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CLINICAL TRIAL

A phase 2 study of everolimus combined with trastuzumab and paclitaxel in patients with HER2-overexpressing advanced breast cancer that progressed during prior trastuzumab and taxane therapy

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Abstract Increased activation of the PI3K/Akt/mTOR pathway is a common factor in putative mechanisms of trastuzumab resistance, resulting in dysregulation of cell migration, growth, proliferation, and survival. Data from preclinical and phase 1/2 clinical studies suggest that adding everolimus (an oral mTOR inhibitor) to trastuzumab plus chemotherapy may enhance the efficacy of, and restore sensitivity to, trastuzumab-based therapy. In this phase 2 multicenter study, adult patients with HER2-positive advanced breast cancer resistant to trastuzumab and pretreated with a taxane received everolimus 10 mg/day in combination with paclitaxel (80 mg/m^2 days 1, 8, and 15 every 4 weeks) and trastuzumab (4 mg/kg loading dose followed by 2 mg/kg weekly), administered in 28-day cycles. Endpoints included overall response rate (ORR), progression-free survival (PFS), overall survival (OS), and safety. Fifty-five patients were enrolled; one remained on study treatment at the time of data

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cutoff. The median number of prior chemotherapy lines for advanced disease was 3.5 (range 1–11). The ORR was 21.8 %, the clinical benefit rate was 36.4 %, the median PFS estimate was 5.5 months (95 % confidence interval [CI]: 4.99–7.69 months), and the median OS estimate was 18.1 months (95 % CI: 12.85–24.11 months). Hematologic grade 3/4 adverse events (AEs) included neutropenia (25.5 % grade 3, 3.6 % grade 4), anemia (7.3 % grade 3), and thrombocytopenia (5.5 % grade 3, 1.8 % grade 4). Nonhematologic grade 3/4 AEs included stomatitis (20.0 %), diarrhea (5.5 %), vomiting (5.5 %), fatigue (5.5 %), and pneumonia (5.5 %), all grade 3. These findings suggest that the combination of everolimus plus trastuzumab and paclitaxel is feasible, with promising activity in patients with highly resistant HER2-positive advanced breast cancer. This combination is currently under investigation in the BOLERO-1 phase 3 trial.

Keywords Advanced breast cancer · Everolimus · Human epidermal growth factor receptor 2-positive · mTOR inhibitor · Paclitaxel · Trastuzumab resistant

Abbreviations

AE	Adverse event
Akt	Protein kinase B
CBR	Clinical benefit rate
CI	Confidence interval
CT	Computed tomography
DLCO	Carbon monoxide diffusing capacity
HER2	Human epidermal growth factor receptor-2
mTOR	Mammalian target of rapamycin
ORR	Overall response rate
OS	Overall survival
PFS	Progression-free survival
PI3K	Phosphatidylinositol 3-kinase
PTEN	Phosphatase and tensin
RECIST	Response evaluation criteria in solid tumors
T	Paclitaxel
TRAS	Trastuzumab
WHO	World Health Organization

Introduction

Overexpression of the tyrosine kinase human epidermal growth factor receptor-2 (HER2) due to amplification of the *HER2*/neu proto-oncogene is reported in nearly 25 % of all breast cancers and is associated with aggressive breast cancer cell growth [1, 2]. In clinical practice, HER2 protein overexpression or amplification is linked to poor prognosis, increased risk of metastases, reduced disease-free survival, and increased mortality [3, 4]. Agents that target HER2, such as trastuzumab, pertuzumab, T-DM1, and lapatinib, have been shown to improve clinical outcomes in patients with HER2-positive breast cancer via down-regulation or inhibition of HER2 activity [5–7]. Trastuzumab, which targets the extracellular domain of HER2, has become the cornerstone of combination therapies for HER2-positive early and advanced breast cancer based on the results of numerous clinical trials [6, 8, 9].

Despite the success of these HER2-directed therapies, the majority of patients with advanced breast cancer relapse or progress [10]. Resistance to trastuzumab is a major clinical concern with room for improvement, considering that the response rates to initial trastuzumab treatment range between 33 and 50 % in patients with HER2-positive advanced breast cancer [11, 12]. In addition, 70 % of patients with initial response to trastuzumab treatment experience disease progression within 1 year [13]. Therefore, additional treatment options are needed to effectively manage HER2-positive advanced breast cancer. Trastuzumab resistance is hypothesized to occur via a number of mechanisms, including increased signaling mediated by upstream growth factor receptors, alterations of the HER2 receptor, phosphatase and tensin (PTEN) deficiency, and constitutive activation of phosphatidylinositol 3-kinase (PI3K) [3, 5, 13].

Increased activation of the PI3K/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) pathway results in dysregulation of cell migration, growth, proliferation, and survival [14]. Therefore, agents that inhibit the PI3K/Akt/mTOR pathway may restore sensitivity to trastuzumab-based therapy. This hypothesis is supported by preclinical studies in which PI3K inhibitors overcame PTEN loss-induced trastuzumab resistance and slowed the growth of HER2-positive breast cancer cells in vitro and in vivo [15, 16].

Everolimus is an oral mTOR inhibitor approved for several oncology indications, including the treatment of patients with hormone-receptor-positive advanced breast cancer recurring or progressing during or after treatment with nonsteroidal aromatase inhibitors [17, 18]. A phase 1b/2 study was conducted in patients with taxane-pretreated and trastuzumab-resistant HER2-positive advanced breast cancer to determine the optimal dose and schedule of everolimus in combination with weekly trastuzumab and paclitaxel. The phase 1b portion of the study reported encouraging antitumor activity and generally good tolerability when everolimus (5 mg/day, 10 mg/day, or 30 mg/week) was combined with weekly trastuzumab and paclitaxel [19]. The overall response rate (ORR) was 44 %, and median progression-free survival (PFS) was approximately 8 months. The current report from the phase 2 portion of this study provides further insights into the efficacy and safety of daily everolimus (10 mg) in combination with weekly paclitaxel and trastuzumab in patients with trastuzumab-resistant HER2-positive advanced breast cancer.

Patients and methods

Study design and patients

J2101 was an open-label, multicenter, phase 2 study with a Simon 2-stage design, involving patients with HER2-positive advanced breast cancer whose disease progressed on or after trastuzumab and taxane therapy. The Simon 2-stage design was considered optimal to allow for early termination of the study after stage 1 in case of lack of efficacy. In the first stage, 30 patients were to be enrolled. If two or more patients achieved a response in the first stage, an additional 22 patients were to be enrolled; otherwise, the study was to be stopped.

Adult patients (≥18 years of age) with histologically confirmed HER2-positive advanced breast cancer (immunohistochemistry 3^+ or positive fluorescence in situ hybridization) whose disease progressed during or within 3 months of receiving the last dose of trastuzumab for advanced disease or recurred within 12 months of completing trastuzumab-based (neo)adjuvant therapy were eligible to enroll. In addition, patients were required to have experienced disease progression during or within 6 months of the last taxane dose (with or without documented response) for advanced disease, or recurrence within 12 months of completing taxane-based chemotherapy as (neo)adjuvant therapy. Other eligibility criteria included a World Health Organization (WHO) performance status of 0 or 1, measurable disease according to response evaluation criteria in solid tumors (RECIST) 1.0, left ventricular ejection fraction greater than 50 %, and adequate bone marrow, liver, and renal function. Exclusion criteria included uncontrolled or symptomatic central nervous system metastases; prior chemotherapy, immunotherapy, or radiotherapy within 4 weeks or prior lapatinib within 2 weeks of study entry; prior treatment with endocrine therapy for breast cancer (endocrine therapy had to have either failed in patients with hormone-receptor-positive disease within 2 weeks of randomization or patients had to be deemed unsuitable for endocrine therapy); or previous exposure to mTOR inhibitors.

All patients provided written informed consent before registration. The J2101 study was conducted in accordance with the Declaration of Helsinki and with local ethics committee approval at each participating center (16 centers in Belgium, France, The Netherlands, Spain, and the United States).

Treatment

For the current single-arm phase 2 study, enrolled patients received daily oral everolimus 10 mg plus weekly trastuzumab and paclitaxel administered in 28-day cycles. The 10 mg/day dose of everolimus used was determined based on the efficacy and safety findings in the 3-arm phase 1b dose-escalation portion of this study, in which two daily regimens (5 and 10 mg/day) and 1 weekly regimen (30 mg/week) were examined [19]. Among these, everolimus 10 mg/day was chosen for further development on the basis of observed dose-limiting toxicity (grade 1/2 stomatitis at the end of cycle 1) and overall safety.

In addition to daily everolimus, all patients in this phase 2 study received weekly trastuzumab and paclitaxel infusion (80 mg/m^2 on days 1, 8, and 15 of each cycle for at least six cycles). Trastuzumab was administered intravenously at a loading dose of 4 mg/kg on day 1 of cycle 1, with a maintenance dose of 2 mg/kg thereafter. Following completion of the first six cycles, patients continued to receive everolimus and trastuzumab, while continuation of paclitaxel was allowed at the investigator's discretion. For those patients who did not continue paclitaxel beyond the first six cycles, trastuzumab was administered once every 3 weeks at a dose of 6 mg/kg. Patients could receive everolimus alone (if trastuzumab was discontinued early because of toxicity) or with trastuzumab until disease progression or unacceptable toxicity occurred.

In addition to the study treatments, prophylactic use of antiemetics was recommended. Patients were allowed to continue baseline bisphosphonate or analgesic therapy. In the event of documented cytopenia (after the first cycle of treatment), the use of hematopoietic growth factors was permitted.

Study objectives

The primary endpoint of the study was ORR as assessed by RECIST 1.0. Secondary endpoints included PFS, overall survival (OS), clinical benefit rate (CBR), and safety.

Study assessments

All baseline assessments were obtained at visit 1 (within 3 weeks before start of treatment). These evaluations included physical and neurologic examinations, WHO performance status, vital signs, height, weight, electrocardiogram, chest X-ray, cardiac imaging, pulmonary function tests, demographics, medical history, confirmation of HER2-positive metastatic breast cancer diagnosis, extent of cancer, documentations of prior therapy, tumor assessment, laboratory evaluations, and a pregnancy test.

Assessment of clinical response

Radiologic assessments (computed tomography [CT] scan, magnetic resonance imaging) and/or physical examination

were performed every 8 weeks during the first six cycles of study treatment. Patients continuing on everolimus and trastuzumab beyond the first six cycles had radiologic assessments performed every 9 weeks (every 8 weeks if paclitaxel was continued) until disease progression or study discontinuation for any other reason.

Assessment of safety

Adverse events (AEs) and laboratory assessments for safety were evaluated throughout the study. AEs were assessed according to National Cancer Institute/National Institutes of Health Common Terminology Criteria for AEs, version 3.0. Cardiac imaging (multigated angiogram or echocardiogram) and electrocardiograms were repeated every 12 weeks (or as clinically indicated). Chest X-rays were performed every two cycles and, if clinically indicated, chest CT scans were performed to monitor for interstitial pneumonitis. Pulmonary function tests (spirometry, carbon monoxide diffusing capacity [DLCO], and room air oxygen saturation at rest) were performed at baseline and on suspicion of noninfectious pneumonitis.

Everolimus dose interruptions and/or reductions were allowed for specific AEs such as grade 2/3 pneumonitis, grade $\geq$2 neurotoxicity, and grade $\geq$3 AEs (except hyperlipidemia and hyperglycemia) until the AE resolved to grade $\leq$1. If necessary, the everolimus dose was reduced from 10 to 5 mg/day or from 5 to 2.5 mg/day. The dose of paclitaxel could be reduced by 10 mg/m^2 (for grade $\geq$2 pneumonitis or other intolerable grade $\geq$2 AEs except neurotoxicity) or by 20 mg/m^2 (for hematologic toxicities or grade $\geq$2 neurotoxicity) if dose interruption did not result in AE resolution to grade $\leq$1. No dose reductions were allowed for trastuzumab; however, dose interruption was permitted to control infusion-related symptoms and for AEs such as a decrease in left ventricular ejection fraction of $\geq$10 points from baseline or $<$50 %, or development of grade $\geq$3 pneumonitis. Treatment discontinuation was recommended for any toxicity requiring interruption for more than 3 weeks and was required for grade 4 pneumonitis.

Statistical analysis

The Simon 2-stage analysis was based on the intent-to-treat population and was designed with 80 % power to reject the null hypothesis when the true response rate is 15 %. Overall response rates are presented with exact 2-sided 95 % confidence interval (CI), as determined via the Clopper–Pearson method. PFS and OS were estimated using the Kaplan–Meier method and are presented with the 2-sided 95 % CI.

Results

Patients

A total of 55 women were enrolled from February to November 2009. The median age of patients enrolled was 56 years, and 83.6 % of the patients were under 65 years of age. Sixty-six percent had a WHO performance status of 0 and 78 % had visceral disease. These patients were heavily pretreated, with a median of 3.5 prior lines of chemotherapy for metastatic disease. All patients had previously received trastuzumab and taxane; 40 (73 %) and 35 (64 %) patients had received prior anthracycline or lapatinib, respectively. Resistance to trastuzumab was documented in 52 (95 %) patients; resistance to taxane or lapatinib was reported in 40 (73 %) and 33 (60 %) patients, respectively (Table 1).

Of the 55 patients enrolled in the study, 28 (50.9 %) completed six cycles of treatment, and 24 of these patients continued study treatment beyond six cycles (Table 2). At the cutoff date (March 15, 2012), one patient was still receiving study treatment. Among the 55 enrolled patients, 37 (67.3 %) discontinued treatment due to progressive disease: 16 (29.1 %) patients during the first six cycles and 21 patients who continued treatment beyond the first six cycles. A total of 10 patients discontinued study treatment because of AEs; eight of these patients discontinued during the first six cycles of study treatment. AEs resulting in discontinuation from the study during the first six cycles included ascites and general physical health deterioration ($n = 1$); decreased performance status and fatigue ($n = 1$); febrile neutropenia and sepsis ($n = 1$); decreased total lung capacity and lung disorder ($n = 1$); and pneumonitis, pneumonia, interstitial lung disease, and decreased DLCO ($n = 1$ each). Two patients discontinued treatment because of AEs after the first six cycles: brain edema ($n = 1$) and decreased forced expiratory volume and dysphonia ($n = 1$). Most of these AEs were suspected to be related to study treatment.

The median duration of exposure to both everolimus and trastuzumab was 24.0 weeks, whereas the median duration of exposure to paclitaxel was 21.7 weeks (Table 3). Patients were followed for a median of 32.6 months (median treatment start date to data cutoff date). The median dose intensity of everolimus was 8.75 mg/day; median dose intensities of trastuzumab and paclitaxel were 0.29 and 7.09 mg/day, respectively (Table 3). The relative dose intensities of everolimus, trastuzumab, and paclitaxel were 0.88, 1.00, and 0.83, respectively. Overall, 47.3 and 89.1 % of patients required at least one dose reduction or interruption of any study drug, respectively (Table 3). The proportions of patients requiring at least one dose reduction were 34.5 % for everolimus (30.9 % for AEs), 25.5 % for

Table 1 Demographic and baseline disease characteristics of patients

	Everolimus + trastuzumab + paclitaxel ($N = 55$)
Age, years	
Median (range)	56.0 (31–83)
Age, *n* (%)	
<65 years	46 (83.6)
≥65 years	9 (16.4)
WHO performance status, *n* (%)	
0	36 (65.5)
1	19 (34.5)
Predominant race, *n* (%)	
Caucasian	48 (87.3)
Black	4 (7.3)
Asian	0
Native American	0
Pacific Islander	0
Other	3 (5.5)
Visceral disease, *n* (%)	
Any	43 (78.2)
Lung	24 (43.6)
Liver	29 (52.7)
Target lesions, *n* (%)	
Yes	52 (94.5)
No	3 (5.5)
Current stage of cancer, *n* (%)	
IV	55 (100)
Disease-free interval, *n*[a]	32
Median weeks (range)	108.9 (3–439)
Number of previous chemotherapy lines for advanced disease	
Median (range)	3.5 (1–11)
Pretreatment for advanced disease, *n* (%)	
Trastuzumab	55 (100.0)
Taxane	55 (100.0)
Lapatinib	35 (63.6)
Anthracycline	40 (72.7)
Resistance, *n* (%)	
Trastuzumab	52 (94.5)
Taxane	40 (72.7)
Lapatinib	33 (60 0)

WHO World Health Organization

[a] Disease-free interval is calculated as the interval between date of first surgery with no residual disease and date of first recurrence of disease

Table 2 Patient disposition (full analysis set; $N = 55$)

	Everolimus + trastuzumab + paclitaxel, *n* (%)
First six cycles	
Enrolled	55 (100)
Completed six cycles	28 (50.9)
Continued beyond six cycles[a]	24 (43.6)
Discontinued	27 (49.1)
Disease progression	16 (29.1)
Adverse events(s)	8 (14.5)
Patient withdrew consent	3 (5.5)
Continued beyond six cycles[b]	0
Beyond first six cycles	
Continued beyond six cycles	24
Ongoing	1
Discontinued	23
Disease progression	21
Adverse event(s)	2

[a] Percentage of patients continuing beyond six cycles who completed first six cycles

[b] Percentage of patients continuing beyond six cycles who discontinued during first six cycles

paclitaxel (21.8 % for AEs), and none for trastuzumab per protocol. At least one everolimus dose interruption was required for 70.9 % of patients (61.8 % for AEs), 67.3 % of patients required a paclitaxel dose interruption (65.5 % for AEs), and 45.5 % of patients required a trastuzumab dose interruption (34.5 % for AEs).

Clinical activity

The trial met its primary endpoint with 12 partial responses, resulting in an ORR of 21.8 % (Table 4). Disease stabilization was achieved in 26 (47.3 %) patients, whereas eight (14.5 %) patients had progressive disease. The disease control rate (defined as ORR plus stable disease) was 69.1 % and the CBR (defined as ORR plus stable disease lasting 24 weeks or longer) was 36.4 %. Overall, at the time of data cutoff, there were 36 PFS events (34 progressive disease and two deaths) based on local radiology review, and the median PFS estimate was 5.5 months (95 % CI: 4.99–7.69 months; Fig. 1). The median OS estimate since start of the study was 18.1 months (95 % CI: 12.85–24.11 months; Fig. 2).

Tolerability

The incidences of AEs were consistent with the known safety profiles of the study drugs (Table 5). All 55 patients reported AEs while on study treatment. Of these, 48 patients (87.3 %) experienced grade 3/4 AEs, 26 patients (47.3 %) had serious AEs, and 16 patients (29.1 %) had AEs leading to discontinuation of at least one of the study treatments. Three deaths occurred on treatment (during study treatment or within 28 days of last treatment): two

Table 3 Treatment characteristics (safety population; $N = 55$)

	Everolimus	Trastuzumab	Paclitaxel	Any
Mean total dose, mg (SD)	1045.5 (541.56)	37.2 (13.96)	906.7 (415.78)	
Median duration of exposure, weeks	24.00[a]	24.00[a]	21.71[a]	24.00[b]
Range	1.7–122.7	2.0–95.0	2.7–68.0	2.7–122.7
Mean dose intensity, mg/day (SD)	7.69 (2.243)	0.28 (0.027)	6.85 (1.536)	
Median dose intensity, mg/day	8.75	0.29	7.09	
Range	3.4–10.0	0.2–0.3	2.5–8.8	
Median relative dose intensity[c]	0.875	1.003	0.827	
Range	0.34–1.00	0.69–1.19	0.30–1.02	
≥1 Dose reduction, *n* (%)	19 (34.5)	0	14 (25.5)	26 (47.3)
≥1 Dose interruption, *n* (%)	39 (70.9)	25 (45.5)	37 (67.3)	49 (89.1)

SD standard deviation

[a] Duration of exposure (weeks) = (date of last dose + X − date of first dose + 1)/7, where X is the number of days remaining to complete the exposure time of the last dose

[b] Duration of exposure for combination treatment (weeks) = date of last dose + X − date of first dose + 1)/7, where X is the number of days remaining to complete the exposure time of the last dose

[c] Relative dose intensity = dose intensity/planned dose intensity

Table 4 Best overall response (full analysis set; $N = 55$)

	Everolimus + trastuzumab + paclitaxel
Best overall response, *n* (%)	
Complete	0
Partial	12 (21.8)
Stable disease	26 (47.3)
Progressive disease	8 (14.5)
Unknown	9 (16.4)
Overall response rate, *n* (%)[a]	12 (21.8)
95 % CI	11.8–35.0
Disease control rate, *n* (%)[b]	38 (69.1)
95 % CI	55.2–80.9
Clinical benefit rate, *n* (%)[c]	20 (36.4)
95 % CI	23.8–50.4

[a] Complete and partial responses

[b] Complete and partial responses plus stable disease

[c] Complete and partial responses plus stable disease ≥24 weeks

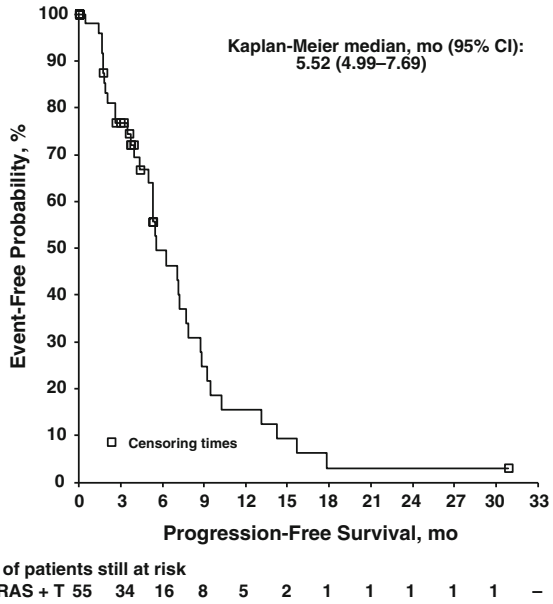

Fig. 1 Kaplan–Meier analyses of progression-free survival in patients with human epidermal growth factor receptor-2-positive advanced breast cancer treated with everolimus (*EVE*), trastuzumab (*TRAS*), and paclitaxel (*T*). *CI* confidence interval

were attributable to disease progression and one to sepsis (suspected by the investigator to be related to study drug). The most common AEs were stomatitis (76.4 %), diarrhea (56.4 %), asthenia (50.9 %), rash (47.3 %), headache (43.6 %), and pyrexia (40.0 %). The most common grade 3/4 AEs (regardless of study treatment) reported in at least 5 % of patients were neutropenia, stomatitis, lymphopenia, leukopenia, anemia, thrombocytopenia, diarrhea, vomiting, fatigue, and pneumonia.

Most of the clinically notable AEs associated with everolimus were grade 1 or 2; among those that were grade 3 or 4, most were manageable by dose adjustments and/or concomitant medications. Cytopenias were reported in 30 (54.5 %) patients and were suspected to be related to study treatment; these were mostly managed by dose interruption and/or dose reduction. Only one patient discontinued paclitaxel early due to thrombocytopenia. Stomatitis, the most frequently reported nonhematologic, clinically notable AE, was primarily grade 1/2 (56.4 %) and the remainder grade 3 (20 %). None of these patients discontinued study

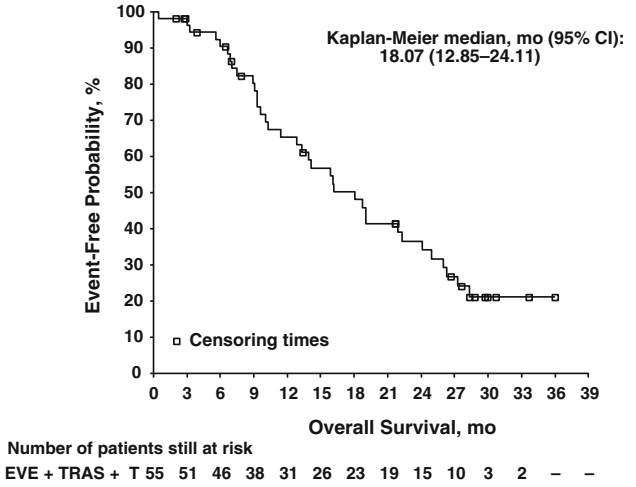

Fig. 2 Kaplan–Meier analyses of overall survival in patients with human epidermal growth factor receptor-2-positive advanced breast cancer treated with everolimus (*EVE*), trastuzumab (*TRAS*), and paclitaxel (*T*). *CI* confidence interval

treatment due to stomatitis, and all 11 patients who experienced grade 3 stomatitis were brought to complete resolution with dose interruption and/or dose reduction, along with concomitant medication. Noninfectious pneumonitis (including interstitial lung disease) was reported in 4 (7.3 %) patients. Grade 2 noninfectious pneumonitis events were diagnosed in two patients and resolved completely following dose interruption and dose reduction; grade 3 events were diagnosed in two patients and resulted in study treatment discontinuation. Both patients who discontinued were treated with steroid therapy and supplemental oxygen; one event resolved 20 days after last study treatment, and one was ongoing at the time of the last available report. No grade 4 noninfectious pneumonitis events were reported (Table 5).

Of the concomitant treatments allowed on study, hematopoietic growth factors were used in 7.2 % of patients who had documented cytopenia during/after cycle 1; baseline bisphosphonate treatment and analgesic medication (including opioids) were continued in 5.4 and 25.4 % of patients, respectively. Use of prophylactic antiemetic medications was continued from baseline in 5.4 % of patients.

Table 5 Adverse events irrespective of relation to study treatment with ≥10 % incidence (any grade) or ≥5 % incidence (grade 3 or 4)

Adverse event (%)[a]	Everolimus + trastuzumab + paclitaxel ($N = 55$)			
	Grade 1	Grade 2	Grade 3	Grade 4
Hematologic disorders				
Neutropenia	0	7.3	25.5	3.6
Anemia	7.3	20.0	7.3	0
Leukopenia	0	3.6	16.4	1.8
Lymphopenia	0	0	18.2	1.8
Thrombocytopenia	1.8	9.1	5.5	1.8
Nonhematologic disorders				
Stomatitis	23.6	32.7	20.0	0
Diarrhea	34.5	16.4	5.5	0
Asthenia	20.0	27.3	3.6	0
Rash	27.3	18.2	1.8	0
Headache	27.3	14.5	1.8	0
Pyrexia	21.8	16.4	0	1.8
Nausea	20.0	18.2	0	0
Cough	23.6	12.7	0	0
Peripheral neuropathy	27.3	5.5	3.6	0
Epistaxis	27.3	1.8	0	0
Vomiting	12.7	9.1	5.5	0
Constipation	21.8	1.8	0	0
Peripheral edema	18.2	5.5	0	0
Fatigue	9.1	7.3	5.5	0
Arthralgia	10.9	9.1	0	0
Nasopharyngitis	10.9	7.3	0	0
Erythema	10.9	7.3	0	0
Decreased appetite	10.9	7.3	0	0
Myalgia	14.5	3.6	0	0
Dyspnea	10.9	5.5	0	0
Muscle spasms	12.7	1.8	0	0
Dry skin	10.9	0	0	0
Insomnia	10.9	0	0	0
Hypophosphatemia	0	0	5.5	1.8
Pneumonia	0	0	5.5	0

The safety analyses excluded events that occurred >28 days after last dose of study treatment

[a] A patient with multiple occurrences of an adverse event was counted only once in the adverse event category. A patient with multiple severity ratings for an adverse event was only included under the maximum rating

Discussion

The results from this phase 2 study provide additional clinical support for the use of mTOR inhibitors to improve the benefit of trastuzumab-based treatment strategies in women with HER2-positive advanced breast cancer. In this study, everolimus added to weekly trastuzumab and paclitaxel yielded a 22 % ORR and 36 % CBR in heavily pretreated patients with tumors exhibiting resistance to trastuzumab (defined as disease progression within 3 months of their last trastuzumab-containing regimen for advanced cancer, or within 12 months after neo/adjuvant therapy). The median PFS of 5.5 months was also encouraging given that the majority of patients had progressed within 3 months of their last regimen for advanced disease. In addition, the median

OS of 18 months was similar to the 23 months reported in a phase 2 study of weekly trastuzumab and paclitaxel in heavily pretreated patients (two or more prior lines of chemotherapy) with trastuzumab-sensitive advanced HER2-positive breast cancer (i.e., no prior exposure to trastuzumab) [20].

Data from primary breast tumors suggest a causal relationship between PI3K/Akt/mTOR pathway activation and overexpression of HER2, implicating the pathway in progression of HER2-positive breast cancer [21]. These findings led to the examination of mTOR inhibition as a strategy for improving sensitivity to trastuzumab-based therapy [22]. This approach was found to be effective in overcoming resistance to trastuzumab as well as in restoring lapatinib sensitivity in several preclinical models of HER2-positive breast cancer [15, 16, 23, 24]. Moreover, in prospective data collected from patients with trastuzumab-refractory breast cancer, loss of HER2 overexpression was rare, whereas activation of the PI3K/Akt/mTOR pathway through loss of PTEN or *PIK3CA* mutation was frequently observed. Interestingly, a recent biomarker analysis from the phase 3 CLEOPATRA study of pertuzumab in combination with trastuzumab and docetaxel showed that *PIK3CA* mutations were associated with a poor prognosis in patients with HER2-positive advanced breast cancer in [25, 26]. However, this analysis did not identify any predictive markers (other than HER2) for response to anti-HER2 therapy; i.e., these biomarker analyses did not provide any insight that would allow refinement of the HER2-positive target population treated with this drug regimen [26]. Analysis of potential biomarkers (e.g., PTEN, HER2, and PI3K) that might predict benefit from everolimus is beyond the scope of the current phase 2 trial because of its small sample size. However, these biomarkers are currently being analyzed in a large, randomized, phase 3 trial of everolimus plus trastuzumab in combination with paclitaxel (BOLERO-1), and this trial may provide future insights into selecting the appropriate patient population to treat with everolimus in combination with available HER2-targeting agents.

Previously reported studies provide further support for the activity of everolimus when combined with trastuzumab in heavily pretreated patients with HER2-positive advanced breast cancer. In the 3-arm phase 1 dose-escalation portion of this study, the ORR was 44 % in 27 evaluable patients from the whole study population and was 31 % among 13 patients who received 10 mg/day everolimus [19]. Likewise, another phase 1/2 study of everolimus in combination with weekly trastuzumab reported a CBR of 34 % in a similar patient population with a high burden of visceral disease that had received at least two prior chemotherapy regimens for advanced breast cancer [27].

In the current study, most of the patients had received prior trastuzumab in the advanced setting and progressed within 3 months. Only seven patients had disease recurrence/progression within 12 months of completing (neo)adjuvant therapy. Therefore, the 5.5-month median PFS should be interpreted in the context of the patient population, which received a median of 3.5 prior lines of therapy, and the majority had progressed rapidly on or after their last trastuzumab-containing regimen for advanced disease. Notably, among 52 patients with tumors considered resistant to trastuzumab-based therapy, 29 % had not progressed at a median follow-up of 32.6 months. Taken together, these data suggest that the combination of a HER2-independent anticancer agent, such as everolimus, with HER2-targeted treatment may delay disease progression in patients who develop trastuzumab-resistant disease. With respect to taxane resistance, 73 % of eligible patients had resistant tumors, and seven patients experienced disease progression during or within 4 months of their last taxane-containing regimen. Thus, despite evidence of resistance to both trastuzumab and taxane therapy, clinical benefit was achieved with everolimus in combination with trastuzumab and paclitaxel.

Safety findings from the phase 2 portion of the trial were similar to observations during the phase 1 portion and consistent with the reported safety profile of each of the study drugs. No new safety signals were observed. As expected, stomatitis was the most frequently reported nonhematologic AE. By comparison, the most frequently reported AEs in the everolimus plus exemestane arm in the phase 3 BOLERO-2 study in hormone-receptor-positive advanced breast cancer were stomatitis, fatigue, asthenia, diarrhea, cough, pyrexia, and hyperglycemia. These are consistent with the AEs previously reported with everolimus monotherapy in other oncology indications [17, 28–30]. Safety data from BOLERO-3, a phase 3 trial of everolimus and trastuzumab paired with vinorelbine instead of paclitaxel for the treatment of patients with HER2-positive advanced breast cancer, revealed a very similar AE profile to that observed in the current phase 2 study. In the initial report from BOLERO-3, the most frequently reported nonhematologic AEs in the everolimus arm in BOLERO-3 were stomatitis, fatigue, pyrexia, diarrhea, nausea, and decreased appetite; commonly reported hematologic AEs for the everolimus arm were neutropenia, anemia, and leukopenia. The most common grade 3/4 nonhematologic AEs in the everolimus arm were stomatitis, fatigue, increased gamma-glutamyltransferase, and asthenia; common hematologic grade 3/4 AEs were neutropenia, leukopenia, anemia, and febrile neutropenia [31]. Overall, the nonhematologic AE profile of the combination of trastuzumab and vinorelbine with everolimus in the BOLERO-3 study was consistent with the known safety

profiles of the individual drugs, and no new unexpected AEs were observed [31]. Final publication of the study outcomes is awaited.

In the current study, AEs were generally manageable with appropriate symptomatic treatment, dose interruptions and reductions, and implementation of the management guidelines per protocol, such as the introduction of hematopoietic growth factors in patients who experienced neutropenia in cycle 1. AEs prompted at least one dose reduction of everolimus in approximately one-third of patients and at least one everolimus dose interruption in 62 % of patients. Similar proportions of patients required at least one paclitaxel dose interruption or reduction because of AEs. Notably, the most common AEs leading to discontinuation were lung-related AEs (six of ten patients who discontinued study treatment because of AEs). However, several patients had a history of underlying respiratory disorders at study entry such as exertional dyspnea, pneumothorax, and pulmonary embolism ($n = 1$ for each). Of the remaining four patients who discontinued study treatment because of AEs, two discontinued, in part, because of febrile neutropenia and brain edema, which have not been reported in breast cancer trials of everolimus in the absence of a chemotherapy partner [32]. For all patients, diligent screening as well as careful monitoring and appropriate dose modifications are recommended to maximize clinical benefit and minimize toxicity.

In conclusion, combining everolimus with trastuzumab and paclitaxel provided substantial clinical benefit in this heavily pretreated population of patients with HER2-positive advanced breast cancer who had progressed rapidly during or after their last treatment regimen, the majority of whom were resistant to trastuzumab and taxane treatment. The promising results achieved with this regimen are currently being confirmed in the randomized, phase 3, BOLERO–1 trial in the first-line setting.

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Conflict of interest S. Hurvitz has received speaker travel reimbursement from Roche and Novartis. M. Campone has received remuneration from Novartis and reports a consultant/advisory role with Novartis-Servier. R.M. O'Regan reports a consultant/advisory role with Novartis and funding from Genentech. F. André reports a consultant/advisory role and funding from Novartis. J. Gligorov has received remuneration from Novartis and reports a consultant/advisory role with Roche. V.C. Tjan-Heijnen has received funding from Novartis, Amgen, Roche, and Sanofi-Aventis NL. H. Mirshahidi reports a consultant/advisory role with Genentech. F. Dalenc reports a consultant/advisory role with Novartis. J.-C. Soria and A. Llombart report no conflicts of interest. M. El-Hashimy is a Novartis employee and reports Novartis stock ownership. S. Miao, J. Lincy, T. Taran, and T. Sahmoud are Novartis employees.

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