A Phase II Study of the Polyamine Analog N^1,N^{11}-Diethylnorspermine (DENSpm) Daily for Five Days Every 21 Days in Patients with Previously Treated Metastatic Breast Cancer

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ABSTRACT

Purpose: **Polyamines are ubiquitous intracellular polycationic molecules essential for cell growth and differentiation. Polyamine analogs down-regulate ornithine decarboxylase, induce spermidine/spermine N^1-acetyltransferase, deplete natural polyamine pools, inhibit growth, and induce programmed cell death in breast cancer models. This study evaluated the activity of the first-generation analog DENSpm in women with metastatic breast cancer.**

Experimental Design: **The overall accrual goal was 34 patients (30 evaluable) in a two-stage design. The second stage of accrual was to proceed if ≥2 among first 15 evaluable patients were progression free at 4 months. The primary objective was to determine whether ≥20% of metastatic breast cancer patients treated with DENSpm as second- or third-line therapy remained progression free after 4 months.**

Results: **Sixteen patients (median age, 52 years; range, 34–65; median performance status, 1; range, 0–1) enrolled in the first stage received 43 cycles (median, 2; range, 1–6) of 100 mg/m^2 DENSpm as a 15-min infusion i.v. on days 1–5 every 21 days. All 16 patients were evaluable for toxicity; 15 were evaluable for response. All patients had disease progression by 4 months, and the study closed after the first stage of accrual. The main toxicities included grade 1–2 abdominal pain, transient perioral numbness, nausea, and grade 1 thrombocytopenia. Two patients had grade 3 abdominal pain during cycle 2 infusion: one was hospitalized, and another was subsequently retreated at 80% dose without pain recurrence.**

Conclusions: **Although this dose and administration schedule of DENSpm was quite tolerable, no evidence of clinical activity was detected. Encouraging preclinical activity of polyamine analogs alone and in combination with cytotoxic drugs supports the continued evaluation of newer-generation polyamine analogs for the treatment and prevention of breast cancer.**

INTRODUCTION

Although many patients diagnosed with metastatic breast cancer benefit at first from systemic therapy with endocrine and chemotherapy strategies, most will develop progressive disease that is refractory to standard therapy. Thus, there is much interest in identifying novel therapeutic targets. Polyamines are ubiquitous intracellular positively charged aliphatic amines, and their association with cancer was first reported in the early 1970s (1). Natural polyamines such as putrescine, spermidine, and spermine are essential for cell growth and differentiation of normal and malignant cells (2). Increased levels of polyamines have been observed in many cancers compared with normal tissue; this observation has led to the exploration of the polyamine enzymatic pathway as a potential therapeutic target (Fig. 1; Ref. 3).

The first such agent, DFMO[3] (or eflornithine), irreversibly inhibits ODC, the first enzyme in the polyamine biosynthetic pathway, and leads to the depletion of putrescine and spermidine (3). It is used for the treatment of African sleeping sickness (4) and is also under investigation as a chemopreventive agent (5). A small study in women with breast cancer suggested a possible correlation between increased tumoral ODC activity and decreased survival (6). O'Shaughnessy *et al.* (7) treated 21 patients with metastatic breast cancer with 4800 mg of oral DFMO three

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[3] The abbreviations used are: DFMO, α-difluoromethylornithine; ODC, ornithine decarboxylase; SSAT, spermidine/spermine N^1-acetyltransferase; PAOh1/SMO, polyamine oxidase h1; CNS, central nervous system; ECOG, Eastern Cooperative Oncology Group; PS, performance status; CT, computerized tomography; CR, complete response; PR, partial response; MTD, maximum tolerated dose; DLT, dose-limiting toxicity.

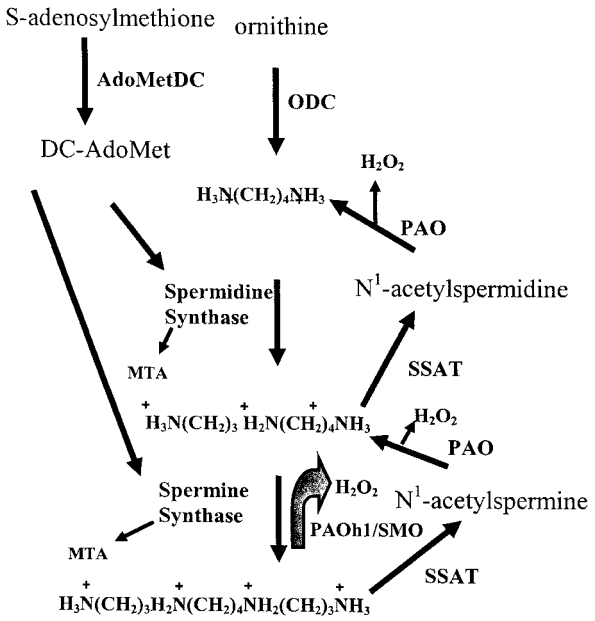

Fig. 1 The polyamine metabolic pathway. *AdoMetDC*, s-adenosylmethionine decarboxylase; *DC-AdoMet*, decarboxylated AdoMet; *MTA*, methylthioadenosine; *PAO*, N^1-acetylpolyamine oxidase.

times a day for 14 days of a 28-day cycle. Although no patient had an objective response, one patient with heavily pretreated liver metastases achieved stable disease for 18 months, and urinary levels of putrescine, spermine, and spermidine were suppressed, suggesting biological activity (7). Levin *et al.* (8) showed a possible survival advantage with the addition of DFMO to a standard chemotherapy regimen in patients with anaplastic gliomas. These preliminary studies with DFMO and other single enzyme inhibitors offer proof of principle that the inhibition of the polyamine pathway could serve as a potential therapeutic target; but in all likelihood, there will be a need to act on several levels of the pathway.

Polyamine analogs down-regulate synthetic enzymes such as ODC and S-adenosylmethionine decarboxylase and stimulate the catabolic enzyme SSAT and PAOh1/SMO (9, 10). This multistep interference with the polyamine pathway depletes the pools of natural polyamines and leads to the intracellular accumulation of analogs that do not support cell growth and differentiation. The first generation of polyamine analogs consisted of symmetrical terminally alkylated analogs of either spermine or spermidine, such as N^1, N^{11}-bis(ethyl)norspermine (DENSpm or BENSpm), bis(ethyl)spermine (DESpm or BESpm), and bis(ethyl)homospermine (DEHSpm or BEHSpm). These analogs demonstrated evidence of antitumor activity in several preclinical tumor models, such as melanoma and lung cancer (11–16). One analog, DESpm, is a potent growth inhibitor of both estrogen receptor-positive and -negative human breast cancer cell lines, and its growth-inhibitory effects were not diminished by the acquisition of resistance to doxorubicin or antiestrogens (17). Similar growth inhibition against MDA-MB-468 and MCF-7 human breast cancer cell lines was seen with another analog, DENSpm, with IC_{50} achievable in serum in Phase I human trials (1–10 μM after 120 h of chronic exposure; Ref. 18). In addition, DENSpm is a particularly potent inducer of SSAT, the rate-limiting step in the two-step eukaryotic catabolism of polyamines. Thus, it was selected for clinical development.

Preclinical toxicology studies identified hypotension with rapid infusions, gastrointestinal mucositis, and lethargy, but not myelosuppression, as potential toxicities of DENSpm. On the basis of promising preclinical activity, several human Phase I trials were conducted (19, 20). A Phase I trial in our institution evaluated the effects of DENSpm given as a 15-min daily i.v. infusion for 5 consecutive days and repeated every 21 days (one cycle; Ref. 21). Nine dose levels from 15.6 to 145 mg/m^2/day i.v. (doses reflecting the free base component of DENSpm; 1 mg of free base = 1.59 mg of salt) were explored in 29 patients, who received 83 complete courses of therapy. DLTs included gastrointestinal bleeding, diarrhea, nausea, abdominal pain, asthenia, and CNS toxicity. The MTD was 116 mg/m^2/day i.v. on days 1–5 (free base). A short half-life of up to 3.7 h and a disproportional increase in maximum serum concentrations *versus* dose increase were observed. Transient, reversible elevations of creatinine occurred, but no patient developed neutropenia, thrombocytopenia, infectious complications, or immunosuppression at any of the dose levels. A Phase II dose and schedule of 100 mg/m^2/day (free base) on days 1–5 by i.v. infusion every 21 days was recommended.

On the basis of these data, we conducted an open-label, single-center, Phase II study of DENSpm in patients with measurable metastatic breast cancer who had received one or two prior chemotherapy regimens. The primary objective of this study was to estimate the proportion of patients who were free of disease progression at 4 months. Secondary objectives were to evaluate toxicities of DENSpm and to determine the overall response rate, duration of response, survival, and clinical benefit.

PATIENTS AND METHODS

Patient Eligibility. Eligible patients were women >18 years of age with histologically confirmed adenocarcinoma of the breast, evidence of metastatic progression within 3 months before study registration, and at least one site of measurable disease. Patients could have received a minimum of one, but no more than two, prior chemotherapy regimens for metastatic disease, including cytotoxic agents and/or trastuzumab. Continuation (but not initiation) of bisphosphonates was permitted. Previous hormonal therapy for metastatic disease, prior adjuvant systemic therapy of any type, and prior radiation therapy were allowed if completed ≥28 days before starting protocol therapy. Patients with a previous diagnosis of malignancy other than invasive breast cancer were allowed to enter only if they were disease free for >5 years, with the exception of curatively treated basal or squamous cell carcinoma of the skin or carcinoma *in situ* of the cervix. Patients were required to have an ECOG PS of ≤2 and normal laboratory parameters (serum creatinine, ≤1.5 mg/dl; absolute neutrophil count, ≥1,500/mm^3; platelet count, ≥100,000/mm^3; aspartate aminotransferase, ≤2 times the upper limit of normal; total bilirubin, ≤1.5 mg/dl). Pregnant or lactating women were not eligible, and women of childbearing potential had to agree to use an effective

method of contraception. Patients with a known history of CNS metastases or any active serious medical illness were not eligible. The study was conducted in accordance with the Declaration of Helsinki. The protocol document and informed consent form were approved by the Institutional Review Board of the Johns Hopkins University and by the Surgeon General's Human Subjects Research Review Board, as required by the United States Army Medical Research and Materiel Command (Fort Detrick, MD). All patients were required to provide written informed consent.

Dosage and Drug Administration. DENSpm was provided free of charge for this study by Parke-Davis Pharmaceutical Research. Parke-Davis was then acquired by Pfizer, Inc., and the license for this compound was transferred to GelTex Pharmaceuticals Inc. (now Genzyme Corporation). The drug was supplied as a lyophilized powder packaged in 10-ml clear glass vials containing 150 mg of free base. When reconstituted with 5 ml of Water for Injection (USP), the resulting solution contained 30 mg/ml DENSpm (free base) and was chemically and physically stable for 96 h at room temperature. The appropriate dose was withdrawn and diluted further in 50 ml of normal saline and infused over 15 min into a peripheral vein once a day for 5 consecutive days every 21 days (one cycle). Toxicity was evaluated using the National Cancer Institute Common Toxicity Criteria version 2.

Pretreatment and Follow-up Studies. At baseline, investigators identified one to three bidimensionally measurable lesions within each patient. The same disease sites were reassessed during the treatment phase using the same methodology. Evaluations were conducted after every other cycle of treatment. These consisted of a minimum of a CT scan of the chest and abdomen every other cycle and a bone scintigraphy every fourth cycle.

Treatment Plan. The initial dose of DENSpm was fixed for all patients at 100 mg/m^2/day (free base). Patients were premedicated with a 5-hydroxytryptamine$_3$ receptor antagonist before each daily infusion. If a course of treatment were interrupted for any reason, the remaining daily doses (maximum of five) were to be completed by day 7 or any remaining doses would be skipped. The next cycle of DENSpm would begin no sooner than 21 days after day 1 of the previous cycle, assuming that all associated nonhematological toxicity had recovered to grade $\leq$1 (or to the patient's baseline) with normal neutrophil, platelets, and serum creatinine values. If these parameters were not met, the start of the next course would be delayed by weekly intervals.

The daily dose of DENSpm would be reduced permanently by 20% if the following occurred in a previous course: grade $\geq$2 treatment-related CNS adverse event; any grade $\geq$3 treatment-related adverse event (except grade 3 nausea and vomiting in the absence of antiemetics); less than five doses given because of treatment-related adverse event; platelet count $<$50,000/mm^3 or platelet transfusion; grade 4 absolute neutrophil count toxicity $\geq$5 days; or a microbiologically documented infection while neutropenic-treated with i.v. antibiotics.

Definitions and Statistical Design. Available data suggest that an increase in response rate is unlikely to impact on overall survival. Studies that allowed crossover of patients from standard therapy to the investigational arm have not shown an improvement in overall survival, even if a higher response rate and longer time to treatment failure were observed after initial therapy with the investigational regimen (22). This Phase II study used a design adopted by ECOG based on the hypothesis that an agent capable of stabilizing breast cancer for $\geq$4 months in 20% of patients with progressive metastatic disease would be worthy of additional investigation in larger efficacy studies.

A Simon two-stage design was used (23) with early termination of the trial if a predetermined minimum level of activity was not observed after the first stage of accrual. Seventeen patients were to be enrolled in the first stage of this trial, expecting 15 to be eligible. A patient would have to complete one full course of treatment (21 days) with response assessment to be considered evaluable for response. If two or more patients were progression free at 4 months on study among 15 eligible patients, the second stage of accrual would enroll an additional 17 patients (expecting 15 eligible). At the end of the study, DENSpm would be considered worthy of additional study if six or more patients ($\sim$20%) were found to be free of disease progression after 4 months on study. The probability that this study would terminate at the end of the first stage of accrual (*i.e.*, $<$2 patients progression free in the 15 eligible patients) was 8% if the true underlying proportion of patients that was progression free was 20%. However, the probability of terminating the study increased to 72% if the true underlying proportion of patients that was progression free was only 7%. The compound and schedule of administration would be considered promising and worthy of additional investigation if $\geq$6 among 30 eligible patients were progression free at 4 months. At the end of the study, the probability of concluding that the drug and schedule were effective would be 87% if the true rate is 25%, but only 5% if the true rate is 7%. All enrolled patients would be evaluable for the secondary end point of toxicity. If the true probability of rare toxicity among 34 patients was 3%, then the probability of observing one or more rare toxicities would be 64%. If the true probability was 5%, then this probability would increase to 83%. The 90% confidence interval for any grade 3 or higher toxicity would be $\leq$30%.

Time to progression is the interval (number of days) between the 1st day of study treatment and the first date of progression or development of new disease sites. Time to objective response was defined as the interval between the first study treatment and the start of the confirmed CR or PR. The duration of objective response among responders was defined as the interval between the start of the confirmed CR or PR and the first date of disease progression. Survival duration was defined as the interval between the date of first treatment and the date of death from any cause. CR was defined as disappearance of all measurable disease confirmed by a second evaluation done at least 3 weeks later with resolution of all sites of evaluable disease (*e.g.*, ascites and pleural effusion), no new sites of disease, and resolution of all symptoms including pain. PR was defined as a $\geq$50% decrease in the sum of the products of the sites of measurable disease identified at baseline and confirmed by a second evaluation done at least 3 weeks later, with no increase $\geq$25% in other areas of measurable disease, significant worsening of tumor-related evaluable disease, new lesions, or clinically significant worsening of tumor-related symptoms. Progressive disease was defined as appearance of a new lesion,

increase in the sum of the products of any single lesion by ≥50% from baseline or best response or of multiple lesions by ≥25% each from baseline or best response, clinically significant increase in sites of evaluable disease, or clinically significant increase in tumor-related symptoms. Clinical benefit was defined as improvement in PS and disease-related symptoms, reduction in narcotic analgesic requirements, or mixed objective tumor response (reduction in size of some lesions without progression at any other site) that were considered to be related to the administration of DENSpm. Patients discontinued protocol treatment in case of disease progression, unmanageable adverse event, refusal to receive additional therapy, or a decision by the investigator based on patient safety or any new information about the compound.

RESULTS

General. Sixteen patients were enrolled from April 2000 to April 2001. The patients' characteristics are listed in Table 1. Despite the overall good PS, this was a heavily pretreated population. All patients were eligible and evaluable for toxicity and response. A total of 43 cycles of DENSpm was administered, and the median number of cycles administered per patient was two (range, 1–6). Two patients received only one cycle of therapy: one died during cycle 1 from complications of her disease without evidence of disease progression and another had evidence of clinical and radiological progression before cycle 2. The additional 14 patients included 8 patients who received two cycles of therapy, 1 who received three cycles, 4 who received four cycles, and 1 who received six cycles.

Antitumor Activity. Fifteen patients were evaluable for response, and no responses were observed. One patient did not complete cycle 1 of therapy. This patient had developed ipsilateral supraclavicular recurrence while on adjuvant chemotherapy with doxorubicin and cyclophosphamide and did not respond to first-line therapy with paclitaxel. She received the first 5 days of cycle 1 without incident, was admitted on day 8 with acute onset of dyspnea at rest and severe hypoxia, and died on day 12. A CT scan on admission showed no evidence of disease progression, but an autopsy showed severe tumoral encasement and obstruction of the pulmonary vessels. Fourteen patients received a minimum of two cycles of therapy, and 6 (40%) among the 15 evaluable patients had radiological evidence of stable disease after completing cycles 1 and 2. Six patients received a minimum of three cycles of therapy, but only 1 (6.6%) among 15 evaluable patients had radiological evidence of stable disease after completing cycles 3 and 4. This patient previously had a PR with prolonged disease stabilization with 175 mg/m^2 paclitaxel i.v. every 21 days and then another prolonged period of stable disease with letrozole. She was enrolled in this trial and had evidence of disease progression after receiving cycles 5 and 6 (4-month evaluation) and went off study. Therefore, no patients among 15 patients evaluable for response received more than six cycles of therapy, and none were progression free at 4 months. Because it would be impossible to observe two or more patients free of disease progression at 4 months to fulfill the minimum requirement to proceed into the second stage of accrual, the study was closed in August 2001 after enrolling 16 patients and fulfilling its predetermined objectives.

Table 1 Study population

Characteristics	Number
No. of patients	16
Age, median (range)	52 (34–65) years
ECOG PS, median (0/1/2)	1 (6/10/0)
ER-positive and/or PR-positive disease	12
Sites of metastatic involvement	
Limited to soft tissue/soft tissue and bone	3/1
Limited to visceral disease	3
Both	9
Prior therapy for metastatic disease	
Chemotherapy (one/two regimens)	1/15
Endocrine therapy	12
Both	12

Toxicities. All 16 patients were evaluable for toxicity (Table 2). No patients discontinued protocol therapy because of toxicity, and there were no treatment-related deaths. One patient (described above) died from pulmonary vascular complications from the disease within 30 days of receiving study drug. No hematological toxicity was observed aside from two patients with grade 1 thrombocytopenia. Two patients had grade 3 abdominal pain, but there were no other episodes of grade 3 or 4 toxicity.

The main toxicity observed was grade 3 abdominal pain (cramps) occurring after the third and fourth daily infusion during cycle 2 in two patients, respectively. The first patient was admitted to the hospital, and a CT scan showed small bowel wall thickening suggestive of jejunitis. Her symptoms of pain and constipation subsided with low doses of narcotics and i.v. hydration. She was not retreated because she then developed evidence of disease progression. A second patient developed abdominal cramps after cycle 2 on day 4, and her day 5 infusion was held. Her symptoms required no intervention and subsided within the next 48–72 h. She then received four additional cycles of therapy with a permanent 20% dose reduction without recurrence of abdominal symptoms or treatment delay.

Transient grade 1 or 2 perioral numbness occurred in 8 of 16 patients during drug infusion at approximately cycle 1 on day 3 and in only 2 of 14 patients during cycle 2. No patients developed confusion, somnolence, or any other evidence of CNS toxicity. No patients reported tinnitus or symptoms suggestive of ototoxicity. Nine patients had grade 1/2 nausea and/or vomiting with cycle 1, but only one with cycle 2; all patients were premedicated with 16 mg ondansetron i.v. Two patients each complained of grade 1 flushing and peripheral neuropathy during cycle 1. Previously described mid-cycle creatinine elevation or hypomagnesemia was not observed.

DISCUSSION

The primary end point of this Phase II clinical trial was to determine whether at least 20% of patients with metastatic breast cancer would be progression free after 4 months of treatment with DENSpm given as a 15-min daily infusion repeated for 5 days every 21 days. This trial used a two-stage design to minimize the risks of exposing an excessive number of patients to a potentially ineffective therapy. The design requires

Table 2 Nonhematological toxicities

Cycle no.	No. of patients/ cycles	No. of patients with toxicity[a]															
		Cardiology		Dermatology				Gastrointestinal						Neurological			
		Edema		Flushing		Pruritus		Diarrhea		Nausea/ vomiting		Abdominal pain		Perioral numbness		Peripheral neuropathy	
		Grade 1	Grade 2–3	Grade 1	Grade 2–3	Grade 1–2	Grade 3	Grade 1–2	Grade 3	Grade 1–2	Grade 3	Grade 1–2	Grade 3	Grade 1–2	Grade 3	Grade 1	Grade 2–3
Cycle 1	16/16	0	0	2	0	0	0	0	0	9	0	1	0	8	0	2	0
Cycle 2	14/14	0	0	0	0	2	0	1	0	1	0	1	2	2	0	0	0
Cycle ≥3	6/13	1	0	0	0	0	0	0	0	2	0	2	0	0	0	0	0

[a] Common Toxicity Criteria, version 2.

a minimum predetermined level of activity during the first stage of accrual to allow expansion into the second stage. Because none among 15 evaluable patients enrolled in the first stage of accrual were progression free at 4 months, it was considered unlikely that the predetermined minimum level of activity of interest would be observed at the end of the trial (*i.e.*, 20%, or 6 among 30 evaluable patients free of disease progression at 4 months). Therefore, the primary objective of the clinical trial was achieved, and the study was closed after enrolling 16 patients.

Secondary objectives of this study were also fulfilled. This study confirmed the favorable toxicity profile of the administration schedule selected in the Phase I study (21). Although 2 of 16 patients developed grade 3 abdominal pain, none withdrew from the trial because of complications from the study drug. Several patients presented with minor perioral numbness during the infusion, a toxicity also observed with other agents targeting the polyamine pathway (24). None had transient mid-cycle creatinine or electrolyte abnormalities. No patients developed confusion and somnolence, symptoms commonly seen in the Phase I trials that used a twice or three times daily schedule. Ototoxicity (*e.g.*, tinnitus) was reported in previous studies of DFMO in glioma patients using an oral 2-week administration schedule every 8 h, followed by either 1 or 2 weeks off (8, 25), despite a suggestion from a smaller Phase II study of DFMO in breast cancer patients that ototoxicity could be prevented by a 2-week drug holiday (7). Preclinical data suggest that this neurosensory toxicity may result from polyamine depletion induced by DFMO in cochlear hair cells (26). However, ototoxicity has not been described in any of the reported clinical trials of DENSpm (19–21).

Several factors may explain the lack of observed clinical activity in this trial. They include the possibility that modulation of the polyamine pathway may not be a meaningful target for the treatment of breast cancer, patient selection, choice of DENSpm as the polyamine analog for Phase I/II testing, selection of the administration schedule, and the potential need for combination therapy with existing drugs.

First, although their primary mechanism of action is not fully understood, natural polyamines are essential for cell growth and differentiation, and levels are increased in malignant *versus* normal tissues. Polyamine analogs are incomplete substitutes that seem to displace natural polyamines from their DNA-binding sites and induce enzymes in the catabolic pathway such as SSAT and PAOh1/SMO, resulting in the depletion of intracellular pools, cessation of cell growth, and cell death. Preclinical studies with the second-generation compounds have offered additional insight on the role of polyamine analogs as anticancer drugs. Our group has shown that treatment with the unsymmetrically substituted analog N^1-ethyl-N^{11}-[(cyclopropyl)methyl]-4,8-diazaundecane (CPENSpm) leads to accumulation of the analog and depletion of natural polyamine pools, with secondary growth inhibition and induction of programmed cell death in breast cancer models (27). Despite promising preclinical data, these data must be confirmed in clinical studies.

Second, the patients enrolled in this study may have been more heavily pretreated and less likely to respond to investigational therapies. Only a few drugs, such as capecitabine (28), have been shown to be active in patients treated previously with anthracyclines and taxanes. Despite the original intent to recruit a less heavily pretreated population, all patients had received two previous chemotherapy regimens, often including an anthracycline and a taxane. This pattern of late referral to studies with novel agents is commonly seen in tertiary centers. Breast cancer is a disease with a diverse biological behavior, and patients with advanced disease often gain some clinical benefit from third- or fourth-line cytotoxic regimens. Therefore, patients and their physicians are reluctant to consider clinical trials testing novel agents earlier in the course of their metastatic disease, despite evidence indicating that this approach does not impact negatively on their chances of benefiting from subsequent treatment with available standard regimens (29).

Third, our choice of DENSpm among the first generation of symmetrically substituted polyamines analogs for additional testing was based on preclinical data. DENSpm is a homologue of DESpm, a prototypical spermine analog initially evaluated in preclinical studies. Although antitumor activity was seen with both compounds in several human tumor models (11–16), DENSpm showed greater antitumor activity and less associated toxicity in a human melanoma xenograft model (14).

Fourth, the administration schedule in this Phase II trial was based on previous preclinical and Phase I studies. DENSpm given as a single daily administration repeated for 6 days in a human melanoma xenograft model showed similar levels of drug accumulation, reduction of natural polyamine pools, and antitumor activity compared with thrice-daily doses for 6 days (15). Excessive CNS toxicity was detected in Phase I trials that used administration schedules with dosing more frequent than

once daily. Investigators at Roswell Park Cancer Institute evaluated DENSpm as a 1-h, twice-daily infusion for 5 consecutive days repeated every 28 days in 15 patients treated over six dose levels (19). The highest dose level was 78 mg/m^2/day (free base), and the MTD was limited to 52 mg/m^2/day (free base) because of neurological DLTs such as headache, slurred speech, ataxia, dysphagia, dysarthria, and paresthesias. Another group of investigators examined the administration of DENSpm as a 15-min infusion given thrice daily for 6 consecutive days and repeated every 28 days in 28 patients treated over six dose levels (20). Once again, the highest dose level given was 74 mg/m^2/day (free base), and the MTD was 52 mg/m^2/day (free base) because of similar neurological DLTs. Other toxicities observed in these two studies included asthenia, injection site reaction, and anemia but not leukopenia or thrombocytopenia. No responses were observed with schedules using more than once a day dosing, and our daily single-dose schedule was selected for additional investigation (21).

Pharmacokinetic data from our previous Phase I study showed a short half-life for DENSpm between 0.5 and 3.7 h, which might suggest the need for a more frequent drug administration than 5 days every 21 days. However, a preclinical model by Porter *et al.* (15) showed that DENSpm drug levels in tumor tissues were still retained (40%) 2 weeks after six single daily doses of DENSpm, whereas levels were almost gone in normal tissues (15). Therefore, serum pharmacokinetic observations noted in our Phase I study may not truly reflect intratumoral pharmacodynamic exposure.

Finally, it is plausible that interference with the polyamine pathway may not be an effective therapeutic approach as a single agent as in this trial. Our data show the ability of CPENSpm and N^1-ethyl-N^{11}-[(cycloheptyl)methyl]-4,8-diazaundecane (CHENSpm) to synergize with commonly used cytotoxic agents in human breast cancer cell lines (30). Therefore, the favorable human safety profile observed with this class of compounds as single agents supports the evaluation of polyamine analogs in combination regimens with standard therapies, and human trials will properly examine the impact of combinatorial strategies being developed in preclinical models. Prevention studies of polyamine analogs in women at high risk for breast cancer and their evaluation in the preoperative setting in patients with operable breast cancer will also permit tissue acquisition for assessment of the *in vivo* effect of these compounds on the polyamine pathway.

Newer analogs are under study and may be more effective. Indeed, a third generation of polyamine analogs has been synthesized (31, 32). Our group recently examined the preclinical *in vitro* and *in vivo* antineoplastic efficacy of SL-11144, a leading compound among the new generation of polyamine analogs designated as oligoamines. These compounds seem to have a more potent antiproliferative activity against breast cancer cells than previously observed with DENSpm, CPENSpm, and CHENSpm, with activation of multiple apoptotic pathways (33).

In summary, this Phase II study of the first-generation polyamine analog DENSpm, given once daily for 5 days and repeated every 21 days, showed no evidence of clinical activity in women with previously treated metastatic breast cancer. Our trial highlights the difficulties of successfully recruiting a less heavily treated breast cancer patient population for studies with novel investigational compounds. These results confirm the tolerability and safety profile of polyamine analogs. Encouraging preclinical data with these compounds justify additional clinical evaluation of other polyamine analogs, alone and in combination with standard cytotoxic regimens, for the treatment, and possibly prevention, of breast cancer.

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Clinical Cancer Research

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