NCI, DCPC
Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN:

2-DIFLUOROMETHYLORNITHINE (DFMO)

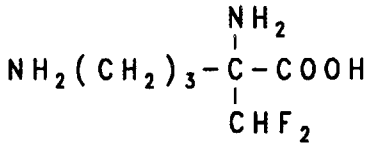
DRUG IDENTIFICATION

CAS Registry No.: 70052-12-9

CAS Name (9CI): 2-(Difluoromethyl)ornithine

Synonyms: α-Difluoromethylornithine
Eflornithine
Ornidyl®
RMI 71782

Structure:



EXECUTIVE SUMMARY

DFMO is a potent, irreversible inhibitor of the activity of the enzyme ornithine decarboxylase (ODC) [1]. ODC catalyzes the conversion of ornithine to putrescine, a critical step in the synthesis of polyamines. Polyamine synthesis is implicated in cell proliferation, as evidenced by the high levels of ODC and polyamines seen in tumor and other proliferating cells [2,3]. Presumably, inhibition of polyamine synthesis in such cells may inhibit proliferation. In fact, DFMO inhibits both the growth of tumor cells and the promotion and progression phases of carcinogenesis [2,3]. Based on the hypothesis that DFMO would be active against cancers with pronounced proliferative phases such as those in colon, bladder, and breast, the development of DFMO as a chemopreventive drug was undertaken (see attached time line). Because the tissues receiving the greatest exposure to DFMO are colon and bladder, they are considered to be primary targets.

Chemopreventive efficacy has been observed in animal models of bladder [4–7], colon [*e.g.*, 8–13], mammary gland [*e.g.*, 5,14–19], liver [20], stomach [21,22], and skin [23–26] cancer. The available studies are adequate to support the development of DFMO as a chemopreventive agent; additional animal efficacy studies are in progress. Developmental research on intermediate biomarkers and their modulation by DFMO is also being carried out in animals [*e.g.*, 27–29].

Preclinical toxicology studies for DFMO have been completed through one-year studies in rats and dogs. Additional specialized animal studies are ongoing to assess thrombocytopenia and ototoxicity identified in early clinical studies. Reproductive and carcinogenicity studies will be required.

The clinical trials for DFMO are summarized in Table I. Completed CB-funded Phase I studies have found a baseline, well-tolerated dose of 0.5 g/m^2 qd, as a single dose or in divided doses, for oral administration ≥10 months [30]. Single- and multi-

ple-dose pharmacokinetics study results are consistent with established findings. No additional Phase I studies are planned. Adverse effects have been associated with clinical administration of DFMO—most significantly, loss of hearing acuity [2,30–34]. This effect is reversed when DFMO treatment is stopped.

The identification of a reliably effective dosing regimen with acceptable side effects in Phase II studies will be a criterion for continued development of DFMO. A Phase II study of DFMO in colon cancer has the goal of identifying the optimal chronic dose that is effective without producing side effects, especially hearing loss.

An additional significant aspect of the Phase II evaluations will be the identification and validation of intermediate biomarkers as surrogate endpoints for cancer incidence reduction in chemoprevention trials. A Phase II trial in uterine cervix to evaluate the reversal of CIN III by DFMO is ready to begin, as are Phase II studies in prevention of bladder and prostate cancers. An additional Phase II trial for oral cavity (changes in leukoplakia histopathology, recurrence, and regression began in 1994; additional trials for prostate (presurgical intervention to follow intermediate biomarkers) and bladder (cancer recurrence and intermediate biomarker characterization) are planned for 1995. A Phase II trial of the combination of DFMO and piroxicam in colorectal adenomatous polyp patients is also under consideration for 1995 (polyp reduction, other intermediate endpoints).

Results of Phase II studies should be available within 2–6 years of award. Thus, decisions to commission additional Phase II studies, or initiate Phase III trials based on the Phase II results, will be made in 1996–2001 in anticipation of awards for late 1996–2002.

Based on the evaluation of the clinical efficacy of DFMO in Phase II trials in colon, bladder, breast, and prostate, as well as on CIN III and oral leukoplakia, the agent may be further developed in Phase III trials in one or more of these targets. The specific target cancers and patient populations selected for these trials will depend on the efficacy seen in Phase II trials.

The sole manufacturer of DFMO is Marion-Merrell Dow Research Institute (MMD); the chemical is not produced currently. The company holds an NCE patent that expires in 2000. It has been produced in three formulations: injectable, oral solution, and oral sachet. Ornidyl® Injection (NDA 19-879 held by MMD) is approved by the FDA for treatment of the meningoencephalitic state of *Trypanosoma brucei* var. *gambiense*; the oral formulations are not approved under the NDA. Oral forms have been investigated clinically by the NCI, DCT for treatment of colon and small-cell lung cancers, metastatic melanoma, brain cancers, and acute leukemia. For chemoprevention studies, the compound has been available as an oral solution (200 mg/ml). Development of a capsule formulation is planned by the CB.

MMD has provided supplies of oral solution, placebo, and bulk drug sufficient for conducting Phase II studies. However, the supply may not be adequate to conduct a large Phase III study. Moreover, the maximum shelf life of DFMO oral solution is 5 years, so that the DFMO solution supplied by MMD in Fall 1993 will expire by Fall 1998. Contracts for future supplies will be negotiated.

PRECLINICAL EFFICACY STUDIES

In CB-sponsored studies, DFMO has demonstrated chemopreventive activity in numerous efficacy tests in animal carcinogenesis models. It has inhibited AOM-induced colon carcinoma in rats (400 ppm, or *ca.* 0.1 mmol/kg-bw/day) [12,13], DMBA- (3.2 g/kg diet, or *ca.* 0.9 mmol/kg-bw/day) [5] and MNU-induced mammary gland tumors in rats (2.0 g/kg diet, or *ca.* 0.6 mmol/kg-bw/day), and OH-BBN-induced bladder tumors in mice (0.6 g/kg diet, or *ca.* 0.4 mmol/kg-bw/day) [5–7]. Further evidence of the chemopreventive efficacy of DFMO comes from studies reported in the literature of the inhibition of tumor induction in rat mammary glands [14–19], mouse skin [23–26], rat intestine [9–10], mouse colon [8], rat bladder [4], rat stomach [21], and rat liver [20]. The results of animal efficacy studies are more than adequate to support the clinical development of DFMO. Besides the completed studies, the CB is sponsoring additional animal efficacy studies in transgenic mice (lymphoma-prone), rat prostate, rat colon, mouse skin, hamster trachea, and hamster pancreas.

The chemopreventive efficacy of DFMO in combination with other agents is also being evaluated to decrease toxicity while retaining or enhancing cancer inhibition. For example, DFMO plus oltipraz synergistically inhibited mouse bladder tumors [6,35]. In contrast, DFMO plus piroxicam was not more efficacious than piroxicam alone in the same model [6,7]; however, the combination enhanced inhibition of rat colon adenomas and adenocarcinomas [12,13]. DFMO significantly inhibited hamster lung tumors only when offered in combination

with 4-HPR, β-carotene or both [36]. Combinations with DFMO currently on test include 4-HPR in the transgenic lymphoma model, and *N*-acetyl-*l*-cysteine in mammary glands, skin and lung cancer models.

A significant effort in the CB program is to identify and validate intermediate biomarkers of cancer and evaluate the potential for chemopreventive agents to modulate these markers. Such studies in animals contribute to the development of more efficient screens for identifying new chemopreventive agents, as well as identifying biomarkers to be used to evaluate specific agents in clinical trials. DFMO has demonstrated activity against several putative biomarkers of colon cancer in AOM-treated rats [27–29,37], including the formation of foci of aberrant crypts, activated c-Ha-*ras* oncogene expression, and mucosal cell proliferation. It is currently on study against biomarkers in mouse skin and rat urinary bladder carcinogenesis. The results already obtained in colon will be useful in designing the protocol for the Phase II trial planned for colon; those in bladder may influence the Phase II trial in bladder.

PRECLINICAL SAFETY STUDIES

Toxicity Except for reproductive and carcinogenicity studies, preclinical toxicology tests completed or in progress under CB contract are sufficient for regulatory filings.

One-year chronic oral toxicity studies in rats and dogs have been completed [38]. In dogs, significant toxicities, including alopecia, dermatitis, and conjunctivitis, were seen at all dose levels (50–200 mg/kg-bw/day, or 0.3–1.0 mmol/kg-bw/day) and a NOEL was not determined. In rats, a NOEL of 400 mg/kg-bw/day (2.2 mmol/kg-bw/day, or the lowest dose tested) was determined, since alopecia, dermatitis, trace to mild liver necrosis in males, and trace to mild inflammation in the glandular stomach (primarily in males) occurred only at the two higher doses tested (800, 1600 mg/kg-bw/day, or 4.4, 8.8 mmol/kg-bw/day).

Two 90-day studies in rats and dogs are nearing completion. Thrombocytopenia and hearing loss, the two major adverse effects seen in clinical studies, were not observed in the chronic animal study. These effects were monitored closely in one of the 90-day studies; effects on hearing were studied in dogs (brainstem evoked auditory response, histopathology of auditory nuclei, and surface morphology examination of the cochlea), and blood coagulation effects were studied in both dogs and rats. No overt evidence of toxicity was found; analysis of cochlear surface morphology is in progress.

In genotoxicity assays, DFMO did not significantly increase SCE frequency in CHO cells *in vitro* or micronucleated cell frequency in bone marrow of mice treated *in vivo*. DFMO was also negative in the Ames mutagenicity assay in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537.

Reproductive and carcinogenicity studies will be contracted, if needed, after Phase II clinical trials have been completed (1996–2002). Studies reported in the literature indicate that DFMO is embryotoxic [39–44], as would be expected from its antiproliferative activity. No published studies are of sufficient duration to be adequate carcinogenicity tests, although no DFMO-induced tumors were seen in multidose studies reported in the literature (lasting up to 34 weeks) [*e.g.*, 4,8,15,25], and none were seen in the CB-funded, one-year toxicity studies in rats and dogs.

ADME Studies in rats and dogs suggest that DFMO is not metabolized in these species. DFMO is well-absorbed following oral administration in rats (78%) and dogs (100%). In one of the 90-day studies, peak plasma levels were 31 μg/ml at one hour post-treatment in dogs dosed with 25 mg DFMO/kg-bw and 66–80 μg/ml at 1–4 hrs post-treatment in rats dosed with 400 mg DFMO/kg-bw. These results are comparable to those reported in the literature [3,45].

Animal studies reported in the literature indicate that DFMO approximates linear pharmacokinetics after oral administration [3,45]. By this route, the highest concentrations of DFMO are found in the intestine, liver, and kidney, but preferential accumulation does not occur in any tissue. The excretion of DFMO is rapid and occurs mainly via the urine. It has rapid clearance in rodents, with a serum and tissue $t_{1/2}$ of about 6 hrs [45].

CLINICAL SAFETY: PHASE I STUDIES

Four Phase I and one Phase I/IIa studies have been and are being carried out under IND 33,018 in patients previously treated for cancer or otherwise at high risk for cancer. Two Phase I studies have been completed under CB awards. Both of these studies were directed primarily at establishing a non-toxic, chronic, oral dose that could be used for chemoprevention studies. In one of these studies, (Dr. P. Carbone, University of Wisconsin) patients previously treated for colorectal, prostate, or bladder cancer received doses of 0.125–0.75 g/

m^2 qid and 0.5–1.0 g/m^2 qd for up to 6 months. From these various doses, a single daily dose of 0.5 g/m^2 (*ca.* 0.07 mmol/kg-bw) was selected for further evaluation of up to 12 months of treatment. The design for the second study (Dr. P.J. Creaven, Roswell Park Memorial Institute) involved 6 months of continuous treatment with DFMO. Patients at higher-than-normal risk for developing colorectal or bladder cancer, or who had been treated previously for bladder, lung, or breast cancer, were treated initially with 0.2 g DFMO/m^2 qd for one month. The dose was doubled monthly thereafter (modified Fibonacci dose-escalation scheme) until either a toxic dose or a maximum dose of 6.4 g/m^2 qd (0.9 mmol/kg-bw) was achieved. Patients were continued on their MTD until the end of the study.

In one of the CB-funded Phase I studies still in progress (Dr. D.S. Alberts, University of Arizona), patients with actinic keratosis received either DFMO (1 or 2 g/m^2 qd, or *ca.* 0.1 or 0.3 mmol/kg-bw qd) or placebo for 6 months. The treatment period has been completed, and the study remains blinded pending completion of analysis of collected samples.

A Phase I/IIa study (Dr. G.D. Luk, Dallas V.A. Medical Center) is in progress in patients with resected, histologically proven colon cancer (Dukes' A, B1, and B2) who were ineligible for adjuvant chemotherapy and patients with adenomatous polyposis coli with histologically proven adenomatous polyps of ≥0.5 cm. The patients were to be treated initially with DFMO for two cycles of 21 days on treatment and 7 days off. Doses were to be escalated in 0.5 g/m^2 qd increments to establish the MTD. Similarly, a de-escalation phase was to be conducted to establish the LED. The third study (Dr. C. Loprinzi, Mayo Clinic) still in progress is being conducted in patients previously treated for superficial bladder cancers. These patients are to receive DFMO doses of 0.125, 0.25, 0.5, or 1.0 g/m^2 qd (0.02–0.3 mmol/kg-bw) for up to two years.

Three Phase II studies are in progress or ready to start that will potentially provide additional relevant data for optimizing the DFMO dosing regimen. One study (Dr. M.F. Mitchell, University of Texas, M.D. Anderson Cancer Center) will be carried out in patients with CIN III and includes a one-month treatment dose-titration; ODC activity in cervical tissue will be used as a drug effect measurement [46]. The IIa portion of the second study (Dr. R.R. Love, University of Wisconsin), recently completed, was a one-month dose-titration in patients with prior colon polyps; polyamine levels in rectal mucosa was the drug effect measurement [47]. Phase IIb of this study will have the specific goal of evaluating the effect on hearing loss of chronic (one-year) treatment with low doses (0.06–0.25 g/m^2 qd, or *ca.* 0.008–0.03 mmol/kg-bw) of DFMO. The third study (Dr. F. Meyskens, University of California, Irvine), which is also directed at colon cancer, will evaluate toxicity, especially hearing loss, in patients treated with total cumulative doses of >100 g/m^2 or >150 g/m^2 (in daily doses of 0.5 g/m^2). Additional Phase II studies will include dosing optimization protocols, as appropriate.

Drug Effect Measurement In the two completed Phase I studies, TPA-induced ODC activity in skin punch biopsies [30] and polyamine levels in urine [31,32] were selected as drug effect measurements. As noted for all studies in which it was used as a drug effect measurement, the methods for determining ODC activity require some additional development—particularly, optimizing sampling and storage techniques to prevent loss of ODC activity and standardizing the activity measurement. Polyamine and ODC measurements in white blood cells and erythrocytes were not reliable drug effect measurements and should not be pursued in future studies. Likewise, SAM decarboxylase activity in lymphocytes was not a reliable measurement. Reportedly, ODC activity in colorectal mucosa is highly variable and may not prove to be a reliable drug effect. In one of the ongoing studies [46], polyamine levels in colorectal mucosa were found to be reliable, while neither ODC activity nor polyamine levels could be used in exfoliated buccal mucosa because of contamination with bacteria containing DFMO-resistant ODC [47]. Methods and standardization of measurements will be monitored early in Phase II studies to insure their reliability.

Safety In the two completed Phase I studies and one of those in progress, the dose-limiting side effect observed was reversible ototoxicity. In one of the completed studies, an MTD was determined of 0.5 g/m^2 qd for ≥10 months, either as a single dose or divided into four doses per day [30]. At this dose level, a significant decrease in TPA-induced ODC activity in skin occurred and no ototoxicity was observed. This dose will serve as the baseline for Phase II studies. The progress and results of dosing studies in Phase II studies will be reviewed; additional steps may be added to these trials to further define optimal dosing regimens.

In studies reported in the literature, DFMO generally has been found to be well-tolerated in hu-

mans, showing only mild to moderate reversible toxicity when administered orally [2,33,34,48]. Reported side effects include anorexia, gastrointestinal symptoms, anemia, thrombocytopenia, and decreased hearing acuity. Each of these complications appears to be transient and reversible upon withdrawal of the drug. Thrombocytopenia has been observed primarily in cancer patients with advanced disease [33,34]. The available information is insufficient to determine if toxicity increases with accrued dose on chronic administration. As noted above, the effects of cumulative dose on toxicity will be assessed in Phase II studies in colon. The results of one of the Phase I studies suggests that C_{max} is more important than cumulative dose [30].

ADME In the two completed Phase I studies, single-dose pharmacokinetics were linear at all dose levels tested [30–32]. Likewise, steady-state trough plasma concentrations were proportional to dose. The values obtained in both studies generally agreed with those reported in the literature [33, 48,49]. For the 0.5 g/m^2 qd dose, the overall plasma values were C_{max}=47.1±5.1 μM, C_{min}= 14.5±5.2 μM, AUC=311±39 μM·hr, and $t_{½}$=3.5 hrs [30]. In the same study, repeat single-dose pharmacokinetics after 10 months of treatment suggested changes in ADME resulting in higher C_{max}, AUC, and $t_{½}$.

Clinical data reported in the literature show that DFMO does not bind significantly to human plasma proteins [48]. These data also indicate that 54–58% of orally administered DFMO is absorbed, and 86% of that absorbed is eliminated unchanged in urine.

CLINICAL EFFICACY: PHASE II STUDIES

A significant aspect of the Phase II evaluations will be the identification and validation of intermediate biomarkers as surrogate endpoints for cancer chemoprevention trials at various target sites. To this end, the CB is sponsoring a Phase II trial in uterine cervix to evaluate the reversal of CIN by DFMO; the trial has just begun, and the results are expected by 1995. The study is being carried out in women with CIN III; treatment with DFMO oral solution will be for 6 months at the optimal dose determined from the Phase IIa portion of the study. CIN lesions will be surgically removed at the end of treatment. Besides CIN, the patients will be monitored for modulation of other putative intermediate biomarkers to assess their correlation with modulation of CIN. Examples of parameters to be monitored are ploidy, micronucleated cell frequency, DNA content, PCNA, *ras* oncogene, EGFR, keratins, and involucrin.

A CB-funded Phase II study of DFMO on the prevention of bladder cancer recurrence was awarded in 1993, with results expected by 2000. The study population will be patients with completely resected Ta,T1 transitional cell carcinoma of the bladder. Patients will be treated with DFMO oral solution for one year, and will be monitored during treatment and for one year following treatment. In addition to recurrence of superficial bladder cancer, potential intermediate biomarkers will be evaluated, including Lewis^x antigen and EGFR. The optimal dose for use in the study will be determined by results of a preliminary dose-finding study (Phase IIa) and the Phase I study in bladder cancer patients described above.

A CB award has been made recently for a Phase II trial of DFMO in prostate. In the prostate study, patients with prostatic carcinoma are being treated with DFMO for 14 days prior to surgery, and patients with PIN will be treated for one year. Changes in histology, PSA level, and prostatic acid phosphatase will be monitored throughout the study. Results are expected by 1996–1997. An additional Phase II trial is planned for 1995 in a prostate cancer cohort (presurgical intervention to follow intermediate biomarkers) and bladder (cancer recurrence and intermediate biomarker characterization). An additional Phase II trial in bladder cancer patients is under consideration for 1995. Finally, a Phase II trial contract was awarded this year for dysplastic oral leukoplakia. Endpoints include changes in leukoplakia histopathology, recurrence, and regression. A Phase II trial of the combination of DFMO and piroxicam in colorectal adenomatous polyp patients is also under consideration for 1995 (polyp reduction, other intermediate endpoints).

PHARMACODYNAMICS

An optimal dosing regimen which reduces ototoxicity and retains efficacy will be investigated in future Phase II trials. Based on rat data, lower doses of DFMO may be effective against colon and bladder cancer in humans. The lowest effective DFMO dose in the AOM-induced rat colon assay (0.1 mmol/kg-bw/day) was approximately 20-fold lower than the one-year rat NOEL (2.2 mmol/kg-bw/day). This suggests that Phase II trials could titrate ototoxicity and efficacy biomarkers down to a dose an order of magnitude less than

the chronic (>10 months) well-tolerated dose of 0.5 g/m^2 qd (0.07 mmol/kg-bw qd).

A single dose study will be required to demonstrate the equivalence of a new capsule formulation with oral solution. Depending on the results of the single dose study, it also may be necessary to evaluate the bioequivalence of the two formulations under steady-state conditions (multiple dosing).

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

In the clinical development of DFMO to date, the modulation of urine, blood, and tissue endpoints by DFMO has been evaluated. The advantages and limitations of these should be considered in future studies and are as follows:

1. Reduction in TPA-induced ODC activity in skin punch biopsies showed potential as a drug effect measurement in subjects with adequate baseline values. However, the methods for measuring this activity in skin and other tissues require further development and standardization, especially optimizing sampling and storage techniques to minimize loss and variability of activity. For example, storing skin biopsies for batch analysis resulted in significant loss of activity.

2. Both ODC activity and polyamine levels in leukocytes/lymphocytes and erythrocytes have proven too low and too variable to be used as drug effect measurements and should not be considered in future trials. Likewise, SAM decarboxylase activity in lymphocytes is too low to be used as a drug effect measurement.

3. Measurements of polyamines in buccal mucosa are unreliable because of contamination with oral bacteria containing DFMO-resistant ODC.

4. The level of ODC activity in colorectal mucosa is reportedly variable, suggesting that it may not be a reliable drug effect measurement in this tissue.

5. Polyamine levels in urine and colorectal mucosa may have value as measurements, particularly changes in putrescine, spermine, and spermidine.

Special care and consideration must be given to the standardization and quality control of all analytical and biochemical methods proposed in order to satisfy regulatory review and support drug development. Such work must precede sample acquisition. Proficiency testing of plasma drug monitoring will be required; the program is administered by NIST.

A principal obstacle to be overcome in the clinical development of DFMO is its effect on hearing acuity. Mechanistic toxicology investigations in guinea pigs have shown that when DFMO is administered for 12 weeks, a loss of hair cells occurs in the Organ of Corti [50]. Additional special studies in rats and dogs are underway to further characterize this effect. Evidence from a Phase I clinical study cited above indicates that 0.5 g DFMO/m^2 qd for up to 10–12 months is tolerated without hearing loss. However, it is not yet known with certainty if the toxic threshold is due to a plasma C_{max} or a total cumulative dose. These are ongoing areas of investigation. Drug combinations which have shown pharmacological synergy in preclinical studies (*e.g.*, DFMO plus NSAIDs in colon and bladder models) will also be considered for development in order to reduce the daily DFMO dose, thereby reducing the risk of ototoxicity.

In the clinical studies carried out to date, hearing loss has been measured against a variety of criteria. Even when hearing loss has been measured quantitatively by audiogram, the frequencies tested and the grading of the severity of the decibel loss have differed. In all future studies, standardized criteria for hearing loss will be used.

Pharmacodynamics Issues

Colon and bladder are primary targets for DFMO chemoprevention as evidenced by clinical ADME studies showing that these tissues receive by far the most exposure to orally administered DFMO. That is, a little more than 50% of an oral dose of DFMO is absorbed and more than 80% of that absorbed is excreted in urine unchanged.

Regulatory Issues

There are no current regulatory issues in studies involving DFMO alone. However, there are regulatory issues related to a CB-sponsored Phase I study of DFMO in combination with the NSAID piroxicam. While FDA has allowed single-dose pharmacokinetics studies on the combination to

proceed, multiple-dose studies have been delayed pending the availability of results of toxicity studies on the combination in the rat and dog, as well as ototoxicity and mutagenicity studies on DFMO alone and in combination with piroxicam. The Ames *Salmonella* assay, a mouse bone marrow micronucleus test, and a CHO cell SCE assay have been performed on the drug combination, as well as on DFMO alone. The results of these tests were negative for both DFMO (as noted above) and the combination.

Brainstem-evoked auditory response, histopathology of auditory nuclei, and surface morphology examination of the cochlea were added to 90-day dog toxicity studies of DFMO and the combination. Coagulation studies were added to 90-day rat and dog toxicity studies. These studies have been performed, and the final report on the combination is in preparation for submission to the FDA. No evidence of toxicity was found for the combination; analysis of cochlear surface morphology is in progress for the animals treated with DFMO alone.

Supply and Formulation Issues

Twelve patents on manufacture or use of DFMO issued by the U.S. Patent Office and World Intellectual Properties Organization expire between 1997 and 2010. Four of these patents are held by MMD for treatment of tumors.

A significant portion of the DFMO supply to be provided to NCI by MMD will be unformulated bulk drug. The bulk drug will be used for any DFMO carcinogenicity studies required and for toxicology studies on any new DFMO combinations proposed for human clinical studies. Also, capsule formulations of DFMO and DFMO combinations will be developed by the CB. An oral capsule dosage form will provide improved patient compliance over the oral solution and will simplify study blinding, particularly in the studies involving DFMO in combination with another agent. Moreover, a capsule product manufactured two years hence will extend the period of use of the DFMO supply beyond the Fall 1998 expiration date of the current supply of solution. A clinical study to establish the bioavailability of solid dosage formulations compared with the oral solution will be performed.

Intermediate Biomarker Issues

Most intermediate biomarkers have not yet been established as acceptable predictors or measures of cancer chemoprevention. Logically, the validation of the biomarkers will occur during Phase II studies on drugs such as DFMO which are earmarked for initial and priority development. For histopathological biomarkers, emphasis will be placed on those that can be measured quantitatively and reproducibly by techniques such as computer-assisted cytomorphometry and cytophotometry.

Clinical Studies Issues

Phase III clinical studies will be required to establish the safety and efficacy of DFMO for each cancer site for which FDA approval is sought. The design and initiation of the Phase III trials await the completion of appropriate Phase II studies identifying the apparent optimal safe and effective dosing regimen for chemoprevention. These Phase II studies will consist of collecting data on the effects of various dosing regimens on a variety of candidate surrogate endpoints and drug effect measurements that appear to be relevant to precancer progression or its control.

Because of the pharmacodynamics data cited above, the greatest effort in Phase II investigations of DFMO will be to support NDAs for the prevention of colon and bladder cancers. Concurrent trials will support NDAs for the prevention of breast, prostate, cervical, and/or oral cancers. Any additional Phase II trials proposed will be evaluated critically for relevance, priority, and need.

REFERENCES

1. Pegg, A.E., McGovern, K.A., and Wiest, L. Decarboxylation of α-difluoromethylornithine by ornithine decarboxylase. *Biochem. J.* 241: 305–307, 1987.
2. Luk, G.D. and Casero, R.A. Polyamines in normal and cancer cells. *Adv. Enzyme Regul.* 26: 91–105, 1987.
3. Pegg, A.E. Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. *Cancer Res.* 48: 759–774, 1988.
4. Homma, Y., Kakizoe, T., Samma, S., and Oyasu, R. Inhibition of *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine-induced rat urinary bladder carcinogenesis by α-difluoromethylornithine. *Cancer Res.* 47: 6176–6179, 1987.
5. Ratko, T.A., Detrisac, C.J., Rao, K.V.N., Thomas, C.F., Kelloff, G.J., and Moon, R.C. Interspecies analysis of the chemopreventive efficacy of dietary α-difluoromethylornithine. *Anticancer Res.* 10: 67–72, 1990.
6. Moon, R.C., Detrisac, C.J., Thomas, C.F., and Kelloff, G.J. Chemoprevention of experimental bladder cancer. *J. Cell. Biochem.* 16I (Suppl.): 134–138, 1992.

7. Moon, R.C., Kelloff, G.J., Detrisac, C.J., Steele, V.E., Thomas, C.F., and Sigman, C.C. Chemoprevention of OH-BBN-induced bladder cancer in mice by piroxicam. *Carcinogenesis* 14: 1487–1489, 1993.
8. Kingsnorth, A.N., King, W.W.K., Diekema, K.A., McCann, P.P., Ross, J.S., and Malt, R. A. Inhibition of ornithine decarboxylase with 2-difluoromethylornithine: Reduced incidence of dimethylhydrazine-induced colon tumors in mice. *Cancer Res.* 43: 2545–2549, 1983.
9. 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.
10. 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.
11. Nigro, N.D., Bull, A.W., and Boyd, M.E. Importance of the duration of inhibition on intestinal carcinogenesis by difluoromethylornithine in rats. *Cancer Lett.* 35: 153–158, 1987.
12. Reddy, B.S., Nayini, J., Tokumo, K., Rigotty, J., Zang, E., and Kelloff, G. Chemoprevention of colon carcinogenesis by concurrent administration of piroxicam, a nonsteroidal antiinflammatory drug with D,L-α-difluoromethylornithine, an ornithine decarboxylase inhibitor, in diet. *Cancer Res.* 50: 2562–2568, 1990.
13. Rao, C.V., Tokumo, K., Rigotty, J., Zang, E., Kelloff, G., and Reddy, B.S. Chemoprevention of colon carcinogenesis by dietary administration of piroxicam, α-difluoromethylornithine, 16-α-fluoro-5-androsten-17-one, and ellagic acid individually and in combination. *Cancer Res.* 51: 4528–4534, 1991.
14. Fozard, J.R. and Prakash, N.J. Effects of D,L-α-difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase, on the rat mammary tumour induced by 7,12-dimethylbenz[*a*]anthracene. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 320: 72–77, 1982.
15. Thompson, H.J., Herbst, E.J., Meeker, L.D., Minocha, R., Ronan, A.M., and Fite, R. Effect of D,L-α-difluoromethylornithine on murine mammary carcinogenesis. *Carcinogenesis* 5: 1649–1651, 1984.
16. Thompson, H.J., Meeker, L.D., Herbst, E.J., Ronan, A.M., and Minocha, R. Effect of concentration of D,L-2-difluoromethylornithine on murine mammary carcinogenesis. *Cancer Res.* 45: 1170–1173, 1985.
17. Thompson, H.J., Ronan, A.M., Ritacco, K.A., and Meeker, L.D. Effect of tamoxifen and D,L-2-difluoromethylornithine on the growth, ornithine decarboxylase activity and polyamine content of mammary carcinomas induced by 1-methyl-1-nitrosourea. *Carcinogenesis* 7: 837–840, 1986.
18. 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* 7: 2003–2006, 1986.
19. Abou-El-Ela, S.H., Prasse, K.W., Farrell, R.L., Carroll, R.W., Wade, A.E., and Bunce, O.R. Effects of D,L-2-difluoromethylornithine and indomethacin on mammary tumor promotion in rats fed high n-3 and/or n-6 fat diets. *Cancer Res.* 49: 1434–1440, 1989.
20. Moore, M.A., Tsuda, H., Ogiso, T., Mera, Y., and Ito, N. Enhancement of phenotypic instability by α-difluoromethylornithine and butylated hydroxyanisole in rapidly induced rat liver lesions. *Cancer Lett.* 25: 145–151, 1984.
21. Newberne, P.M., Charnley, G., Adams, K., Cantor, M., Suphakarn, V., Roth, D., and Schrager, T.F. Gastric carcinogenesis: A model for the identification of risk factors. *Cancer Lett.* 38: 149–163, 1987.
22. Lehnert, T., Buhl, K., and Ivankovic, S. Inhibition of gastric tumorigenesis by α-diflоromethylornithine in rats treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. *J. Cancer Res. Clin. Oncol.* 119: 594–598, 1993.
23. Weeks, C.E., Herrmann, A.L., Nelson, F.R., and Slaga, T.J. α-Difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase, inhibits tumor promoter-induced polyamine accumulation and carcinogenesis in mouse skin. *Proc. Natl. Acad. Sci. USA* 79: 6028–6032, 1982.
24. Takigawa, M., Verma, A.K., Simsiman, R.C., and Boutwell, R.K. Polyamine biosynthesis and skin tumor promotion: Inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-promoted mouse skin tumor formation by the irreversible inhibitor of ornithine decarboxylase α-difluoromethylornithine. *Biochem. Biophys. Res. Commun.* 105: 969–976, 1982.
25. Takigawa, M., Verma, A.K., Simsiman, R.C., and Boutwell, R.K. Inhibition of mouse skin tumor promotion and of promoter-stimulated epidermal polyamine biosynthesis by α-difluoromethylornithine. *Cancer Res.* 43: 3732–3738, 1983.
26. Verma, A.K., Duvick, L., and Ali, M. Modulation of mouse skin tumor promotion by dietary 13-*cis*-retinoic acid and α-difluoromethylornithine. *Carcinogenesis* 7: 1019–1023, 1986.
27. Kulkarni, A., Zanz, E., Kelloff, G., and Reddy, B.S. Effect of chemopreventive agents, piroxicam and D,L-α-difluoromethylornithine, on intermediate biomarkers of colon carcinogenesis. *Carcinogenesis* 13: 995–1000, 1992.
28. Singh, J., Kelloff, G., and Reddy, B.S. Effect of chemopreventive agents on intermediate biomarkers during different stages of azoxymethane-induced colon carcinogenesis. *Cancer Epidemiol. Biomarkers Prev.* 1: 405–411, 1992.
29. Singh, J., Kelloff, G., and Reddy, B.S. Intermediate biomarkers of colon cancer: Modulation of expression of *ras* oncogene by chemopreventive agents during different stages of azoxymethane-induced colon carcinogenesis. *Carcinogenesis* 14: 699–704, 1993.
30. 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: 732–737, 1993.
31. 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.

32. Creaven, P.J., Pendyala, L., and Petrelli, N.J. Evaluation of α-difluoromethylornithine as a potential chemopreventive agent: Tolerance to daily oral administration in humans. *Cancer Epidemiol. Biomarkers Prev.* 2: 243–247, 1993.
33. 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 synthesis. *J. Clin. Oncol.* 2: 124–130, 1984.
34. 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.
35. Moon, R.C., Kelloff, G.J., Detrisac, C.J., Steele, V.E., Thomas, C.F., and Sigman, C.C. Chemoprevention of OH-BBN-induced bladder cancer in mice by oltipraz, alone and in combination with 4-HPR and DFMO. *Anticancer Res.* 14: 5–11, 1994.
36. Moon, R.C., Rao, K.V.N., Detrisac, C.J., Kelloff, G.J., Steele, V.E., and Doody, L.A. Chemoprevention of respiratory tract neoplasia in the hamster by oltipraz, alone and in combination. *Int. J. Oncol.* 4: 661–667, 1994.
37. Singh, J., Kulkarni, N., Kelloff, G., and Reddy, B.S. Modulation of azoxymethane-induced mutational activation of *ras* protooncogenes by chemopreventive agents in colon carcinogenesis. *Carcinogenesis* 15: 1317–1323, 1994.
38. Crowell, J.A., Goldenthal, E.I., Kelloff, G.J., Malone, W.F., and Boone, C.W. Chronic toxicity studies of the potential cancer preventive 2-(difluoromethyl)-*dl*-ornithine. *Fundam. Appl. Toxicol.* 22: 341–354, 1994.
39. Fozard, J.R., Grove, J., Part, M.L., and Prakash, N.J. Studies on the mechanism of the contragestational effects of α-difluoromethylornithine, an irreversible inhibitor of *l*-ornithine decarboxylase. *Br. J. Pharmacol.* 69: 335P–336P, 1979.
40. Fozard, J.R., Part, M.-L., Prakash, N.J., Grove, J., Schechter, P.J., Sjoerdsma, A., and Koch-Weser, J. L-Ornithine decarboxylase: An essential role in early mammalian embryogenesis. *Science* 208: 505–508, 1980.
41. Reddy, P.R.K. and Rukmini, V. α-Difluoromethylornithine as a postcoitally effective antifertility agent in female rats. *Contraception* 24: 215–221, 1981.
42. Luzzani, F., Colombo, G., and Galliani, G. Evidence for a role of progesterone in the control of uterine ornithine decarboxylase in the pregnant hamster. *Life Sci.* 31: 1553–1558, 1982.
43. Galliani, G., Colombo, G., and Luzzani, F. Contragestational effects of D,L-α-difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase, in the hamster. *Contraception* 28: 159–170, 1983.
44. Méndez, J.D., Diaz-Flores, M., Durán, G., and Hicks, J.J. Inhibition of rat embryonic development by the intrauterine administration of α-difluoromethylornithine. *Contraception* 28: 93–98, 1983.
45. Romijn, J.C., Verkoelen, C.F., and Splinter, T.A.W. Problems of pharmacokinetic studies on alpha-difluoromethylornithine in mice. *Cancer Chemother. Pharmacol.* 19: 30–34, 1987.
46. Meyskens, F.L., Jr., Pelot, D., Meshkinpour, H., Plezia, P., Gerner, E., and Emerson, S. Preliminary results of a Phase IIA trial of difluoromethylornithine (DFMO) to prevent colon cancer. In: Wattenberg, L., Lipkin, M., Boone, C.W., and Kelloff, G.J. (eds.) *Cancer Chemoprevention*, Boca Raton: CRC Press, pp. 541–555, 1992.
47. Meyskens, F.L., Jr., Gerner, E., Emerson, S., Pelot, D., Boyle, J., Alberts, D., and Dutton, C. Phase IIa trial of DFMO and its effect on polyamine levels in buccal mucosal cells and rectal colonic biopsies. (meeting abstract). *Second International Cancer Chemoprevention Conference*, Berlin, Germany, 1993, p. 133.
48. Haegele, K.D., Alken, R.G., Grove, J., Schechter, P.J., and Koch-Weser, J. Kinetics of α-difluoromethylornithine: An irreversible inhibitor of ornithine decarboxylase. *Clin. Pharmacol. Ther.* 30: 210–217, 1981.
49. Griffin, C.A., Slavik, M., Chien, S.C., Hermann, J., Thompson, G., Blanc, O., Luk, G.D., Baylin, S.B., and Abeloff, M.D. Phase I trial and pharmacokinetic study of intravenous and oral alpha-difluoromethylornithine. *Invest. New Drugs* 5: 177–186, 1987.
50. Salzer, S.J., Mattox, D.E., and Brownell, W.E. Cochlear damage and increased threshold in α-difluoromethylornithine (DFMO) treated guinea pigs. *Hear. Res.* 46: 101–112, 1990.

Table I. Clinical Trials of DFMO Sponsored/Funded by NCI, DCPC

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase I (Safety, ADME)					
NO1-CN-85102-01 Tolerance to Long Term Administration of α-Difluoromethylornithine (DFMO): A Phase I, Pharmacokinetic and Biochemical Study in Familial Polyposis, Superficial Bladder, and Other Cancer or High Risk Groups (Dr. Patrick J. Creaven, Roswell Park Memorial Institute) 8/7/89–10/30/91 IND 33,018	----	FAP or high risk for colorectal cancer or other cancers; or previously treated for bladder, breast, colon, gastric, lung, or tongue cancer 27 patients	Oral 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 g/m^2 qd for 6 months	Drug effect measurements: Polyamine levels in urine and erythrocytes; SAM decarboxylase and TPA-induced ODC activity in lymphocytes [Polyamines in erythrocytes and ODC and SAM decarboxylase activity in lymphocytes not useful—levels too low]	Study complete; adequate safety and ADME Ototoxicity in 16/27 subjects; 1.6 g/m^2 qd suggested MTD; no otoxicity at 0.8 g/m^2 qd Steady-state C_{max} (2.8 ± 0.7 – 58.2 ± 19.5 μg/ml) and AUC (13.8 ± 5.47 – 240.1 ± 100.1 μg/ml·hr) linear with dose; single-dose and steady-state $t_{½}$=4.1– 5.6 hrs and t_{max} = 3–4 hrs Published reports: [31,32]
NO1-CN-85106-01 Phase I and Pharmacokinetic Study of Difluoromethylornithine (DFMO) (Dr. David Alberts, Univ. of Arizona) 7/89– IND 33,018	----	Actinic keratosis patients 36 patients (12/dose)	Oral 0, 0.25–2.0 g/m^2 qd for 3 weeks–6 months	Drug effect measurements: Polyamine levels in leukocytes and buccal mucosa; TPA-induced ODC activity in skin	Study near completion

Table I. Clinical Trials of DFMO Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase I (Safety, ADME) *(continued)*					
NO1-CN-85109-01 Phase I Chemoprevention and Pharmacokinetic Study of DFMO in Subjects with Colorectal, Bladder, and Prostate Carcinoma and Individuals at Increased Risk for Colorectal Cancer (Dr. Paul Carbone, Univ. of Wisconsin) 7/7/89–9/30/91 IND 33,018	----	Previously treated colorectal, prostate, superficial bladder cancer, or adenomatous polyps; family history of colorectal cancer 32 patients	Oral 0.5, 1.0, 2.0, 3.0 g/m^2 daily for 6–12 months	Drug effect measurement: TPA-induced ODC activity in skin and lymphocytes [ODC lymphocyte activity not used; inhibition did not correlate with DFMO dose]	Study complete; adequate safety and ADME Dose limiting otoxicity at 2 highest doses; 0.5 g/m^2 qd suggested MTD Steady-state C_{min} linear with dose (8.7 ± 0.59 – 50.5 ± 7.2 g/m^2) At 0.5 g/m^2 qd, C_{max} = 47.1 ± 5.1 μM, C_{min} = 14.5 ± 5.2 μM, AUC = 311 ± 39 μM·hr, and $t_{½}$ = 3.5 hrs Single-dose C_{max}, AUC, and $t_{½}$ increase after 10 months, suggesting change in ADME Published report: [30]

Table I. Clinical Trials of DFMO Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance *IND No.*	Cancer Target	Study Population *No. of Subjects*	Dose(s) *Study Duration*	*Endpoints*	*Remarks*
Phase I (Safety, ADME) ***(continued)***					
CCOP/NCCTG 89-51-51 Phase I Toxicity Trial of Difluoromethylornithine (DFMO) in Patients with Superficial and Superficially Invasive Bladder Cancer (Dr. Charles Loprinzi, Mayo Clinic) 5/9/91– IND 33,018	----	Previously treated superficial and superficially invasive bladder cancer 120 patients (30/dose)	Oral 0.125, 0.25, 0.5, 1.0 g/m^2 qd for 2 years	Drug effect measurements: TPA-induced ODC activity in skin, bladder, and urine; SAM decarboxylase activity in urine; polyamine levels in urine and bladder	Study in progress. Insufficient data available for evaluation. Patient accrual reportedly slow
UO1-CA-50399 Phase I Chemoprevention Using DFMO to Modulate ODC in Colon Cancer (Dr. Gordon D. Luk, Dallas V.A. Medical Center) 12/31/91– IND 33,018	Colon	Previously treated colon cancer, history of colorectal adenomatous polyps 60 patients	Oral 0.5, 1.0, 1.5 g/m^2 qd for 21 days, then 7-day holiday; other doses to be determined Up to 6 months	Drug effect measurement: TPA-induced ODC activity in colorectal mucosa Efficacy: Prevention of new polyps or cancers	Phase I/IIa study. Dose-titration and dosing regimen determinations. Study still in progress. Insufficient data available for evaluation

Table I. Clinical Trials of DFMO Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers)					
NO1-CN-25434-01 Phase II Chemoprevention Trials of DFMO in Superficial Bladder Cancer—Step 1, Pilot Study (Dr. Edward M. Messing, Univ. of Wisconsin) 1993–1994 IND 33,018	Bladder	Scheduled for bladder surgery, without bladder TCC 30 patients	Oral 0.5, 1.0 g/m^2 qd for 14 days 1 year	Drug effect measurements: TPA-induced ODC activity and polyamine levels in bladder, and urinary polyamines	Study to assess the effects of surgery on drug effect measurements and determine the intersubject variability in these parameters Preliminary results show highly significant decrease in polyamine and ODC activity following bladder surgery but still measurable
NO1-CN-25434-01 Phase II Chemoprevention Trials of DFMO in Superficial Bladder Cancer—Step 2, Phase IIa (Dr. Edward M. Messing, Univ. of Wisconsin) 1993–1996 IND 33,018	Bladder	Scheduled for bladder surgery, with or without bladder TCC 60 patients (20/dose)	Oral 0.5, 1.0 g/m^2 qd for 14 days 3 years	Drug effect measurements: TPA-induced ODC activity and polyamine levels in bladder Efficacy: EGF, EGFR, Lewis^x antigen	Dose-titration and preliminary evaluation of biomarkers. Accrual has begun; treatment will start after completion of Step 1

Table I. Clinical Trials of DFMO Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers) ***(continued)***					
NO1-CN-25434-01 Phase II Chemoprevention Trials of DFMO in Superficial Bladder Cancer—Step 3, Phase IIb (Dr. Edward M. Messing, Univ. of Wisconsin) 1994–1999 IND 33,018	Bladder	Previously treated Ta, T1 bladder cancer 240 patients	Oral (dose to be determined) for 12 months 6 years	Drug effect measurements: TPA-induced ODC activity and polyamine levels in bladder Efficacy: EGF, EGFR, Lewis^x antigen, histopathology	Efficacy (tumor recurrence) and evaluation of intermediate biomarkers. Study will start after completion of Steps 1 and 2 and Phase I trial in bladder cancer patients cited above (CCOP/NCCTG 89-51-51)
Planned Study Clinical Trials of DFMO in Patients Previously Treated with BCG for Superficial Bladder Cancer 1995 IND 33,018	Bladder	Previously resected superficial bladder cancer (Ta, T1, TIS) and intravesical BCG treatment	Oral (doses to be determined) for 1 month (Phase IIa), 1 year (Phase IIb)	Drug effect measurements: Urinary polyamines, others Efficacy: Tumor recurrence, histopathology, intermediate biomarkers (to be determined)	Dose-titration/biomarker determinations. Efficacy and evaluation of intermediate biomarkers as surrogate endpoints. Emphasis on quantitative evaluation of biomarkers by computer-assisted cytomorphometry and cytophotometry

Table I. Clinical Trials of DFMO Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers) *(continued)*					
NO1-CN-25433-02 Phase IIa A Reverse Phase I Trial of α-Difluoromethylornithine (DFMO) in Patients with Cervical Intraepithelial Neoplasia (CIN) Grade III (Dr. Michele F. Mitchell, Univ. of Texas, M.D. Anderson Cancer Center) 1993–1994 IND 33,018	Cervix	CIN III patients 24 patients (6/dose)	Oral 0.125, 0.25, 0.5, 1.0 g/m^2 qd for 1 month	Drug effect measurements: Polyamine levels and TPA-induced ODC activity in cervical tissue Intermediate biomarker: PCNA	Dose-titration; drug effect measurements
NO1-CN-25433-02 Phase IIb A Randomized Double-blind Study of α-Difluoromethylornithine (DFMO) in Patients with Cervical Intraepithelial Neoplasia (CIN) Grade III (Dr. Michele F. Mitchell, Univ. of Texas, M.D. Anderson Cancer Center) 1994-1995 IND 33,018	Cervix	CIN III patients 50 patients	Oral (determined from Phase IIa) 1 year	Efficacy: CIN regression, DNA ploidy, micronucleated cell frequency, *ras* expression, EGFR, keratins, involucrin	Efficacy (regression of CIN) and evaluation of other intermediate biomarkers Study not yet started

Table I. Clinical Trials of DFMO Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers) *(continued)*					
PO1-CN-41108-Project III Phase IIa Trial—Polyamine Contents of Colonic and Buccal Mucosa in Individuals Treated with Oral Difluoromethylornithine (Dr. Frank Meyskens, Univ. of California, Irvine) Near completion IND 31,143	Colon	Previous history of colorectal adenomatous polyps 89 patients	Oral 0.25, 0.5, 0.75, 1.0, 1.5, 3.0 g/m^2 qd for 1 month	Drug effect measurements: Polyamine levels in colorectal and oral buccal mucosa [Polyamine levels in oral buccal mucosa not reliable due to oral bacteria with DFMO-resistant ODC]	Dose-titration study still in progress. Insufficient data available for evaluation Published report: [47]
RO1-CA59024 Phase IIb (Dr. Frank Meyskens, Univ. of California, Irvine) 1993– IND 31,143	Colon	Previous history of colorectal adenomatous polyps 125 patients	Oral (to be determined from Phase IIa) for 1 year	Drug effect measurement: Polyamine levels in colorectal mucosa	Dose-titration to identify optimal chronic low dose. Chemopreventive efficacy not measured

Table I. Clinical Trials of DFMO Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers) *(continued)*					
UO1-CA59352 Phase IIa Chemoprevention Study of Difluoromethylornithine (DFMO) in Subjects with or at Risk for Colorectal Cancer (Dr. Richard R. Love, Univ. of Wisconsin) 1993-1995 IND 33,018	Colon	Subjects with history of colorectal adenomatous polyps or otherwise at high risk for colorectal cancer 40 subjects (20/arm)	Oral 0.5 g/m^2 qd for 1 year	Drug effect measurements: TPA-induced ODC activity and polyamine levels in colorectal tissue	Effect of chronic (1-year) DMFO treatment on otoxicity and drug effect measurements
Planned Study Phase II Chemoprevention Trials of DFMO and Piroxicam in Colon Cancer 1995 IND 33,018	Colon	Colorectal adenomatous polyp patients	Oral dose for 3 years	Efficacy: Histopathology, intermediate biomarkers	Efficacy (polyp reduction) and evaluation of intermediate biomarkers
NO1-CN-25436-02 Difluoromethylornithine (DFMO) in Oral Dysplastic Leukoplakia (Dr. Margie L. Clapper, Fox Chase Cancer Center 9/94– IND 33,018	Oral Cavity	Clinical evidence of oral leukoplakia with histopathological evidence of dysplasia ≥50 patients/arm	Oral 0.5 g/m^2 qd for 6 months	Efficacy: Histopathology, recurrence, regression Other intermediate biomarkers: DNA ploidy, PCNA, Ki-67, BrdU, MIB-1, KS-5	Modulation of dysplastic lesions and intermediate biomarkers with emphasis on quantitative evaluation of biomarkers by computer-assisted cytomorphometry and cytophotometry

Table I. Clinical Trials of DFMO Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers) *(continued)*					
UO1-CA59C52 Phase IIa Phase I Chemoprevention Study of Difluoromethylornithine (DFMO) in Subjects with/or at Risk for Prostate Cancer (Dr. Richard R. Love, Univ. of Wisconsin) 1993–1994 IND 33,018	Prostate	Scheduled for prostatectomy (stage A or B prostatic carcinoma or bladder cancer without prostatic carcinoma and scheduled for cystoprostatectomy) 15 patients	Oral 0.5 g/m^2 qd for 14 days	Drug effect measurements: TPA-induced ODC activity in skin and prostate; polyamine levels in prostate Efficacy: Serum PSA, PAP, testosterone; histopathology (TRUS-guided biopsies)	Drug tolerance/effective dose determination. Preliminary intermediate biomarker evaluation. Study will start as soon as FDA approves protocol
UO1-CA59C52 Phase IIb Phase II Chemoprevention Study of Difluoromethylornithine (DFMO) in Subjects with/or at Risk for Prostate Cancer (Dr. Richard R. Love, Univ. of Wisconsin) 1994?–1995? IND 33,018	Prostate	Serum PSA 3–10 ng/ml (includes patients with prostatic carcinoma and PIN) 46 patients (30 in treatment arm and 16 in placebo arm)	Oral 0.5 g/m^2 qd for 14 days–1 year	Drug effect measurements: TPA-induced ODC activity in prostate; polyamine levels in prostate Efficacy: Serum PSA, PAP, testosterone; histopathology (TRUS-guided biopsies) Toxicity	Efficacy and evaluation of intermediate biomarkers and toxicity. Study will start at completion of Phase IIa

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers) ***(continued)***					
Planned Study Phase II Clinical Trials of DFMO in Patients with Prostate Cancer in the Period Prior to Radical Prostatectomy (Presurgical Period). Modulation of Surrogate Endpoint Biomarkers (SEB) 1995 IND 33,018	Prostate	Patients with prostate cancer scheduled for prostatectomy 100 patients (50/arm)	Oral 0.5 g/m^2 qd for 14 days–1 month	Efficacy: Modulation of PIN, other histopathology Other intermediate biomarkers (to be determined)	Evaluation of intermediate biomarkers with emphasis on those that can be measured quantitatively by computer-assisted cytomorphometry and cytophotometry

DFMO DEVELOPMENT STATUS