Minireview

Development of Difluoromethylornithine (DFMO) as a Chemoprevention Agent[1]

Frank L. Meyskens, Jr.[2] and Eugene W. Gerner

Departments of Medicine (Hematology/Oncology) and Biological Chemistry and the Chao Family Comprehensive Cancer Center, University of California, Irvine, Orange, California 92668 [F. L. M.], and Departments of Radiation Oncology and Biochemistry, Arizona Cancer Center, Tucson, Arizona 85724 [E. W. G.]

Abstract

DL-α-difluoromethylornithine (DFMO) was synthesized over 20 years ago. It was hoped that this enzyme-activated, irreversible inhibitor of ornithine decarboxylase, the first enzyme in polyamine synthesis, would be effective as a chemotherapy for hyperproliferative diseases, including cancer and/or infectious processes. DFMO was generally found to exert cytostatic effects on mammalian cells and tissues, and its effectiveness as a therapeutic agent has been modest. DFMO was also found to cause treatment-limiting (but reversible) ototoxicity at high doses. This side effect, along with its minimal therapeutic activity, contributed to the loss of interest by many clinicians in further developing DFMO as a cancer therapeutic agent. However, DFMO was subsequently shown to inhibit carcinogen-induced cancer development in a number of rodent models, and interest in developing this compound as a preventive agent has increased. The rationale for the inhibition of ornithine decarboxylase as a cancer chemopreventive agent has been strengthened in recent years because this enzyme has been shown to be transactivated by the c-*myc* oncogene in certain cell/tissue types and to cooperate with the *ras* oncogene in malignant transformation of epithelial tissues. Recent clinical cancer chemoprevention trials, using dose de-escalation designs, indicate that DFMO can be given over long periods of time at low doses that suppress polyamine contents in gastrointestinal and other epithelial tissues but cause no detectable hearing loss or other side effects. Current clinical chemoprevention trials are investigating the efficacy of DFMO to suppress surrogate end point biomarkers (*e.g.*, colon polyp recurrence) of carcinogenesis in patient populations at elevated risk for the development of specific epithelial cancers, including colon, esophageal, breast, cutaneous, and prostate malignancies.

Early Rationale for the Development of Inhibitors of Polyamine Metabolism

Studies on the diamine putrescine and its polyamine products spermidine and spermine date to the 17th century with the observation by Leeuwenhoek of spermine phosphate crystals in human semen (1). The strong association between high levels of the polyamines and rapid proliferation in prokaryotes and eukaryotes was recognized more than 25 years ago (2–4). These investigations led scientists at the Merrell Research Institute in Strasbourg to synthesize specific inhibitors of ODC[3] (5), the first enzyme in mammalian polyamine synthesis, and of other enzymes involved in polyamine metabolism (6–7). It was hoped that the inhibition of polyamine metabolism would be a successful strategy for chemotherapy for cancer and/or other hyperproliferative diseases or infectious diseases such as protozoal parasiticism (8).

Subsequent studies by the Merrell group and others, using specific ODC inhibitors (9–14) or genetic approaches (15, 16) to manipulate levels of endogenous polyamines, confirmed that amines derived from ornithine are essential for mammalian cell viability, and high levels are necessary for optimal mammalian cell growth. Corroborative results, demonstrating the importance of the polyamines for viability and growth, were also obtained in nonmammalian systems. Mutant strains of *Escherichia coli* and *Saccharomyces cerevisiae*, incapable of synthesizing the diamine putrescine, the first amine in the polyamine pathway, do not grow (17, 18). Null mutants of *S. cerevisiae*, which makes putrescine but not the triamine spermidine because of the deletion of the gene encoding the *S*-adenosylmethionine decarboxylase, also do not grow (19). *E. coli* apparently lack this spermidine requirement for growth (20).

Misconceptions Regarding DFMO Effects on Cells and Tissues

Because polyamines are ubiquitous and, apparently, essential molecules in cells, it was reasonable to presume that the inhibition of polyamine synthesis might be toxic. In some protozoal parasites, this hypothesis is true (21). The mechanism of cell death induced by DFMO in *Trypanosoma brucei* involves the limitation of the production of an essential antioxidant, trypanothione (22). The parasites die because of their inability to eliminate endogenous reactive oxygen species. The suppression of polyamine synthesis does not, however, generally cause cell death in mammalian cells (23–25). Although DFMO has been reported to kill some human tumor cells, concentrations required

Received 12/22/98; revised 2/10/99; accepted 2/10/99.
[1] Supported in part by National Cancer Institute Grants P30CA 62203, N01-CN-75019, and CA-72008.
[2] To whom requests for reprints should be addressed, at Chao Family Comprehensive Cancer Center, University of California-Irvine Medical Center, 101 The City Drive, Orange, California 92868-2675. Phone: (714) 456-6310; Fax: (714) 456-2240; E-mail: flmeyske@uci.edu.

[3] The abbreviations used are: ODC, ornithine decarboxylase; DFMO, difluoromethylornithine; SEB, surrogate end point biomarker; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; APC, adenomatous polyposis coli; min, multiple intestinal neoplasia; NSAID, nonsteroidal anti-inflammatory drug.

for cytotoxicity are greatly in excess of those required to suppress ODC activity (26). DFMO is usually cytostatic, causing a reduction in the rate of cell proliferation in the absence of cell death.

Several recent exceptions to this generalization have been described. Treatment of human colon cancer derived CaCo-2 cells, (constitutively expressing an activated Ki-*ras* oncogene) with DFMO suppressed colony formation.[4] However, DFMO suppressed the growth, but not the colony formation, of non-transfected CaCo-2 cells. DFMO also caused regression of epidermal papillomas induced by low doses of the chemical carcinogen 7,12-dimethylbenz[*a*]anthracene in transgenic mice overexpressing ODC (27). It has been shown that the activation of the oncogene c-*myc* influences cell proliferation and apoptosis by separable pathways (some involving ODC expression), presumably by modulating the production of cell survival and cell death factors (28). Mutations in the *ras* oncogene are prevalent in the transgenic skin carcinogenesis model (29). Thus, a plausible mechanism for selective cytotoxicity of DFMO in cells overexpressing an activated *ras* is that polyamines are required for either the formation of cell survival factors or the inhibition of cell death factors in cells expressing an activated *ras* oncogene. The suppression of polyamine pools would lead to a loss of viability.

In the few models in which DFMO seems to induce cell death [*e.g.*, in Lawson *et al.*[4] and Peralta Soler (27)], the mechanism of death is not apoptosis. In fact, DFMO has been shown to suppress apoptosis in several cell culture models (28, 30, 31). In these models, apoptosis induction requires overexpression of ODC.

Rationale for DFMO as an Inhibitor of Carcinogenesis

Polyamine contents are often elevated in rodent and human neoplastic cells/tissues, compared with relevant normal cells/tissues (32). A well-documented example of this relationship involves colonic polyps and cancers, compared with adjacent normal colonic mucosa (33–35). The mechanism of this elevation likely involves activation of signaling pathways influencing processes affecting intracellular polyamine pools. For example, nearly 70% of human colon cancers are associated with the activation of the c-*myc* oncogene (36). *ODC* is one target gene for the transcriptional transactivating activity of c-*myc* (37, 38). We have recently found that the loss of function of the *APC* tumor suppressor gene in the *min* mouse model of gastrointestinal cancer (39) causes steady-state levels of ODC RNA to increase 6–10-fold in both small and large intestinal tissue.[5] The increased ODC RNA expression is associated with an increase in especially small intestinal polyamine contents. DFMO suppresses both the increased polyamine contents and tumorigenesis in the small intestines of *min* mice. These data suggest a signaling pathway between the tumor suppressor gene *APC* and ODC. Others have recently shown that *APC* acts to suppress expression of c-*myc* (40). Because ODC is one of the transcriptional activation targets of c-*myc*, it is likely that the elevation of ODC RNA in the *min* mouse, a model in which the loss of functional APC is associated with gastrointestinal tumorigenesis, is mediated by activation of c-*myc*.

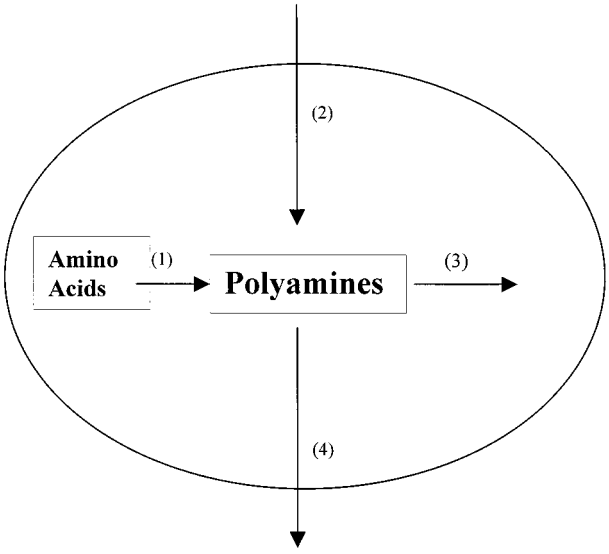

Fig. 1 Four general processes affect intracellular polyamine pool sizes. Polyamines can be (*1*) synthesized from amino acids; (*2*) transported from extracellular sources; (*3*) catabolized to shorter chain di- or polyamines or other molecules [*e.g.*, γ-aminobutyric acid (GABA)]; and/or (*4*) excreted.

Intracellular polyamine pool sizes are determined by a number of factors in addition to *ODC*, as depicted in Fig. 1. The identities of other oncogenes and tumor suppressor genes influencing the expression of ODC and/or other proteins affecting polyamine contents, as described in Fig. 1, remain to be elucidated for specific tissues.

Effects of DFMO on Cell and Tissue Polyamine Contents

Treatment of mammalian cell cultures, rodents, or humans with DFMO generally causes a suppression of putrescine and spermidine contents in cells/tissues in which intracellular pools depend on ODC activity (see Fig. 1), without affecting spermine pool sizes (41, 42). A notable exception to this finding is in human prostate tissue, in which spermine is the major polyamine. The administration of DFMO to men who are scheduled for surgical interventions to treat some form of prostate hyperplasia or neoplasia causes a suppression in all prostate polyamine pools, including the spermine pool.[6] Supplying cells or

[4] K. R. Lawson, N. A. Ignatenko, G. A. Piazza, and E. W. Gerner. Sulindac and difluoromethylornithine induce cytotoxicity by independent mechanisms, submitted for publication.

[5] S. H. Erdman, N. A. Ignatenko, M. B. Powell, K. Blohm, H. Holubec, and E. W. Gerner. Alterations in polyamine metabolism in the *min* mouse model of gastrointestinal carcinogenesis, submitted for publication.

[6] A. R. Simoneau, E. W. Gerner, R. B. Nagle, C. E. McLaren, and F. L. Meyskens, Jr. Human prostate polyamine levels and the response to α-difluoromethylornithine, manuscript in preparation.

Table 1 Side effects of DFMO (dose, g/m^2/day)

Except for hearing loss, the information on side effects is largely qualitative; in our more recent low-dose chemoprevention trials, the approach has been more quantitative (42, 67, 76). A detailed analysis of side effects and hearing changes of the most recent long-term, 1-year randomized trial of several doses of DFMO (42) is currently underway.[a]

	High (>3)	Intermediate (1–3)	Low (<1)
Diarrhea	Frequent, severe	Occasional, mild	Uncommon
Abdominal pain/bloating	Frequent, severe	Occasional, mild	Uncommon
Nausea/Vomiting	Frequent, moderate	Uncommon	Rare
Hematological	Modest	Not seen	Not seen
Reversible hearing loss	Common, cumulative dose-related	Occasional, dose-related	Uncommon, may be absent at <0.5 g/m^2/day

[a] Unpublished data.[8]

animals with sufficient amounts of exogenous polyamines to restore normal intracellular pools can reverse most of the effects of DFMO (8, 10, 41).

Several groups reported that polyamine metabolism was an integral component of the mechanism of carcinogenesis, especially in epithelial tissues. Inhibitors of ODC were found to suppress tumor formation in experimental models of bladder, breast, colon, and skin carcinogenesis (32, 43–45). Inactivation of the FAD-dependent polyamine oxidase (PAO), the second enzyme in polyamine catabolism, impeded colon carcinogenesis in the dimethylhydrazine-treated rat model (46).

The mechanism of cancer prevention by DFMO probably involves more than simple inhibition of cell proliferation. Studies in animals suggested that DFMO acts late in models of chemical carcinogenesis, affecting the transition of noninvasive tumors to invasive cancers (47). Consequently, several groups have demonstrated that the expression of genes affecting tumor invasion, including the matrix metalloproteinases, are dependent on polyamines and inhibited by DFMO in several cell types (48–50).

Validation of SEBs for DFMO Effect

To assess DFMO effects in humans, we sought to validate specific markers of effects of this drug in specific tissues. ODC is the target for DFMO, and consequently should be an appropriate SEB for DFMO effect. However, ODC is a highly regulated and labile protein. Consequently, measurement of its activity in uninduced tissues is difficult. Because polyamines are stable molecules, we reasoned that the measurement of ODC products may be a meaningful measure of DFMO effect in some cases, and we have used high performance liquid chromatography (HPLC) techniques to measure tissue polyamine contents, in addition to ODC enzyme activity. We evaluated our ability to measure ODC activity and polyamine contents in colonic and rectal mucosa under a number of conditions relevant to our methods of obtaining colorectal mucosal biopsies (51). These conditions included bowel preparation procedures, size of the biopsy, number of biopsies evaluated in a single measure, and biopsy location in the bowel. Our results indicated that the bowel preparation method did not influence our measurements. Polyamine contents, and especially the spermidine/spermine ratio, were less variable than the measurement of ODC enzyme activity. Spermidine/spermine ratios were least variable, because this parameter did not depend on a second measurement (*e.g.*, tissue protein or DNA content) for normalization. Consequently, we routinely measure polyamine contents as primary SEBs of DFMO effects in human colonic tissues (*e.g.*, see Ref. 42).

Clinical Studies of DFMO in Malignant and Precancerous Conditions

Early clinical cancer therapeutic trials with DFMO were disappointing, and at high doses (greater than 3 g/m^2/day), several side effects occurred, including diarrhea, abdominal pain, and emesis, as well as moderate anemia, leukopenia, and thrombocytopenia (52–57). Some responses were noted in Phase I toxicity and uncontrolled Phase II efficacy studies, but controlled studies failed to establish DFMO as a useful agent in specific disease sites. In addition, DFMO treatment was associated with treatment-limiting ototoxicity (58), which curtailed its utility as a cancer therapeutic agent. Recently DFMO combined with BCNU has been shown to have considerable effect on glioblastomas (59), and a reexploration of the drug in combination may be worthwhile.

We and several other groups have been actively involved in the development of DFMO as a chemoprevention agent with a systematic emphasis on the skin (60, 61), cervix (62–64), and colon (42, 65, 66). The side effects of DFMO at intermediate (1–3 g/m^2/day) doses are few and limited to mild gastrointestinal upset and reversible hearing changes. At the doses (less than 0.50 g/m^2/day) of DFMO being proposed for long-term chemoprevention trials, no systematic side effects (including hearing loss), have been seen (discussed below). A comparison of the side effects seen with DFMO at low, intermediate, and high doses is shown in Table 1.

Two major issues have been of prime importance in consideration of the development of DFMO as a chemoprevention agent: (*a*) its ability to lower polyamine levels in the tissue of interest; and (*b*) its effect on hearing; and the key elements of the major chemoprevention trials with DFMO are summarized in Table 2. We have performed a series of studies that have demonstrated that DFMO lowers polyamines in rectal mucosa (42, 65, 66) and does so in a dose-response manner without a rebound increase of polyamine levels after discontinuation of the drug (42). Additionally, at a dose below 0.40 g/m^2/day, side effects and hearing changes did not occur more frequently than

Table 2 Chemoprevention trials of DFMO

Phase	Organ Site	Dose of DFMO (g/m^2/day)	Comment	Reference
Pilot	Colon	(0.50)	Polyamines in buccal mucosal cells was not a surrogate for rectal mucosa	Boyle (65) 1992
IIA (1 month, de-escalation)	Colon	(0.075–0.50)	Polyamines in rectal mucosa suppressed down to dose of 0.20 and perhaps lower, well-tolerated	Meyskens (67) 1994
IIb (12 months, randomized)	Colon	(0.20–0.40)	Polyamines in rectal mucosa suppressed without rebound. No side effects or hearing loss	Meyskens (42) 1998
IIb (12 months)	Colon	(0.5)	Polyamine suppressed, infrequent reversible hearing loss	Love (67) 1998
I (1 month)	Skin	(0.125–1.0)	TPA-induced ODC suppressed and no side effects at dose <0.5	Love (60) 1993
I (1 month)	Skin	(0.50)	Combined with piroxidam, TPA induced ODC suppressed	Carbone (61) 1998
I (1 month, de-escalation)	Cervix	(0.06–1.0)	Polyamines suppressed in cervix tissue; responses of CINIII documented	Mitchell (64) 1998
II (12 months, randomized)	Bladder	(0.25–1.0)	Well-tolerated at all doses. No side effects or hearing changes noted	Loprinzi (76) 1996

in the placebo group, even after 1 year of therapy. Because the overall effect of DFMO on rectal mucosal levels of polyamines (putrescine levels, spermidine:spermine ratio) was equivalent at daily doses of 0.20 or 0.40 g/m^2/day, these studies suggested that a dose of DFMO of 0.20 g/m^2/day would be effective in lowering colon mucosal polyamines without producing side effects, including audiometric decreases in hearing threshold (see Ref. 42). In a smaller study, Love *et al.* has reported on the effect of 0.50 g/m^2/day of DFMO on rectal mucosal polyamines (67). Compared with the placebo group, polyamines were decreased after 3 and 12 months of therapy. A recent case-control study of patients with colon cancer (68) indicates that increases in mucosal polyamine measurements were significantly associated with risk (odds ratio, 4.8). This study provides additional evidence for polyamines as biomarkers for identifying high-risk individuals and/or as intermediate end points in colon prevention trials.

In a complex Phase I study of oral DFMO for 1 month at multiple doses, an effect of the drug on TPA-induced ODC activity in the skin biopsies was demonstrated (60). On the basis of limited data, the investigators concluded that a dose of 0.50 g/m^2/day produced this biochemical response and that no side effects were demonstrated. In a two-step Phase I study of DFMO, piroxicam, or the combination in 31 subjects the combination of DFMO alone at 0.50 g/m^2/day significantly reduced cutaneous TPA-induced ODC levels (61). In this study, no objective changes in hearing were demonstrated (61). Mitchell *et al.* (64) have also reported the results from a 1 month dose de-escalation Phase I trial of oral DFMO in grade 3 cervical intraepithelial neoplasia. A dose of 1.0 g/m^2/day produced a significant decrease in the spermidine:spermine ratio in the cervix tissue. Surprisingly, 15 patients experienced a complete or partial histological response that was not dose-dependent. Lower doses and longer-term randomized trials will be necessary to determine whether lower doses also produce these effects because the effect on tissue polyamines takes time and, except for one agent [topical *trans*-retinoic acid (69)], evaluation of chemoprevention agents in the Phase III setting has not borne out promising Phase II results in cervical intraepithelial neoplasia (70–73). We have also measured the effect of 1 month of oral DFMO on polyamines in the prostate in patients undergoing a definitive surgical procedure, and we have demonstrated a marked lowering of polyamines;[6] (R. Love of Wisconsin has also obtained similar results.[7])

Although DFMO has been highly effective as a chemoprevention agent in combination in preclinical models, to date only one clinical study has been reported using DFMO in combination (61). Using a complicated but rational two-step approach, the effect of 6 months of oral daily DFMO and piroxicam alone and in combination on TPA-induced ornithine ODC in skin biopsies and urinary 11-dehydrothromboxane B_2 was measured, and an effect on these biomarkers demonstrated. On the basis of these responses and a favorable clinical profile, doses of DFMO of 0.50 g/m^2 daily and piroxicam 10 mg every other day was recommended for Phase IIa and IIb trials.

In therapeutic trials, hearing loss was frequent and considerable, although reversible (58). However, the doses being used in chemoprevention trials are considerably lower. There are three reports that have examined the issue of hearing loss from DFMO in detail (42, 74, 75). Our original meta-analysis of patients receiving DFMO for therapeutic reasons suggested that hearing loss rarely occurred below a total cumulative dose of about 150 g and that above this dose, the hearing loss was cumulative but reversible (74). However, these patients were receiving doses of DFMO above 1 g/m^2 daily, and, therefore, the direct relevance of this finding to hearing changes at the lower doses used in chemoprevention trials is problematic.

Pasic *et al.* (75) has done an analysis of hearing changes in 66 patients entered into their Phase I and II trials. The oral doses of DFMO ranged between 0.5 and 5.0 g/m^2 daily. A complex analysis was performed, and the conclusions were made that small predictable shifts in auditory thresholds occurred, which

[7] R. Love, personal communication.

increased as the daily dose of DFMO increased, but that the changes were not related to cumulative dosage. However, an analysis of the mean thresholds at the beginning and end of the study for all of the subjects receiving a dose of 0.50 g/m^2 of DFMO indicated that there was no discernible shift of audiometric threshold at any frequency measured. The most relevant study addressing the issue of hearing loss by DFMO is our 1-year placebo-controlled randomized trial of DFMO (42). The doses of DFMO were low but effective in lowering tissue polyamines. Pretreatment and serial audiometry were performed. There was no evidence for a dose-related effect of DFMO on hearing at the three doses tested, 0.075, 0.20, and 0.40 g/m^2/day. Subsequent detailed analyses of the data indicate that there is no evidence to suggest that hearing loss at any frequency at the lowest and intermediate dose occurred.[8] At the highest dose tested (0.40 g/m^2/day), there may be a 3-dB decrease (which was clinically unimportant) at the two lowest of the eight frequencies tested.

Overall, we conclude that the effect of DFMO on hearing at doses relevant for usage as a chemoprevention agent is not significant. In a study of different doses (0.125–1.0 g/m^2/day) of DFMO given to patients with superficial bladder cancers, Loprinzi *et al.* (76) have found that little to no side effects were demonstrated. We have also found that the effect of DFMO on nonaudiological side effects at doses below 0.40 g/m^2/day is not greater than placebo (41), thereby providing considerable strength for its usage at low doses as a chemoprevention agent. Recently, two detailed studies of aging and hearing have been published (77, 78), which will help considerably in the long-term evaluation of subtle hearing changes in response to DFMO and other potentially ototoxic drugs; a set of guidelines for hearing changes and chemoprevention drug development is currently being developed by the National Cancer Institute.[9]

Current Lessons and Future Development

Several important lessons have emerged from the development of DFMO that have relevance to the development of chemoprevention agents in general, particularly those which are currently used for other indications.

These key issues include:

(1) The relevance of *in vitro* and preclinical models to identify appropriate SEBs for the intervention, and to predict consequences for the intervention, in humans (*e.g.*, inhibit proliferation, induce apoptosis, inhibit invasion).

(2) Side effects that occur at high therapeutic doses of the drug may not be present or relevant at lower doses.

(3) A dose de-escalation design is a powerful method by which to determine the lowest dose of an agent that can consistently modulate the relevant biochemical markers without side effects.

(4) A dose of DFMO 0.20–40 g/m^2 daily is probably the best estimate of the proper dose for subsequent colon cancer chemoprevention trials. DFMO doses required to suppress polyamine contents in other tissues need to be verified for each tissue under study.

(5) Although DFMO is a potent inhibitor of epithelial carcinogenesis, it does not totally suppress tumorigenesis in animal models. Consequently, combinations of DFMO with other agents, such as the NSAIDs should be considered. Our group is conducting both preclinical and clinical investigations combining DFMO with the NSAID sulindac at this time.

At the clinical level, interest in the exploration of DFMO as a chemoprevention agent has recently increased markedly. Currently, we are aware of the following clinical trials using DFMO as a chemoprevention agent: breast (C. Fabian, University of Kansas), Barrett's esophagus (D. Brenner, University of Michigan), cervix (M. Follen Mitchell, M. D. Anderson Cancer Center, Houston, TX), and prostate (A. Simoneau, University of California-Irvine). Additionally, DFMO is being studied in combination with piroxican in a Phase II nonmelanoma skin cancer trial (P. Carbone, University of Wisconsin) and with sulindac in a Phase IIb colon cancer prevention study (F. Meyskens, University of California-Irvine, and E. Gerner, University of Arizona).

[8] S. Emerson, unpublished data.
[9] Jaye L. Viner, personal communication.

References

1. Cohen, S. S. A Guide to the Polyamines. New York: Oxford University Press, 1998.

2. Bachrach, U., and Weinstein, A. Effect of aliphatic polyamines on growth and macromolecular syntheses in bacteria. J. Gen. Microbiol., *60:* 159–165, 1970.

3. Pohjanpelto, P., and Raina, A. Identification of a growth factor produced by human fibroblasts *in vitro* as putrescine. Nature New Biol., *235:* 247–249, 1972.

4. Russell, D. H. The roles of the polyamines, putrescine, spermidine and spermine in normal and malignant tissues. Life Sci., *13:* 1635–1647, 1973.

5. Bey, P., Danzin, C., Van Dorsselaer, V., Mamont, P., Jung, M., and Tardif, C. Analogues of ornithine as inhibitors of ornithine decarboxylase. New deductions concerning the topography of the enzyme's active site. J. Med. Chem., *21:* 50–55, 1978.

6. Kolb, M., Danzin, C., Barth, J., and Claverie, N. Synthesis and biochemical properties of chemically stable product analogues of the reaction catalyzed by *S*-adenosyl-L-methionine decarboxylase. J. Med. Chem., *25:* 550–556, 1982.

7. Bolkenius, F. N., Bey, P., and Seiler, N. Specific inhibition of polyamine oxidase *in vivo* is a method for the elucidation of its physiological role. Biochim. Biophys. Acta, *838:* 69–76, 1985.

8. McCann, P. P., Pegg, A. E., and Sjoerdsma, A. (eds.), Inhibition of Polyamine Metabolism. Biological Significance and Basis for New Therapies. New York: Academic Press, Inc., 1987.

9. Mamont, P. S., Bohlen, P., McCann, P. P., Bey, P., Schuber, F., and Tardif, C. α-methyl ornithine, a potent competitive inhibitor of ornithine decarboxylase, blocks proliferation of rat hepatoma cells in culture. Proc. Natl. Acad. Sci. USA, *73:* 1626–1630, 1976.

10. Mamont, P. S., Duchesne, M. C., Grove, J., and Bey, P. Antiproliferative properties of D,L-α-difluoromethylornithine in cultured cells: a consequence of the irreversible inhibition of ornithine decarboxylase. Biochem. Biophys. Res. Commun., 81: 58–66, 1978.

11. Prakash, N. J., Schechter, P. J., Grove, J., and Koch-Weser, J. Effect of α-difluoromethylornithine, an enzyme-activated irreversible inhibitor of ornithine decarboxylase, on L1210 leukemia in mice. Cancer Res., *38:* 3059–3062, 1978.

12. Danzin, C., Jung, M. J., Grove, J., and Bey, P. Effect of α-difluoromethylornithine, an enzyme-activated irreversible inhibitor of ornithine decarboxylase, on polyamine levels in rat tissues. Life Sci., *24:* 519–524, 1979.

13. Kato, Y., Inoue, H., Gohda, E., Tamada, F., and Takeda, Y. Effect of DL-α-hydrazino-δ-aminovaleric acid, an inhibitor of ornithine decarboxylase, on polyamine metabolism and growth of mouse sarcoma-180. Gann, *67:* 569–576, 1976.

14. Newton, N. E., and Abdel-Monem, M. M. Inhibitors of polyamine biosynthesis. 4. Effects of α-methyl-(+/−)-ornithine and methylglyoxal bis(guanylhydrazone) on growth and polyamine content of L1210 leukemic cells of mice. J. Med. Chem., *20:* 249–253, 1977.

15. Steglich, C., and Scheffler, I. E. An ornithine decarboxylase-deficient mutant of Chinese hamster ovary cells. J. Biol. Chem., *257:* 4603–4609, 1982.

16. Pohjanpelto, P., Holtta, E., and Janne, O. A. Mutant strain of Chinese hamster ovary cells with no detectable ornithine decarboxylase activity. Mol. Cell. Biol., *5:* 1385–1390, 1985.

17. Tabor, H., Tabor, C. W., Cohn, M. S., and Hafner, E. W. Streptomycin resistance (rpsL) produces an absolute requirement for polyamines for growth of an *Escherichia coli* strain unable to synthesize putrescine and spermidine [Δ(speA-speB) Δ specC]. J. Bacteriol., *147:* 702–704, 1981.

18. Tabor, C. W., Tabor, H., Tyagi, A. K., and Cohn, M. S. The biochemistry, genetics, and regulation of polyamine biosynthesis in *Saccharomyces cerevisiae*. Fed. Proc., *41:* 3084–3088, 1982.

19. Balasundaram, D., Tabor, C. W., and Tabor, H. Spermidine or spermine is essential for the aerobic growth of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA, *88:* 5872–5876, 1991.

20. Xie, Q. W., Tabor, C. W., and Tabor, H. Deletion mutations in the speED operon: spermidine is not essential for the growth of *Escherichia coli*. Gene (Amst.), *126:* 115–117, 1993.

21. Bacchi, C. J., Nathan, H. C., Hutner, S. H., McCann, P. P., and Sjoerdsma, A. Polyamine metabolism: a potential therapeutic target in trypanosomes. Science (Washington DC), *210:* 332–334,1980.

22. Fairlamb, A. H., Henderson, G. B., Bacchi, C. J., and Cerami, A. *In vivo* effects of difluoromethylornithine on trypanothione and polyamine levels in bloodstream forms of *Trypanosoma brucei*. Mol. Biochem. Parasitol., *24:* 185–191, 1987.

23. Fuller, D. J., and Gerner, E. W. Delayed sensitization to heat by inhibitors of polyamine-biosynthetic enzymes. Cancer Res., *42:* 5046–5049, 1982.

24. Oredsson, S. M., Deen, D. F., and Marton, L. J. Decreased cytotoxicity of *cis*-diamminedichloroplatinum(II) by α-difluoromethylornithine depletion of polyamines in 9L rat brain tumor cells *in vitro*. Cancer Res., *42:* 1296–1299, 1982.

25. Claverie, N., and Mamont, P. S. Comparative antitumor properties in rodents of irreversible inhibitors of L-ornithine decarboxylase, used as such or as prodrugs. Cancer Res., *49:* 4466–4471, 1989.

26. Barranco, S. C., Ford, P. J., and Townsend, C. M., Jr. Heterogeneous survival and cell kinetics responses of human astrocytoma clones to α-difluoromethylornithine *in vitro*. Invest. New Drugs, *7:* 155–161, 1989.

27. Peralta Soler, A., Gilliard, G., Megosh, L., George, K., and O'Brien, T. G. Polyamines regulate expression of the neoplastic phenotype in mouse skin. Cancer Res., *58:* 1654–1659, 1998.

28. Packham, G., Porter, C. W., and Cleveland J. L. c-Myc induces apoptosis and cell cycle progression by separable, yet overlapping, pathways. Oncogene, *13:* 461–469, 1996.

29. Megosh, L., Halpern, M., Farkash, E., and O'Brien, T. G. Analysis of *ras* gene mutational spectra in epidermal papillomas from K6/ODC transgenic mice. Mol. Carcinog., *22:* 145–149, 1998.

30. Packham, G., and Cleveland, J. L. The role of ornithine decarboxylase in c-Myc-induced apoptosis. Curr. Top. Microbiol. Immunol., *194:* 283–290, 1995.

31. Tome, M. E., Fiser, S. M., Payne, C. M., and Gerner, E. W. Excess putrescine accumulation inhibits the formation of modified eukaryotic initiation factor 5A(eIF-5A) and induces apoptosis. Biochem. J., *328:* 847–854, 1997.

32. Verma, A. K. Inhibition of tumor promotion by DL-α-difluoromethylornithine, specific irreversible inhibitor of ornithine decarboxylase. Basic Life Sci., *52:* 195–204, 1990.

33. Hixson, L. J., Garewal, H. S., McGee, D., Sloan, D., Fennerty, M. B., Sampliner, R. E., and Gerner, E. W. Ornithine decarboxylase and polyamines in colorectal neoplasia and adjacent mucosa. Cancer Epidemiol. Biomark. Prev., *2:* 369–374, 1993.

34. Rozhin, J., Wilson, P. S., Bull, A. W., and Nigro, N. D. Ornithine decarboxylase activity in the rat and human colon. Cancer Res., *44:* 3226–3230, 1984.

35. Tempero, M. Bile acids, ornithine decarboxylase, and cell proliferation in colon cancer: a review. Dig. Dis., *4:* 49–56, 1986.

36. Augenlicht, L. H., Wadler, S., Corner, G., Richards, C., Ryan, L., Multani, A. S., Pathak, S., Benson, A., Haller, D., and Heerdt, B. G. Low-level c-*myc* amplification in human colonic carcinoma cell lines and tumors: a frequent, p53-independent mutation associated with improved outcome in a randomized multi-institutional trial. Cancer Res., *57:* 1769–1775, 1997.

37. Bello-Fernandez, C., Packham, G., and Cleveland, J. L. The ornithine decarboxylase gene is a transcriptional target of c-Myc. Proc. Natl. Acad. Sci. USA, *90:* 7804–7808, 1993.

38. Pena, A., Reddy, C. D., Wu, S., Hickok, N. J., Reddy, E. P., Yumet, G., Soprano, D. R., and Soprano, K. J. Regulation of human ornithine decarboxylase expression by the c-Myc.Max protein complex. J. Biol. Chem., *268:* 27277–27285, 1993.

39. Su, L. K., Kinzler, K. W., Vogelstein, B., Preisinger, A. C., Moser, A. R., Luongo, C., Gould, K. A., and Dove W. F. Multiple intestinal neoplasia caused by a mutation in the murine homolog of the *APC* gene. Science (Washington DC), *256:* 668–670, 1992.

40. He, T. C., Sparks, A. B., Rago, C., Hermeking, H., Zawel, L., da Costa, L. T., Morin, P. J., Vogelstein, B., and Kinzler, K. W. Identification of c-MYC as a target of the APC pathway. Science (Washington DC), *281:* 1509–1512, 1998.

41. Gerner, E. W., and Mamont, P. S. Restoration of polyamine contents in rat hepatoma (HTC) cells after inhibition of polyamine biosynthesis: relationship with cell proliferation. Eur. J. Biochem., *156:* 31–35, 1986.

42. Meyskens, F. L., Jr., Gerner, E., Emerson, S., Pelot, D., Durbin, T., Doyle, K., and Lagerberg, W. A randomized double-blind placebo controlled Phase IIb trial of difluoromethylornithine for colon cancer prevention. J. Natl. Cancer Inst., *90:* 1212–1218, 1998.

43. Nigro, N. D., Bull, A. W., and Boyd, M. E. Inhibition of intestinal carcinogenesis in rats: effect of difluoromethylornithine with piroxicam or fish oil. J. Natl. Cancer Inst., *77:* 1309–1313, 1986.

44. Thompson, H. J., and Ronan, A. M. Effect of D,L-2-difluoromethylornithine and endocrine manipulation on the induction of mammary carcinogenesis by 1-methyl-1-nitrosourea. Carcinogenesis (Lond.), *7:* 2003–2006, 1986.

45. Nowels, K., Homma, Y., Seidenfeld, J., and Oyasu R. Prevention of inhibitory effects of α-difluoromethylornithine on rat urinary bladder carcinogenesis by exogenous putrescine. Cancer Biochem. Biophys., *8:* 257–263, 1986.

46. Halline, A. G., Dudeja, P. K., Jacoby, R. F., Llor, X., Teng, B. B., Chowdhury, L. N., Davidson, N. O., and Brasitus, T. A. Effect of polyamine oxidase inhibition on the colonic malignant transformation process induced by 1,2-dimethylhydrazine. Carcinogenesis (Lond.), *11:* 2127–2132, 1990.

47. Slaga, T. J. Multistage skin carcinogenesis: a useful model for the study of the chemoprevention of cancer. Acta Pharmacol. Toxicol., *55* (Suppl. 2)*:* 107–124, 1984.

48. Wallon, U. M., Shassetz, L. R., Cress, A. E., Bowden, G. T., and Gerner, E. W. Polyamine-dependent expression of the matrix metalloproteinase matrilysin in a human colon cancer cell line. Mol. Carcinog., *11:* 138–144, 1994.

49. Kubota, S., Kiyosawa, H., Nomura, Y., Yamada, T., and Seyama, Y. Ornithine decarboxylase overexpression in mouse 10T1/2 fibroblasts: cellular transformation and invasion. J. Natl Cancer Inst., *89:* 567–571, 1997.

50. Smith, M. K., Goral, M. A., Wright, J. H., Matrisian, L. M., Morris, R. J., Klein-Szanto, A. J., and Gilmour, S. K. Ornithine decarboxylase overexpression leads to increased epithelial tumor invasiveness. Cancer Res., *57:* 2104–2108, 1997.

51. Hixson, L. J., Emerson, S. S., Shassetz, L. R., and Gerner, E. W. Sources of variability in measurements of ornithine decarboxylase activity and polyamine contents in colorectal mucosa. Cancer Epidemiol Biomark. Prev., *3:* 317–323, 1994.

52. Dunzendorfer, U., and Knoner, M. Therapy with inhibitors of polyamine biosynthesis in refractory prostatic carcinoma: an experimental and clinical study. Onkologie, *8:* 196–200, 1985.

53. 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.

54. 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.

55. 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.

56. Talpaz, M., Plager, C., Quesada, J., Benjamin, R., Kantajian, H., and Gutterman, J. Difluoromethylornithine and leukocyte interferon: a Phase I in cancer patients. Eur. J. Cancer & Clin. Oncol., *22:* 685–689, 1986.

57. Harari, P. M., Fuller, D. J. M., Carper, S. W., Croghan, M. K., Meyskens, F. L., Jr., Shimm, D. S., and Gerner, E. W. Polyamine biosynthesis inhibitors combined with systemic hyperthermia in cancer therapy. Int. J. Radiat. Oncol. Biol. Phys., *19:* 89–96, 1990.

58. Schweitzer, V. G. Ototoxicity of chemotherapeutic agents. Otolaryngol. Clin. N. Am., *26:* 759–789, 1993.

59. Prados, M., Rodriguez, L., Chamberlain, M., Silver, P., and Levin, V. Treatment of recurrent gliomas with 1,3-bix(2-chloroethyl-1-nitrosourea and α-difluoromethylornithine. Neurosurgery (Baltimore), *24:* 806–809, 1989.

60. Love, R. R., Carbone, P. P., Verma, A. K., Gilmore, D., Carey, P., Tutsch, K. D., Pomplun, M., and Wilding, G. Randomized Phase I chemoprevention dose-seeking study of α-difluoromethylornithine. J. Natl. Cancer Inst., *85:* 732–736, 1993.

61. Carbone, P. P., Douglas, J. A., Larson, P. O., Verma, A. K., Blair, I. A., and Tutsch, K. Phase I chemoprevention study of piroxicam (PXM), and α-difluoromethylornithine (DFMO). Cancer Epidemiol. Biomark. Prev., *7:* 907–912, 1998.

62. Nishioka, K., Melgarejo, A. B., Lyon, R. R., and Mitchell, M. F. Polyamines as biomarkers of cervical intraepithelial neoplasia. J. Cell. Biochem., *23* (Suppl.)*:* 87–95, 1995.

63. Mitchell, M. F., Hittelman, W. K., Lotan, R., Nishioka, K., Tortolero-Luna, G., Richards-Kortum, R. R., Wharton, J. T., and Hong, W. K. Chemoprevention trials and surrogate end point biomarkers in the cervix. Cancer (Phila.), *76* (Suppl)*:* 1956–1977, 1995.

64. Mitchell, M. F., Tortolero-Luna, G., Lee, J. J., Hittelman, W. N., Lotan, R., Wharton J. T., Hong, W. K., and Nishioka, K. Phase I dose de-escalation trial of α-difluoromethylornithine in patients with grade 3 cervical intraepithelial neoplasia. Clin. Cancer Res., *4:* 303–310, 1998.

65. Boyle, J. O., Meyskens, F. L., Jr., Garewal, H. S., and Gerner, E. W. Polyamine contents in rectal and buccal mucosae in humans treated with oral difluoromethylornithine. Cancer Epidemiol. Biomark. Prev., *1:* 131–135, 1992.

66. Meyskens, F. L., Jr., Emerson, S. S., Pelot, D., Meshkinpour, H., Shassetz, R., Einspahr, J., Alberts, D. S., and Gerner, E. W. Dose de-escalation chemoprevention trial of α-difluoromethylornithine in patients with colon polyps. J. Natl. Cancer Inst., *86:* 1122–1130, 1994.

67. Love., R. R., Jacoby, R., Newton, M. A., Tutsch, K. D., Simon, K., Pomplun, M., and Verma, A. K. A randomized, placebo-controlled trial of low dose α-difluoromethylornithine in individuals at risk for colorectal cancer. Cancer Epidemiol. Biomark. Prev., *7:* 989–992, 1998.

68. Wang, W., Liu, L. Q., and Higuchi, C. M. Mucosal polyamine measurements and colorectal cancer risk. J. Cell. Biochem., *63:* 252–257, 1996.

69. Meyskens, F. L., Jr., Surwit, E., Moon, T. E., Childers, J. M., Davis, J. R., Dorr, R., Johnson, C. S., and Alberts, D. S. Enhancement of regression of cervical intraepithelial neoplasia II (moderate dysplasia) with topically applied all-*trans*-retinoic acid: a randomized trial. J. Natl. Cancer Inst., *86:* 539–543, 1994.

70. Butterworth, C. E., Jr., Hatch, K. D., Macaluso, M., Cole, P., Sauberlich, H. E., Soong, S. J., Borst, M., and Baker, V. V. Folate deficiency and cervical dysplasia. J. Am. Med. Assoc., *267:* 528–533, 1992.

71. Childers, J. M., Chu, J., Voigt, L. F., Tamimi, H. K., Franklin, E. W., Alberts, D. S., and Meyskens, F. L., Jr. Chemoprevention of cervical cancer with folic acid: a Phase III Southwest Oncology Group Intergroup study. Cancer Epidemiol. Biomark. Prev., *4:* 155–159, 1995.

72. Romney, S. L., Ho, G. Y., Palan, P. R., Basu, J., Kadish, A. S., Klein, S., Mikhail, M., Hagan, R. J., Chang, C. J., and Burk, R. D. Effects of β-carotene and other factors on outcome of cervical dysplasia and human papillomavirus infection. Gynecol. Oncol., *65:* 483–492, 1997.

73. Keefe, K. A., Wilczynski, S., Lagerberg, W., Brewer, C., Chapman, J., Upsani, S., and Berman, M. Effects of β-carotene on cervical intrepithelial neoplasia (CIN): a Phase II trial. Twenty-ninth Annual Society of Gynecologic Oncologists Meeting. Gynecol. Oncol., *68:* 110, 1998.

74. Croghan, M. K., Aicken, M. G., and Meyskens, F. L., Jr. Dose-related α-difluoromethylornithine (DFMO) ototoxicity (reversible hearing loss). Am. J. Clin. Onc., *14:* 331–335, 1991.

75. Pasic, T. R., Heisey, D., and Love, R. R. α-Difluoromethylornithine ototoxicity: chemoprevention clinical trial results. Arch. Otolaryngol. Head Neck Surg., *123:* 1281–1286, 1997.

76. Loprinzi, C. L., Messing, E. M., O'Fallon J. R., Poon, M. A., Love, R. R., Quella, S., K., Trump, D. L., Morton, R. F., and Novotny, P. Toxicity evaluation of difluoromethylornithine: doses for chemoprevention trials. Cancer Epidemiol. Biomark. Prev., *5:* 371–374, 1996.

77. Morrell, C. H., Gordon-Salant, S., Pearson, J. D., Brant, L. J., and Fozard, J. L. Age- and gender-specific reference ranges for hearing level and longitudinal changes in hearing level. J. Acoust. Soc. Am., *100:* 1949–1967, 1996.

78. Wiley, T. L., Cruickshanks, K. J., Nondahl, D. M., Tweed, T. S., Klein R., and Klein, B. E. K. Aging, and high-frequency hearing sensitivity. J. Speech Lang. Hear. Res., *41:* 1061–1072, 1998.

Clinical Cancer Research

Development of Difluoromethylornithine (DFMO) as a Chemoprevention Agent

Frank L. Meyskens, Jr. and Eugene W. Gerner

Clin Cancer Res 1999;5:945-951.