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CLINICAL TRIAL

A Phase I dose-escalation study of the VEGFR inhibitor tivozanib hydrochloride with weekly paclitaxel in metastatic breast cancer

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Abstract Tivozanib is a potent selective tyrosine kinase inhibitor (TKI) of vascular endothelial growth factor receptors (VEGFRs) 1, 2, and 3. This Phase Ib study investigated the safety/tolerability, pharmacokinetics (PK), and activity of tivozanib with weekly paclitaxel in metastatic breast cancer (MBC). MBC patients with no prior VEGFR TKI treatment received daily oral tivozanib (3 weeks on, 1 week off) with weekly paclitaxel 90 mg/m^2. Standard 3 + 3 dose escalation was used; tivozanib cohorts (C) included C1 0.5 mg, C2 1.0 mg, and C3 1.5 mg. Assessments included Response Evaluation Criteria in Solid Tumors response, PK, and vascular function. Eighteen patients enrolled. Toxicities in >20 % of patients included fatigue, alopecia, nausea, diarrhea, peripheral sensory neuropathy, and hypertension. Grade 3/4 toxicities in >15 % of patients included fatigue and neutropenia. Maximum tolerated dose was tivozanib 1.5 mg with paclitaxel 90 mg/m^2. Four patients withdrew because of toxicity and one due to progressive disease. Thirteen patients were evaluable for response: four (30.8 %) had confirmed partial response; four had stable disease ≥6 months (30.8 %). PK data suggest no influence of paclitaxel on tivozanib concentrations. Tivozanib plus weekly paclitaxel was tolerable at all dose levels, supporting their combination at full dose. Activity in this small population was encouraging.

Keywords Metastatic breast cancer · Paclitaxel · Tivozanib · VEGFR inhibitor

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Introduction

Angiogenesis is a defining feature of cancer growth [1]. Neovascularization is necessary for sustained tumor growth and metastatic progression [2]. Vascular endothelial growth factor (VEGF) plays a critical role in oncologic angiogenesis [3]. The various isoforms of VEGF bind to three tyrosine kinase VEGF receptors (VEGFRs). Specific targeting of VEGFRs offers a rational approach for anticancer therapy.

Tivozanib hydrochloride (tivozanib; previously AV-951) is an oral, potent, and selective small-molecule tyrosine kinase inhibitor (TKI) designed to provide optimal blockade of the VEGF pathway by inhibiting VEGFRs 1, 2, and 3. In cell-based models, tivozanib has inhibitory activity against these VEGFR kinases at subnanomolar concentrations (half maximal inhibitory concentrations of 0.21, 0.16, and 0.24 nM, respectively) [4]. A Phase I study demonstrated clinical response to tivozanib in multiple tumors and determined a maximum tolerated dose (MTD)

of 1.5 mg/day [5]. A Phase II trial in advanced renal cell carcinoma (RCC) showed that tivozanib had anti-tumor activity and a favorable safety profile [6]. The most common treatment-related toxicities included hypertension and dysphonia, with a low incidence of gastrointestinal toxicity. A Phase III trial investigated the efficacy and safety of tivozanib compared with a less-selective VEGFR TKI, sorafenib, as initial targeted treatment for advanced RCC. Tivozanib significantly improved progression-free survival (PFS) and showed a differentiated safety profile compared with sorafenib. Hypertension was the predominant adverse event (AE) for tivozanib, and certain off-target toxicities (e.g., hand–foot syndrome) and dose adjustments were fewer with tivozanib than sorafenib [7].

Weekly paclitaxel is an active and tolerable regimen for treating metastatic breast cancer (MBC). The combination of the anti-VEGF antibody bevacizumab and chemotherapy, including paclitaxel, showed activity in MBC [8–13]. Sorafenib improved PFS when added to capecitabine in human epidermal growth factor receptor 2 (HER2)-negative MBC, although this produced high rates of hand–foot syndrome [14]. As tivozanib is a potent and selective VEGFR inhibitor, it was hypothesized that combining tivozanib and weekly paclitaxel would provide activity with non-overlapping toxicity. This Phase Ib study investigated the safety/tolerability, pharmacokinetics (PK), and activity of tivozanib combined with weekly paclitaxel in MBC.

Methods

Patient population

Eligible patients were females aged ≥18 years with histologically or cytologically documented invasive breast cancer, either metastatic disease or locally advanced unresectable tumor with progressive disease (PD) despite neoadjuvant/adjuvant chemotherapy. Measurable or evaluable disease by Response Evaluation Criteria in Solid Tumors (RECIST), Eastern Cooperative Oncology Group performance status of 0–2 with a life expectancy of ≥3 months, ≤4 prior chemotherapy regimens in the adjuvant and/or metastatic settings, and ≤1 prior taxane-based regimen for metastatic disease were required.

Patients with HER2-positive disease were eligible if they had PD on, or were not candidates for, trastuzumab therapy. Patients with hormone-receptor (HR)-positive disease were eligible after progression on endocrine therapy. There was no limit on the number of prior endocrine/biological treatments; however, prior treatment with VEGFR TKIs was prohibited, and no prior bevacizumab was allowed within 4 weeks of the study start. Patients could not have symptomatic central nervous system metastases, baseline >Grade 1 neuropathy, or significant cardiovascular disease, uncontrolled hypertension, or myocardial infarction within 3 months. Adequate hepatic, renal, and bone marrow function was required. This study was conducted in accordance with the Declaration of Helsinki and approved by the responsible institutional review board or ethics committee of all participating centers. All patients gave written informed consent.

Study design and treatment

This was a Phase Ib, open-label, multicenter, dose-escalation study of tivozanib combined with weekly paclitaxel. Tivozanib was administered orally once daily for 3 weeks followed by 1-week off (1 cycle = 4 weeks). A single dose of tivozanib was provided 5 days before the start of combination dosing for PK sampling. Paclitaxel was administered weekly as a 1-h intravenous infusion of 90 mg/m^2 for 3 weeks followed by 1-week off. To reduce hypersensitivity reactions with paclitaxel, patients were pretreated with corticosteroids, antihistamines, and/or histamine 2-receptor antagonists. A standard 3 + 3 dose-escalation design was used. Tivozanib doses were 0.5 mg/day (Cohort 1 [C1]), 1.0 mg/day (Cohort 2 [C2]), and 1.5 mg/day (Cohort 3 [C3]); doses were selected based on data from the prior Phase I monotherapy study [5]. During Cycle 1, if 1 of the 3 patients within a cohort experienced a dose-limiting toxicity (DLT), the cohort was expanded to a minimum of 6 patients. If 0 of 3 or 1 of 6 patients experienced a DLT during Cycle 1, the dose was escalated to the next dose level. If ≥2 of the 6 patients at a dose level experienced a DLT during Cycle 1, dose escalation was stopped, and the prior dose level was considered the MTD. The MTD was defined as the maximum dose at which no more than 1 patient experienced a DLT (e.g., Grade 3 non-hematologic toxicity lasting ≥3 days despite supportive care, Grade 4 non-hematologic toxicity, Grade 3 aminotransferase elevation lasting ≥1 week, Grade 3/4 febrile neutropenia or Grade 4 neutropenia lasting ≥5 days, or toxicity of any grade that resulted in treatment interruption for ≥2 weeks). Dose interruption or modification of either agent was available based on predetermined toxicity criteria. Concurrent endocrine- and/or HER2-directed therapy was prohibited. Treatment was continued until unacceptable toxicity or PD.

Study assessments

Safety and response assessments

Patients were evaluated for AEs from informed consent to 1 month after the last study dose. The National Cancer

Institute Common Terminology Criteria for Adverse Events version 3.0 was used to grade toxicities. Physical examination was performed weekly for Cycle 1, Cycle 2, days 1 and 15, day 1 for all subsequent cycles, at study end, and at 1-month follow-up. Laboratory assessments were performed on chemotherapy administration days. Electrocardiograms were obtained at screening, Cycle 1 day 22, end of treatment, and 1-month follow-up. Patients completing ≥2 cycles of treatment were considered evaluable for response using RECIST version 1.0. Patients who developed early PD, irrespective of study treatment duration, prior to response evaluation were considered to have progressed on study. Radiologic disease assessment was performed at baseline and after every two cycles. Responses were confirmed by a repeat evaluation at least 4 weeks after the criteria for response were first met. For stable disease (SD), follow-up measurements had to meet the SD criteria at least once after study entry at an interval of 4 weeks.

Pharmacokinetic analysis

Tivozanib levels were assessed at day 5 (before dosing with tivozanib), and at 1, 2, 4, 8, and 24 h post-dose. In addition, samples for tivozanib and paclitaxel concentrations were collected at Cycle 1, days 1, 2, 8, 15, and 22 and Cycle 2, days 1, 15, and once during days 22–28. Tivozanib serum concentrations were determined using a validated HPLC–MS/MS assay [5].

Vascular assessments

Vascular evaluation was planned in patients at one institution to explore the effect of tivozanib on endothelium-derived nitric oxide bioavailability and vascular dysfunction. Evaluation consisted of plasma nitrotyrosine [15] and flow-mediated vasodilation [16] measurements before the initiation of Cycles 1–3. To assess endothelium-dependent vasodilation, brachial artery diameter was measured using high-resolution B-mode ultrasound at baseline and 5 min after an ischemic stimulus. Endothelium-independent vasodilation was assessed at baseline and 3 min after administration of nitroglycerin (0.4 mg). Data for each patient at each time point was averaged, and standard deviation was calculated. Comparisons between time points were performed using a paired *t* test.

Study objectives and statistical design

The study's primary objective was to determine the safety, tolerability, and MTD of tivozanib when administered with paclitaxel. Secondary objectives included evaluation of anti-tumor activity, PK, and vascular reactivity to tivozanib exposure. Descriptive statistics were used for continuous variables, and frequency and percentages were used for discrete variables. Changes in vascular parameters before and after exposure to tivozanib were evaluated using a paired *t* test.

Results

Patients

Eighteen patients with MBC were enrolled February–December 2009: C1, 7; C2, 4; and C3, 7 (Table 1). More than 70 % had received chemotherapy in the metastatic setting (median of two prior lines of therapy). All patients had received prior taxane in the adjuvant/neoadjuvant or metastatic setting, and ≥50 % of patients received prior bevacizumab, with a median time since last bevacizumab dose of 3.9 months (range 1.1–17.6 months). All patients were evaluable for toxicity; 13 were evaluable for efficacy.

Table 1 Patient demographics

Characteristic	$N = 18$
Mean age (range), years	48 (32–65)
Race, *n* (%)	
White	16 (89)
Other	2 (11)
ECOG performance status, *n* (%)	
0	13 (72)
1	5 (28)
2	0
Mean time since diagnosis (range), months	76.2 (18–254)
Receptor status, *n* (%)[a]	
ER/PR-positive	10 (56)
HER2-positive	4 (22)
ER-negative/PR-negative/HER2-negative	7 (39)
Median no. of prior metastatic chemotherapy regimens (range)	2 (0–4)
Prior treatment by setting, *n* (%)	
Adjuvant	14 (78)
Metastatic	13 (72)
Neoadjuvant	6 (33)
Prior treatment, *n* (%)	
Taxanes	18 (100)
Bevacizumab	10 (56)
Trastuzumab	5 (28)
Radiotherapy	14 (78)

ECOG Eastern Cooperative Oncology Group, *ER* estrogen receptor, *HER* human epidermal growth factor receptor, *PR* progesterone receptor

[a] Three patients were ER-positive/PR-positive and HER2-positive

Safety and tolerability

Two patients experienced a DLT during the study. The first, in C1, experienced Grade 1 palpitations leading to withdrawal of consent and expansion of that cohort. The second, in C3, was found to have asymptomatic Grade 2 pneumoperitoneum on an imaging study; this resolved with conservative management but led to patient removal from the study and expansion of that cohort. With no further DLT events in C3, the MTD was identified as tivozanib 1.5 mg/day with paclitaxel 90 mg/m^2 weekly for 3 weeks in a 4-week cycle.

All patients had ≥1 treatment-emergent AE (TEAE; 94.4 % with tivozanib-related AEs and 88.9 % with paclitaxel-related AEs). The most frequently reported TEAEs (in >20 % of patients) for all cycles included fatigue (77.8 %); alopecia (50.0 %); and diarrhea, nausea, and peripheral sensory neuropathy (44.4 % each) (Table 2). Ten patients (55.6 %) had Grade 3/4 AEs; the most common (in >15 % of patients) were fatigue (3 patients, 16.7 %) and neutropenia (3 patients, 16.7 %) (Table 3). Two Grade 4 events (lumbar compression fracture and hip fracture) were reported for one patient in C1; no other Grade 4 events were reported. When all neuropathy categories were combined (peripheral sensory neuropathy and polyneuropathy), the overall frequency of all-grade neuropathy was 66.7 % (12 reports of neuropathy in 12 distinct patients for all cycles) with only one case of Grade 3/4 neuropathy. The frequency of Grade 3/4 TEAEs was higher in C3 (6 patients, 85.7 %) than in C1 (3 patients, 42.9 %) and C2 (1 patient, 25.0 %); however, there were no Grade 4 AEs in this cohort and no Grade 5 AEs in this study. Two patients died; one death involved a patient with PD (death occurred 31 days after the last dose of study drug); the second death was considered tumor-related and occurred 54 days after the last dose of study drug. Both deaths were considered secondary to underlying disease, not to study medication.

Seven patients experienced AE-related tivozanib and/or paclitaxel dose interruptions; five had a TEAE leading to withdrawal (three in C1: lumbar compression fracture, palpitations, and superior vena cava syndrome; two in C3: pneumoperitoneum and shortness of breath). Two patients in C3 developed Grade 3 hypertension, leading to tivozanib dose reduction in one patient. Both patients were controlled with antihypertensive medication.

Efficacy

Patients were exposed to a median of six cycles (range 0–13) of tivozanib combined with paclitaxel, with a median duration of exposure of 5.4 months (range 0–12.0).

Thirteen patients were evaluable for efficacy (C1, 5; C2, 3; C3, 5); five withdrew from the study before completion

Table 2 Most frequently reported treatment-emergent adverse events (occurring in >20 % of patients) for all cycles

Toxicity[a]	Tivozanib dose level in combination with paclitaxel[b]			
	Cohort 1 dose level 1 ($n = 7$)	Cohort 2 dose level 2 ($n = 4$)	Cohort 3 dose level 3 ($n = 7$)	All ($n = 18$)
Fatigue	5	3	6	14 (77.8 %)
Alopecia	3	2	4	9 (50.0 %)
Diarrhea	1	2	5	8 (44.4 %)
Nausea	2	1	5	8 (44.4 %)
Peripheral sensory neuropathy	3	2	3	8 (44.4 %)
Cough	2	2	3	7 (38.9 %)
Hypertension	2	2	3	7 (38.9 %)
Vomiting	3	2	2	7 (38.9 %)
Stomatitis	1	1	4	6 (33.3 %)
Headache	2	2	1	5 (27.8 %)
Neutropenia	2	0	3	5 (27.8 %)
Back pain	2	1	1	4 (22.2 %)
Constipation	1	0	3	4 (22.2 %)
Dyspepsia	2	0	2	4 (22.2 %)
Edema peripheral	1	1	2	4 (22.2 %)
Epistaxis	2	0	2	4 (22.2 %)
Flatulence	0	1	3	4 (22.2 %)
Polyneuropathy	1	1	2	4 (22.2 %)
Pyrexia	3	0	1	4 (22.2 %)

[a] Adverse events are listed in decreasing frequency for all patients

[b] Cohort 1 (dose level 1): tivozanib dose 0.5 mg/day, paclitaxel dose 90 mg/m^2 per week; Cohort 2 (dose level 2): tivozanib dose 1.0 mg/day, paclitaxel dose 90 mg/m^2 per week; Cohort 3 (dose level 3): tivozanib dose 1.5 mg/day, paclitaxel dose 90 mg/m^2 per week

of Cycle 2 because of toxicity, as detailed above. Of 13 efficacy-evaluable patients, none had confirmed complete response (CR), and four (30.8 %) had confirmed partial response (PR) (C1, HR-positive/HER2-positive [1]; C2, HR-negative/HER2-negative [1]; C3, HR-positive/HER2-negative [1] and HR-positive/HER2-positive [1]). One patient had an unconfirmed PR. Six patients (46.2 %) had confirmed SD; four had SD > 6 months (30.8 %). One patient had an unconfirmed SD. One patient (7.7 %) had PD as best overall response (Table 4). Of note, three of the four patients with PR had prior exposure to bevacizumab. For responses confirmed by RECIST, the objective response rate (CR + PR) was 30.8 % (95 % confidence interval [CI] 9.1–61.4 %) and the disease control rate (CR + PR + SD) was 76.9 % (95 % CI 46.2–95.0 %). Figure 1 represents a waterfall plot of maximum tumor change from baseline for the 12 patients for whom maximum change in tumor size was available. In the four patients with confirmed PR, the median duration of response was 9.0 months (range 5.6–11.2). For patients with confirmed SD, the median duration was 8.2 months (range 3.5–11.5).

Pharmacokinetics

Mean tivozanib concentration versus time profiles for all doses evaluated show the expected increase in concentrations with dose, with accumulation of drug approximating steady-state levels over the 21-day treatment period (Fig. 2). Mean predose tivozanib serum concentrations ± standard deviation on day 15 of Cycle 1 were 19.9 ng/mL (±10.7 ng/mL; $n = 5$), 36.2 ng/mL (±27.6 ng/mL; $n = 3$), and 107.3 ng/mL (±59.7 ng/mL; $n = 7$) for the 0.5, 1.5, and 2.0 mg groups, respectively. These concentrations are similar to those determined in prior tivozanib monotherapy trials [5, 6]. Tivozanib PK parameters obtained before the start of combination dosing also were consistent with previously reported values. Mean concentrations of paclitaxel 5 min post-infusion were 2,032 ng/mL (±706 ng/mL; $n = 14$) for day 1, 2,311 ng/mL (±979 ng/mL; $n = 15$) on day 8, and 1,865 ng/mL (±858 ng/mL; $n = 11$) on day 15, consistent with previously reported values [17, 18].

Vascular analysis

Three patients completed the vascular physiological assessment. Data from Cycle 1 day 1 (baseline) and Cycle 3 day 1 visits were compared. Baseline arterial diameter did not change with tivozanib exposure with a mean ± standard deviation of 2.88 ± 0.14 mm at Cycle 1 day 1 and 2.89 ± 0.26 mm at Cycle 3 day 1 ($P = 0.97$). Similarly, there was no change in the reactive hyperemia stimulus. In contrast, at these same time points, flow-mediated vasodilation decreased significantly from a mean percentage of 19.7 ± 4.7 to 9.1 ± 3.9 % ($P = 0.02$). There was no difference in the vasodilatory response to nitroglycerin as a result of tivozanib administration at these same time points, with a mean of 17.4 ± 12.8 versus 16.8 ± 4.9 % ($P = 0.95$).

Table 3 Summary of Grade 3/4 treatment-emergent adverse events for all cycles

Toxicity[a]	Tivozanib dose level in combination with paclitaxel[b]			
	Cohort 1 dose level 1 ($n = 7$)	Cohort 2 dose level 2 ($n = 4$)	Cohort 3 dose level 3 ($n = 7$)	All ($n = 18$)
Fatigue	0	1	2	3 (16.7 %)
Neutropenia	0	0	3	3 (16.7 %)
Back pain	1	0	1	2 (11.1 %)
Diarrhea	0	1	1	2 (11.1 %)
Hypertension	0	0	2	2 (11.1 %)
Alopecia	0	0	1	1 (5.6 %)
Anemia	1	0	0	1 (5.6 %)
Hip fracture	1	0	0	1 (5.6 %)
Leukopenia	0	0	1	1 (5.6 %)
Lumbar vertebral fracture	1	0	0	1 (5.6 %)
Palmar-plantar erythrodysesthesia syndrome	0	0	1	1 (5.6 %)
Palpitations	0	0	1	1 (5.6 %)
Polyneuropathy	0	1	0	1 (5.6 %)
Stomatitis	0	0	1	1 (5.6 %)
Superior vena cava occlusion	1	0	0	1 (5.6 %)

[a] Adverse events are listed in decreasing frequency for all patients

[b] Cohort 1 (dose level 1): tivozanib dose 0.5 mg/day, paclitaxel dose 90 mg/m^2 per week; Cohort 2 (dose level 2): tivozanib dose 1.0 mg/day, paclitaxel dose 90 mg/m^2 per week; Cohort 3 (dose level 3): tivozanib dose 1.5 mg/day, paclitaxel dose 90 mg/m^2 per week

Table 4 Summary of best overall response

	Tivozanib dose level in combination with paclitaxel[a]			
	Cohort 1 dose level 1 ($n = 5$)	Cohort 2 dose level 2 ($n = 3$)	Cohort 3 dose level 3 ($n = 5$)	All ($n = 13$)
Best overall response				
CR	0	0	0	0
PR (confirmed)	1 (20.0 %)	1 (33.3 %)	2 (40.0 %)	4 (30.8 %)
PR (confirmed + unconfirmed)	2 (40.0 %)	1 (33.3 %)	2 (40.0 %)	5 (38.5 %)
SD (confirmed)	2 (40.0 %)	2 (66.7 %)	2 (40.0 %)	6 (46.2 %)
SD (confirmed + unconfirmed)	2 (40.0 %)	2 (66.7 %)	3 (60.0 %)	7 (53.8 %)
SD (confirmed) >6 months	0	2 (66.7 %)	2 (40.0 %)	4 (30.8 %)
PD	1 (20.0 %)	0	0	1 (7.7 %)
ORR (CR + PR)	20.0 %	33.3 %	40.0 %	30.8 %
95 % CI for ORR	0.5–71.6 %	0.8–90.6 %	5.3–85.3 %	9.1–61.4 %
DCR (CR + PR + SD)	60.0 %	100 %	80.0 %	76.9 %
95 % CI for DCR	14.7–94.7 %	29.2–100 %	28.4–99.5 %	46.2–95.0 %

CI confidence interval, *CR* complete response, *DCR* disease control rate, *ORR* overall response rate, *PD* progressive disease, *PR* partial response, *SD* stable disease

[a] Cohort 1 (dose level 1): tivozanib dose 0.5 mg/day, paclitaxel dose 90 mg/m^2 per week; Cohort 2 (dose level 2): tivozanib dose 1.0 mg/day, paclitaxel dose 90 mg/m^2 per week; Cohort 3 (dose level 3): tivozanib dose 1.5 mg/day, paclitaxel dose 90 mg/m^2 per week

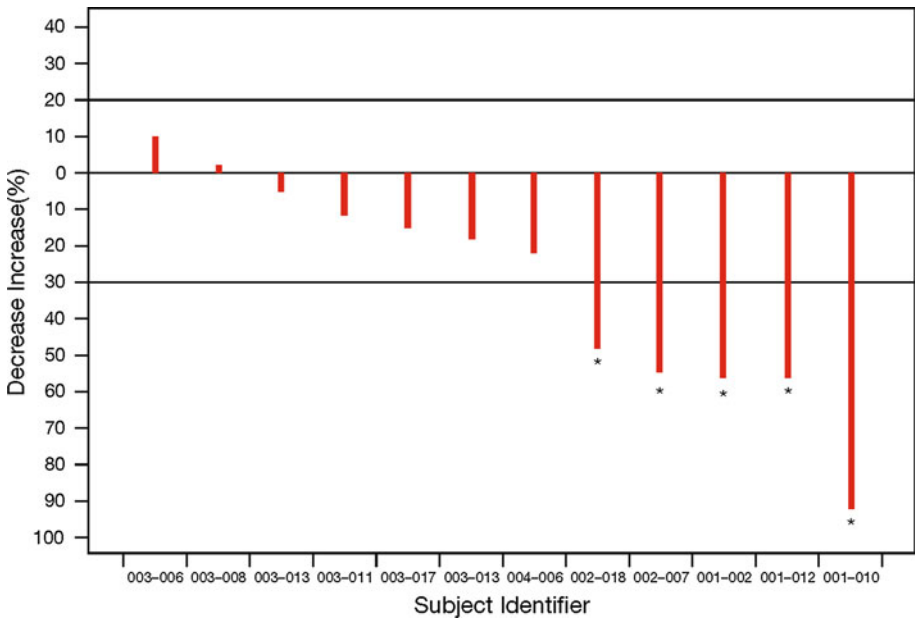

Fig. 1 Waterfall plot of maximum tumor change from baseline. Maximum change in tumor size from baseline was available for 12 patients. Thirteen patients were evaluable for efficacy, but one patient did not have a post-baseline assessment for change in tumor size and was not included in the waterfall plot. *Patients 001–002, 001–010, 001–012, and 002–018 had a confirmed partial response (PR). Patient 002–007 had an unconfirmed PR

Discussion

In this Phase Ib trial, tivozanib combined with weekly paclitaxel was acceptable at all dose levels, with MTD identified at the full dose and schedule of the two agents (tivozanib 1.5 mg/day and paclitaxel 90 mg/m^2 weekly). Therapy was well tolerated; the most common AEs were fatigue, diarrhea, nausea, and neuropathy. PK data suggested no influence of paclitaxel on circulating tivozanib levels or of tivozanib on paclitaxel concentration.

Inhibitors of VEGF/VEGFRs have been studied extensively in MBC. Adding bevacizumab to chemotherapy in patients with metastatic HER2-negative breast cancer increased response rate and prolonged PFS, although improvements in overall survival were not observed in unselected Phase III trial populations [8, 10–13]. Also, adding sorafenib to capecitabine chemotherapy improved PFS in a Phase II trial, but dermatologic toxicity was significant [14]. Tivozanib offers greater affinity for the three VEGFRs compared with poly-targeted inhibitors (e.g., sorafenib) [4, 19]. This favorable target specificity likely may contribute to less off-target toxicity. In this study, tivozanib plus weekly paclitaxel were tolerable at all doses, with evidence of clinical activity.

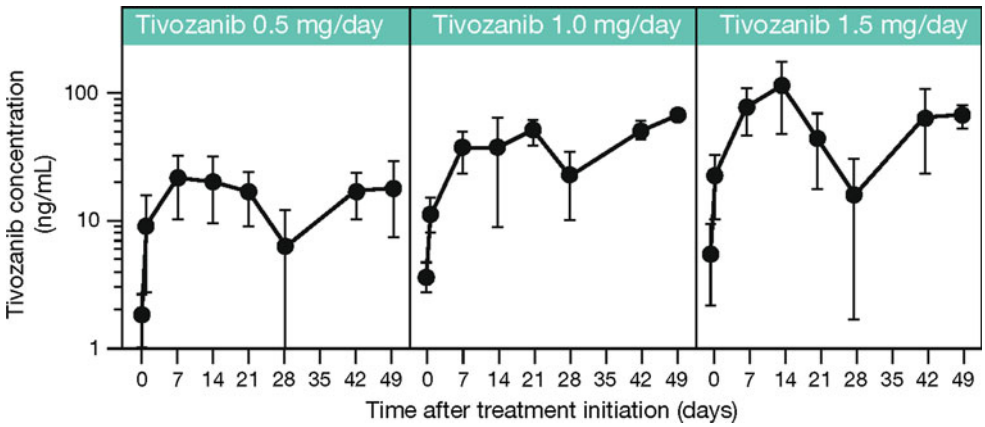

Fig. 2 Mean (±standard deviation) tivozanib concentration–time profiles during the first two cycles

Development of angiogenesis inhibitors in breast cancer has focused on identifying a subset with preferential sensitivity to VEGF/VEGFR inhibition. One strategy includes examining clinico-pathologic features, as it was suggested that the triple-negative subset (TNBC: estrogen receptor-negative/progesterone receptor-negative/HER-negative) may preferentially benefit from angiogenesis inhibition. Analysis of basal-like breast cancer tissue (frequently triple-negative) often identifies microvascular glomeruloid tufts and/or immunohistochemistry-identified vascular proliferation, which are associated with poor prognosis, and may suggest sensitivity to targeted vascular disruption [20, 21]. Examination of TNBC subsets from large clinical trials of bevacizumab has suggested possible preferential activity in these patients. The RIBBON-2 study, which added bevacizumab to chemotherapy in the second-line setting for MBC, showed improved PFS from 2.7 to 6.0 months (hazard ratio 0.494, $P < 0.001$) in the prespecified TNBC subset, compared with the hazard ratio of 0.78 for the entire study population of HER2-negative MBC [22]. In the GeparQuinto neoadjuvant study of chemotherapy with/without bevacizumab, a prespecified analysis within the TNBC subgroup showed a significant improvement in pathologic CR from 27.9 to 39.3 % ($P = 0.003$) with the addition of bevacizumab; results in the HR-positive subset did not show a significant differential [23]. However, pooled analyses of the Phase III first-line MBC studies has not demonstrated preferential benefit from adding bevacizumab for patients with TNBC [10]. A neoadjuvant study by the National Surgical Adjuvant Breast and Bowel Project also showed no significant improvement in the rate of pathological CR with the addition of bevacizumab to chemotherapy in the HR-negative subgroup [24]. The adjuvant BEATRICE study recently demonstrated no PFS improvement with the addition of bevacizumab to standard chemotherapy for TNBC [25]. In this study of tivozanib and paclitaxel, no tumor type response pattern was observed; however, the small sample size limits interpretation.

Multiple other efforts have attempted to identify markers predictive of response to antiangiogenic therapy. Biomarker analyses as part of larger trials have suggested higher baseline levels of VEGF-A and/or VEGFR-2 may predict benefit from bevacizumab [26–28]. The ongoing MERiDiAN study is prospectively evaluating the performance of baseline VEGF-A as a marker predictive of benefit from bevacizumab. There has been interest in whether small nucleotide polymorphisms in VEGF are associated with response to bevacizumab therapy in breast cancer. However, results of pharmacogenomic analyses have been conflicting [27, 29]. Additionally, baseline levels of circulating endothelial cells and progenitors, and dynamic change during treatment, may serve as predictive biomarkers for benefit from angiogenesis inhibition [30]. The development of toxicity may be predictive, as some patients taking bevacizumab or VEGFR inhibitors who develop significant hypertension, an on-target toxicity of VEGF inhibition, appear to derive greater clinical benefit [31–33]. Novel imaging techniques, including dynamic contrast-enhanced magnetic resonance imaging, are of interest in evaluating response to antiangiogenic therapy [34]. Microenvironmental conditions, such as hypoxia response, have been shown to differ among HER2-related breast tumor subgroups and may provide predictive value regarding sensitivity to antiangiogenic therapy [35]. Other biomarkers are under evaluation with further analysis of existing Phase III studies [36].

The vascular function results in our study suggest that tivozanib reduced the bioavailability of endothelium-derived nitric oxide leading to endothelial dysfunction. These results should be interpreted with caution, as the cohort size ($n = 3$) is smaller than commonly required for a vascular physiological study. Nevertheless, the results are consistent with the expected effect of potent VEGFR-2 inhibition, a crucial component of the nitric oxide synthase response to increases in shear stress [37]. These results differ from work with another VEGFR inhibitor, vandetanib [38], suggesting variability in VEGFR inhibitor effects on vascular function and possibly clinical outcomes. More

investigation into the vascular effects of these medications is needed.

The results from this trial support further evaluation of tivozanib with paclitaxel at their full recommended doses in MBC. Given the history of angiogenesis inhibitors in breast cancer, additional development of this therapeutic class requires careful clinical trial design. Future study of tivozanib and related agents should involve a priori identification of subgroups of interest, as well as integration of prospective scientific biomarker analyses, to best determine the role of a VEGFR inhibitor/chemotherapy combination in breast cancer treatment.

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Conflict of interest E. L. Mayer has served as a consultant for Amgen. M. N. Dickler participated in a compensated advisory board for AVEO Oncology. S. Agarwal and L. Steelman are full-time employees of AVEO Oncology. M. M. Cotreau and A. L. Strahs are full-time employees and stockholders of AVEO Oncology. All remaining authors have declared no conflicts of interest.

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