Phase 1b Study of Trebananib Plus Paclitaxel and Trastuzumab in Patients With HER2-Positive Locally Recurrent or Metastatic Breast Cancer

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Abstract

Given the potential interactions between human epidermal growth factor receptor 2 (HER2) signaling and angiogenesis, we investigated the angiopoietin (Ang) 1/Ang2 inhibitor trebananib plus trastuzumab and paclitaxel in HER2-positive breast cancer. Forty women received trebananib (10 or 30 mg/kg) plus trastuzumab and paclitaxel. The combination demonstrated acceptable toxicity and antitumor response in HER2-positive locally recurrent/metastatic breast cancer.

Introduction: Trebananib, a peptide-Fc fusion protein, blocks angiogenesis by inhibiting binding of angiopoietin-1/2 to the receptor tyrosine kinase Tie2. Trebananib plus trastuzumab and paclitaxel was evaluated in human epidermal growth factor receptor 2–positive breast cancer in an open-label phase 1b clinical study. **Patients and Methods:** Women with human epidermal growth factor receptor 2–positive breast cancer received weekly paclitaxel (80 mg/m^2), trastuzumab (8 mg/m^2 then 6 mg/kg every 3 weeks), and intravenous trebananib (10 mg/kg or 30 mg/kg weekly) beginning week 2. The primary end point was the incidence of dose-limiting toxicities. Secondary end points included incidence of adverse events (AEs), pharmacokinetics, and tumor response (objective response and duration of response). **Results:** Forty women were enrolled; 2 experienced dose-limiting toxicities (grade 3 ocular transient ischemic attack [10 mg/kg cohort] and grade 3 elevation in γ-glutamyl transferase [30 mg/kg cohort]). The most common treatment-emergent AEs were peripheral edema (n = 28), diarrhea (n = 27), alopecia (n = 26), fatigue (n = 24), and nausea (n = 24). Maximum observed concentration and area under the concentration–time curve increased proportionally with the trebananib dose. Objective response was confirmed in 31 patients. In the 10 mg/kg cohort, 16 patients (80%) experienced partial response, and none experienced complete response. In the 30 mg/kg cohort, 12 patients (71%) experienced partial response and 3 (18%) experienced complete response. Median (95% confidence interval) duration of response in the 10 and 30 mg/kg cohorts was 12.6 (4.3-20.2) and 16.6 (8.2-not estimable) months, respectively. **Conclusion:** This phase 1b study showed that trebananib was tolerated with manageable AEs at a dose up to 30 mg/kg weekly. Trebananib demonstrated anticancer activity, as indicated by objective response and duration of response.

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Introduction

Breast cancer is a multifactorial disease, with development, progression, and metastasis regulated by a variety of pathologic processes and signaling pathways, which include tumor angiogenesis[1] and activation of human epidermal growth factor receptor (EGFR) pathways.[2] Human epidermal growth factor receptor 2 (HER2) is a member of the EGFR (also called erbB/HER) family of transmembrane tyrosine kinase receptors, which include EGFR1 (erbB1/HER1), HER2 (erbB2/HER2-neu), HER3 (erbB3), and HER4 (erbB4). These receptors are capable of signal transduction causing activation of the Ras/MAP kinase pathway, the PI3K/Akt pathway, src family kinases, and signal transducer and activator of transcription proteins. In addition, HER2 is capable of signal transduction without ligand activation through homodimerization or heterodimerization with other members of the HER family.[3] Activation of these pathways promotes cell proliferation, survival, and angiogenesis.[3,4] Overexpression of HER2 in human tumor cells has been shown to be associated with increased angiogenesis and expression of vascular endothelial growth factor (VEGF).[5] Trastuzumab, a humanized monoclonal antibody directed against HER2, has been shown to inhibit tumor cell growth and VEGF expression.[6]

The HER2 gene is amplified in approximately 20% to 25% of patients with breast cancer, and overexpression of HER2 has been shown to be associated with a clinically aggressive form of the disease, leading to poor patient outcomes and prognosis prior to the development of HER2-targeted therapies.[2,7] In clinical studies, trastuzumab significantly improved overall survival in patients with metastatic HER2-positive breast cancer when administered in combination with chemotherapy.[8-10] Consequently, trastuzumab has become a standard-of-care treatment in women with HER2-positive metastatic breast cancer.[11,12] Additional agents acting through HER2 and through interaction with other members of the EGFR/HER family such as lapatinib (a dual HER2 and EGFR inhibitor), pertuzumab (which impairs HER2 dimerization with other HER family members), and trastuzumab emtansine (an antibody–drug conjugate) are more recently introduced in the standard of care[13,14]; others, such as neratinib (which targets EGFR, HER2, and HER4) have also shown activity in HER2-positive breast cancer.[15] Recent data have demonstrated a meaningful therapeutic impact of pertuzumab, in combination with trastuzumab, both in metastatic breast cancer and early stage disease.[14,16]

Angiogenesis has been shown to play a key role in tumor growth and metastasis in breast cancer.[1,17,18] Many factors, including VEGF and angiopoietin (Ang) 1 and Ang2 regulate tumor angiogenesis.[19,20] The angiopoietin axis is distinct from the VEGF pathway, with Ang1 and Ang2 regulating angiogenesis and vascular remodeling by interacting with the tyrosine kinase receptor Tie2.[19] Ang2 is up-regulated at sites of tumor angiogenesis,[21] is prevalent in tumors of patients with invasive breast cancer, and is associated with poor survival in breast cancer.[22,23]

There is also evidence that cross talk exists between the HER signaling pathway in breast cancer and angiogenic modulation of tumor vasculature, particularly that signaling by HER2 (and other EGFR/HER family members) regulates expression of several angiogenic factors. As a result, there has been a great deal of interest in dual targeting of HER2 and VEGF, and a number of clinical studies have investigated the combination of the VEGF-A inhibitor bevacizumab with trastuzumab and chemotherapy in patients with HER2-positive breast cancer.[24,25] Although expression of Ang2 in normal tissues appears to be largely limited to endothelial cells, several studies suggest that Ang2 is expressed by tumor cells, including breast cancer cells.[6,26,27] Furthermore, HER2 overexpression has been shown to up-regulate Ang2 in breast cancer cell lines,[26,27] suggesting that HER2 tumors might be particularly susceptible to therapies that target angiopoietins.

Trebananib is a peptide-Fc fusion protein that binds Ang1 and Ang2 and prevents their interaction with the Tie2 receptor.[28] This inhibition has been shown to suppress tumor cell growth in preclinical studies,[28,29] and trebananib has shown antitumor activity in clinical studies when administered alone,[30] in combination with VEGF inhibitors,[31-34] or in combination with chemotherapy.[35-37] Trebananib, in combination with various chemotherapy regimens at doses up to 15 mg/kg weekly, has demonstrated tolerability and efficacy in large phase 3 trials in ovarian cancer.[38-41]

Given the potential interactions between HER2 signaling, the angiopoietin axis, and other modulators of angiogenesis, we investigated the combination of trebananib, trastuzumab, and paclitaxel in patients with HER2-positive breast cancer in this phase 1b study. This is the first published report on the use of an angiopoietin inhibitor, trebananib (AMG 386), in combination with trastuzumab and paclitaxel in patients with HER2-positive metastatic breast cancer. This study also extends upon previous safety data of administering trebananib at doses up to 30 mg/kg weekly, in combination with paclitaxel.

Patients and Methods

Study Design and Eligibility

This open-label phase 1b trial, conducted at 16 centers in North America and Europe, started on March 9, 2009, and was completed on October 15, 2015. The study was designed to evaluate trebananib in 2 groups of patients with metastatic breast cancer: one group consisted of patients receiving trebananib with trastuzumab and paclitaxel in a first-line setting, and the second group consisted of patients receiving trebananib with lapatinib and capecitabine after previously failed trastuzumab in a first-line setting. This article reports results only from the trebananib plus trastuzumab and paclitaxel groups. Patients were enrolled sequentially to 2 dose cohorts of trebananib, 10 and 30 mg/kg weekly. Only after 20 patients had been enrolled in the 10 mg/kg weekly trebananib dose cohort were patients enrolled in the 30 mg/kg weekly trebananib dose cohort. Trial registration: ClinicalTrials.gov, NCT00807859.

For the trebananib plus trastuzumab and paclitaxel cohorts, women ($\geq$ 18 years) were eligible if they had a histologically or cytologically confirmed diagnosis of adenocarcinoma of the breast with locally recurrent or metastatic disease; had measurable or nonmeasurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0[42]; had an Eastern Cooperative Oncology Group performance status $\leq$ 1; and had not received trastuzumab, lapatinib, or chemotherapy for metastatic or locally recurrent disease (trastuzumab in adjuvant or neoadjuvant therapy was allowed). HER2 positivity was confirmed through fluorescence

in situ hybridization, chromogenic in situ hybridization, or 3+ staining by immunohistochemistry. Patients were excluded if they had inflammatory breast cancer; history of arterial or venous thrombosis within 1 year; clinically significant cardiovascular disease within 1 year; bleeding diathesis or clinically significant bleeding within 6 months; central nervous system metastasis; nonhealing wound, ulcer, or fracture; history of interstitial pulmonary disease; uncontrolled hypertension (diastolic blood pressure $>$ 90 mm Hg or systolic blood pressure $>$ 140 mm Hg); inadequate hepatic, renal, cardiac, or hematologic function; major surgical procedure within 28 days; open breast biopsy within 14 days; minor surgical procedure/fine-needle aspiration within 7 days; radiation therapy within 14 days; prior radiation therapy, radiofrequency ablation, percutaneous cryotherapy, or hepatic chemoembolization on all sites of disease unless disease progression was documented; peripheral neuropathy grade 2 or higher; chemotherapy within 3 weeks; treatment with a VEGF receptor multikinase inhibitor within 21 days; bevacizumab within 45 days; anticoagulation therapy within 1 week; treatment with immune modulators (eg, systemic cyclosporine or tacrolimus) within 30 days; hormonal agent within 14 days; or treatment with any inhibitor of the angiopoietin pathway. The protocol was approved by each center's independent ethics committee; all patients provided written informed consent.

Study Procedures

Trebananib, trastuzumab, and paclitaxel were administered by intravenous infusion. Patients received paclitaxel 80 mg/m^2 once a week. Trastuzumab was administered at a dose of 8 mg/m^2 in week 1 followed by 6 mg/kg once every 3 weeks. Trebananib 10 or 30 mg/kg once a week was administered beginning at week 2. Initially, 6 patients were administered trebananib 10 mg/kg once a week and standard 6 + 3 dose-limiting toxicity (DLT) enrollment criteria were employed. If $\leq$ 1 of 6 or $\leq$ 2 of 9 patients experienced a DLT, the expansion phase was initiated; alternatively, if $\geq$ 3 of 9 patients had a DLT, lower doses of trebananib could be evaluated. Patients who dropped out during the first 28 days of treatment for reasons other than DLT were replaced. In the expansion phase, additional patients were enrolled for a total of 20 patients at the 10 mg/kg once a week dose. After all 20 subjects in the 10 mg/kg once a week expansion phase were enrolled, the 30 mg/kg once a week cohort was opened following the same 6 + 3 enrollment and DLT rules as for the 10 mg/kg once a week dose. This cohort was also expanded to a total of 20 patients at the 30 mg/kg once a week dose.

Treatment continued until disease progression, unacceptable toxicity, or withdrawal of consent. Dose modifications for trebananib were not permitted. If trebananib was withheld for $>$ 28 days or more than twice for treatment-related toxicity, treatment with trebananib was permanently discontinued. Dose modifications were not permitted for trastuzumab. Doses of paclitaxel could be modified based on protocol-specified rules consistent with the prescribing information for each drug.

End Points

The primary end point was the incidence of adverse events (AEs) and clinical laboratory abnormalities, defined as DLTs, of the trebananib cohorts. Patients who experienced an event before initiation of trebananib were not evaluated for a DLT. DLTs were defined as any grade $\geq$ 3 hematologic or nonhematologic toxicities as defined by Common Terminology Criteria for Adverse Events (CTCAE) version 3.0,[43] with the following modifications: grade 3 infusion reactions lasting $>$ 2 hours, grade 3 fatigue persisting for $>$ 7 days, grade 4 diarrhea that persists despite maximum supportive care, grade 3 or 4 nausea/vomiting for $>$ 72 hours, grade 3 or 4 neutropenia with fever ($>$ 38.5°C), grade 4 neutropenia lasting $>$ 7 days, grade 4 thrombocytopenia, grade 4 anemia, grade 4 hypertension, or aspartate aminotransferase/alanine aminotransferase $>$ 10$\times$ upper limit of normal. DLTs were evaluated for all patients who received at least 2 doses of trebananib and 1 dose of trastuzumab. The evaluation period for DLTs was 21 days from the first dose of study drug. Secondary end points included incidences of other AEs and clinical laboratory abnormalities not defined as DLTs, pharmacokinetic profiles of trebananib, trastuzumab, and paclitaxel, objective response (OR), duration of response (DOR), change in tumor burden, progression-free survival (PFS), and incidence of anti-trebananib antibodies.

Assessments

AEs occurring from the start of treatment to 30 days after the last dose were recorded and graded using CTCAE version 3.0. Potential relationship of AEs to study treatment was assessed by the investigators. In addition to routine physical examinations, vital signs, electrocardiograms, and safety laboratory results, left ventricular ejection fraction was evaluated by multigated acquisition scan or echocardiogram (at baseline, week 9, week 18, then every 18 weeks, and at safety follow-up).

Radiologic imaging (computed tomography or magnetic resonance imaging) of at least the chest, abdomen, and pelvis was done before cycle 1 and every 9 $\pm$ 1 weeks after the first dose of study treatment until disease progression or the safety follow-up visit. Imaging was evaluated by investigators per RECIST version 1.0. Patients with a partial response or complete response had a confirmatory radiographic evaluation conducted at least 28 days from the initial evaluation of response.

Pharmacokinetics

Serum samples for evaluation of trebananib and trastuzumab pharmacokinetics were collected from all patients before infusion as well as within 10 minutes after infusion of trebananib, trastuzumab, and paclitaxel on day 1 of weeks 7 and 19 and every 9 $\pm$ 1 weeks thereafter. Serum samples were also taken 6, 24, 48, 96, and 168 hours after the end of trebananib infusion on day 1 of week 7, or at 6, 24, 48, 96, 168, 336, and 504 hours after the end of trastuzumab infusion on day 1 of week 7. Plasma samples for evaluation of the pharmacokinetics of paclitaxel were collected before paclitaxel and trebananib/trastuzumab infusion on day 1 of weeks 1, 7, 19, 28, 37, and 46 and within 5 minutes after the end of infusion of paclitaxel on day 1 of weeks 1 and 7. Analysis of individual serum trebananib, individual plasma paclitaxel, and individual serum trastuzumab was made by comparing concentrations before treatment (day 1 of week 1) and after treatment (steady state at week 7). The pharmacokinetics of paclitaxel when administered with trastuzumab on day 1 of week 1 (without trebananib) and week 7 (paclitaxel with trastuzumab and trebananib at steady state) were compared.

Table 1 Baseline Demographics and Clinical Characteristics

Characteristic	Trebananib 10 mg/kg + Trastuzumab/ Paclitaxel (n = 20)	Trebananib 30 mg/kg + Trastuzumab/ Paclitaxel (n = 20)
Age (years), median (range)	54.5 (29-76)	51.5 (33-74)
Race		
White	17 (85)	19 (95)
Black/African American	1 (5)	0
Hispanic/Latino	1 (5)	1 (5)
Other	1 (5)	0
ECOG PS		
0	11 (55)	11 (55)
1	9 (45)	9 (45)
2	0	0
No. of metastatic sites ≤ 3	17 (85)	18 (90)
Time (months) since LR/MBC diagnosis, median (range)	1.1 (0.3-90.1)	1.1 (0.1-7.0)
Prior adjuvant or neoadjuvant chemotherapies	8 (40)	8 (40)
Prior trastuzumab (neoadjuvant setting)	2 (10)	7 (35)

Data are presented as n (%) unless otherwise indicated.
Abbreviations: ECOG PS = Eastern Cooperative Oncology Group performance status; LR/MBC = locally recurrent or metastatic breast cancer.

Immunogenicity

Serum samples for assessment of anti-trebananib binding and neutralizing antibodies (as previously described)[44] were collected predose; at weeks 1, 5, and 10; every 9 ± 1 weeks thereafter; and at the safety follow-up visit.

Statistical Analyses

OR, DOR, and tumor burden were determined for the subset of patients with at least one unidimensionally measurable lesion at baseline for each cohort. Safety analyses and analysis of PFS included all patients who received ≥ 1 dose of trebananib and reported by descriptive statistics (eg, percentage). Exact binomial 2-sided 80% confidence intervals (CIs) were generated for OR for each cohort. For PFS and DOR, medians with 95% CIs were calculated using the Kaplan-Meier method for each cohort. Noncompartmental pharmacokinetic analyses for detection of trebananib, trastuzumab, and paclitaxel were done using Phoenix WinNonlin 6.3 software (Pharsight, St. Louis, MO).

Results

Patient Characteristics

A total of 40 women were enrolled at 16 centers in Belgium, France, and the United States. Twenty were in the trebananib 10 mg/kg once a week plus trastuzumab/paclitaxel cohort, and 20 were in the trebananib 30 mg/kg once a week plus trastuzumab/paclitaxel cohort (Table 1). At the time of this analysis, all but one patient in the trebananib 30 mg/kg cohort had discontinued study treatment (Figure 1). Key reasons for discontinuation of trebananib were disease progression (trebananib 10 mg/kg, n = 9; trebananib 30 mg/kg, n = 6) and AEs (trebananib 10 mg/kg, n = 6; trebananib 30 mg/kg, n = 9). Median (range) number of trebananib infusions was 29.5 (1-173) in the trebananib 10 mg/kg cohort and 25.5 (13-138) in the trebananib 30 mg/kg cohort. Median (range) number of trastuzumab cycles was 12.0 (2-60) in the trebananib 10 mg/kg cohort and 15.0 (5-77) in the trebananib 30 mg/kg cohort. Patients received a median (range) of 6.0 (2-11) paclitaxel cycles in the trebananib 10 mg/kg cohort and 8.0 (3-19) in the trebananib 30 mg/kg cohort.

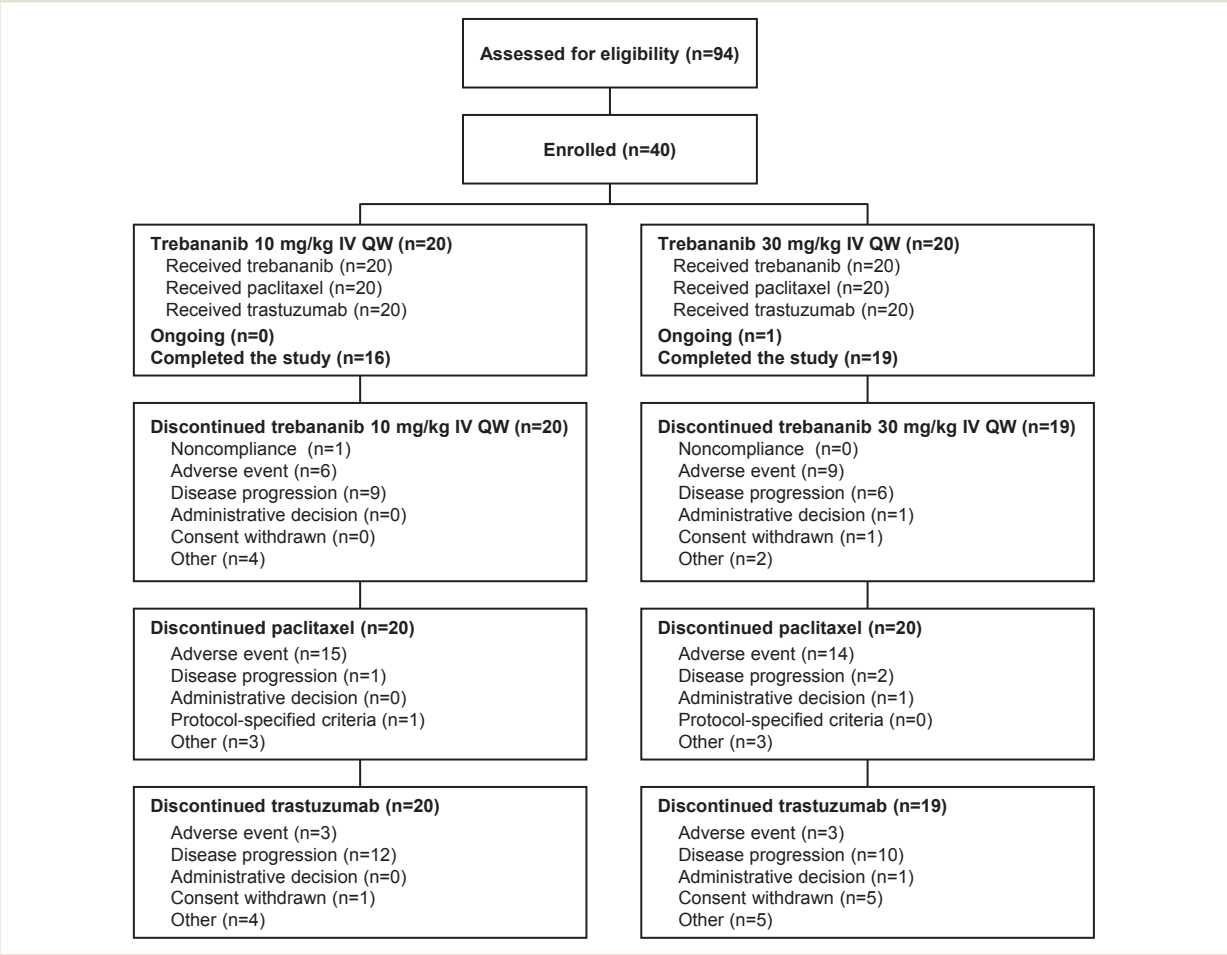
DLTs and AEs

Two patients experienced DLTs during the first 21 days of the study. One patient in the trebananib 10 mg/kg cohort experienced a grade 3 ocular, arterial thromboembolic transient ischemic attack on day 11 and discontinued the study. The event was considered to be related to trebananib by the investigator but was not deemed serious. One patient in the trebananib 30 mg/kg cohort experienced a grade 3 elevation in γ-glutamyl transferase on day 21 of the study. The DLT was considered to be related to trebananib and paclitaxel but was not considered serious by the investigator. Treatment was delayed by 3 days, the event resolved by day 25, and the patient was not removed from the study. Given the low incidence of DLTs, both cohorts were expanded to 20 patients to further evaluate toxicity and efficacy of trebananib in combination with trastuzumab and paclitaxel.

All patients experienced at least one treatment-emergent AE. Grade 3 or higher AEs were reported in 16 patients (80%) in the trebananib 10 mg/kg cohort and 13 patients (65%) in the trebananib 30 mg/kg cohort. Six (30%) and 9 (45%) patients had serious treatment-emergent AEs in the trebananib 10 and 30 mg/kg cohorts, respectively; 6 (30%) and 8 (40%) patients in the trebananib 10 and 30 mg/kg cohorts, respectively, had treatment-emergent AEs that led to discontinuation of trebananib. The only AE that led to discontinuation of trebananib in more than one patient was peripheral edema (n = 2 in the trebananib 30 mg/kg cohort).

The most frequently occurring AEs were peripheral edema, fatigue, diarrhea, alopecia, nausea, nail disorder, and rash (Table 2). Most events were mild to moderate in severity. The most frequently occurring grade 3 or higher AEs were peripheral neuropathy,

Figure 1 Disposition of Patients in the Study



peripheral sensory neuropathy, and dyspnea (Table 2). Two patients had grade 4 events (neutropenia and ankle fracture, both in the trebananib 10 mg/kg cohort). Five patients (25%) in the trebananib 30 mg/kg cohort and 2 (10%) in the trebananib 10 mg/kg cohort had pleural effusion. One pleural effusion event in the trebananib 30 mg/kg cohort was grade 3; all others were grade 1/2. There were no fatal AEs.

AEs of interest are shown in Table 3. Two patients (10%) in the trebananib 30 mg/kg cohort had grade 3 peripheral edema. There were no other grade 3 or higher peripheral edema events. Five patients (3 in the trebananib 10 mg/kg cohort and 2 in the trebananib 30 mg/kg cohort) had grade 1 blurred vision. No patient had ascites during the study. Incidences of hypertension and arterial and venous thromboembolic events were low (Table 3). Three patients in the trebananib 30 mg/kg cohort experienced decreased left ventricular ejection fraction, one of which was grade 3.

Efficacy

At the time of this analysis, 12 patients (60%) in the trebananib 10 mg/kg cohort and 9 patients (45%) in the trebananib 30 mg/kg cohort had disease progression or died. Median (95% CI) PFS for patients in the trebananib 10 and 30 mg/kg trebananib cohorts was 14.5 (6.9-20.6) and 18.5 (10.4-21.9) months, respectively (Figure 2A). OR was confirmed in 31 (84%) of 37 patients with measurable disease at baseline. Among the 20 patients in the trebananib 10 mg/kg cohort, none had a complete response and 16 patients (80%) had a partial response (OR, 80%; 95% CI, 56-94). In the trebananib 30 mg/kg cohort, 17 patients had measurable disease. Of these 17, 3 (18%) had a complete response and 12 (71%) had a partial response (OR, 88%; 95% CI, 64-98). Median (95% CI) DOR for responders in the trebananib 10 and 30 mg/kg cohorts was 12.6 (4.3-20.2) and 16.6 (8.2—not estimable) months, respectively. Mean (standard deviation [SD]) decrease in the sum of the longest diameters of target lesions from baseline was 61% (25%) and 69% (24%) in the trebananib 10 and 30 mg/kg cohorts (Figure 2B).

Pharmacokinetics

For trebananib, mean (SD) maximum observed concentration (C_{max}) and area under the concentration—time curve within a dosing interval (AUC_{tau}), respectively, at steady state were 242 (82.6) μg/mL and 8.22 (3.59) mg•h/mL following 10 mg/kg

Table 2 Treatment-Emergent AEs Occurring in ≥ 20% of Patients

AE	Trebananib 10 mg/kg + Trastuzumab/Paclitaxel (n = 20)[a]		Trebananib 30 mg/kg + Trastuzumab/Paclitaxel (n = 20)[b]	
	Any Grade	Grade 3 or Higher	Any Grade	Grade 3 or Higher
Any AE	20 (100)	16 (80)	20 (100)	13 (65)
AEs occurring in ≥ 20% of patients				
Peripheral edema	13 (65)	0	15 (75)	2 (10)
Fatigue	15 (75)	2 (10)	9 (45)	0
Diarrhea	13 (65)	0	14 (70)	0
Alopecia	13 (65)	0	13 (65)	0
Nausea	11 (55)	0	13 (65)	0
Nail disorder	12 (60)	2 (10)	7 (35)	0
Rash	12 (60)	1 (5)	6 (30)	0
Dyspnea	10 (50)	3 (15)	7 (35)	1 (5)
Peripheral sensory neuropathy	10 (50)	4 (20)	4 (20)	0
Paresthesia	3 (15)	1 (5)	10 (50)	2 (10)
Headache	9 (45)	0	8 (40)	0
Neutropenia	9 (45)	1 (5)	8 (40)	1 (5)
Epistaxis	5 (25)	0	9 (45)	0
Pain in extremity	9 (45)	0	5 (25)	0
Arthralgia	9 (45)	0	3 (15)	0
Decreased appetite	9 (45)	0	2 (10)	0
Constipation	7 (35)	0	8 (40)	0
Cough	8 (40)	0	6 (30)	0
Insomnia	5 (25)	0	8 (40)	0
Peripheral neuropathy	8 (40)	4 (20)	5 (25)	4 (20)
Edema	8 (40)	0	5 (25)	0
Asthenia	2 (10)	0	8 (40)	1 (5)
Back pain	6 (30)	0	5 (25)	0
Dysgeusia	5 (25)	0	6 (30)	0
Vomiting	6 (30)	0	5 (25)	0
Hypertension	6 (30)	1 (5)	4 (20)	0
Abdominal pain	3 (15)	0	6 (30)	0
Dyspepsia	6 (30)	0	3 (15)	0
Lymphedema	3 (15)	0	6 (30)	0
Pain	6 (30)	0	2 (10)	0
Weight increased	2 (10)	0	6 (30)	0
Dizziness	5 (25)	0	4 (20)	0
Oropharyngeal pain	5 (25)	0	4 (20)	0
Pyrexia	4 (20)	0	5 (25)	0
Breast pain	5 (25)	0	3 (15)	0
Urinary tract infection	5 (25)	0	3 (15)	0

Data are presented as n (%).
Abbreviation: AE = adverse event.
[a]Two patients in the trebananib 10 mg/kg cohort had grade 4 AEs (neutropenia and ankle fracture, respectively); no patients had grade 5 AEs.
[b]None of patients in the trebananib 30 mg/kg cohort had grade 4 or 5 AEs.

weekly infusion of trebananib and 781 (170) μg/mL and 31.1 (8.92) mg•h/mL following 30 mg/kg weekly infusion of trebananib (Table 4). Mean (SD) concentration–time profiles for trebananib at steady state (week 7) are shown in Figure 3. Clearance and volume of distribution at steady-state estimates of trebananib were similar between treatment groups. For trastuzumab, C_{max} and AUC_{tau} were similar between dose cohorts, suggesting that the higher trebananib dose did not influence the pharmacokinetics of trastuzumab. Trastuzumab C_{max} at week 7 was 156 (30.5) μg/mL and 158 (34.2) μg/mL in the trebananib 10 mg/kg cohort and 30 mg/kg cohorts,

Table 3 Summary of AEs of Interest

AE	Trebananib 10 mg/kg (n = 20)		Trebananib 30 mg/kg (n = 20)	
	Any Grade	Grade 3 or Higher	Any Grade	Grade 3 or Higher
Edema (all types)[a]	18 (90)	0	20 (100)	3 (15)
Peripheral edema[b]	13 (65)	0	15 (75)	2 (10)
Generalized edema[c]	1 (5)	0	3 (15)	1 (5)
Lymphedema	3 (15)	0	6 (30)	0
Hemorrhage[d]	6 (30)	0	10 (50)	0
Hypertension	6 (30)	1 (5)	5 (25)	0
Pleural effusion	2 (10)	0	5 (25)	1 (5)
Blurred vision	3 (15)	0	2 (10)	0
Thromboembolic events				
Venous	1 (5)	0	2 (10)	1 (5)
Arterial	1 (5)	1 (5)[e]	0	0
Decreased left ventricular ejection fraction	0	0	3 (15)	1 (5)

Abbreviation: AE = adverse event.
[a]Specific subtypes of edema listed were chosen based on incidence in ≥ 25% of patients in either cohort.
[b]Defined as edema confined to single body area.
[c]Defined as edema with contiguous extension to more than single body area.
[d]Eighty-seven percent of events were epistaxis; 80% in the trebananib 10 mg/kg cohort and 100% in the trebananib 30 mg/kg cohort were grade 1.
[e]This event, an ocular transient ischemic attack, was noted as a dose-limiting toxicity.

respectively. Trastuzumab AUC_{tau} was 21.7 (4.39) mg•h/mL and 24.0 (6.30) mg•h/mL in the trebananib 10 and 30 mg/kg cohorts, respectively. The pharmacokinetics of paclitaxel was also not affected by the coadministration of trebananib. For the trebananib 10 mg/kg cohort, mean (SD) paclitaxel C_{max} was 2310 (1100) ng/mL at week 1 and 2490 (728) ng/mL at week 7; mean (SD) area under the concentration−time curve to the last measurable concentration (AUC_{inf}) of paclitaxel was 5440 (1830) ng•h/mL at week 1 and 5220 (1470) ng•h/mL at week 7. For the trebananib 30 mg/kg cohort, mean (SD) paclitaxel C_{max} was 1940 (919) ng/mL at week 1 and 2020 (1040) ng/mL at week 7, and mean (SD) paclitaxel AUC_{inf} was 4550 (1180) ng•h/mL at week 1 and 4310 (1020) ng•h/mL at week 7.

Anti-Trebananib Antibodies

Three (11%) of 27 patients had anti-trebananib binding, but nonneutralizing, antibodies at baseline (n = 1, trebananib 10 mg/kg; n = 2, trebananib 30 mg/kg). No patients developed binding or neutralizing anti-trebananib antibodies during the study. Positivity for anti-trebananib binding antibodies did not appear to affect the pharmacokinetics of trebananib.

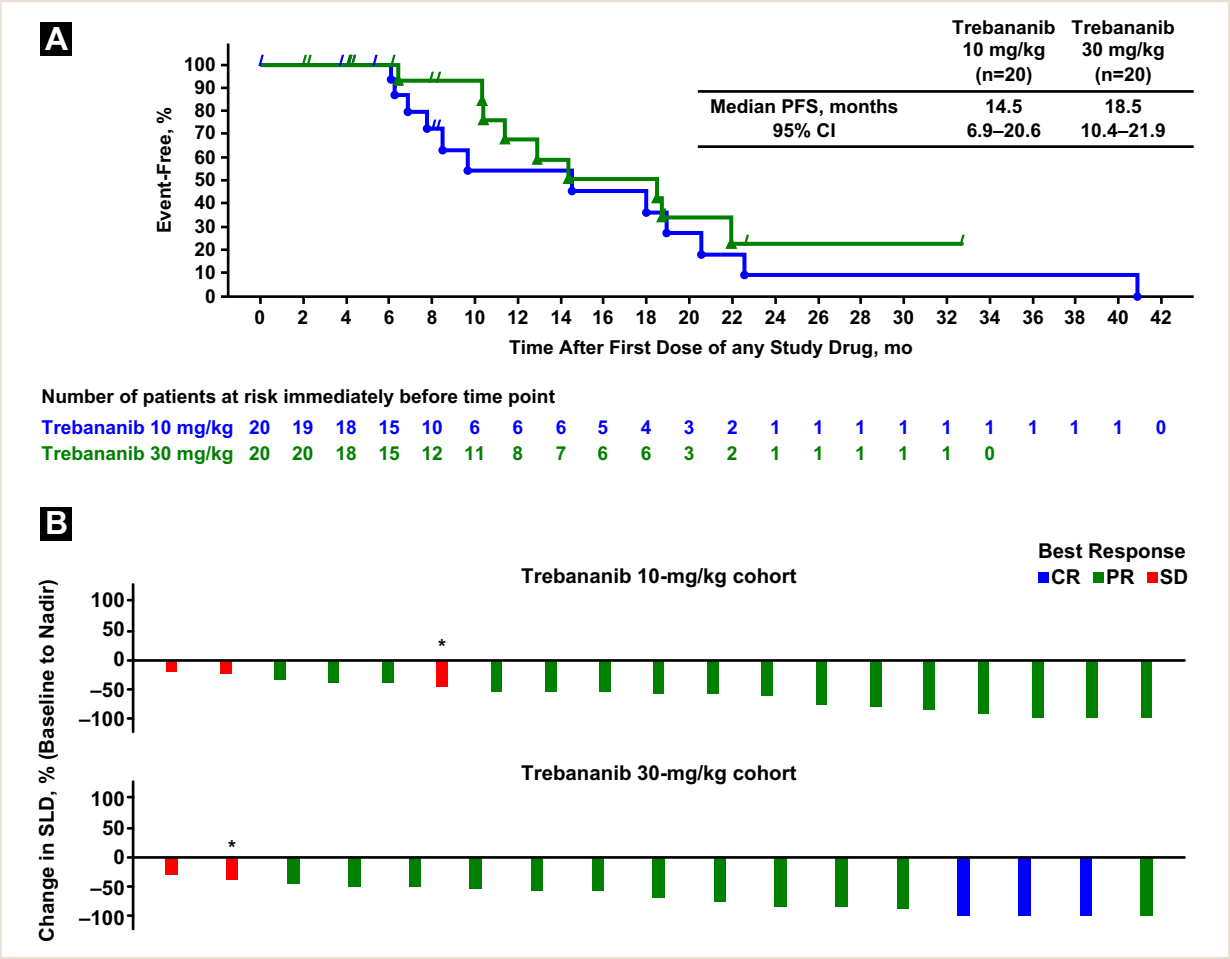
Discussion

In this phase 1b study, administration of trebananib (10 or 30 mg/kg once a week) in combination with paclitaxel and trastuzumab in women with HER2-positive locally recurrent or metastatic breast cancer resulted in a toxicity profile consistent with that anticipated for the combination. Most treatment-related AEs were grade 1 or 2, and there was no evidence of pharmacokinetic interactions between these agents. The activity of the combination was notable: overall, more than 80% of patients had an OR; PFS and DOR results were promising in these patients when compared with historical controls.

AEs occurring in this study were consistent with previously reported findings[38-41] and showed that a combination of trebananib, trastuzumab, and paclitaxel did not result in unanticipated toxicity. The most frequently occurring AE was peripheral edema; this was grade 1 or 2 in most cases and infrequently resulted in discontinuation of study treatment. Edema and pleural effusion have been associated with trebananib and occurred at similar rates in this study as those seen in previous reports. Pleural effusion occurred in 5% of patients given trebananib 3 or 10 mg/kg, and edema occurred in 13% to 61% of patients.[45,46] Because trebananib binds to and inactivates angiopoietins 1 and 2, the vascular permeability and the normal flow of the lymphatic and venous circulation can be perturbed, which may lead to accumulation of extracellular fluid in general and (lymph) edema. In most cases, edema is generally mild and reversible but usually takes 4 to 12 months to resolve after stopping trebananib.[45] Other AEs, such as fatigue, rash, diarrhea, and headache, were mild and were consistent with previously published findings from studies in which trebananib was administered as a single agent[30] or in combination[37] with paclitaxel. Previous studies in combination with paclitaxel were conducted at doses of trebananib 10 or 15 mg/kg once a week. This study suggests that doses of trebananib of up to 30 mg/kg once a week in combination with paclitaxel (and trastuzumab) are tolerable. AEs that are typically associated with anti-VEGF pathway agents (eg, hypertension, impaired wound healing, and thrombosis)[47] were infrequent, demonstrating the distinct toxicity profile of trebananib.

The pharmacokinetics of trebananib appeared to be dose proportional at the doses tested, and exposure was similar to that in previous monotherapy studies at the same doses[30] and for the combination of trebananib 10 mg/kg and paclitaxel.[41] This study did not identify an effect of trebananib on the pharmacokinetic profile of either trastuzumab or paclitaxel. Pharmacokinetic

Figure 2 Analysis of PFS and Lesion SLD. (A) Kaplan-Meier Analysis of Median PFS. (B) Change in SLD of Target Lesions by Best Response. Data From all Patients With at Least One Measurable Lesion per Modified RECIST are Shown. One Patient in Each Cohort (*) had a > 30% Reduction in SLD of Target Lesions With No Confirmatory Tumor Assessment and Was Therefore Assigned Best Response of SD



Abbreviations: CR = complete response; PFS = progression-free survival; PR = partial response; RECIST = Response Evaluation Criteria in Solid Tumors; SD = stable disease; SLD = sum of longest diameter.

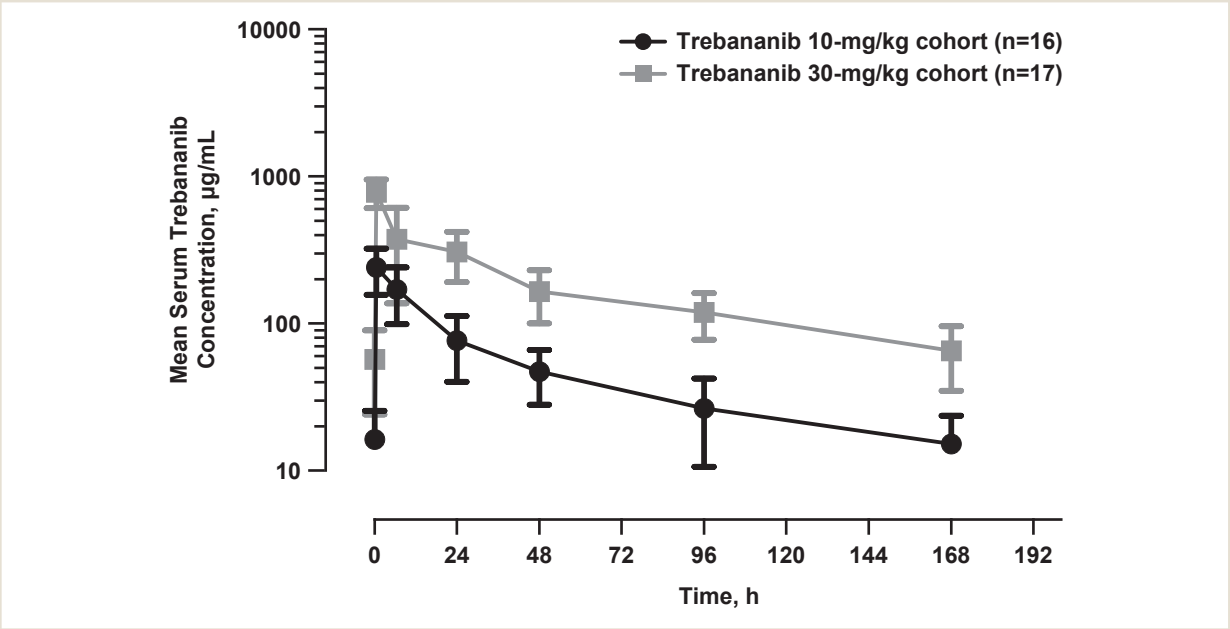
interactions for the 3 agents evaluated were not anticipated because trebananib is cleared by the neonatal Fc receptor and via the kidney,[48] whereas trastuzumab is metabolized following recycling by the neonatal Fc receptor[49] and paclitaxel is metabolized by cytochrome P450 enzymes.[50] As reported previously for other combinations,[35,41] the presence of anti-trebananib antibodies did not affect the pharmacokinetic profile of trebananib, trastuzumab, or paclitaxel.

Table 4 Steady-State Trebananib Pharmacokinetic Parameter Estimates at Week 7

Parameter	Trebananib 10 mg/kg Cohort			Trebananib 30 mg/kg Cohort		
	n	Mean (SD)	CV (%)	n	Mean (SD)	CV (%)
$C_{max,ss}$, µg/mL	18	242 (82.6)	34.1	17	781 (170)	21.8
$AUC_{tau,ss}$, mg•h/mL	17	8.22 (3.59)	43.7	11	31.1 (8.92)	28.7
C_L, mL/h/kg	18	1.39 (0.454)	32.7	11	1.07 (0.537)	50.1
V_{ss}, mL/kg	18	55.7 (21.5)	38.6	11	51.2 (21.4)	41.9
C_{min}, µg/mL	16	15.3 (8.46)	55.5	12	65.3 (30.4)	46.6

Abbreviations: $AUC_{tau,ss}$ = area under the concentration–time curve from time 0-168 hours at steady state; C_L = serum clearance after intravenous infusion; $C_{max,ss}$ = maximum observed drug concentration at steady state; C_{min} = minimum observed drug concentration during dosing interval; CV = coefficient of variation; SD = standard deviation; V_{ss} = volume of distribution at steady state.

Figure 3 Mean (Standard Deviation) Steady-State Serum Trebananib Concentrations at Week 7



This was a small phase 1b study and as such has a number of limitations. Evaluation of antitumor activity was exploratory, and results must be interpreted cautiously when compared with historical controls. Furthermore, because the 10 and 30 mg/kg once a week cohorts were nonrandomized and sequentially enrolled, comparison of the safety and efficacy data of the 2 cohorts must be viewed with caution. Nevertheless, the results provide preliminary evidence of antitumor activity in patients given either the 10 or 30 mg/kg once a week dose of trebananib in combination with trastuzumab and paclitaxel. OR in this study (80% in the trebananib 10 mg/kg cohort; 88% in the trebananib 30 mg/kg cohort) compares favorably with previous phase 3 studies that have reported response rates from 36% to 62% with the combination of trastuzumab and paclitaxel in women with HER2-positive advanced breast cancer.[8-10] Similarly, PFS in this study (14.5 months in the trebananib 10 mg/kg cohort; 18.5 months in the trebananib 30 mg/kg cohort) appears longer than time to progression or PFS times observed in previous studies (7.4-14.5 months).[8-10] It is important to note that the combination of pertuzumab, trastuzumab, and docetaxel is now considered standard treatment for HER2-positive metastatic breast cancer.[51,52] In the phase 3 CLEOPATRA study, PFS was prolonged by 6.1 months, from 12.4 months in the control group (placebo/docetaxel/trastuzumab) to 18.5 months in the pertuzumab/docetaxel/trastuzumab group (hazard ratio = 0.62; 95% CI, 0.51-0.75; $P < .001$). Similarly, AEs in the CLEOPATRA clinical study were mild and included diarrhea, rash, mucosal inflammation, febrile neutropenia, and dry skin.[14] These findings suggest that the addition of trebananib to trastuzumab/paclitaxel/pertuzumab may provide further benefit.

Several phase 2 and 3 clinical studies have evaluated the combination of the VEGF inhibitor bevacizumab with trastuzumab plus chemotherapy in different lines of treatment in patients with HER2-positive breast cancer. In the AVEREL phase 3 study of bevacizumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer, median PFS was 16.5 and 13.7 months in the bevacizumab and nonbevacizumab arms, respectively; however, the difference was not statistically significant (hazard ratio = 0.82; 95% CI, 0.65-10.02; $P = .0775$).[25] In the adjuvant phase 3 BETH trial, the addition of bevacizumab to adjuvant trastuzumab and chemotherapy also did not significantly improve invasive disease-free survival or overall survival in patients with high-risk HER2-positive breast cancer.[53] In a neoadjuvant phase 2 clinical trial (BEVERLY-2), which was conducted specifically in patients with inflammatory nonmetastatic HER2-positive breast cancer, bevacizumab, trastuzumab, and chemotherapy produced a complete pathologic response in 33 of 52 patients with nonmetastatic HER2-positive primary breast cancer, which was higher than historical data on this population of patients.[54] Furthermore, a neoadjuvant randomized phase 2 trial in noninflammatory HER2-positive breast cancer has also failed to demonstrate a substantial benefit of the addition of bevacizumab to trastuzumab and chemotherapy because of increased AEs.[55] A phase 2 study of lapatinib in combination with bevacizumab in heavily pretreated patients with HER2-positive breast cancer has shown activity with the addition of bevacizumab,[56] but no randomized trials have been completed to date. Finally, multiple studies in other tumor types (eg, colorectal, non–small-cell lung cancer, squamous cell carcinoma of the head and neck) have investigated the value of dual inhibition of the EGF/HER and VEGF pathways; several have demonstrated antitumor activity, with some showing improvements in PFS.[57-59] Hence, the clinical value of the addition of the VEGF inhibitor bevacizumab to EGF/HER targeted therapies in breast and other tumor types remains unclear. This study of trebananib in combination with trastuzumab in metastatic breast cancer suggests that inhibition of

Ang1/Ang2 modulated angiogenesis may be an important alternative approach to combined blockade of HER2 signaling and angiogenesis. A more definitive assessment of efficacy of the combination of HER and Ang1/Ang2 inhibition would require larger randomized, placebo-controlled studies, such studies are not currently planned.

Conclusion

This phase 1b study showed that the combination of trebananib (10 mg/kg or 30 mg/kg weekly), trastuzumab, and paclitaxel has acceptable toxicity in women with HER2-positive locally recurrent or metastatic breast cancer. Preliminary evidence suggests that the combination had antitumor activity in patients with HER2-positive metastatic breast cancer. The data suggest that further investigation of Ang1/Ang2 inhibition in combination with HER2 (and other members of the HER family of receptors) may be warranted.

Clinical Practice Points

- HER2 is amplified in approximately 20% to 25% of patients with breast cancer, and overexpression has been shown to be associated with a clinically aggressive form of the disease. Furthermore, HER2 overexpression has been shown to up-regulate Ang2 in breast cancer cell lines, suggesting that HER2 tumors might be particularly susceptible to therapies that target angiopoietins.
- Trebananib is a peptide-Fc fusion protein that binds Ang1 and Ang2 and prevents their interaction with the Tie2 receptor, and has demonstrated tolerability and efficacy in combination with chemotherapy in large phase 3 trials in ovarian cancer. This study is the first to report data on the use of an angiopoietin inhibitor, trebananib, in combination with trastuzumab and paclitaxel in HER2-positive metastatic breast cancer.
- Results demonstrated an acceptable toxicity profile consistent with that anticipated for the combination. Most treatment-related AEs were grade 1 or 2, and there was no evidence of pharmacokinetic interactions between these agents. Additionally, the activity of the combination was notable: overall, more than 80% of patients had an OR; PFS and DOR were promising when compared with historical controls.
- The data suggest that further investigation of Ang1/Ang2 inhibition in combination with HER2 (and other members of the HER family of receptors) may be beneficial.

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Disclosure

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