A Neoadjuvant, Randomized, Open-Label Phase II Trial of Afatinib Versus Trastuzumab Versus Lapatinib in Patients With Locally Advanced HER2-Positive Breast Cancer

Mothaffar F. Rimawi,1 Sabina B. Aleixo,2 Ashley Alarcon Rozas,3 João Nunes de Matos Neto,4 Maira Caleffi,5 Alicardo Cesar Figueira,6 Sulene Cunha Souza,7 Andre B. Reiriz,8 Carolina Gutierrez,1 Heloïsa Arantes,9 Martina M. Uttenreuther-Fischer,10 Flavio Solca,11 C. Kent Osborne1

Abstract
Neoadjuvant therapy is used to shrink tumors to facilitate surgery. In this phase II neoadjuvant trial we compared the efficacy and safety of the oral irreversible ErbB family blocker afatinib with lapatinib and trastuzumab, in patients with untreated, locally advanced (LA) HER2-positive breast cancer (BC). Although recruitment was stopped early, 8 of 10 patients who received afatinib monotherapy achieved objective responses. Adverse events were manageable.

Background: Chemotherapy is standard neoadjuvant treatment of LA BC. Patients with HER2-positive BC require targeted therapy. Trastuzumab and pertuzumab, which target HER2, with chemotherapy are approved as neoadjuvant therapy, however, treatments with different mechanisms of action might provide a broader range of activity. In this study we evaluated the efficacy and safety of the irreversible ErbB family blocker afatinib, versus trastuzumab or lapatinib in the neoadjuvant treatment of HER2-positive, LA BC. Patients and Methods: Treatment-naïve, HER2-positive BC patients with stage IIIA, B, C or inflammatory disease were randomized 1:1:1 to daily afatinib (50 mg), lapatinib (1500 mg), or weekly trastuzumab (4 mg/kg loading dose, then 2 mg/kg/wk) for 6 weeks until surgery or follow-up neoadjuvant treatment. The primary end point was objective response rate according to Response Evaluation Criteria in Solid Tumors (version 1.0).

Results: Recruitment was stopped early because of slow patient enrollment; 29 patients were randomized to afatinib (n = 10), lapatinib (n = 8), or trastuzumab (n = 11). Objective response was seen in 8 afatinib-, 6 lapatinib-, and 4 trastuzumab-treated patients. Eleven patients had stable disease (best response); 1 lapatinib- and 1 trastuzumab-treated patient had progressive disease. All 10 afatinib-treated patients experienced drug-related adverse events (commonly diarrhea, dermatitis aciform, and paronychia) versus 6 of 8 lapatinib- (diarrhea and rash) and 5 of 11 trastuzumab-treated patients (vomiting and arthralgia).

Conclusion: Afatinib demonstrated clinical activity that compared favorably to trastuzumab and lapatinib for neoadjuvant treatment of HER2-positive BC, with a safety profile consistent with epidermal growth factor receptor tyrosine kinase inhibitors.

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Neoadjuvant Anti-HER2 Therapy in Breast Cancer

Introduction

HER2 (ErB2) is a member of the ErbB family of receptors, and approximately 20% to 25% of breast cancer (BC) patients have HER2-positive disease. Trastuzumab is a humanized murine monoclonal antibody (mAb) that inhibits HER2 signal transduction extracellularly and induces antibody-dependent cellular cytotoxicity. It was the first targeted therapy approved for the treatment of HER2-positive metastatic BC (MBC) in combination with paclitaxel. Since then, other agents have been approved for this indication, including lapatinib, a reversible, dual epidermal growth factor receptor (EGFR [ErbB1])/HER2 tyrosine kinase inhibitor (TKI). Lapatinib (with capecitabine) is indicated for HER2-positive MBC patients, whose disease has progressed during previous trastuzumab-based therapy. Lapatinib (with letrozole) is also recommended for postmenopausal women with hormone receptor-positive, HER2-positive MBC. Pertuzumab, a HER2-targeting mAb that blocks its dimerization is approved for HER2-positive MBC (in combination with trastuzumab and docetaxel) as is the HER2 antibody drug conjugate trastuzumab emtansine. Clinical benefits of HER2-directed therapy were subsequently shown in early-stage BC and led to the approval of trastuzumab for the adjuvant treatment of early-stage HER2-positive BC.

Neoadjuvant chemotherapy is the standard of care for women with BC who present with large, locally advanced (LA) tumors, to reduce tumor size and to allow for breast conservation surgery. Patients treated with neoadjuvant chemotherapy who achieve a pathological complete response (pCR) have significant improvements in disease-free survival (DFS; \( P < .0001 \)) and overall survival (OS; \( P < .0001 \)). The neoadjuvant setting also allows for direct assessment of response to therapy according to pCR at the time of surgery in previously untreated patients. Several neoadjuvant studies of trastuzumab in combination with chemotherapy have documented increased pCR rates and improved DFS in HER2-positive early-stage disease. Based on the observations from the Neoadjuvant Herceptin (NOAH) trial, trastuzumab with neoadjuvant chemotherapy was approved for use in HER2-positive patients with LA BC, followed by adjuvant trastuzumab monotherapy, and this combination has been recommended in this setting since 2011.

Secondary (acquired) resistance to HER2-directed therapies is well recognized as a challenge to second-line treatment, however, primary (intrinsic) resistance has also been described, and this might have an effect on neoadjuvant treatment. With respect to trastuzumab, resistance mechanisms might include weakened access of trastuzumab to HER2 because of truncation of the extracellular domain of the protein (termed p95HER2), alternative signaling from insulin-like growth factor receptor-1, and other EGFR family members, and aberrant activation of downstream signaling pathways, such as phosphatidylinositol 3-kinase (PI3K)/protein kinase B. BC patients with primary resistance to HER2-directed therapy might benefit from a broader range of ErbB family inhibition in neoadjuvant and adjuvant settings.

Evidence that EGFR is important in HER2-positive BC was described by Rimm and colleagues, who showed that levels of EGFR expression correlated with decreased benefit from adjuvant concurrent trastuzumab and chemotherapy treatment. DFS rates of 90% (EGFR low) versus 74% (EGFR high) were observed in operable BC patients, after concurrent trastuzumab and chemotherapy treatment. In the neoadjuvant setting, the Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimisation (NeoALTTO) and Neoadjuvant Study of Pertuzumab and Herceptin in an Early Regimen Evaluation (NeoSphere) studies, in which a broader ErbB family inhibition was used, clearly showed favorable results compared with single-agent targeted approaches.

Afatinib is an irreversible ErbB family blocker that covalently binds to EGFR, HER2, and ErbB4, and thus irreversibly blocks signaling from all homo- and heterodimers formed by the ErbB family members. Of note, human epidermal growth factor receptor-3 (HER3) is unique in the family because its intrinsic kinase activity is weak if at all present. However, the lack of activity does not diminish its importance in ErbB signaling because HER3 receptors are strong mediators of PI3K pathway activation. HER3-mediated signaling functions exclusively through heterodimerization and afatinib has been shown to block transphosphorylation of ErbB3 in BC cells. This agent differs from lapatinib and trastuzumab by its broader scope of ErbB receptor inhibition and by its covalent mode of binding resulting in potent and long-lasting activity. In preclinical models, afatinib demonstrated potent antitumor activity in HER2-positive, trastuzumab-resistant SUM190 xenografts and in the HER2-positive MAXF1162 patient-derived xenografts. Afatinib also showed clinical activity in patients with HER2-positive MBC whose disease relapsed after previous trastuzumab therapy in an open-label, phase II trial. Objective responses (ORs) were experienced by 4 (11%) evaluable afatinib-treated patients, 19 patients (46%) achieved clinical benefit, median progression-free survival was 15.1 weeks, and median OS was 61.0 weeks.

This phase II study was performed to determine the efficacy, safety, and effect on biomarkers of the irreversible ErbB family blocker afatinib, compared with the reversible dual EGFR/HER2 TKI lapatinib, or the HER2-targeted mAb trastuzumab, in the neoadjuvant treatment of HER2-positive, treatment-naïve BC patients with LA disease. All 3 agents were used as monotherapy.

Patients and Methods

Study Design

This was an international, multicenter, open-label, phase II, parallel, randomized, 3-arm, exploratory trial in treatment-naïve patients with a pathologically confirmed diagnosis of HER2-positive, LA BC. The study consisted of a 2-week screening period, followed by 6 weeks of treatment. The study was conducted between September 2009 and August 2011 at 15 centers in Latin America (Brazil, Colombia, and Peru) and 1 center in the United States, and was performed in compliance with the Declaration of Helsinki, the International Conference on Harmonization Tripartite Guideline for Good Clinical Practice, and in accordance with applicable regulatory and relevant local guidelines, with all patients providing written informed consent before study participation.

Study Population

Eligible female patients (aged ≥ 18 years) with histologically confirmed LA, nonmetastatic HER2-positive BC (including stage IIIA, B, and C, and inflammatory BC) who were treatment-naïve, had a life expectancy of ≥ 6 months, had at least 1 tumor lesion...
of ≥ 5 cm in diameter, and an Eastern Cooperative Oncology Group performance status of 0 or 1 were included. Patients had to undergo fresh tumor biopsies for HER2 and biomarker analyses; HER2-positive disease was assessed using immunohistochemistry and a score of 3+ was considered positive. Patients with an equivocal score of 2+ were deemed HER2-positive if their tumors were also HER2-positive according to fluorescence in situ hybridization. Patients with an absolute neutrophil count of < 1500/mm³, a platelet count of < 100,000/mm³, hemoglobin levels of < 9.0 g/dL, bilirubin levels of > 1.5 mg/dL (≥ 26 μmol/L, SI unit equivalent), aspartate amino transferase or alanine amino transferase levels greater than twice the upper limit of normal, serum creatinine greater than 1.5 times the upper limit of normal, and/or calculated/measured creatinine clearance of ≤ 45 mL/min were excluded, as were patients with significant or recent acute gastrointestinal disorders or any serious active infection.

**Treatment**

Patients were randomized 1:1:1 to receive 50 mg afatinib orally daily, 1500 mg lapatinib orally daily, or trastuzumab as a once-weekly infusion starting with a loading dose of 4 mg/kg in week 1, followed by 2 mg/kg per week. Patients were randomized to treatment using a centralized interactive voice response system. Treatment duration was 6 weeks in two 21-day treatment courses or until tumor progression (progressive disease [PD] according to Response Evaluation Criteria in Solid Tumors [RECIST] version 1.0) or drug-related adverse events (AEs). After 6 weeks, patients still receiving therapy could be treated by their local physician according to the applicable standard of care, which could include surgery or neoadjuvant chemotherapy. Recommendations for the management of AEs and dose reductions were provided to ensure that patients received the most effective treatment at the highest tolerated dose. In the event of a first afatinib-related AE of Common Terminology Criteria for Adverse Events (CTCAE) Grade ≥ 3, the afatinib dose was reduced to 40 mg. A second afatinib-related AE of Grade ≥ 3 led to a reduction to 30 mg afatinib, and a third event led to the discontinuation of afatinib treatment. Furthermore, if the AE of Grade ≥ 3 did not resolve to Grade ≤ 1 or baseline within 14 days after stopping afatinib treatment, the patient was permanently discontinued from the study. In the event of a first drug-related AE to lapatinib or trastuzumab, dose reductions were performed as specified in the respective Summary of Product Characteristics/Prescribing Information. Concomitant medications for underlying diseases and to treat tumor-associated symptoms were permitted; treatment with bisphosphonates was allowed, and corticosteroids were only permitted as short-term therapy (< 14 days). Localized radiation therapy was not allowed.

**Efficacy and Safety Assessments**

The primary end point was OR during treatment, determined according to RECIST version 1.0. OR was evaluated based on clinical assessment and was defined as complete response (CR) or partial response (PR), confirmed by 2 consecutive repeat assessments performed 3 weeks apart after the criteria for response were first met. Secondary end points included clinical benefit (CR, PR, stable disease [SD]), determined according to RECIST version 1.0 criteria, at any time during the study, and the sum of the longest diameters of the target lesions at 3 and 6 weeks; this was assessed for the primary breast lesion only and was restricted to maximum decrease from baseline at any time point. The safety of afatinib was assessed according to intensity and incidence of AEs, graded according to the US National Cancer Institute CTCAE version 3.0.

**Pharmacokinetic and Biomarker Analyses**

Additional objectives were to evaluate the pharmacokinetics (PKs) of afatinib and to assess biomarkers in all treated patients. For quantification of afatinib plasma concentrations, venous blood was collected at course 1 visit 2 (day 8), course 2 visit 1 (day 22), and course 2 visit 2 (day 29), and afatinib plasma concentrations were determined using a validated high-performance liquid chromatography tandem mass spectroscopy assay. Biopsy tissue was used to assess treatment-induced modulation of biomarkers, including phospho-mitogen-activated protein kinase (MAPK), total MAPK, EGFR, and HER2, phospho-EGFR and -HER2, cell proliferation markers (Ki-67 and p27), cleaved caspase 3, phosphate and tensin homologue (PTEN), PI3K, and HER2 homodimerization. Fresh tumor tissue was obtained at baseline, during treatment at cycle 2 visit 1, and at the end of treatment. Tissue was obtained with a core needle biopsy (up to 4 core biopsies were taken through a single incision). A portion of the breast biopsy specimen was placed in fixative for hematoxylin and eosin and immunohistochemical staining. The remaining specimens were snap-frozen at −80°C for future analysis. Immunohistochemical evaluation of each biomarker based on the percentage of positive cells and intensity of staining was performed; both were combined to determine an expression score for each biomarker. Examination of the HER2:HER2 dimerization state was assessed with VeraTag technology from Monogram Biosciences (South San Francisco, CA). Descriptive statistics were calculated to summarize each molecular marker. Paired comparisons between baseline and other time points were performed using either Wilcoxon signed rank test for continuous levels of expression scores or the McNemar test for dichotomized levels of expression scores.

**Statistical Analyses**

All analyses performed in this study were exploratory. The sample size calculation (40 patients per arm) was based on the Sargent and Goldberg design. Based on an assumption of a 30% overall response rate (ORR) with trastuzumab and lapatinib, and a 45% ORR for afatinib, the probability of observing an afatinib ORR larger than trastuzumab or lapatinib was 90%. The ORR according to RECIST version 1.0 was estimated along with the exact 95% Clopper—Pearson confidence interval for each treatment regimen. Fisher’s exact test was used to compare ORR between treatment groups: afatinib versus trastuzumab and afatinib versus lapatinib. Because of the exploratory nature of the trial, no adjustments were made for multiple treatment comparisons.

**Results**

**Patient Population**

A total of 73 patients were screened and 29 patients whose tumors tested positive for HER2 were randomized and treated: 10 with afatinib, 8 with lapatinib, and 11 with trastuzumab. Because of slow patient enrollment, this trial stopped recruitment early. All but
1 of the 29 randomized patients completed the study; 1 patient treated with afatinib permanently discontinued because of the AE, dermatitis acneiform. Baseline patient demographics and tumor characteristics are shown in Table 1. The median age of women participating in the study was 49 years; most patients had Stage IIIA (n = 16; 55.2%) or IIIB (n = 8; 27.6%) BC. The median exposure to study medication was similar across treatment groups (44-45 days) and ranged from 12 to 53 days.

**Efficacy**

Of 29 patients eligible for efficacy assessment, an OR (clinical assessment) was observed in 80.0% (n = 8) of women treated with afatinib compared with 75.0% (n = 6) of patients treated with lapatinib and 36.4% (n = 4) of patients who received trastuzumab. All responding patients had a PR; no patients presented with a CR at any time during the 6-week study. The remaining 11 patients had SD as the best response at any time during the study across all treatments. Table 2 provides a summary of ORs and overall tumor response at week 3 and week 6. At week 3, 5 (50.0%) afatinib-treated patients had a PR, as did 2 (25.0%) lapatinib- and 2 (18.2%) trastuzumab-treated patients. At week 6, 7 (70.0%) patients treated with afatinib had a PR compared with 6 (75.0%) lapatinib- and 4 (36.4%) trastuzumab-treated patients. One patient who received afatinib and had a PR at week 3 discontinued study medication on day 11 because of the AE dermatitis acneiform, still had a PR at week 6 but was counted as missing at week 6. PD was not observed in any patient treated with afatinib; 1 (12.5%) lapatinib- and 1 (9.1%) trastuzumab-treated patient showed evidence of PD at week 6. Of

<table>
<thead>
<tr>
<th>Table 1 Baseline Patient Demographics and Tumor Characteristics</th>
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<tbody>
<tr>
<td><strong>Total Patients Treated, n (%)</strong></td>
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<tr>
<td>---------------------------------</td>
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<tr>
<td>Race, n (%)</td>
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<tr>
<td>American Indian/Alaskan Native</td>
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<tr>
<td>Black/African-American</td>
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<tr>
<td>White</td>
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<tr>
<td>Hispanic/Latino, n (%)</td>
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<tr>
<td>Yes</td>
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<tr>
<td>No</td>
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<tr>
<td>Age, Years</td>
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<tr>
<td>Mean (range) (median)</td>
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<tr>
<td>Tumor Stage (UICC/AJCC), n (%)</td>
</tr>
<tr>
<td>0</td>
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<tr>
<td>III</td>
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<tr>
<td>IIIA</td>
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<td>IIIB</td>
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<tr>
<td>Inflammatory Breast Cancer Indicator, n (%)</td>
</tr>
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<tr>
<td>Yes</td>
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<tr>
<td>Missing</td>
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<tr>
<td>Time Since First Diagnosis, Months</td>
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<tr>
<td>Mean (range) (median)</td>
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<tr>
<td>HER2 Status, n (%)</td>
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<tr>
<td>0</td>
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<tr>
<td>1+</td>
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<tr>
<td>2+</td>
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<tr>
<td>3+</td>
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<tr>
<td>Missing</td>
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<td>ER Status</td>
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<tr>
<td>Positive</td>
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<tr>
<td>Negative</td>
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<tr>
<td>Not evaluable</td>
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<tr>
<td>Median Baseline Tumor Diameter of Primary Target Lesion (Range), mm</td>
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</tbody>
</table>

Abbreviations: AJCC = American Joint Committee on Cancer; ER = estrogen receptor; UICC = International Union Against Cancer.

aPatients received a loading dose of trastuzumab of 4 mg/kg in week 1.

HER2 status according to immunohistochemistry in this patient was missing; the tumor was HER2-positive according to fluorescent in situ hybridization.

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patients with estrogen receptor (ER)-positive tumors at baseline. At week 6, PR was achieved in 4 afatinib-treated patients, 3 lapatinib-treated patients, and 0 trastuzumab-treated patients. The adjusted mean maximum decrease from baseline in the diameter of the primary target lesion was −27.5 mm for afatinib therapy, −31.0 mm for lapatinib therapy, and −20.9 mm for trastuzumab therapy. A total of 80.0% (n = 8) of patients treated with afatinib had a maximum decrease in tumor diameter of ≥30% versus lapatinib (n = 6; 75.0%) or trastuzumab (n = 4; 36.4%; Figure 1).

Safety
All 10 patients in the afatinib group experienced drug-related AEs versus 75.0% (n = 6) and 45.5% (n = 5) of lapatinib- or trastuzumab-treated patients. The incidence of drug-related AEs observed in ≥2 patients per treatment group is presented in Table 3. The most frequently reported drug-related AEs for afatinib were diarrhea (n = 10; 100%), dermatitis acniform (n = 6; 60.0%), paronychia (n = 5; 50.0%), mucosal inflammation (n = 4; 40.0%), and rash (n = 3; 30.0%), with 3 (30.0%) patients experiencing drug-related AEs of Grade 3 (diarrhea, dermatitis acniform, and mucosal inflammation). No Grade 4 AEs were reported for patients treated with afatinib. The most frequently reported drug-related AE for lapatinib was diarrhea (n = 3; 37.5%) with 1 patient reporting Grade 3 diarrhea, and for trastuzumab were vomiting and arthralgia (n = 2 patients each; 18.2%; no Grade 3 or 4 AEs were reported for patients treated with trastuzumab).
Two patients treated with afatinib had drug-related AEs that led to single dose reductions to 40 mg (diarrhea and rash), 1 additional patient had a second dose reduction to 30 mg based on the protocol-specified dose-reduction scheme for the AE mucosal inflammation, and 1 patient had a drug-related AE that led to treatment discontinuation (Grade 3 dermatitis acneiform); no patients treated with lapatinib or trastuzumab had dose reductions.

**Pharmacokinetics**

Pharmacokinetic (PK) analyses were performed for afatinib only, with 10 afatinib-treated patients providing at least 1 PK sample. Steady state seemed to be reached at day 8 in patients who received 50 mg afatinib and individual predose plasma concentrations appeared to remain stable over the observed treatment periods. The geometric mean predose values were 32.1 ng/mL on day 8 of course 1, 35.6 ng/mL on day 22 of course 2, and 27.2 ng/mL on day 29 of course 2. The overall variability of predose plasma concentrations of patients who received 50 mg was moderate to high, with geometric coefficient of variation values of 35.6% to 74.7% (Figure 2). The data from 3 patients who had afatinib dose reductions to 40 mg (as allowed by the study protocol) showed that afatinib plasma concentrations decreased when dose reductions were performed. Data were too limited to draw further conclusions.

**Biomarkers**

Examination using VeraTag technology of HER2:HER2 dimerization as a surrogate for functional activation revealed that dimerization was increased two- to ten-fold in the lapatinib group in all of the evaluable (4/8) paired biopsies from which baseline and cycle 2 visit 1 samples were suitable for review (Figure 3). In the afatinib group, the HER2:HER2 dimerization signal was consistently decreased with treatment in the 3 evaluable pairs and no trend was observed in the 4 evaluable patients in the trastuzumab group. No meaningful correlations were observed for any of the other biomarkers (ER, PR, EGFR, phosphorylated MAPK, phosphorylated protein kinase B, PTEN, p27, Ki-67), which is likely related to the small sample size available and the interpatient variability in expression at baseline for some of these markers. The small number of available biomarker samples in this study did not allow for a correlation analysis between EGFR expression and clinical response.

**Discussion**

Afatinib showed signs of clinical activity in the neoadjuvant treatment of HER2-positive LA BC and the efficacy results from this exploratory phase II trial compared favorably with the HER2-targeted therapies trastuzumab or lapatinib. All 3 treatments demonstrated a safety profile consistent with previous monotherapy trials; although afatinib was associated with a higher rate of drug-related AEs, these were mostly Grade 2 and manageable. Afatinib has previously shown clinical activity in HER2-negative and HER2-positive MBC and is currently being assessed in other phase II/III trials in the MBC setting including LUX-Breast 1 (NCT01125566) (recruitment was stopped and the experimental arm recently discontinued because of a low likelihood of meeting study criteria for efficacy and a higher rate of AEs), LUX-Breast 2 (NCT01271725), and LUX-Breast 3 (NCT01441596).

![Figure 2](image-url)

*Abbreviations: Cpre,ss = Steady State Predose Concentrations; gMean = Geometric Mean.*

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**Table 3** Incidence of Drug-Related AEs Observed in 2 or More Patients in Any Treatment Group

<table>
<thead>
<tr>
<th></th>
<th>Afatinib 50 mg Once Daily</th>
<th>Lapatinib 1500 mg Once Daily</th>
<th>Trastuzumab 2 mg/kg Weeklya</th>
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</thead>
<tbody>
<tr>
<td>Total Patients Treated, n (%)</td>
<td>10 (100.0)</td>
<td>8 (100.0)</td>
<td>11 (100.0)</td>
</tr>
<tr>
<td>Total With Drug-Related AEs, n (%)</td>
<td>10 (100.0)</td>
<td>6 (75.0)</td>
<td>5 (45.5)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>10 (100.0)</td>
<td>3 (37.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Dermatitis Acneiform</td>
<td>6 (60.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Paronychia</td>
<td>5 (50.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Mucosal Inflammation</td>
<td>4 (40.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Rash</td>
<td>3 (30.0)</td>
<td>1 (12.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Hypocalcemia</td>
<td>2 (20.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1 (10.0)</td>
<td>0 (0.0)</td>
<td>2 (18.2)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>2 (18.2)</td>
</tr>
</tbody>
</table>

aPatients received a loading dose of trastuzumab of 4 mg/kg in week 1.
Although this phase II trial was terminated early because of a low number of patients recruited, the ORR observed among 8 of 10 afatinib-treated patients, of which 2 had inflammatory BC and 6 of 8 lapatinib-treated patients, shows that targeting multiple ErbB family members is a feasible approach to the neoadjuvant treatment of HER2-positive BC.

The concept of targeting more than 1 ErbB family member using multiple agents such as lapatinib and trastuzumab in the treatment of HER2-positive BC has been shown in preclinical studies. Using HER2-positive models, the triple combination of gefitinib, trastuzumab, and pertuzumab improved tumor shrinkage to a greater extent than single-agent or dual therapy. Results from the neoadjuvant NeoALTTO, NeoSphere, and the Chemotherapy, Herceptin and Lapatinib in Operable Breast cancer (CHER-LOB) studies have confirmed these preclinical observations. In combination with chemotherapy, trastuzumab and lapatinib, and trastuzumab and pertuzumab, achieved higher pCRs within the range of 46% to 51% compared with lower pCRs of 24% to 30% with a single-agent combined with the same chemotherapy regimen. More recently, the Translational Breast Cancer Research Consortium 006 trial of trastuzumab and lapatinib, without chemotherapy, reported a pCR rate of 36% in ER-negative/HER2-positive patients versus 21% in ER-positive/HER2-positive patients, suggesting that some patients with LA HER2-positive BC might not require concurrent chemotherapy to elicit a treatment response. This idea is further supported by data from the NeoALTTO trial after 6 weeks of therapy, which showed that lapatinib monotherapy elicited high objective clinical response rates of 52.6% before surgery and paclitaxel treatment. In treatment-naïve primary HER2-positive BC patients, the chemotherapy-free trastuzumab with pertuzumab combination in the phase II, NeoSphere trial, reported a pCR rate of 16.8%. Overall, these clinical findings, combined with the clinical activity observed with afatinib as a single agent, support the rationale that targeting more than 1 ErbB family member, with or without chemotherapy, could have some merit in the treatment of HER2-positive LA BC.

It is unclear as to why recruitment was slow in our study; however, we assume that the positive data being published from the neoadjuvant combination trials with trastuzumab or lapatinib (discussed previously herein) at the time this trial was recruiting might have had an effect. In some centers, clinicians were concerned that patients with these large BC tumors would have been undertreated if they did not receive chemotherapy, and the time taken to prescreen patients for HER2-positive status and the study eligibility criteria might have hindered patient recruitment. However, Filion and colleagues recently established, in 9 neoadjuvant and adjuvant phase II or III BC studies conducted at the same institution, that no specific eligibility criterion consistently prohibited recruitment, and there was no criterion precluding recruitment that was shared by some or all of the trials.

Results from the biomarker analyses showed that afatinib treatment consistently resulted in HER2 downregulation in the clinical samples evaluated. This observation is in line with previous preclinical data that showed that canertinib (irreversible ErbB inhibitor) can modulate HER2 expression by targeting internalization, ubiquitylation, and proteasomal degradation in N87 gastric cancer cells. The increased HER2:HER2 dimerization observed in tissue samples obtained from patients treated with lapatinib is consistent with previously reported data that showed lapatinib-induced HER2 accumulation at the cell surface results in the stabilization of inactive HER2 homo- and heterodimers in the plasma membrane. The lack of HER2 dimer modulation by trastuzumab observed in this study is in line with a previous study showing no downregulation of HER2 in LA HER2-overexpressing BC patients treated with trastuzumab, but contrasts with the enhanced HER2 phosphorylation, ubiquitylation, and degradation of the receptor observed after treatment in HER2-overexpressing SKBR3 and MCF7-HER2 BC cell lines.

Abbreviations: C2_V1 = Cycle 2 Visit 1 (Day 22); H22D = HER2 Dimerization State.
Although the small number of samples did not allow for any meaningful statistical correlations, biomarker analyses continue to play an important role in clinical trials to identify and evaluate predictive markers of treatment response.

Other irreversible inhibitors that target various members of the ErbB family currently under investigation include neratinib, an EGFR and HER2 dual inhibitor, and dacomitinib, a pan-HER inhibitor. Neratinib is being investigated for the treatment of BC in the first- and second-line setting, and a phase II study in advanced HER2-positive BC patients demonstrated ORRs of 24% in patients who received previous trastuzumab treatment and 56% in patients without previous trastuzumab treatment.52 A neo-adjuvant phase II study is under way to assess neratinib with or without trastuzumab followed by postoperative trastuzumab treatment in women with LA HER2-positive BC.53 Dacomitinib is currently in phase III trials in non—small-cell lung cancer and has demonstrated antiproliferative activity in vitro in HER2-positive BC cell lines resistant to trastuzumab and lapatinib.54 No BC studies are currently ongoing with dacomitinib.

It is unlikely that a single agent is capable of achieving efficacy comparable with combination therapy in the neo-adjuvant treatment of LA HER2-positive BC. However, in light of afatinib’s potent inhibition of the ErbB family and the efficacy signal observed for this agent as monotherapy versus trastuzumab and lapatinib in this study, further investigation is warranted in the neo-adjuvant setting to identify the potential role of afatinib in combination with other agents targeting HER2 or in combination with chemotherapy. Of note, recently reported results showed that afatinib in combination with trastuzumab and chemotherapy was associated with a pCR rate of 49.2% in the neo-adjuvant setting.55

**Conclusion**

Afatinib demonstrated clinical activity in this phase II trial of a 6-week, preoperative treatment window, and compared favorably with trastuzumab or lapatinib. Although higher rates of the drug-related AEs, diarrhea and dermatitis acniform, were observed, they were mostly Grade 2 and manageable. Because this trial was terminated early because of slow recruitment, further trials are needed to identify the potential role of afatinib, likely in combination with other agents in HER2-positive BC in the neo-adjuvant setting, with longer treatment times.

**Clinical Practice Points**

- Afatinib is a potent and selective, irreversible ErbB family blocker which covalently binds to and irreversibly blocks signaling from all homo- and heterodimers formed by the ErbB family members.
- A total of 29 patients were recruited in a phase II clinical trial to determine the efficacy and safety of afatinib versus trastuzumab versus lapatinib in HER2-positive, LA BC patients. The study was terminated early because of recruitment challenges.
- Objective responses were observed in 8 (80%) afatinib-treated patients, 6 (75%) lapatinib-treated patients, and 4 (36.4%) trastuzumab-treated patients; all responders had a PR (no CRs were reported). The other 11 patients had SD as their best response.
- All patients who received afatinib (n = 10) experienced drug-related AEs. Drug-related AEs were reported in 6 (75%) patients who received lapatinib and 5 (45.5%) patients who were treated with trastuzumab. Diarrhea was the most common drug-related AE reported in afatinib-treated (n = 10) and lapatinib-treated (n = 3) patients, with vomiting (n = 2) being the most common event during trastuzumab treatment. Grade 3 drug-related AEs were observed in 3 patients who received afatinib and in 1 lapatinib-treated patient.
- Results reported herein suggest that afatinib might be an effective treatment in HER2-positive, LA BC patients in the neo-adjuvant setting, with a safety profile consistent with other EGFR TKIs.

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**Disclosure**

C.K.O. has been a member on advisory committees for bioTheranostics, NanoString Technologies, and Pfizer. H.A., M.M.U.-F. and F.S. are employees of Boehringer Ingelheim. The remaining authors have stated that they have no conflicts of interest.

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