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ORIGINAL REPORT

Phosphorylated ERα, HIF-1α, and MAPK Signaling As Predictors of Primary Endocrine Treatment Response and Resistance in Patients With Breast Cancer

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

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The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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ABSTRACT

Purpose
We aimed to identify signaling pathways involved in the response and resistance to aromatase inhibitor therapy in patients with breast cancer.

Patients and Methods
One hundred fourteen women with T2-4 N0-1, estrogen receptor (ER) α–positive tumors were randomly assigned to neoadjuvant letrozole or letrozole plus metronomic cyclophosphamide. Twenty-four tumor proteins involved in apoptosis, cell survival, hypoxia, angiogenesis, growth factor, and hormone signaling were assessed by immunohistochemistry in pretreatment samples (eg, caspase 3, phospho- mammalian target of rapamycin, hypoxia-inducible factor 1α [HIF-1α], vascular endothelial growth factor, mitogen-activated protein kinase [MAPK], phosphorylated epidermal growth factor receptor, phosphorylated ERα [pERα]). A multivariate generalized linear regression approach was applied using a penalized least-square minimization to perform variable selection and regularization. Ten-fold cross-validation and iterative leave-one-out were employed to validate and test the model, respectively. Tumor size, nodal status, age, tumor grade, histological type, and treatment were included in the analysis.

Results
Ninety-one patients (81%) attained a disease response, 48 achieved a complete clinical response (43%) whereas 22 did not respond (19%). Increased pERα and decreased p44/42 MAPK were significant factors for complete response to treatment in all leave-one-out iterations. Increased p44/42 MAPK and HIF-1α were significant factors for treatment resistance in all leave-one-out iterations. There was no significant interaction between these variables and treatment.

Conclusion
Activated ERα form was an independent factor for sensitivity to chemoendocrine treatment, whereas HIF-1α and p44/42 MAPK were independent factors for resistance. Although further confirmatory analyses are needed, these findings have clear potential implications for future strategies in the management of clinical trials with aromatase inhibitors in the breast cancer.

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INTRODUCTION

Aromatase inhibitors (AIs) have increased response rates compared with tamoxifen[1-3] in breast cancer (BC) but like tamoxifen a significant number of patients treated with AIs fail to attain a disease response. Research into the mechanism of endocrine responsiveness and resistance in BC has revealed that growth factor pathways and oncogenes involved in cell signaling bypass the effects of endocrine treatment.[4] There are data to support central roles for the epidermal growth factor receptor (EGFR) and HER2 in the development of such resistance.[5,6] It is likely that they induce an autocrine loop leading to an induction of key regulators of cell proliferation and cell survival,[6,7] such as mitogen activated protein kinase (MAPK),[8-10] phoshatidylinositol 3′-kinase (PI3K), and its downstream effector the mammalian target of rapamycin (mTOR).[11]

Primary systemic therapy with AIs is commonly used in postmenopausal women with estrogen receptor (ER) α–positive tumors.[12,13] In this light, we considered that primary systemic therapy with the AI letrozole could be used to identify the signaling pathways responsible for endocrine sensitivity and resistance that might be targeted with combination strategies to individualize treatment in

the future. We have used such an approach to evaluate the activation status of cell signaling pathways potentially related to endocrine response in a series of BC specimens obtained before letrozole-based therapy.[14] Our aims were to test which markers were correlated with response to endocrine treatment and to assess which were associated with resistance. The studied pathways include phospho-EGFR (pEGFR) and HER2 growth factor receptors, MAPK (p38 and p44/p42) pathways,[15,16] metabolic pathways (PI3K, phospho-AKT and phospho-mTOR),[7] apoptosis and proliferation (caspase 3, p53, bcl2, bNIP3, cyclin D1, Ki67),[17] the hypoxia-related proteins (hypoxia-inducible factor 1α [HIF-1α], CAIX, prolyl hydroxylase (PHD) 1, PHD2, and PHD3),[18-20] angiogenesis (vascular endothelial growth factor [VEGF], cyclooxygenase [COX-2],[21,22] immunosuppressive tumor infiltration by regulatory T cells [T_{Reg}]),[23] and ER signaling (ERα, phospho-ERα, ERβ, progesterone receptor [PgR], and forkhead box protein [FOXP1][24-26]).

PATIENTS AND METHODS

Patients and Treatment Management

This was a single center, randomized, phase II trial in which patients were assigned to one of two treatment arms on a 1:1 ratio (letrozole alone [LET arm] or letrozole and metronomic chemotherapy with cyclophosphamide [LET-CYC arm] given continuously for 6 months until definitive surgery) as previously described.[27] Between November 2000 and January 2004, elderly women (age $>$ 70 years) or women unfit for chemotherapy between 65 and 70 years old with T2-4 N0-1 and ER-positive BC were considered eligible.[27] The study was approved by the local ethical committee. Written informed consent was obtained from all patients before random assignment.

Treatment Evaluation

On first presentation, an incisional biopsy (0.5 to 0.8 cm^3) was performed. Tumor size and response was assessed by the same specialist, according to the WHO criteria[28] by the clinical measurement of the changes in the product of the two largest diameters recorded in two successive evaluations. Tumor progression (PD) was defined as an increase of at least 25% in tumor size; stable disease (SD) as an increase of less than 25%, or a reduction of less than 50%; partial response (PR) as a tumor shrinkage greater than 50%; and complete response (CR)[30] as the complete disappearance of all clinical signs of disease. Pathologic complete response (pCR) was defined as the absence of neoplastic cells in the breast and in the axillary lymph nodes after histology. Surgery (quadrantectomy or modified radical mastectomy in association with full axillary node dissection) was planned after clinical reassessment. All patients subjected to quadrantectomy underwent irradiation of the residual breast (60 Gy delivered over 6 weeks).

Histopathologic Grade and Immunohistochemistry

Immunohistochemical evaluation for routine markers was performed on paraffin-embedded tumor samples of whole tumor sections obtained at diagnosis for bcl2, p53, HER2, ERα, PgR, and Ki67 as described elsewhere.[31]

The antibodies, sources, and protocols used for the other markers are referenced in Table 1.[32-38] Immunohistochemistry for all these markers was performed on 5-μ sections of tissue microarray containing two 1-mm diameter cores taken from selected morphologically representative tumor regions from the incisional biopsy. Quality control was assessed on each block by hematoxylin and eosin staining. The Envision HRP kit (Dako; Cambridgeshire, United Kingdom) system was used for subsequent visualization.

Staining was assessed in the nucleus for HIF1-α, FOXP3, Ki67, ERα, pERα, ERβ, PgR, and p53, nucleus and cytoplasm for PHD1, PHD2, PHD3, FOXP1, phospho-AKT (pAKT), BNIP3, phospho-p38 MAPK, and phospho-p44/42; membrane for CAIX, pEGFR, and HER2; cytoplasm for bcl-2, VEGF, phospho-mTOR, PI3K, Cyclin D1, COX-2, and caspase-3. All sections had a negative control slide (no primary antibody) of an adjacent section to preclude nonspecific staining. Positive controls included breast carcinomas known to exhibit high levels of each marker. A single pathologist, blinded to patient outcome and to the origin of the samples, used a semi-quantitative method. Intensity was semi-quantitively assessed: 0 (no staining), 1 (weak staining), 2 (moderate staining), or 3 (strong staining) for HIF1-α, ERβ, p53, PHD1, PHD2, PHD3, phospho-AKT (pAKT), phospho-p38, MAPK, and phospho-p44/42, CAIX, pEGFR, VEGF, phospho-mTOR, PI3K, Cyclin D1, COX-2. The cutoff for FOXP3, FOXP1, PgR, ERα, HER2, bcl-2, Ki67, and BNIP3 was as previously reported.[26,27,39,40] For pERα, the Allred score was used as a continuous variable.

Some scores of the markers were missing due to insufficient tumor or unsatisfactory staining. The approach to missing cases was to exclude cases where the value of the covariate under study was missing in the univariate analysis and to exclude cases when 1 or more covariates were missing in the multivariate analysis.

Table 1. List of Antibodies Used for the Immunohistochemical Markers

Marker	Clone
pERα (serine 118)[32]	16J4
Cyclin D1[32]	DCS6
pmTOR (Ser2448) [33]	49F9
HIF-1α[20]	ESEE 122
COX-2[34]	
p38 MAPK (Thr180/Tyr182)[19,35]	12F8
p44/42 MAPK (Thr202/Tyr204)[33]	20G11
PI3 kinase p110α[32]	
phospho-Akt (Ser473)[24]	736E11
VEGF[30]	VG1
PHD1[36]	PHD112
PHD2[20]	366G76
PHD3[20]	EG188e
FOXP1[20]	JC12
FOXP3[26]	236A/E7
BNIP3[34]	
Carbonic anhydrase IX[37]	M75
Caspase-3[38]	84,803
Estrogen receptor beta[34]	ERβ
pEGFR[32]	

Abbreviations: pER, phosphorylated estrogen receptor; pmTOR, phosphorylated mammalian target of rapamycin; HIF-1α, hypoxia-inducible factor 1α; COX, cyclooxygenase; MAPK, mitogen-activated protein kinase; PI3 kinase, phoshatidylinositol 3′-kinase; VEGF, vascular endothelial growth factor; PHD, prolyl hydroxylase; FOXP, forkhead box protein; BNIP, BCL2/adenovirus E1B 19kDa interacting protein 3; pEGFR, phosphorylated epidermal growth factor receptor.

Statistical Methodology

A regression approach was chosen, the elastic-net method,[36] that addresses the problem of variable selection and regularization in multivariate analyses by using least-square penalization. This method is particularly powerful with respect to standard linear regression when the size of the study (ie, the number of cases) is small with respect to the number of covariates. It applies a combination of the L1 and L2 penalty; that is, it performs least-square minimization while enforcing a constrain on a combination term including the sum of the absolute values of the regression coefficients and the sum of their squares (for details, see Zou and Hastie[36]). This enables efficient variable selection and encourages a grouping effect, where strongly correlated predictors tend to stay in or out of the model together. A cross-validation approach was used to build and validate the model as described in Zou and Hastie.[36] Specifically, the L1 and L2 parameters in the penalized least-square were tuned using the following steps: select a grid of values for the L2 parameter ranging from 0 to 100, for each of these values tune the L1 parameter using 10-fold cross-validation, finally choose the value of the L2 parameter which provides the smallest cross-validation error.

Table 2. Molecular Markers Considered in This Study

Molecular Marker	Localization	Median	Mean	95% CI
Tissue microarray				
pERα intensity	Nucleus	3.0	3.6	0.0 to 8.0
Cyclin D1 intensity	Cytoplasm	1.0	1.0	0.0 to 3.0
Phospho-mTOR intensity	Cytoplasm	2.0	1.8	0.0 to 3.0
HIF-1α intensity	Nucleus	1.0	1.2	0.0 to 3.0
COX-2 intensity	Cytoplasm	2.0	1.6	0.0 to 3.0
p38 MAPK intensity	Cytoplasm	1.0	0.9	0.0 to 2.0
p38 MAPK intensity	Nucleus	1.0	1.2	0.0 to 3.0
p44/42 MAPK intensity	Cytoplasm	1.0	1.1	0.0 to 2.8
p44/42 MAPK intensity	Nucleus	1.0	1.1	0.0 to 3.0
pEGFR intensity	Membrane	1.0	1.3	0.0 to 3.0
PI3K intensity	Cytoplasm	2.0	2.2	1.0 to 3.0
pAKT intensity	Cytoplasm	2.0	2.0	0.0 to 3.0
VEGF intensity	Cytoplasm	2.0	2.2	1.0 to 3.0
Routine markers (whole slide staining)				
ERα		310.0	301.6	164.0 to 410.0
PgR		170.0	145.2	0.0 to 343.0
bcl2		100.0	83.2	0.0 to 100.0

NOTE. Markers considered in the analysis had < 20% of missing values (ie, the marker was assessed in at least or more than 80% of the patients) or ≥ 20% positivity in sample staining (ie, the marker stained positive in at least 20% of the samples).

Abbreviations: pER, phosphorylated estrogen receptor; mTOR, mammalian target of rapamycin; HIF-1α, hypoxia-inducible factor 1α; COX, cyclooxygenase; MAPK, mitogen-activated protein kinase; pEGFR, phosphorylated epidermal growth factor receptor; PI3 kinase, phoshatidylinositol 3′-kinase; VEGF, vascular endothelial growth factor; ER, estrogen receptor; PgR, progesterone receptor.

The markers introduced in the model are described in Table 2 where median, mean, and 95% quantiles are provided. The categorization of the markers was based on the variables as described earlier. The clinical variables introduced in the model were tumor size (0 if T ≤ 2, 1 if T > 2), nodal status (negative *v* positive), age, tumor grade, histological type (1 = lobular, 2 = ductal), and treatment. All scores and clinical variables were standardized before being introduced in the model by applying location and scale transformation as described by Zou and Hastie.[36]

Markers responsible for endocrine response were studied by contrasting all responders (ie, patients with PR and CR) with nonresponders (NR; ie, patients with SD and PD).[29,30] To further define markers responsible for chemoendocrine sensitivity, CR was also compared with PR and NR combined.

An iterative leave-one-out approach was used to test the model. At each step, one case (ie, a single patient) was left out from the analysis, a fit of the model was produced for the remaining cases using 10-fold cross-validation and a treatment response prediction was made for the left-out case. This allowed testing of the models' ability to predict treatment response, specificity and sensitivity, and respective area under the receiver operator characteristic (ROC) curve, were estimated using these predictions. The ROC analysis was done using the nonparametric option for estimation of the SE of the area under the curve in SPSS version 15 (SPSS, Chicago, IL); the cross-validation and leave-one-out analyses were implemented in R 2.5.1 (http://cran.r-project.org) and the R package elasticnet 1.0 to 3 was used to perform elastic-net regression.[36]

RESULTS

One hundred fourteen patients were enrolled, 57 were randomly assigned to receive 6 months of primary LET and 57 to LET-CYC. Patient characteristics are outlined in Table 3.

Table 3. Patient Characteristics

Characteristic	Letrozole		Letrozole + Cyclophosphamide	
	No.	%	No.	%
Median age, years	79		75	
Range	64-89		62-94	
TNM				
T2	42	73.7	44	77.2
T3-4	15	26.3	13	22.8
N0	35	61.4	41	71.9
N1	22	38.6	16	28.1
Primary histology				
Ductal carcinoma	44	77.2	47	82.5
Lobular carcinoma	13	22.8	10	17.5
Grade				
2	22	38.6	22	39.3
3	35	61.4	34	60.7
Not assessable	—		1	

Clinical Response to Treatment

One hundred thirteen patients were evaluated for disease response; one patient was not evaluated due to discontinuation of treatment. Ninety-one of 113 patients (80.5%) attained a disease response: 41 (73.2%) of 56 in LET arm and 50 (87.7%) of 57 in LET-CYC arm. Clinical CR was obtained in 48 patients (42.5%), 23 (41.1%) in LET arm, and 25 (43.8%) in LET-CYC arm, respectively.

To study markers involved in chemoendocrine response we compared any responders (ie, PR and CR, 91 [79.8%] *v* the NR, 22 [19.3%]). Furthermore, to determine markers responsible for chemoendocrine sensitivity, we compared the CR, 48 patients (42.1%) versus PR or NR, 65 patients (57%). Mosaic plots (Fig 1) and immunohistochemistry (Fig 2) are shown for the significant factors associated with response to chemoendocrine treatment.

Factors Associated With Endocrine Sensitivity (CR)

The elastic-net reduced model for sensitivity included nuclear expression of pERα, which was significantly positively correlated with endocrine sensitivity in all leave-one-out iterations, and cytoplasmic expression of p44/42 MAPK, which showed significant negative correlation with CR in all iterations (Figs 3A and 3B). pEGFR was significant in 43 of 62 (≈69%) of the fits; where increased membrane pEGFR corresponded with a lower CR. When present, pEGFR grouped with p44/42 MAPK expression (Fig 3C). None of these factors showed significant interaction with treatment in any of the leave-one-out iterations. All other variables were significant in less than 50% of the leave-one-out models or never significant (Fig 3C). The test of the model on the cases which were left out from the model building (one at each iteration) gave an area under the ROC curve of 0.5 with 95% confidence limits of 0.35 to 0.65; thus, the null hypothesis of ROC = 0.5 (random prediction) could not be rejected.

Factors Associated With Endocrine Response (nonresponders v responders)

p44/42 MAPK cytoplasmic expression intensity was the only prognostic factor that was significant in the reduced model of endocrine resistance in all leave-one-out iterations showing a consistent

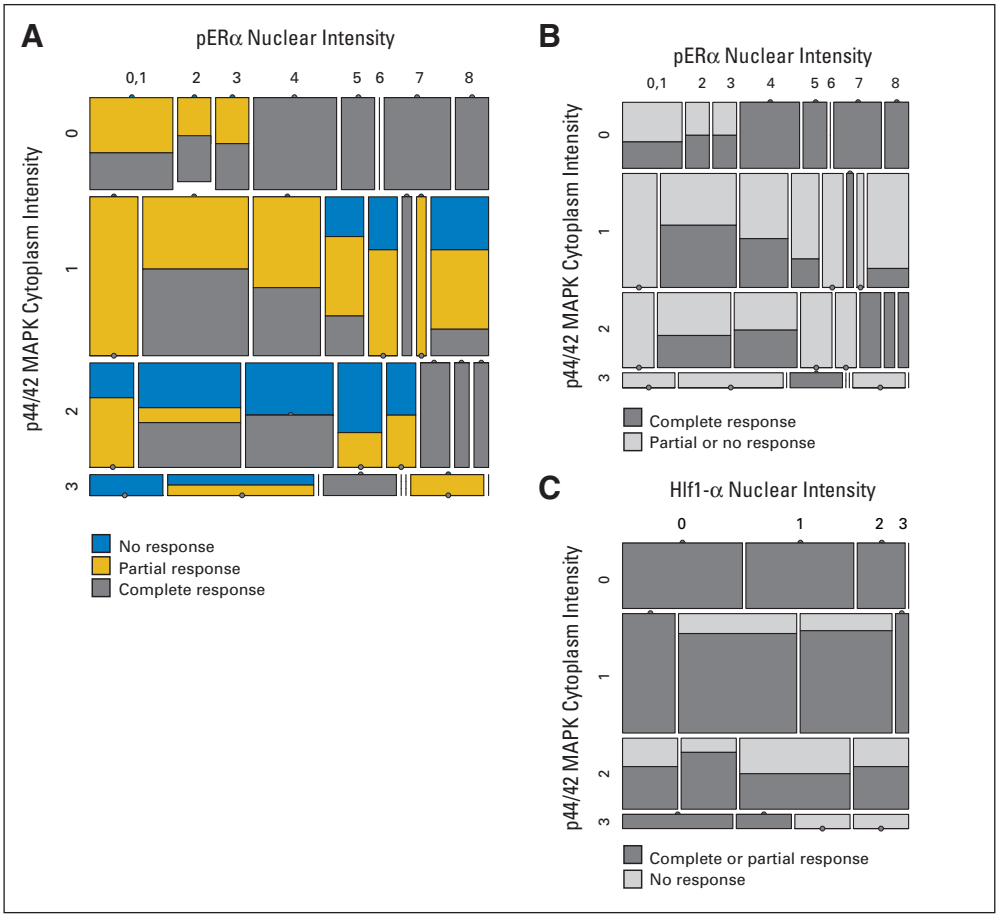

Fig 1. Mosaic plots of the factors that were consistently associated with endocrine response. (A) A mosaic plot of p44/42 MAPK cytoplasmic expression and pERα nuclear expression with treatment response; gray corresponds to complete response (CR), gold to partial response (PR), and blue to no response (NR; ie, PD and SD). (B) A mosaic plot of p44/42 MAPK cytoplasmic expression and pERα nuclear expression with CR (gray) or PR/NR (light gray). A mosaic plot of p44/42 MAPK cytoplasmic expression and HIF-1α nuclear expression with any response (ie, CR and PR [gray]) or NR [light gray]).

negative correlation with clinical response (Fig 3D). Nuclear HIF-1α was also significant in all leave-one-out fits and grouped with cytoplasmic p44/42 MAPK showing a similar negative correlation with response (Fig 3E and 3F). Neither of these factors showed significant interaction with treatment in any of the leave-one-out iterations.

The test of the model on the cases which were left out from the model building (one at each iteration) gave an area under the ROC curve of 0.69 with 95% confidence limits of 0.53 to 0.85; thus, the null hypothesis of ROC = 0.5 (random prediction) could be rejected with $P = .037$.

Analysis of Post-Treatment Ki67 and Post-Treatment T-Stage As Markers of Relapse-Free Survival

Due to the relatively short follow-up analysis of relapse-free survival was possible and thus post-treatment ki67 expression and high post-treatment stage that have been shown to correlate with relapse-free survival in previous studies[34,41] have been used as surrogates. Post-treatment Ki67 showed a significant inverse correlation with clinical response (NR *v* PR *v* CR; $\chi^2 = 10.85$, $P = .001$; Appendix Table A1, online only). A full multivariate regression analysis of the markers was not performed as the distribution of post-treatment Ki67 is highly skewed (Appendix Fig A1, online only). However, the three main significant factors for treatment response, p44/42 MAPK, HIF-1α, and pERα, showed a significant positive ($P < .001$), positive ($P < .001$), and negative ($P = .004$) association , respectively, with high post-treatment stage (Moses test for extreme tendencies). Post-treatment T-stage was also significantly correlated with clinical response ($\chi^2 = 14.41$; $P = .006$), with a significantly lower number of CR present in stages higher than 1 after treatment with respect to NR or PR (Appendix Table A2, online only). As with Ki67 expression, a full covariate analysis of the markers was not performed as the distribution of post-treatment stage is highly skewed (Appendix Fig A2, online only). However, the three main significant factors for treatment response, p44/42 MAPK, HIF-1α, and pERα, showed a significant a positive ($P < .001$), positive ($P < .001$), and negative ($P = .005$) association, respectively, with high post-treatment stage (Moses test for extreme tendencies).

DISCUSSION

The neoadjuvant model provides a valuable approach for testing biologic hypotheses and to study the predictive role of signaling pathways. We have used such a model to measure the basal expression of different putative markers of the kinase signaling and hypoxia regulated pathways involved in endocrine responsiveness/resistance that may predict clinical response.

To analyze these data, we have employed a generalized linear regression method that addresses the problem of dimensionality reduction and variable selection in multivariate regression by using penalized least-square methods.[36]

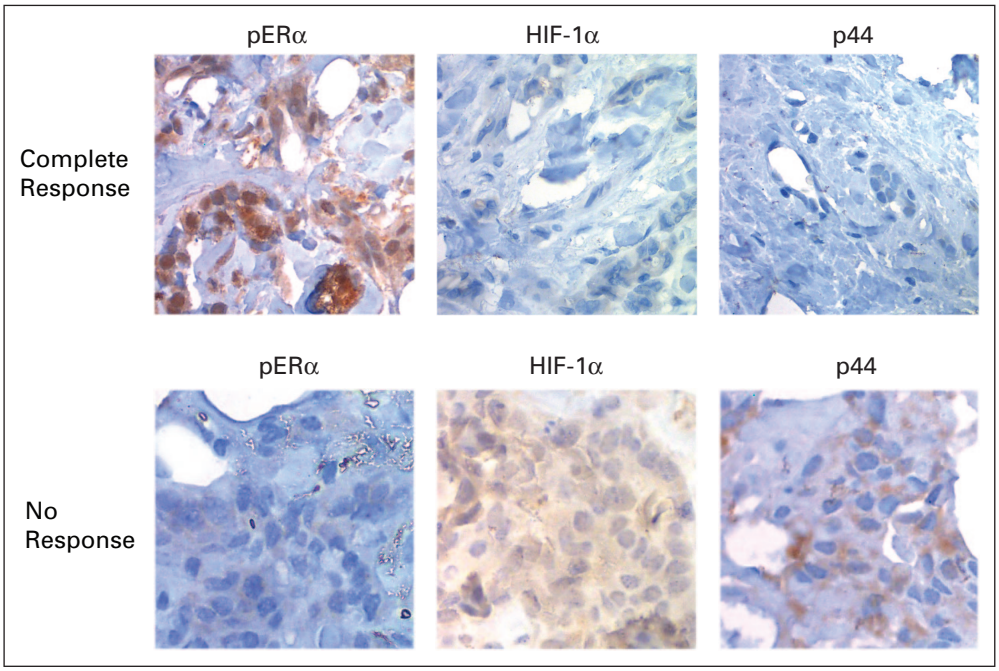

Fig 2. Expression of markers of resistance (hypoxia-inducible factor 1α, p44/p42, mitogen-activated protein kinase) and responsiveness (phosphorylated estrogen receptor α) in a tumor with no clinical response[11] and a tumor with a complete clinical response.[30]

This analysis identified nuclear pERα as a significant independent factor for sensitivity to chemoendocrine treatment. Moreover, providing further support to the potential of nuclear pERα as a marker of response, nuclear pERα was significantly negatively associated with high post-treatment ki67 expression and with high post-treatment stage, both of which have been shown to correlate with clinical outcome in previous studies.[34,41] The identification of nuclear pERα as related to CR is in keeping with the findings of Murphy et al[25] who reported phosphorylated serine[118] ERα expression correlated with an improved disease outcome in patients with BC treated with hormone therapy.[42] The role of phosphorylated serine[118] ERα being a marker of response to antiestrogen therapy is further supported by its enhanced expression that develops during the hypersensitive phase that occurs after long-term estrogen deprivation in ERα-positive BC cells.[9,43] Thus, since the phosphorylated serine[118] ERα may be a more precise marker of endocrine-based treatment response (unlike ERα, ERβ, and PgR in our multiple logistic regression) it should be further evaluated for determining its use in endocrine therapy.[44] Indeed, this is an important issue since conventional ERα as assessed by immunohistochemistry predicts response to hormone therapy in only 36% to 55% of patients, although this can be improved with the use of PgR.[2,3] Nevertheless, although this study showed that the model of endocrine sensitivity was valid during cross-validation it lacked generality when tested on patients who did not contribute to the model building. Thus, a larger series of patients is required to refine and confirm the endocrine sensitivity model.

Emerging evidence suggests that other adaptive changes occur during prolonged endocrine therapy with crosstalk between growth factor pathways and ER.[10,16,37] Thus, EGFR, HER2, insulin growth factor, and transforming growth factor family members result in phosphorylation of serine 118 (and 167) of ER through MAPK and other intermediates including PKC and AKT.[5,33,35] However, it is presently unclear whether this ligand-independent activation of ERα contributes to resistance. Although EGFR through MAPK may result in serine[118] ERα phosphorylation, we observed no association between MAPK and pERα suggesting that MAPK may preferentially phosphorylate other targets that contribute to antiestrogen resistance, independent of ER signaling. One such alternative mechanism is constitutive activation of MAPK pathway which alters p27 phosphorylation leading to a reduction of cdk2 inhibitory activity.[32]

Although letrozole is able to reduce estrogen levels by 90% in breast tumors,[38] tumors may acquire resistance and thus an ability to grow in the presence of AIs. In vivo models suggest that while letrozole initially prevents tumor growth, after long exposure it induces an activation of several growth factor cascades, such as MAPK pathways, that might be responsible for reducing/inhibiting AI activity.[45] In this study, p44/42MAPK and HIF-1α were found to be significantly associated with endocrine resistance and were significantly positively associated with high post-treatment stage. These data together with a significant positive correlation between these two factors provide strong evidence for a central role for p44/42 MAPK in endocrine resistance.

Although growth factor signaling mediates a significant proportion of this resistance, the results indicate that HIF-1α may also be responsible. The hypoxic microenvironment favors malignant progression of cancer (reviewed by Harris)[46] and hypoxia, via HIF-1α, may promote estrogen-independent growth and a more aggressive BC phenotype.[18] Indeed, ERα expression is lower in HIF-1α positive than in negative BC[18] and hypoxia has been shown to reduce the expression of ERα and the inhibitory effects of antiestrogens in vitro.[18,47] Tumor hypoxia also activates the MAPK pathway[48] which in turn phosphorylates HIF-1α,[49] suggesting hypoxia and growth factor signals may synergize to augment the HIF-1α mediated response. The upregulation of pathways involved in glycolysis, angiogenesis, pH regulation, and downregulation of ERα lead to endocrine resistance.[14,50] Our results suggest that a HIF-1α and MAPK may define patients resistant to AIs and thus identify patients who may benefit from other therapeutic strategies including MAPK and HIF inhibitors for which several trials are planned.[51] Conversely, an alternate strategy is to use the HIF

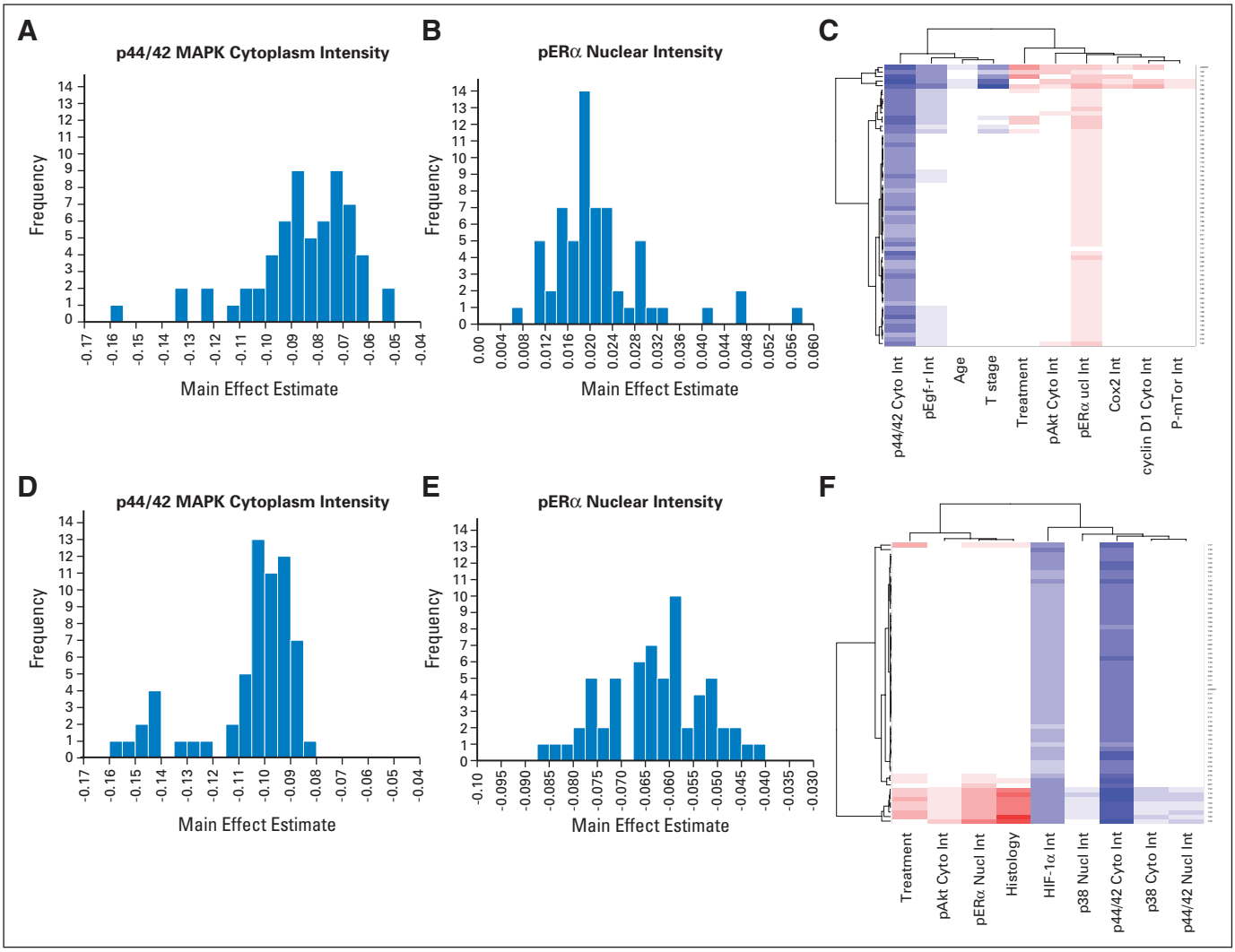

Fig 3. Main effect estimate for significant factors in the reduced model of endocrine response. The complete distribution of the main effect estimate in the leave-one-out iterations is shown for the factors that were consistently significant in the reduced model of (A, B, C) endocrine sensitivity and (D, E, F) response, respectively. The heatmaps on the right panels (C and F) display the leave-one-out model results at each iteration (y-axis) for factors (x-axis) that were significant in at least 5% of the iterations. In these heatmaps, double-clustering is performed just for the visual purpose of grouping together similar model results and similar main effects; standard correlation was used. Red indicates positive association; blue indicates negative association; and white indicates no significant association.

pathway to activate bioreductive drugs, such as tirapazamine, that inhibit DNA repair under hypoxic conditions[52] and has been shown to have an antiangiogenic effect as well as direct antitumor activity.[53] It would also be of interest to examine the role of these pathways in response to bevacizumab in patients with BC treated with endocrine therapy.

In conclusion, to our knowledge, this is the first report in a clinical data set demonstrating the role of cytoplasmic p44/42 MAPK and nuclear HIF-1α in endocrine resistance and of nuclear pERα in endocrine responsiveness in patients receiving letrozole-based treatment. Furthermore, no significant interaction with treatment was observed for these factors, suggesting that this profile might be only related to the AI effect. Nevertheless, an additional effect for cyclophosphamide cannot be discounted and confirmatory studies will be needed. Thus, the results support the development of new treatment strategies based on the combination of AI with signal transduction inhibitors as a means to prevent endocrine resistance with signal transduction inhibitor(s) targeting mainly MAPK (but also HIF1α) administered with an AI being more effective than using an AI alone.[8,47] They also stress the heterogeneity of baseline signaling pathways and suggest that trials might benefit from selecting patients with combinations of AIs.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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Glossary Terms

Aromatase inhibitors: Used in treating breast cancer in postmenopausal women, aromatase inhibitors inhibit the conversion of androgens to estrogens by the enzyme aromatase, thus depriving the tumor of estrogenic signals. Because of decreased production of estrogen, estrogen receptors, which are important in the progression of breast cancer, cannot be activated.

HIF (hypoxia-inducible factor): HIF is a transcriptional factor that regulates the adaptive responses of mammalian cells to low oxygen (hypoxia: oxygen concentration below normal physiological limits in a specific tissue. Under these circumstances). It is composed of HIF-1α, which is upregulated in conditions of hypoxia, and HIF-1α (or aryl hydrocarbon receptor nuclear translocators), which is expressed constitutively. Dimerization of HIF-1α with HIF-1α leads to transcription of genes such as VEGF and PDGF.

VEGF (vascular endothelial growth factor): VEGF is a cytokine that mediates numerous functions of endothelial cells including proliferation, migration, invasion, survival, and permeability. VEGF is also known as vascular permeability factor. VEGF naturally occurs as a glycoprotein and is critical for angiogenesis. Many tumors overexpress VEGF, which correlates to poor prognosis. VEGF-A, -B, -C, -D, and -E are members of the larger family of VEGF-related proteins.

Pathologic complete response (pathCR): The absence of any residual tumor cells in a histologic evaluation of a tumor specimen is defined as a complete pathologic response.

Metronomic chemotherapy: A schedule of chemotherapy given at lower doses to allow more frequent administration without the induction of myelosuppression seen with maximum tolerated dose (MTD) regimens. This type of regimen is also called antiangiogenic scheduling due to the fact that slowly proliferating (angiogenic) endothelial cells are more efficiently targeted by metronomic chemotherapy than by MTD regimens, resulting in inhibition of tumor growth due to insufficient neovascularization.

mTOR: The mammalian target of rapamycin belongs to a protein complex (along with raptor and G > L) that is used by cells to sense nutrients in the environment. mTOR is a serine/threonine kinase that is activated by Akt and regulates protein synthesis on the basis of nutrient availability. It was discovered when rapamycin, a drug used in transplantation, was shown to block cell growth presumably by blocking the action of mTOR.

MAPK (mitogen-activated protein kinase): MAPKs are a family of enzymes that form an integrated network influencing cellular functions such as differentiation, proliferation, and cell death. These cytoplasmic proteins modulate the activities of other intracellular proteins by adding phosphate groups to their serine/threonine amino acids.

EGFR (epidermal growth factor receptor): Also known as HER-1, EGFR belongs to a family of receptors (HER-2, HER-3, HER-4 are other members of the family) and binds to the EGF, TGF-α, and other related proteins, leading to the generation of proliferative and survival signals within the cell. It also belongs to the larger family of tyrosine kinase receptors and is generally overexpressed in several solid tumors of epithelial origin.

ER (estrogen receptor): Belonging to the class of nuclear receptors, estrogen receptors are ligand-activated nuclear proteins present in many breast cancer cells that are important in the progression of hormone-dependent cancers. After binding, the receptor-ligand complex activates gene transcription. There are two types of estrogen receptors (α and β). ERα is one of the most important proteins controlling breast cancer function. ERβ is present in much lower levels in breast cancer and its function is uncertain. Estrogen-receptor status guides therapeutic decisions in breast cancer.

Regulatory T cells (known as suppressor T cells): are a specialized subpopulation of T cells that act to suppress activation of the immune system and thereby maintain immune system homeostasis and tolerance to self-antigens. This is an important "self-check" built into the immune system so that responses do not go haywire. Regulatory T cells come in many forms, including those that express the CD8 transmembrane glycoprotein (CD8+ T cells), those that express CD4, CD25 and Foxp3 (CD4+CD25+ regulatory T cells or "Tregs") and other T cell types that have suppressive function. These cells are involved in closing down immune responses after they have successfully tackled invading organisms and also in keeping in check immune responses that may potentially attack one's own tissues (autoimmunity).

