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A phase II study of 2-methoxyestradiol nanocrystal colloidal dispersion alone and in combination with sunitinib malate in patients with metastatic renal cell carcinoma progressing on sunitinib malate

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Summary

Background—Current treatment for metastatic renal cell cancer with vascular endothelial growth factor (VEGF) tyrosine kinase inhibitors (TKI) have provided improved overall survival, but complete responses are rare. We conducted a multicenter phase II study to evaluate the objective response rate of 2-methoxyestradiol (2ME2 NCD) alone and in combination with sunitinib for patients with metastatic renal cell carcinoma who have progressed on sunitinib alone.

Methods—Adults with metastatic kidney cancer were stratified depending on whether they were still taking sunitinib or had discontinued sunitinib therapy at the time of registration. Patients were treated with 2ME2 NCD alone or in combination with sunitinib. The primary endpoint was objective response rate.

Results—In total, 17 patients were enrolled, and 12 were evaluable for response (arm A, $n=7$; arm b, $n=5$). In arm A, four patients had the best response of stable disease, and three patients developed disease progression. In arm B, three patients had a best response of stable disease, and two patients had disease progression. One patient continued to receive treatment for a total of 14 cycles before developing disease progression. Fatigue was the most common observed toxicities. Thirty five percent of patients required discontinuation of therapy secondary to toxicities.

Conclusions—2ME2 NCD had minimal anti-tumor activity, with no observed objective responses. The study was terminated because 2ME2 NCD was not found to be tolerable at the recommended phase 2 dose in this patient population. A newer 2ME2 analog is in development with a more favorable toxicity profile and increased potency.

Keywords

Renal cell carcinoma; Clinical trials; Phase II; 2-methoxyestradiol; Sunitinib malate; Antiangiogenic agent

Introduction

Approximately 58,240 cases of kidney cancer will be diagnosed in the US in 2010 alone, resulting in approximately 13,040 deaths [1]. Many patients present with advanced or unresectable disease, and nearly 30% of patients treated with a nephrectomy with curative intent will eventually relapse.

Clear cell kidney cancer is a highly vascular tumor with overexpression of vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) surface receptors. Traditional chemotherapies have largely been ineffective in the treatment of this disease, and the use of cytokine therapy (interleukin-2 or interferon-alpha) had been the standard of care for many years given their reported response rates of approximately 10–15% [2, 3]. However, these treatments only offer a modest improvement in overall survival with significant associated risks and toxicities during treatment. Because of this, other treatment options have been limited to palliative care or participation on a clinical trial. The use of an anti-VEGF/PDGF agent has been a focus of clinical research for antitumor and antiangiogenic activity against clear cell kidney cancers. Within the last 5 years, there have been four antiangiogenic agents that have been FDA approved for use in renal cell carcinoma (sunitinib, sorafenib, bevacizumab and pazopanib) [4–7]. Although encouraging responses are evident, complete responses are rare with the median time to progression being 5.5 months for sorafenib and 11 months for sunitinib [4, 5].

2-methoxyestradiol (2ME2; EntreMed, Inc., Rockville MD) is a non-estrogenic derivative of estradiol with antiproliferative and antiangiogenic activity that downregulates hypoxia inducible factor alpha (HIF-1 α). HIF-1 α is a key regulatory protein responsible for a cell's ability to respond to stress, including hypoxic conditions. Nonclinical studies have indicated that 2ME2 has a dual mechanism of action as: (1) an antiproliferative drug acting directly on the tumor cell compartment, and (2) an antiangiogenic drug, acting on tumor vasculature [8–13]. 2ME2 has been shown in chick chorioallantoic model systems to have an anti-angiogenic effect. Because 2ME2 is an orally active agent, it is proposed to be an effective alternative to existing therapies for the treatment of cancer. Several clinical trials have been

developed to evaluate 2ME2 for the treatment of prostate, breast, and multiple myeloma [14].

2ME2 was originally formulated as a capsule, but pharmacokinetic results from clinical trials showed that the capsule formulation did not result in acceptable sustained plasma levels of 2ME2 to adequately evaluate the therapeutic potential of 2ME2 [15]. For this reason, 2ME2 was reformulated using a nanocrystal dispersion system (NCD) that involved reducing the compound into nanometer sized particles [16]. In a phase I trial using 2ME2 NCD, there was evidence of prolonged disease stabilization in a renal cell cancer patient who previously progressed on an VEGFR tyrosine kinase inhibitor (TKI), and another subject demonstrated stable disease for greater than 1 year [17].

Because of the promising results from the phase I study of 2ME2 NCD regarding disease stabilization of renal cell cancer patients and because 2ME2 decreases expression of HIF-1 α , we undertook a study to determine the efficacy of 2ME2 for patients who have progressed on VEGFR TKI agents.

Methods

Eligibility

All patients were required to have histologically confirmed renal cell cancer with a component of clear cell carcinoma. Patients must also have had evidence of metastases that are measurable, prior or current treatment with sunitinib malate, and evidence of disease progression while receiving sunitinib. Patients were ambulatory with a performance status of 2 or lower according to the criteria of the Eastern Cooperative Oncology Group. Other requirements included: adequate function of major organs (indicated by a hemoglobin of greater than 9 g/dl, a creatinine less than 1.5 times the upper limit of normal (ULN), a total bilirubin within normal institutional limits, a serum aspartate aminotransferase and alanine aminotransferase less than 2.5 times ULN, a normal cardiac ejection fraction, and the provision of a written informed consent.

Patients were excluded if they had any major surgery, radiotherapy, chemotherapy, cytokine therapy, or other experimental therapy within 4 weeks of treatment initiation. Any adverse events experienced during those prior therapies must have resolved to baseline or a grade 1 effect prior to treatment initiation. Other exclusion criteria included active brain metastases, uncontrolled intercurrent illness such as symptomatic congestive heart failure, unstable angina pectoris, or cardiac arrhythmias. Patients who had previously treated brain metastasis were allowed provided that the patient was clinically stable off systemic steroids and antiepileptic agents with repeat imaging studies confirming stable disease. Because tumor metabolic activity was to be evaluated using ¹⁸F- fluorodeoxyglucose positron emission tomography (FDG-PET) imaging, patients with type I insulin-dependent diabetes or poorly controlled type II insulin dependent diabetes were excluded.

Informed consent was obtained from all subjects who participated in this clinical trial.

Study design

All patients had documented evidence of disease progression while receiving treatment with sunitinib malate. This study was conducted with two arms to distinguish whether patients were to receive treatment with 2ME2 NCD alone versus continue sunitinib in conjunction with 2ME2 NCD. Patients on Arm A (for patients previously treated with sunitinib malate but now were discontinued from the therapy) were treated with single agent 2ME2 NCD to evaluate the activity of 2ME2 NCD alone. Patients on Arm B (for patients currently receiving on sunitinib malate but with demonstrated progressive disease) continued with

their current sunitinib dose in conjunction with 2ME2 NCD to evaluate the anti-tumor effect of combining these two agents. An open-label, Simon optimal two-stage design was used for both arms.

Patients on Arm A were instructed to take 2ME2 NCD at a dose of 1500 mg by mouth three times daily with at least 6 h duration between dosages. Patients on Arm B were also given 2ME2 NCD at a dose of 1500 mg by mouth three times daily. However, these patients also continued their sunitinib therapy at the highest tolerable dose. For patients receiving less than the highest dose of sunitinib malate (50 mg), the dose was increased if no significant grade ≥ 2 toxicity was observed after two cycles of therapy. For instance, a dose escalation from 25 mg to 37.5 mg, or from 37.5 mg to 50 mg of sunitinib was allowed during treatment of patients on Arm B.

The study was monitored by the sponsor, medical monitor, and the investigators at each site. The study was approved by the institutional review board of each participating site.

Treatment plan

Patients were stratified depending on whether they were still taking sunitinib or had discontinued sunitinib therapy at the time of registration. For patients who had discontinued treatment with sunitinib malate, a four week washout period was required prior to treatment initiation. These patients were treated with 2ME2 NCD alone. Patients who were still taking sunitinib at the time of registration were continued on their current dose and schedule of sunitinib; these patients were started on 2ME2 NCD concurrently with their sunitinib. No drug washout of sunitinib malate was required for these patients.

2ME2 NCD was supplied as a 100 mg/mL colloidal dispersion in 8 ounce bottles. Patients were counseled to take 2ME2 NCD by mouth three times daily with at least 6 h between dosages. Patients were instructed to refrain from eating for 1 h prior to and 30 min after all doses of 2ME2 NCD. For any missed doses of 2ME2 NCD, patients were instructed to take a dose of 2ME2 NCD as soon as possible, but not more than three doses of 2ME2 NCD were to be taken within a 24-h period.

Sunitinib malate was administered orally as the highest tolerable dose, which was the current dose that the patient had been taking prior to enrollment on this trial. Sunitinib was given at the authorized dose daily for four consecutive weeks followed by a 2 week rest period to comprise a complete cycle of 6 weeks. The lowest dosage of sunitinib allowed was 25 mg and the highest was 50 mg. For patients receiving less than the highest dose of sunitinib, a dose escalation of sunitinib was allowed if the patient did not have any significant grade ≥ 2 toxicity after two cycles of therapy at the current dose of sunitinib (for example, an increase from 25 to 37.5 mg or from 37.5 to 50 mg of sunitinib).

Use of growth factors were allowed during the study at the discretion of the treating physician.

A subset of patients at the lead site also underwent FDG-PET scanning at baseline and also during cycle 1 of treatment. Selected patients for the FDG-PET scanning underwent a baseline scan within 7 days of the first 2ME2 NCD dose. For subsequent FDG-PET scans, patients on arm A underwent a scan at cycle 1, week 6 while patients on arm B underwent a scan at cycle 1, week 4 and also cycle 1, week 6. These imaging studies were performed to examine the metabolic response rate to 2ME2 NCD.

Dose modifications

Development of subjectively intolerable grade 2, or any grade ≥ 3 toxicity to be at least possibly related to 2ME2 NCD required discontinuation of the study drug until symptoms returned to baseline or a tolerable grade 2. If the toxicity resolved within 21 days, therapy may be resumed at the next lowest dose level. Serum phosphate levels have been observed to decrease with 2ME2 NCD, so dose modification was necessary only if the serum phosphate was deemed to be clinically significant by the treating physician and was unresponsive to oral supplementation. All dose modifications were permanent for the duration of the study.

For patients on Arm B (receiving concurrent sunitinib and 2ME2 NCD) who experienced an adverse event requiring holding of treatment, both agents were held until recovery. In general, dose modification for only the most-likely offending agent was performed. For subjects at the lowest dose of one agent, dose reduction for the other agent was performed for unacceptable toxicity. If patients were on the lowest dose for both agents and still required additional dose modification, they were taken off the study.

Follow up of patients

Follow-up of both groups occurred at 6 week intervals, which consisted of physical examination, a complete blood count, and serum chemistries. Disease assessments were performed in week four during each even-numbered cycle of therapy, which consisted of CT scans. Patients were treated until disease progression or unacceptable toxicity.

Statistical analysis

This study was conducted as two parallel, multicenter phase II studies. The primary endpoint was objective response rate of single agent 2ME2 NCD (arm A) and 2ME2 NCD in combination with sunitinib (arm B) in patients with metastatic renal cell carcinoma who have progression on prior therapy with sunitinib. Arm A examined patients with metastatic or unresectable renal cell cancer who had failed prior therapy with sunitinib and were treated with 2ME2 NCD alone. In arm B, patients who had progressed on current therapy with single agent sunitinib were treated with 2ME2 NCD in combination with sunitinib. For each arm, we used an open label, Simon optimal two-stage design. We expected to see a response rate of 2ME2 NCD alone or in combination with sunitinib to be at least 20% in order to be considered sufficient evidence for further investigation of this clinical regimen. We considered 2ME2 NCD alone or in combination with sunitinib to be ineffective in generating an objective response if the true response probability is at most 5%. This allowed the incorporation of two stages with decisions to continue after the first 21 in each arm were accrued and evaluable for objective response using RECIST criteria. If at least two responses would have been observed among these first 21 patients within an arm, then an additional 20 patients would have been accrued for a total of 41 evaluable patients. This study design had 90% power to detect an effective treatment if the true response rate was at least 20% versus the null hypothesis that it was at most 5% with a 5% significance level. If both arms continued to the full sample size, we would require a total of 82 evaluable patients.

Standard descriptive statistics were used to summarize demographic variables and pharmacodynamic parameters. Toxicities were summarized in tabular format by type and severity (grade). Percentage changes in standardized update values from baseline to the 6 weeks assessment were calculated. A paired t-test was used to evaluate mean changes from baseline to the week 4 and week 6 assessments.

Results

Patient characteristics

From February 2007 until March 2008, 17 patients with metastatic renal cell carcinoma were enrolled in the study by three participating cancer centers. The median age was 58 years with various performance status, and sites of disease as shown in Table 1. All of the patients were Caucasian. The patient characteristics are depicted in Table 1. All patients were evaluable for toxicities. Of the 17 patients enrolled on the study, five patients were unevaluable for response. Patients were considered to be unevaluable if they were not able to complete one cycle of therapy. Of the five unevaluable patients, two patients developed disease progression prior to completion of one cycle of therapy (one developed a new metastatic lesion to the eye that prompted radiation therapy; the other patient developed a new brain metastasis), one patient developed grade 3 dyspnea related to a pleural effusion that was not related to 2ME2, one patient developed a toxicity of bilateral rash to the arms, and one patient developed grade 2 fatigue and refused additional therapy.

Treatment

Patients received 1500 mg of 2ME2 NCD orally three times daily (4500 mg/day) alone in Arm A or in combination with sunitinib malate (Arm B). Each treatment cycle was 6 weeks in duration, and the median number of cycles administered was one (range 0–14). One patient on the sunitinib plus 2ME2 NCD arm required a dose reduction of 2ME2 NCD to 1000 mg/day due to grade 3 fatigue that was persistent.

Toxicity

All patients were evaluable for toxicities. Three patients (all in Arm A) developed unacceptable toxicity (adverse events) after completion of at least 1 cycle, which resulted in discontinuation from the trial (see Table 2). One patient developed grade 4 increase in the ALT level that improved to a grade 3 after holding drug, but continued to have grade 3 elevation in ALT and grade 2 elevation in AST. Two patients developed weight decrease and fatigue, which were judged to be definitely related to the study drug.

Two patients were discontinued from therapy secondary to the discretion of the treating physician. One patient developed grade 3 dyspnea secondary to a pleural effusion prior to completion of the first cycle. The second patient completed four cycles of therapy of 2ME2 NCD alone (arm A), but developed signs and symptoms of clinical disease progression, although the subject did not meet the protocol definition of disease progression. A third patient developed bilateral rash on the arms and was taken off study after an extensive discussion between the patient and the treating physician.

Overall, the main toxicities associated with this drug were fatigue (60%), diarrhea (53%), increased AST (41%), anorexia (35%), arthralgia and myalgia (35%). A list of observed toxicities is shown in Table 3. Several patients experienced transient changes in liver function tests (increase in AST and ALT). Based on phase I clinical trials using 2ME2 NCD, transient changes in liver function tests were expected. In most cases, grade 2 increases in liver function tests decreased with continued drug administration. Other toxicities observed in the study included myalgias, arthralgias, and anorexia. Hypophosphatemia, increases in GGT values, and thrombosis were not seen in this study, although they have been reported in prior phase I trials.

Pharmacokinetics (PK)

Pharmacokinetic variables were determined for patients who received at least one dose of 2ME2 NCD. Variations in intra- and inter-patient trough plasma concentrations of 2ME2

and its metabolite 2ME1 were moderate with a coefficient of variation averaging around 50% in this study. The overall trough concentrations of 2ME2 and 2ME1 after 1500 mg 2ME2 NCD alone averaged 12.91 ng/mL and 276.40 ng/mL, respectively. In patients administered 1500 mg 2ME2 NCD in combination with 50 mg sunitinib malate, the overall trough concentrations of 2ME2 and 2ME1 averaged 17.96 ng/mL and 443.17 ng/mL, respectively.

The average trough plasma concentrations of 2ME2 and 2ME1 appeared to be higher when 2ME2 NCD was administered in combination with sunitinib malate compared to 2ME2 NCD administered alone, but the differences were not statistically significant. The average trough plasma concentrations of the metabolite 2ME1 were greater than 20 times higher than the average trough plasma concentrations of 2ME2, as expected from previous clinical studies.

Efficacy, overall and relapse-free survival

Of the 17 patients enrolled on this study, 12 patients were evaluable for response. Ten patients (seven were evaluable) were enrolled on arm A, and seven patients (five were evaluable) were enrolled on arm B (see Table 4). In arm A, four patients had the best response of stable disease, and three patients developed disease progression. Of the four patients with stable disease, two patients discontinued the study secondary to adverse events, and two patients demonstrated disease progression after receiving four cycles of therapy. One unevaluable patient on Arm A demonstrated a 28% decrease in target lesions, which would have been classified as stable disease based on RECIST criteria, but since this patient developed an adverse event unrelated to the study drug and was deemed to be unevaluable, the response could not be confirmed.

In arm B, three patients had a best response of stable disease, and two patients had disease progression. Of the three patients with stable disease, two patients developed disease progression after receiving four cycles of therapy. Only one patient continued to receive treatment for a total of 14 cycles before developing disease progression. It is difficult to determine if this patient had slow disease progression versus true disease stabilization, but he had both bone metastases as well as soft tissue disease (lymphadenopathy), suggesting that he likely derived some benefit from the combination of sunitinib plus 2ME2 NCD.

Pharmacodynamic analysis using FDG-PET imaging

We also evaluated patients using FDG-PET imaging as an exploratory endpoint to determine the pharmacodynamic response of patients receiving 2ME2 NCD alone or in combination with sunitinib (see Table 5). Patients who completed their predetermined FDG-PET imaging (Arm A=2 scans, Arm B=3 scans) were deemed to be evaluable for analysis. The FDG metabolic response was assessed based on the European Organization for Research and Treatment of Cancer (EORTC) guidelines where progressive metabolic disease is an increase of greater than 25% within the tumor region or the appearance of new FDG uptake in metastatic lesions [18]. A partial metabolic response was defined as a reduction of at least 25% in the tumor SUV. Complete metabolic response was defined as the complete resolution of FDG uptake within the tumor volume so that uptake is indistinguishable from the surrounding normal tissue.

In Arm A, six patients were evaluable for a metabolic response, and three were unevaluable. All three unevaluable patients underwent baseline FDG-PET imaging, but did not complete FDG-PET imaging at cycle 1, week 6. Five out of the six evaluable patients were determined to have a decrease in the mean value of maximum standardized uptake value (SUV) ranging from -0.7% to -83.6%.

In Arm B, five patients were evaluable for a metabolic response, and two patients were unevaluable for a metabolic response. Of the two unevaluable patients, one patient did not complete FDG-PET imaging at cycle 1, week 6, and the second patient did not undergo FDG-PET imaging during cycle 1, week 4 and at week 6. Four of the five evaluable patients were determined to have a decrease in mean value of maximum SUV (ranging from -4.0% to -45.3%) after 4 weeks of treatment. One patient with a mean decrease in maximum SUV of -45.3% had the longest duration of stable disease, receiving a total of 14 cycles. There were no significant decreases detected in the maximum SUVs from baseline to the week 4 or week 6 assessments.

Discussion

We conducted a multicenter phase II study to evaluate the objective response rate of 2ME2 NCD alone and in combination with sunitinib malate for patients with metastatic renal cell carcinoma who have progressed on sunitinib malate alone. Other endpoints in this study included the safety and tolerability of 2ME2 NCD alone and in combination with sunitinib, progression free survival of patients, and pharmacodynamic response using FDG-PET scanning. This novel metabolite of estradiol has anti-angiogenic and anti-tumor effects by targeting the colchicine binding site on tubulin, and downstream has effects due to modulation of transcription of genes dependent on HIF-1 α .

2ME2 NCD alone and in combination with sunitinib malate did not appear to be tolerable when given at 1500 mg three times daily, since 35% patients enrolled on this study experienced unacceptable toxicities that required discontinuation of therapy. As reported in prior clinical trials using 2ME2, the main toxicities included transient elevation in liver function tests: aspartate aminotransferase (AST), and alanine aminotransferase (ALT). In most cases, the LFT elevations were grade 2. Liver dysfunction has been reported in patients treated with prostate cancer and ovarian cancer, which was reversible after discontinuation of therapy [19, 20]. Because six out of 17 patients discontinued therapy secondary to side effects before meeting any objective response endpoint, 2ME2 NCD was deemed not to be tolerable in this patient population and the clinical trial was closed early.

Fifty eight percent of our patients experienced fatigue, which was the most common observed toxicity. Forty seven percent of patients experienced grade 1 or 2 fatigue, while twelve percent experienced grade 3 fatigue. Seventy percent of the patients experiencing fatigue were treated with 2ME2 NCD alone, which is consistent with other clinical trials using 2ME2 [19].

Forty eight percent of our patients developed grades 1 or 2 diarrhea, which was the most common toxicity in this trial. This side effect can be attributed to either sunitinib or 2ME2 NCD, but the majority of patients (63%) affected by diarrhea were in the combination arm of sunitinib plus 2ME2 NCD. The toxicity profile otherwise was mild, which is consistent with observation from previous trials.

We utilized FDG-PET imaging as a pharmacodynamic tool to assess early responses to therapy, but there was not a consistent correlation between changes in SUV and the RECIST disease status. For instance, five out of the six patients in Arm A were determined to have a decrease in the mean value of maximum standardized uptake value (ranging from -0.7% to -83.6%), but these did not correlate with the RECIST disease status. However, in Arm B, four patients were determined to have a decrease in mean value of maximum standardized uptake value (ranging from -4.0% to -45.3%) after four weeks of treatment, and three of these patients did have evidence of stable disease by RECIST criteria after two cycles of therapy. One patient with a mean decrease in maximum SUV of -45.3% had the longest

duration of stable disease, receiving a total of 14 cycles. It appears that FDG-PET imaging potentially can be used as an early pharmacodynamic measure of tumor response, but changes in maximum SUV may not be the best measurement, but rather other parameters such as the peak of SUV or total SUV may be more useful.

Developing antiangiogenic drugs in renal cell cancer is challenging, especially given the number of VEGF signaling pathway (VSP) inhibitors approved for use. While sequential use of VSP inhibitors is commonly utilized in clinical practice, the clinical utility and optimal sequence for these agents are unknown. Patients experiencing disease progression on one VEGFR TKI may still respond to treatment with an alternative VEGFR TKI or eventual rechallenge with the same agent [21–23]. This is especially true if the period between VEGFR TKI treatment is prolonged [24]. What this implies is that in response to VEGFR TKI-induced hypoxia, the tumor/host develops a compensatory increase in other proangiogenic factors, which eventually overcompensates for the VSP inhibition, and that if the VEGF inhibition is withdrawn, host compensatory factors will normalize leading to renewed sensitivity to VSP inhibition. Unlike with cytotoxic chemotherapy where true therapy resistance may develop, we do not know whether true VSP inhibitor resistance can occur and if responses to subsequent VSP inhibitors results in a meaningful prolongation of survival, or simply a transient decrease in tumor burden. As a result, second-line trials in renal cell cancers utilizing VSP inhibitors need to be interpreted cautiously, as the radiographical response may be increased simply due to the drug washout study requirements and pharmacokinetics of the prior therapy received.

This trial is unique in its study design, examining both 2ME2 NCD as a single anti-angiogenic agent for metastatic renal cell carcinoma and in combination with a tyrosine kinase inhibitor. Since 2ME2 NCD has both antiproliferative and antiangiogenic effects, single-agent activity was assessed although the presumption was that combination therapy would likely result in improved benefit. As one potential mechanism of disease progression after VEGFR tyrosine kinase inhibitor treatment may be upregulation of HIF-1 α , Arm B of the study assessed continued inhibition of the VEGF receptor tyrosine kinase with active downregulation of HIF-1 α by 2ME2 NCD. The advantages of this study design is that it provides a novel way to screen for drug activity, both alone and in combination, which minimizes potential confounding issues seen with sequential antiangiogenic agent use, as no drug washout period was required in Arm B for the sunitinib, and patients continued to receive sunitinib at their previous prescribed dose.

In conclusion, 2ME2 NCD had minimal anti-tumor activity, with no observed objective responses. Only one evaluable patient had stable disease for longer than 4 months (for a total of 14 months). The study was terminated after 17 patients had been enrolled (Arm A=10; Arm B=7) because 2ME2 NCD was not found to be tolerable at the recommended phase 2 dose in this patient population as 35% of patients required discontinuation of therapy secondary to toxicities. In addition, no objective responses were observed for those patients, which also prompted early termination of the study. A newer 2ME2 analog is in development with a more favorable toxicity profile and increased potency. This compound is ENMD-1198, which has been examined in a phase I clinical trial and found to have activity and prolonged disease stabilization in ovarian, prostate, and neuroendocrine carcinoma of the pancreas. Further testing with this newer 2ME2 analog is warranted, and this particular trial design should be utilized in future renal cell carcinoma studies [25].

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References

1. American Cancer Society. Cancer facts and figures 2010. Atlanta: American Cancer Society; 2010.
2. Interferon-alpha and survival in metastatic renal carcinoma: early results of a randomised controlled trial. Medical Research Council Renal Cancer Collaborators. *Lancet*. 1999; 353(9146):14–17. [PubMed: 10023944]
3. Pyrhonen S, Salminen E, Ruutu M, Lehtonen T, Nurmi M, Tammela T, Juusela H, Rintala E, Hietanen P, Kellokumpu-Lehtinen PL. Prospective randomized trial of interferon alfa-2a plus vinblastine versus vinblastine alone in patients with advanced renal cell cancer. *J Clin Oncol*. 1999; 17(9):2859–2867. [PubMed: 10561363]
4. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, Oudard S, Negrier S, Szczylik C, Kim ST, Chen I, Bycott PW, Baum CM, Figlin RA. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med*. 2007; 356(2):115–124. [PubMed: 17215529]
5. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, Negrier S, Chevreau C, Solska E, Desai AA, Rolland F, Demkow T, Hutson TE, Gore M, Freeman S, Schwartz B, Shan M, Simantov R, Bukowski RM. Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med*. 2007; 356(2):125–134. [PubMed: 17215530]
6. Rini BI, Halabi S, Rosenberg JE, Stadler WM, Vaena DA, Ou SS, Archer L, Atkins JN, Picus J, Czaykowski P, Dutcher J, Small EJ. Bevacizumab plus interferon alfa compared with interferon alfa monotherapy in patients with metastatic renal cell carcinoma: CALGB 90206. *J Clin Oncol*. 2008; 26(33):5422–5428. [PubMed: 18936475]
7. Sternberg CN, Davis ID, Mardiak J, Szczylik C, Lee E, Wagstaff J, Barrios CH, Salman P, Gladkov OA, Kavina A, Zarba JJ, Chen M, McCann L, Pandite L, Roychowdhury DF, Hawkins RE. Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. *J Clin Oncol*.
8. Mabjeesh NJ, Escuin D, LaVallee TM, Pribluda VS, Swartz GM, Johnson MS, Willard MT, Zhong H, Simons JW, Giannakakou P. 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. *Cancer Cell*. 2003; 3(4):363–375. [PubMed: 12726862]
9. Attalla H, Makela TP, Adlercreutz H, Andersson LC. 2-Methoxyestradiol arrests cells in mitosis without depolymerizing tubulin. *Biochem Biophys Res Commun*. 1996; 228(2):467–473. [PubMed: 8920936]
10. Yue TL, Wang X, Loudon CS, Gupta S, Pillarisetti K, Gu JL, Hart TK, Lysko PG, Feuerstein GZ. 2-Methoxyestradiol, an endogenous estrogen metabolite, induces apoptosis in endothelial cells and inhibits angiogenesis: possible role for stress-activated protein kinase signaling pathway and Fas expression. *Mol Pharmacol*. 1997; 51(6):951–962. [PubMed: 9187261]
11. LaVallee TM, Zhan XH, Johnson MS, Herbst CJ, Swartz G, Williams MS, Hembrough WA, Green SJ, Pribluda VS. 2-methoxyestradiol up-regulates death receptor 5 and induces apoptosis through activation of the extrinsic pathway. *Cancer Res*. 2003; 63(2):468–475. [PubMed: 12543804]
12. Fotsis T, Zhang Y, Pepper MS, Adlercreutz H, Montesano R, Nawroth PP, Schweigerer L. The endogenous oestrogen metabolite 2-methoxyoestradiol inhibits angiogenesis and suppresses tumour growth. *Nature*. 1994; 368(6468):237–239. [PubMed: 7511798]
13. Mooberry SL. Mechanism of action of 2-methoxyestradiol: new developments. *Drug Resist Updat*. 2003; 6(6):355–361. [PubMed: 14744499]
14. Lakhani NJ, Sarkar MA, Venitz J, Figg WD. 2-Methoxyestradiol, a promising anticancer agent. *Pharmacotherapy*. 2003; 23(2):165–172. [PubMed: 12587805]
15. Dahut WL, Lakhani NJ, Gulley JL, Arlen PM, Kohn EC, Kotz H, McNally D, Parr A, Nguyen D, Yang SX, Steinberg SM, Venitz J, Sparreboom A, Figg WD. Phase I clinical trial of oral 2-methoxyestradiol, an antiangiogenic and apoptotic agent, in patients with solid tumors. *Cancer Biol Ther*. 2006; 5(1):22–27. [PubMed: 16357512]
16. Takeuchi H, Yamamoto H, Kawashima Y. Mucoadhesive nanoparticulate systems for peptide drug delivery. *Adv Drug Deliv Rev*. 2001; 47(1):39–54. [PubMed: 11251244]

17. Tevaarwerk AJ, Holen KD, Alberti DB, Sidor C, Arnott J, Quon C, Wilding G, Liu G. Phase I trial of 2-methoxyestradiol NanoCrystal dispersion in advanced solid malignancies. *Clin Cancer Res.* 2009; 15(4):1460–1465. [PubMed: 19228747]
18. Young H, Baum R, Cremerius U, Herholz K, Hoekstra O, Lammertsma AA, Pruim J, Price P. Measurement of clinical and subclinical tumour response using [18 F]-fluorodeoxyglucose and positron emission tomography: review and 1999 EORTC recommendations. European Organization for Research and Treatment of Cancer (EORTC) PET Study Group. *Eur J Cancer.* 1999; 35(13):1773–1782. [PubMed: 10673991]
19. Matei D, Schilder J, Sutton G, Perkins S, Breen T, Quon C, Sidor C. Activity of 2 methoxyestradiol (Panzem NCD) in advanced, platinum-resistant ovarian cancer and primary peritoneal carcinomatosis: a Hoosier Oncology Group trial. *Gynecol Oncol.* 2009; 115(1):90–96. [PubMed: 19577796]
20. Sweeney C, Liu G, Yiannoutsos C, Kolesar J, Horvath D, Staab MJ, Fife K, Armstrong V, Treston A, Sidor C, Wilding G. A phase II multicenter, randomized, double-blind, safety trial assessing the pharmacokinetics, pharmacodynamics, and efficacy of oral 2-methoxyestradiol capsules in hormone-refractory prostate cancer. *Clin Cancer Res.* 2005; 11(18):6625–6633. [PubMed: 16166441]
21. Eichelberg C, Heuer R, Chun FK, Hinrichs K, Zacharias M, Huland H, Heinzer H. Sequential use of the tyrosine kinase inhibitors sorafenib and sunitinib in metastatic renal cell carcinoma: a retrospective outcome analysis. *Eur Urol.* 2008; 54(6):1373–1378. [PubMed: 18692304]
22. Dudek AZ, Zolnieriek J, Dham A, Lindgren BR, Szczylik C. Sequential therapy with sorafenib and sunitinib in renal cell carcinoma. *Cancer.* 2009; 115(1):61–67. [PubMed: 19051290]
23. Tamaskar I, Garcia JA, Elson P, Wood L, Mekhail T, Dreicer R, Rini BI, Bukowski RM. Antitumor effects of sunitinib or sorafenib in patients with metastatic renal cell carcinoma who received prior antiangiogenic therapy. *J Urol.* 2008; 179(1):81–86. discussion 86. [PubMed: 17997441]
24. Rini, BI.; Hutson, TE.; Elson, P.; Heng, DY.; Knox, JJ.; Michaelson, D.; Choueiri, TK.; Escudier, B. Clinical activity of sunitinib rechallenge in metastatic renal cell carcinoma; ASCO GU Cancer Symposium (Meeting article) 145 (abstr 319); 2010.
25. Zhou Q, Gustafson D, Nallapareddy S, Diab S, Leong S, Lewis K, Gore L, Messersmith WA, Treston AM, Eckhardt SG, Sidor C, Camidge DR. A phase I dose-escalation, safety and pharmacokinetic study of the 2-methoxyestradiol analog ENMD-1198 administered orally to patients with advanced cancer. *Invest New Drugs.*

Table 1

Patient characteristics

Patient characteristics	N=17	Arm A	Arm B
Age	Number		
Median age: 58 years		61 years	54 years
Range: 42–89		44–89	42–66
Gender			
Male	11 (65%)	6 (35%)	5 (29%)
Female	6 (35%)	4 (24%)	2 (12%)
Performance Status (ECOG)			
0	7 (41%)	3 (18%)	4 (24%)
1	8 (47%)	5 (29%)	3 (18%)
2	2 (12%)	2 (12%)	0 (0%)

ECOG Eastern Cooperative Oncology Group

Table 2

Reasons for discontinuation

Reason	All	Arm A	Arm B
Disease Progression	10	5	5
Adverse Event	3	3	0
Physician discretion	2	2	0
Patient and physician discretion	1	0	1
Noncompliance	1	0	1

Table 3

Toxicities by type and severity

Toxicities	Grade 1	Grade 2	Grade 3	Grade 4	Total
Fatigue	3	5	2	0	10
Diarrhea	7	2			9
Increased AST	1	3	3		7
Anorexia/decreased weight	5	1			6
Arthralgia/myalgia	4	2			6
Increased ALT	1	0	3	1	5
Anemia/decreased Hemoglobin		3	1		4
Constipation	1	2			3
Dysgeusia	3				3
Pruritus	3				3
Nausea	2				2
Abdominal pain		2			2
Hypothyroidism		1			1
Pleural effusion			1		1

AST aspartate aminotransferase, ALT alanine aminotransferase

Table 4

Best response by RECIST

Best Response by RECIST	All	Arm A	Arm B
Complete or partial response	0	0	0
Stable disease	7	4	3
Disease progression	5	3	2
Unknown or unevaluable	5	4	1

Table 5

Changes from baseline in maximum SUV

	Mean	Standard deviation	Median	Range	p-value [^]
Arm A (N=6)					
Baseline to Week 6	-4%	58%	-12%	-84%—97%	0.438
Arm B (N=5)					
Baseline to Week 4	-12%	20%	-7%	-45%—24%	0.130
Baseline to Week 6	4%	22%	1%	-18%—41%	0.353

[^] paired t-test (one-sided)