

Molecular Changes in Tamoxifen-Resistant Breast Cancer: Relationship Between Estrogen Receptor, HER-2, and p38 Mitogen-Activated Protein Kinase

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Terms in blue are defined in the glossary, found at the end of this issue and online at www.jco.org.

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A B S T R A C T

Purpose

To evaluate growth factor receptor cross talk with the estrogen receptor (ER) in paired clinical breast cancer specimens and in a xenograft model before tamoxifen and at tumor progression as a possible mechanism for tamoxifen resistance.

Methods

Specimen pairs from 39 patients were tissue arrayed and stained for ER, progesterone receptor (PgR), Bcl-2, c-ErbB2 (HER-2), and phosphorylated (p) p38 mitogen-activated protein kinase (MAPK), p-ERK1/2 MAPK, and p-Akt. Xenograft MCF-7 tumors before and after tamoxifen resistance were assessed for levels of p-p38.

Results

Pretreatment, there were strong correlations between ER, PgR, and Bcl-2, and an inverse correlation between ER and HER-2. These correlations were lost in the tamoxifen-resistant tumors and replaced by strong correlations between ER and p-p38 and p-ERK. ER expression was lost in 17% of resistant tumors. Three (11%) of the 26 tumors originally negative for HER-2 became amplified and/or overexpressed at resistance. All ER-positive tumors that overexpressed HER-2 originally or at resistance expressed high levels of p-p38. In the pretreatment and tamoxifen-resistant specimens, there were strong correlations between p-p38 and p-ERK. In the tamoxifen-resistant xenograft tumors, like the clinical samples, there was a striking increase in p-p38.

Conclusion

The molecular pathways driving tumor growth can change as the tumor progresses. Crosstalk between ER, HER-2, p38, and ERK may contribute to tamoxifen resistance and may provide molecular targets to overcome this resistance.

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INTRODUCTION

Tamoxifen, a selective **estrogen receptor (ER)** modulator, is the most used drug for the treatment of ER-positive breast cancer. Adjuvant therapy studies of tamoxifen show a 40% to 50% reduction in the odds of recurrence and reduced mortality.¹ Tamoxifen also provides temporary remissions in 30% to 50% of patients with metastatic disease, and it is effective in prevention.² Although **aromatase inhibitors**

may be slightly more effective than tamoxifen, it remains an important drug because of its documented benefits in sequence with these agents for adjuvant therapy, and because it will continue to have a role in metastatic disease.³⁻⁵ De novo and acquired resistance to tamoxifen remain problems, however, and clarification of the mechanisms for resistance would have important clinical implications.

There are several potential causes for resistance to tamoxifen. Both preclinical

and clinical studies suggest that one such mechanism involves cross talk between ER and growth factor and/or **stress kinase signaling** pathways.⁶ The transcriptional (nuclear) and the newly recognized rapid membrane effects of ER can activate growth factor signaling pathways. Furthermore, the kinase cascade generated by the **insulin-like growth factor (IGF)** and the **epidermal growth factor receptor (EGFR)** families can activate ER and its coregulatory proteins, causing a vicious cycle of cross talk that leads to enhanced tumor cell survival and proliferation.⁶⁻¹⁰ Recent *in vivo* laboratory studies demonstrate that overexpression of **HER-2**, a transmembrane tyrosine kinase amplified in about 10% of ER-positive breast cancers, results in loss of tamoxifen's estrogen antagonist activity and the acquisition of tamoxifen-stimulated growth.¹¹ Tamoxifen, like estrogen, activates HER-2 via the membrane functions of ER, which, in turn, phosphorylate both ER and **AIB1 (SRC3)**,¹¹ an important ER coactivator.¹²⁻¹⁴ The molecular cross talk is blocked and the antagonist activity of tamoxifen is restored by inhibiting the growth factor signaling pathway.¹¹ Acquired resistance to tamoxifen has also been causally related to increasing levels and activity of EGFR, HER-2, and **Akt**.^{15,16} The cumulative data from clinical studies support the hypothesis that overexpression of HER-2 and/or EGFR, and high levels of phosphorylated Akt or **ERK**, do contribute to tamoxifen resistance in some patients.¹⁷⁻²¹ Aromatase inhibitors (estrogen deprivation), on the other hand, may be more effective in such tumors since ligand deprivation would shut off both nuclear and membrane ER activity, thereby eliminating the cross talk generated in the presence of estrogen or tamoxifen.^{11,22-24}

Stress and/or cytokine signaling pathways may also contribute to resistance to tamoxifen. Laboratory²⁵ and clinical²⁶ studies suggest that elevated levels of phosphorylated **jun N-terminal kinase (JNK)** are associated with tamoxifen resistance, and preliminary data also implicate activated p38.²⁷ p38 is a member of the **mitogen-activated protein kinase (MAPK)** family that is activated by environmental stresses including ionizing radiation, heat, oxidative stress, inflammatory cytokines (tumor necrosis factor family), growth factors, and tissue ischemia.^{28,29} In endometrial cancer cells, estrogen and tamoxifen both stimulate p38 activity.³⁰ In turn, p38 signaling can phosphorylate ER α (Thr³¹¹), inhibit ER nuclear export, enhance ER's interaction with coactivators, and increase the estrogen agonist activity of tamoxifen.³¹ These mechanisms may well be important for tamoxifen resistance in clinical breast cancer.

A major hindrance to the identification of molecular pathways involved in tamoxifen resistance in patients is the difficulty of obtaining paired tumor tissues for biomarker studies immediately before treatment and again at the time that resistance develops. Although local recur-

rence is not uncommon, most patients with breast cancer recur in bone or in visceral organs, which are relatively inaccessible for biopsy. Furthermore, the majority of patients with breast cancer today never suffer recurrence, and others do so only after tamoxifen adjuvant therapy has been completed. In our study we present the results of an analysis of such paired breast cancer specimens from 39 patients receiving tamoxifen adjuvant therapy. We also report data from a xenograft model of acquired tamoxifen resistance that, together with the clinical data, suggest a potential role for p38 signaling in addition to HER-2 in the development of resistance.

METHODS

Patient and Tissue Specimens

Clinical records of the Royal Marsden Hospital (London, UK) were reviewed to identify appropriate patients treated between 1982 and 2000. Patients had to have their initial surgery at the Royal Marsden Hospital and had to have relapsed and had a repeat tissue biopsy while still taking tamoxifen adjuvant therapy. Archived paraffin blocks from each biopsy were retrieved from 54 eligible patients and 0.6-mm cores of each block were placed into triplicate tissue microarrays containing 121 specimens per slide. The arrays also contained multiple marker cores to orient the analysts (M.C.G. and S.D.) scoring the slides, who were blinded to the outcome of the patients. This study was approved by the ethics committee and institutional human subjects committees at the Royal Marsden Hospital and Baylor College of Medicine (Houston, TX).

Immunohistochemical Analysis

Immunohistochemical (IHC) staining was performed on 4- μ m thick sections of tissue microarrays in triplicate. ER, **progesterone receptor (PgR)**, and **Bcl-2** IHC staining was done as previously described.³² For HER-2, staining was performed using Herceptest (DakoCytomation, Carpinteria, CA) and **fluorescent in situ hybridization (FISH)**; Vysis PathVysion, Downers Grove, IL) kits following the manufacturer's instructions. Phosphorylated (p) -p38, **p-Akt**, and **p-ERK** rabbit polyclonal antibodies (Cell Signaling, Beverly, MA) were used at 1:100, 1:80, and 1:80 dilutions, respectively, with 1-hour incubation. The antigen retrieval methods included ficin predigestion for p-p38, and Tris-EDTA pH 8.0 in pressure-cooker for 10 minutes each for p-Akt and p-ERK. The IHC signal for these three antibodies was developed using labeled streptavidin-peroxidase and DAB+ chromogen (DakoCytomation). p-p38 IHC signals were seen in the cytoplasm as well as in the nucleus of the tumor cells and were scored separately. Nuclear staining was scored for p-ERK. Appropriate positive and negative controls were run with each batch. ER and PgR were scored by the percentage of positively staining cells. IHC staining for p-p38 and p-ERK was interpreted using the Allred score.³³ p-Akt was scored on the basis of intensity of staining (0 to 4+). For HER-2 FISH, a ratio of ≥ 2 was considered gene amplification.

MCF-7 Cell Line and Xenograft Model System

MCF-7 human breast cancer cells were used and treated with 12-*O*-tetradecanoylphorbol 13-acetate as previously described as a control for p-p38.²⁵ Animal care was followed

according to institutional guidelines. The MCF-7 xenograft model of acquired tamoxifen resistance has been previously reported.^{34,35} In brief, estrogen-supplemented (0.25-mg estradiol pellets; Innovative Research, Rockville, MD) 4- to 6-week old female ovariectomized athymic nude mice (Harlan, Sprague, Dawley Inc, Madison, WI) were inoculated in the mammary fat pad with 5×10^6 MCF-7 cells. After 2 to 4 weeks, when tumors had reached a diameter of approximately 8 mm, each mouse was randomly allocated to continuing estrogen or to removal of the estrogen pellet alone or combined with tamoxifen treatment (500 μ g of tamoxifen citrate; Sigma, St. Louis, MO) in peanut oil subcutaneously daily Monday through Friday. Tumor growth was assessed and tumor volumes were measured as described previously.³⁴ Some tumors were harvested for molecular studies during estrogen treatment, during the growth inhibitory phase induced by estrogen deprivation or by tamoxifen, or after 3 to 4 months when the tumors started to progress as tamoxifen resistance developed. Mice were sacrificed according to institutional guidelines and the tumor was resected and immediately frozen in liquid nitrogen or fixed in 10% formalin overnight.

For Western blot analysis, cell and tumor extracts and immunoblots with the phosphorylated and total p38 antibody (Cell Signaling) were performed as previously described.²⁵

RESULTS

Patient Characteristics

Fifty-four patients with paired specimens from the primary and recurrent breast cancers were identified. IHC analyses were obtained on both samples from over 39 patients because there was insufficient malignant tissue in at least one of the pairs of 0.6-mm cores in some cases. The median age of the 39 patients was 61 years (range, 28 to 81 years). Median time on adjuvant tamoxifen was 25 months (range, 7 to 149 months). All pretreatment specimens were taken from the primary tumor. The sites of recurrence included the ipsilateral breast and/or chest wall in 27 patients, the ipsilateral axilla in 11, and a distant skin nodule in one patient.

Correlations of ER With Other Biomarkers Before Treatment and After Tamoxifen Resistance

We first examined correlations between ER content and expression levels of the other six molecular markers (Table 1). In the pretreatment specimens, as expected, there were strong direct correlations (Spearman rank) between ER and PgR and between ER and the estrogen-regulated gene product Bcl-2. Also, consistent with other reports, there was an inverse correlation between ER and HER-2 expression. No significant correlations were evident between ER and levels of p-p38, p-Akt, or p-ERK.

In contrast, in the tumor specimens taken at the time of recurrence on tamoxifen (tamoxifen resistance), the significant correlations between ER and PgR and between ER and Bcl-2 were lost. This is consistent with prior reports showing that PgR loss is relatively common as tumors progress over time, especially following tamoxifen treat-

Table 1. Correlations Between Biomarker Scores With ER Score in Breast Carcinomas at Presentation and at Relapse on Tamoxifen

	Correlation Coefficients (N = 39)			
	Pretreatment		Relapse	
	Rho	P	Rho	P
PgR	0.48	.002	0.29	NS
HER-2	-0.41	.012	0.04	NS
Bcl-2	0.72	< .0001	0.27	NS
p-p38	0.19	NS	0.50	.001
p-Akt	0.30	NS	0.11	NS
p-ERK	0.12	NS	0.39	.017

Abbreviations: PgR, progesterone receptor; NS, not significant; p, phosphorylated.

ment.³⁶ The inverse relationship between ER and HER-2 also disappeared with tamoxifen resistance. Tamoxifen resistance was associated with a strong direct correlation of ER with p-p38 and with p-ERK but not with p-Akt. When the analysis was restricted to only the 29 patients whose tumors were ER-positive on the pretreatment biopsy specimen, the positive relationship between ER and p-p38 persisted in the specimens taken at the time of recurrence (Rho = 0.48, $P = .01$; data not shown). These data suggest that tamoxifen resistance may be accompanied by activation of the p38 and ERK signaling pathways.

Our data in this small series are consistent with prior observations showing that ER loss occurs in 15% to 30% of tumors at tamoxifen resistance (Table 2).³⁷⁻³⁹ Of the 10 ER-negative tumors pretreatment, none converted to ER-positive. In contrast, of the 29 ER-positive tumors, five (17%) became ER-negative at the time of recurrence.

HER-2 status also changed when tamoxifen resistance developed (Table 3). Thirty tumors were HER-2-negative pretreatment and three converted to HER-2-positive at relapse. All nine tumors that were HER-2-positive at presentation remained so at relapse. Of the 10 ER-negative patients pretreatment, six were HER-2-positive (IHC 3+; not shown). All of these remained positive in the tamoxifen-resistant specimens. Of the 29 originally ER-positive patients pretreatment, three were HER-2-positive. However, at the time of recurrence, three additional patients (11%) of the remaining 26 converted from

Table 2. ER Status of Breast Carcinomas at Presentation and at Relapse on Tamoxifen

	ER Status at Presentation (N = 39)		
		Positive (n = 29)	
		Negative (n = 10)	No. of Patients
ER status at relapse			
Negative	10	5	17
Positive	0	24	83

Abbreviation: ER, estrogen receptor.

Table 3. HER-2 Status of Breast Carcinomas at Presentation and Relapse

	HER-2 Status at Presentation (N = 39)	
	Negative (n = 30)	Positive (n = 9)
HER-2 status at relapse		
Negative	27	0
Positive	3	9

HER-2-negative to HER-2-positive. One of these patients (patient No. 228) became ER-negative and HER-2-positive by both IHC and FISH (amplification increase from 1.0 to 2.2) at the time of tamoxifen resistance. The two others remained ER-positive and one (patient No. 221) converted to HER-2-positive by FISH (amplification increase from 1.0 to 3.0) and IHC and the other (patient No. 259) by IHC alone (amplification 1.1 to 1.3). Thus, in this small study, approximately 11% of patients with ER-positive, HER-2-negative tumors converted from low HER-2 expression to high HER-2 expression at the time of their first recurrence of disease on adjuvant tamoxifen. The HER-2 immunohistochemical staining for these three patients is shown in Figure 1.

Phosphorylated p38 MAPK

p-p38 staining was detected in both the cytoplasm and the nucleus. There was excellent agreement in the triplicate

determinations (not shown) and in the scores for cytoplasmic compared to nuclear staining. Therefore, we present only the mean scores for nuclear staining for p-p38 (Fig 2). In the 29 ER-positive specimens, one did not have sufficient tumor in the core to assess p-p38. In the remaining 28 tumors before tamoxifen, the Allred score ranged from 0 to 8. At the time of relapse on tamoxifen, the Allred score increased one or more units in seven patients, decreased in eight, and remained stable in 13. Four of the five tumors that converted from ER-positive to ER-negative at the time of tamoxifen resistance had no staining for p-p38 and the other specimen decreased from an IHC score of 7.5 to only 2. The patient in this group that converted from HER-2-negative to HER-2-positive showed no staining for p-p38 before treatment or at the time of relapse (Fig 2). All three ER-positive, HER-2-positive tumor specimens in the pretreatment samples had relatively high scores of p-p38, and the two ER-positive patients who converted from HER-2-negative to HER-2-positive had a striking increase in the expression of p-p38. In the tumors that were originally ER-positive and HER-2-negative and remained so at relapse, there was a wide range of p-p38 IHC scores. Four of these showed a reduction in p-p38 staining at the time of relapse, five showed an increase, and the rest showed no significant change. There were no clearly discernable patterns

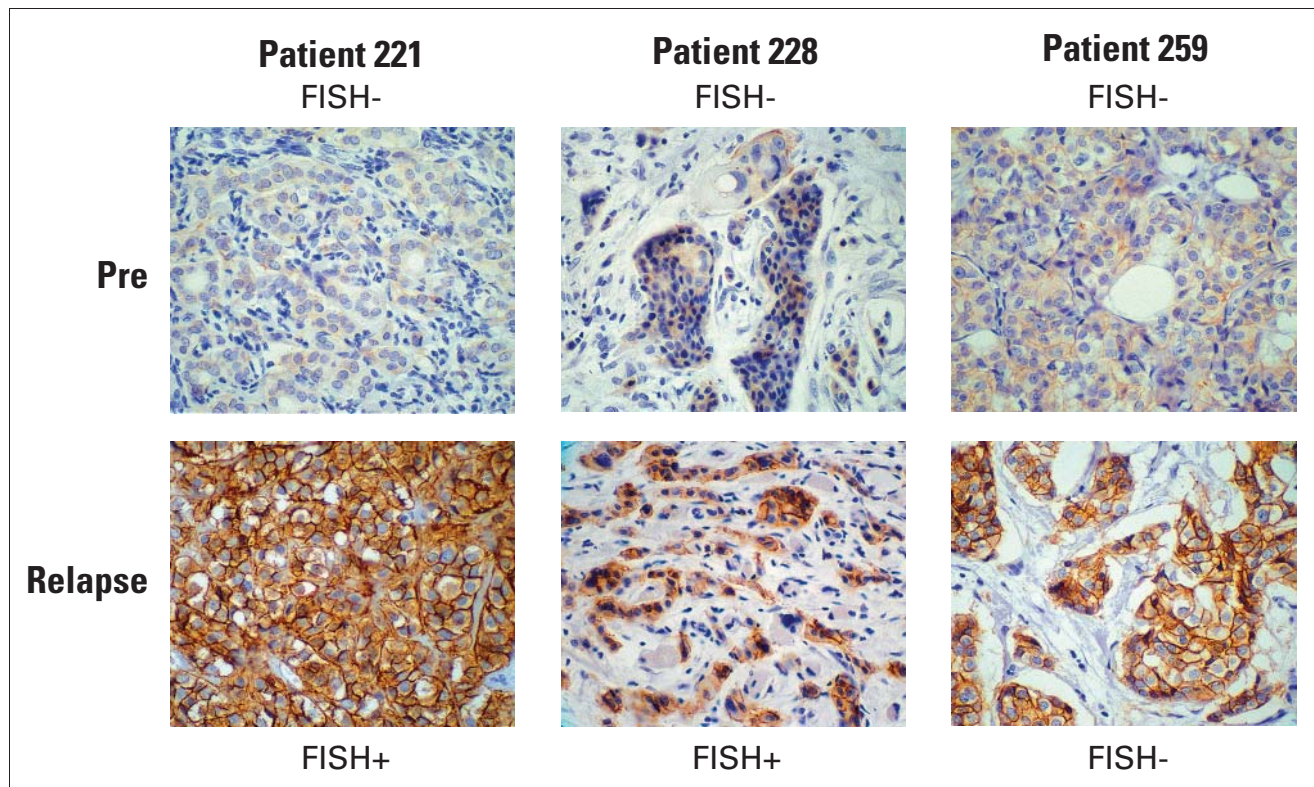


Fig 1. HER-2 immunohistochemical staining of three cases that had a HER-2-negative presentation sample and a HER-2-positive relapse sample. FISH, fluorescent in situ hybridization.

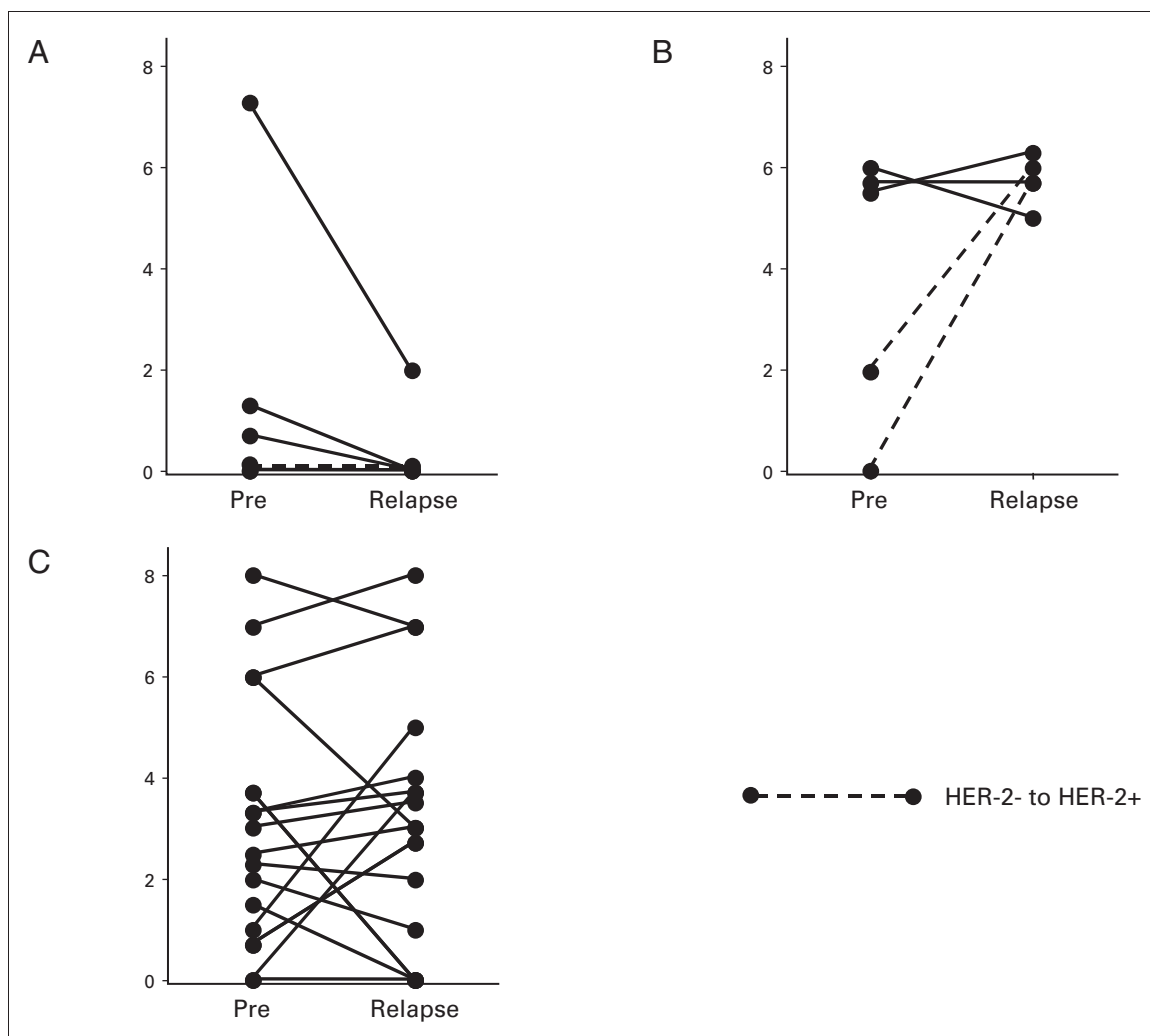


Fig 2. Differences in p38 mitogen-activated protein kinase levels in estrogen receptor (ER) -positive breast carcinomas at presentation and relapse. The data are grouped according to the ER and HER-2 status in the relapse specimens. (A) Relapse ER-negative; (B) relapse ER-positive and HER-2-positive; (C) relapse of ER-positive and HER-2-negative.

between the expression of p-Akt and p-ERK, and there was no significant difference in the expression scores between the pre- and post-treatment samples (data not shown).

Correlations Between p-p38, p-Akt, and p-ERK

In the pretreatment specimens there was a significant correlation between p-p38 and p-Akt ($Rho = 0.43$; $P = .02$; Table 4). This direct correlation was lost in the tamoxifen-resistant specimens. A direct positive correlation was also observed between p-p38 and p-ERK ($Rho = 0.48$; $P = .01$) in the pretreatment specimens, and this was preserved in specimens taken at the time of relapse. While there was no significant correlation between p-MAPK and p-Akt in the pretreatment specimens, there was a relatively strong correlation in the specimens taken at the time of tamoxifen resistance.

Time to recurrence appeared to vary according to the phenotype of the recurrent disease (Table 5).

Activation of p38 MAPK in a Model of Acquired Tamoxifen Resistance

We have previously reported a xenograft model of acquired tamoxifen resistance³⁴ using MCF-7 cells, and an equivalent acquired resistance model was also described for this cell line in vitro.¹⁵ The MCF-7 tumors in vivo become growth-stimulated by tamoxifen after a 3- to 4-month period of growth suppression. Resistant tumors still express high levels of ER³⁴ but they also show evidence of oxidative stress and increased levels of phosphorylated c-Jun and JNK,²⁵ important molecules in AP-1 mediated gene expression. As these cells progress to the tamoxifen-stimulated phenotype, they also express increasingly higher levels of EGFR and HER-2.^{15,40} Now we show

Table 4. Correlation Between Phosphorylated p38 Mitogen-Activated Protein Kinase (MAPK), and ERK1/2 MAPK in ER-Positive Breast Carcinomas at Presentation and at Relapse on Tamoxifen

	Correlation Coefficients (N = 29)			
	Pretreatment		Relapse	
	Rho	P	Rho	P
p-p38 v p-Akt	0.43	.002	0.22	NS
p-p38 v p-ERK1/2	0.38	.06	0.41	.04
p-ERK 1/2 v p-Akt	0.25	NS	0.48	.01

Abbreviations: p, phosphorylated; NS, not significant.

that coincident with the increase in these growth factor receptors is a progressive increase in p-p38 (Fig 3). Compared with tumors from mice treated by estrogen deprivation alone, where the levels of p-p38 were very low, those from mice treated with estrogen deprivation plus tamoxifen had slightly increased levels of activated p38, even during the tumor growth inhibitory phase, and they had a striking increase in the tamoxifen-resistant phase. There was no change in total p38. Thus, while changes in ER expression do not explain tamoxifen resistance in this model, resistant growth is accompanied by a marked increase in p-p38, similar to the data in some patient's tumors, where both HER-2 and p-p38 increased coincident with tamoxifen resistance.

DISCUSSION

Clues to the mechanisms for resistance to tamoxifen in patients with breast cancer are few due to the lack of tumor tissue for molecular studies during treatment with the drug or at the time of resistance. We, and others, have shown in such sequential paired specimens that ER loss over time accounts for tamoxifen resistance in, at most, a minority of patients.³⁷⁻³⁹ PgR loss occurs more frequently, and when this occurs, the tumor takes a more aggressive course.³⁶ Recent laboratory studies suggest that transcriptional repression of the *PgR* gene by signaling through the IGF and EGFR families may be the cause of PgR downregulation in some tumors.^{41,42} Other laboratory studies suggest that tamoxifen can activate growth factor receptor signaling, which can also then reduce ta-

Table 5. Median TTR and Range for Patients on Adjuvant Tamoxifen by Receptor Phenotype

	TTR (months)	Range (months)
ER+ (29 patients)	32.5	7–149
ER– (10 patients)	15.5	6–30
ER+ → ER– (5 patients)	19	11–46
ER+/HER-2+ (5 patients)	23	18–28
ER+/HER-2– (19 patients)	51	7–149

Abbreviations: TTR, time to recurrence; ER, estrogen receptor.

moxifen's estrogen antagonist profile and increase its agonist activity on gene expression by functional activation of ER, thereby leading to tamoxifen resistance.^{11,15}

Although the number of patients in our study with pre- and post-treatment tissue biopsy specimens is small and needs to be expanded, the data do provide further support to the hypothesis that cross talk between ER and growth-factor or stress-response pathways may contribute to tamoxifen resistance in some patients. Except for the inverse correlation between ER and HER-2 that has been well documented in the pretreatment primary tumor biopsies, we found no correlation between ER expression and levels of several growth-factor signaling molecules known to activate ER, such as Akt, ERK, and p38. However, at the time of tamoxifen resistance, strong correlations between ER and p-p38 and p-ERK, both strong activators of ER function, emerged. Furthermore, the inverse correlations between ER and HER-2 and the direct correlation with PgR and Bcl-2 in the pretreatment biopsy disappeared in the tamoxifen-resistant tumors. Interestingly, HER-2 amplification and/or overexpression was observed in three tumors that were negative pretreatment, consistent with a recent report that it is not uncommon for HER-2 to become amplified over time as tumors progress.⁴³ The cumulative data clearly indicate that the molecular signaling pathways used by the cell can change over time. This will necessitate serial biopsies in patients to define the active pathways and appropriate targeted therapy.

It is interesting that tumors that were ER-positive/HER-2-positive pretreatment and those that became HER-2-positive at relapse invariably also expressed high

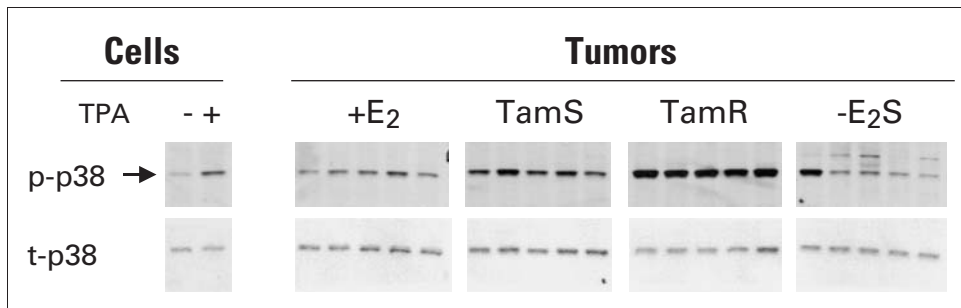


Fig 3. Western blot analysis of phosphorylated (p)-p38. Left panel: MCF-7 cells left untreated (–) or treated with (+) 12-O-tetradecanoylphorbol 13-acetate (TPA) to induce p-p38. Right panel: Xenograft tumor extracts from estrogen-treated (E₂), tamoxifen-sensitive (TamS), tamoxifen-resistant (TamR), and E₂-deprived-sensitive (–E₂S) groups were analyzed with antibodies that recognize the phosphorylated form of and total p38 MAPK. The arrow shows p-p38 MAPK form.

levels of p-p38. p38 is activated by a variety of cellular stresses, cytokines, and growth factors.²⁸ Importantly, p38 signaling has a variety of effects on ER function and, like high levels of p-ERK, can enhance the agonist activity of tamoxifen-bound ER, perhaps contributing to tamoxifen resistance.^{8,30,31} Interestingly, there was also a strong correlation between p-p38 and p-ERK in both the pretreatment and the tamoxifen-resistant tumors, suggesting that they may be regulated by similar upstream signaling in these tumors.

The clinical data demonstrating a possible association between ER, HER-2, and p38 in the tamoxifen-resistant specimens is further supported by data from the xenograft model. When resistance develops in this model, ER is still expressed at high levels, PgR is lost, and the levels of EGFR and HER-2 increase.^{25,34,44} We now find that, as in the clinical specimens from patients, the development of tamoxifen resistance is also associated with markedly increased p-p38. The levels rise slightly with tamoxifen treatment even before resistance is observed, perhaps due to the oxidative stress induced by tamoxifen in these xenografts.²⁵ Whether this increase in p-p38 levels by tamoxifen, in comparison to the complete inhibition of these levels by estrogen deprivation therapy, accounts in part for the partial agonist activity of tamoxifen still remains to be investigated. Both ERK and p38 can phosphorylate and activate ER.^{11,30,31,45} Also, like ERK,⁸ a recent report demonstrates that p38 phosphorylates AIB1.⁴⁶ This phosphorylation is blocked by the selective p38 inhibitor SB203580. Functionally, phosphorylation of AIB1 by p38 enhances its interaction with ER. In addition, AIB1 is a promiscuous coactivator, important for other transcription factors such as **nuclear factor kappa B** and AP-1. The functional significance of p38-induced AIB1 phosphorylation on these other pathways needs clarification. The data suggest the possibility that as an adaptive mechanism to bypass the cytotoxic effects of tamoxifen, some tumors may upregulate growth factor or stress kinase signaling, which activates ERK and p38, and in turn modulate the functions of ER and perhaps other transcription factors to render tamoxifen less effec-

tive. In addition, these networks activate their own downstream cell proliferation and cell survival signals.

Previous clinical studies have suggested that p-p38 may be associated with poor outcome in some breast cancer patients,⁴⁷ and laboratory studies have further demonstrated the importance of p38 in mediating heregulin-induced progression of breast cancer cells to a more invasive phenotype.^{48,49} This is, however, the first report regarding the potential role of p38 in tamoxifen-resistant breast cancer. This model of tamoxifen resistance offers several potential clinical applications. First, HER-2 and other growth factor receptors, their downstream signaling targets, and ER coactivators such as AIB1 may help to predict tamoxifen resistance in some patients. Second, these molecules represent possible therapeutic targets to prevent or overcome the tamoxifen-resistant phenotype. Growth factor pathway inhibitors and/or inhibitors of p38 deserve clinical trials in this setting. Finally, clinical research in the era of targeted therapy requires the serial acquisition of tumor tissue from each patient to define modes of action, to identify pathways leading to treatment resistance, and to select the correct targeted therapy.

Authors' Disclosures of Potential Conflicts of Interest

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. Consultant/Advisory Role: D. Craig Allred, AstraZeneca; Mitchell Dowsett, AstraZeneca. Honoraria: D. Craig Allred, AstraZeneca; Mitchell Dowsett, AstraZeneca. Research Funding: M. Carolina Gutierrez, AstraZeneca; Stephen Johnston, AstraZeneca; D. Craig Allred, AstraZeneca; Mitchell Dowsett, AstraZeneca. For a detailed description of these categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and Disclosures of Potential Conflicts of Interest found in Information for Contributors in the front of each issue.

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