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Activity of 2 methoxyestradiol (Panzem[®] NCD) in advanced, platinum-resistant ovarian cancer and primary peritoneal carcinomatosis: A Hoosier Oncology Group trial

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ABSTRACT

Background. 2-Methoxyestradiol (Panzem[®], 2ME2) is an endogenous metabolite of estradiol that destabilizes microtubules and exerts anti-angiogenic properties. This study was conducted to determine the activity and safety of 2ME2 administered as a NanoCrystal[®] dispersion (NCD) formulation in patients with recurrent, platinum-resistant epithelial ovarian cancer (EOC).

Methods. Eligible patients had relapsed, platinum-resistant or refractory EOC with measurable or detectable disease. There was no limit on the number of prior treatment regimens. 2ME2 NCD 1000 mg orally four times daily (q.i.d.) was administered continuously during 4 week cycles. The primary endpoint was objective response rate (ORR). Secondary endpoints were assessment of toxicity, rate of clinical benefit defined as the number of patients experiencing an objective response, a CA125 response or stable disease (SD) >3 months, mean change in CA-125, progression-free survival (PFS), and pharmacokinetic analyses of 2ME2.

Results. Eighteen patients were enrolled. Median age was 65.5 (range 40–73). Patients had received a median of five prior treatments. The most common adverse events were fatigue (78%), nausea (78%), diarrhea (39%), neuropathy (50%), edema (39%), and dyspnea (44%), the majority being grade 1–2. There were no objective responses, but seven patients had SD as best response. Of those, two patients had SD for greater than 12 months. The rate of clinical benefit was 31.3%. Fairly stable plasma levels of 2ME2 ranging within the predicted therapeutic window were observed.

Conclusions. The NCD formulation of 2ME2 is well tolerated in patients with heavily pretreated EOC. Few of these heavily pretreated patients had sustained stable disease.

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Introduction

Epithelial ovarian cancer (EOC) is the leading cause of mortality among gynecological malignancies [1]. Treatment relies on surgical debulking and platinum-based chemotherapy. Unfortunately, most patients relapse and become resistant to platinum and subsequent chemotherapy [2,3]. There is a need for more effective therapies based

on understanding of the biological mechanisms driving ovarian tumor growth [4].

Efficient tumor growth and metastasis requires the formation of a vascular network that enables cancer cells to nest, expand, and invade. The process of angiogenesis is tightly regulated by several pro- and anti-angiogenic molecules, of which the vascular endothelial growth factor (VEGF) is the best described [5–7]. Clinico-pathologic correlations confirm an important role for angiogenesis in ovarian cancer progression. Microvessel density (MVD) [8,9], expression of VEGF [10–14] and its receptors, or expression of other pro angiogenic peptides (angiopoietin [15,16] or matrix metalloproteinases MMPs [17,18]) correlate with an invasive ovarian cancer phenotype and worse clinical outcome. Three recent studies of bevacizumab, a monoclonal

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antibody binding VEGF, recorded objective responses in 17% of patients and disease stabilization in 54% of patients [19,20] with recurrent EOC. These data suggest that targeting budding vessel formation has anti-tumor efficacy and support the study of other anti-angiogenic agents in EOC.

2ME2 is a naturally-occurring metabolite of estradiol that exhibits anti-angiogenic and anti-tumor effects in preclinical models. Endogenous 2ME2 is hydroxylated and O-methylated at the 2-position. Because of these modifications, 2ME2 binds poorly to the estrogen receptor [21] and does not exert estrogenic effects in tissues. It has been demonstrated that in tumors, 2ME2 inhibits microtubule formation in endothelial cells, disrupting their proliferation, migration and formation of vascular networks [22]. Additionally 2ME2 inhibits the expression of hypoxia induced factor (HIF)-1 α [23,24], a critical mediator of angiogenesis under hypoxia. Decreased levels of HIF-1 α caused by 2ME2 treatment block secretion of VEGF [24] and this translates into decreased vessel formation. In addition, 2ME2 exerts direct anti-tumor effects through disruption of microtubules [25,26], alteration in expression levels and activity of stress kinases [27], cyclin dependent kinases [28], and other regulators of the cell cycle [29] or of apoptosis [30]. It has also been shown that 2ME2 upregulates death receptor 5 (DR5), inducing apoptosis through the extrinsic pathway [31]. Such effects mediate growth arrest or apoptosis of different types of cancer cells [28] and result in inhibition of xenograft growth in preclinical models [22,32,33].

Previous trials in solid tumors, prostate and breast cancer had shown that an oral capsule formulation of 2ME2 had modest anti-tumor activity [34–36]. This was at least in part attributed to limited bioavailability, resulting in wide inter-patient variability and sub-optimal plasma levels [34]. Therefore the agent was reformulated as a NanoCrystal® dispersion (NCD) using technology from Elan Drug Delivery, Inc. This formulation was tested in a preclinical model of lung cancer. Mice treated with four fractionated daily doses of 2ME2-NCD had significant inhibition of pulmonary metastases compared to mice receiving one daily dose [37]. The fractionated administration of 2ME2-NCD compared to one daily dose resulted in steady-state plasma levels, rather than high peak levels. These observations suggested that steady plasma levels are required for 2ME2's anti-tumor activity [38]. In subsequent phase I clinical trials, the maximum tolerated dose was defined at 1000 mg orally q.i.d., dose limiting toxicities being fatigue and hypophosphatemia [39,40]. At this dose, the mean plasma level was 21.6 ng/mL, a therapeutically active concentration based on preclinical models [41].

In the current trial we studied the effects of this NCD formulation of 2ME2 in patients with recurrent platinum-resistant or refractory EOC. The primary objective of this study was to determine the ORR and the secondary objectives were to measure the rate of clinical benefit, tolerability, and pharmacokinetic distribution.

Patients and methods

Patient population

Patients with advanced, histologically documented EOC or primary peritoneal carcinomatosis (PPC) who recurred within 6 months after platinum-based chemotherapy were eligible. Eligibility included both measurable and detectable disease. Measurable disease was defined according to Response Evaluation Criteria in Solid Tumor (RECIST) [42]. Patients with non-measurable disease could enroll if they had clinically or radiologically detectable disease (e.g. ascites, mesenteric thickening) plus two consecutive rising pre-treatment serum CA-125 levels over two-fold the nadir, or one CA-125 measurement over 100 IU/mL. All patients were at least 18 years old with an Eastern Cooperative Oncology Group (ECOG) perfor-

mance status (PS) of 0 to 2 and a life expectancy of at least 3 months. Other eligibility criteria included no upper limit to the number of prior therapies allowed, adequate hematologic, hepatic, and renal function. Key exclusion criteria included prior treatment with chemotherapy, radiotherapy or experimental anticancer agent within 4 weeks of Day 1 of 2ME2 treatment; prior treatment with 2ME2; history of brain metastases; clinical evidence of small bowel obstruction, and use of oral anticoagulation. All patients gave written informed consent and the protocol was approved by institutional review boards.

Treatment plan

Treatment consisted of 2ME2 orally at a dose of 1000 mg q.i.d. continuously. Each cycle was 4 weeks and treatment was continued until disease progression or intolerable toxicity. Patients discontinued therapy if they had recurrent Grade 3 or Grade 4 toxicity as defined by the National Cancer Institute Common Terminology Criteria for Adverse Event (NCI-CTCAE) Version 3.0, any subjectively intolerable toxicity; or progressive disease.

Efficacy and toxicity assessment

Tumor burden was evaluated by clinical examination at baseline and prior to each cycle, and radiographically at baseline, before each odd cycle, and at the end of treatment. CA-125 measurements were obtained from all patients on Day 1 of each cycle. Investigator-determined best overall response was defined using RECIST for measurable tumors and Gynecological Cancer Intergroup CA-125 response criteria for non-measurable tumors [43]. Adverse events were assessed on Day 1 of each cycle and graded according to NCI-CTCAE Version 3. Pharmacokinetic analyses included determination of the steady-state concentrations of 2ME2 and of the major metabolite 2-methoxyestrone (2ME1) in plasma collected at the beginning of each treatment cycle.

Immunohistochemistry (IHC) analyses

To identify potential markers of response to 2ME2, the protocol was amended to include archival tumor IHC analysis. Separate consent for tissue analysis was obtained from seven patients and these tumor samples were immunostained for known targets of 2ME2: tubulins (β I, II, III and IV tubulin, acetylated tubulin) and HIF-1 α expression level. In addition the expression of several HIF-1 α target genes (CA IX, PAI-1, VEGF, and KDR) was assessed by IHC. Activation of Akt (pSer⁴⁷³ Akt) was also investigated, as this is an established biomarker of resistance to therapy in ovarian malignancies [44]. Details of experimental IHC conditions and antibodies used are included in [Supplementary Table 1](#).

Statistical analysis

This was an open-label, multi-center phase II study performed through the Hoosier Oncology Group (protocol Gyn # 111). A sample size of 18 was chosen for this pilot study. No previous studies with this agent in patients with recurrent or resistant ovarian cancer had been conducted at the time when this study was designed to be used as a basis for sample size calculation. A sample of size 16 would have provided 80% power to detect a difference between the null hypothesis that the response rate was less than or equal to 5% vs. the alternative that the response rate was greater than or equal to 25% using a .05 level of significance. Two more subjects were enrolled which allowed for a 10% non-evaluable rate. The objectives were measurement of activity (with the primary outcome being overall response rate), safety, and partial pharmacokinetic analysis of 2ME2 administered as NCD. Efficacy was measured as overall response rate

(ORR), including complete (CR) and partial responses (PR) by RECIST in patients with measurable disease and CA-125 responses in patients with detectable disease. Additionally, we measured the relative change from baseline in CA-125 levels, progression-free survival (PFS), and the rate of clinical benefit. PFS was defined as the time from Day 1 of treatment to the time of documented disease progression or death. The rate of clinical benefit was defined as the number of patients experiencing an objective tumor response, or a CA-125 response, in the absence of disease progression by clinical or radiographic criteria, or sustained stable disease (>3 months) by clinical and radiographic criteria. Safety was measured as the frequency and severity of treatment-emergent adverse events (AEs). Demographic and baseline characteristics were summarized using medians (with ranges) for continuous variables, and proportions for categorical variables. Median PFS was estimated using the method of Kaplan–Meier. The results of these descriptive analyses for this pilot study were used to determine if additional clinical studies of 2ME2 are warranted.

Results

Patients

Eighteen consenting patients with recurrent platinum-resistant or refractory EOC or PPC were enrolled. Of the 18 patients who entered the study, all received at least one dose of study drug. Patient characteristics presented in Table 1 indicate that 15 patients recurred during or within 6 months of a prior platinum containing regimen. Of those, 4 patients had platinum-refractory disease (progression through a platinum-based chemotherapy regimen) and 11 had platinum-resistant disease (progression within 6 months from completing platinum-based chemotherapy). Two patients were allergic to platinum and for one patient platinum sensitivity status was unknown. Fifteen patients had measurable and 3 patients had detectable disease.

Table 1
Patient characteristics.

Patients enrolled (n = 18)	Number
Age	
Median	65.5
Range	40–73
ECOG PS	
0	13
1	5
Race	
Caucasian	18
Primary site of tumor	
Ovary	14
Fallopian tube	1
Primary peritoneal	3
Histological subtype	
Serous papillary	14
Clear cell	2
Endometrioid	1
Other	1
Stage at diagnosis	
I	1
II	2
III	12
IV	3
Number of prior therapies	
Median	5
Range	1–16
Platinum-sensitivity	
Refractory	4
Resistant	11
Allergic	2
Unknown	1
Measurable disease (RECIST)	15
Detectable disease	3

Table 2
Toxicity.

Toxicity (CTCAE v3)	Grade 3 No (%)	Grade 4 No (%)	All grades No (%)
Fatigue	1 (5.5%)	2 (11.1%)	14 (77.8%)
Nausea	3 (16.7%)	–	14 (77.8%)
Abdominal pain	2 (11.1%)	–	10 (55.6%)
Vomiting	1 (5.5%)	–	10 (55.6%)
Neuropathy	–	–	9 (50.0%)
Dyspnea	–	2 (11.1%)	8 (44.4%)
Edema	1 (5.5%)	–	7 (38.9%)
Diarrhea	1 (5.5%)	–	7 (38.9%)
Constipation	–	–	6 (33.3%)
Rash	1 (5.5%)	–	3 (16.7%)
Cough	–	–	5 (27.7%)
Depression	–	–	5 (27.7%)
Dyspepsia	–	–	5 (27.7%)
Anorexia	1 (5.5%)	–	3 (16.7%)
Mucositis	1 (5.5%)	–	3 (16.7%)
Arthritis	–	–	3 (16.7%)
Liver panel abnormalities	1 (4.3%)	–	2 (11.1%)

Note. Adverse events encountered in at least 15% of patients, irrespective of relatedness.

The median number of prior chemotherapy regimens was 5 (range 1–16), and the median age was 65.5 (range 40–73). The majority of patients had tumors originating in the ovary of serous papillary histological pattern. Two patients with clear cell histology were enrolled.

Treatment administration and safety

Sixty-six cycles of chemotherapy were administered; 13 of the 66 cycles were given without dose reductions or delays. All missed doses recorded in patients' diaries were considered dose errors or delays. The mean number of cycles administered per patient was 3.67 and the median number of cycles was 2 (range 1 to 14). Causes for dose modifications were: dosing errors (52%); dose modifications due to toxicity (34%); or intercurrent illness leading to treatment interruption (14%). Causes for treatment discontinuation were: disease progression (12 patients); toxicity (4 patients); and withdrawal of consent (2 patients).

Table 2 lists adverse events occurring during study, irrespective of relatedness. The most common adverse events encountered on trial were fatigue (78%), nausea (78%), diarrhea (39%), neuropathy (50%), edema (39%), and dyspnea (44%). The majority of these events were graded as grade 1–2. Grade 3–4 toxicities occurring in more than one patient included: fatigue (n = 3), dyspnea (n = 2), bowel obstruction (n = 2); pain (n = 3); and nausea (n = 3). Two patients were deceased within 30 days of receiving treatment on this protocol, both deaths being attributed to disease progression and assessed as unrelated to treatment.

Efficacy

Sixteen patients were included in the analysis of efficacy; as two patients were not evaluable because of early discontinuation from treatment and incomplete follow-up evaluation. There were no responses by RECIST. See Fig. 1 for the waterfall plot of best response. Seven patients had stable disease as best response documented. Of those patients, five had stable disease lasting more than 3 months, with two of these 5 maintaining stable disease for more than 1 year. One patient had a confirmed partial response by CA-125 (251 IU/mL at baseline to 110 IU/mL) at Cycle 7; however, this patient also had an objective response of stable disease by RECIST, and in this protocol CA-125 responses were counted only in the absence of measurable response.

Other objectives of the study were to determine the relative change from baseline in CA-125 levels, the rate of clinical benefit, and the PFS. There were no CA-125 mean differences from baseline to

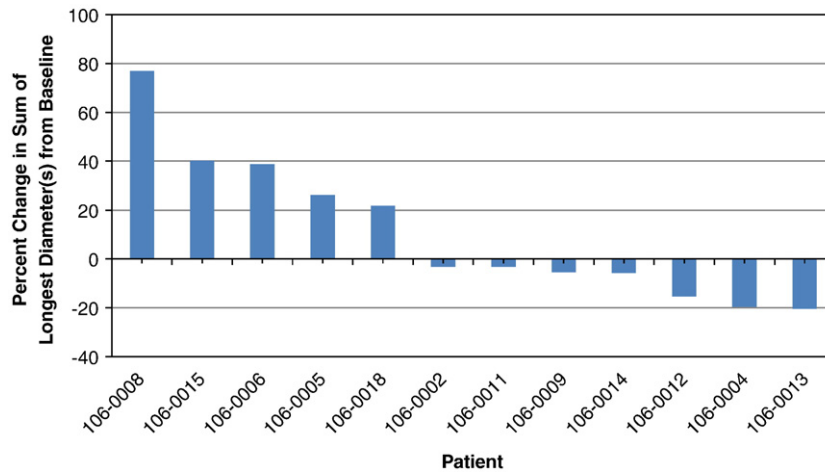


Fig. 1. Waterfall plot of the percent change in the sum of the longest diameter(s) for all target tumors for each patient (best response).

Cycle 2 for the 11 patients for whom values at both time-points were available. The rate of clinical benefit among 16 patients who were treated and had the first disease evaluation performed was 31.3% (five out of 16 patients evaluable); with 16.1% as the 90% lower confidence boundary. The median PFS was 1.77 months (95% C.I.: 1.08 to 12.62 months, see Fig. 2).

Pharmacokinetic analyses

Trough plasma concentrations of 2ME2 and its metabolite, 2-methoxyestrone (2ME1) were measured in the plasma obtained at the beginning of each cycle of therapy from 14 out of the 18 patients enrolled. The mean \pm standard deviation trough plasma level of 2ME2 was 30.27 ± 20.18 ng/mL, and the geometric mean (95% confidence interval) was 25.03 (17.4–36.0) ng/mL. 2ME2 is significantly oxidized at the 17 position to 2ME1. The mean \pm standard deviation trough plasma level of 2ME1 was 475.66 ± 226.14 , and the geometric mean (95% confidence interval) was 433.74 (337.7–557.2) ng/mL. Overall, the average trough levels of 2ME1 were about 15 times higher than the trough levels of 2ME2, indicating extensive metabolism of the parent drug (see Fig. 3). Animal studies suggest that plasma levels of 2ME2 between 3 and 17 ng/mL are needed for efficient anti-tumor activity [37].

Molecular predictors of response

To identify molecular predictors of response, archival tumors from seven consenting patients were analyzed retrospectively by IHC for

expression of target proteins of 2ME2, particularly β tubulins and HIF-1 α . In addition, activation of Akt in tumors was measured by IHC for pSer⁴⁷³Akt. Hypothesizing that the two patients who were on treatment for over 1 year had a distinct profile, we analyzed the IHC results by grouping samples in two categories: one including these two patients, and the other including the rest of patients. Interestingly, and contrary to previous preclinical data predicting that patients responding to 2ME2 would have tumors expressing lower levels of tubulin, both patients with prolonged disease stabilization expressed high levels of β 1–IV tubulin and one patient expressed high level of acetylated tubulin. Expression of HIF-1 α was detected at moderate levels in both responders' samples, but also in 2 of 5 non-responding

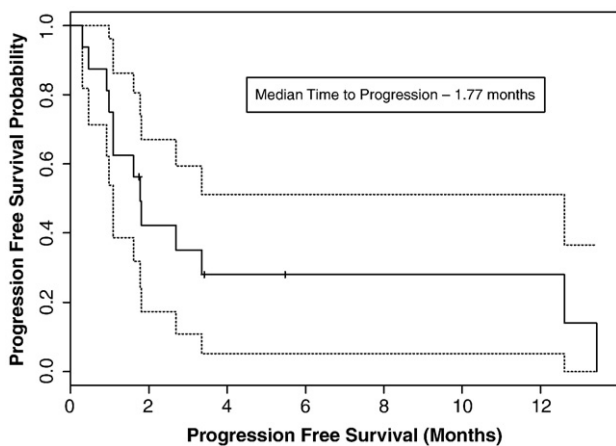


Fig. 2. Kaplan–Meier curve for progression-free survival (PFS) (evaluable patients).

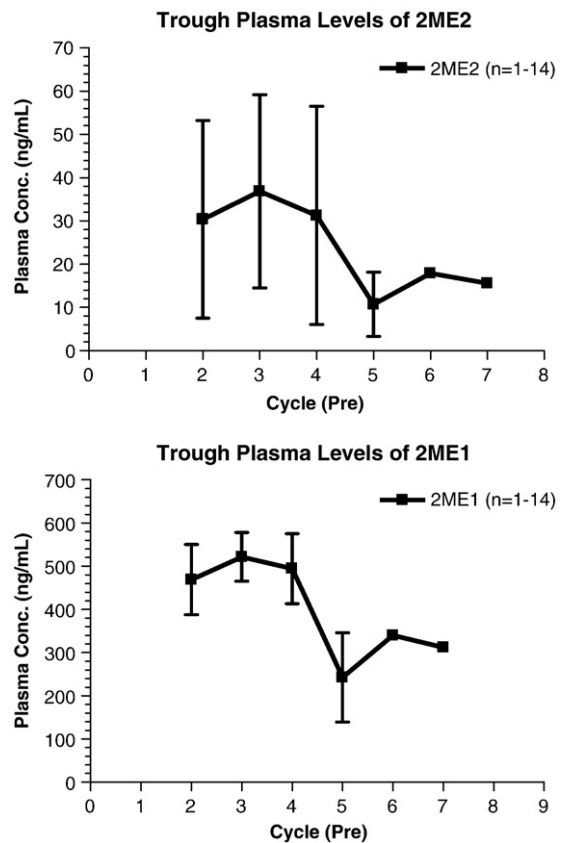


Fig. 3. Trough plasma levels of 2ME2 and 2ME1 during treatment.

Table 3
Results of IHC analyses.

IHC staining	Staining score						
	Patient ID						
Antigen	02	04*	06	08	09	13	14*
βI tubulin	4	2	4	3	4	4	3
βII tubulin	1	2	4	4	2	4	3
βIII tubulin	1	4	0	0	4	3	4
βIV tubulin	3	4	2	2	4	4	3
Acetylated tubulin	1	4	2	1	0	4	1
HIF-1α	0	2	0	0	1	2	1
CA IX	1	1	2	2	4	3	3
KDR	1	2	1	4	2	0	2
PAI-1	0	1	3	2	2	4	3
VEGF	1	3	2	1	2	4	2
pAKT(ser473)	0	3	1	4	0	2	0

Note. 0 = no stain; 1 = light stain; 2 = light medium stain; 3 = medium stain; 4 = dark stain. Gradation in staining was based on an increase in the number of staining cells and an increase in the staining intensity.

*Denotes patient who experienced clinical benefit >1 year.

patients. There was no correlation between the expression level of HIF-1α and expression levels of its target genes (CA IX, PAI-1, VEGF and KDR), or between these target genes and benefit from 2ME2 treatment. One of the two responding patients had low level of pAkt, however the other patient who benefited from 2ME2 had a tumor staining strongly for pAkt. Results of IHC stains are summarized in Table 3.

Discussion

The goal of this phase II trial was to assess safety and efficacy of 2ME2-NCD in patients with recurrent, platinum-resistant or refractory ovarian cancer. This novel steroid analog targets tubulins and transcription of genes dependent on HIF-1α. Preclinical models predict that 2ME2 inhibits cancer cell proliferation and induces disruption of endothelial networks [45]. The dosage of 2ME2-NCD chosen for this study was based on two prior phase I studies with this formulation of 2ME2 [39,40]. The maximum tolerated dose was 1000 mg administered orally four times daily. At this dose the average minimum trough concentration was within the target range of 3 to 17 ng/mL of 2ME2. Based on several animal tumor models it is known that this concentration exerts anti-tumor effect [46–48]. These levels were reached and maintained in our study. Fairly stable trough plasma concentrations of 30.27 ± 20.18 ng/mL and geometric means ranging from 17.4 to 36 ng/mL were achieved during treatment, suggesting better bioavailability of the NCD suspension as compared to the original oral capsule formulation of 2ME2. However, we observed persistent and significant inter-patient variability. Part of this variability may be due to the multiple dose delays or errors noted during this trial due to the schedule (q.i.d.) as recorded by patients in their diaries.

The toxicity profile observed was mild, which is consistent with observations from previous trials [35,36]. Notably, there was only one case with grade 3 hepatotoxicity, which was reversible after study drug discontinuation. Liver dysfunction had been reported in patients with prostate cancer treated with 2ME2 [34] and in a minority of patients with metastatic breast cancer treated with docetaxel and 2ME2 [36]. The majority of other toxicities were mild, consisting mostly of gastrointestinal effects and fatigue. Notably, no myelosuppression was observed.

2ME2 had modest anti-tumor activity, with no observed objective responses. However, 5 of 16 evaluable patients had stable disease for longer than three months and two of these 5 patients achieved stable disease for greater than 1 year. The rate of clinical benefit, defined variably in different protocols, has become increasingly recognized as a valuable endpoint particularly for trials investigating biological agents. These are more likely to induce disease stability rather than objective responses, thus translating into prolonged progression-free

interval. Evaluating clinical benefit may therefore be a more relevant endpoint in such settings [49].

Interestingly, we did not observe responses or clinical benefit in patients with clear cell histology, as previously speculated based on results from a phase I investigation, where one patient with clear cell carcinoma of the ovary achieved a durable partial response [35]. However, only two women with this histology were enrolled in this study.

In an effort to identify molecular predictors of clinical benefit from 2ME2, a subset of tumors from patients enrolled in this trial were retrospectively analyzed for expression of β-tubulins, HIF-1α and some of its targets, and phosphoAkt. Unfortunately, only seven archived specimens could be analyzed; and this small number precluded identification of a predicting marker or signature. We found relatively high expression levels for β I–IV tubulins in tumors from the two patients who experienced prolonged stable disease. This was surprising, as high level of tubulins had been linked to resistance to other microtubule targeting agents (e.g. paclitaxel [50–52] and vinorelbine [53,54]) and β-tubulin mutations have been associated with resistance to 2ME2 in breast cancer and leukemia cells [55,56]. Secondly, we observed that both patients with prolonged stable disease had tumors expressing moderate levels of HIF-1α [45,57,58]. One of these two tumor samples also expressed several HIF-1α target genes: PAI-1, VEGF and CA IX. Given the small number of specimens analyzed and the fact that other tumors from non-responding patients expressed HIF-1α, this finding is at most, hypothesis-generating and should be re-tested in future tumor sets.

In conclusion, this trial demonstrates that the NCD formulation of 2ME2 was well tolerated and had better bioavailability, resulting in therapeutic target levels with the dose and schedule administered. However anti-tumor activity was modest in this heavily pretreated group of patients, with two patients experiencing clinical benefit exceeding one year. To further exploit the unique anti-angiogenic and anti-tumor properties of this natural estrogen derivative, new analogues of 2ME2 with less metabolism and more potent anti-tubulin properties are in clinical development [59].

Conflict of interest statement

Dr. Carolyn Sidor is an Employee of EntreMed Inc., the sponsor of the trial. The rest of the authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ygyno.2009.05.042.

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