Evaluation of α-Difluoromethylornithine as a Potential Chemopreventive Agent: Tolerance to Daily Oral Administration in Humans[1]

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Abstract

An initial clinical trial of α-difluoromethylornithine given p.o. daily for 6 months was carried out in 27 subjects free of disease following surgery for malignancy or in a defined high-risk group for cancer. The aim was to determine the highest nontoxic dose, principal side effects, and pharmacokinetic parameters. The starting dose was 200 mg/m²/day in divided doses with escalation each month in the absence of toxicity to 6400 mg/m²/day or to the highest nontoxic dose, whichever was lower. When the highest nontoxic dose was reached, this dose was continued to complete 26 weeks of treatment. Twenty-two subjects completed 26 weeks of α-difluoromethylornithine treatment of whom 20 reached a nontoxic dose of at least 1600 mg/m²/day. The dose-limiting toxicity was loss of high-tone auditory acuity on an audiogram. Other side effects included diarrhea, fatigue, joint pain, insomnia, and rash. Pharmacokinetics were linear with dose. Area under the plasma concentration × time curve and maximum plasma concentration of α-difluoromethylornithine did not predict for development of ototoxicity. The dose for phase II chemoprevention studies should not exceed 1600 mg/m²/day.

Introduction

Biosynthesis of polyamines in eukaryotic cells requires the decarboxylation of ornithine by the enzyme ODC.[3] Analogues of ornithine have been synthesized as enzyme-activated inhibitors of ODC, and these include DFMO (eflornithine, Ornidyl) (1). Because of its selective effect on ODC, this compound has been investigated for its biochemical effects on polyamine biosynthesis, for its antitumor effects *in vitro* and *in vivo*, and its effect as a chemopreventive agent in animal systems. DFMO induces a depletion of intracellular putrescine and spermidine pools. However, it does not cause total depletion of intracellular polyamines and may even lead to an increase in spermine pools (2–5). The effect of DFMO is usually cytostatic rather than cytotoxic, and it has been suggested that this is related to the incomplete depletion of polyamines (2–6). Although DFMO produces an irreversible inhibition of ODC, the enzyme has an extremely rapid regeneration time, so that the metabolic blockade can be quickly abrogated by the cell (6).

In humans it has been studied at the phase I and phase II levels as an antitumor agent (7–11) and is clinically used against infections with *Trypanosoma brucei gambiense* (West African sleeping sickness). The drug has very low toxicity. In phase I studies, doses of 12 g/m²/day for 28 days produced thrombocytopenia and gastrointestinal disturbances (7), 64 g/m²/day i.v. for 25–43 days by continuous infusion produced vomiting and metabolic acidosis (8); 27 g/m²/day 5 times every 2 weeks produced diarrhea and mucositis (9). In phase II studies hearing loss was also seen (10, 11).

Several studies have demonstrated that DFMO will reduce both the incidence and the frequency of tumors in animals treated with carcinogens. Fozard and Prakash (12) gave 2% DFMO in drinking water to rats pretreated with dimethylbenzanthracene. He found that a 30–73-day administration of the drug led to a 69% reduction in incidence and a 90% reduction in frequency of tumors. Moon (13) gave large doses of DFMO (either 3.2 or 6.4 g/kg), starting up to 1 week before the administration of dimethylbenzanthracene and continuing for as much as 6 months. Up to a 40% decrease in incidence and up to an 80% decrease in frequency were noted with this treatment. In rats given methylnitrosourea s.c., DFMO in the drinking water in concentrations up to 1%, starting 1 week before the injection of the carcinogen and continuing for 6 months, produced an 82% reduction in the incidence of tumors (14).

On the basis of its low toxicity, its demonstrated effectiveness as a chemopreventive agent, and the existence of a potential biochemical test for biological effect (inhibition of polyamine biosynthesis), DFMO was entered into initial clinical evaluation as a potential chemopreventive agent.

This trial was designed to study subject acceptability, tolerance, and side effects of daily administration p.o. of DFMO for 6 months. Biochemical measurements were conducted in parallel and are reported separately (15). Preliminary reports of some of these data have appeared (16–18).

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[3] The abbreviations used are: ODC, ornithine decarboxylase; DFMO, α-difluoromethylornithine; AUC, area under the plasma concentration × time curve; C_{max}, maximum plasma concentration.

Materials and Methods

Subjects. Subjects were adult volunteers (*a*) who had previously had a malignancy, were free of clinically detectable disease, but were at high risk for recurrence, or (*b*) who belonged to high-risk groups for the development of a primary malignancy. They were required to be ambulatory; to have normal renal, hepatic, and bone marrow function; and to be free of acute medical problems. Each subject gave written informed consent to entry into the study. The study was approved by the Institutional Review Board of Roswell Park Cancer Institute.

Drug Administration. DFMO was supplied by the Chemoprevention Branch of the National Cancer Institute. Subjects were given DFMO as an aqueous solution containing 200 mg/ml of DFMO. The appropriate dose was diluted in water or fruit juice before ingestion. Monitoring of compliance was by measuring unused amounts of the drug. Initially the drug was administered four times daily. However, it was quickly found that the complexity of this administration militated against full compliance. The administration schedule was therefore changed to a twice-daily schedule.

The starting dose was 200 mg/m^2/day in all subjects. This was given for 4 weeks. In the absence of side effects, the dose was doubled to 400 mg/m^2/day for 4 weeks. This procedure was repeated for a total of 26 weeks, the final dose being given for 6 weeks. The highest dose explored was 6400 mg/m^2/day. In the event of toxicity, the drug was discontinued for 4 weeks to allow toxicity to clear. It was then resumed at the next lowest dose (half of the dose that induced toxicity) and continued for the remainder of the 6 months of treatment. If symptoms occurred which were felt to be possibly but not probably drug related, the drug was stopped until the symptoms had cleared and then restarted at the same dose. If the symptoms recurred, they were presumed to be drug related and the procedure above was adopted. If they did not recur, dose escalation was continued.

Study Monitoring. The following were done pretreatment, monthly during treatment, at the end of the drug administration period and three months later: complete blood count, blood chemistry profile,[4] urinalysis, and audiogram. Unmasked air conduction audiograms were carried out on a GSI-16, 2-channel audiometer (Grason Stadler, Littleton, MA) at 250, 500, 1000, 2000, 4000, and 8000 Hz. For assessment of high-frequency hearing loss the mean decrease (in dB) at 4000 and at 8000 Hz was calculated for each ear. Blood was also drawn for pharmacokinetic studies of DFMO and for biochemical studies (see below).

Subjects were contacted weekly by telephone, and a telephone questionnaire was filled out to evaluate possible toxicity. If any responses to the phone questionnaire indicated the possibility of drug toxicity, the subjects were asked to visit for examination.

Pharmacokinetic Monitoring. For DFMO pharmacokinetics, blood was drawn after an initial 50 mg/m^2 dose on day 1, pretreatment, and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 12 h. Blood was drawn again at the end of each month on a given chronic administration of a dose, before the morning dose, and after the morning dose at 1, 2, 3, 4, 5, and 6 h. The monthly sampling was taken prior to the dose escalation. DFMO in plasma was assayed by the method of Bitonti *et al.* (19). The DFMO measurements were carried out in perchloric acid extracts of plasma with a gradient ion exchange high-performance liquid chromatography procedure. The column used was Bakerbond-SCX with a gradient mobile phase of acetic acid (0.2 M) to sodium acetate (0.2 M). Detection was by fluorescence after post-column derivatization of DFMO with *o*-phthalaldehyde.

Data Analysis. A noncompartmental program, Lagran (20), was used to calculate the half-life and AUC. C_{min}, the minimum plasma concentration before the next dose, and the time to reach maximum plasma concentration are the observed values. The computer program Minitab was used to explore correlations between DFMO pharmacokinetics and ototoxicity.

Table 1 Subject characteristics

Subjects entered	27
Male	16
Female	11
Age range (years)	22–71
Mean (years)	53
Risk factors	
Prior malignancy but clinically free of disease	17
Colon polyps	5 (familial polyposis, 3)
History of heavy smoking	3
Miscellaneous	2

Results

The characteristics of the subjects entered are shown in Table 1. Five subjects were removed from study prior to completion. One subject developed restlessness and insomnia at the lowest dose. These symptoms cleared on stopping the drug, but returned on restarting it. The subject was therefore removed from the study. One subject developed recurrent joint pain and was subsequently diagnosed as having Lyme disease. One subject was removed for noncompliance, and two subjects developed overt malignancy during the course of the study. Thus, 22 subjects completed a full 26 weeks of DFMO. Of these 22 subjects, 6 were escalated to 6400 mg/m^2/day, 9 to a maximum dose of 3200 mg/m^2/day, and 6 to a maximum dose of 1600 mg/m^2/day. One subject reached a dose of only 800 mg/m^2/day. Toxicities were mild. No severe toxicities were seen.

The following probably or possibly drug-related side effects were seen in more than one subject: loss of high-frequency auditory acuity on audiogram, which in some subjects was accompanied by hearing changes; diarrhea, fatigue, joint pain, swelling, and stiffness; insomnia accompanied by restlessness; and skin rash.

Changes on audiogram and or auditory symptoms were the most important side effect seen. If all changes of any magnitude or any auditory symptoms were included, 17 of 27 subjects could be identified as having

[4] Serum Na^+, K^+, Cl^-, HCO_3^-, CO_2, PO_4^{---}, blood urea nitrogen, glucose, creatinine, uric acid, total protein, albumin, total bilirubin, alkaline phosphatase, lactate dehydrogenase, serum glutamic oxalacetic transaminase, γ-glutamyl transferase, and cholesterol.

Table 2 Ototoxicity[a]

Subject no.	Ototoxicity score (dB)[b]	Auditory symptoms	Total cumulative dose resulting in ototoxicity (g)	Maximum dose reached (mg/m²/day)	Final dose (mg/m²/day)	Comments
1	11.5	None	417.5	3200	800	Dose reduced to 1600 mg/m²; further dose reduction due to diarrhea (subject has ileo-anal anastamosis)
5	12.5	None	115.0	1600	1600	Dose reduced to 800 mg/m²/day; change in audiogram later found to be due to noisy work environment. Dose reescalated without toxicity
7	12.5	Tinnitus	273.0	3200	1600	
8	12.5	Tinnitus	601.9	6400	6400	Toxicity produced by final dose, study completed
9	14.5	None	70.7	800	400	
16	43	None	683.4	6400	6400	Toxicity produced by final dose, study completed
19	13.5	None	377.2	3200	1600	

[a] Ototoxicity score ≥10 dB (see text).
[b] Mean of the hearing loss (dB) in each ear at 4000 and 8000 Hz.

possible ototoxicity. Sixteen subjects had changes noted on the audiogram. Of these, 6 also had auditory symptoms including tinnitus, muffled sounds, and roaring in the ears; one subject had auditory symptoms only with no change noted on the audiogram. The most common change seen was a loss of auditory acuity at the highest frequencies measured (4000 and 8000 Hz). All values had returned to baseline by the time of the next audiogram (1 month) in the absence of drug administration. To define more precisely the occurrence of probable ototoxicity, we used an arbitrary cutoff of a mean decrease in auditory acuity at 4000 and 8000 Hz, of 10 dB or greater in either ear. The 7 subjects who met this more rigorous criterion are listed in Table 2 with the total cumulative dose of drug received, the dose at which ototoxicity was observed, and the final dose in the study.

Other abnormalities seen are listed in Table 3, which includes all reported symptoms and all noted abnormalities whether or not these were considered to be drug related. As noted in the table, some subjects in whom these abnormalities occurred subsequently tolerated higher doses of DFMO without recurrence, and other subjects had other bases for developing these abnormalities that were unrelated to DFMO.

One subject with familial polyposis had a marked diminution in the number and size of polyps in the rectal stump and was maintained on 6400 mg DFMO/m²/day for 10 months beyond the planned 6 months of treatment, without toxicity.

Pharmacokinetic measurements were carried out in 23 of 27 subjects at all of the doses at which complete or nearly complete blood sampling could be obtained. Plasma concentration × time curves for one subject are shown in Fig. 1. The pharmacokinetics are linear with dose, as shown in Fig. 2. The correlation coefficient of pharmacokinetic parameters with dose were $r = 0.88$ for C_{max}, 0.89 for AUC, and 0.9 for the minimum plasma concentration before the next dose. At the highest nontoxic dose (1600 mg/m²/day) the values for these parameters were 26.4 ± 9.2 μg/ml, 115.4 ± 32.2 μg/ml·h, and 9.11 ± 5.3 μg/ml, respectively. Plasma decay half-life was 4.7 ± 1.9 h, and time to reach maximum plasma concentration was 3.2 ± 1.1 h at this dose.

Since the pharmacokinetic parameters AUC and C_{max} were measured each month and audiograms were also performed monthly, several values for dose, AUC, C_{max}, and change in auditory acuity at high frequency were available for each subject. Thus, it was possible to plot changes in auditory acuity at high frequency against dose, AUC, and C_{max} to explore possible correlations. When this was done for all doses in all subjects, the correlation was poor (r = 0.55, 0.6, and 0.48, respectively for ΔdB versus dose, AUC, and C_{max}). When the procedure was repeated using only those subjects who developed ototoxicity, the correlations were significantly better

Table 3 Other toxicities[a]

Toxicity	No. of subjects	Comments
Diarrhea	8	Two subjects had underlying bowel disease associated with diarrhea
Fatigue	5	All 5 subjects tolerated higher doses of DFMO without recurrence of symptoms
Joint pain, swelling, and stiffness	5	One subject later shown to be serologically positive for Lyme disease
Sleeplessness/restlessness	4	Three subjects tolerated higher doses without recurrence of symptoms
Rash	4	Two subjects had previously noted allergic skin rash
Nausea	1	
Leukopenia	1	Nadir 3.5 × 10⁹/liter; subject removed from study
Electrocardiogram changes	1	Thought to be probably related to underlying cardiac disease
Stomatitis	1	

[a] All abnormalities noted are reported whether thought to be drug related or not.

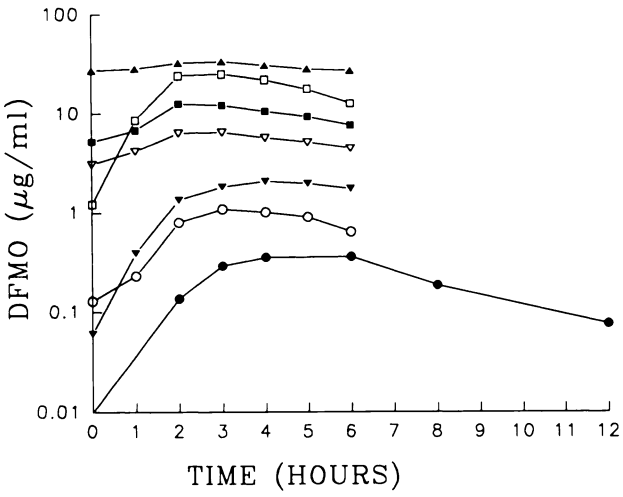

Fig. 1. Concentration of DFMO with time in the plasma of a subject receiving escalating doses of the agent: ●, initial single dose of 50 mg/m²; ○, 200 mg/m²; ▼, 400 mg/m²; ▽, 800 mg/m²; ■, 1600 mg/m², □, 3200 mg/m²; ▲, 6400 mg/m².

(r = 0.86, 0.9, and 0.88, respectively). Multivariate analysis indicated no effect of age or sex on ototoxicity. However, when the AUC and C_{max} after single doses of 50 and 800 mg/m² were compared in subjects developing ototoxicity and those not developing it, no significant difference in these parameters was observed between the 2 groups by the Wilcoxan rank-sum test. Moreover, the total drug needed to induce ototoxicity was highly variable between patients (Table 2).

Discussion

This was not a standard phase I study to evaluate the maximum tolerated dose. The objective of the study was to establish the highest acceptable nontoxic or minimally toxic dose for chronic daily administration in a population of subjects at higher-than-normal risk for developing malignancy. As noted above, large daily doses of DFMO are tolerated when the drug is used in the treatment of disease. The toxic effects that have been described include anemia, leukopenia, thrombocytopenia, nausea, vomiting, mucositis, diarrhea, and hearing loss. The total doses used have been in the range of 2–12 g/m²/day for variable periods. The present study was designed to establish the highest nontoxic dose rapidly, by doubling the dose each month, starting with the safe dose of 200 mg/m²/day. A dose of 1600 mg/m²/day could be administered without toxicity in 20 of the 27 subjects entered onto the study. Of significance is the observation that this and lower doses could produce a measurable modulation of polyamine excretion in urine. These data are reported separately (15).

Carbone *et al.* (21, 22) have recently reported a phase I and pharmacokinetic study of DFMO as a potential chemopreventive. They explored 125, 250, 500, and 750 mg/m² four times a day (total daily doses of 500, 1000, 2000, and 3000 mg/m²/day) and 500 and 1000 mg/m² single daily doses. They noted dose-limiting ototoxicity at total daily doses of 1000 mg/m² and cumulative doses of >90 g/m². However, 5 of 6 subjects were able

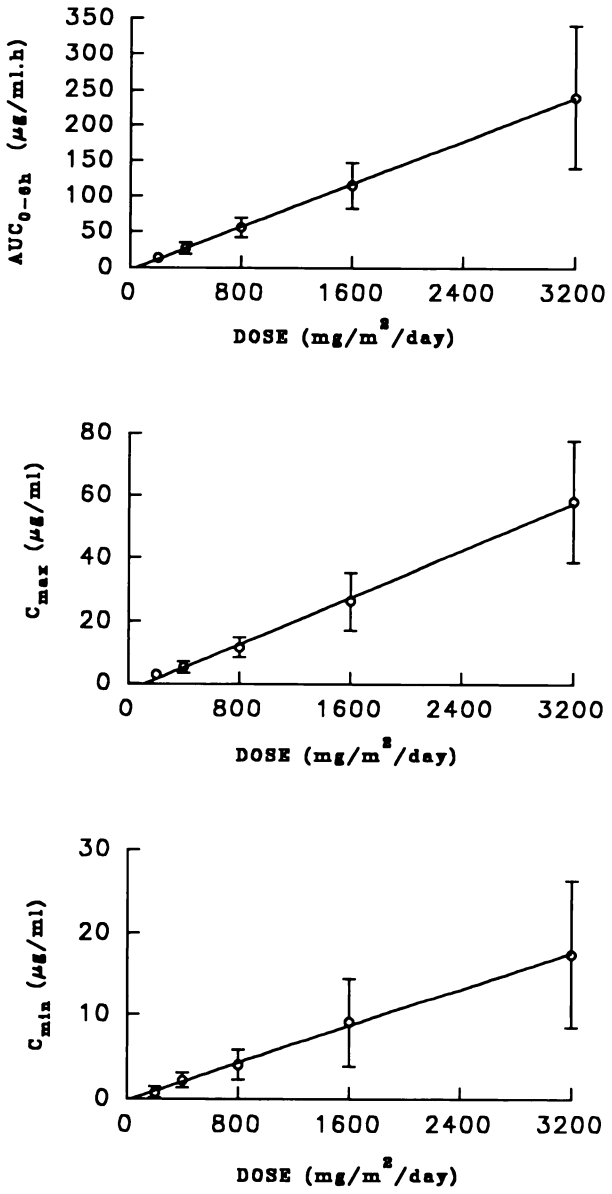

Fig. 2. AUC, C_{max}, and minimum plasma concentration before the next dose plotted against dose of DFMO for all subjects studied at each dose level. Values are means ± SD.

to tolerate 500 mg/m² for 10 months without ototoxicity. At 10 months 4 of 5 of these subjects had a >50% decrease in 12-*O*-tetradecanoylphorbol-13-acetate-induced ODC activity in punch biopsies of the skin. These authors were thus also able to demonstrate a biochemical effect of DFMO at doses which are well tolerated on chronic administration.

It is of interest that one subject with familial polyposis had a marked diminution in polyps in the rectal stump (she had had a colectomy) on DFMO. She reached the highest dose without toxicity and was continued on the drug. She also demonstrated an effect of DFMO on polyamine excretion in the urine (15). Increased levels of polyamine biosynthetic activity in rectal polyps com-

pared to adjacent colonic mucosa have been demonstrated by Porter *et al.* (23).

The failure of DFMO to prevent development of malignancy in 2 subjects is perhaps related to the markedly lower effect of this drug on already transformed cells compared to cells undergoing malignant transformation. One subject had a recurrence of colon carcinoma 5 months after initiating treatment with DFMO and presumably had microscopic disease at the time of entry into the study. The other had had a right modified radical mastectomy 4 years before and a left modified radical mastectomy 5 months before entry into the study, both for infiltrating ductal carcinoma. Three months after starting on DFMO she complained of epigastric pain, anorexia, and a 4-kg weight loss. Investigation revealed pancreatic enlargement, and laparotomy showed this to be extensive infiltrating adenocarcinoma of the pancreas involving liver and lymph nodes. It must be assumed that this lesion was present at the time of entry into the study. Thus, neither of these cases represents failure of DFMO as a chemopreventive agent.

The pharmacokinetics of DFMO was linear with dose. In this small group of subjects it was not possible to predict ototoxicity from either the AUC or C_{max} because of large interpatient variability in susceptibility to ototoxicity. Further studies of a larger group of subjects will be necessary to determine the parameters predictive for ototoxicity, which was dose limiting in this study.

On the basis of the findings in the present study, it is recommended that a dose of 1600 mg/m^2/day should not be exceeded for chronic administration in chemoprevention studies. However, it should be noted that except in one subject the maximum period of administration of this or higher doses was 3 months in the present study. Moreover, measurable biochemical effects were seen with doses as low as 200 mg/m^2/day (15), so the appropriate dose for long-term chemoprevention trials may be considerably lower than 1600 mg/m^2/day.

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References

1. Metcalf, B. W., Bey, P., Danzin, C., Jung, M. J., Casara, P., and Vevert, J. P. Catalytic irreversible inhibition of mammalian ornithine decarboxylase (E.C. 4.1.1.17) by substrate and product analogues. J. Am. Chem. Soc., *100:* 2551-2553, 1978.

2. Pegg, A. E., and McCann, P. P. Polyamine metabolism and function. Am. J. Physiol., *243:* C212-C221, 1982.

3. Pegg, A. E. Recent advances in the biochemistry of polyamines in eukaryotes. Biochem. J., *234:* 2249-2262, 1986.

4. Porter, C. W., and Sufrin, J. R. Interference with polyamine biosynthesis and/or function by analogs of polyamines or methionine as a potential anticancer chemotherapeutic strategy. Anticancer Res., *6:* 525-542, 1986.

5. Pera, P. J., Kramer, D. L., Sufrin, J. R., and Porter, C. W. Comparison of the biological effects of four irreversible inhibitors of ornithine decarboxylase in two murine lymphocytic leukemia cell lines. Cancer Res., *46:* 1148-1154, 1986.

6. Pegg, A. E. Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. Cancer Res., *48:* 759-774, 1988.

7. Abeloff, M.D., Slavik, M., Luk, G. D., Griffin, C. A., Hermann, J., Blanc, O., Sjoerdsma, A., and Baylin, S. B. Phase I trial and pharmacokinetic studies of α-difluoromethylornithine—an inhibitor of polyamine biosynthesis. J. Clin. Oncol., *2:* 124-130, 1984.

8. Maddox, A. M., Keating, M. J., McCredie, K. E., Estey, E., and Freireich, E. J. Phase I evaluation of intravenous difluoromethylornithine—a polyamine inhibitor. Invest. New Drugs, *3:* 287-292, 1985.

9. Natale, R. B., Meyer, K., and Forastiere, A. Phase I trials of α-difluoromethyl-ornithine alone and in combination with methylglyoxal bisguanylhydrazone. Proc. Am. Soc. Clin. Oncol., *4:* 137, 1985.

10. Meyskens, F. L., Kingsley, E. M., Glattke, T., Loescher, L., and Booth, A. A phase II study of α-difluoromethylornithine (DFMO) for the treatment of metastatic melanoma. Invest. New Drugs, *4:* 257-262, 1986.

11. Abeloff, M. D., Rosen, S. T., Luk, G. D., Baylin, S. B., Zeltzman, M., and Sjoerdsma, A. Phase II trials of α-difluoromethylornithine, an inhibitor of polyamine synthesis, in advanced small cell lung cancer and colon cancer. Cancer Treat. Rep., *70:* 843-845, 1986.

12. Fozard, J. D., and Prakash, N. J. Effects of D,L-α-difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase on the rat mammary tumor induced by 7,12,-dimethylbenz[α]anthracene. Naunyn-Schmiedebergs Arch. Pharmacol., *320:* 72-77, 1982.

13. Moon, R. C. Report no. IITRI L06173. Prepared for the National Cancer Institute by IIT Research Institute under Contract NO1-CN-55448-01, 1987.

14. Thompson, H. J., Herbst, E. J., and Meeker, L. D. Chemoprevention of mammary carcinogenesis: a comparative review of the efficacy of a polyamine antimetabolite, retinoids and selenium. J. Natl. Cancer Inst., *77:* 595-598, 1986.

15. Pendyala, L., Creaven, P. J., and Porter, C. W. Urinary and erythrocyte polyamines during the evaluation of oral α-difluoromethylornithine in a phase I chemoprevention clinical trial. Cancer Epidemiol., Biomarkers & Prev., *2:* 235-241, 1993.

16. Pendyala, L., Creaven, P. J., and Porter, C. Polyamine levels in red blood cells of subjects receiving α-difluoromethyl ornithine in a phase I chemoprevention study. *In:* R. H. Dowling, U. R. Folsch, and C. Loser (eds.), Polyamines in the Gastrointestinal Tract, pp. 193-202. Kluwer Academic Publishers, Dordrecht, the Netherlands: 1992.

17. Creaven, P. J., Pendyala, L., Petrelli, N., Douglass, H., Herrera, L., Porter, C., and Solomon, J. Phase I study of difluoromethylornithine (DFMO) as a chemopreventive agent. Proc. Am. Soc. Clin. Oncol., *11:* 144, 1992.

18. Pendyala, L., Creaven, P. J., Porter, C., Bhargava, A. K., Murphy, M. J., and Zdanowicz, J. R. Urinary and erythrocyte polyamines during oral difluoromethylornithine. Proc. Am. Assoc. Cancer Res., *33:* 208, 1992.

19. Bitonti, A. J., Bacchi, C. J., McCann, P. P., and Sjoerdsma, A. Uptake of α-difluoromethylornithine by *Trypanosoma brucei brucei*. Biochem. Pharmacol., *35:* 351-354, 1986.

20. Rocci, M. L., Jr., and Jusko, W. J. LAGRAN program for area and moments in pharmacokinetic analysis. Comput. Programs Biomed., *16:* 203-216, 1983.

21. Carbone, P. P., Love, R. R., Carey, P., Tutsch, K., Verma, A. K., Wilding, G., and Gilmore-Cunningham, D. Phase I and pharmacokinetics study of difluoromethyl-ornithine (DFMO), a potential chemopreventive. Proc. Am. Assoc. Cancer Res., *32:* 203, 1991.

22. Love, R. R., Carbone, P. P., Verma, A. K., Tutsch, K. D., Carey, P., Pomplun, M., Wilding, G., and Gilmore, D. Phase I study of difluoromethylornithine (DFMO): a chemopreventive agent. Proc. Am. Assoc. Cancer Res., *33:* 207, 1992.

23. Porter, C. W., Herrera-Ornelas, L., Pera, P., Petrelli, N. F., and Mittelman, A. Polyamine biosynthetic activity in normal and neoplastic human colorectal tissues. Cancer (Phila.), *60:* 1275-1281, 1987.