α-Difluoromethylornithine as Treatment for Metastatic Breast Cancer Patients

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ABSTRACT

DFMO (α-difluoromethylornithine) is an oral irreversible inhibitor of ornithine decarboxylase, the first rate-limiting enzyme in polyamine synthesis. DFMO has been shown to have antiproliferative effects against several human cancers, and some studies have suggested that DFMO may have pro-apoptotic and anti-invasive properties as well. DFMO is well tolerated with minimal toxicity but has been associated with ototoxicity with prolonged daily administration. We conducted a Phase I/II tolerability, pharmacokinetic, and efficacy study of high-dose DFMO in metastatic breast cancer patients. Twenty-one patients were treated with 4800 mg of DFMO p.o. three times a day for 14 days, followed by a 2-week drug holiday on a 28-day cycle. Urinary polyamine and blood DFMO levels were measured at multiple time points during therapy. High-dose DFMO was well tolerated, and no clinically significant ototoxicity was noted. No patient achieved an objective antitumor response; however, one patient with heavily pretreated liver metastases has achieved stable disease for 18 months to date on DFMO. Putrescine, spermine, and spermidine urinary levels were suppressed with DFMO treatment and remained low during the 2-week drug holiday. High-dose DFMO on a schedule of 2 weeks on treatment followed by 2 weeks off is well tolerated, is not associated with ototoxicity, and leads to sustained suppression of urinary polyamine levels. Although not an active cytotoxic agent for metastatic breast cancer, the intriguing prolonged growth arrest of liver metastases in one patient highlights the potential clinical growth inhibitory properties of DFMO. We believe that DFMO is worthy of study as adjuvant therapy in primary breast cancer patients and as a chemopreventive agent.

INTRODUCTION

The mainstays of treatment for metastatic breast cancer remain hormonal therapies and chemotherapy. These cytotoxic therapies modestly prolong the survival of metastatic breast cancer patients (1); however, the primary goals of treatment are preventing and palliating tumor-related symptoms. There is increasing interest in developing therapies for metastatic disease that can control the growth of breast cancer while maintaining patients' performance status and quality of life.

Polyamines (*e.g.*, putrescine, spermidine, and spermine) are small, aliphatic amines involved in cellular proliferation and differentiation (2). ODC[2] is the first rate-limiting enzyme involved in polyamine synthesis, and increased activity of this enzyme with accumulation of intracellular polyamines has been shown to play an important role in the development and growth of many cancers, including breast cancer (3, 4). There is increasing evidence that polyamines support breast cancer cell proliferation (5) as well as tumor progression to a hormone-independent, aggressive phenotype (6). Manni *et al.* (7) recently have shown that increased ODC activity in primary breast cancer specimens was an independent negative prognostic factor and was superior to lymph node status in predicting disease-free and overall survival.

DFMO is an oral irreversible inhibitor of ODC and acts as a "suicide substrate" for ODC. DFMO interferes with polyamine biosynthesis, and this depletion of polyamines inhibits DNA synthesis by reducing the rate of DNA elongation (8). Inhibition of polyamine biosynthesis by DFMO has been shown to thwart proliferation of both hormone-dependent and -independent breast cancers *in vitro* and *in vivo* (9, 10). In mice bearing estrogen receptor-positive MCF-7 human breast cancer cells, DFMO treatment inhibited tumor growth by 73% (11). Human studies of DFMO have demonstrated reductions in polyamine content of rectal mucosa cells after DFMO treatment in patients with a history of adenomatous colorectal polyps (12). In a recent Phase II trial, Levin *et al.* (13) treated 98 recurrent glioma patients with oral DFMO at 3.6 g/m^2 every 8 h on days 1–14, 22–35, and 43–56 until disease progression. Antitumor activity was seen in 45% of patients with anaplastic gliomas and 17% of patients with glioblastoma multiforme. The major toxicities reported were ototoxicity [grade 3 (defined as patient perception of hearing loss), 14%], mild diarrhea, and leukopenia.

Because of the demonstrated safety of high-dose DFMO and the promising antiproliferative effects of DFMO against human breast cancer *in vivo*, we conducted a Phase I/II study of DFMO as treatment for patients with metastatic breast cancer.

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[2] The abbreviations used are: ODC, ornithine decarboxylase; DFMO, α-difluoromethylornithine; CT, computed tomography; dc-SAM, decarboylated *S*-adenosylmethionine; HPLC, high-performance liquid chromatography; DFI, disease-free interval.

Our objectives were to study whether 2 weeks of high-dose DFMO treatment followed by a 2-week drug holiday was tolerable and not associated with hearing loss in breast cancer patients, to investigate how this drug schedule would affect urinary polyamine levels, and to study antitumor activity.

PATIENTS AND METHODS

The primary objectives included evaluating the safety of high-dose DFMO in this patient population and monitoring changes in urinary levels of polyamines and decarboxylated 5-adenosylmethionine. The secondary objective was to determine the objective tumor response rate (complete and partial responses) of DFMO in hormone-refractory advanced breast cancer patients. This study was designed as a two-step study in which 20 patients were treated initially with DFMO. If one objective response was seen in the first 20 patients, an additional 15 patients would be enrolled and treated with the same DFMO regimen. Patients who were at least 18 years of age and who had documented metastatic breast cancer and at least one site of bidimensionally measurable disease were eligible for treatment with DFMO. Patients were eligible regardless of their estrogen receptor status; estrogen receptor-positive patients must have had evidence of progressive disease with at least one prior hormonal therapy. Patients may have been treated with previous chemotherapy in the adjuvant or metastatic setting. Patients were required to have a Karnofsky performance status of at least 70% and to have a serum creatinine $\leq$2.0 mg/dl, serum bilirubin $\leq$2.0 mg/dl as well as a granulocyte count of at least 1500/μl and platelet count of at least 100,000/μl. Patients with childbearing potential were required to have a negative serum pregnancy test within 7 days prior to study entry. All patients gave written informed consent as part of this Institutional Review Board-approved study.

DFMO was supplied by Ilex Oncology, Inc. (San Antonio, TX) as a clear, colorless, aqueous solution containing 200 mg/ml. In this open-label study, the patients were treated with 4800 mg of DFMO three times daily p.o. for 2 weeks followed by a 2-week drug holiday on a 28-day treatment cycle. The 2-week drug holiday was chosen based on promising data from Levin showing that a 2-week drug holiday may decrease ototoxicity[3] and based on data suggesting that polyamines may be repleted in cochlear hair cells during a 2-week drug holiday (14). This dose was based on 3000 mg/m^2, using an average body surface area of 1.6 m^2 with a total volume per dose of 24 ml. Concomitant treatment with any other antitumor agents except for pamidronate for patients with bony metastasis was not allowed.

Patients underwent complete staging evaluation with imaging studies including chest X-ray, bone scan, bone survey (in case of positive bone scan), and CT scans. The patients also had baseline blood studies drawn to assess their complete blood count with a differential and complete chemistry profile as well as a CA-27.29 tumor marker serum level. Patients also underwent baseline audiometry testing.

While on study, patients had complete blood counts with differential and complete chemistry profiles repeated monthly as well as physical examinations. Repeat audiometry testing was planned only in the event of the development of tinnitus (grade 2) or patient perception of hearing loss (grade 3). Patients underwent repeat tumor assessments according to their baseline evaluation techniques every 2 months. CA-27.29 tumor marker serum levels were also measured every 2 months in patients whose levels were elevated at baseline.

In 11 patients enrolled, DFMO blood levels were measured pretreatment and at 2, 4, 6, and 8 h after the initial dosing (day 1). These pharmacokinetic blood levels were measured on days 14, 28, 42, and 56 for as long as the patients remained on treatment. Nine of the 11 patients also collected 24-h urine specimens before and at various intervals on treatment for evaluation of polyamine levels (putrescine, spermidine, and spermine). dc-SAM in the 24-h urine specimens was also measured in four of these patients. dc-SAM was measured to further document the efficacy of our treatment protocol to inhibit ODC activity. As a result of ODC suppression by DFMO, a compensatory rise in *S*-adenosylmethionine decarboxylase occurs, leading to increased production of dc-SAM.

Tumor responses (partial and complete) were assessed using standard criteria (15). NCI Common Toxicity Criteria were used to evaluate and grade adverse events.

DFMO and Polyamine Levels.

Polyamine Determinations. Putrescine, spermidine, and spermine levels in urine were measured by HPLC after partial purification of the samples with a Waters Silica plus SepPak cartridge. HPLC was performed with an ISCO model 2350 using a C_{18} Bondapak radial cartridge and an acetonitrile gradient at a flow rate of 1.7 ml/min. The polyamines were detected using fluorescence after postcolumn derivatization with *o*-phthalaldehyde. Results are expressed in nmol/ml.

dc-SAM. dc-SAM was determined by HPLC and fluorescence after elution with an acetonitrile gradient on a Spherisorb 250 $\times$ 4.6 mm ODS Column (C_{18}) following derivatization with chloroacetaldehyde. The dc-SAM standards were a gift from Keijiro Sameljima, Josai University, Tokyo, Japan. Results are expressed in ng/ml.

DFMO. DFMO was measured by HPLC and fluorescence using a Spherisorb 250 $\times$ 4.6 mm ODS Column (C_{18}) and a acetonitrile gradient. Results are expressed as μg/ml using standards supplied by Ilex Oncology, Inc.

Statistical Analysis

Repeated measures ANOVA was used to assess changes in urinary polyamines and dc-SAM levels. To better meet distributional assumptions of the analysis, all data were analyzed on the log scale. When ANOVA revealed an overall significant effect, pairwise comparisons were made; significant differences are reported in the figure legends.

RESULTS

Twenty-one patients were entered on study between December 1997 and August 1998 from multiple sites within the US Oncology network. The patients' characteristics are summarized

[3] Victor Levin, personal communication.

Table 1 Patient characteristics

Number of patients	21
Median age (years)	55 (37–80)[a]
Median Karnofsky performance status	90%
Estrogen receptor status	
Positive	17
Negative	3
Unknown	1
Prior therapy	
Median number of regimens	
Chemotherapy	3 (0–7)[a]
Hormonal therapy	3 (0–4)[a]
Metastatic disease	
Median number of sites	2
No. of patients with metastatic sites	
Bone	10 (48%)
Liver	8 (38%)
Lung	10 (48%)
Soft tissue	7 (33%)

[a] Range.

Table 2 Toxicities during all cycles of DFMO treatment (n = 21 patients)

	Grade			
Toxicity	1	2	3	4
Diarrhea	2	3	2	
Nausea	4	1		
Heartburn	2	3		
Fatigue	3	1		
Hot flashes		1		
Decreased hearing		1		
Allergic reaction	1			
Anorexia	2			
Vomiting	1			
Stomatitis	1			
Alopecia	1			
Anemia	8	4		
Thrombocytopenia		2		
Neutropenia		1		

in Table 1. The median age of the patients was 55 years (range, 37–80 years). Five patients were <50 years of age, whereas five were between the ages of 72 and 80. Three patients' breast cancers were estrogen receptor negative, 1 was estrogen receptor unknown, and 17 were estrogen receptor positive as assessed on their primary breast cancers. The median DFI from diagnosis of primary breast cancer to the development of metastatic disease for the 21 patients was 37 months (range, 14–158 months). Three patients had received no prior chemotherapy but had received prior hormonal therapy. Five patients had received only one prior chemotherapy regimen, with four of these receiving only prior adjuvant therapy. All patients had bidimensionally measurable disease and had a median of two sites of metastatic disease.

The 21 patients were treated with a median of 2 cycles (range, 1–17) of DFMO. All 21 patients were evaluable for toxicity. Three patients received three or more cycles, 10 received two, and 5 received one cycle. Only one patient received more than 3 cycles of therapy (18 cycles). Three patients were removed from the study prior to completing one cycle of therapy; one because of noncompliance, one because of an allergic reaction to DFMO (hives), and one because of concomitant treatment with another anticancer agent.

Toxicity. Only one patient was removed from study for toxicity (allergic reaction with diffuse hives). One additional patient was unable to tolerate the full dose of DFMO because of grade 3 diarrhea with dehydration. Her dose was reduced to 3600 mg of DFMO three times a day. She then received an additional two cycles, which were associated with the development of grade 2 diarrhea.

The toxicities associated with DFMO over the course of therapy are summarized in Table 2. The most common nonhematological toxicities observed were diarrhea, nausea, heartburn, and fatigue. Hematological toxicity was observed but was mild. Twelve patients developed mild anemia, 8 patients grade 1 anemia, and 4 patients grade 2 anemia. No patient required RBC transfusions. Two patients developed grade 1 thrombocytopenia. There was no significant leukopenia or any changes in the complete chemistry profile. Three patients had baseline audiometry studies that showed mild loss of hearing in the middle to high frequency range. No worsening in these patients' hearing was observed with DFMO. A mild deterioration in hearing at the upper frequencies (5–10 decibels) was noted on repeat audiometry in the one patient who has received 18 cycles of DFMO. This patient was asymptomatic without hearing loss or tinnitus. After 7 months on DFMO, however, repeat audiometry was performed to monitor her hearing. Her hearing was felt to be essentially normal even with the observed mild decrease at the upper frequencies. She remains asymptomatic with clinically normal hearing after 18 months on DFMO. No other patients complained of changes in their hearing on DFMO.

Antitumor Activity. None of the 18 patients who were evaluable for an antitumor response achieved an objective response. Fifteen patients developed progressive disease after one to three cycles of DFMO. Two patients achieved stable disease during two and three cycles of DFMO, respectively, and then developed progressive disease. Of interest, however, is the one patient who has achieved stable disease in her extensive liver metastases for 18 months and is still receiving DFMO. This patient is a 40-year-old woman with widely metastatic disease to her bones and liver (not previously biopsied) who had been treated previously with two hormonal therapies and three chemotherapy regimens for metastatic disease including CAF (cyclophosphamide, doxorubicin, and 5-fluorouracil), docetaxel, and capecitabine. When the patient began treatment with DFMO in December 1997, her abdominal CT scan showed innumerable low-density metastatic lesions within both lobes of her liver, the largest measuring 5 × 5 cm, which represented progression of her liver disease compared with the September 1997 CT scans (tumor volume of the three liver indicator lesions, 33.7 cm^2 in September and 42.3 cm^2 in November 1997). While on DFMO, her abdominal CT scans from April, June, August, October, and December 1998 and March 1999 have shown stable liver disease with no change in the bidimensional measurements of the indicator liver lesions (*e.g.*, the tumor volume of the three liver lesions in October 1998 was 32 cm^2). Her bone scan has also been stable over this interval. In addition, her CA-27.29 level decreased from a high of 1206 in April 1998 to 494 in October 1998 and was 460 in March 1999.

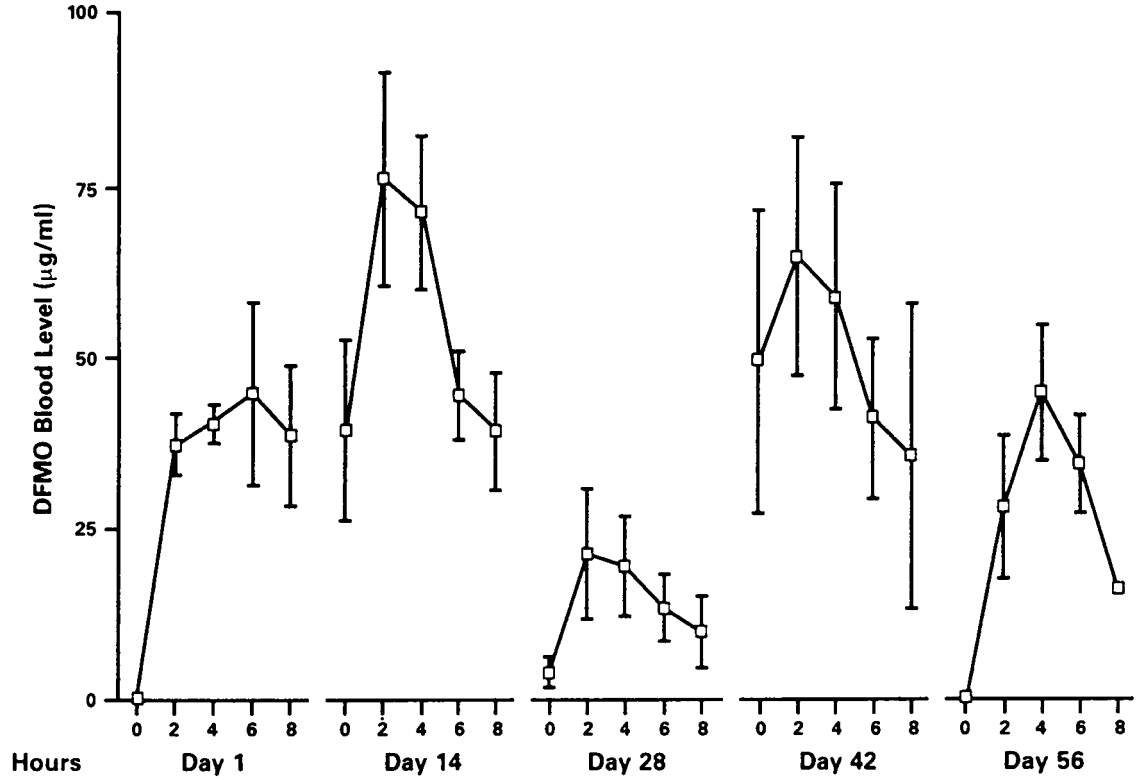

Fig. 1 DFMO blood levels after a dose of the drug given at the beginning (days 1, 28, and 56) or at the end (days 14 and 42) of a treatment cycle. The numbers of determinations were 11 (day 1), 8 (day 14), 6 (day 28), 5 (day 42), and 3 (day 56). Data represent means; *bars*, SD.

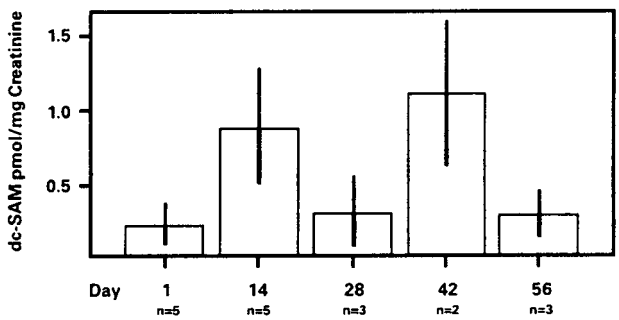

Fig. 2 Levels of dc-SAM in 24-h urine specimens collected at the times indicated. Data represent means; *bars*, SD.

DFMO and Polyamine Levels. Fig. 1 shows the 8-h profiles of DFMO blood levels following a dose of the drug given at the beginning (days 1, 28, and 56) or at the end (days 14 and 42) of a 14-day cycle. As expected, after 14 days off DFMO, blood levels of the drug had essentially declined to undetectable values (day 28 pre, day 56 pre). The higher pretreatment DFMO blood level observed on days 14 and 42 obviously reflects the effect of the previous dosing, 8 h earlier.

Administration of DFMO according to our protocol had a definite effect on the polyamine milieu of the host. We clearly demonstrated an increase in the 24-h urine levels of dc-SAM after each cycle of DFMO administration (Fig. 2, *days 14* and *42*; $P < 0.05$, day 1 *versus* day 14). This is a reflection of the compensatory increase in *S*-adenosylmethionine decarboxylase resulting from effective blockade of ODC.

The effect of DFMO treatment on urinary polyamine levels is illustrated in Fig. 3. The putrescine concentration was significantly reduced (Fig. 3*A*). It is remarkable that despite discontinuation of DFMO for 2 weeks, putrescine levels continued to remain suppressed, although to a lesser extent than during treatment (Fig. 3*A*, *days 28* and *56*). Spermidine concentrations were reduced to a lesser degree (not statistically significant) and still remained below baseline during the 2-week drug holiday (Fig. 3*B*). Spermine levels were very low (Fig. 3*C*, note change in scale) and tended to follow a similar pattern.

DISCUSSION

This Phase I/II study represents the first clinical evaluation of high-dose DFMO in metastatic breast cancer. The high-dose DFMO schedule used in this trial, 4800 mg three times a day for 2 weeks followed by a 2-week drug holiday, was associated with very mild toxicity and only 1 of 21 patients withdrew from the study because of toxicity. Importantly, despite the high doses of DFMO administered, no clinically apparent diminution in patients' hearing was observed, in contrast to the high-dose DFMO study by Levin *et al.* (13) where patients received high-dose DFMO for 2 weeks followed by a 1-week drug holiday. However, because only three patients were treated for at least 3 months with

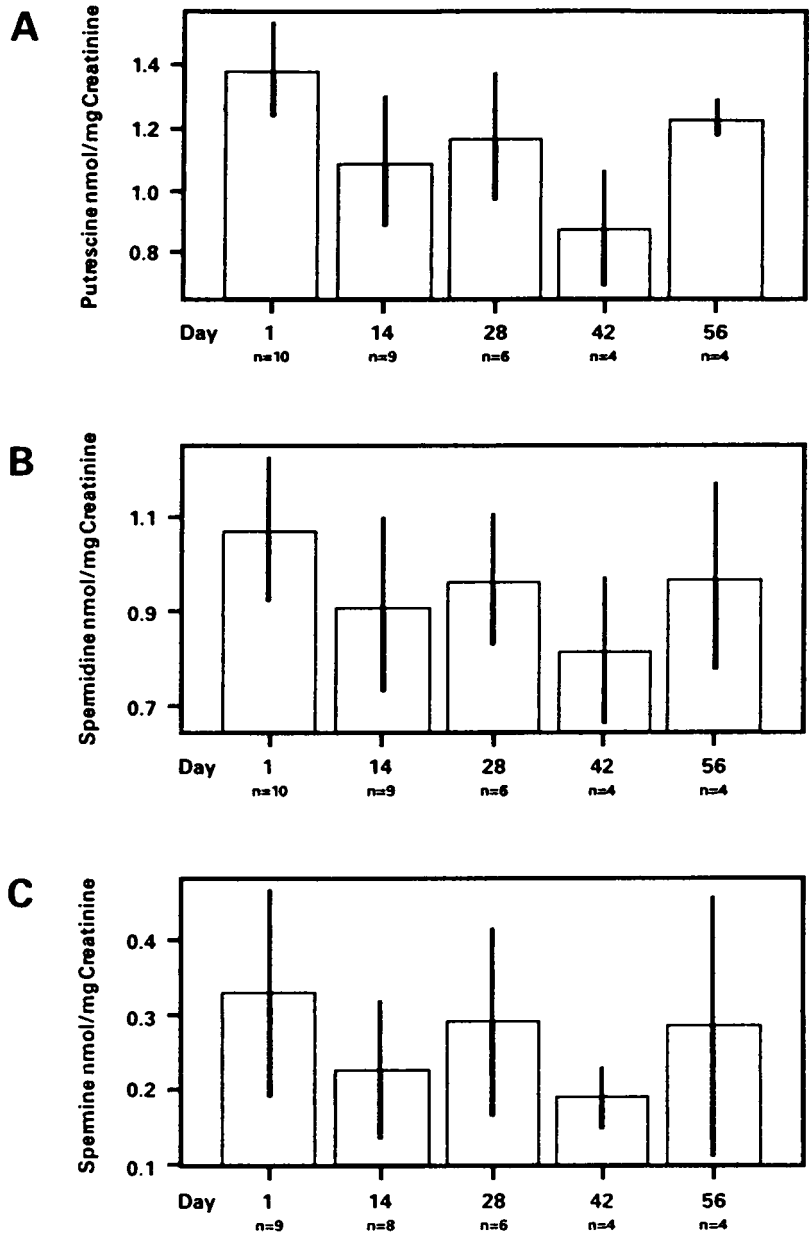

Fig. 3 Levels of putrescine (*A*), spermidine (*B*), and spermine (*C*) in 24-h urine specimens collected at the times indicated. Data represent means; *bars*, SD. Statistically significant differences in *panel A*: day 1 *versus* day 42, $P < 0.01$; day 42 *versus* day 56, $P < 0.02$.

DFMO, we cannot conclude at this time that the 2-week drug holiday each month was effective in reducing the incidence of significant ototoxicity that was observed by Levin *et al.* (13) in their study of high-dose DFMO in glioma patients. It is of interest in our trial, however, that the one patient who has received 18 months of high-dose DFMO treatment with a 2-week drug holiday each month has developed only very mild hearing loss at the upper frequencies that was not clinically significant. Levin[3] conducted a second study of high-dose DFMO in 500 patients with glioma, administering 3600 mg three times a day for 2 weeks followed by 2 weeks off. With >50% of patients receiving 6 months of DFMO, only one patient developed clinical hearing loss (grade 3 ototoxicity), and 4% of patients developed tinnitus. Thus, our findings of lack of ototoxicity associated with high-dose DFMO for 2 weeks followed by 2 weeks off are consistent with those of Levin.[3]

The 2-week DFMO drug holiday likely allows repletion of polyamines in normal tissues, including the cochlear hair cells, thereby avoiding ototoxicity (14). However, because DFMO is an irreversible inhibitor of ODC and because the decrease in urinary polyamine levels is sustained during the 2-week drug holiday, the drug holiday may not substantially decrease the potential antitumor effects of DFMO.

DFMO was not found to be an active antitumor agent in treating metastatic breast cancer patients. Even in the seven

patients who had received either no prior chemotherapy (three patients) or only prior adjuvant chemotherapy (four patients), no objective tumor responses were seen with DFMO. In this study, the overall treated population did not benefit from DFMO with the median time to disease progression of only 2 months. It should be noted, however, that despite the sustained suppression of urinary polyamine levels (even during the drug holiday period), the decrease induced by DFMO treatment was only moderate and was statistically significant only in the case of putrescine. It is quite likely that the small number of patients in some of the pharmacokinetic groups may have precluded the finding of a more significant effect. Nevertheless, our study again emphasizes the well known difficulty in inducing optimal suppression of polyamines "*in vivo*" with DFMO because of the multiple compensatory pathways present in the regulation of polyamine metabolism. It is possible that combination of DFMO treatment with a low polyamine diet may achieve a superior suppressive effect.

Of significant interest is the 40-year-old woman with extensive liver and bony metastasis who has achieved 18 months to date of stable disease with DFMO after progression of her disease through three prior chemotherapy and two prior hormonal therapy regimens. Although the median DFI of 37 months for patients treated with DFMO on this study suggests some selection of patients with more indolent disease, this patient's DFI was only 21 months. Although depletion of polyamine levels in human breast cancer cells has been shown to induce programmed cell death (16), the absence of a decrease in her tumor volume argues against increased apoptosis as the mechanism for her prolonged stable disease. Increased ODC protein levels are associated with enhanced tumor invasiveness in murine tumors, which suggests that ODC inhibitors such as DFMO may decrease local metalloproteinase activity or other molecules that contribute to the invasive phenotype (17–19). It has been suggested that the observed antimetastatic activity of DFMO in an animal model may be due to inhibition of angiogenesis (20). It is interesting to speculate about whether possible anti-invasive and/or antiangiogenic effects of DFMO in this patient with aggressive disease may explain the striking clinical finding of her prolonged stable liver disease.

The plasma steady-state levels of DFMO measured on days 14 and 42 are in accordance with other reported pharmacokinetic studies (21, 22). Several chemoprevention studies of DFMO have demonstrated significant decreases in polyamine levels in rectal mucosa, precancerous lesion of the cervix, and in Barrett's mucosa with lower daily doses (0.25–3.0 g/m^2/day; Refs. 12, 23, 24). Our study has shown that high-dose DFMO administered for 2 weeks of a 4-week cycle decreases urinary polyamine levels to a degree comparable to that reported by Pendyala *et al.* (25) in a drug-escalation phase 1 chemoprevention trial where the maximum dose of DFMO was 3200 mg/m^2/day given continuously. Our study shows that it is possible to maintain suppression of urinary polyamines during the 2-week drug holiday, thus supporting the feasibility of an intermittent regimen, which may be ultimately less toxic. This prolonged although moderate suppression of urinary polyamine levels following high-dose DFMO administration has not been reported previously and suggests that this schedule of high-dose DFMO is associated with prolonged inhibition of ODC. Meyskens *et al.* (12) have shown that the polyamine levels in rectal mucosal biopsies had returned to normal 3 months after low-dose DFMO was stopped. As has been demonstrated previously by Haegele *et al.* (26), the urinary excretion of dc-SAM increased in our study with DFMO treatment, reflecting suppression of polyamine synthesis by ODC.

This study has demonstrated the short-term tolerability of high-dose DFMO with a monthly 2-week drug holiday, sustained decreases in urinary polyamine levels with this dose and schedule, and intriguing clinical activity in a patient with aggressive breast cancer. We currently are administering preoperative DFMO to patients who have been diagnosed with ductal carcinoma *in situ* or early invasive breast cancer by core needle biopsy to study changes in breast cancer cell proliferation, apoptosis, and metalloproteinase expression before and after DFMO administration. Plans are also underway to study high-dose DFMO with a 2-week drug holiday on a 4-week cycle as adjuvant therapy after standard chemotherapy in high-risk node-positive breast cancer patients. It is possible that the moderate suppression of polyamines observed in this study, although not sufficient to induce remission of advanced disease, may be quite effective in the adjuvant or chemopreventive setting. This possibility is suggested by the lower doses of DFMO required to inhibit rodent mammary carcinogenesis than that needed to induce regression of established tumor (27, 28).

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