Original Study

Use of Everolimus and Trastuzumab in Addition to Endocrine Therapy in Hormone-Refractory Metastatic Breast Cancer

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Abstract

Patients with endocrine-refractory, hormone receptor-positive, HER2-negative metastatic breast cancer were treated with everolimus (n = 30) or trastuzumab (n = 24) in addition to their existing endocrine therapy. We hypothesized that the combination could restore sensitivity to endocrine therapy. Progression-free survival was 5.7 months, and 2.2 months, and clinical benefit rate at 24 weeks was 48% and 11% for patients who received everolimus or trastuzumab, respectively.

Background: Increased signaling through growth factor receptor pathways, including HER2, plays a role in resistance to endocrine therapy (ET) in patients with hormone receptor (HR)-positive metastatic breast cancer (MBC). Inhibition of mechanistic target of rapamycin improves outcomes when used in addition to ET in patients with HR-positive MBC, who previously received ET. We hypothesized that the additional use of trastuzumab (T) or everolimus (E) could restore sensitivity to ET in patients with endocrine-resistant, HR-positive, HER2-negative MBC. **Patients and Methods:** Patients with endocrine-resistant HR-positive, HER2-negative MBC continued the ET during which they had experienced disease progression, and were randomized to receive T or E. At disease progression, patients could continue the therapy they were receiving and have E or T used in addition. **Results:** Fifty-four patients were randomized to the additional use of E (n = 30) or T (n = 24) with existing ET. Progression-free survival (PFS) was 5.7 months, and 2.2 months, respectively, and clinical benefit rate at 24 weeks was 48% and 11% for patients receiving E or T, respectively. PFS was 4.5 months and 3.1 months for patients in whom E (n = 16) or T (n = 12) was used post progression, respectively. There were no new safety signals apart from 2 patients who had a decreased ejection fraction while receiving E with ET. **Conclusion:** These results suggest that E, but not T, can potentially reverse resistance to ET in patients with endocrine-resistant HR-positive, HER2-negative MBC. Further, the additional use of E with an ET to which the cancer has already been exposed might offer the possibility of delaying time to use of chemotherapy.

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Introduction

The outcome for patients with all stages of hormone receptor (HR)-positive breast cancer, which constitute most breast cancers diagnosed, has improved with the use of endocrine therapies (ETs).[1] However, all metastatic HR-positive breast cancers eventually develop resistance to ET, which negatively affects outcome

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for these patients. There are multiple mechanisms of resistance to ET.[2-4] One such mechanism includes increased signaling through growth factor pathways, including human epidermal growth factor pathways.[5-7] Despite encouraging preclinical studies,[8-10] clinical trials using agents that inhibit these growth factor receptors, such as epidermal growth factor receptor, insulin growth factor receptor-1, and vascular endothelial growth factor, in patients with metastatic HR-positive breast cancer have not in general shown promising efficacy.[11-17] This might be in part because in most cases they were evaluated in patients treated in the first-line setting, who might not have had endocrine-resistant breast cancers (ERBCs). In support of this concept, inhibition of the downstream protein, mechanistic target of rapamycin (mTOR), improved outcome when used in addition to ET in patients with endocrine-pretreated metastatic breast cancer,[18,19] but did not improve outcome in the first-line setting.[20]

Several preclinical studies have shown a role for HER2 in resistance to ET.[7,10] Long-term exposure of HR-positive breast cancers to selective estrogen receptor (ER) modulators (SERMs) in vivo results in endocrine resistance and in an increase in the expression of HER2.[10,21] Although these SERM-resistant cancers exhibit only moderate expression of HER2 (2+ expression using immunohistochemistry [IHC]), and do not exhibit HER2 gene amplification, SERM-stimulated tumor growth is inhibited by trastuzumab.[21] HER2 protein expression is increased in recurrent HR-positive breast cancers, in patients treated with tamoxifen, compared with primary cancers.[6] Despite these preclinical findings, the additional use of trastuzumab with chemotherapy was reported to be ineffective in patients with HER2-negative breast cancer, regardless of HR status.[22,23] Likewise, a randomized trial showed that the additional use of lapatinib with letrozole, in patients with HR-positive, HER2-negative metastatic breast cancer was not associated with an improved outcome.[11] However, a subset of patients in this trial who were determined to have ERBC, defined as disease relapse less than 6 months from discontinuing tamoxifen, had a 5-month improvement in progression-free survival (PFS) when lapatinib was used in addition to letrozole, despite the fact that they had known HER2-negative cancers at diagnosis.[11] Taken together these data suggest there might be a role for targeting HER2 signaling in metastatic ERBC.

Despite the lack of success in targeting individual growth factor receptors in HR-positive metastatic disease, inhibition of mTOR has been shown to be effective in 4 randomized trials.[18,19,24,25] All of these trials accrued patients who had received previous ET. The median PFS in the ET control groups was <6 months suggesting that most, but not all, of patients in these trials had ERBC. All of these trials[18,19,24,25] showed a significant improvement in PFS when everolimus was used in addition to a previously unused ET; thus, benefit observed might have been in part to simply treating with a new ET.

We hypothesized that inhibition of HER2 with trastuzumab, or of mTOR with everolimus, could resensitize metastatic ERBC to the endocrine agent during which they had experienced disease progression. Further, we hypothesized that dual inhibition of HER2 and mTOR with continued inhibition of ER could be effective in metastatic ERBC.

Patients and Methods

Patient Eligibility and Selection

Patients 18 aged years or older with metastatic HR-positive ERBC, defined as disease progression within 6 months of starting their most recent line of ET were eligible. Patients had to receive at least 1 line of ET in the metastatic setting and could have received any number of previous lines of systemic therapy. Patients could not have received previous HER2-directed or mTOR inhibitor therapy. Either measurable or evaluable/unmeasurable disease was allowed.

Tumors were HR-positive and HER2-negative at initial primary diagnosis. All patients had to have a biopsy in the metastatic setting that was HR-positive with moderate expression of HER2, defined as 1+ or 2+ using IHC. Patients with 2+ expression of HER2 had to have fluorescent in situ hybridization to prove nonamplification as per American Society of Clinical Oncology-College of American Pathologists (ASCO-CAP) guidelines. Patients with metastatic cancers that were HER2-positive according to ASCO-CAP guidelines defined as 3+ using IHC or HER2-amplified using fluorescent in situ hybridization, or those with cancers that were HER2 0 using IHC were not eligible. Key exclusion criteria included uncontrolled central nervous system metastases, or life-threatening visceral metastases.

All participants provided written informed consent before enrollment. The protocol and informed consent were approved by an institutional review board at Emory and Northwestern Universities.

Treatment Plan and Study Design

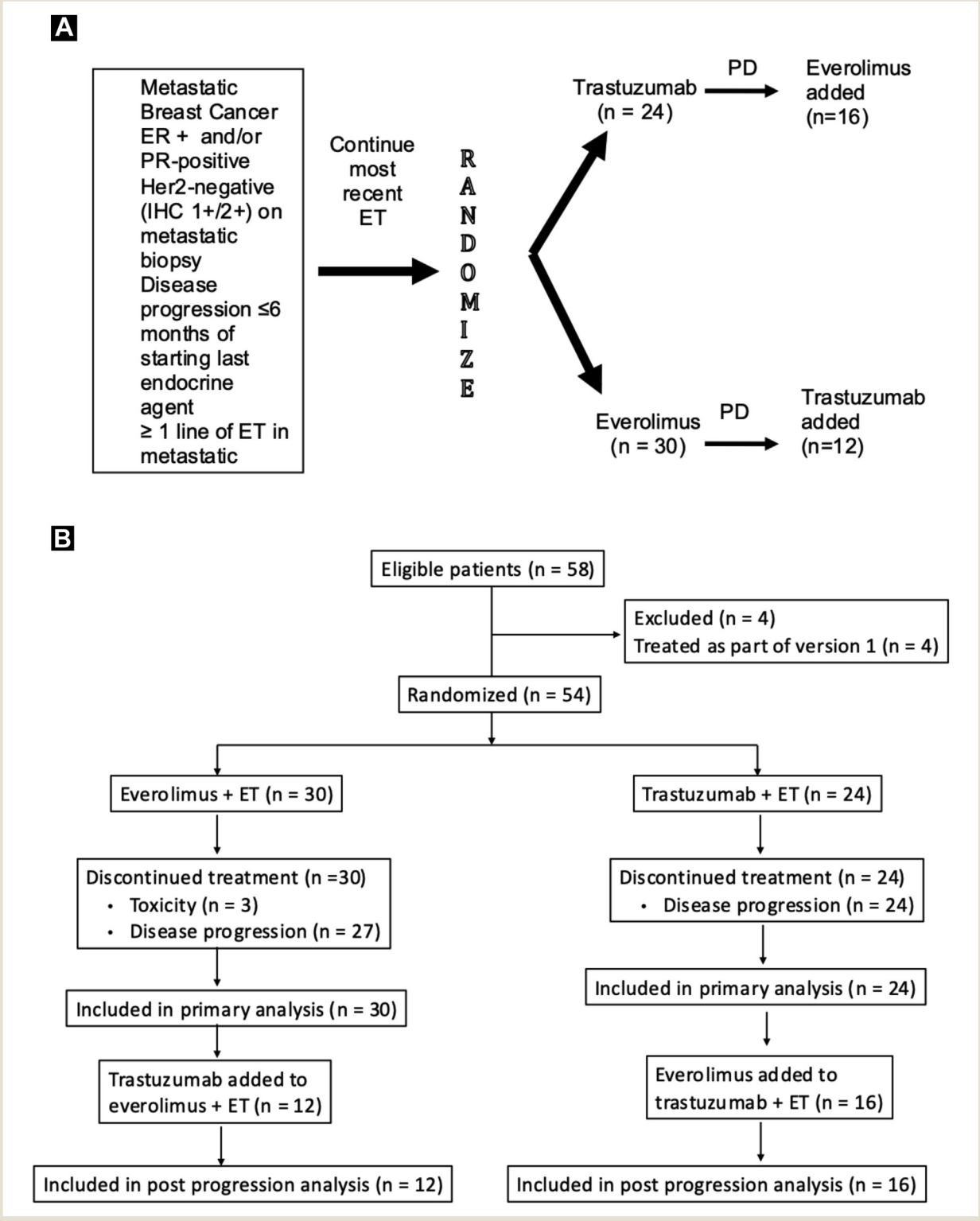
Patients continued the most recent ET during which they had experienced disease progression within 6 months of starting the ET and were randomized to receive trastuzumab or everolimus (Figure 1A). Trastuzumab was administered as an 8 mg/kg loading dose, followed by a dose of 6 mg/kg every 3 weeks. Everolimus was given at a starting dose of 10 mg daily. Staging was performed at 6 weeks and then every 12 weeks. Treatment was continued until disease progression, toxicity, or patient withdrawal.

At the time of disease progression, patients in the upfront trastuzumab arm could continue trastuzumab and ET and start everolimus and patients in the upfront everolimus arm could start trastuzumab in addition to everolimus and ET (post progression period; Figure 1A). Staging was performed 6 weeks after initiation of the combined treatment and then every 12 weeks. Treatment was continued until disease progression, toxicity, or patient withdrawal. An assessment of ejection fraction (EF) was required at baseline and then every 12 weeks if clinically indicated.

Study End Points

The primary end point was overall response (OR) rate according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST) criteria to everolimus or trastuzumab in addition to ET in patients with measurable disease. Secondary end points included clinical benefit rate (CBR) at 24 weeks in patients with measurable disease and PFS, overall survival (OS), and safety in all patients in both arms. The primary end point of the post progression period was OR with secondary end points of CBR at 24 weeks, PFS, OS, and safety.

Figure 1 Trial Participants. (A) Patients With Endocrine-Resistant HR-Positive, HER2-Negative Breast Cancer Were Treated With Trastuzumab or Everolimus While Continuing Treatment With the Endocrine Agent During Which They Had Experienced Disease Progression. At the Time of Disease Progression, Everolimus Treatment Was Started in Patients Who Had Received Trastuzumab Initially; and Trastuzumab Treatment Was Started in Patients Who Were Initially Treated With Everolimus. (B). Consort Diagram of Patient Accrual



Abbreviations: ER = estrogen receptor; ET = endocrine therapy; IHC = immunohistochemistry; PR = progesterone receptor.

Protocol Amendments

The initial study protocol randomized patients to trastuzumab with ET versus trastuzumab and everolimus with ET. Because it was believed that this design would not allow the evaluation of everolimus alone in addition to ET the protocol was amended such that patients were randomized to trastuzumab or everolimus along with ET. Eight patients who were randomized in the initial design to receive trastuzumab with ET were included in the analysis. The trial was amended once more when an interim analysis showed the futility of the trastuzumab arm, and that arm was closed. Accrual continued onto the everolimus arm, and those patients were eligible for the additional use of trastuzumab at disease progression.

Correlative Studies

Patients were required to have had a biopsy in the metastatic setting, which was performed immediately before study entry or could be archival and collected within 1 year of study entry when possible. Total RNA was prepared from available formalin-fixed paraffin-embedded (FFPE) samples from pretreatment tissues as previously described.[26] RNA was amplified and analyzed using the Affymetrix Clariom D Pico microarray platform (Thermo Fisher Scientific, Waltham, MA) at the Emory Integrated Genomics Core according to the manufacturer's protocols. RNA levels were analyzed for association with PFS using significance analysis of microarrays (SAM) survival analysis[27] using univariate analysis and corrected for false discovery rate.

Statistical Analysis

During the initial randomization part, the trial was powered as 2 phase II trials, and the primary objective of this study was to evaluate but not to compare the OR in each of the 2 arms. OR was defined using RECIST criteria in patients with measurable disease: complete response (CR) was disappearance of all disease seen radiologically, with partial response (PR) defined as at least a 30% decrease in target lesions, and progressive disease (PD) as at least a 20% increase in longitudinal diameter of target lesions. CBR was defined as the sum of stable disease, PR, or CR at 24 weeks. PFS was defined as months from date of randomization or treatment initiation to date of progression or death, whichever happened first, or date of last follow-up if alive without progression. OS was defined as months from date of randomization or treatment initiation to date of death, or date of last follow-up if alive.

Simon's 2-stage design was adopted. For each arm, a proportion of patients with favorable outcome <1.0% would be of no interest. The new treatment would be of interest if the proportion of response was at least 10.0%. Thirty-nine patients would be needed in each arm to test the null hypothesis: $P \leq .01$ against the alternative hypothesis: $P \geq .10$ at the 4.1% level of significance and with 80.2% power. Although patients were randomized to receive everolimus or trastuzumab, the trial was not powered to compare outcomes between the 2 arms.

After an interim analysis showed the futility of the trastuzumab arm, the trial continued to accrue to the everolimus arm alone without randomization. A sample size of 34 patients was needed to achieve 86% power to detect a difference of 0.09 using a 1-sided binomial test.

Statistical analysis was conducted using SAS version 9.4 (SAS Institute Inc, Cary, NC), and SAS macros or software developed at the Biostatistics and Bioinformatics at Winship Cancer Institute. Descriptive statistics for each baseline covariate were reported for all study populations and according to study arm. Kaplan–Meier plots were produced to compare the survival curves between the 2 groups for the first progression and post progression separately. No formal statistical tests were carried out for *P* values because the study was underpowered per protocol.

Table 1 Characteristics of Patients at Randomization

Characteristic (%)	Everolimus Arm (n = 30)	Trastuzumab Arm (n = 24)
Age Mean (Range)	57 (34-76)	54 (31-70)
Median Age, Years	59.5	53
AA/Other, n (%)	4 (14.29)	9 (37.5)
Caucasian, n (%)	24 (85.71)	15 (62.5)
ER, n (%)	30 (100)	24 (100)
PR, n (%)	14 (46.7)	9 (37.5)
HER2 IHC +1 −2, n (%)	30 (100)	24 (100)
Lung/Pleura, n (%)	10 (33.33)	5 (20.83)
Liver, n (%)	17 (56.6)	11 (45.8)
Bone, n (%)	24 (80)	20 (83)
Soft Tissue, n (%)	10 (33.33)	11 (45.8)
Chemotherapy, n (%)	15 (50)	13 (54)
ET, n (%)	17 (56.67)	13 (54.17)
Median Number of Lines (Range)	2 (1-9)	3 (1-7)
Previous Chemotherapy in the Metastatic Setting, n (%)	17 (56.67)	10 (41.7)
Median Number of Lines of ET in the Metastatic Setting (Range)	2 (1-5)	2.5 (1-4)
Nonsteroidal AI, n (%)	5 (16.7)	0
Tamoxifen, n (%)	5 (16.7)	7 (29.2)
Fulvestrant, n (%)	10 (33.3)	8 (33.3)
Exemestane, n (%)	8 (26.7)	9 (37.5)
Megestrol Acetate, n (%)	2 (6.7)	0

Abbreviations: AA = African American; AI = aromatase inhibitor; ER = estrogen receptor; ET = endocrine therapy; IHC = immunohistochemistry; PR = progesterone receptor.

Results

Patient and Treatment Characteristics

From June 2009 to October 2015, 58 patients were accrued. Four patients were initially treated with the combination of trastuzumab and everolimus and were not included in the final analysis. After an interim analysis, the trastuzumab arm was closed because of futility. Subsequently 9 patients were recruited and received everolimus with ET without randomization with the option to use trastuzumab in addition upon progression. Overall, 30 and 24 patients continued the ET during with they experienced disease progression and were treated with everolimus or trastuzumab, respectively (Figure 1B).

Patient characteristics for the 2 arms at initial randomization are shown in Table 1. Median age was 56 years and most patients were Caucasian, with 4 (14%) and 9 (38%) African-American/other patients in the everolimus and trastuzumab arms, respectively. All patients had ER and/or progesterone receptor-positive disease with HER2 expression of 1+ or 2+ using IHC on a metastatic specimen. Forty-three of 54 patients (80%) had measurable disease; 25

Table 2 Response Rate and Clinical Benefit Rate Among Patients With Measurable Disease

End Point	Treatment	
	Everolimus With ET (n = 25)	Trastuzumab With ET (n = 18)
Initial Randomization		
ORR	7 (28)	0
PR	7 (28)	0
SD	12 (48)	8 (44.4)
PD	6 (24)	10 (55.6)
CBR at 12 weeks	18 (72)	6 (33.3)
CBR at 24 weeks	12 (48)	2 (11.1)
CBR at 52 weeks	6 (24)	0
After Crossover	**Everolimus Added (n = 11)**	**Trastuzumab Added (n = 11)**
ORR	1 (9.1)	1 (9.1)
PR	1 (9.1)	1 (9.1)
SD	6 (54.6)	4 (36.4)
PD	4 (36.4)	6 (54.6)
CBR at 12 weeks	8 (72.7)	6 (54.6)
CBR at 24 weeks	5 (45.6)	2 (18.2)
CBR at 52 weeks	1 (9.1)	0

Data are presented as n (%). After crossover all patients (n = 22) received everolimus, trastuzumab, and endocrine therapy.
Abbreviations: CBR = clinical benefit rate; ET = endocrine therapy; ORR = overall response rate; PD = progressive disease; PR = partial response; SD = stable disease.

of 30 (83%), and 18 of 24 (75%) in the everolimus and trastuzumab arms, respectively. The median previous lines of therapy for metastatic disease was 2 (range, 1-9) and 3 (range, 1-7) in the everolimus and trastuzumab arms, respectively. All patients had received at least 1 line of ET in the metastatic setting and all had experienced disease progression within 6 months of starting an ET. The median number of lines of previous ET overall was 2 (range, 1-5). The ET used in combination with everolimus or trastuzumab was exemestane (31.5%), letrozole (7.4%), anastrozole (1.9%), tamoxifen (22.2%), fulvestrant (33.3%), and megestrol acetate (3.7%; Table 1).

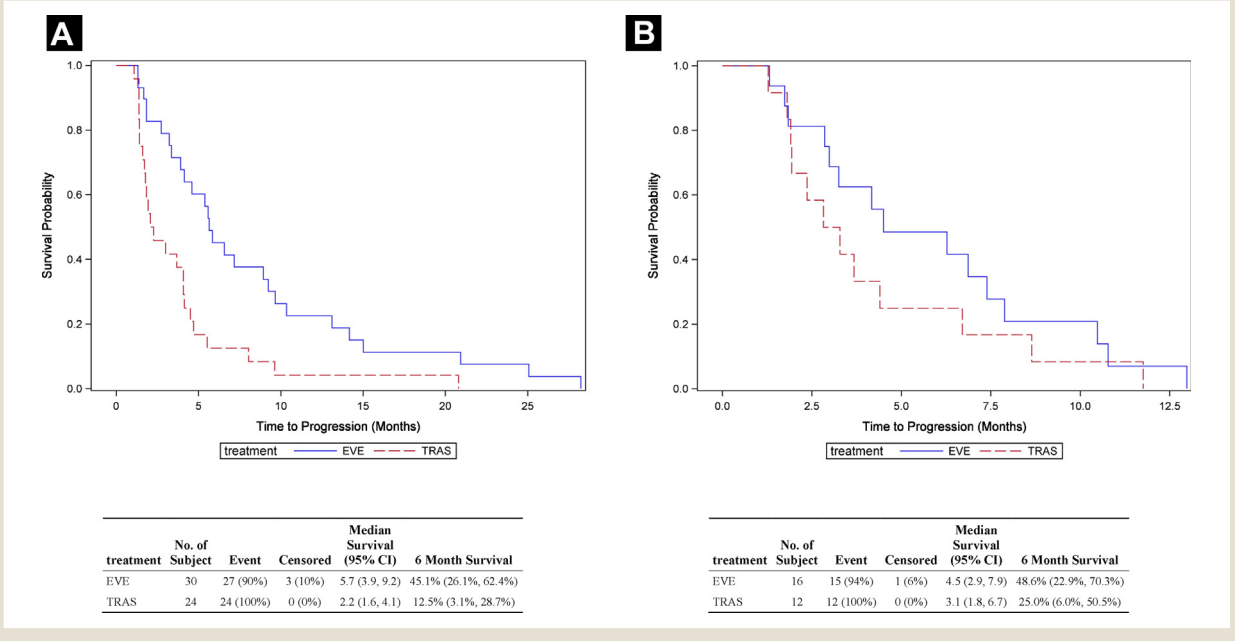
Among the 50 patients who had disease progression while enrolled in the initial arms, everolimus was used in addition in 16 patients who were originally treated with trastuzumab, and trastuzumab was used in addition in 12 patients who were originally treated with everolimus. The rest of the patients were removed from the study because of investigator or patient preference.

Efficacy

Among patients with measurable disease (n = 43; 25 and 18 in the everolimus and trastuzumab arms, respectively), the OR was 28% and 0% in the everolimus and trastuzumab arms, respectively, with no CRs noted (Table 2). The CBR among patients with measurable disease at 12, 24, and 52 weeks for everolimus was 72%, 48%, and 24%, and for trastuzumab was 33%, 11%, and 0% respectively. The median PFS, in patients with or without measurable disease (n = 54; 30 and 24 in the everolimus and trastuzumab arms, respectively) was 5.7 months (95% confidence interval [CI], 3.9-9.2) and 2.2 months (95% CI, 1.6-4.1) for the everolimus and trastuzumab arms, respectively (Figure 2A).

In patients with measurable disease (n = 22) who received everolimus, trastuzumab, and ET post progression, OR was 9% (Table 2). The CBR among patients with measurable disease at 12, 24, and 52 weeks for patients in whom everolimus was used in

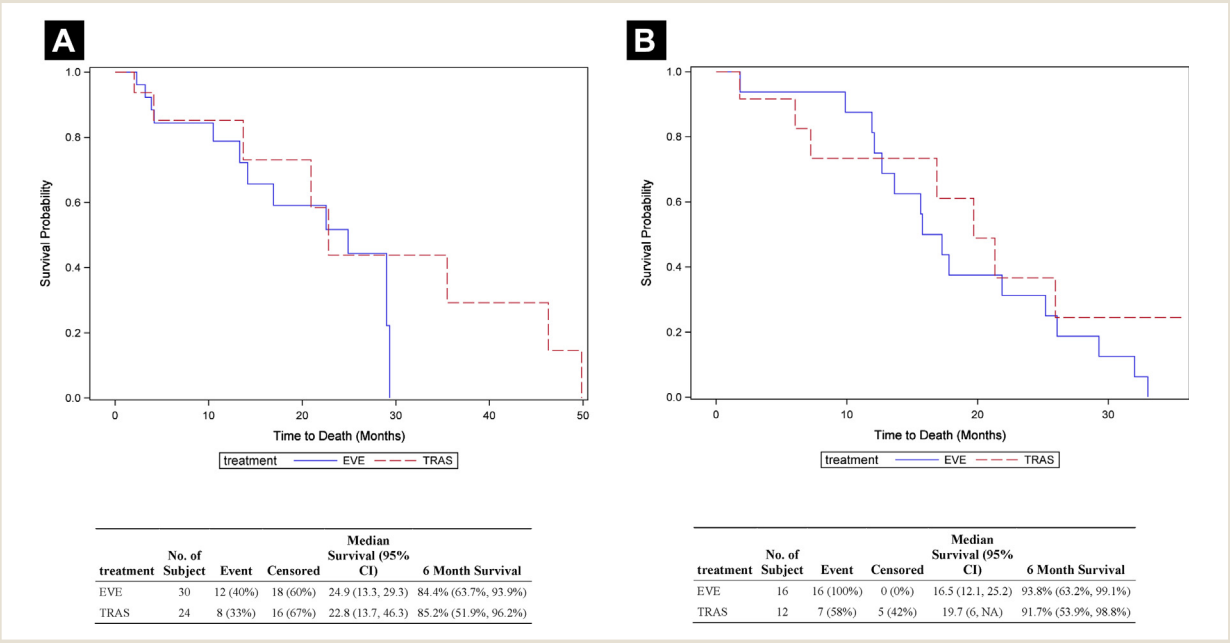
Figure 2 Progression-Free Survival. Progression-Free Survival for Patients Treated With (A) Everolimus (EVE) or Trastuzumab (TRAS) at Initial Randomization; and (B) for Patients Treated With the addition of EVE or TRAS at the Time of Disease Progression



treatment	No. of Subject	Event	Censored	Median Survival (95% CI)	6 Month Survival
EVE	30	27 (90%)	3 (10%)	5.7 (3.9, 9.2)	45.1% (26.1%, 62.4%)
TRAS	24	24 (100%)	0 (0%)	2.2 (1.6, 4.1)	12.5% (3.1%, 28.7%)

treatment	No. of Subject	Event	Censored	Median Survival (95% CI)	6 Month Survival
EVE	16	15 (94%)	1 (6%)	4.5 (2.9, 7.9)	48.6% (22.9%, 70.3%)
TRAS	12	12 (100%)	0 (0%)	3.1 (1.8, 6.7)	25.0% (6.0%, 50.5%)

Figure 3 Overall Survival. Overall Survival for Patients Treated With (A) Everolimus (EVE) or Trastuzumab (TRAS) at Initial Randomization; and (B) for Patients Who Received EVE, TRAS, and Endocrine Therapy From the Time of Disease Progression



treatment	No. of Subject	Event	Censored	Median Survival (95% CI)	6 Month Survival
EVE	30	12 (40%)	18 (60%)	24.9 (13.3, 29.3)	84.4% (63.7%, 93.9%)
TRAS	24	8 (33%)	16 (67%)	22.8 (13.7, 46.3)	85.2% (51.9%, 96.2%)

treatment	No. of Subject	Event	Censored	Median Survival (95% CI)	6 Month Survival
EVE	16	16 (100%)	0 (0%)	16.5 (12.1, 25.2)	93.8% (63.2%, 99.1%)
TRAS	12	7 (58%)	5 (42%)	19.7 (6, NA)	91.7% (53.9%, 98.8%)

addition to trastuzumab and ET (n = 11) was 72%, 46%, and 9%, and for patients in whom trastuzumab was used in addition to everolimus and ET (n = 11) was 54%, 18%, and 0%, respectively. In patients with or without measurable disease (n = 28), the median time to second progression was 4.5 months (95% CI, 2.9-7.9) for the arm when everolimus was used in addition (n = 16) and 3.1 months (95% CI, 1.8-6.7) in the arm when trastuzumab was used in addition (n = 12; Figure 2B).

Overall survival was 24.9 months (95% CI, 13.3-29.3) for the everolimus arm, and 22.8 months (95% CI, 13.7, 46.3) for the trastuzumab arm for all patients treated (Figure 3). The 6-month survival probability was approximately 85% for both arms.

Safety

Most patients were removed from the study because of disease progression (Figure 1B, Table 3). Most common Grade 3 or 4

Table 3 Treatment-Related Grade 3/4 Adverse Events

	Everolimus Arm (n = 30)		Trastuzumab Arm (n = 24)		After Crossover (n = 28)	
Adverse Event	**Grade 3**	**Grade 4**	**Grade 3**	**Grade 4**	**Grade 3**	**Grade 4**
Hypertension	2 (6.7)	0	0	0	0	0
Mucositis/Stomatitis	2 (6.7)	0	0	0	0	0
Hypertriglyceridemia	1 (3.3)	1 (3.3)	0	0	0	0
Fatigue	2 (6.7)	0	0	0	0	0
Angioedema	1 (3.3)	0	0	0	0	0
Colitis	1 (3.3)	0	0	0	0	0
Creatinine Clearance Decreased	1 (3.3)	0	0	0	0	0
Dyspnea	1 (3.3)	0	0	0	1 (3.6)	0
Decreased EF	2 (6.7)	0	0	0	2 (7.2)	0
Increased ALT	1 (3.3)	0	0	0	1 (3.6)	0
Increased AST	1 (3.3)	0	0	0	2 (7.2)	0
Edema	1 (3.3)	0	0	0	0	0
Neutropenia	0	0	0	0	1 (3.6)	0
Abdominal pain	0	0	0	0	1 (3.6)	0

Data are presented as n (%).
Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; EF = ejection fraction.

toxicities are shown in Table 3. Three patients were removed from the study specifically because of everolimus toxicity: Grade 4 hypertriglyceridemia, Grade 3 fatigue, and prolonged treatment interruption for elective spine surgery because of bone metastases. Three other patients who were withdrawn because of toxicity were found to have simultaneous PD and for the purpose of the efficacy analysis were not censored. Four patients experienced a decrease in EF, 2 of whom were taking everolimus without trastuzumab, and 2 who were receiving everolimus and trastuzumab. All of the patients were asymptomatic and the lowest EF reported was 35%.

Among the 46 patients who were exposed to at least 1 dose of everolimus either initially (n = 30) or in combination with trastuzumab after progression (n = 16), 20 (44%) patients required modification of therapy because of toxicities, which included pneumonitis, elevated transaminases, thrombocytopenia, and mucositis, all of which are consistent with previous studies.

Correlative Studies

Total RNA was prepared from 33 FFPE samples (29 metastases and 4 matching primary tumors). Of the 29 patients for whom metastatic tissue was available, 15 were treated with ET and everolimus and 14 were treated with ET and trastuzumab. Twenty-four samples passed quality control metrics (11 in the everolimus arm, and 13 in the trastuzumab arm). Within the everolimus arm, 8 patients had PD at 12 weeks, and 3 did not. Univariate analysis for genes associated with PFS during everolimus treatment using SAM survival analysis[27] identified 48 genes associated with PFS including myosin light chain 2, tropomyosin 3, sarcolipin, xin actin binding repeat containing 2, myotilin, titin, Kelch like family member 41, and myosin light chain 1 (false discovery rate <0.20; see Supplemental Table 1 in the online version). Ingenuity pathway analysis[28] determined that these 48 genes were associated with calcium signaling, epithelial adherens junction signaling, Ras homolog gene family, member A signaling, tight junction signaling, integrin linked kinase signaling, and actin-based motility signaling (see Supplemental Table 2 in the online version). Upstream regulators of these genes that were implicated included myocyte enhancer factor 2C, signal transducer and activator of transcription 5B, myogenic differentiation 1, GATA binding protein 4, lysine demethylase 5A, and TEA domain transcription factor 3 (see Supplemental Table 3 in the online version). There were no genes significantly associated with PFS in the trastuzumab arm.

In the everolimus arm, there was no association between efficacy and previous therapy, including lines of ET in the metastatic setting or ET used, although patient numbers were too small to definitively exclude an association. In the trastuzumab arm, the level of HER2 expression was not associated with benefit from trastuzumab.

Discussion

Increased activation of growth factor receptor pathways has been shown to occur in HR-positive ERBC,[5-7] and, because of the availability of agents, inhibiting these pathways represents a logical approach to potentially reverse resistance to ET. In this trial we evaluated inhibition of HER2 or mTOR, or both, in patients with HR-positive, metastatic ERBC, who continued the ET during which they had experienced disease progression. We found that the additional use of everolimus, but not trastuzumab, to ET appeared reasonably effective in these ERBCs. The toxicity profile was as expected with stomatitis being common in the everolimus arm, along with hyperglycemia, hypertension, and fatigue. Four patients experienced a decrease in EF, 2 of whom were being treated with everolimus and 2 with everolimus and trastuzumab.

We believe this trial is unique for several reasons. First, we focused on patients with ERBC on the basis of documented progression within 6 months of starting ET in the metastatic setting. This is in contrast to previous randomized trials[11,18,19] in the pretreated setting in which patients with progression during adjuvant ET were allowed, and is supported by the very short median PFS of 2 months in the ineffective trastuzumab arm. Second, to our knowledge, this is the first reported trial to evaluate the use of everolimus while continuing the ET during which disease progression had occurred. In previous randomized trials,[11,18,19] patients could not have received an ET that they had previously received, and therefore were exposed to a new ET along with everolimus. Last, although trastuzumab has been evaluated in HR-positive, HER2-positive cancers, this is the only trial we are aware of that evaluated HER2 inhibition in patients with metastatic, HR-positive ERBC with low or moderate expression of HER2.

Despite preclinical[7,10,21] and clinical data[11] supporting a role for HER2 in endocrine resistance, we found that inhibition of HER2 with trastuzumab in HR-positive, HER2-negative ERBC was not effective, resulting in closure of this arm after an interim analysis. The futility of trastuzumab might be because only 1 component of the HER2 pathway was targeted using this approach allowing potentially compensatory activation of other growth factor signaling pathways. In general, attempts to target a single growth factor have been unsuccessful in enhancing the activity of ET in HR-positive metastatic breast cancer.[11-13,15-17] The concept of targeting more than 1 growth factor receptor pathway is supported by the fact that inhibiting mTOR, which represents a common node for these pathways, significantly improves outcome when used in addition to ET in patients with metastatic breast cancer.[18,19,24] It is additionally possible that dual inhibition of HER2 or the use of a tyrosine kinase inhibitor rather than a monoclonal antibody would have been more effective than trastuzumab alone.[29,30]

We hypothesized that everolimus might be able to resensitize ERBC to ET. Although randomized trials[18,19,24] showed that PFS is improved with the additional of everolimus with ET, patients in these trials were not permitted to have received an ET used previously. We found that the additional use of everolimus with the same ET during which the patient had developed disease progression resulted in a PFS of approximately 6 months (range, 1-25 months). This is somewhat shorter than the median PFS noted in the randomized trials,[18,19,24] which can perhaps be explained by the fact that all patients in our trial had definitive ERBC using clinical criteria, as evidenced by the shorter PFS in the futile trastuzumab arm, compared with the control arms in the randomized trials. These results, although not conclusive and requiring validation, suggest that targeting mTOR can resensitize ERBC to ET. Because of the availability of cyclin dependent kinase (CDK) inhibitors, it is possible that patients might receive several lines of ET before being candidates for everolimus, and these results suggest that reuse of an ET that the cancer was previously exposed to might be an option. The combination of everolimus and trastuzumab appeared

somewhat effective in patients who had previously received trastuzumab, but was not effective in patients who had previously received everolimus, suggesting that inhibition of mTOR rather than HER2 might be the more effective strategy in ERBC.

We found 48 genes that appeared to be associated with PFS in patients treated with everolimus that were related to key oncogenic pathways (see Supplemental Tables 1-3 in the online version). A number of the genes we identified are associated with stromal muscle cells, and could reflect reduced tumor content in patients with better responses. Several of these genes are associated with breast cancer, including TEA domain transcription factor 3, a key mediator of Hippo signaling, GATA binding protein 4, which is silenced in many breast cancers, suggesting a tumor suppressor role, and lysine demethylase 5A, which is amplified in many breast cancers.[31,32] Although these findings require further validation, these limited data suggest that predictive genes for a benefit of everolimus in ERBC exist. Other studies that have attempted to identify genomic predictive biomarkers for everolimus in breast cancer have produced inconsistent results,[33,34] although importantly most of the specimens used were primary, rather than metastatic, ERBC samples as was used in our trial.

There are a number of limitations to our study. The patient numbers were too small to definitely determine whether everolimus can truly resensitize ERBC to a given ET. Additionally, we cannot rule out that similar results would have been noted with everolimus alone, as was used in the BOLERO 6 trial.[35] Several patients were withdrawn from the trial after the first progression, thus the numbers in the post progression arms were even smaller. Furthermore, we cannot rule out the possibility that other HER2-directed agents, such as tyrosine kinase inhibitors might have been a better choice than trastuzumab in targeting ERBC, because of the emerging data with neratinib in HER2-mutated cancers.[36] Our correlative studies are clearly affected by small numbers and require further validation. Last, this trial was designed before the widespread use of CDK inhibitors, and our results might not be applicable to patients who have received these agents. The use of everolimus after use of CDK inhibitors is being evaluated in ongoing clinical trials.

Conclusion

The use of everolimus while continuing the most recent ET is feasible, resulting in a median PFS of 6 months, in patients with metastatic ERBC. In contrast, inhibition of HER2 with trastuzumab appeared ineffective. We showed efficacy of everolimus regardless of concomitant ET. Because of the widespread use of CDK inhibitors, patients may receive multiple lines of ET before being considered for everolimus. This trial suggests that a previous ET could be revisited in these patients, further delaying the use of chemotherapy, although the efficacy of everolimus in patients who have previously received CDK inhibitors is currently unclear.

Clinical Practice Points

- Induction of growth factor signaling has been shown in endocrine-resistant HR-positive breast cancers.
- In this phase II trial, we showed that inhibition of mechanistic target of rapamycin appears to enhance the activity of, and potentially reverse resistance to, endocrine agents.
- In contrast, in keeping with other clinical trials, inhibition of a single growth factor receptor, in this case, HER2, dis not improve outcome when used in addition to endocrine therapy in endocrine-resistant, HR-positive, HER2-negative metastatic breast cancers.
- With the approval and ongoing development of targeted agents used in addition to endocrine therapy in earlier lines of therapy for HR-positive, metastatic breast cancer, the finding that everolimus is effective when used in addition to previously used endocrine agents could expand the therapeutic options for patients with HR-positive metastatic breast cancer, thereby prolonging the time for use of chemotherapy.

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Disclosure

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Supplemental Data

Supplemental tables accompanying this article can be found in the online version at https://doi.org/10.1016/j.clbc.2018.12.017.

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