Results of a Phase II Double-Blinded Randomized Clinical Trial of Difluoromethylornithine for Cervical Intraepithelial Neoplasia Grades 2 to 3

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ABSTRACT

Purpose: **Our purpose was to conduct a double-blinded randomized trial of difluoromethylornithine (DFMO) at 0.125, 0.5 gm/m^2, versus placebo in the treatment of cervical intraepithelial neoplasia (CIN) grades 2 to 3. A promising phase I study has shown histopathologic responses at these dose levels.**

Experimental Design: **Patients with histopathologically confirmed CIN 2-3 lesions were recruited from a colposcopy clinic and underwent Papanicolaou testing, human papillomavirus testing, and colpophotography. They took oral contraception and DFMO or placebo elixir for 28 days and filled out the National Cancer Institute common toxicity calendars. They returned for follow-up and a repeat Papanicolaou smear, colpophotograph, and loop excision of the cervix.**

Results: **There were no statistically significant differences among the arms in histopathologic response. This could not be explained by any biases in risk factors. The prominent toxicities were diarrhea, dizziness, nausea, and headaches. There were no differences in the toxicities among arms. The Papanicolaou smear was a poor biomarker of response and correlated poorly with the histopathology.**

Conclusions: **DFMO is not active at 0.125 and 0.5 gm/m^2 for 28 days when given orally in CIN 2-3. Higher oral doses or longer administration is necessary, supporting data from breast trials. Alternatively, a trial of topical DFMO might merit attention as activity has been noted in trials of actinic keratoses.**

INTRODUCTION

D,L-α-Difluoromethylornithine (DFMO) is an enzyme-activated, irreversible inhibitor of ornithine decarboxylase. It was hoped to be an important chemotherapeutic and antimicrobial agent when it was synthesized some 25 years ago (1). DFMO has had some success as a chemotherapeutic agent (1) and some failures. O'Shaughnessy (2) reported one response in a trial of DFMO in 21 patients with metastatic breast cancer; the patient had liver metastases and responded for over 18 months. Levin reported that DFMO added survival advantage when added to a nitrosurea-based regimen post-chemotherapy and radiotherapy in a phase III study of anaplastic gliomas (3). Two further phase III studies of glioblastoma multiforme showed no benefit from DFMO (4, 5). Due to these modest responses and the ototoxicity seen at high doses, DFMO was not pursued further as a chemotherapeutic agent.

Ornithine decarboxylase, the enzyme suppressed by DFMO, has been shown to be "transactivated by the c-*myc* oncogene and to cooperate with the ras oncogene in malignant transformation" (1). Once investigators realized that these actions could be mitigated in cell and animal models with low doses of DFMO, the idea occurred that it could serve as a chemoprevention agent. Several studies were undertaken to see if low doses of DFMO were absorbed into various organs, and if so, what surrogate end point biomarkers they modulated.

Our group conducted a study in human papillomavirus (HPV)–positive immortalized cell lines and HPV-positive and HPV-negative cervical cancer cell lines, establishing that the doses used in the phase I study were able to cause cell death. The mechanism in some cases was by apoptosis (6). This study established the biological model that DFMO could suppress, and even stop cell growth of the precancerous and cancerous model of interest. Moreover, this phenomenon occurred irrespective of the presence of the etiologic agent HPV.

We designed a phase I study and applied for a competitively awarded chemoprevention contract from the National Cancer Institute. The phase I study was conducted in patients with grade 3 cervical intraepithelial neoplasia (CIN). The details of the study have been published elsewhere (7). First, a confirmatory biopsy was taken and read by the study pathologist. The patients were instructed to take DFMO elixir, provided by the National Cancer Institute, for 28 days. Six patients were treated at each of five dose levels given ranging from 1.0 to 0.06 gm/m^2 (1.0, 0.5, 0.25, 0.125, and 0.06). Significant clinical and histopathologic

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regression was noted at observed at all dose levels. Results are summarized in Table 1. Extensive polyamine biomarker testing was done to test modulation, but significant modulation of the plasma arginine and the tissue spermidine/spermine ratio biomarkers was noted only at 1.0 gm/m^2. Because of the significance of the histopathologic regression and the lack of ornithine decarboxylase–based biomarker modulation, we elected to design a phase II study testing two dose levels against placebo, 0.5, and 0.125 gm/m^2.

PATIENTS AND METHODS

Subjects and Recruitment. The recruitment of patients began in July 1999, and ended in July 2002. We screened for eligibility all women 18 years and older who were not pregnant, with no prior malignancy, and who agreed to use contraception for the duration of the study. The study was conducted at the colposcopy clinic of three sites at the University of Texas: the M.D. Anderson Cancer Center, the University of Texas Health Science Center-Houston, and the Lyndon Baines Johnson Hospital. Exclusion criteria included HIV-positivity and/or a positive pregnancy test. The sites attract different patient populations allowing for a blend of patients of different economic and ethnic backgrounds. The Cancer Center sees patients of many ethnicities, but most are insured. The University of Texas Health Science Center-Houston sees many Black patients who have Medicaid funding. The Lyndon Baines Johnson site sees many Hispanic, Black, and White patients who are uninsured.

Further, to be considered eligible, each patient had a biopsy-proven high-grade lesion of squamous intraepithelial neoplasia [CIN grade 2 or 3, or carcinoma *in situ* (CIS)]. The lesion had to involve an area roughly four to five times the size of a biopsy; the biopsies measure 2 × 2 mm. Each patient underwent Papanicolaou smear, colposcopically directed biopsy, and endocervical curettage prior to study entry. All these materials were reviewed by M.D. Anderson Cancer Center study pathologists. No patients were enrolled if their Papanicolaou smear, colposcopically directed biopsy, or endocervical curettage had any signs suspicious of invasive cancer.

Table 1 Review of data from phase I study*

Patient no	Dose (gm/m^2/d)	Age (y)	Path	Lesion size	Response
1	1.0	24	CIN 3	> 2/3	partial
2	1.0	41	CIN 3	1/3	partial
3	1.0	30	CIN 3	1/3–2/3	partial
4	0.5	27	CIN 3	1/3–2/3	partial
25	0.5	37	CIN 3	1/3	partial
7	0.25	25	CIN 3	1/3–2/3	partial
8	0.25	23	CIN 3	1/3	partial
9	0.25	27	CIN 3	1/3–2/3	partial
22	0.25	29	CIN 3	1/3	complete
11	0.125	25	CIN 3	1/3	complete
19	0.125	26	CIN 3	1/3–2/3	complete
20	0.125	22	CIN 3	1/3	partial
21	0.125	40	CIN 3	1/3–2/3	partial
13	0.06	22	CIN 3	1/3	complete
18	0.06	40	CIN 3	1/3	complete

NOTE: * Non-responders not included in this study. Total = 30 patients.

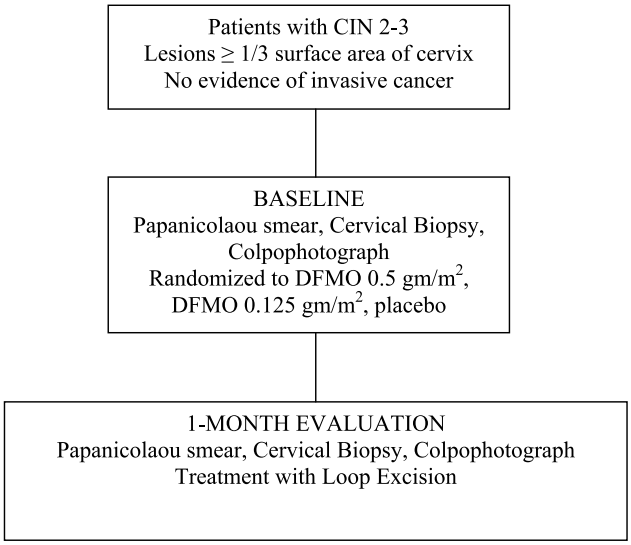

Fig. 1 Study schema.

After informed consent, patients were randomly assigned by computer in the research pharmacy to placebo or DFMO at one of two dose levels. Because the two dose levels resulted in different quantities of elixir in the bottles distributed, our research pharmacy created a placebo group that had two different amounts of placebo elixir in the bottles distributed. This allowed for true blinding of the groups. Both placebo and DFMO were supplied by the National Cancer Institute Chemoprevention Branch.

The principal investigator (M.F.), A.T.V., L.W., and the nurse practitioners saw all the patients. The group (M.F. and nurse practitioners) has been working together for 20 years. Two research nurses worked on this trial. National Cancer Institute auditors reviewed the data at 6-month intervals. The M.D. Anderson Cancer Center Internal Review Board staff audited the trial at routine intervals (6–12 months) as is institutional policy. The research staff members are fluent in Spanish and English. Telephone calls were made to the patients by the research nurses at weekly intervals.

After randomization, the patients underwent colposcopy and colpophotography by the doctors and nurse practitioners to establish baseline measurements. All of the colpophotographs (for lesion size) and pathology slides [baseline, 1 month loop electrosurgical excision procedure (LEEP) for diagnostic response] of every participant were reviewed thrice blinded for response. The colpophotographs were reviewed by the practicing physician/gynecologic oncologist and nurse practitioners blinded to the study outcome. The cytology slides were reviewed by a study cytologist blinded to the study outcome.

Study Plan. The study schema is presented in Fig. 1. This study was conducted and half-complete just prior to the adverse drug reaction involving decreased hearing at a higher dose level of DFMO. In our phase I study, all audiograms were unchanged pretreatment and posttreatment at these same doses. With the permission of the Chemoprevention Branch, we did not include audiograms in this study.

Patients were randomized to placebo, 0.125, and 0.5 gm/m^2 per day. They were instructed to drink the elixir daily, usually

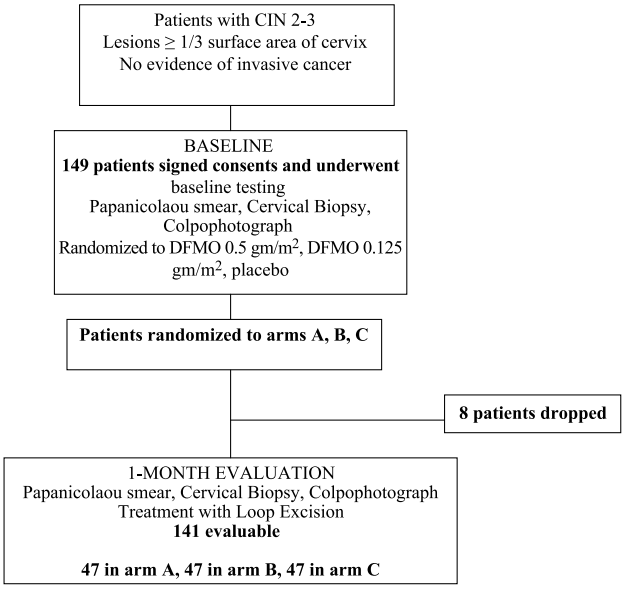

Fig. 2 Flow of patients in study.

mixed with orange juice. At study start, they were biopsied and at study finish, they underwent a LEEP that removed the entire area at risk. We defined the area at risk as removing the area 4 to 5 mm outside the transformation zone, or 4 to 5 mm outside the lesions if the lesion was located in the transformation zone, and then 4 to 5 mm around the remainder of the uninvolved transformation zone with the 20 × 8 mm loop. The patient had an additional specimen of the canal taken if the endocervical curettage was positive. Usually, this specimen was obtained with the 10 × 10 mm loop. All specimens' margins were inked for the pathologists and sutures marked the 12 o'clock position.

Response was evaluated histologically by the loop excision specimen taken at the 1 month visit. Histologic response was classified as: no change, partial response, complete response, and progressive disease. Lesions were considered to have progressed if they were CIN 2-3 and they progressed to CIS, if they were CIN 2 and they progressed to CIN 3/CIS. Although some pathologists make no distinction between CIN 3 and CIS, ours followed the WHO classification. If there was full thickness involvement by undifferentiated cells, the biopsy was read as CIS. For lesions involving at least 2/3 of the distance from the basement membrane to the surface, the pathologists read these as CIN 3. For lesions > 1/3 and < 2/3, the lesions were graded as CIN 2. Dr. Malpica's kappa for readings on the same set of slides three times is 0.85.

Patients' biopsies were considered to have "no change" if the diagnosis did not change. They were considered to have a "partial response" if they regressed to HPV or CIN 1. They were considered a complete response if they regressed to normal or atypia only in the LEEP specimen. All LEEP specimens that were judged partial or complete responders, were re-cut until the blocks were exhausted, that is until no further tissue remained.

Compliance was evaluated by counting the unused elixir in the bottle at the end of the month. All of the patients were asked to fill out the National Cancer Institute toxicity criteria daily. These sheets were collected at the end of 1 month, the end of study. Patients were also called weekly by the research nurses and reminded to fill out the toxicity calendars as well as asked how they were doing.

Sample Size and Power Calculations. The sample size and interim analyses were planned in advanced and based on the end point of histologic outcome. The sample was based on a formula using a logistic regression: Log $[\theta/(1-\theta)] = \beta_0 + \beta_1 I_{trt} + \beta_2 I_{rec} + \beta_3 I_{CIN\ 2}$. In this formula, $I_{trt} = 0$ if the patient was on placebo, and $I_{trt} = 1$ if the patient was on DFMO, $I_{rec} = 0$ if the patient has a new lesion, and $I_{rec} = 1$ if the patient has a recurrent lesion. $I_{CIN\ 2} = 0$ if the patient has CIN 3, and $I_{CIN\ 2} = 1$ if the patient has CIN 2. We assumed that patients with CIN 2 and 3 would be represented equally in the trial, as would patients with new and recurrent lesions. Initially, we assumed that recurrence would have no effect on response, that is $\beta_2 = 0$. We assumed a regression rate of 0.10 for CIN 3 and 0.30 for CIN 2, based on the pathologic reviews cited in ref. (8); two studies of the natural history of CIN. We judged that a response rate of 0.30 would be a significant finding. So that the response rate under therapy of 0.40 for CIN 3 and 0.70 for CIN 2, a sample size of 57 patients per group renders a power of 80. If we assume that recurrent lesions are more recalcitrant, and use the value $\beta_2 = -0.75$, a sample size of 64 patients per group renders a power of 80. In this trial, we attempted to accrue 60 patients in each of the three groups and planned interim analyses at 60, 120, and 180 or anytime asked by the sponsor.

Statistical Analysis. Simple descriptive statistics were carried out using SAS. χ^2 tests were applied to compare categories of response: no change, partial response, complete response, and progressive disease. Patients were further classified as *responders* or *nonresponders*. Responders were those who had a complete response or a partial response. Nonresponders were those who had no change or progressive disease.

Patients were then further stratified by potential confounders such as age, grade of CIN (grade 3 versus 2), HPV status (positive by Digene Virapap versus negative), oral contraceptive use (user versus nonuser), and smoking (smoker versus nonsmoker). χ^2 tests were used to compare the distribution of these variables among the three groups. All of the tests were two-sided at the 5% level of significance. Computations were made using StatView, Mathematica, and SAS. Grade 1 and 2 toxicities were tallied and compared in the three arms. χ^2 analyses were used to compare the toxicity summaries.

A futility analysis was carried out using Mathematica, at the request of our Internal Review Board, using the prior probabilities of response and nonresponse and the probability of being CIN 2 or 3, the probability of being randomized to treatment or placebo, assuming a uniform prior distribution and the posterior beta distributions were calculated based on the response rates found in RESULTS. These posteriors were then used as priors for 10,000 simulations of the remaining portion of the trial. For each simulated outcome, the probability of a difference in response of proportions between treated and control patients was calculated. This difference was at least 0 and at most 0.30. In addition to the Bayesian analysis, we conducted a frequentist analysis. The results of both simulations were tested using Fisher's exact test or the

χ^2 with Yates' continuity correction. Significance was set at $P < 0.05$ level.

RESULTS

Demographic Data. All eligible patients were interviewed and invited to be part of the study, they were considered eligible based on biopsy. Further review excluded patients because of breast-feeding, HIV positivity, and other immunodepressive illnesses.

One hundred and forty-nine patients were randomized (Fig. 2), they ranged in age from 18 to 75 with a mean of 31 years and a median of 29 years, most patients were premenopausal. There were 51 White patients (34.2%), 40 Black patients (26.8%), 56 Hispanic (not White) patients (37.6%), and 2 Asian patients (1.4%) in this trial. The breakdown of smokers, CIN 2-3 distribution, and lesion size can be seen in Table 2. The groups were balanced as to these prognostic factors, as expected by the randomization. The HPV testing revealed that 70% were positive by Hybrid Capture II (Digene, Gaithersburg, MD). HPV positivity was also not different among the groups, this agrees with data from 1,800 patients in our program project (data not shown). PCR from the same group is positive 95% of the time.

Compliance. Of the 141 patients who remained in the trial, most returned their bottles of elixir empty. Two patients admitted to not taking all of their medication. Most of the patients returned with empty bottles. The bottles were turned in to the research pharmacy.

Histologic Response. The biopsies and the LEEP excision specimens were reviewed by both study pathologists (I.B., A.M.) thrice blinded to the study arm. The final results are presented in Table 3. This analysis shows no statistically significant differences among the arms.

One patient who had CIN 2-3 on all baseline studies including the Papanicolaou smear, endocervical curettage, and biopsy did in fact have 4 mm of microinvasive cancer on the LEEP specimen. She was treated with repeat cone biopsy, radical hysterectomy, and lymph node dissection. Fortunately, the end result showed favorable pathology, without further need for additional therapy. It is highly unlikely that she progressed during the month, but rather that her lesion, which was high up in the endocervical canal, was not detected, despite two Papanicolaou smears and an endocervical curettage.

Futility Analysis. The results of the Bayesian and Frequentist analyses were similar. If the patients had continued to be accrued up to the projected sample size of 180 and showed the same pattern of response that existed among the three arms at the time of the interim analysis, the probability that there would be a statistically significant difference among them would have been 0.0406. This figure was based on an analysis of 10,000 simulated trials. For each simulation, the response rate for each arm was generated from the beta distribution with the parameters of the distribution based on the uncertainty about the response rates in each arm. The equality of the response rates was tested in each simulation using a χ^2 analysis of the appropriate 3×2 contingency table.

Given this analysis, our Internal Review Board suggested that we close the trial at 149 and not accrue to the sample size of 180. We discussed this with the National Cancer Institute and the Chemoprevention Branch, and they were agreeable.

Table 2 Prognostic variables

	Placebo	0.5 gm/m^2	0.125 gm/m^2	*P* value*
Smokers (+)	12/40 = 30%	13/46 = 28%	9/40 = 23%	NS
CIN 2	12/40 = 30%	17/45 = 38%	16/41 = 39%	NS
CIN 3	28/40 = 70%	28/45 = 62%	25/41 = 61%	NS
Lesion 1/3	20/41 = 49%	28/46 = 61%	19/43 = 44%	NS
Lesion > 1/3	21/41 = 51%	18/46 = 39%	24/43 = 56%	NS
Recurrent CIN	7/47 = 15%	6/47 = 13%	4/47 = 9%	NS

**P* value for columns 1 versus 2, 1 versus 3, 2 versus 3, and overall for each row run.

Table 3 Results of a phase II clinical trial of DFMO as treatment of cervical high-grade squamous intraepithelial lesions

Histologic outcome	Placebo	0.5 gm/m^2	0.125 gm/m^2	*P**
Complete regression	4	2	4	NS
Partial regression	13	14	13	NS
No change/progression	30	31	30	NS
Total	47	47	47	

**P* value for columns 1 versus 2, 1 versus 3, 2 versus 3, and overall for each row run.

Colpophotographs. In the 141 patients, the colpophotographs were not a reliable means of detecting response. Judged three times, independently, they corresponded poorly to lesions and disease status. We plan to re-analyze the digitized images and report further if results are different.

Pap Smears. The results of the Pap smears were not a reliable biomarker and were not in agreement with the histology. The results are detailed in Table 4. Up to 30% to 40% of the time, the Papanicoloau smear showed improvement. Because we know that there was no statistically significant difference in the arms, this is clearly misleading. However, what is more misleading is that the patients in whom the Papanicolaou smears showed improvement were not those in whom there was pathologic evidence of no change or partial regression (data not shown). Because the Pap smear has a false-negative rate of 40%, it is not considered a reliable end point. Additionally, clinical experience from this trial would suggest that clinicians do not scrape hard enough for the Pap smear when they are about to perform colposcopy because the Pap smear could cause bleeding, which might obscure the colposcopy.

Toxicities. Grade 1 and 2 toxicities were noted in the study. Patients report toxicities daily over the course of the month. In Table 5, all grade 1 and 2 toxicities for the study are shown. There were no grade 3 or 4 toxicities. As shown in Table 6, they were equally distributed by arm. Arm A was placebo, arm B was 0.5, and arm C was 0.125 g/m^2. As expected, DFMO causes diarrhea, fatigue, headache, and nausea in a significant number of patients. Otherwise, the drug was well tolerated. In Table 7, the major toxicities are subjected to analysis to see if they differ by arm. The overall toxicities are balanced, and there are no statistically significant differences among the arms. This may be explained by zealous data collection by the research nurses or by the fact that there is indeed a "placebo effect".

Table 4 Papanicolaou smears as biomarkers pretreatment versus posttreatment

	Placebo	0.5 gm/m^2	0.125 gm/m^2	*P**
Better	15/37 = 41%	19/44 = 43%	13/41 = 32%	NS
No change	16/37 = 43%	22/44 = 50%	20/41 = 49%	NS
Worse	6/37 = 16%	3/44 = 7%	8/41 = 19%	NS

**P* value for columns 1 versus 2, 1 versus 3, 2 versus 3, and overall for each row run.

CONCLUSIONS

DFMO has had many successes as a chemoprevention agent in the literature, most of these have been in modulation of biomarkers. As for trials that report clinical responses, there are few. Besides our phase I study (7), there has been a successful phase II study of topical DFMO for actinic keratoses (9). The exact dose for the topical DFMO is difficult to ascertain. A phase II study of DFMO in breast cancer prevention was designed and conducted to test reducing risk defined by biomarker change and mammographic density change. Of 119 subjects who entered, 96% completed the study, took DFMO at 0.5 gm/m^2, or placebo for 6 months. There were no statistically significant changes in surrogate endpoint biomarkers or mammographic density (10).

DFMO seemed such a promising compound that many studies have appeared in the literature. As with all chemoprevention trials, investigators often publish biomarker development prior to clinical response. Table 8 lists studies that show biomarker modulation with or without reporting a clinical response (11–24).

Why was this phase II trial in the cervix negative? The usual reasons for negative chemoprevention trials are: lack of a phase I trial, lack of a phase I trial in that organ site, insufficient enrollment of patients, no uniform biopsies at study entry and exit, lack of pretrial evidence for biological effect, and lack of surrogate endpoint biomarker validation (8).

Our phase I trial was in the cervix, it showed encouraging results, it was the same population, and explored the doses of interest (7). The phase I study used the same doses, from the same supplier, for the same period of time (1 month). How was that phase I study different? We don't know. Many an investigator has had a promising phase I and a disappointing phase II. The setting was the same, the drug was from the same batch, the pharmacy the same, the research group the same. If anything was different, all of the patients in the phase I had CIN 3, whereas in this phase II study, patients had CIN 2-3. This should have helped the response rates not hurt it.

Did this study enroll an insufficient number of patients? We wanted to enroll 60 patients per arm, although we did not enroll 180 patients, the futility analysis strongly suggested that there was little chance that 31 more patients would have changed our outcome. It is difficult to know. There is some controversy in the statistical community concerning application

Table 5 Toxicities

	Grade 1	Grade 2
Alopecia	14	0
Anorexia	26	2
Anxiety	15	0
Cheilitis	29	0
Chest pain	22	1
Constipation	13	0
Depression	12	0
Diarrhea	36	2
Dizziness	37	1
Dry nose	27	0
Fatigue	46	1
Headache	61	7
Indigestion	22	0
Insomnia	23	1
Mood	16	0
Muscle weakness	20	0
Myalgia	21	0
Nausea	55	1
Photosensitivity	18	0
Skin reaction	16	1
Stomatitis	23	0
Vomiting	23	0

Table 6 Toxicity frequency table

Group	Frequency	Percentage (%)
A	327	36.2
B	306	33.8
C	271	30.0
Total	904	100.0

Table 7 Demonstrating no statistical significance of the major toxicities among the arms the six categories of toxicity × ARM cross-tabulation

Toxity			ARM			Total
			A	B	C	
AE6	Diarrhea	count	29	15	11	55
		percentage within toxicity	52.7%	27.3%	20.0%	100.0%
		percentage within ARM	8.9%	4.9%	4.1%	6.1%
	Dizziness	count	23	10	19	52
		percentage within toxicity	44.2%	19.2%	36.5%	100.0%
		percentage within ARM	7.0%	3.3%	7.0%	5.8%
	Fatigue	count	19	23	18	60
		percentage within toxicity	31.7%	38.3%	30.0%	100.0%
		percentage within ARM	5.8%	7.5%	6.6%	6.6%
	Headache	count	35	53	35	123
		percentage within toxicity	28.5%	43.1%	28.5%	100.0%
		percentage within ARM	10.7%	17.3%	12.9%	13.6%
	Nausea	count	26	26	24	76
		percentage within toxicity	34.2%	34.2%	31.6%	100.0%
		percentage within ARM	8.0%	8.5%	8.9%	8.4%
	Other	count	195	179	164	538
		percentage within toxicity	36.2%	33.3%	30.5%	100.0%
		percentage within ARM	59.6%	58.5%	60.5%	59.5%
Total		count	327	306	271	904
		percentage within all toxicity	36.2%	33.8%	30.0%	100.0%
		percentage within ARM	100.0%	100.0%	100.0%	100.0%

Table 8 DFMO biomarker trials reported in the literature with or without clinical responses

Author (ref)	No. of patients	Tissue(s)	DFMO Dose	Drug Administration	Biomarkers modulated
Carbone (11)	18 (organ transplant)	skin	1.0 and 0.5 gm/m^2	28 d	12-*O*-tetradecanoylphorbol-13-acetate induced ornithine decarboxylase in skin, putresine in skin
Simoneau (12)	9 (TURP)	prostate	0.5 gm/m^2	28 d	putresine, spermidine, spermine, spermidine/spermine ratio
Bacus (13)	14 (CIN 3)	cervix	0.06–1.0 gm/m^2	28 d	mean nuclear grade, standard deviation of mean nuclear grade
Poulin (14)	25 (CIN 3)	cervix	0.06–1.0 gm/m^2	28 d	57 nuclear morphometric features
Meyskens (15)	125 (adenomatous polyp patients)	rectal mucosa	0.075, 0.20, 0.4 gm/m^2 and placebo	15 months	putresine, spermidine, spermine, spermidine/spermine ratio
Messing (16)	25 (prostate cancer)	prostate	0.5 gm/m^2	14 d versus placebo	putresine only
Bozzo (17)	10 (solar keratoses)	skin	topical	?	nuclear morphometry
Boiko (18)	25 (CIN 3)	cervix	0.06–1.0 gm/m^2	28 d	epidermal growth factor receptor
Boiko (19)	25 (CIN 3)	cervix	0.6–1.0 gm/m^2	28 d	DNA index
Meyskens (20)	111 (adenomatous polyp)	colon	0.1–3.0 gm/m^2	28 d	putresine, spermidine/spermine ration
Boyle (21)	5 (mouth)	mouth	3 gm/m^2	28 d	putresine (rectum only), spermidine/spermine ratio (rectum only)
Love (22)	45 (personal history of colon cancer, personal history of adenomatous polyp)	colon	0.5 gm/m^2 and placebo	1 y	putresine, spermidine
Hu (23)	25 (CIN)	cervix	0.06–1.0 gm/m^2	28 d	proliferating cell nuclear antigen
Hu (24)	25 (CIN)	cervix	0.06–1.0 gm/m^2	28 d	MPM-2

of the Bayesian analyses to the data after the study was designed to fit a logistic equation or frequentist sample size. Given the paucity of resources and the concerns of sponsors these days, it is hard to justify continuing studies for which the projections seem negative.

Did we have biological support for the study? Yes, we did. Biological evidence for dose and effect was present in cell lines, both precancerous and cancerous. Our laboratory work agreed with other work in the literature (6, 25–28). Very substantial and well-known groups have documented mechanisms by which DFMO induces apoptosis. More work could be done exploring the interactions of DFMO and HPV. However, our work shows that DFMO is active irrespective of the presence of HPV.

Was there insufficient validation of biomarkers for a trial? Our group worked extensively on biomarker validation in the phase I study (7). Tissue, red cell, and plasma ornithine decarboxylase, arginine, putrescine, spermidine, spermine, and the spermidine/spermine ratio were explored. The polyamine levels are of such variability that large numbers of patients are required to validate increases and decreases of these biomarkers. However, we were able to show that other biomarkers of ploidy, proliferation, dysregulation, and genomic alteration were statistically significantly modulated in tissue. Our group had validated several biomarkers from archival tissue and then used them to test tissue from the phase I trial (13, 14, 18, 19, 23, 24). Thus, sufficient biomarkers have been developed.

Our plans are to complete the biomarker assessments for this trial blinded to outcome, analyze them, and be certain they agree with the histopathologic outcome. These will provide end points to characterize the lesions in biological terms not "seen" by histology. Clearly, CIN 3 lesions need to be treated with a higher dose or for a longer period of time. Perhaps a higher local dose would be of interest. A topical application would be possible in the cervix. Meyskens et al. (29) reported statistically significant results using *trans*-retinoic acid in a cervical cap. The responses seen in the studies of actinic keratoses are certainly encouraging and would support the concept of a topical trial in the cervix.

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