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ORIGINAL REPORT

Double-Blind, Placebo-Controlled, Multicenter, Randomized, Phase IIB Neoadjuvant Study of Letrozole-Lapatinib in Postmenopausal Hormone Receptor–Positive, Human Epidermal Growth Factor Receptor 2–Negative, Operable Breast Cancer

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See accompanying article on page 1084

ABSTRACT

Purpose
This is a randomized, double-blind, placebo-controlled study aimed to evaluate the clinical and biologic effects of letrozole plus lapatinib or placebo as neoadjuvant therapy in hormone receptor (HR) –positive/human epidermal growth factor receptor 2 (HER2) –negative operable breast cancer.

Methods
Ninety-two postmenopausal women with stage II to IIIA primary breast cancer were randomly assigned to preoperative therapy consisting of 6 months of letrozole 2.5 mg orally daily plus lapatinib 1,500 mg orally daily or placebo. Surgery was performed within 2 weeks from the last study medication. Clinical response was assessed by ultrasonography. Pre- and post-treatment samples were evaluated for selected biomarkers. Fresh-frozen tissue samples were collected for genomic analyses.

Results
Numerically similar clinical response rates (partial + complete response) were observed (70% for letrozole-lapatinib and 63% for letrozole-placebo). Toxicities were generally mild and manageable. A significant decrease in Ki-67 and pAKT expression from baseline to surgery was observed in both arms. Overall, 34 patients (37%) had a mutation in *PIK3CA* exon 9 or 20. In the letrozole-lapatinib arm, the probability of achieving a clinical response was significantly higher in the presence of *PIK3CA* mutation (objective response rate, 93% *v* 63% in *PIK3CA* wild type; $P = .040$).

Conclusion
The combination of letrozole-lapatinib in early breast cancer was feasible, with expected and manageable toxicities. In unselected estrogen receptor–positive/HER2-negative patients, letrozole-lapatinib and letrozole-placebo resulted in a similar overall clinical response rate and similar effect on Ki-67 and pAKT. Our secondary end point findings of a significant correlation between *PIK3CA* mutation and response to letrozole-lapatinib in HR-positive/HER2-negative early breast cancer must now be independently confirmed.

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INTRODUCTION

Preoperative chemotherapy is the standard treatment for locally advanced and inflammatory breast cancer and is currently adopted for treatment of larger primary tumors to improve the chance for breast conservation. In patients with hormone receptor (HR) –positive/human epidermal growth factor receptor 2 (HER2) –negative tumors, however, the rate of pathologic complete response (pCR) after neoadjuvant chemotherapy is generally less than 10%, questioning the effectiveness of chemotherapy in this breast cancer subtype and the value of pCR as a surrogate end point for outcomes.[1-3] With the growing understanding of tumor biology and molecular subtypes, endocrine manipulation

has become the cornerstone of therapy for HR-positive tumors, and neoadjuvant hormonal therapy is recently emerging as an attractive option for patients with this breast cancer subtype.[4-6] However, a significant proportion of patients show primary or acquired resistance to hormonal therapy. The cross-talk between estrogen receptor and other growth factor receptor families, including the epidermal growth factor receptor (EGFR) family, is suggested to play a crucial role in the development of endocrine resistance.[7] Several studies combining EGFR-targeted agents with hormonal therapy have been conducted in early and advanced disease, producing conflicting results.[8-11]

Lapatinib is a small-molecule, dual inhibitor of the tyrosine kinase activity of EGFR and HER2 and is currently approved in patients with HER2-positive advanced breast cancer who experience treatment failure with trastuzumab-based therapy.[12,13] We designed this phase II, randomized study to evaluate the role of adding blockade of both EGFR and HER2 to endocrine therapy in patients with HR-positive/HER2-negative primary breast cancer. The aims of this study were to evaluate the activity, safety, and biologic effects of letrozole-lapatinib and letrozole-placebo.

METHODS

Study Design

This was a double-blind, placebo-controlled, randomized phase IIB study. Postmenopausal women with HR-positive/HER2-negative, stage II to IIIA primary breast cancer were randomly assigned to receive letrozole-lapatinib (arm A) or letrozole-placebo (arm B). The primary end point was breast objective response rate (complete plus partial response), as assessed by ultrasonography.

Secondary aims included pCR rate in the breast and axillary lymph nodes; percentage of breast-conserving surgery (BCS); safety profile of study treatments; time to treatment failure (defined from start of primary therapy to the occurrence of local or distant tumor progression, permanent treatment discontinuation, or death from any cause); the percent inhibition of biomarkers of proliferative and apoptosis pathways; and the relationship between gene expression profile, copy number variations, somatic mutations (including but not limited to *PI3KCA*), and protein biomarkers and clinical/biologic response. The protocol was approved by ethical committees of participating institutions.

Patients

Patients were eligible if they met the following criteria: previously untreated, infiltrating primary breast cancer of more than 2.0 cm in largest clinical diameter; estrogen and/or progesterone receptor positivity ($\geq$ 10% of positive cancer cells by immunohistochemistry [IHC]); HER2 negativity (either IHC 0 to 1+ or fluorescent in situ hybridization negativity by local assessment); postmenopausal status; Eastern Cooperative Oncology Group performance status of 0 to 1; left ventricular ejection fraction (LVEF) within the normal range; normal organ and marrow function; ability to swallow and retain oral medication; and written informed consent.

Study Procedures

At diagnosis, patients underwent a complete staging to exclude distant metastases. Local disease extent was evaluated by mammography, breast ultrasonography, and clinically by caliper. Random assignment was performed centrally at the Modena University Hospital Trial Office, through a dedicated Web site with password-restricted access. Random assignment sequence was generated using R software (http://www.r-project.org/). Patients were randomly assigned 1:1 in a double-blind manner to letrozole 2.5 mg orally (PO) daily plus lapatinib 1,500 mg PO daily (arm A) or letrozole 2.5 mg PO daily plus lapatinib-matched placebo daily (arm B). Treatment was administered over a 28-days cycle for six cycles. LVEF evaluation was repeated every third cycle. Surgery was planned within 2 weeks after last study drug administration. After surgery, hormonal therapy was given for up for 5 years, whereas adjuvant chemotherapy was administered at the discretion of the treating physician. Radiation therapy was planned according to local guidelines. Clinical examination, hematology, complete blood chemistry, carcinoembryonic antigen, CA 15-3, chest radiogram, and liver ultrasound were planned every 6 months for the first 3 years, and then yearly up to 5 years. Mammography was repeated yearly. An independent data monitoring committee periodically evaluated adverse events (AEs) and patient outcomes.

Assessment of Response and Toxicity

The clinical response was defined comparing the tumor size (largest breast tumor diameter) before and at the end of cycle 6, as assessed by ultrasound examination. Response was evaluated according to criteria based on modified RECIST guidelines. Complete response (CR) is defined as disappearance of primary tumor. Partial response (PR) is defined as at least a 30% decrease of the tumor's longest diameter. Progressive disease (PD) is defined as a more than 20% increase of the tumor's longest diameter. Stable disease (SD) is defined as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD. pCR is defined as complete absence of infiltrating tumor cells in the breast and axillary lymph nodes.

Toxicities were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 and reported as cumulative incidence. All the analyses were conducted according to the intention-to-treat (ITT) principle. The ITT population comprised all enrolled patients who received at least one dose of study drug.

Sample Size Calculation and Statistics

The study used Simon's two-stage design in each arm, based on an expected rate of clinical objective response (cOR) of 50% with letrozole. The combination of lapatinib-letrozole was considered worthwhile if the cOR rate reached 70%. Setting $\alpha = 10\%$ and $\beta = 10\%$, the resulting sample sizes were 46 patients for arm A and 45 patients for arm B. A formal comparison of the two arms was not planned. Random assignment was used to reduce bias as a result of patient selection into treatment arms. All data were presented descriptively as medians, means, or proportions. Biomarker changes from baseline to surgery were evaluated using the Wilcoxon signed rank matched-pair test. The association between biomarker expression and response was evaluated using Pearson's χ^2 test. Differences in mean biomarker inhibition were evaluated using the *t* test. Statistical analyses were performed using STATA software V.10 (STATA, College Station, TX).

Biomarkers and Genomic Analyses

Formalin-fixed paraffin-embedded tumor blocks from diagnostic core biopsies and from surgical specimen after preoperative study therapy were centralized and reviewed for quality and tumor content. Fresh-frozen samples from the diagnostic core biopsy were centralized for genomic studies.

IHC and Assessment of the Apoptotic Index

HER2 was centrally re-evaluated on diagnostic core biopsies. The following biomarkers were evaluated on diagnostic core biopsy and on surgical specimen following preoperative therapy: Ki-67, PTEN, pAKT, and pEGFR. The antibodies used were as follows: HER2 (MoAb HER2, clone 4B5; Ventana, Tucson, AZ), Ki-67 (clone Ki-67-MIB-1; DAKO, Carpinteria, CA), pAKT (Ser 473; Cell Signaling Technology, Beverly, MA), PTEN (clone 28H6; Novocastra, Buffalo Grove, IL), and pEGFR (Tyr 1068; Cell Signaling Technology). IHC staining was performed according to the avidin-biotin method, using tissue sections of 3 μm in thickness. The following parameters were recorded: presence or absence of immunoreactivity (diffuse or focal), cell types exhibiting a positive reaction (tumor, endothelial, stromal, and inflammatory cells), and percentage of immunostained tumor cells. HER2 fluorescent in situ hybridization analysis (PathVysion HER-2 DNA Probe Kit; Vysis, Downers Grove, IL) was performed in any case of HER2 IHC 2+ and in cases of discordance between the local and central laboratory.

Tissue sections were stained using the terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling method, according to the standard procedure included in the Apop Tag Plus In Situ Apoptosis Detection Kit (Chemicon International, Temecula, CA). The percentage of apoptotic events per cells population, by counting at least 3,000 malignant cells randomly selected at $\times$400 magnification, was recorded.

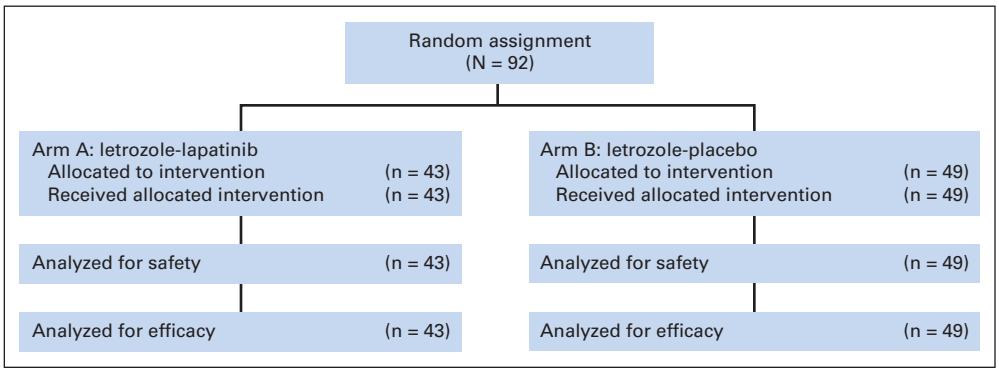

Fig 1. CONSORT diagram.

PI3KCA *Mutation Analysis*

Three 5-μm, formalin-fixed paraffin-embedded sections of a primary lesion containing at least 50% tumor cells were deparaffinized and incubated in lysis buffer with proteinase K (50 mmol/L Tris, 1 mmol/L EDTA, 05% Tween-20) at 56°C overnight. Genomic DNA was extracted with QIAmpl DNA Mini Kit (Qiagen, Hilden, Germany). DNA concentration was determined using the NanoDrop 2000 spectrophotometer (Nano Drop Products, Wilmington, DE). Genetic analysis of the *PIK3CA* gene was performed using a commercially available *PIK3CA* status kit (certified European Conformity in Vitro Diagnostic for diagnostic use; Diatech Pharmacogenetics, Jesi, Ancona, Italy). The kit permits the identification of mutations in codons 542, 545, and 546 of exon 9 (E542K, E545K, E545A, E545G, Q546E, Q546K) and codons 1043, 1047, and 1049 of exon 20 (M1043I, H1047Y, H1047R, H1047L, G1049R, G1049S) of the *PIK3CA* gene. A real-time polymerase chain reaction (RotorGene 6000, Qiagen) was carried out using 30 ng of DNA as template. Specific mutations were subsequently identified by pyrosequencing on PyroMark Q96 ID (Qiagen).

Molecular Studies

Genomic DNA and total RNA were extracted from pretreatment frozen core biopsies of the primary tumor. RNA samples that met the quality requirements were processed according to the Affymetrix GeneChip 3′ IVT Express Kit user's manual (Affymetrix, Santa Clara, CA).

DNA samples were processed by using the Affymetrix Genome-wide Human SNP array. See Data Supplement for detailed description of sample processing and analysis.[14]

RESULTS

Ninety-two patients were randomly assigned, 43 patients to letrozole-lapatinib (arm A) and 49 patients to letrozole-placebo (arm B; Fig 1). Baseline patient characteristics were balanced between the two arms (Table 1).

Compliance With Treatment and Toxicity

Overall, nine patients prematurely discontinued treatment, five in arm A (PD, n = 1; AEs, n = 2; informed consent withdrawn, n = 2) and four in arm B (PD, n = 2; suspected metastatic disease, n = 1; informed consent withdrawn, n = 1). Ten patients required lapatinib or placebo dose reduction, nine in the letrozole-lapatinib arm and one in the letrozole-placebo arm.

The safety analyses included all randomly assigned patients. As expected, skin disorders, diarrhea, and liver function test abnormalities occurred more frequently in lapatinib-treated patients. One episode of grade 4 skin toxicity was reported in one patient randomly assigned to the letrozole-lapatinib arm. In arm B (letrozole-placebo), AEs of grade ≥ 2 were infrequent (Table 2).

Cardiac function was preserved over time. The mean LVEF at baseline and at weeks 12 and 24 from starting therapy were 61%, 60.1%, and 58.6%, respectively, in the letrozole-lapatinib arm and 61%, 62.6%, and 60.5%, respectively, in the letrozole-placebo group.

Table 1. Patient Clinical and Tumor Characteristics

Characteristic	Arm A, Letrozole-Lapatinib (n = 43)		Arm B, Letrozole-Placebo (n = 49)	
	No. of Patients	%	No. of Patients	%
Age, years				
Median	70		70	
Range	49-88		47-88	
ECOG PS				
0	41	95.35	45	91.8
1	2	4.65	3	6.12
NA	—	—	1	2
Clinical stage				
IIA	21	48.8	26	53
IIB	17	39.5	21	42.9
IIIA	5	11.6	2	4.1
Histology				
Ductal	29	67.4	38	77.6
Lobular	8	18.6	7	14.3
Mucinous	2	4.7	1	2
Other/not specified	4	9.3	3	6.12
Histologic grade				
1	1	2.3	—	—
2	16	37.2	19	38.8
3	13	30.2	19	38.8
NA	13	30.2	11	22.4
HER2 status				
IHC 0	23	53.5	24	49.0
IHC 1+	8	18.6	13	26.5
IHC 2+/FISH not amplified	5	11.6	4	8.2
FISH not amplified	7	16.3	8	16.3
ER expression, %				
Mean	89		90	
Range	40-100		30-100	
PgR expression, %				
Mean	56		52	
Range	0-100		0-100	

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; ER, estrogen receptor; FISH, fluorescent in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; PgR, progesterone receptor; NA, not available.

Table 2. Treatment Toxicity: Adverse Events Occurring in at Least 10% of the Study Population and Grade 3 to 4 Adverse Events

Adverse Event	Letrozole-Lapatinib (n = 43)								Letrozole-Placebo (n = 49)							
	Overall		Grade 2		Grade 3		Grade 4		Overall		Grade 2		Grade 3		Grade 4	
	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
Skin disorders	25	58.1	10	23.2	4	9.3	1	2.3	3	6.1	1	2.0	0	0	0	0
Nail toxicity	5	11.6	1	2.3	2	4.6	0	0	0	0	0	0	0	0	0	0
Diarrhea	26	60.5	4	9.3	6	13.9	0	0	5	10.2	2	4.1	0	0	0	0
Increased transaminases	8	18.6	2	4.6	2	4.6	0	0	2	4.1	0	0	1	2.0	0	0
Increased γ-glutamyltransferase	3	6.9	0	0	2	4.6	0	0	0	0	0	0	0	0	0	0
Mucositis	7	16.3	5	11.6	0	0	0	0	1	2.0	0	0	0	0	0	0
Fatigue	7	16.3	1	2.3	1	2.3	0	0	6	12.2	1	2.0	0	0	0	0
Musculoskeletal disorders	6	13.9	1	2.3	1	2.3	0	0	9	18.4	3	6.1	0	0	0	0
Dyspepsia	5	11.6	2	4.6	0	0	0	0	5	10.2	0	0	0	0	0	0
Nausea	2	4.6	0	0	0	0	0	0	6	12.2	0	0	0	0	0	0
Congestive heart failure	1	2.3	0	0	1	2.3	0	0	0	0	0	0	0	0	0	0

Table 3. Biomarker Expression Analyses

Biomarker	Baseline			Surgery			
		Expression (%)			Expression (%)		
	No. of Patients	Mean	Range	No. of Patients	Mean	Range	*P**
Arm A, letrozole-lapatinib							
Ki-67	41	18.7	2-48	39	12.8	1-50	.002†
pAKT	41	12.2	0-90	36	2.36	0-60	.0148†
PTEN	38	70.3	0-100	33	75.1	0-100	.4843
pEGFR	41	0.49	0-20	39	0.77	0-30	.9845
TUNEL	36	0.47	0.05-1.2	27	0.33	0.05-1.4	.0158†
Arm B, letrozole-placebo							
Ki-67	49	19.2	2-60	46	12.8	1-45	< .001†
pAKT	47	16.8	0-90	46	7.98	0-80	.0323†
PTEN	40	71.4	0-100	40	71.6	0-100	.5373
pEGFR	48	0.31	0-15	46	0.43	0-20	.9873
TUNEL	40	0.44	0.05-1.7	30	0.33	0.05-2	.1142

Abbreviations: pAKT, phosphorylated AKT; pEGFR, phosphorylated epidermal growth factor receptor.
*Wilcoxon signed rank test (from baseline).
†Significant value.

One patient in the letrozole-lapatinib arm who experienced clinical symptoms of congestive heart failure not considered related to study drug fully recovered from the event.

Surgery and Responses

Eighty-one of 92 patients were evaluable by ultrasonography; eight patients were assessed by mammography and/or palpation. Three patients discontinued therapy and withdrew consent and were counted as nonresponders according to the ITT analysis.

Central radiology review was performed. The cOR rate (CR+PR) was similar in the two arms (70% and 63% in the letrozole-lapatinib and the letrozole-placebo arms, respectively).

The distribution of responses in the two arms was as follows: arm A: CR, 12%; PR, 58%; SD, 23%; and PD, 2%; and arm B: CR, 2%; PR, 61%; SD, 29%; and PD, 6%. When limiting the analysis to patients evaluable by ultrasound, the cOR rate was 69.2% in the letrozole-lapatinib arm and 57.8% in the letrozole-placebo arm.

Forty-two of 43 patients randomly assigned to arm A underwent surgery; 27 patients (62.8%) received BCS, and 15 patients (34.9%) received mastectomy. The conversion rate from mastectomy to BCS was 46% (six of 13 patients). Forty-seven of 49 patients randomly assigned to arm B underwent surgery; 35 patients (71.4%) received BCS, and 12 patients (24.5%) received mastectomy. The conversion rate from mastectomy was 58.9% (10 of 17 patients). No pCR was observed in the two arms.

Biomarkers and Genomic Analyses

All primary tumor samples were confirmed as HER2 negative by central review. Biomarker analysis results are listed in Table 3. A significant reduction in Ki-67 and pAKT expression was observed between pre- and post-treatment samples (Fig 2). In particular, mean Ki-67 expression decreased from 18.7% to 12.8% in the letrozole-lapatinib arm ($P = .002$) and from 19.2% to 12.8% in the letrozole-placebo arm ($P < .001$). No differences in the mean Ki-67 suppression were observed between the two arms, consistent with the similar cOR rates. The mean Ki-67 suppression tended to be higher in responders versus nonresponders and in patients with *PIK3CA* mutation versus wild type (WT) in the letrozole-lapatinib arm. The Data Supplement summarizes the mean Ki-67 suppression overall, according to response and to *PIK3CA* status in the two arms. A significant decrease in apoptosis was also observed in the letrozole-lapatinib arm.

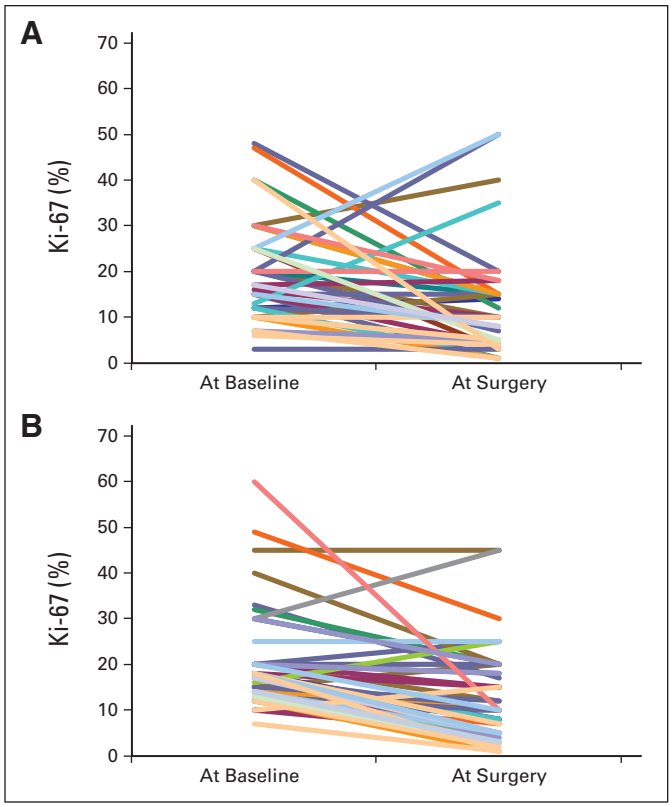

Fig 2. Individual Ki-67 changes from baseline to surgery in (A) arm A (letrozole-lapatinib) and (B) arm B (letrozole-placebo).

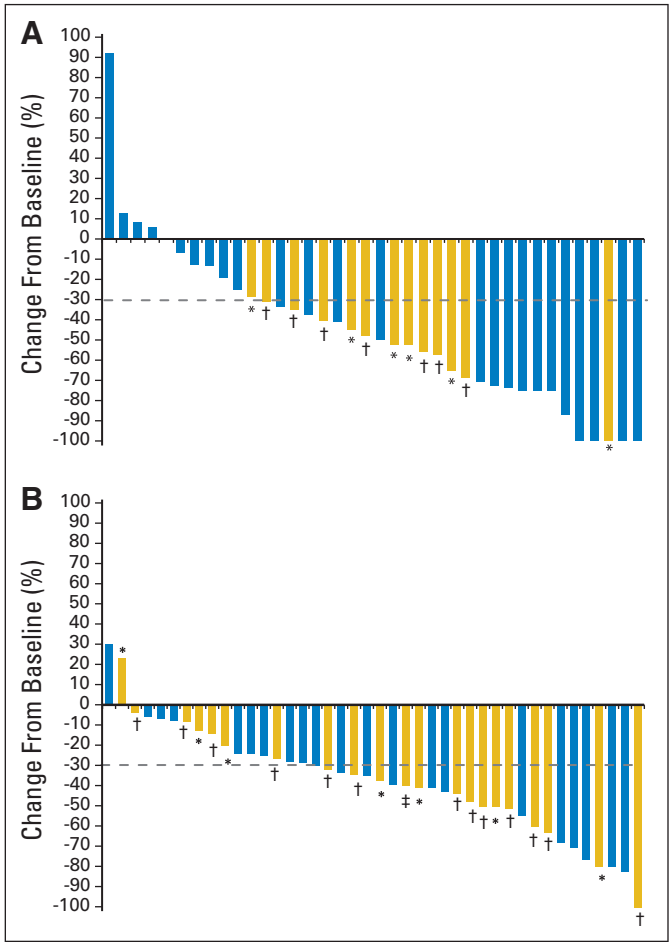

Fig 3. Individual response and *PI3KCA* mutations. Waterfall plots of individual tumor response (percent change from baseline) in (A) arm A (letrozole-lapatinib) and (B) arm B (letrozole-placebo). Blue bars represent patients with *PI3KCA* wild type; gold bars represent patients with *PI3KCA* mutations. (*) Mutations in exon 9. (†) Mutations in exon 20. (‡) Mutations in both exons.

A mutation in *PIK3CA* exon 9 or 20 was observed in 37% of the patients. Specifically, 14 patients (15.2%) had mutation in exon 9, and 22 patients (23.9%) had mutation in exon 20. One patient was confirmed as having mutations in both exons (Data Supplement). The cOR rate tended to be higher in patients with *PI3KCA*-mutated tumors, despite not reaching statistical significance (cOR rate, 76% in *PI3KCA*-mutated *v* 59% in *PI3KCA* WT tumors; $P = .09$). In the letrozole-lapatinib arm, this difference was more marked (93% in mutated *v* 63% in WT tumors; $P = .037$), whereas in the letrozole-placebo arm, the cOR rate was similar between mutated and WT tumors (63% *v* 66%, respectively; $P = .79$). Individual responses according to *PI3KCA* status are presented in Figure 3. Results of gene expression analyses and copy number data are available in the Data Supplement.

DISCUSSION

The EGFR and HER2 cross-talk with estrogen receptor signaling represents a potential mechanism of escape to endocrine therapy. On these premises, we have designed this study to evaluate the clinical and biologic effects of cotargeting both these pathways in HR-positive/HER2-negative patients.

The overall response rate was similar in the two arms (70% for letrozole-lapatinib and 63% for letrozole-placebo). The overall response rate in the letrozole-lapatinib arm reached the prespecified 70% target; however, the letrozole-placebo arm outperformed the expected 50% objective response rate. The inhibition of the HER2-EGFR pathway does not seem to provide additional benefit in endocrine therapy–naive HR-positive/HER2-negative disease.

These results are consistent with those observed in the metastatic setting. Indeed, in the HER2-negative population of EGF30008 study, no difference was observed in median progression-free survival. However, according to a preplanned exploratory analysis, a trend in progression-free survival improvement with the combination of lapatinib-letrozole was observed in the HER2-negative subgroup, which experienced progression within 6 months from prior tamoxifen, suggesting activation of the EGFR pathway as an escape mechanism to endocrine blockade.[13]

The conversion rate from mastectomy to BCS tended to be higher in the placebo arm; however, the decision to offer BCS depends on several parameters, such as breast size, tumor location, presence of ductal carcinoma in situ, contraindication to radiation therapy, and patient decision. No patient achieved a pCR, as expected from neoadjuvant hormonal therapy trials.[4-6,15,16]

The biomarker analyses showed a significant decrease of Ki-67 and of pAKT expression in both arms. Inhibition of Ki-67 was similar in the two treatment arms, which was consistent with the similar response rates observed. Indeed, previous studies have suggested a correlation between a decreased Ki-67 and treatment efficacy.[17,18]

Akt is a serine/threonine kinase that plays an important role in survival when cells are exposed to different apoptotic stimuli, and constitutive activation of the PI3K/Akt pathway has been implicated as one of the mechanisms of resistance to hormonal therapy.[19-21] We have previously reported a significant decrease of pAKT after neoadjuvant chemotherapy plus trastuzumab and/or lapatinib in HER2-positive breast cancer.[22,23] The decrease of pAKT in both arms is consistent with the high clinical response rate observed in these patients.

The more interesting finding is the relationship between *PI3KCA* mutation and response. Although not statistically significant, overall, the presence of *PI3KCA* deregulation was associated with a numerically higher clinical response rate. This difference is mainly driven by the letrozole-lapatinib arm, where the presence of *PI3KCA* mutations predicted for higher chance of responding to treatment. These data suggest a potential benefit of adding lapatinib to letrozole when a deregulation of the PI3K/Akt pathway is documented. Indeed, at least in the HER2-positive setting, preclinical and clinical evidence suggests that deregulation of this pathway may be a marker of trastuzumab resistance while sensitivity to lapatinib is retained.[24-26] In HER2-negative disease, the hypothesis is that tumors harboring *PI3KCA* mutations rely more on GFR/PI3K signaling than on estrogen for growth; thus, blocking growth factor receptor signaling with lapatinib might restore hormonal sensitivity. Indeed, the PI3K pathway influences estrogen and progesterone receptor levels and activity, and a large body of experimental and clinical evidence suggests that hyperactivation of the PI3K pathway promotes antiestrogen resistance.[27]

The combination of letrozole-lapatinib was feasible. Toxicities, generally mild and manageable, are expected with the use of lapatinib

and other tyrosine kinase inhibitors (skin rash, diarrhea, and liver function test alterations). Nevertheless, these toxicities are clinically relevant even if low grade, because these AEs can persist for the entire treatment duration. Definitively, given the similar clinical activity and the greater toxicity, the combination of letrozole-lapatinib cannot be considered a potential interesting option for HR-positive/HER2-negative patients. However, our data suggest that patients whose tumors harbor *PI3KCA* mutations might benefit more from letrozole-lapatinib, thus justifying the higher toxicities. Nevertheless, our findings are based on a limited sample size, and an independent validation on a larger cohort is necessary.

Other studies have explored the role of combining targeted agents to endocrine therapy in the neoadjuvant setting. In a randomized trial conducted in women with HR- and EGFR-positive tumors, gefitinib monotherapy induced tumor shrinkage in 54% of patients, whereas the combination of gefitinib plus anastrozole versus gefitinib alone resulted in a greater inhibition of tumor proliferation.[8]

In another preoperative trial, everolimus added to letrozole significantly increased the clinical response rate and was associated with a higher inhibition of Ki-67 after 15 days of therapy compared with letrozole-placebo.[28] These and other studies underline the importance of conducting relatively small neoadjuvant studies with mandatory tissue sample collection.

Other studies have addressed the role of cross-talk between HR and growth factor receptor pathways; these studies, however, have been conducted in different patient populations and/or by using different tyrosine kinase inhibitors. Our study is the first prospective randomized phase II trial conducted in the neoadjuvant setting to explore the role of lapatinib in HR-positive/HER2-negative operable breast cancer and to relate biomarker expression and/or modulation with treatment efficacy.

In conclusion, our study demonstrated that, in HR-positive/HER2-negative patients, letrozole-lapatinib and letrozole-placebo resulted in a similar clinical response rate and similar effects on Ki-67 and pAKT. Our secondary end point findings of a significant correlation between *PIK3CA* mutation and response to letrozole-lapatinib in HR-positive/HER2-negative early breast cancer must now be independently confirmed.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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GLOSSARY TERMS

EGFR (epidermal growth factor receptor): (also known as HER1) belongs to a family of receptors (HER2, HER3, HER4 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.

gene expression profile: the expression of a set of genes in a biologic sample (eg, blood, tissue) using microarray, reverse-transcriptase polymerase chain reaction, or other technology capable of measuring gene expression.

Ki-67: a marker of proliferation. Ki-67 is a protein that is expressed in the nucleus of proliferating cells. It is absent only in resting cells. Cells in the G1, S, G2, and M phase of the cell cycle express this marker.

phospho-AKT: the phosphorylated form of AKT, which is the activated form of the molecule.

phospho-EGFR: the phosphorylated (ie, activated) form of the epidermal growth factor receptor, which is the activated form of the molecule. See EGFR (epidermal growth factor receptor).

PIK3CA: the catalytic subunit of phosphatidylinositol 3-kinase involved in the generation of PIP3 which, in turn, leads to the activation of AKT and other oncogenic kinases. Mutations in the PIK3CA gene have been found in a number of cancers, including ovarian, breast, colon, and lung carcinomas. See PI3K and AKT/PKB.

PTEN: a tumor suppressor gene with a gamut of regulatory activities. The gene product is a multifunctional molecule. The predominant activity identified for PTEN is its lipid phosphatase activity that converts inositol trisphosphates into inositol bisphosphates, thus inhibiting survival and proliferative pathways that are activated by inositol trisphosphates. PTEN acts to maintain arrest in the G1 phase of the cell cycle and enable apoptosis through an AKT-dependent mechanism.

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