversely, we show no relationship between expression of HER4 and early relapse on tamoxifen. Previously, we have shown that expression of HER1-3 is linked to high proliferation indices in breast cancer, whereas HER4 is associated with a low proliferation index (34). Furthermore, preliminary data suggested that patients with ER-positive disease were at greater risk of early relapse due to HER1-3 expression than ER-negative cases (30).

Several mechanisms have been proposed by which the type I receptor tyrosine kinases may modify response to estrogens and tamoxifen. There is a considerable body of biological evidence suggesting that crosstalk between the HER family signaling pathways and the ER at several levels may be responsible. Signaling pathways activated by the type I receptor tyrosine kinases activate the ER by phosphorylation at sites including those on the AF1 region of the ER. The serine phosphorylation site S118 on the AF1 region of ER is a target for phosphorylation by mitogen-activated protein kinase (35). Mitogen-activated protein kinase is a component of the Ras/Raf/mitogen-activated protein kinases pathway and overexpression or activation of this pathway has been associated with a poor clinical response to tamoxifen therapy (36) and to tamoxifen resistance in vitro (11, 14). In addition, the phosphatidylinositol-3 kinase/Akt pathway has been shown to mediate activation of the ER at Ser 167 (also in the AF1 region; ref. 37) resulting in reduced sensitivity to tamoxifen in vitro. The consequence of phosphorylation of the ER in breast cancers may be to promote the known receptor agonistic activity of tamoxifen and to enhance transcription of genes involved in proliferation and genes which block apoptosis leading to tumor growth.

Table 2. Relationship between PR and HER status

<table>
<thead>
<tr>
<th></th>
<th>HER1</th>
<th>HER2</th>
<th>HER3</th>
<th>HER1-3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>PR</td>
<td>141</td>
<td>2</td>
<td>120</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>236</td>
<td>4</td>
<td>213</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>377</td>
<td>6</td>
<td>333</td>
<td>50</td>
</tr>
</tbody>
</table>

NOTE: Patients positive for HER1, HER2, HER3, or HER1-3 combined (cases positive for one or more of HER1 HER2 or HER3) with respect to PR status. Cutoffs for positivity are defined in the text. HER3 shows a significant inverse relationship with PR \( (P = 0.001, \chi^2) \). There is no significant relationship between PR and either HER1 \( (P = 0.600, \text{Fishers}) \) or HER2 \( (P = 0.277, \chi^2) \), although when the group is combined as HER1-3, the significant inverse relationship persists \( (P = 0.001, \chi^2) \).
Crosstalk between the HER family and ER may also occur by disturbing the balance between ER and its coactivators and corepressors. One particular coactivator AIB1, which may be phosphorylated by growth receptor pathways (38), has been linked to poor prognosis in tamoxifen treated ER-positive patients (39). More recently, it has also been shown that membrane-associated ER may directly signal via activation/cross talk with the membrane growth factor HER1 (40). This concept provides a mechanism for a positive feedback loop whereby subsequent activation of downstream growth factor pathway members such as mitogen-activated protein kinase is active through nongenomic mechanisms either in the cytoplasm or at the membrane where it may interact with the membrane growth factor HER1 (40). This concept provides a mechanism for a possible mechanism by which heterodimers may produce a more marked effect than homodimers. The lack of any association we have shown for the HER4 isoforms, and potentially ER transcrip-
tion pathway. It is possible that the ER in these PR-negative patients is not functioning as a nuclear receptor but instead is active through nongenomic mechanisms either in the cytoplasm or at the membrane where it may interact with the HER family or other active signal transduction pathways. However, evidence relating to the expression of different PR isoforms, and potentially ERβ, as predictors of tamoxifen response may also be of importance (46–48). Further work is required to investigate these potential explanations. However, our data suggests that in both HER1-3-positive and PR-negative breast cancers there is evidence of resistance to tamoxifen (this group represents almost 50% of all ER-positive cancers in this study). In these patients, based on published evidence from the ATAC trial and from Ellis’ group (29), we hypothesize enhanced response rates could be obtained by use of aromatase inhibitors in place of tamoxifen at an early stage. Conversely, we hypothesize, other patients, particularly those with HER1-3-negative and PR-negative tumors, may continue to derive significant benefit from tamoxifen without the need to switch to aromatase inhibitors. However, this conclusion would seem

Data presented here also confirm that ER-positive/PR-negative tumors are significantly less likely to respond to tamoxifen treatment than ER-positive/PR-positive tumors (31, 44–46). This is of particular current interest because early results from the ATAC suggest that PR-negative tumors derive increased clinical benefit from early treatment with aromatase inhibitors (3).

The inverse relationship shown here between PR and HER 1-3 expression might support supposition that PR negativity may be acting as a surrogate marker for HER1-3 overexpression in the ATAC trial. However, closer examination of the evidence presented here confirms that ER-positive/PR-negative tumors and HER1-3-positive tumors represent distinct patient subgroups with relatively little overlap. This suggests that the association between HER1-3 expression and loss of PR expression, whereas real, is weak and that alternative, as yet unexplained, mechanisms must underpin tamoxifen resistance in PR-negative tumors. PR expression has long been thought to reflect a functional, ER transcription pathway. It is possible that the ER in these PR-negative patients is not functioning as a nuclear receptor but instead is active through nongenomic mechanisms either in the cytoplasm or at the membrane where it may interact with the HER family or other active signal transduction pathways. However, evidence relating to the expression of different PR isoforms, and potentially ERβ, as predictors of tamoxifen response may also be of importance (46–48). Further work is required to investigate these potential explanations. However, our data suggests that in both HER1-3-positive and PR-negative breast cancers there is evidence of resistance to tamoxifen (this group represents almost 50% of all ER-positive cancers in this study). In these patients, based on published evidence from the ATAC trial and from Ellis’ group (29), we hypothesize enhanced response rates could be obtained by use of aromatase inhibitors in place of tamoxifen at an early stage. Conversely, we hypothesize, other patients, particularly those with HER1-3-negative and PR-negative tumors, may continue to derive significant benefit from tamoxifen without the need to switch to aromatase inhibitors. However, this conclusion would seem

### Table 3. Time-dependent analysis of variables

<table>
<thead>
<tr>
<th>Years</th>
<th>HER1-3 Positive (%)</th>
<th>HER1-3 Negative (%)</th>
<th>PR Positive (%)</th>
<th>PR Negative (%)</th>
<th>HER1-3-positive/PR negative</th>
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</thead>
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<tr>
<td>1-3</td>
<td>21/98 (21.4)</td>
<td>25/251 (10)</td>
<td>2.18 (1.13-4.21)</td>
<td>0.0189</td>
<td>39/197 (19.8)</td>
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<tr>
<td></td>
<td>0.0254</td>
<td></td>
<td>0.48 (0.26-0.92)</td>
<td>0.0052</td>
<td>11/173 (6.4)</td>
</tr>
<tr>
<td>P</td>
<td>0.0189</td>
<td></td>
<td>0.0254</td>
<td>0.0052</td>
<td>3.08 (1.40-6.8)</td>
</tr>
<tr>
<td>4-5</td>
<td>3/70 (4.3)</td>
<td>7/204 (3.4)</td>
<td>0.96 (0.18-5.05)</td>
<td>0.5016</td>
<td>8/147 (5.4)</td>
</tr>
<tr>
<td></td>
<td>1.62 (0.39-6.68)</td>
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<td>0.91 (0.23-3.64)</td>
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</tbody>
</table>

NOTE: HER1-3-positive, cases expressing one or more of HER, HER2, HER3 above the cutoffs defined in the text. HER1-3-negative, cases negative for all three receptors. PR positive, cases expressing PRs. PR negative, cases lacking progesterone receptor expression. Combined high risk, cases either HER1-3 positive and/or PR positive; Low risk, cases both HER1-3 negative and PR positive. Hazard ratio, relative increased hazard (with 95% confidence intervals in parentheses) from Cox’s regression analysis for HER1-3-positive versus HER1-3-negative cases. PR-positive versus PR-negative and high risk versus low-risk cases, respectively. Years 1-3 (4-5), patients at risk (denominator) and numbers of relapses (numerator) within different subgroups during years 1-3 (4-5), in years 4 and 5 of tamoxifen treatment only. Percentages, percentage relapse rates in at risk population during time period in question. P’s derived from Cox’s multiple regression analysis.
This suggests that PR status is a time-dependent predictor of diagnosis; as per the ATAC trial). We suspect these patients aromatase inhibitors if treated early (i.e., from time of diagnosis; as per the ATAC trial). We suspect these patients are in effect excluded from the IES study by virtue of their having relapsed before the randomization point of this study. This suggests that PR status is a time-dependent predictor of early relapse due to de novo tamoxifen resistance. The relatively small number of events, which occurs after 3 years, suggests that this finding should be confirmed in a larger data set. Further evidence for this supposition is provided for the moderate association between PR status and outcome in the ARNO 95/ABCsG 8 Trials (49), which randomized patients following only 2 years of tamoxifen treatment. However, data from the BIG1-98 study, which is comparable with ATAC in design, does not seem to support this argument (50). Whereas the most likely explanation of this is underpowering of the subanalysis relating to PR status, caution should be exercised before extrapolating results from ATAC and those presented here to treatment of patients.

Data presented here strongly support the conclusion that the predictive value of PR, and possibly HER1-3, expression is time dependent and identifies patients at high risk of de novo tamoxifen resistance. Ultimately, the underlying mechanisms behind later recurrences will need to be investigated as they may reflect up-regulation of previously dormant HER1-3 pathways or alternatively it may involve other, as yet unknown pathways, which may also be detectable at diagnosis.

From a clinical perspective, data from the ATAC trial, if confirmed, may provide sufficient support to encourage early switching of ER-positive patients from tamoxifen to aromatase inhibitors if their tumors do not express PR. Despite the importance of these findings however, additional information from prospective clinical trials is required before recommendations relating to patient management can be made. The data presented here supports extending preplanned prospective analysis, such as that defined within the multinational TEAM trial (prospectively testing for interactions between HER1-3 and outcome relating to tamoxifen or exemestane treatment), to incorporate measurement and reporting of PR status. The ability to select, at diagnosis, patients at high risk of early relapse on tamoxifen could provide the opportunity to tailor their adjuvant therapy differently, either in terms of an aromatase inhibitor or by supplying them with a HER family inhibitor such as Iressa or Herceptin to be used in conjunction with their endocrine treatment (particularly, if they are premenopausal and hence not suitable for aromatase inhibitors).

In conclusion, this study shows that both HER1-3 and PR expression, in ER-positive primary breast cancers, can be used to identify patients who exhibit de novo tamoxifen resistance, as evidenced by a high risk of relapse during tamoxifen therapy. Furthermore, both PR and HER1-3 seem to be time-dependent predictors of risk of relapse with risk declining markedly after 3 years of tamoxifen treatment. These data, taken in context with data from the recent ATAC and IES studies, strongly support the hypothesis that HER1-3 and PR status identifies separate tamoxifen resistant tumor subsets, and that patients with HER1-3 expression or lacking PR expression are likely to benefit from early implementation of therapy with aromatase inhibitors. Conversely, this data would also suggest that ER/PR-positive tumors lacking HER1-3 expression (over 50% of cases), could be treated by switching to an aromatase inhibitor following 2 to 3 years of tamoxifen treatment. Further research to determine the mechanisms relating to tamoxifen resistance and to test this hypothesis in the context of the redesigned TEAM trial will provide a valuable insight into the most appropriate future therapeutic options for differing sets of breast cancer patients.

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