Published in final edited form as:
Clin Cancer Res. 2011 March 1; 17(5): 1147–1159. doi:10.1158/1078-0432.CCR-10-1869.

Gefitinib or Placebo in Combination with Tamoxifen in Patients with Hormone Receptor-Positive Metastatic Breast Cancer: a Randomized Phase II Study

C. Kent Osborne[1], **Patrick Neven**[2], **Luc Y. Dirix**[3], **John R. Mackey**[4], **Jean Robert**[5], **Craig Underhill**[6], **Rachel Schiff**[1], **Carolina Gutierrez**[1], **Ilenia Migliaccio**[1], **Valsamo K. Anagnostou**[7], **David L. Rimm**[7], **Patrick Magill**[8], and **Mark Sellers**[8]

[1]Lester and Sue Smith Breast Center and Department of Pathology, Baylor College of Medicine, Houston, TX, USA

[2]Multidisciplinary Breast Centre, UZ Leuven, Leuven, Belgium

[3]AZ Sint-Augustinus, Antwerp, Belgium

[4]Cross Cancer Institute, Edmonton, Canada

[5]CHAUQ Hospital du St-Sacrement, Quebec, Canada

[6]Border Medical Oncology, Wodonga, Australia

[7]Department of Pathology, Yale University School of Medicine

[8]AstraZeneca, Macclesfield, UK

Abstract

Purpose—Increased growth factor signaling may contribute to tamoxifen resistance. This randomized Phase II trial assessed tamoxifen plus placebo or the EGFR inhibitor gefitinib in ER-positive metastatic breast cancer.

Experimental Design—Patients with newly metastatic disease or recurring after adjuvant tamoxifen (Stratum 1, St1), or recurred during/after adjuvant aromatase inhibitor (AI) or after failed first-line AI (Stratum 2, St2) were eligible. Primary variables were progression-free survival (PFS) (St1) and clinical benefit rate (CBR) (St2). A ≥ 5% improvement in response variables with gefitinib was considered to warrant further investigation. Outcome was correlated with biomarkers measured on the primary tumor.

Corresponding author: C. Kent Osborne, MD, Lester and Sue Smith Breast Center, Baylor College of Medicine, One Baylor Plaza, MS 600, Houston, TX 77030, USA, Tel: +1 713 798 1641, Fax: +1 713 798 1642, kosborne@bcm.edu.

INVESTIGATORS: *Argentina*— M. Abraham, E. Batagelj, C. Blajman, A. Ferro, G. López, M. Matwiejuk, N. Pilnik, R. Wainstein, J.J. Zarbá; *Australia*— M. Friedlander, J. Stewart, C. Underhill, M. White, N. Wilken; *Belgium*— L. Dirix, P. Neven, M. Piccart; *Brazil*— A. Anelli, J. Biazus, J. Bines, M. Caleffi, B. Ferrari, N.G. Skare, C. Tosello de Oliveira, N.H. Yamaguchi; *Canada*— M. Burnell, P. Dube, R. Haq, J. Mackey, A. Paterson, J. Robert, S. Verma; *Denmark*— G. Tuxen; *France*— I. Ray-Coquard, E. Teissier, C. Veyret; *Germany*— M. Clemens, W. Eiermann, W. Jonat, M. Kaufmann, S. Loibl, K. Ruffert, A. Schneeweiss; *South Africa*— J. Apffelstaedt, S. Fourie, J. Jordaan, G. McMichael, E. Murray, J. Raats, B. Smit, D. Vorobiof, D. Werner; *Spain*— E.A. Aguilar, H. Cortes-Funes, C. Crespo, R. Cubedo, L.G. Estevez, X.F. Mayol, A.A. Torres; *United Kingdom*— J. Dewar, R. Leonard, P. Murray, J. Robertson, A. Wardley; *United States*—F. Arena, W. Audeh, D. Hyams, D. Irwin, R. Kerr, P. Klein, R. Kramer, J. Kroener, E. Lester, A. Lyss, G. Miletello, Y. Novik, K. Osborne, D. Rinaldi, J. Thropay, I. Wiznitzer.

Patrick Magill and Mark Sellers are salaried employees of AstraZeneca and own equity in AstraZeneca. David L Rimm is a co-founder of, consultant to, and stockholder in HistoRx Inc. (Branford, CT, USA). C. Kent Osborne and Rachel Schiff have received minor honoraria as consultants to AstraZeneca.

Results—In St1 (n=206), the PFS hazard ratios (HR, gefitinib:placebo) were 0.84 (95% CI, 0.59 to 1.18; median PFS 10.9 *v* 8.8 months). In the St1 endocrine therapy naïve subset (n=158) the HR was 0.78 (95% CI, 0.52 to 1.15), and the prior endocrine-treated subgroup (n=48) 1.47 (95% CI, 0.63 to 3.45). In St1, CBRs were 50.5% with gefitinib and 45.5% with placebo. In St2 (n=84), CBRs were 29.2% with gefitinib and 31.4% with placebo. Biomarker analysis suggested that in St1 there was greater benefit with gefitinib in patients who were ER negative or had lower levels of ER protein.

Conclusions—In St1, the improved PFS with gefitinib plus tamoxifen met the protocol criteria sufficient to warrant further investigation of this strategy. In St2, there was a numerical disadvantage for gefitinib; additional investigation after AI therapy is not warranted. Studies of predictive biomarkers are needed to subset appropriate patients.

Keywords

gefitinib; tamoxifen; hormone-receptor positive metastatic breast cancer; biomarkers; estrogen receptor

Introduction

Targeting the estrogen receptor (ER) is a very effective adjuvant therapy for patients with ER-positive tumors (1,2). Tamoxifen, one of the most frequently used endocrine therapies, is a selective ER modulator that displays ER antagonist activity in the breast and ER agonist activity in other tissues (3). It is widely used for adjuvant endocrine therapy in pre-menopausal patients and is still used frequently in post-menopausal patients. Aromatase inhibitors (AIs) target ER by reducing estrogen levels in postmenopausal patients. These drugs are slightly more effective than tamoxifen in both the metastatic and adjuvant therapy settings (2,4). Currently AIs are prescribed after several years of tamoxifen adjuvant therapy or instead of tamoxifen in some patients. Despite the effectiveness of endocrine therapy, *de novo* and acquired resistance remain problematic.

Compelling data suggest that increased growth factor signaling, in particular the epidermal growth factor receptor (EGFR)/cErbB2 (HER2) pathway, contributes to resistance to ER targeting therapies (5-12). Clinical studies have reported that breast cancers overexpressing EGFR or HER2 are less likely to benefit from tamoxifen or AIs (5-8). Preclinical studies also support this hypothesis. We reported that HER2 overexpression in a xenograft model results in tamoxifen-stimulated growth as a mechanism of *de novo* resistance (9-12). Blocking the EGFR/HER2 pathway with gefitinib, an EGFR tyrosine kinase inhibitor, or with the anti-HER2 antibody trastuzumab restores tamoxifen's antagonist activity and inhibits tumor growth (9,10). These data suggest that ER and the HER family cooperate to provide the dominant survival signals in some tumors and that effective treatment requires blockade of both pathways.

EGFR/HER2 signaling may also be important for acquired resistance to tamoxifen and other endocrine therapies in ER-positive tumors which do not initially overexpress EGFR/HER2. Several small biomarker studies in patients progressing after receiving endocrine therapy have shown increased levels of HER2 in post-endocrine therapy tumor biopsies, circulating tumor cells, or serum (13-15). Laboratory studies have also shown that long-term tamoxifen treatment of ER-positive cells results in higher levels of both EGFR and HER2 at the time of drug resistance (16-21). We reported that increasing levels of EGFR/HER2 caused acquired tamoxifen resistance in a xenograft model by a mechanism similar to the *de novo* resistance observed in tumors initially expressing high HER2 levels (16). Furthermore, the addition of gefitinib to tamoxifen significantly delayed the onset of acquired resistance in these tumors

just as it did in tumors initially overexpressing HER2. Similar data were reported using an *in vitro* model system (18).

These data provide a rationale for combining HER inhibitors with tamoxifen or other endocrine therapies not only in patients with ER-positive, HER2-overexpressing tumors but even in those with tumors initially expressing low levels of EGFR/HER2. We therefore initiated the first clinical study of this new strategy, evaluating tamoxifen alone versus tamoxifen plus gefitinib in patients with ER- or progesterone receptor (PgR)-positive, HER2-positive or negative metastatic breast cancer. A variety of receptors and signaling molecules expressed in the original primary tumors were also investigated for a relationship to gefitinib benefit.

Materials and Methods

Study Design

This Phase II, randomized, double-blind, stratified, multicenter, parallel-group study (NCT00229697) evaluated the efficacy and safety of adding gefitinib to tamoxifen among women with ER/PgR-positive metastatic breast cancer regardless of HER2 status. Randomization was stratified by country. The study was conducted in 54 centers in 12 countries (Argentina, Australia, Belgium, Brazil, Canada, Denmark, France, Germany, South Africa, Spain, United Kingdom, and United States).

Patients were stratified into two groups based on prior hormonal therapy. Stratum 1 included women with newly-diagnosed metastases or those who had recurred ≥ 1 year after stopping adjuvant therapy with tamoxifen. Stratum 2 included women with recurrent disease during or after adjuvant AI or those progressing after first-line AI treatment for metastatic disease. Both groups were included in Stratum 2 because the mechanisms of AI resistance would likely be similar.

Patients

Pre- or post-menopausal women aged ≥ 18 years with metastatic breast cancer and a World Health Organization (WHO) performance status (PS) of 0, 1, or 2 were eligible. Patients with either non-measurable or measurable disease as per Response Evaluation Criteria in Solid Tumors (RECIST) were eligible.

Patients were not eligible if a sample from the original tumor (or metastatic site) was unavailable and/or could not be submitted for central determination of ER and PgR status, or if they had received prior chemotherapy for metastatic disease. Other exclusion criteria included: recurrence within 1 year of adjuvant treatment with tamoxifen; current hormonal replacement therapy or therapy with a luteinizing hormone releasing hormone analog; rapidly progressive visceral, central nervous system or liver metastases or significant symptomatic lymphangitic pulmonary metastases; known hypersensitivity to either study drug; other malignancies diagnosed within the last 5 years with the exception of basal cell carcinoma or cervical cancer *in situ*; pregnancy or breastfeeding; treatment with an investigational drug within 30 days or prior treatment with a TKI. Concomitant use of phenytoin, carbamazepine, rifampicin, barbiturates, or St John's Wort was prohibited.

All patients provided written, informed consent, and approval for the study was obtained from independent ethics committees. The study was conducted in accordance with the Declaration of Helsinki and applicable International Conference on Harmonisation/Good Clinical Practice requirements.

Study Procedures

Patients were randomized (1:1) to receive tamoxifen (20 mg/day orally) plus gefitinib (250 mg/day orally) or tamoxifen (20 mg/day orally) plus placebo until clinical or objective progression (RECIST), unacceptable toxicity or patient withdrawal from the study.

The randomization was prepared by AstraZeneca (Shared Services Group in Statistical Programming). Once a center had confirmed patient eligibility, the center assigned the next sequential randomization patient code for the patient's stratification group and notified a Centralized Registration Desk who monitored recruitment and notified centers if a stratum was complete. Tablets and packaging of gefitinib and placebo were visually identical to ensure blinding. The treatment code, indicating the treatment randomization for each patient, was not to be broken except in a medical emergency. Dose reductions of randomized treatment or tamoxifen were not permitted; dose interruptions of up to 14 days were allowed if necessary for treatment toxicity.

Plasma concentrations of study drug were determined using validated high performance liquid chromatography methods with tandem mass spectrometric detection.

In Stratum 1, the primary response variable was progression-free survival (PFS; defined as time to progressive disease or death) an endpoint similar to our xenograft model showing benefits to the addition of gefitinib to tamoxifen (16). In Stratum 2, the primary response variable was clinical benefit rate (CBR; defined as complete response [CR], partial response [PR], or stable disease [SD] for ≥ 24 weeks using RECIST). This variable was chosen because of the high frequency of *de novo* resistance to tamoxifen after progression on AIs. Secondary response variables included CBR in Stratum 1, PFS in Stratum 2. PFS was also assessed in the subset of patients with tumors overexpressing HER2. Secondary variables included safety and pharmacokinetic analysis with steady-state trough concentrations (C_{min}) determined for tamoxifen and gefitinib. All of these analyses were preplanned. In addition, a retrospective analysis was performed in patients in Stratum 1 according to prior endocrine therapy treatment. Analysis of PFS and ORR were performed in endocrine therapy-naïve patients and patients with prior endocrine therapy. Tumor assessments were performed at baseline and every 3 months until progression. Adverse events (AEs) were assessed according to the Common Toxicity Criteria (CTC) of the National Cancer Institute (Version 3.0).

Paraffin blocks from the primary tumor and/or a metastasis were assessed for ER, PgR, and HER2 expression by methods and scoring system described previously in a central laboratory (Breast Center, Baylor College of Medicine, Houston, TX) (22,23). ER and PgR Allred scores ≥ 3 were considered positive. Because the level of HER2 expression needed to reduce benefit from endocrine therapy is not known and may not require the very high levels associated with gene amplification and response to tratuzumab, in a preplanned analysis we chose an Allred score of ≥ 3 out of 8 (equated to 2+ and 3+ by other methods) as positive for this study. In our preclinical model, increases in EGFR and HER2 expression were observed on tamoxifen but the level of expression was still below levels in cell lines amplified for the gene (16). In another analysis we restricted HER2 positivity to the subset of tumors with an Allred score of ≥ 6, a level that correlates with amplification. Six patients whose tumors were negative for both ER and PgR on central laboratory evaluation were discontinued from the study, although they were included in the intent-to-treat (ITT) analysis. Only 18% of patients had metastatic tumors available for assay.

Blood samples were collected from each patient at 1, 3, 6, and 9 months for pharmacokinetic analyses

Exploratory Biomarker Analyses

After marking cancer-containing areas of the paraffin blocks from the original tumor sample, triplicate 0.6 mm punches were taken and placed in three separate tissue microarrays containing up to 121 specimens per slide. The arrays also contained multiple marker cores to orient the pathologists (I.M., C.G.) scoring the slides who were blinded to patient treatment and outcome. All biomarker studies were pre-planned.

Immunohistochemistry Analyses—In addition to ER, PgR and HER2, p-ERK 1/2 MAPK, p-Akt, and p27 were measured as previously described (13,23). For ERK, Akt and p-27 only the nuclear signal was scored.

Fluorescence Assays—ER and EGFR were also quantified by a more sensitive quantitative fluorescence assay (24,25). Because non-nuclear ER has been hypothesized to play a role in growth factor receptor signaling, fluorescence in both the nuclear and non-nuclear compartments was measured (9). EGFR is predominantly a membrane protein, and, therefore, only the non-nuclear fluorescence was considered. For these analyses the arrays were deparaffinized, rehydrated, and antigen-retrieved by pressure cooking in 10mM citrate (pH=6) for ER; proteinase K (Dako, Carpinteria, CA) digestion was preformed for the EGFR assay. Slides were incubated with a cocktail of the primary antibody and a polyclonal rabbit cytokeratin antibody (Z0622, Dako Carpinteria, CA) for 1 hour at room temperature and overnight at 4°C for ER and EGFR, respectively. This was followed by a 1-hour incubation with Alexa 546-conjugated goat anti-rabbit secondary antibody (A11010, Molecular Probes, Eugene, OR) diluted 1:100 in mouse EnVision (K4001, Dako, Carpinteria, CA). Cyanine 5 (Cy5)-tyramide (FP1117, Perkin-Elmer, Boston, MA) diluted 1/50 was used for target detection. Prolong mounting medium (Prolong Gold, P36931, Molecular Probes, Eugene, OR) containing 4′, 6-Diamidino-2-phenylindole (DAPI) was used to identify nuclei. Serial sections of a smaller specialized TMA (control array) were stained along side all cohorts to confirm assay reproducibility. MCF-7 cells, and A431 and EGFR transfected CHO cells were used as positive controls for ER and EGFR assays, respectively. Negative control sections, in which the primary antibody was omitted, were used for each immunostaining run.

Image collection and quantitative analysis were performed using the AQUA® system (HistoRx, New Haven, CT). AQUA® Analysis allows exact measurement of protein concentration within subcellular compartments (24,25). In brief, high resolution DAPI, cytokeratin-Alexa 546 and target-Cy5 monochromatic images were captured by the PM-2000™ microscope. Tumor was distinguished by creating an epithelial tumor “mask” from the cytokeratin signal on the basis of an intensity threshold set by visual inspection of histospots. AQUA score of target proteins in the tumor mask for EGFR and nuclear compartment for ER were calculated by dividing the sum of the target compartment pixel intensities by the area of the compartment in which they were measured. Specimens with less that 5% tumor area per histospot were not included in AQUA analysis for not being representative of the corresponding tumor specimen.

Statistical Analysis

The sample size for each stratum was calculated from the effect of gefitinib relative to placebo considered at trial design to be sufficiently large to warrant further investigation of this strategy. In Stratum 1,196 patients to yield ≥ 118 progression events was estimated sufficient to detect a ≥5% improvement in the PFS hazard ratio (HR) of gefitinib to placebo with a probability of 0.90. In Stratum 2, 78 patients were estimated to yield a probability of 0.84 of observing ≥5% higher CBR for gefitinib compared with placebo using the Sargeant selection design (26).

Primary efficacy analyses were performed in the ITT population (all patients who received study treatment). PFS was also analyzed on a subset of the ITT population with HER2-expressing tumors and in the retrospective analysis of Stratum 1 according to prior endocrine therapy (see above). The PFS HR and associated 95% confidence interval (CI) and *P*-value for gefitinib relative to placebo were estimated using the Cox proportional hazards model. An interaction test for difference in PFS was also performed in the retrospective analysis of the prior endocrine therapy subsets. The CBR odds ratio, 95% CI and *P*-value for gefitinib relative to placebo were determined using logistic regression. The analysis of ORR was performed for the ITT population and the odds ratio, 95% CI and *P*-value were determined using logistic regression. HER2 status (positive [Allred score ≥ 3] *v* negative), PgR status (positive *v* negative), WHO performance status (0-1 *v* 2), and presence/absence of visceral metastases were the intended covariates in all analyses, but as most patients in Stratum 2 were HER2-negative and WHO performance status 0 or 1 it was not possible to include these covariates in Stratum 2 analyses. Analyses of all safety parameters were performed on the ITT population. For pharmacokinetic analyses, steady-state plasma C_{min} were summarized using the geometric mean and coefficient of variation (CV). Overall survival was not assessed since the trial closed before a sufficient number of deaths had occurred.

Where sufficient data were available, possible relationships between the biomarkers and PFS were explored using Cox's proportional hazards regression. Cox proportional hazards models were fitted including terms for the level of the biomarker, treatment, biomarker treatment interaction, WHO PS and visceral metastases. Where appropriate, the model was fitted both for the level of the biomarker as a continuous variable and as a categorical response (positive, negative). A significance level of 10% was used as the threshold to assess the level of evidence for an interaction between the biomarker status and the relative treatment effect.

Results

Patient Population

A total of 290 patients was registered (206 in Stratum 1; 84 in Stratum 2, Fig. 1) between October 14, 2003 and June 30, 2006. One Stratum 2 patient never started treatment leaving 289 patients. Table 1 shows the baseline characteristics. In total 153 patients were randomized to tamoxifen plus gefitinib and 136 to tamoxifen plus placebo. Patients were mostly post-menopausal (mean age 62.3 years). More than 93% were Caucasian and >98% were ER-positive. In Stratum 1, 105 patients were randomized to tamoxifen plus gefitinib and 101 to tamoxifen plus placebo. The proportion of HER2-positive and PgR-positive patients tended to be slightly higher in the gefitinib arm than the placebo arm, while slightly more patients in the placebo arm were PS 0-1. In Stratum 2, 48 patients were randomized to tamoxifen plus gefitinib and 35 to tamoxifen plus placebo. The proportion of patients who were PgR-positive also tended to be higher in the gefitinib arm than the placebo arm. No patients had received prior trastuzumab. 28% of patients in Stratum 1 and 40% in Stratum 2 had received prior adjuvant chemotherapy.

Overall, treatment compliance was over 98% in both treatment groups and only three patients (1.0%) discontinued study treatment as a result of non-compliance (two in the tamoxifen plus gefitinib arm and one in the tamoxifen plus placebo arm).

Stratum 1

There was a numerical advantage in PFS for tamoxifen plus gefitinib compared with tamoxifen plus placebo (Table 2): median PFS was 10.9 versus 8.8 months, with an HR

(gefitinib:placebo) of 0.84 (95% CI, 0.59 to 1.18; $P = 0.314$). This represents a numerical advantage for tamoxifen plus gefitinib which exceeded the predefined criteria of interest of ≥ 5%. The PFS Kaplan-Meier curves (Fig. 2A) showed similar PFS in both arms over the first 200 days, after which the curves began to diverge compatible with a delay in the development of resistance with gefitinib similar to that in preclinical models. The secondary response variable CBR also showed a numerical advantage for tamoxifen plus gefitinib with an odds ratio of 1.10 (95% CI, 0.62 to 1.95).

The HER2-positive (Allred score ≥3) subset (n = 37) in Stratum 1 also showed a numerical advantage in PFS for the gefitinib arm compared with the placebo arm that was greater than for the overall Stratum 1 population (HR 0.54; 95% CI, 0.25 to 1.15; $P = 0.111$) (Table 2, Fig. 2B). When we defined HER2 positive as an Allred score of ≥ 6 (only 16 patients) the HR was 0.82 (95% CI, 0.29 to 2.33).

In the retrospective analysis of Stratum 1, 106 PFS events were observed in the 158 endocrine therapy-naïve patients, with median PFS of 12.1 months in the gefitinib arm and 8.9 months in the placebo arm (HR 0.78; 95% CI, 0.52 to 1.15) (Table 2 and Fig. 2C). A total of 29 PFS events were observed in the 48 patients who received prior endocrine therapy; median PFS was 9.4 months in the gefitinib arm and 10.9 months in the placebo arm (HR 1.47; 95% CI, 0.63 to 3.45) (Table 2 and Fig. 2C). In the interaction test for treatment by prior endocrine therapy, P was 0.13. ORR in the endocrine-therapy naïve subgroup was 13.4% in the gefitinib arm and 17.1% in the placebo arm. In the prior endocrine therapy subgroup, ORR was 8.7% in the gefitinib arm and 8.0% in the placebo arm.

Stratum 2

No objective responses were reported in either treatment arm in Stratum 2 (Table 2). The CBR was numerically higher for the placebo group than the gefitinib group (31.4% *v* 29.2%, odds ratio [gefitinib:placebo] 0.72, 95% CI, 0.26 to 1.95; $P = 0.517$). The median PFS was also numerically higher for placebo compared with gefitinib. (Table 2, Fig. 2D).

Exploratory Biomarker Analyses

Figure 3 shows a progression free survival forest blot for the various biomarkers analyzed including EGFR, p-27, p-Akt, and p-ERK. None of these biomarkers performed on the primary tumor taken sometimes years before the patient was entered onto this study showed a statistically significant correlation with gefitinib benefit. ER determined by the quantitative fluorescent AQUA assay did show a significant inverse relationship with gefitinib PFS benefit (Fig. 4) as the level of ER fluorescence in the nucleus declined, PFS benefit from gefitinib increased (Fig. 4A), with similar results observed when non-nuclear ER fluorescence was considered (Fig. 4b). Although these analyses are only exploratory, these results are compatible with the idea that gefitinib benefit may be more pronounced in tumors with lower ER levels. No relationship between PgR and outcome was observed (data not shown).

Safety

The most common AEs are shown in Table 3. Diarrhea, rash, and alopecia were much more common in patients treated with gefitinib, and vomiting and anorexia occurred slightly more frequently in the gefitinib arm. Grade 3/4 toxicity was also more frequent with gefitinib (41% *v* 15%). Four patients treated with tamoxifen and gefitinib died on treatment (none considered treatment-related) and one patient receiving tamoxifen and placebo died (pulmonary embolism considered tamoxifen-related). Twenty-five (16.3%) patients withdrew prematurely from the gefitinib arm due to an AE compared with five (3.7%) on

the placebo arm. The incidence of serious AEs was 26.1% (40 patients) and 15.4% (21 patients), in the gefitinib and placebo arms, respectively.

Pharmacokinetics

The C_{min} of tamoxifen was similar both in the absence and presence of gefitinib, with no difference between strata indicating that gefitinib does not affect the pharmacokinetics of tamoxifen (Table 4). Tamoxifen C_{min} was estimated to be 126 ng/mL for months 3-9. The C_{min} for gefitinib did not change over time and was estimated to be 223 ng/mL, similar to that in prior studies.

Discussion

This was the first clinical trial initiated to explore the hypothesis that activation of the HER pathway during tamoxifen treatment in ER-positive tumors contributes to acquired resistance. It was further hypothesized that simultaneous blockade of HER receptors and ER may delay this resistance even in tumors with low initial levels of EGFR/HER2 because expression of these proteins can increase when ER is blocked. The EGFR inhibitor gefitinib was chosen since it inhibits signaling from EGFR homo- and heterodimers and since it was effective in preclinical studies in which upregulation of EGFR was a dominant characteristic of tamoxifen-resistant tumors (16-18,20,21).

This randomized Phase II trial was not designed to show a statistically significant advantage for the addition of gefitinib to tamoxifen, but rather to determine if the addition of gefitinib resulted in a numerical advantage compared with placebo of sufficient interest to warrant further study. This proof-of-concept trial included two different studies that differ with regards to the patient population, primary endpoints, and sample size. In Stratum 1, patients receiving tamoxifen plus gefitinib did have a numerical advantage compared with placebo in PFS; this numerical advantage was greater than the predefined criterion (≥ 5% advantage predefined as being the minimum to warrant further investigation of this strategy). Stratum 1 patients also had a slight numerical advantage for gefitinib in CBR (10%). Although there was a greater numerical PFS advantage for gefitinib in patients whose initial primary tumor was HER2-expressing (Allred score ≥ 3), this did not explain the entire PFS advantage of gefitinib seen in this stratum since a small minority of the patients were HER2 positive. Furthermore, the HR for the subset with Allred scores ≥ 6 which correlates with gene amplification was 0.82, very similar to that for the entire group in Stratum 1. This suggests that patients whose tumors were initially low/negative for HER2 and EGFR may also have received some benefit compatible with the clinical and preclinical observations that EGFR and HER2 levels can increase during tamoxifen treatment in some tumors (13-18,20,21).

The retrospective analysis by previous endocrine therapy provided further insight into which groups of patients in Stratum 1 may obtain the greatest benefit from gefitinib and tamoxifen combination therapy. Numerically prolonged PFS was observed in endocrine therapy-naïve patients compared to a numerical disadvantage in patients who had received prior endocrine therapy, although the interaction test did not reach statistical significance at the conventional 5% level. ORRs were not numerically superior with gefitinib in either the endocrine therapy-naïve or those with prior endocrine therapy. Similar findings have also been reported in a subset analysis of a second placebo-controlled trial evaluating gefitinib plus anastrozole *v* anastrozole alone (NCT00077025) in postmenopausal women with newly-diagnosed hormone receptor-positive metastatic breast cancer (27). PFS was prolonged in the endocrine therapy-naïve patient subgroup when gefitinib was combined with anastrozole compared with anastrozole alone (median PFS 20.2 months versus 8.4 months [HR 0.39; 95% CI 0.16, 0.97]); while in the prior endocrine therapy subgroup there appeared to be less

benefit from adding gefitinib (median PFS 11.2 vs 7.1 months [HR 0.65; 95% CI 0.32, 1.33]).

The results for Stratum 2 (patients progressing after AI treatment) demonstrated no objective responses in either arm of this stratum, but in the placebo arm slightly more patients had SD and there was also a slight advantage in PFS compared with the gefitinib arm. This result suggests that tamoxifen plus gefitinib given after resistance to an AI is not effective.

It could be argued that the 5% improvement in PFS with gefitinib established before the study began was too low to provide a meaningful indicator that this strategy was worth additional study. However, because of difficulties inherent in biopsying metastatic breast cancer just before treatment to assess EGFR/HER2 status and not knowing how often EGFR/HER2 would increase over time with treatment, a relatively low bar was set so as not to miss a hint of activity that might be important for a small subset of patients. Furthermore, the response rate of gefitinib in prior studies in unselected patients with metastatic disease was very low (28).

Both regimens were reasonably well tolerated, although there were more AEs in the gefitinib arm that led to a higher withdrawal rate. The AEs reported in this study are consistent with the established safety profile of gefitinib and the clinical characteristics of the patients did not suggest any difference in safety between the two strata.

The results in Stratum 1 lend support to the idea that signaling through the EGFR/HER2 pathway may contribute to acquired resistance to tamoxifen in some patients. ER-positive patients rarely express detectable levels of EGFR, and only overexpress HER2 in about 12% of cases. The 18% reported in this study may be due to the definition used (Allred score ≥ 3). It is possible that tumors with initially low expression of EGFR and HER2 may upregulate the expression of these receptors when the patient is treated with tamoxifen or other ER-targeted therapies. ER signaling is known to downregulate the expression of EGFR and HER2, and, therefore, it is not surprising that ER blockade might therefore increase expression of these receptors (29). More clinical biomarker data are needed to determine how frequently this occurs in patients, although three small studies evaluating HER2 reported increasing levels in 12-35% of patients treated with endocrine therapy (13-15). It will also be important to determine if molecular profiling of the primary tumor can predict which tumors are likely to increase the expression of EGFR/HER2 when treated with ER-targeted therapy. Our biomarker studies of EGFR, p-ERK, p-Akt, and p-27 were disappointing in this regard. Perhaps the results are not surprising since these biomarkers were determined on the primary tumor before any treatment and would not necessarily predict the pathway driving tumor growth when the tumor recurs sometimes years later or after intervening treatment. The correlation between low ER determined by a sensitive fluorescence method and benefit with gefitinib should be interpreted with great caution and could be due to chance alone. However, tumors with active growth factor receptor signaling or high PI3K activity tend to have lower ER expression, and it is interesting to speculate that such tumors may be those most likely to upregulate EGFR and then be inhibited by EGFR blockade (30). Furthermore the correlation between lower ER and benefit from the addition of lapatinib to letrozole was recently reported lending support to this possibility (31). The results also suggest that to more accurately select patients for this kind of targeted therapy, a tumor biopsy taken just before the start of treatment is likely to be more informative. Preliminary results from a small recently reported study showing an advantage for anastrozole plus gefitinib compared with anastrozole alone and an exploratory analysis of HER2-negative patients in a very large recent randomized trial of letrozole with or without lapatinib lend support to our hypothesis and suggest that the HER pathway may also be important for acquired resistance to AIs (27,32).

The small numerical disadvantage with gefitinib in patients resistant to AIs in Stratum 2 is difficult to explain and may reflect low patient accrual. The results for Stratum 2 (patients progressing after AI treatment) demonstrated no objective responses in either arm of this stratum. The marginally greater number of patients who had SD and the slight advantage in PFS in the placebo arm represented a numerical disadvantage for gefitinib when given to patients who have developed resistance to an AI and indicates that further investigation in this group of patients is not warranted. Perhaps blocking EGFR led to enhanced signaling from other HER receptor dimers that generate more potent survival stimuli in some tumors.

A problem that complicates virtually all metastatic breast cancer studies is that expression levels of biomarkers are usually measured on the primary tumor but are not reassessed in metastatic disease that is not easily biopsiable prior to starting protocol therapy. Although 98% of the primary tumors from patients on this study were ER-positive, some would have been expected to lose ER at the time of recurrence when they entered the study (33), and others might have increased levels of EGFR or HER2 due to prior endocrine therapy. In this era of targeted therapy serial tissue biopsies will be necessary to accurately determine optimal therapy at any timepoint in individual patients. Nevertheless, this study together with others showing similar results lends support to the hypothesis that the EGFR/HER2 pathway may contribute to the development of tamoxifen resistance in some patients and that further investigation of this approach is warranted.

Acknowledgments

We thank the investigators and patients participating in the study and Annette Smith from Complete Medical Communications for editing assistance. The trial itself was funded by AstraZeneca and supported in part by Breast Cancer SPORE P50 CA58183 from the NCI, NIH R33 CA 106709 to DLR, by NIH P50 SPORE CA58183 to CKO, and by NIH P30 NCI Core 125123 to CKO.

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Fig 1.
Study flow.

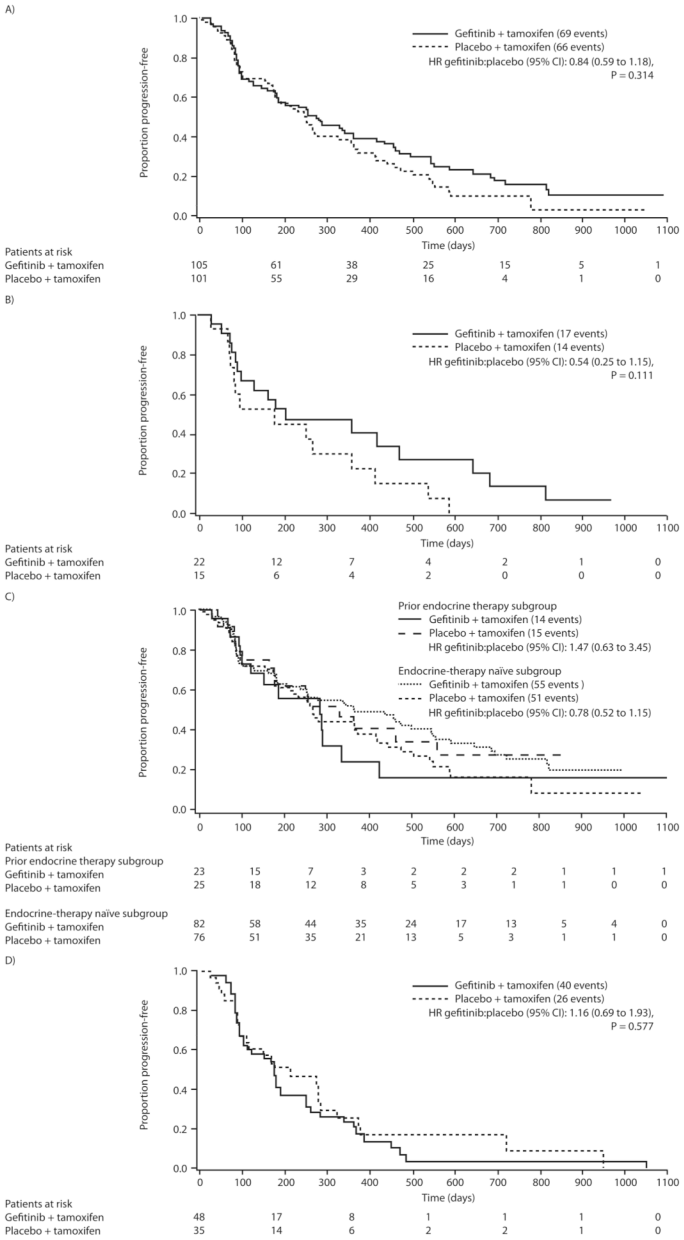

Fig 2.

Kaplan-Meier curves for progression-free survival for (A) Stratum 1 overall, (B) Stratum 1 HER2-positive patients, (C) Stratum 1 endocrine therapy-naïve patients and patients previously treated with endocrine therapy and (D) Stratum 2.

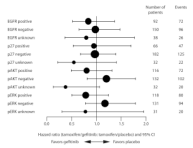

Fig 3.
Forest plot showing hazard ratios (and 95% confidence intervals) for progression-free survival for EGFR (fluorescent) and p27, pAKT and pERK (all IHC nuclear).

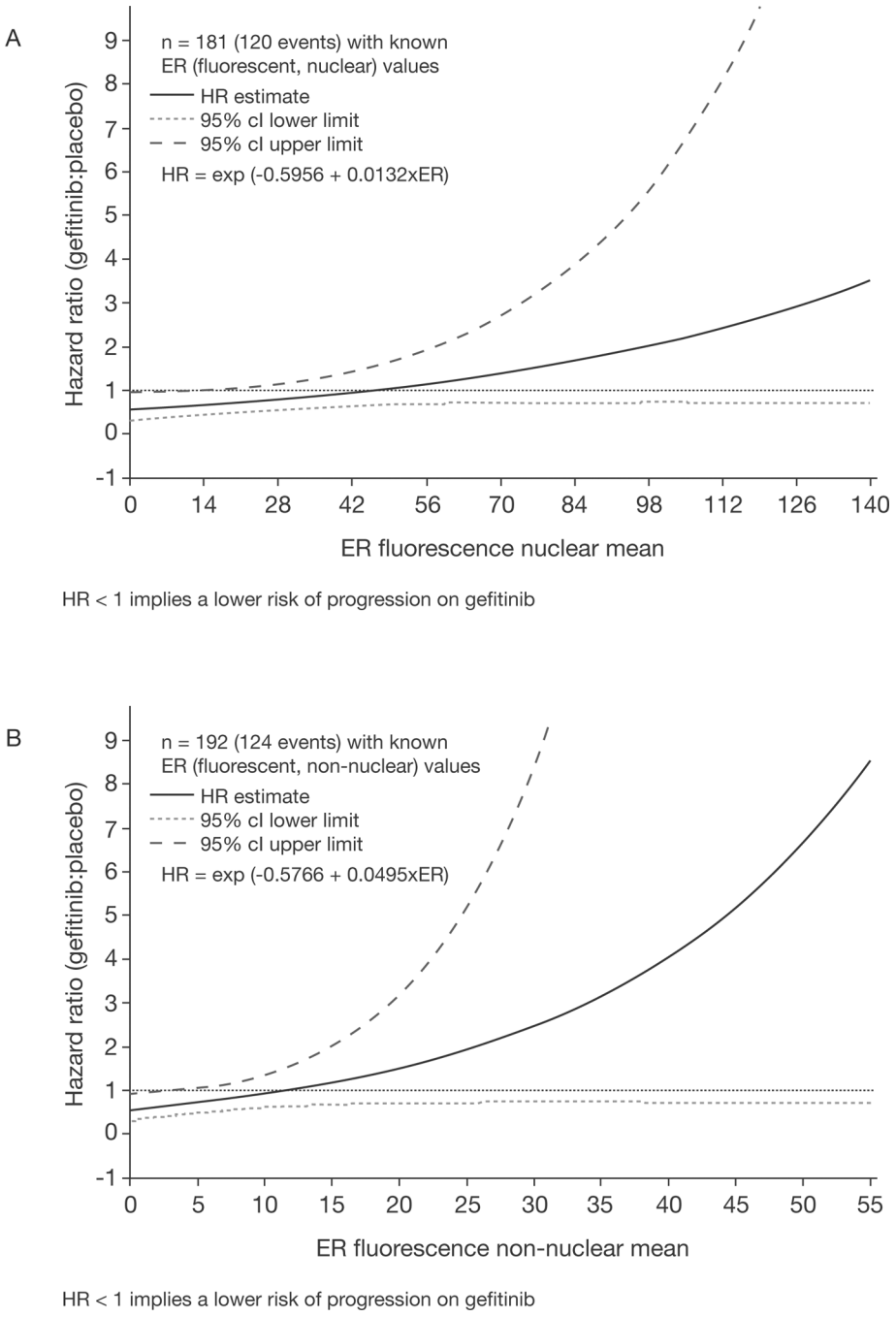

Fig 4.

Progression-free survival hazard ratio as a function of ER (fluorescent nuclear) measured on a continuous scale.

Table 1

Baseline patient characteristics (ITT population).

	Tamoxifen + gefitinib (n = 153)	Tamoxifen + placebo (n = 136)
Mean age (range), years	61.6 (40-89)	63.1 (40-86)
Age group, n (%)		
36-45	11 (7.2)	8 (5.9)
46-55	39 (25.5)	30 (22.1)
56-65	44 (28.8)	34 (25.0)
66-75	41 (26.8)	47 (34.6)
> 75	18 (11.8)	17 (12.5)
Race, n (%)		
Caucasian	143 (93.5)	128 (94.1)
Black	1 (0.7)	3 (2.2)
Oriental	2 (1.3)	0 (0.0)
Other	7 (4.6)	5 (3.7)
Estrogen receptor status, n (%)[a]		
Positive	151 (98.7)	134 (98.5)
Negative	2 (1.3)	2 (1.5)
Progesterone receptor status, n (%)[a]		
Positive	129 (84.3)	102 (75.0)
Negative	24 (15.7)	34 (25.0)
HER2 status, n (%)[a]		
Positive	26 (17.0)	17 (12.5)
Negative	127 (83.0)	119 (87.5)
Stratum 1		
N	105	101
HER2 status, n (%)*		
Positive (Allred score ≥ 3)	22 (21.0)	15 (14.9)
Positive (Allred score ≥ 6)	9 (5.9)	7 (5.1)
Progesterone receptor status, n (%)[a]		
Positive	89 (84.8)	77 (76.2)
Negative	16 (15.2)	24 (23.8)
WHO performance status, n (%)		
0-1	95 (90.5)	98 (97.0)
2	10 (9.5)	3 (3.0)
Visceral disease present, n (%)	55 (52.4)	46 (45.5)

	Tamoxifen + gefitinib (n = 153)	Tamoxifen + placebo (n = 136)
	Stratum 2	
N	48	35
HER2 status, n (%)*		
Positive (Allred score ≥ 3)	4 (8.3)	2 (5.7)
Positive (Allred score ≥ 6)	1 (0.7)	1 (0.7)
Progesterone receptor status, n (%)[a]		
Positive	40 (83.3)	25 (71.4)
Negative	8 (16.7)	10 (28.6)
WHO performance status, n (%)		
0-1	46 (95.8)	33 (94.3)
2	2 (4.2)	2 (5.7)
Visceral disease present, n (%)	25 (52.1)	22 (62.9)

Abbreviations: HER2, human epidermal growth factor receptor 2; WHO, World Health Organization.

*ER, PR, and HER2 status determined in the central reference laboratory at Baylor College of Medicine.

ITT population includes six patients who were hormone receptor negative at central review, 5 in Stratum 1, and 1 in Stratum 2.

Table 2

Summary of key efficacy results (ITT population).

	Tamoxifen plus gefitinib (Stratum 1, n = 105; Stratum 2, n = 48)	Tamoxifen plus placebo (Stratum 1, n = 101; Stratum 2, n = 35)	HR* or OR†	95% CI	*P*-value
Primary response variables					
Stratum 1					
PFS, median (months)	10.9	8.8	0.84	0.59 to 1.18	0.314
Stratum 2					
CBR‡, n (%)	14 (29.2)	11 (31.4)	0.72	0.26 to 1.95	0.517
Secondary response variables					
Stratum 1					
CBR‡, n (%)	53 (50.5)	46 (45.5)	1.10	0.62 to 1.95	0.748§
Objective response rate, n (%)	13 (12.4)	15 (14.9)	0.76	0.33 to 1.75	0.518§
HER2 positive subset‖ (Allred score of ≥ 3 to < 6) PFS, median (months)	6.7	5.8	0.54	0.25 to 1.15	0.111§
HER2 positive subset** (Allred score of ≥ 6) PFS, median (months)	††	††	0.82	0.29 to 2.33	0.707§
Endocrine therapy-naïve subgroup‡‡ PFS, median (months)	12.1	8.9	0.78	0.52 to 1.15	
Prior endocrine therapy subgroup§§ PFS, median (months)	9.4	10.9	1.47	0.63 to 3.45	
Stratum 2					
PFS, median (months)	5.7	7.0	1.16	0.69 to 1.93	0.577§
Objective response rate, n (%)	0	0			

Abbreviations: CBR, clinical benefit rate; CI, confidence interval; HR, hazard ratio; OR, odds ratio; PFS, progression-free survival.

*For PFS, a HR (gefitinib:placebo) of < 1 indicates a reduced hazard (lower risk of progression) with tamoxifen plus gefitinib than with tamoxifen plus placebo.

†For CBR, an OR (gefitinib:placebo) of > 1 indicates higher odds of clinical benefit with the tamoxifen plus gefitinib combination than with tamoxifen plus placebo.

‡Defined as complete response, partial response or stable disease for at least 24 weeks using RECIST criteria.

§*P*-value for illustrative purposes only (the study was only powered for the primary response variables).

//Stratum 1 HER2 positive subset (Allred score of 3 to < 6): tamoxifen + gefitinib, n = 22; tamoxifen + placebo, n = 15.

**Stratum 1 HER2 positive subset (Allred score of ≥ 6): tamoxifen + gefitinib, n = 9; tamoxifen + placebo, n = 7.

††Median PFS values were not calculated due to small patient numbers.

‡‡Retrospective analysis, Stratum 1 endocrine therapy-naïve subgroup: tamoxifen + gefitinib, n = 82; tamoxifen + placebo, n = 76.

§§Retrospective analysis, Stratum 1 prior endocrine therapy subgroup: tamoxifen + gefitinib, n = 23; tamoxifen + placebo, n = 25.

Table 3

Adverse events in the overall population.*

	Tamoxifen + gefitinib (n = 153) N (%)		Tamoxifen + placebo (n = 136) N (%)	
Adverse event	**Any grade**	**CTC grade 3 or 4**	**Any grade**	**CTC grade 3 or 4**
All	150 (98.0)	63 (41.2)	118 (86.8)	21 (15.4)
Diarrhea	93 (60.8)	6 (3.9)	31 (22.8)	0 (0)
Alopecia	76 (49.7)		28 (20.6)	
Rash	67 (43.8)	6 (3.9)	19 (14.0)	0 (0)
Vomiting	34 (22.2)	3 (2.0)	18 (13.2)	2 (1.5)
Nausea	48 (31.4)	2 (1.3)	36 (26.5)	4 (2.9)
Bone pain	12 (7.8)	2 (1.3)	17 (12.5)	2 (1.5)
Dry Skin	34 (22.2)	0 (0)	7 (5.1)	0 (0)
Fatigue	23 (15.0)	1 (0.7)	24 (17.6)	0 (0)
Headache	19 (12.4)	1 (0.7)	12 (8.8)	0 (0)
Muscle spasms	18 (11.8)	0 (0)	4 (2.9)	0 (0)
Pruritis	18 (11.8)	0 (0)	13 (9.6)	0 (0)
Hot flush	15 (9.8)	0 (0)	25 (18.4)	0 (0)
Arthralgia	13 (8.5)	0 (0)	14 (10.3)	0 (0)
Dyspnea	15 (9.8)	1 (0.7)	17 (12.5)	3 (2.2)

Abbreviations: CTC, Common Toxicity Criteria.

*Using a cut-off of 10% in either group.

Table 4

Trough concentrations (C_{min}) of tamoxifen and gefitinib.

Treatment arm	Month	N	Geometric mean (ng/mL)	CV%
Tamoxifen trough concentrations				
Tamoxifen + placebo	1	96	110.3	43.2
	3	79	131.7	50.1
	6	51	124.1	41.0
	9	40	140.3	38.3
	Total	**170**	**131.3**	**44.8**
Tamoxifen + gefitinib	1	108	103.2	41.5
	3	94	122.6	46.0
	6	64	123.1	45.1
	9	54	130.4	52.1
	Total	**212**	**124.7**	**47.2**
Gefitinib trough concentrations				
Tamoxifen + gefitinib	1	108	228.9	72.9
	3	92	224.2	68.3
	6	67	208.9	128.3
	9	56	226.8	94.2
	Total	**323**	**222.9**	**86.4**