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PHASE I STUDIES

Phase I safety, pharmacokinetic and pharmacodynamic studies of 2-methoxyestradiol alone or in combination with docetaxel in patients with locally recurrent or metastatic breast cancer

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Summary *Purpose:* We report the first phase I trials of 2-methoxyestradiol (2ME2, Panzem® Capsules, EntreMed, Rockville, MD), alone and in combination with docetaxel, in patients with metastatic breast cancer (MBC).

Patients and methods: In Trial 001, 2ME2 monotherapy was administered orally once (200–1000 mg/d, cohorts 1–5) or twice daily (200–800 mg/q12h, cohorts 6–9) for 28 days followed by a 14-day observation period, continuously thereafter. In Trial 002, docetaxel 35 mg/m^2 was administered weekly for four of six weeks for a maximum six cycles; 2ME2 (200–1000 mg/d) was given orally once daily for 28 days followed by a 13-day observation period in cycle one, continuously thereafter. In both trials, responding or stable patients continued 2ME2 until progression.

Results: Trial 001 enrolled 31 patients; there were no objective responses. Trial 002 enrolled 15 patients; ORR was 20% including one CR. There were no Grade IV toxicities; MTD was not reached in either study. When combined with docetaxel, three patients had significant transaminase elevations that returned to normal with continued treatment (in two of three patients). There was significant inter-patient variability and extensive metabolism to 2-methoxyestrone (2ME1). Steady-state AUC and trough concentrations of 2ME2 increased linearly up to 400–600 mg/d; doses above 400–600 mg/d did not increase 2ME2 levels. The target trough concentration (3–25 ng/mL) was not attained. Combined administration did not alter docetaxel or 2ME2 pharmacokinetics.

Conclusion: 2ME2, alone or in combination with docetaxel, was well tolerated in patients with MBC but systemic exposure remained below the expected therapeutic range.

Keywords Angiogenesis · Breast cancer · Clinical trial

Introduction

Breast cancers must stimulate angiogenesis, the growth of new blood vessels, in order to grow beyond a few millimeters in diameter [1, 2]. Extensive laboratory data suggests that angiogenesis plays an essential role in breast cancer development, invasion and metastasis [3–6]. Clinicopathologic correlations also confirm the central role of angiogenesis. Breast cancer vascularity, quantified by tumor microvessel density, predicts tumor shedding at the time of surgery [7], bone marrow micrometastases [8] recurrence and overall survival [9–11]. This nascent vascular network provides a unique opportunity for therapy.

2ME2 is naturally formed *in vivo* by the sequential hydroxylation and O-methylation of estradiol at the 2-position. Despite being a natural derivative of estradiol, 2ME2 binds very poorly to the estrogen receptor (0.05% of estradiol binding) [12]. Accordingly, 2ME2 does not exhibit direct estrogenic activity [13–16]. 2ME2 does not sustain the growth of estrogen-dependent breast cancer cell lines and has

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significant cytotoxic activity independent of estrogen responsiveness at sub- and low micromolar concentrations [17]. 2ME2 is a potent inhibitor of tumor growth in many human tumor xenograft and metastases models [17–19] and exhibits antiangiogenic activity *in vitro* and *in vivo* [18–20]. Treatment with 2ME2 *in vitro* inhibits proliferation of endothelial cells stimulated by either vascular endothelial growth factor (VEGF, 60 ng/mL) or basic fibroblast growth factor (bFGF, 20 ng/mL) and enhances the antiproliferative effect of docetaxel [21].

Altogether, these findings suggest that 2ME2 may provide an alternative or addition to existing therapies for the treatment of breast cancer. We therefore conducted separate phase I trials of 2ME2, alone (Trial 001) and in combination with docetaxel (Trial 002), in patients with metastatic breast cancer.

Patients and methods

Patient eligibility

Trial 001

Women with histologically or cytologically confirmed metastatic breast cancer were eligible if they had received at least two prior regimens (one of which may have been hormonal) for metastatic disease. In addition patients were required to have a Karnofsky performance status ≥ 80 and adequate renal (creatinine <1.5 mg/dL), hepatic (total bilirubin <1.5 mg/dL; AST and ALT <2.5 times upper limit of normal) and hematologic (WBC $>3000/mm^3$; platelets $>100{,}000/mm^3$; INR ≤ 1.2 with normal PTT) function. Patients were excluded if they had any history or radiographic evidence of central nervous system disease; screening brain magnetic resonance image (MR) was required prior to entry. In addition patients may not have had any other primary malignancy within 5 years, major surgical procedure or chemotherapy within 21 days, active infection or clinically significant cardiovascular disease including myocardial infarction or unstable angina within 3 months. Women of reproductive potential were required to use an effective barrier means of contraception throughout the study.

Trial 002

Eligibility criteria were identical to Trial 001 except patients were required to have an Eastern Cooperative Oncology Group (ECOG) Performance Status 0 or 1 and could have received no more than one prior non-taxane containing chemotherapy regimen for advanced disease. Adjuvant taxane use was allowed if the disease-free interval was >12 months.

The Indiana University Institutional Review Board approved both protocols; all patients provided individual written informed consent prior to screening and study entry.

Treatment plan

Trial 001

2ME2 monotherapy was administered orally once (200–1000 mg/d, cohorts 1–5) or twice daily (200–800 mg/q12h, cohorts 6–9) for 28 days followed by a 14-day observation period in cycle one. After cycle one, 2ME2 was given continuously until progression; 28 days defined each subsequent cycle.

Trial 002

Docetaxel 35 mg/m^2 was administered weekly for four of six weeks; dexamethasone 4 mg orally every 12 h for 3 doses was initiated 12 h prior to each docetaxel infusion to prevent fluid accumulation. 2ME2 (200–1000 mg/d) was given orally once daily on Days 2–29 followed by a 13-day observation period in cycle one; continuously thereafter (Fig. 1). After a maximum 6 cycles of combined therapy, responding or stable patients continued 2ME2 alone until progression; 28 days defined each subsequent cycle. Docetaxel dose was reduced for hematologic and non-hematologic toxicity.

Safety and efficacy assessments

In both studies patients were evaluated weekly for the first 4 weeks, then on Days 1 and 15 of all subsequent treatment cycles. Complete blood count, serum chemistries and hormone levels (estrogen, progesterone, testosterone, luteinizing hormone and follicle stimulating hormone) were assessed at each evaluation. Complete blood count was measured prior to each docetaxel infusion in patients enrolled in Trial 002. Docetaxel treatment was interrupted for Grade ≥ 3 non-hematological or Grade 4 hematological toxicity according to the National Cancer Institute Common Toxicity Criteria, version 2.0. In both studies 2ME2 was discontinued for Grade 3 or greater non-hematological, and Grade 4 hematological toxicity that did not resolve within 14 days; 2ME2 dose was not otherwise adjusted. Disease status was assessed after cycle 1, cycle 2 and every other cycle thereafter.

Pharmacokinetics

Trial 001

Serum for determination of 2ME2 pharmacokinetics was obtained prior to dosing, 30, 60, and 90 min, 2, 4, 8, 12 h after dosing on Days 1 and 28 of cycle 1. Additional samples were

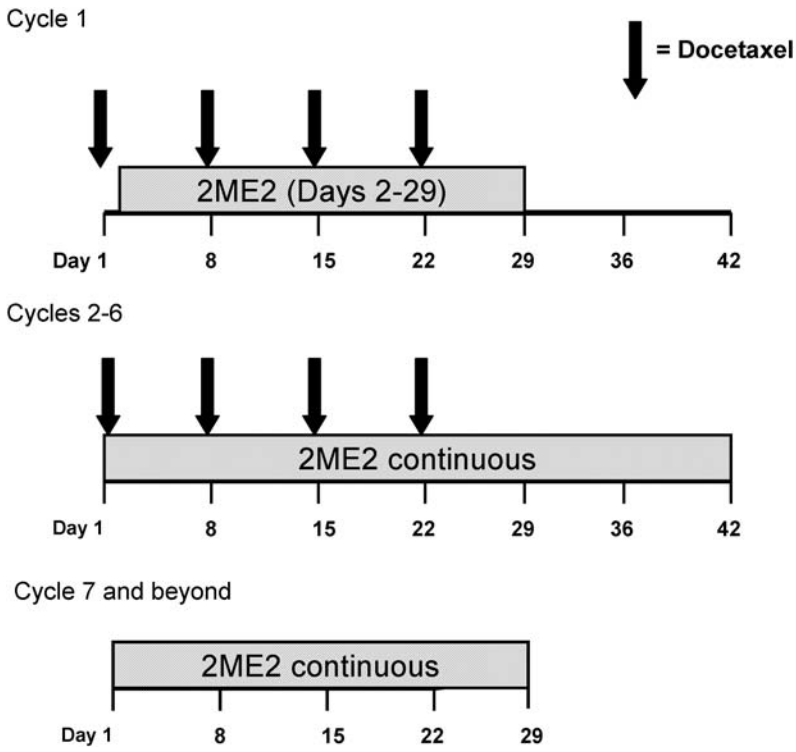

Fig. 1 Docetaxel 35 mg/m^2 was administered weekly for four of six weeks. 2ME2 (200–1000 mg/d) was given orally once daily on Days 2–29 followed by a 13-day observation period in cycle one. 2ME2 was administered continuously in all subsequent cycles. After a maximum 6 cycles of combined therapy, responding or stable patients continued 2ME2 alone until progression; 28 days defined each subsequent cycle

obtained 16 and 24 h after dosing in patients in cohorts 1–5. Serum for analysis of 2ME2 and 2ME1 trough concentrations was obtained prior to dosing on Days 8, 15 and 22 of cycle 1 and on Day 1 of all subsequent cycles.

Trial 002

Serum for determination of docetaxel concentration was obtained prior to dosing, at the end of infusion, 30 and 60 min, 2, 4, 6, 8, 12, and 24 h after infusion on Day 1 of cycle 1. Additional samples were obtained immediately after infusion on Days 8, 15 and 22 of cycle 1. 2ME2 was initiated on Day 2 of cycle 1; serum for determination of 2ME2 pharmacokinetics was obtained prior to dosing, 10, 20, 30, 60, and 90 min, 2, 4, 8, and 24 h after dosing. Additional samples were obtained 1 h after dosing on Days 8, 15, 22, and 29 of cycle 1. Detailed pharmacokinetic sampling for both docetaxel and 2ME2 was repeated on Day 22 of cycle 2.

2ME2 pharmacokinetic analyses included area under the curve (AUC), elimination half-life ($t_{1/2}$), t_{max}, C_{max}, C_{min}, estimated steady-state concentration (C_{ss}), total clearance (C_l), and volume of distribution (V_d). Serum concentrations of 2ME2 and 2ME1 were determined using a validated gas chromatographic/mass spectrometric method using trideuterated 2ME2 and 2ME1 as internal standards. Docetaxel pharmacokinetic analyses included total clearance, volume of distribution, and dose-normalized AUC. Serum concentration of docetaxel was determined using a validated high-performance liquid chromatography—mass spectroscopy method using atmospheric pressure chemical ionization. The lower limit of quantitation was 0.5 ng/mL. The assay was linear over the range of 0.5–1000 ng/mL. Intra-day and interday coefficient of variation was less than 10%.

Pharmacodynamics

Blood and urine samples were collected to monitor changes in the concentrations of the angiogenic proteins VEGF (plasma and urine) and bFGF (serum and urine) at baseline, Day 28 of cycle 1, and Day 1 of all subsequent cycles. Serum only was collected for assessment of VCAM-1, MMP-2 and MMP-9. Samples were obtained in a serum separator or standard red top tube and allowed to stand on ice for 30 min, then separated by centrifugation at 3000g for 30 min. Plasma, serum and urine were stored at -20°C in 1 ml aliquots for later analysis; duplicate aliquots were preserved for each patient at each time point. All samples were measured in duplicate using commercially available enzyme linked immunosorbant assays (ELISA, R & D Systems, Minneapolis, MN and Oncogene Research Products, Cambridge, MA). All samples with a coefficient of variation >10% were repeated. The assays have the following limits of detection: VEGF 9 pg/mL, bFGF <1 ng/mL, VCAM-1 <2 ng/mL, MMP-2 <0.37 ng/mL, and MMP-9 <0.156 ng/mL.

Prostate-specific antigen (PSA) is produced by ~30% of breast cancer and is associated with improved disease-free and overall survival in patients with localized breast

cancer [22, 23]. PSA inhibits bFGF- and VEGF-stimulated endothelial proliferation [24] and is cleaved to an angiostatin-like compound by plasminogen suggesting a potential role in regulation of breast cancer angiogenesis [25]. Serum PSA was measured at baseline, Days 1 and 15 of cycle 1 and Day 1 of all subsequent cycles. PSA assays were performed in the CLIA certified laboratories at Indiana University using standard techniques.

Statistical analysis

The primary objective of these phase I trials was to evaluate the safety and tolerability of 2ME2, alone and in combination with docetaxel, in patients with metastatic breast cancer. Dose limiting toxicity (DLT) was defined as any Grade IV hematologic or Grade III non-hematologic toxicity within the first cycle of therapy. Three patients were enrolled into each successive cohort (Trial 001 cohorts 1–5: 200–1000 mg/d, cohorts 6–9: 200–800 mg twice daily; Trial 002: 200–1000 mg/d). If none of the 3 patients in a cohort experienced DLT, accrual to the next cohort commenced. If 2 or more patients experienced DLT, the previous dose level was considered maximum tolerated dose (MTD). If DLT was observed in 1 of 3 patients, 3 additional patients were to be added to that dose level. If no additional DLTs were reported, accrual to the next cohort commenced. If ≥ 2 of the 6 patients experienced DLT, the previous dose level was considered maximum tolerated dose (MTD).

Secondary endpoints included characterization of 2ME2 pharmacokinetics, impact of 2ME2 on hormone levels, assessment of potential surrogates of response and angiogenesis, and tumor response. A complete response (CR) was defined as the complete disappearance of all clinically detectable disease measured by physical examination and/or radiographic studies for a period of at least 4 weeks. A partial response (PR) was defined as a greater than or equal to a 50% decrease in the sum of the products of the two longest perpendicular diameters of all measurable lesions for a period of at least 4 weeks without an increase greater than 25% in the size of any area known to contain malignant disease and without the appearance of any new areas of malignancy. Progressive disease (PD) was defined as an increase of at least 25% in the size of measurable lesions. Time to treatment failure (TTF) was defined as time from study entry to discontinuation of study therapy for any reason.

Results

Patient populations

Thirty-one patients were enrolled in Trial 001; 15 patients were enrolled in Trial 002. Patient characteristics are shown in Table 1. All patients were evaluable for toxicity. One patient in Trial 001 discontinued treatment without progression prior to the first disease assessment and is not evaluable for response.

Table 1 Patient characteristics

	Trial 001 ($n = 31$)	Trial 002 ($n = 15$)
Median age	48 (30–72)	49 (36–74)
Performance status	Karnofsky 100–11 (35%) 90–11 (35%) 80–9 (30%)	ECOG 0–8 (53%) 1–7 (47%)
ER+	17 (55%)	11 (73%)
HER2+	6 (19%)	1 (7%)
Prior chemotherapy[a]	Median = 3 (1–10)	0/1 = 11/4
Prior HDCT/SCT	13 (42%)	1 (7%)
Visceral dominant disease	22 (71%)	10 (67%)

[a]Includes only cytotoxic regimens for recurrent or metastatic disease. All patients with ER+ disease had received prior hormonal therapy.

Toxicity and treatment delivered

No changes in estradiol, progesterone, testosterone, luteinizing hormone, or follicle stimulating hormone were identified in either study.

Trial 001

2ME2 monotherapy was well tolerated with few patients experiencing Grade 3 or 4 toxicities (Table 2). There was no neutropenia or thrombocytopenia; anemia was limited to patients with extensive prior chemotherapy and/or marrow infiltration. Fatigue was the most commonly reported toxicity; no patient developed increased transaminases or symptoms suggesting significant estrogenic exposure. Non-hematologic toxicity was mild and not clearly associated with dose or duration of 2ME2 therapy. In most cases, toxicity was attributed to the underlying malignancy. Six patients discontinued therapy before completing cycle 1; one patient withdrew consent and five patients experienced rapid disease progression with symptomatic decline. Nine patients received more than 2 cycles of 2ME2 monotherapy (Table 3).

Trial 002

Concurrent docetaxel and 2ME2 was generally well tolerated (Table 2). Transaminase elevations were detected in three patients. Transaminases returned to baseline within 14 days despite continuing 2ME2 and remained normal after resuming a reduced dose (25%) of docetaxel in two patients. Both 2ME2 and docetaxel were held for 14 days in the third

Table 2 Toxicity

	Trial 001 ($n=31$)			Trial 002 ($n=15$)		
	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)
Anemia	3 (10)	1 (3)	1 (3)	1 (7)	0	0
Neutropenia	0	0	0	0	1 (7)	0
Hyperlacrimation	1 (3)	0	0	2 (13)	0	0
Nausea	7 (23)	2 (6)	0	6 (40)	1 (7)	0
Anorexia	5(16)	3(10)	0	3 (20)	1 (7)	0
Diarrhea	2 (6)	2 (6)	0	4 (27)	4 (27)	0
Constipation	4 (13)	2 (6)	0	2 (13)	0	0
Fatigue	7 (23)	7 (23)	3 (10)	3 (20)	4 (27)	1 (7)
Edema	0	0	0	2 (13)	0	0
Increased transaminase	0	0	0	0	2 (13)	1(7)
Arthralgia/myalgia	3 (10)	1 (3)	0	7 (47)	1 (7)	1 (7)
Peripheral neuropathy	0	0	0	2 (13)	1 (7)	0
Headache	1 (3)	1 (3)	0	3 (20)	1 (7)	0
Oncholysis	0	0	0	3 (20)	0	0
Dermatitis	0	0	0	1 (7)	0	0

NCI CTC v2.0 worst grade experienced per patient.

Table 3 Treatment delivered

	Trial 001 ($n=31$)	Trial 002 ($n=15$)[a]	
Cycles completed	2ME2	Docetaxel	2ME2
<1	6	0	0
≤2	16	6	6
3/4	2/2	0/2	0/1
5/6	1/1	1/6	2/0
7/8/9	1/0/2	NA	1/1/1
10		NA	1
20		NA	1
32		NA	1

[a]Patients who discontinued docetaxel due to toxicity or those who completed a maximum 6 cycles of combined therapy continued 2ME2 monotherapy until progression.

Table 4 Mean pharmacokinetic parameters for 2ME2 (Trial 001)

	Day 1		Day 28	
	C_{max} (ng/mL)	AUC_{0-24} (ng*h/mL)	C_{max} (ng/mL)	AUC_{0-24} (ng*h/mL)
200 mg/d	3.9	46.2	3.0	51.9
400 mg/d	9.2	70.9	12.4	175.8
600 mg/d	9.4	77.9	13.2	201.4
800 mg/d	5.7	49.5	10.9	142.3
1000 mg/d	5.6	49.7	11.4	111.6
200 mg q12h	1.4	12.3	4.7	42.4
400 mg q12h	2.8	21.1	12.8	121.5
600 mg q12h	5.9	27.3	ND	ND
800 mg q12h	ND	ND	ND	ND

ND, Not done.

patient who had had an allergic reaction to an antibiotic given for an intercurrent illness; grade 3 transaminase elevation recurred despite docetaxel dose reduction prompting discontinuation of protocol therapy. Other toxicities were similar in frequency and severity to those expected from docetaxel monotherapy.

Patients completed a median 5 cycles of combined therapy; 2 patients continued 2ME2 monotherapy for more than one year without progression (Table 3). Eight patients required docetaxel dose reduction; doses were reduced twice in one patient. Reasons for dose reduction included diarrhea (2), hand-foot syndrome (2), increased transaminase (2), peripheral neuropathy (1), neutropenia (1) and pleural effusion (1).

Pharmacokinetics

Pharmacokinetic parameters from Trial 001 are shown in Table 4. There was large interpatient variability with both the daily and every 12 h schedules (data not shown). Terminal half-life was approximately 10 h with daily dosing. No accumulation was detected with daily dosing; slight drug accumulation was detected with every 12 h dosing. 2ME2 concentrations increased with increasing doses through 600 mg/day and 400 mg q12h; no further increase in exposure was obtained with higher doses. 2ME2 is significantly oxidized at the 17 position to 2-methoxyestrone (2ME1). 2ME1 concentrations were approximately 10-fold higher than 2ME2; both 2ME2 and 2ME1 were extensively conjugated to gluronides and sulfates (data not shown).

Docetaxel pharmacokinetic studies were completed in eight patients. 2ME2 did not induce any major alterations in docetaxel clearance, volume of distribution or dose-normalized AUC (Table 5). As in trial 001, there was significant interpatient variability in 2ME2 clearance with extensive metabolism to 2ME1. 2ME2 peak and trough levels were not altered by concurrent docetaxel administration (data not shown).

Table 5 Docetaxel pharmacokinetics with and without concurrent 2ME2

	Docetaxel	Docetaxel + 2ME2	*p*
Clearance (L/h/m^2)	7.42	8.14	0.59
VOD (L)	3.6	2.6	0.96
Dose-normalized AUC_{0-24} (ng*h/mL)	79.2	76.0	0.20

Pharmacodynamics

Pre-treatment and at least one follow-up sample were available for 22 of 31 patients in Trial 001 and 13 of 15 patients in Trial 002. Plasma VEGF became undetectable in all patients who continued treatment for more than three months in both studies. Baseline serum VCAM-1 was lower in patients with an objective response to combined docetaxel plus 2ME2 than in non-responding patients (372 ± 123 ng/mL vs. 504 ± 221 ng/mL; $p < 0.0001$); serum VCAM-1 did not correlate with time to progression in patients receiving 2ME2 monotherapy. Urine VEGF, serum MMP-2 and MMP-9 concentrations did not predict time to progression or change consistently with treatment or disease status in either study. Serum and urine bFGF were rarely elevated at baseline; PSA was undetectable in all patients at all timepoints.

Efficacy

There were no objective responses in Trial 001. One patient who had had four previous chemotherapy regimens experienced a minor response in liver metastases and subsequently progressed after 9 months. Seven patients remained stable for $\geq$4 months; median time to treatment failure (TTF) was 55 days (range 13–258). Overall response rate in Trial 002 was 20% including one complete response with recalcification of lytic bone lesions; 40% of patients had stable disease. Two patients remained on 2ME2 monotherapy for more than one year without progression. Median TTF was 203 days (range 19–966). There was no obvious correlation between 2ME2 dose and TTF in either study.

Discussion

We report the first phase I study of the naturally occurring antiangiogenic agent 2ME2, alone or in combination with docetaxel. Overall treatment was well tolerated with most toxicities attributed to the underlying malignancy or concurrent docetaxel. Though transient hepatic toxicity has been reported in a minority of men with advanced prostate cancer treated with 2ME2 monotherapy [26], only three patients in our study developed hepatic toxicity—all in combination with docetaxel. The mechanism by which 2ME2 causes or potentiates hepatic toxicity remains unclear. We did not observe any correlation between 2ME2 dose or pharmacokinetic parameters and hepatic toxicity in our patients. Nonetheless, it remains possible that altered 2ME2 metabolism could result in higher systemic exposure to the free drug and its metabolites. Alternatively, patients with a low activity variant of COMT, an enzyme that can revert the metabolism of 2ME2 into 2-hydroxyestradiol, may have higher levels of this metabolite which is known to cause hepatic toxicity in some patients.

As expected based on the preclinical experience, we found no changes in hormone levels in any of our patients but it is important to note that all of our patients were postmenopausal at study entry. Future studies are needed to assess the impact of 2ME2 on hormonal levels in women with intact, functioning ovaries. Similarly, we did not measure sex hormone binding globulin (SHBG), a plasma glycoprotein that binds to certain steroids which was recently shown to increase in hormone refractory prostate cancer patients receiving 2ME2 monotherapy [26].

2ME2 does not directly influence the transcription of any of the angiogenic factors (VEGF, bFGF, VCAM, MMP-2 and MMP-9) we measured [27], thus association with response would be indirect at best. A recent study found significant variability in plasma and urine VEGF levels in the short term in the absence of treatment [28]. Given this variability along with the multiple analyses and modest clinical activity of 2ME2 in our study, the associations we identified can only be viewed as hypotheses to be tested in future studies.

The plasma levels of 2ME2 achieved in our studies were well below that expected for activity based on preclinical models. Nonetheless we saw encouraging signs of activity in some patients. Though there were no objective responses in Trial 001, one patient with liver metastases who had progressed on four prior chemotherapy regimens had a minor response lasting 9 months. While the overall response rate in Trial 002 is similar to that reported in other trials using weekly docetaxel [29–35], the prolonged disease stability experienced by some patients is notable. Two patients continued 2ME2 monotherapy for over a year without progression. One patient with bone metastases had a complete response including recalcification of lytic lesions; she completed 6 cycles of combined therapy and then remained on 2ME2 alone for nearly two and half years before progressing.

In conclusion, 2-methoxyestradiol is well tolerated with evidence of anti-cancer activity in patients with refractory metastatic breast cancer. The maximum tolerated dose was not identified in this study but further dose escalation was abandoned given the pharmacokinetic limitations we encountered. Clearly, improvements in the formulation of 2ME2 to provide greater drug exposure are required. Specifically the oral bioavailability was apparently

dissolution-rate limited and resulted in the non-linearity of the pharmacokinetics. Moreover, the extensive conversion to 2ME1 and conjugation resulted in low plasma levels. Reformulation of the drug has improved the bioavailability with increased activity in relevant pharmacokinetic and efficacy models. Phase I evaluation of these new formulations have begun in separate trials at Indiana University and the University of Wisconsin Comprehensive Cancer Center.

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