VOLUME 27 · NUMBER 24 · AUGUST 20 2009

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Estrogen Receptor, Progesterone Receptor, Human Epidermal Growth Factor Receptor 2 (HER2), and Epidermal Growth Factor Receptor Expression and Benefit From Lapatinib in a Randomized Trial of Paclitaxel With Lapatinib or Placebo As First-Line Treatment in HER2-Negative or Unknown Metastatic Breast Cancer

Richard S. Finn, Michael F. Press, Judy Dering, Michael Arbushites, Maria Koehler, Cristina Oliva, Lisa S. Williams, and Angelo Di Leo

From the Geffen School of Medicine at University of California, Los Angeles; Norris Cancer Center, University of Southern California, Los Angeles, CA; Medicine Development Centre Oncology, GlaxoSmithKline, Middlesex, United Kingdom; and Sandro Pitigliani Medical Oncology Unit, Prato, Italy.

Submitted May 19, 2008; accepted February 24, 2009; published online ahead of print at www.jco.org on July 20, 2009.

Supported by grants from GlaxoSmithKline, and a National Institutes of Health Loan Repayment Program award (R.S.F.).

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical Trials repository link available on JCO.org.

Corresponding author: Richard S. Finn, MD, 10833 Le Conte Ave, 11-934 Factor Bldg, Los Angeles, CA 90095; e-mail: Rfinn@mednet.ucla.edu.

The Acknowledgment and Appendix are included in the full-text version of this article; they are available online at www.jco.org. They are not included in the PDF version (via Adobe® Reader®).

0732-183X/09/2724-3908/$20.00

DOI: 10.1200/JCO.2008.18.1925

ABSTRACT

Purpose

Lapatinib is a dual inhibitor of epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2) with activity in HER2-amplified metastatic breast cancer (MBC). Its role in non–HER2-amplified MBC remains unclear. EGF30001, a phase III trial of lapatinib and paclitaxel versus paclitaxel and placebo, demonstrated lapatinib does not significantly benefit HER2-negative or HER2-unselected patients with MBC. Published data support interactions between steroid hormone and peptide growth factor signaling. We hypothesized that molecular subgroups may exist within EGF30001 that would benefit from lapatinib.

Methods

A blinded, retrospective biomarker evaluation was performed using immunohistochemistry to semiquantitate estrogen (ER), progesterone (PR), and EGFR expression. HER2 amplification was determined by fluorescent in situ hybridization. Effects of these biomarkers on event-free survival (EFS) were examined in patients with available tissue (n = 493).

Results

Lapatinib improved median EFS in HER2-amplified, ER- or PR-positive MBC (n = 36; 5.7 *v* 4.5 months; $P = .351$); benefit was greater and statistically significant in HER2-amplified, ER-negative, PR-negative MBC (n = 42; 8.3 *v* 5.0 months; $P = .007$). In HER2-negative, ER-positive MBC, median EFS improvement varied by degree of PR expression (H-score): no benefit if PR-strong (n = 133; 9.3 *v* 7.3 months; $P = .373$), benefit if PR-weak (n = 50; 7.3 *v* 2.4 months; $P = .026$), and potential antagonism if PR-negative (n = 40; 3.7 *v* 7.2 months; $P = .004$). No benefit was seen in triple-negative MBC (n = 131; median EFS, 4.6 *v* 4.8 months; $P = .255$). EGFR expression was not correlated with benefit from lapatinib.

Conclusion

Although subgroups are small, these analyses support the hypothesis that semiquantitative determination of hormone receptor status may be a surrogate for EGFR and/or HER2 dependency.

J Clin Oncol 27:3908-3915. © 2009 by American Society of Clinical Oncology

INTRODUCTION

The epidermal growth factor receptor (EGFR) family has been pursued as a therapeutic target in breast cancer.[1] Human EGFR 2 (HER2) amplification occurs in 20% to 25% of women and is associated with a poor prognosis.[2,3] Elevated EGFR expression has been correlated with a poor prognosis in breast cancer specimens and in laboratory models.[4] Whereas clinical data with trastuzumab and lapatinib have validated HER2 as a therapeutic target in breast cancer, studies using agents aimed at disrupting EGFR signaling have not shown meaningful activity in breast cancer.[5,6] This raises the question of whether EGFR plays a role in breast cancer pathogenesis or whether the correct patients with EGFR-dependent disease are being selected. The latter is important, as EGFR mutations and amplification were only retrospectively found to be associated with response to EGFR inhibitors in non–small-cell lung cancer.[7-9]

Lapatinib is a small-molecule inhibitor of the EGFR and HER2 tyrosine kinases. Preclinical studies using human breast cancer cell lines have shown that HER2 amplification is predictive of response, whereas EGFR expression is not.[10-12] Clinical activity of lapatinib in advanced HER2-overexpressing breast cancer was confirmed in open-label[13] and randomized clinical trials.[14,15] A randomized, placebo-controlled, phase III study (EGF30001) evaluated the role of lapatinib in combination with paclitaxel in first-line HER2-negative or -unknown metastatic breast cancer.[16] Of the 579 women enrolled, 493 had tissue available for centralized fluorescent in situ hybridization (FISH) and immunohistochemistry (IHC). After central review, 86 patients had evidence of HER2 amplification, and 407 patients had HER2-nonamplified disease. The primary end point was time to progression (TTP). The intent-to-treat population did not show benefit in TTP when lapatinib was added to paclitaxel; however, the distinct subgroup of HER2-amplified patients receiving lapatinib with paclitaxel demonstrated clear clinical benefit.[16] This supports the concept that patient selection and predictive markers are critical in determining certain subgroups of patients who are more likely to respond to treatment. It was hypothesized that a subgroup within the HER2-nonamplified cohort may derive benefit from the addition of lapatinib.

The data supporting EGFR as a target in breast cancer has been difficult to reconcile with the lack of clinical activity of EGFR-targeted agents in this disease. Recent data from a retrospective study of more than 35,000 clinical samples suggested a relationship between peptide growth factor signaling and hormone receptor status,[17] specifically, that progesterone receptor (PR) loss in estrogen receptor (ER) –positive disease was associated with higher expression of EGFR and/or HER2. In addition, a presurgical study demonstrated cell-cycle inhibition in ER-positive and PR-weak/-negative patients with breast cancer who were exposed to a short course of the EGFR inhibitor gefitinib.[18] Other data suggested that breast cancers lacking ER, PR, and HER2 amplification ("triple-negative" breast cancer) have higher levels of EGFR and may be more sensitive to EGFR-targeted agents.[19,20] A blinded, retrospective, semiquantitative analysis of ER, PR, HER2, and EGFR expression was performed to correlate these measurements with benefit from lapatinib.

METHODS

Patient Selection

The eligibility criteria and study design for EGF30001 have been previously reported.[16] Briefly, 579 women with advanced breast cancer (stage III or IV) previously untreated in the metastatic setting were randomly assigned to receive paclitaxel 175 mg/m^2 intravenously every 3 weeks with either oral lapatinib 1,500 mg daily or placebo. Patients had either HER2-negative (per the enrolling site) or HER2-unknown breast cancer.

ER and PR Staining

Tumor specimens from pretreatment or archival tumor biopsies were available for HER2 status (n = 493), ER and PR (n = 455), and EGFR (n = 443) determination. Paraffin-embedded samples were cut to 4 μm, mounted on slides, deparaffinized, and rehydrated through a graded series of alcohol. Endogenous peroxidase was quenched with 3% H_2O_2 in phosphate-buffered saline (PBS). Antigen retrieval was performed for ER only by placing slides in 0.1 mol/L of citrate buffer in a steam bath for 1 hour and allowing them to cool for 20 minutes. Slides were blocked with 10% normal goat serum in PBS at room temperature for 1 hour, and then primary antibody was applied for 1 hour at room temperature. Both ER 1D5 (Immunotech; Marseilles, France) and PgR 636 (Dako; Carpinteria, CA) antibodies were used at 1:50 dilute in 10% normal goat serum. Slides were washed in PBS, followed by antimouse secondary (Dako Polymer Envision Plus, Dako; Carpinteria, CA) for 30 minutes at room temperature. The chromogen used was diaminobenzidine, and slides were counterstained with ethyl green, passed through butanol, and dehydrated in xylene before mounting. Negative controls consisted of eliminating primary antibody, and positive controls were known ER-/PR-positive cell lines that were pelleted, embedded, and cut. Known positive human tumors were used as well. Controls were included in each staining batch. In a blinded fashion, the samples were read and scored by light microscopy.

Calculation of H-Score for ER and PR

ER and PR were semi-quantitated using the H-score. Tissue was scored (H-score) based on the total percentage of positive cells and the intensity of the staining (1+, 2+, or 3+), where H = (% 1+ × 1) + (% 2+ × 2) + (% 3+ × 3). The sample was considered negative if H = 0 and positive if H was more than 0; positive samples were also categorized as weak if H = 1 to 50 and strong if H was more than 50. A minimum of 100 cells were evaluated in calculating the H-score.

HER2 Testing by FISH

FISH assays were performed in a blinded fashion using the HER2 PathVysion FISH assay (Abbott Laboratories, North Chicago, IL) as described elsewhere[21,22] and as approved by the US Food and Drug Administration. Enumerations of red HER2 signals and green chromosome 17 centromeres in each of at least 20 interphase carcinoma cell nuclei were performed by a clinical laboratory scientist/technician and confirmed by a board-certified pathologist. Samples with a HER2:CEP17 ratio ≥ 2.0 were considered FISH positive.

EGFR Immunohistochemistry

EGFR IHC was performed using a commercially available immunohistochemical assay kit (PharmDX; Dako). A monoclonal mouse antibody was used in a peroxidase antiperoxidase immunohistochemical assay to demonstrate the EGFR protein product in tissue sections. The immunohistochemical technique involved incubation of the tissue sections with two different antibodies: a primary antibody specific for the EGFR protein (clone 2-18C9; Dako) and a goat antimouse immunoglobulin conjugated to a horseradish peroxidase–labeled dextran polymer (Envision+; Dako). The site of immunoprecipitates was identified by the use of a chromogen, diaminobenzidine, which was visualized microscopically. Subjective assessment of the amount of membrane staining (0, 1+, 2+, 3+) was performed according to the manufacturer's package insert. Again, a minimum of 100 cells were evaluated in calculating the IHC score.

Statistical Analysis

The primary population was the EGF30001 intent-to-treat population (all randomly assigned patients who received one or more dose of study medication), and the primary end point was TTP (time from random assignment until disease progression or death because of disease under study). However, event-free survival (EFS; time from random assignment until disease progression or death due to any cause) was the clinical end point used for biomarker correlations in this analysis, as it is a more conservative end point. Kaplan Meier curves were generated for EFS and used to calculate median EFS. Estimates of treatment hazard ratios (HR) based on log-rank tests and 95% CIs were calculated. Treatment arms were compared using log-rank tests. The EFS analysis was repeated in the retrospectively defined subpopulations defined by hormone and/or EGFR and HER2 status. Interactions between hormone status and treatment arms were examined using Cox's proportional hazards regression to model EFS.

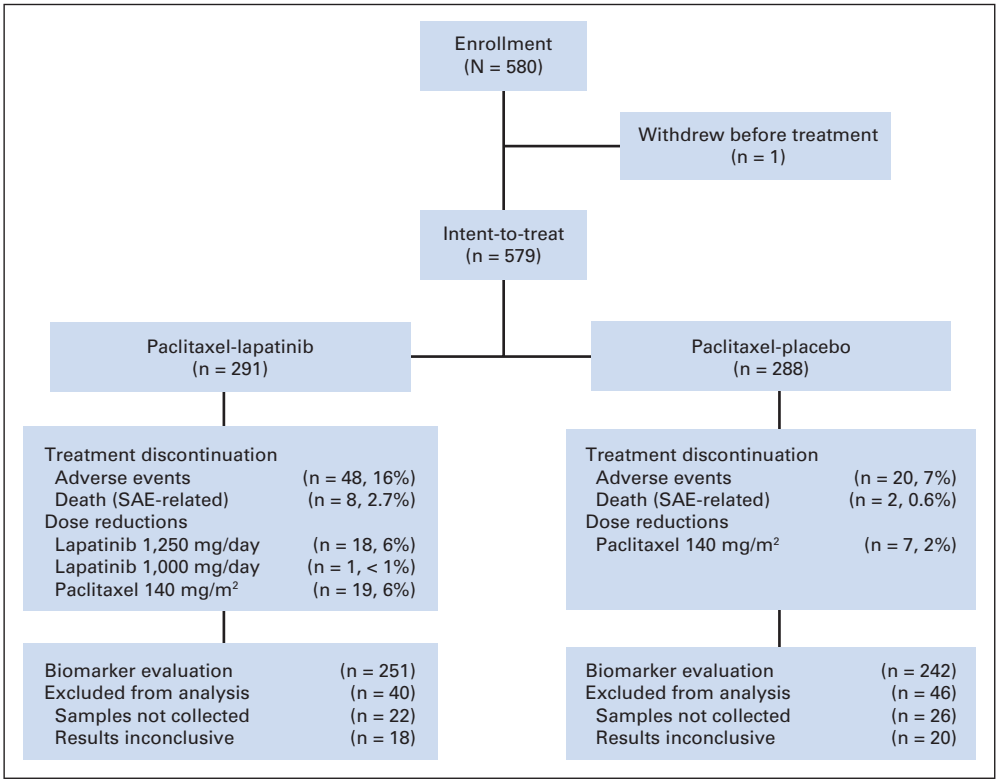

Fig 1. Patient enrollment and movement through the study. SAE, serious adverse event.

RESULTS

Patient Characteristics

Patient enrollment and characteristics have been presented previously (Fig 1; Appendix Table A1 [online only]).[16] Both treatment arms were well balanced on the basis of several clinical parameters.

Hormone Receptor Status and Response to Lapatinib in HER2-Amplified Breast Cancer

EGF30001 enrolled women with HER2-negative or unknown breast cancer. On central review, 86 patients were found to have HER2 amplification. Eighty-three were FISH positive, and three patients had 3+ IHC staining and no material available for FISH. The addition of lapatinib to paclitaxel significantly improved median EFS (8.1 *v* 5.0 months; $P = .008$; HR = 0.49; 95% CI, 0.3 to 0.8; Fig 2A). Benefit from lapatinib was also analyzed on the basis of hormone receptor (ER and PR) status. Whereas lapatinib showed some improvement in median EFS in HER2-amplified and either patients with ER- or PR-positive disease (n = 36; 5.7 *v* 4.5 months; $P = .351$; HR = 0.7; 95% CI, 0.34 to 1.45; Fig 2B), improvement was greater in hormone receptor–negative patients (n = 42; 8.3 *v* 5.0 months; $P = .007$; HR = 0.34; 95% CI, 0.17 to 0.7; Fig 2C). Interaction between treatment arm and hormone status (ER and PR negative, ER or PR positive) was not statistically significant ($P = .170$), but the test of the interaction term lacked power because of the limited sample size. There was no benefit from lapatinib in patients without HER2 amplification (n = 407) on the basis of that biomarker alone.

Hormone Receptor Status and Response to Lapatinib in HER2-Nonamplified Breast Cancer

The role of HER family inhibitors in HER2-nonamplified breast cancer is not defined. We analyzed EFS in patients with HER2-nonamplified disease as a function of ER and PR expression, which were calculated using the H-score as described in Methods. ER and PR expression in the non–HER2-amplified, hormone receptor–positive population is shown in Figure 3A. There was no correlation between ER and PR in this cohort, demonstrating that the expression of one is not a function of the other ($R^2 = 0.031$; Fig 3B). As hypothesized, we sought to determine whether PR expression in the ER-positive cohort was associated with benefit from lapatinib. Patients were subgrouped and blinded to clinical outcome by PR H-score into three predetermined groups: (1) H-score = 0, PR negative (n = 40); (2) H-score 1 to 50, PR weak (n = 50); and (3) H-score more than 50, PR strong (n = 133). These cutoffs were based on data from a presurgical study with gefitinib that identified an H-score in these ranges as being associated with response to an EGFR inhibitor.[17] In HER2-nonamplified, ER-positive patients (n = 223), median EFS improvement with the addition of lapatinib varied based on PR H-score: no improvement if PR-strong (n = 133; 9.3 *v* 7.3 months; $P = .373$; HR = 0.83; 95% CI, 0.55 to 1.25; Fig 4A); improvement if PR-weak (n = 50; 7.3 *v* 2.4 months; $P = .026$; HR = 0.49; 95% CI, 0.25 to 0.92; Fig 4B); and potential antagonism if PR-negative (n = 40; 3.7 *v* 7.2 months; $P = .004$; HR = 3.58; 95% CI, 1.58 to 8.12; Fig 4C). Interaction tests between treatment arm and the three strata of PR status (PR-strong, PR-weak, PR-negative) were highly significant ($P < .001$).

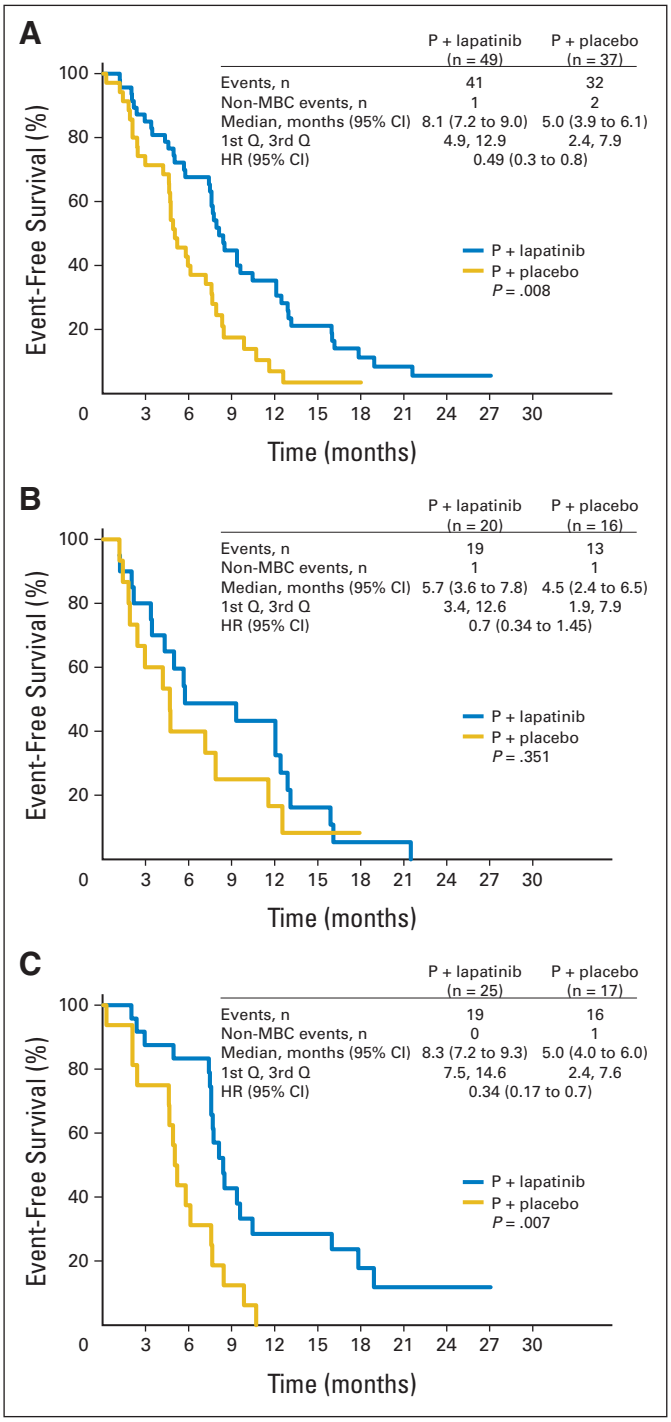

Fig 2. Hormone receptor status and response to lapatinib in human epidermal growth factor receptor 2 (HER2) –amplified breast cancer. (A) Lapatinib in patients with HER2 amplification. (B) Lapatinib in the subset that was either estrogen receptor positive or progesterone receptor positive. (C) Lapatinib in the HER2-amplified, hormone receptor–negative population. P, paclitaxel; MBC, metastatic breast cancer; 1stQ, first quartile; 3rdQ, third quartile; HR, hazard ratio.

Lapatinib in ER-/PR-/HER2-Negative (Triple Negative) Breast Cancer

Triple-negative breast cancer poses a unique clinical challenge, as there are no therapeutic agents tailored to this population.[23] In EGF30001, 131 patients were categorized as triple-negative (HER2

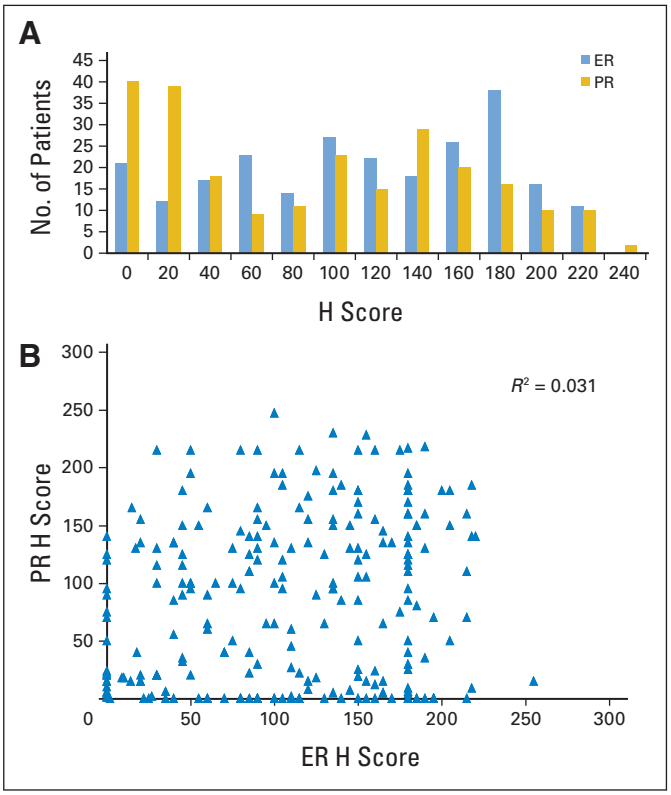

Fig 3. Estrogen receptor (ER) and progesterone receptor (PR) expression in patients with hormone receptor–positive, non–human epidermal growth factor receptor 2–amplified cancer. (A) Distribution of ER and PR in patients with hormone receptor–positive disease (ER or PR H-score > 0). ER: median, 110; mode, 180. PR: median, 90; mode, 0 (n = 246). (B) No relationship between ER and PR expression in this cohort.

FISH negative or IHC 0, 1, or 2+ when FISH was not available and H-score of 0 for ER and PR). There was no EFS improvement with the addition of lapatinib in this subgroup (4.6 *v* 4.8 months; $P = .255$; HR = 1.25; 95% CI, 0.85 to 1.83; Fig 5).

EGFR Expression and Response to Lapatinib

Table 1 shows the frequency of EGFR expression as measured by IHC. The majority (315 of 443; 71%) of patients did not have detectable expression (EGFR = 0). Both treatment arms were balanced for levels of EGFR expression; there were slightly more IHC 3+ patients in the lapatinib arm. Consistent with other EGFR-targeted agents,[24] EGFR expression was not predictive of improved median EFS with lapatinib (EGFR = 0; n = 315; 4.6 *v* 4.8 months; $P = .502$; Fig 6A; EGFR = 1+, 2+, 3+; n = 128; 4.8 *v* 4.9 months; $P = .968$; Fig 6B).

As previously reported, EGFR expression was highest in the triple-negative subgroup (Table 2).[19,20] However, even when the analysis was restricted to triple-negative cases, detectable levels of EGFR were not associated with improved median EFS with the addition of lapatinib (paclitaxel plus lapatinib, n = 67; 5.2 *v* 4.2 months; $P = .064$; Fig 6C; paclitaxel plus placebo, n = 59; 4.3 *v* 4.9 months; $P = .450$; Fig 6D).

DISCUSSION

Patient selection is critical to the clinical success of molecularly targeted agents. As seen from the results of this study, there was no overall

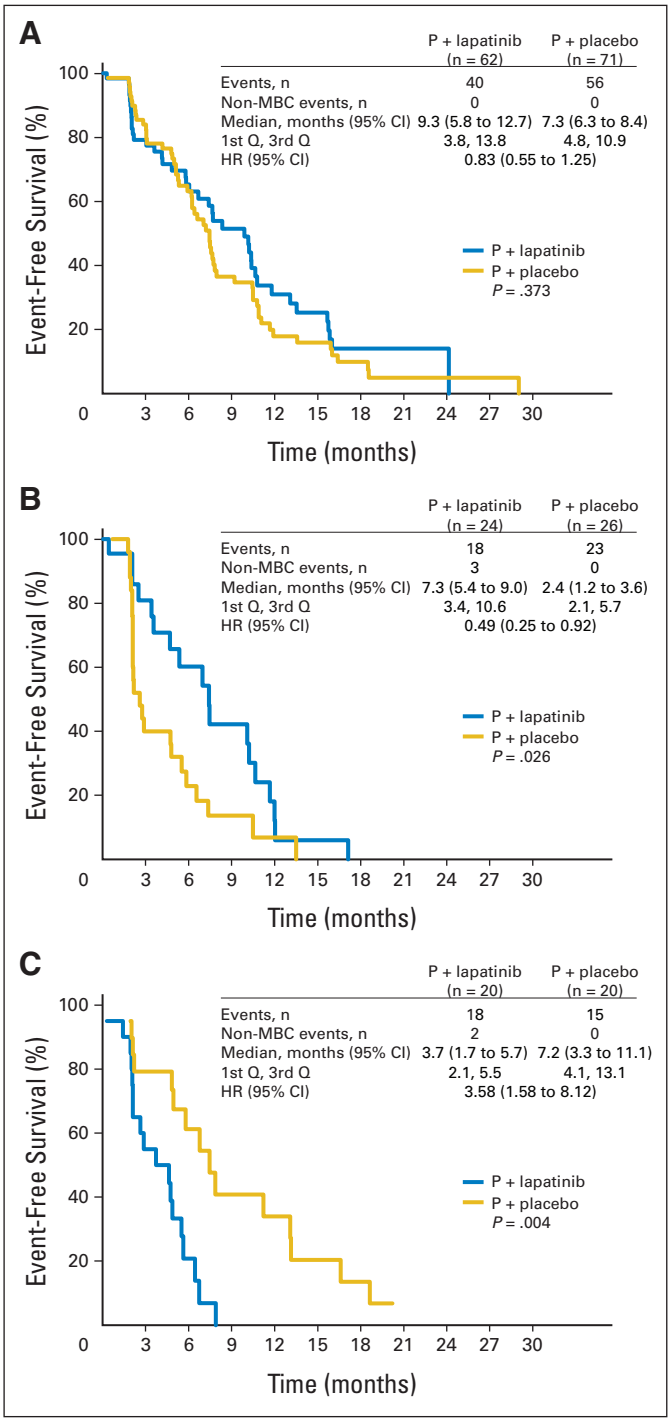

Fig 4. Hormone receptor status and response to lapatinib in women with human epidermal growth factor receptor 2 (HER2) –nonamplified cancer. Subgroup analysis of estrogen receptor–positive patients as a function of progesterone receptor (PR) status reveals a variable response based on PR expression. (A) PR strong, H-score more than 50, (B) PR weak, H-score 1 to 50, and (C) PR negative, H-score = 0. P, paclitaxel; MBC, metastatic breast cancer; 1stQ, first quartile; 3rdQ, third quartile; HR, hazard ratio.

benefit from adding lapatinib to paclitaxel in a mixed population of patients with HER2-negative or -unknown breast cancer. Clearly, when HER2-amplified patients were determined by central review, a significant benefit from lapatinib in the first-line metastatic setting was observed.[15] On the basis of a growing body of literature supporting ER

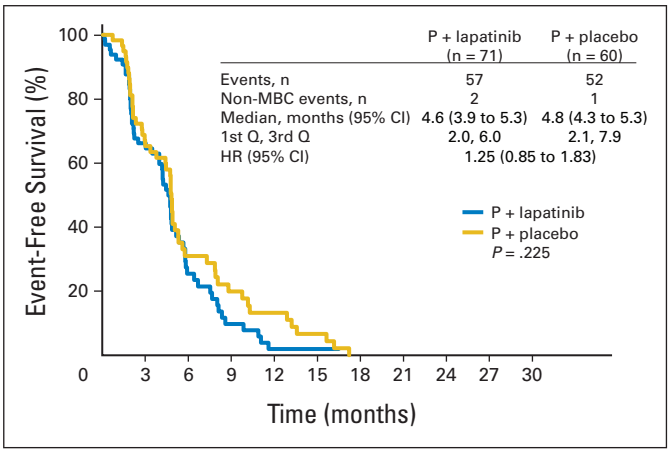

Fig 5. Lapatinib adds no benefit on estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2–negative breast cancer ("triple-negative"), n = 131. Abbreviations: P, paclitaxel; MBC, metastatic breast cancer; 1stQ, first quartile; 3rdQ, third quartile; HR, hazard ratio.

and PR expression and its relationship to EGFR and HER2 signaling, it was hypothesized that measurement of these receptors may identify a subgroup of patients who may benefit from lapatinib.

Significant cross-talk between the HER and steroid receptor families has been described.[25,26] HER2-driven breast cancers may be more aggressive and carry a worse prognosis than HER2-negative cancers, which are usually ER-positive tumors.[27] In addition, elevated EGFR expression and HER2 amplification have been associated with a worse response to hormone blockade,[17,25] and an inverse correlation has been shown between HER2 and ER and PR expression.[28] Analysis of 86 patients with HER2-amplified disease and their response as a function of hormone receptor status demonstrated that patients with hormone receptor–negative tumors derived greater benefit from lapatinib than patients with either ER- or PR-positive tumors. The observation of a potential interaction between hormone receptor status and response (or resistance) to lapatinib has not been previously described. We were able to ask this question in the front-line setting because these patients were thought to be HER2 negative based on local assessment. Whether this relationship between hormone receptor status and response to lapatinib exists in women who have received prior trastuzumab is unknown. Although these subgroups are small, these findings raise the question of whether the complete loss of steroid receptors identifies a group of patients with greater dependence on HER family signaling. A correlation between HER2 status

Table 1. Intensity of EGFR Expression by Immunohistochemistry: Treatment Arm

EGFR IHC Score	P + Lapatinib		P + Placebo		Total	
	No.	%	No.	%	No.	%
0	152	34.3	163	36.7	315	71.1
1+	43	9.7	37	8.4	80	18.1
2+	16	3.6	14	3.2	30	6.8
3+	12	2.7	6	1.2	18	4.1
Total	223	50.3	220	49.7	443	

Abbreviations: EGFR, epidermal growth factor receptor; IHC, immunohistochemistry; P, paclitaxel.

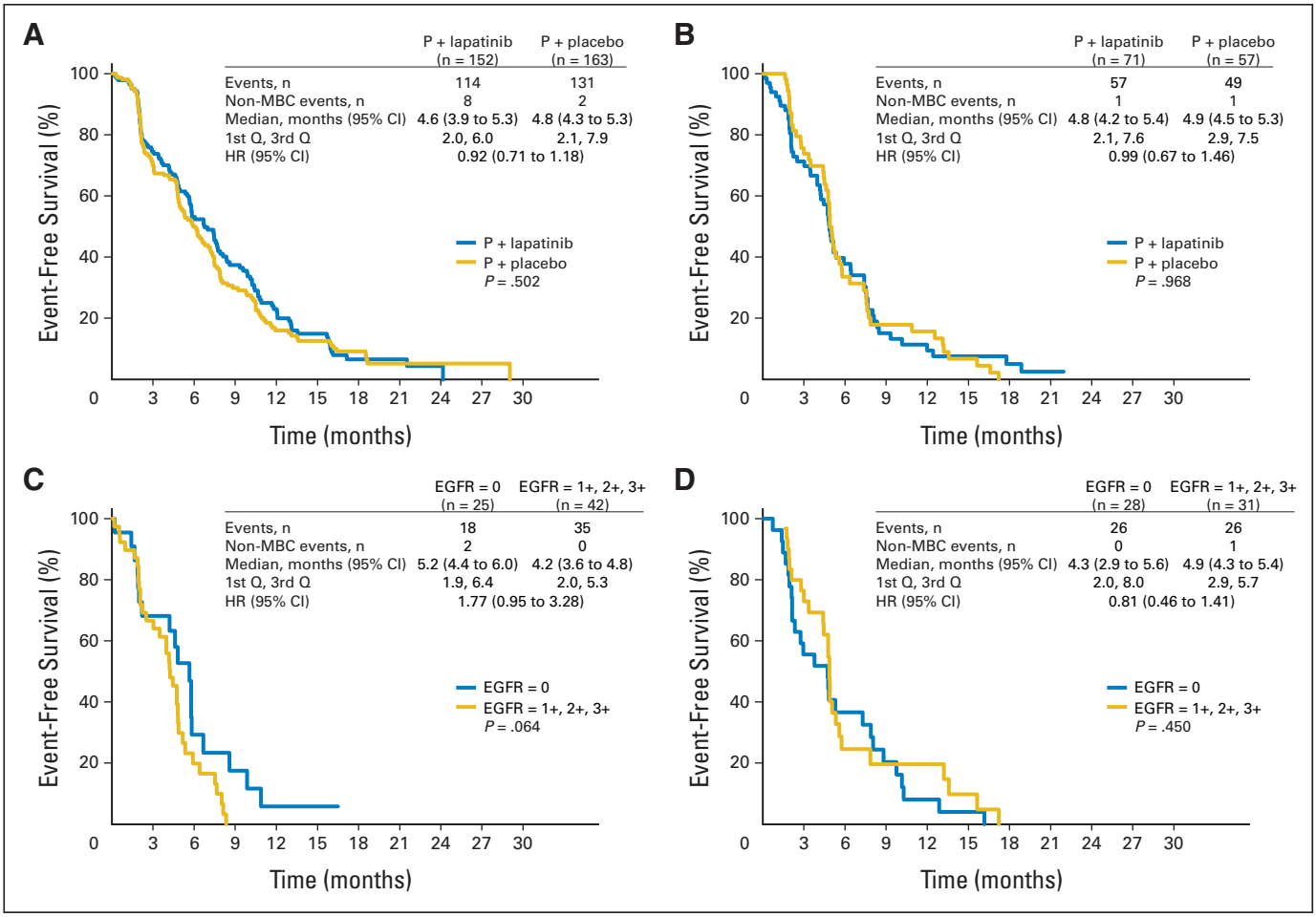

Fig 6. Epidermal growth factor receptor (EGFR) expression does not affect response to lapatinib in a non–human epidermal growth factor receptor 2–amplified cohort. (A) EGFR = 0; (B) EGFR = 1, 2, or 3+. In patients with triple-negative breast cancer, EGFR expression does not predict for benefit in either the (C) paclitaxel (P) + lapatinib group or (D) P + placebo group. MBC, metastatic breast cancer; 1stQ, first quartile; 3rdQ, third quartile; HR, hazard ratio.

and hormone receptors exists, and studies have shown that patients with HER2-positive and hormone receptor–positive tumors are less dependent on hormone signaling and respond less well to hormone manipulation.[25,29] Still, there is a need to identify those patients with HER2-amplified disease who respond to lapatinib, and this hypothesis will be studied in a large, placebo-controlled study of letrozole with or without lapatinib (EGF30008, NCT #00073528). Of note, contrasting recent data suggest that patients with normal HER2 status may benefit from adjuvant trastuzumab in early breast cancer.[30] In EGF30001, there was no evidence of activity of lapatinib in the large unselected HER2-negative population when evaluated in the metastatic setting.

The largest patient group (n = 243) in this study had HER2-negative, hormone receptor–positive tumors. The majority of these

Table 2. Intensity of EGFR Expression by Immunohistochemistry: HER2 and Hormone Receptor Status

EGFR IHC Score	No. of Patients (n = 455)				
	ER Positive or PR Positive (n = 282)		ER Negative and PR Negative (n = 173)		
	HER2 Positive (n = 36)	HER2 Negative (n = 246)	HER2 Positive (n = 42)	HER2 Negative (n = 131)	Total
0	30	207	23	53	313
1+	6	18	10	43	77
2+	0	6	5	18	29
3+	0	4	2	12	18
Total IHC > 0	6	28	17	73	124
Not available	0	11	2	5	18

Abbreviations: EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor; IHC, immunohistochemistry; ER, estrogen receptor; PR, progesterone receptor.

patients were ER positive, with variable levels of PR (*v* only PR positive; n = 20). We hypothesized that PR loss in ER-positive patients may be associated with a benefit from lapatinib in this group of 223 patients. It was initially demonstrated that ER and PR expression were independent variables. Next cutoffs for PR-expression subgroups were blindly selected and designated as described in the Methods section. These designations, as previously experienced,[18] were based on hypotheses that (1) having no detectable PR was biologically significant; (2) there is a unique patient subgroup identified by a lower H-score, as can be seen by the increased number of patients in this range; (3) this subgroup is more likely to benefit from EGFR inhibition, as previously seen.[18] The small subgroup size limits this analysis, and conclusions may reflect sample size rather than true biology; however, the ER-positive, PR-weak group demonstrated a significant benefit from the addition of lapatinib. It is also in ER-positive, PR-negative patients that responses were seen to the EGFR small-molecule inhibitors gefitinib and erlotinib in phase II and presurgical studies,[18,31-33] providing precedent for linking EGFR dependence to hormone receptor status. Laboratory studies have identified a role for RTK signaling via PI3-kinase in modifying PR expression. In HER2-negative breast cancer cells, insulin-like growth factor and the HER family ligands EGF and heregulin downregulated PR expression.[34] Furthermore, a recent study has shown that HER family signaling through PI3-kinase and AKT can decrease PR expression.[35] Together, these data suggest that in the absence of amplification, decreased PR expression may be the readout for EGFR activation and dependence.

The question of whether there is true antagonism in the ER-positive, PR-negative group needs further clarification and may be an artifact of the small subgroup size. Alternatively, this represents a true biologic effect of lapatinib that is unique to those cancers that have no detectable PR expression by immunohistochemistry as compared with those that have weak PR expression. In the neoadjuvant study performed with gefitinib, only six samples were ER positive and PR negative; five of six showed no molecular response to gefitinib, and one showed molecular inhibition.[18]

Despite data supporting EGFR as a therapeutic target in breast cancer, the predictive marker for those patients who will respond to EGFR inhibition remains elusive. To answer this important question, ER, PR, and EGFR were analyzed in the large cohort of cases with HER2-negative tumors. Like other studies, the highest levels of EGFR expression were in ER-, PR-, and HER2-negative patients (triple negatives). These patients have only chemotherapy available as a treatment option and are in need of new approaches. In our analysis, this group did not benefit from the addition of lapatinib to paclitaxel, suggesting that EGFR may not be a good target for this population. Ongoing studies with the EGFR monoclonal antibody cetuximab are in progress in combination with platinum salts.[36,37] It is hoped these patients may respond to an antibody approach or benefit from the potential synergy between platinum salts and EGFR inhibition.[38,39] Furthermore, it is possible that EGFR is still a potential target, but additional selection criteria (ie, more accurate biomarkers) are necessary. There was no group in our study in which EGFR measurements by IHC predicted benefit from the addition of lapatinib.

This study adds to the growing data describing a relationship between the HER family and steroid receptor signaling. The conclusions drawn are limited by the progressively small subgroups analyzed and, even though blinded, the study's retrospective nature. Still, it represents a biomarker analysis of the largest prospective trial of a HER2 or EGFR inhibitor (and the only dual-kinase inhibitor studied) in patients with non–HER2-amplified breast cancer. These data can be used to guide further biomarker assessments of ongoing lapatinib and EGFR inhibitor studies and, if validated in EGF30008, may ultimately guide patient selection in a prospective study.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: Michael Arbushites, GlaxoSmithKline (C); Maria Koehler, GlaxoSmithKline (C); Cristina Oliva, GlaxoSmithKline (C); Lisa S. Williams, GlaxoSmithKline (C) **Consultant or Advisory Role:** Michael F. Press, Genentech (C), GlaxoSmithKline (C); Angelo Di Leo, GlaxoSmithKline (C) **Stock Ownership:** Michael Arbushites, GlaxoSmithKline; Maria Koehler, GlaxoSmithKline; Cristina Oliva, GlaxoSmithKline; Lisa S. Williams, GlaxoSmithKline **Honoraria:** Richard S. Finn, GlaxoSmithKline; Michael F. Press, Genentech, GlaxoSmithKline; Angelo Di Leo, GlaxoSmithKline **Research Funding:** None **Expert Testimony:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Richard S. Finn, Michael F. Press, Judy Dering, Maria Koehler, Angelo Di Leo
Provision of study materials or patients: Richard S. Finn, Michael Arbushites, Maria Koehler, Cristina Oliva, Lisa S. Williams, Angelo Di Leo
Collection and assembly of data: Richard S. Finn, Michael F. Press, Judy Dering, Michael Arbushites, Maria Koehler, Cristina Oliva, Lisa S. Williams, Angelo Di Leo
Data analysis and interpretation: Richard S. Finn, Michael F. Press, Judy Dering
Manuscript writing: Richard S. Finn, Michael F. Press, Judy Dering, Michael Arbushites, Maria Koehler, Cristina Oliva, Lisa S. Williams, Angelo Di Leo
Final approval of manuscript: Richard S. Finn, Michael F. Press, Judy Dering, Michael Arbushites, Maria Koehler, Cristina Oliva, Lisa S. Williams, Angelo Di Leo

REFERENCES

1. Johnston JB, Navaratnam S, Pitz MW, et al: Targeting the EGFR pathway for cancer therapy. Curr Med Chem 13:3483-3492, 2006

2. Slamon DJ, Clark GM, Wong SG, et al: Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235:177-182, 1987

3. Slamon DJ, Godolphin W, Jones LA, et al: Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 244:707-712, 1989

4. Salomon DS, Brandt R, Ciardiello F, et al: Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 19:183-232, 1995

5. Baselga J, Albanell J, Ruiz A, et al: Phase II and tumor pharmacodynamic study of gefitinib in patients with advanced breast cancer. J Clin Oncol 23:5323-5333, 2005

6. Francis P, Green M, Gebski V, et al: Gefitinib has a low clinical benefit rate in advanced breast cancer patients. Presented at the 28th Annual San Antonio Breast Cancer Symposium, San Antonio, TX, December 8-11, 2005 (abstr 4080)

7. Hirsch FR, Varella-Garcia M, Bunn PA Jr, et al: Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non–small-cell lung cancer. J Clin Oncol 24:5034-5042, 2006

8. Lynch TJ, Bell DW, Sordella R, et al: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 350:2129-2139, 2004

9. Paez JG, Janne PA, Lee JC, et al: EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. Science 304:1497-1500, 2004

10. Konecny GE, Pegram MD, Venkatesan N, et al: Activity of the dual kinase inhibitor lapatinib (GW572016) against HER-2-overexpressing and trastuzumab-treated breast cancer cells. Cancer Res 66:1630-1639, 2006

11. Rusnak DW, Alligood KJ, Mullin RJ, et al: Assessment of epidermal growth factor receptor (EGFR, ErbB1) and HER2 (ErbB2) protein expression levels and response to lapatinib (Tykerb, GW572016) in an expanded panel of human normal and tumour cell lines. Cell Prolif 40:580-594, 2007

12. Xia W, Gerard CM, Liu L, et al: Combining lapatinib (GW572016), a small molecule inhibitor of ErbB1 and ErbB2 tyrosine kinases, with therapeutic anti-ErbB2 antibodies enhances apoptosis of ErbB2-overexpressing breast cancer cells. Oncogene 24: 6213-6221, 2005

13. Gomez HL, Doval DC, Chavez MA, et al: Efficacy and safety of lapatinib as first-line therapy for ErbB2-amplified locally advanced or metastatic breast cancer. J Clin Oncol 26:2999-3005, 2008

14. Cameron D, Casey M, Press M, et al: A phase III randomized comparison of lapatinib plus capecitabine versus capecitabine alone in women with advanced breast cancer that has progressed on trastuzumab: Updated efficacy and biomarker analyses. Breast Cancer Res Treat 112:533-543, 2008

15. Geyer CE, Forster J, Lindquist D, et al: Lapatinib plus capecitabine for HER2-positive advanced breast cancer. N Engl J Med 355:2733-2743, 2006

16. Di Leo A, Gomez H, Aziz Z, et al: Phase III, double-blind, randomized study comparing lapatinib plus paclitaxel with placebo plus paclitaxel as first-line treatment for metastatic breast cancer. J Clin Oncol 26:5544-5552, 2008

17. Arpino G, Weiss H, Lee AV, et al: Estrogen receptor-positive, progesterone receptor-negative breast cancer: Association with growth factor receptor expression and tamoxifen resistance. J Natl Cancer Inst 97:1254-1261, 2005

18. Finn RS, Dering J, Ginther C, et al: ER+PR– breast cancer defines a unique subtype of breast cancer that is driven by growth factor signaling and may be more likely to respond to EGFR targeted therapies. J Clin Oncol 24:6s, 2006 (suppl; abstr 514)

19. Hoadley KA, Weigman VJ, Fan C, et al: EGFR associated expression profiles vary with breast tumor subtype. BMC Genomics 8:258, 2007

20. Nielsen TO, Hsu FD, Jensen K, et al: Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 10:5367-5374, 2004

21. Press MF, Sauter G, Bernstein L, et al: Diagnostic evaluation of HER-2 as a molecular target: An assessment of accuracy and reproducibility of laboratory testing in large, prospective, randomized clinical trials. Clin Cancer Res 11:6598-6607, 2005

22. Press MF, Slamon DJ, Flom KJ, et al: Evaluation of HER-2/neu gene amplification and overexpression: Comparison of frequently used assay methods in a molecularly characterized cohort of breast cancer specimens. J Clin Oncol 20:3095-3105, 2002

23. Cleator S, Heller W, Coombes RC: Triple-negative breast cancer: Therapeutic options. Lancet Oncol 8:235-244, 2007

24. Clark GM, Zborowski DM, Culbertson JL, et al: Clinical utility of epidermal growth factor receptor expression for selecting patients with advanced non-small cell lung cancer for treatment with erlotinib. J Thorac Oncol 1:837-846, 2006

25. Arpino G, Wiechmann L, Osborne CK, et al: Crosstalk between the estrogen receptor and the HER tyrosine kinase receptor family: Molecular mechanism and clinical implications for endocrine therapy resistance. Endocr Rev 29:217-233, 2008

26. Yarden Y, Sliwkowski MX: Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2:127-137, 2001

27. Sørlie T, Perou CM, Tibshirani R, et al: Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 98:10869-10874, 2001

28. Konecny G, Pauletti G, Pegram M, et al: Quantitative association between HER-2/neu and steroid hormone receptors in hormone receptor-positive primary breast cancer. J Natl Cancer Inst 95:142-153, 2003

29. Dowsett M, Houghton J, Iden C, et al: Benefit from adjuvant tamoxifen therapy in primary breast cancer patients according oestrogen receptor, progesterone receptor, EGF receptor and HER2 status. Ann Oncol 17:818-826, 2006

30. Paik S, Kim C, Jeong J, et al: Benefit from adjuvant trastuzumab may not be confined to patients with IHC 3+ and/or FISH-positive tumors: Central testing results from NSABP B-31. J Clin Oncol 25:5s, 2007 (suppl; abstr 511)

31. Ciardiello F, Troiani T, Caputo F, et al: Phase II study of gefitinib in combination with docetaxel as first-line therapy in metastatic breast cancer. Br J Cancer 94:1604-1609, 2006

32. Guix M, Granja N, de M, Meszoely I, et al: Short preoperative treatment with erlotinib inhibits tumor cell proliferation in hormone receptor-positive breast cancers. J Clin Oncol 26:897-906, 2008

33. Polychronis A, Sinnett HD, Hadjiminas D, et al: Preoperative gefitinib versus gefitinib and anastrozole in postmenopausal patients with oestrogen-receptor positive and epidermal-growth-factor-receptor-positive primary breast cancer: A double-blind placebo-controlled phase II randomised trial. Lancet Oncol 6:383-391, 2005

34. Cui X, Zhang P, Deng W, et al: Insulin-like growth factor-I inhibits progesterone receptor expression in breast cancer cells via the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin pathway: Progesterone receptor as a potential indicator of growth factor activity in breast cancer. Mol Endocrinol 17:575-588, 2003

35. Tokunaga E, Oki E, Kimura Y, et al: Coexistence of the loss of heterozygosity at the PTEN locus and HER2 overexpression enhances the Akt activity thus leading to a negative progesterone receptor expression in breast carcinoma. Breast Cancer Res Treat 101:249-257, 2007

36. Carey LA, Mayer E, Marcom PK, et al: TBCRC 001: EGFR inhibition with cetuximab in metastatic triple negative (basal-like) breast cancer. Presented at 30th Annual San Antonio Breast Cancer Symposium, San Antonio, TX, December 13-16, 2007 (abstr 307)

37. O'Shaughnessy J, Weckstein DJ, Vukekja SJ, et al: Preliminary results of a randomized phase II study of weekly irinotecan/carboplatin with or without cetuximab in patients with metastatic breast cancer. Presented at 30th Annual San Antonio Breast Cancer Symposium, San Antonio, TX, December 13-16, 2007 (abstr 308)

38. Aboud-Pirak E, Hurwitz E, Pirak ME, et al: Efficacy of antibodies to epidermal growth factor receptor against KB carcinoma in vitro and in nude mice. J Natl Cancer Inst 80:1605-1611, 1988

39. Fan Z, Baselga J, Masui H, et al: Antitumor effect of anti-epidermal growth factor receptor monoclonal antibodies plus cis-diamminedichloroplatinum on well established A431 cell xenografts. Cancer Res 53:4637-4642, 1993

