[CANCER RESEARCH 48, 6584–6586, November 15, 1988]

Phase I Study of Difluoromethylornithine in Combination with Recombinant α2a-Interferon[1]

John H. Edmonson,[2] John S. Kovach, Jan C. Buckner, Larry K. Kvols, and Richard G. Hahn

Mayo Clinic, Rochester, Minnesota 55905

ABSTRACT

24 patients with advanced, histologically proven cancer were treated with difluoromethylornithine 2.25 g/m² orally every 6 h for the first 7 days of each 4-week treatment cycle. These patients also received daily i.m. doses of recombinant human α2a-interferon (IFN) on Days 3 through 7 of each cycle. IFN doses of 3, 6, 12, 24, 36, and 48 × 10⁶ units/m² have been studied utilizing three patients at each daily dose level. Three additional patients have been observed at each of the two highest doses for better toxicity definition.

This combination produced slight transient declines in leukocyte and platelet counts and transient rises in serum aspartate aminotransferase; however, these changes were no more pronounced at the higher IFN doses than at daily doses of 6 × 10⁶ units/m². Mild nausea and vomiting occurred in most patients and mild diarrhea also was common at all IFN dose levels. Chills, fever, myalgia, lethargy and fatigue, and anorexia were also observed at all IFN doses; however, lethargy and fatigue (lassitude) seemed to be the major factor which limited patient tolerance of IFN to 48 × 10⁶ units/m² daily. No ototoxicity was identified clinically or audiometrically and no life-threatening toxicity has occurred. Initial Phase II studies in melanoma are currently in progress.

INTRODUCTION

DFMO[3] is an inhibitor of the biosynthesis of the polyamine putrescine. Its sole biochemical action appears to be the selective irreversible inhibition of ornithine decarboxylase, the enzyme which catalyzes the conversion of ornithine to putrescine (1). Decline in the biosynthesis of putrescine leads to a depletion in intracellular concentrations of putrescine and spermidine and to a much lesser extent spermine (2). Alterations in concentrations of polyamines have been demonstrated to have a variety of effects on mammalian cell proliferation and differentiation and to inhibit the growth of several different kinds of human cancer cells *in vitro* (1, 3–5). Furthermore, DFMO has been demonstrated to modulate the cytotoxic effects of several anticancer drugs *in vitro* (6–10). Following Phase I studies by Abeloff *et al.* (11), Phase II human studies have been done with minimal evidence of activity of DFMO against small cell lung cancer and melanoma but with negative preliminary results against colon cancer (12, 13).

The inhibition of tumor growth by interferons has recently attracted much investigative interest including the development of numerous clinical studies (14). Highly purified human recombinant α2a-interferon has been studied extensively as a single agent in patients with advanced cancer generally on a daily or thrice weekly schedule. Daily doses have commonly ranged from 12 to 50 × 10⁶ units/m², and at the higher doses this treatment has produced significant fatigue, anorexia, and weight loss in addition to transient febrile reactions (15). α2a-interferon treatment in both the toxic higher doses and in the better tolerated lower doses (12 × 10⁶ units/m²) has induced objective tumor regression in 20–25% of patients with advanced malignant melanoma when administered thrice weekly i.m. In addition to melanoma, objective tumor regression has been observed during treatment with α2a-interferon in a variety of other solid tumors and hematological malignancies (16–21). With the possible exception of a rare type of B-cell leukemia (hairy cell leukemia), however, complete remission of human cancer with interferon as a single agent is infrequent.

Our pharmacology group became interested in studying the antiproliferative activity of recombinant human α2a-interferon in combination with DFMO after Sunkara *et al.* reported that mouse type I interferon and DFMO were synergistic against B_{16} murine melanoma *in vitro* and *in vivo* (22). Through a series of *in vitro* studies we have demonstrated that human α2a-interferon combined with DFMO has synergistic antiproliferative activity against a variety of human epithelial tumor cell lines which differ markedly in their sensitivity to interferon as a single agent (23). This synergistic activity of DFMO plus interferon is dependent upon depletion of polyamines by DFMO in that the addition of putrescine eliminates the synergism. The *in vitro* synergistic activity of human recombinant α2a-interferon and DFMO is achieved at concentrations readily achievable in humans. The mean plasma concentration of interferon 4 h following single i.m. injections of 36 × 10⁶ units of human recombinant α2a-interferon exceeds 200 units/ml, a concentration nearly 70 times the concentration of 3 units/ml at which synergism has been documented *in vitro* (24). Similarly, DFMO given orally at 3 g/m² every 6 h produces mean minimum steady state plasma concentrations of 37 × 10^{-5} M (11), a level more than 30 times that required for synergism with interferon *in vitro* against all cell lines studied (23). Even at doses of 1.5 g/m² every 6 h, DFMO produces mean minimum steady state plasma concentrations of 11.3 × 10^{-5} M (11), a level more than 10 times that required for synergism *in vitro*. Because of this attainable opportunity for possible synergism based on both the *in vitro* data and the mouse B_{16} melanoma model *in vivo*, we decided to develop a DFMO-IFN regimen in patients with advanced cancer. The results of that phase I clinical study are presented here.

MATERIALS AND METHODS

Between August 1985 and October 1986, 24 patients with advanced histologically proven cancer were treated with difluoromethylornithine from Merrell Dow Research Institute in combination with recombinant α2a-interferon from Hoffmann-LaRoche, Inc. DFMO was administered orally in doses of 2.25 g/m² every 6 h during the first 7 days of each 4-week treatment cycle. This dose level was selected to ameliorate DFMO toxicity following the recommendations of Abeloff *et al.* (11). The patients also received daily i.m. doses of IFN on Days 3 through 7 of each cycle. IFN doses of 3, 6, 12, 24, 36, and 48 × 10⁶ uts/m² were studied in three new patients at each dose level. Three additional patients were treated at each of the two highest IFN doses as we sought to define the maximal tolerable doses in the combination.

Patients accepted for this study were those for whom more conventional treatment offered no reasonable hope of significant benefit. Each

Received 3/28/88; revised 8/3/88; accepted 8/9/88.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by Grant CA1508302 from the National Cancer Institute.

[2] To whom requests for reprints should be addressed, at Division of Medical Oncology, Mayo Clinic, Rochester, MN 55905.

[3] The abbreviations used are: DFMO, difluoromethylornithine; IFN, interferon.

Table 1 *Toxicity of DFMO + α2a-interferon*

Daily IFN dose ($U \times 10^6/m^2$)	Total no. of courses	No. of patients	Mean lowest WBC $\times 10^3/\mu l$ (range)	Mean lowest Plt ct $\times 10^3/\mu l$ (range)	Audiogram	Mean degree of lassitude (range)[a]	Mean (U/l) increase in AST[b] (range)
3	3	3	2.8 (2.2–3.2)	201 (152–245)	No change	0.33 (0–1)	45 (20–85)
6	10	5[c]	3.7 (2.1–6.2)	211 (140–320)	No change	0.80 (0–2)	57 (17–103)
12	13	7[c]	3.8 (1.5–4.7)	221 (92–495)	No change	0.86 (0–2)	21 (10–54)
24	11	4[c]	3.7 (3.2–4.8)	169.5 (153–201)	No change	1.50 (1–2)	49 (23–68)
36	11	6	4.6 (3.3–9.6)	269 (128–393)	No change	1.83 (0–3)	68 (22–155)
48	12	6	3.5 (1.8–4.8)	167.5 (96–311)	No change	1.83 (0–3)	53 (47–121)

[a] Graded by increasing severity from 0 to 3 (disabling).
[b] AST, aspartate aminotransferase.
[c] Including some who began at lower doses.

patient had life expectancy of at least 2 months, was able to provide informed consent to participate, and maintained nutrition without parenteral supplements. Patients had satisfactory renal, hepatic, and bone marrow function as indicated respectively by serum creatinine no higher than 1.5 mg/dl, direct reacting serum bilirubin no higher than 0.3 mg/dl, and by the presence of standard hematological values (WBC $\geq$4,000/μl, platelets $\geq$130,000/μl and Hgb $\geq$10 g/dl). Patients with history of cardiac disease were excluded, as were pregnant or lactating women and patients with uncontrolled infection or severe nausea and vomiting. All of the patients had Eastern Cooperative Oncology Group performance status scores of 2 or better and all had recovered from any previous irradiation or chemotherapy. No patient had previously received DFMO, IFN, or immunotherapy.

During each 7-day treatment, patients were clinically observed daily for signs and symptoms of toxicity and had routine hematological and biochemical monitoring before each cycle and on treatment Days 3 and 6. Interval weekly hemoglobin, serum creatinine, aspartate aminotransferase, and serum bilirubin values were recorded in addition to twice weekly leukocyte and platelet counts. Urinalysis and audiometry were performed prior to treatment and at each fourth week clinical evaluation. For patients continuing treatment, doses were reduced by 50% for retreatment in cases of severe toxicity, and if severe neurological toxicity had occurred, the treatment was to be stopped. Patients who had experienced no significant toxicity were retreated at the next higher IFN dose level. Although participants were not required to have objectively evaluable disease, those who did were evaluated by standard response criteria.

RESULTS

Fifteen men and 9 women with median age of 50 years (range, 20–67 years) received from one to eight courses of DFMO-IFN without any lethal or life-threatening toxicity but without any objective tumor regression. Among the 24 patients, 10 had adenocarcinoma of kidney origin, three had adenocarcinoma of the colon, two had osteosarcoma, one each had carcinoma arising from the breast, pancreas, stomach, duodenum, esophagus, thyroid, testis, and unknown primary site, and one patient had synovial sarcoma. 12 of these patients had received previous cytotoxic drug therapy; eight had received radiation therapy (several had both); three patients had received megestrol acetate. All except six patients had received some type of nonsurgical treatment.

Toxic effects of the DFMO-IFN regimen included at all IFN doses chills and fever, diarrhea, nausea and vomiting, myalgia, fatigue, transient leukopenia, and transient increases in blood transaminase levels (aspartate aminotransferase). Treatment-related symptoms usually receded within 1 week after each course was completed, and the transient laboratory abnormalities resolved by Day 21. No hearing loss was observed either clinically or by audiometry performed at the time of return for retreatment. Although side effects were slightly more prominent at the higher IFN doses, only fatigue and lethargy were obviously dose related, and the lassitude produced by this regimen at IFN doses of 48 units/m^2/day appeared to be dose limiting (Table 1). In one of the patients treated at this highest IFN dose, lassitude was associated with transient hypotension. No evidence of cumulative toxicity was observed, and toxicity of the combination was similar among patients treated at escalated doses to that observed in patients who began treatment at those doses.

DISCUSSION

Among the 24 advanced cancer patients treated with DFMO combined with IFN in the present study, transient febrile reactions and the anticipated flu-like responses to the treatment were practically universal. Mild nausea and vomiting and diarrhea also were common, perhaps more so than ordinarily would be expected from recombinant α2a-interferon alone. These tolerable gastrointestinal effects were perhaps the only observed toxic activities related to the DFMO portion of the treatment. With the exception of lethargy and fatigue, which became disabling and intolerable in some patients above IFN doses of 24×10^6 units/day on the thrice weekly schedule, this regimen was generally acceptable to both patients and physicians. We have selected for subsequent Phase II studies the lower IFN dose (36×10^6 units/day) of the two evaluated within the "toxic" range.

Since our study began, a number of other DFMO-IFN results have been published. Sunkara has extended his earlier B_{16} mouse melanoma observations of treatment synergy to include metastatic Lewis lung carcinoma (25). In these studies he has also confirmed that α-interferon present in the mouse type 1 interferon preparation is responsible for the potentiation of antitumor activity with DFMO. Enhanced antiproliferative activity between DFMO and α-interferon has also been recently observed in human melanoma cell lines (26), human lung cancer cell lines (27), human renal cell carcinoma xenografts (28), and human lymphoblastoid (Daudi) cells in culture (29). Sunkara *et al.* (30) have also demonstrated potentiation of antiproliferative and antimetastatic effects of DFMO by interferon inducers. In addition to the abstract from our present study (31), two other Phase I human trials have been reported, from the University of Arizona (32), and from the M. D. Anderson Tumor Institute (33), respectively. Using 11-day courses and administering both agents on each day, Croghan *et al.* (32) (University of Arizona) identified maximum tolerated daily doses of 4 g/m^2 for DFMO

and 6×10^6 units/m^2 for IFN. These investigators also observed reversible hearing loss in four of their 17 patients and felt that leukopenia, fatigue, and weight loss were dose limiting. They observed three partial tumor regressions among their 17 melanoma patients. Talpaz *et al.* (33) at M. D. Anderson, utilizing a continuous regimen of DFMO and α-interferon, identified maximum tolerable daily doses of 6 g/m^2 for DFMO and 3.2 $\times 10^6$ units/m^2 for IFN-α. Of 12 patients who had melanoma, two experienced partial tumor regression. Minor responses also were observed in patients, respectively, with melanoma, colon carcinoma and renal carcinoma. Our 1-week-monthly, intermittent regimen utilized DFMO in daily doses of one and one-half to more than twice the daily doses used in these other studies, and we administered IFN in daily doses 8 to 15 times those included in the other two studies. By limiting the duration of continuous exposure of patients to DFMO, we seem to have eliminated the reversible hearing loss observed in patients receiving more prolonged but less intensive courses of treatment. We are currently evaluating the intermittent regimen developed in the present study for activity against malignant melanoma in a formal Phase II trial.

REFERENCES

1. Mamont, P. S., Duchesne, M. C., Grove, J., and Bey, P. Antiproliferative properties of DL-α-difluoromethylornithine in cultured cells. A consequence of the irreversible inhibition of ornithine decarboxylase. Biochem. Biophys. Res. Commun., *81:* 58–66, 1978.
2. Pegg, A. E., and McCann, P. P. Polyamine metabolism and function: brief review. Am. J. Physiol., *243:* C212–C221, 1982.
3. Luk, G. D., Goodwin, G., Marton, L. V., and Baylin, S. B. Polyamines are necessary for the survival of human small-cell lung carcinoma in culture. Proc. Natl. Acad. Sci. USA, *78:* 2355–2358, 1981.
4. Kingsnorth, A. N., Russell, W. E., McCann, P. P., Diekema, K. A., and Malt, R. A. Effects of α-difluoromethylornithine and 5-fluorouracil on the proliferation of a human colon adenocarcinoma cell line. Cancer Res., *43:* 4035–4038, 1983.
5. Luk, G. D., Civin, C. I., Weissman, R. M., and Baylin, S. B. Ornithine decarboxylase: essential in proliferation but not differentiation of human promyelocytic leukemia cells. Science (Wash. DC), *216:* 75–77, 1982.
6. Sunkara, P. S., Fowler, S. K., Nishioka, K., and Rao, P. N. Inhibition of polyamine biosynthesis by α-difluoromethylornithine potentiates the cytotoxic effects of arabinosylcytosine in Hela cells. Biochem. Biophys. Res. Commun., *95:* 423–430, 1980.
7. Hung, D. T., Deen, D. F., Seidenfeld, J., and Marton, L. J. Sensitization of 9L rat brain gliosarcoma cells to 1,3-bis(2-chloroethyl)-1-nitrosourea by α-difluoromethylornithine, an ornithine decarboxylase inhibitor. Cancer Res., *41:* 2783–2785, 1981.
8. Oredson, S. M., Gray, J. W., Deen, D. F., and Marton, L. J. Decreased cytotoxicity and 1-β-D-arabinofuranosylcytosine in 9L rat brain tumor cells pretreated with α-difluoromethylornithine. Cancer Res., *43:* 2541–2544, 1983.
9. Hung, D. T., Marton, L. J., Deen, D. F., and Shafer, R. H. Depletion of intracellular polyamines may alter DNA conformation in 9L rat brain tumor cells. Science (Wash. DC), *221:* 368–370, 1983.
10. Tofilon, P. J., Deen, D. F., and Marton, L. J. α-Difluoromethylornithine-induced polylamine depletion of 9L tumor cells modifies drug-induced DNA crosslink formation. Science (Wash. DC), *222:* 1132–1135, 1983.
11. 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.
12. 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.
13. 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.
14. Creagan, E. T. Interferon trials in the management of malignant melanoma and other neoplasms: an overview. *In:* L. Nathanson (ed.), Basic and Clinical Aspects of Malignant Melanoma, pp. 167–194. Boston: Martinus Nijhoff Publishers, 1987.
15. Creagan, E. T., Ahmann, D. L., Frytak, S., Long, H. J., Chang, M. M., and Itri, L. M. Three consecutive phase II studies of recombinant interferon alpha-2a in advanced malignant melanoma. Cancer (Phila.), *59:* 638–646, 1987.
16. Quesada, J. R., Rios, A., Swanson, D., Trown, P., and Gutteman, J. U. Antitumor activity of recombinant-derived interferon alpha in metastatic renal cell carcinoma. J. Clin. Oncol., *3:* 1522–1528, 1985.
17. Krown, S. E., Real, F. X., Cunningham-Rundles, S., *et al.* Preliminary observations on the effect of recombinant leukocyte A interferon in homosexual men with Kaposi's sarcoma. N. Engl. J. Med., *308:* 1071–1076, 1983.
18. Quesada, J. R., Hersh, E. M., Rueben, J., *et al.* Treatment of hairy cell leukemia with recombinant-DNA derived interferon alpha: a phase II study. Blood, *64* (Suppl. 1)*:* 172a, 1984.
19. Bunn, P. A., Foon, K. A., Idhe, D. C., *et al.* Recombinant leukocyte A interferon: an active agent in advanced cutaneous T-cell lymphomas. Ann. Intern. Med., *101:* 484–487, 1984.
20. Quesada, J. R., Hawkins, M., Horning, S., *et al.* A collaborative phase I-II study of recombinant DNA-produced leukocyte interferon (clone A) in metastatic breast cancer, malignant lymphoma, and multiple myeloma. Am. J. Med., *77:* 427–432, 1984.
21. Foon, K. A., Sherwin, S. A., Abrams, P. G., *et al.* Treatment of advanced non-Hodgkin's lymphoma with recombinant leukocyte A interferon. N. Engl. J. Med., *311:* 1148–1152, 1984.
22. Sunkara, P. S., Prakash, N. J., Mayer, G. D., Sjoerdsma, A. Tumor suppression with a combination of α-difluoromethylornithine and interferon. Science (Wash. DC), *219:* 851–853, 1983.
23. Kovach, J. S., and Svingen, P. A. Enhancement of the antiproliferative activity of human interferon by polyamine depletion. Cancer Treat. Rep., *69:* 97–103, 1985.
24. Horning, S. J., Levine, J. F., Miller, R. A., *et al.* Clinical and immunologic effects of recombinant leukocyte A interferon in eight patients with advanced cancer. J. Am. Med. Assoc., *247:* 1718–1722, 1982.
25. Sunkara, P. S. Murine α and γ interferons potentiate the antitumor activity of α-difluoromethylornithine, an inhibitor of polyamine biosynthesis, against mouse B_{16} melanoma and Lewis lung carcinoma. Proc. Am. Assoc. Cancer Res., *27:* 294, 1986.
26. Bregman, M. D., and Meyskens, F. L., Jr. Difluoromethylornithine enhances inhibition of melanoma cell growth in soft agar by dexamethasone, clone A interferon and trans retinoic acid: a new approach to biological modification. Proc. Am. Assoc. Cancer Res., *26:* 326, 1985.
27. Bepler, G., Carney, D. N., Nau, M. M., Gazdar, A. F., and Minna, J. D. Additive and differential biological activity of α-interferon A, difluoromethylornithine, and their combination on established lung cancer cell lines. Cancer Res., *46:* 3413–3419, 1986.
28. Heston, W. D., Fleischmann, J., Tackett, R. E., and Ratliff, T. L. Effects of α-difluoromethylornithine and recombinant interferon-α2 on the growth of a human renal cell adenocarcinoma xenograft in nude mice. Cancer Res., *44:* 3220–3225, 1984.
29. Rosenbaum, M. G., and Gutterman, J. U. Synergistic antiproliferative activity of leukocyte interferon in combination with α-difluoromethylornithine against human cells in culture. Cancer Res., *44:* 2339–2340, 1984.
30. Sunkara, P. S., Prakash, N. J., Rosenberger, A. L., Hagan, A. C., Lachmann, P. J., and Mayer, G. D. Potentiation of antitumor and antimetastatic activities of α-difluoromethylornithine by interferon inducers. Cancer Res., *44:* 2799–2802, 1984.
31. Edmonson, J. H., Kovach, J. S., Buckner, J. C., Kvols, L. K., and Hahn, R. G. Phase I study of difluoromethylornithine in combination with recombinant alpha 2a interferon. Proc. Am. Soc. Clin. Oncol., *6:* 239, 1987.
32. Croghan, M., Booth, A., and Meyskens, F. A phase I trial of recombinant α-interferon and difluoromethylornithine in metastatic melanoma. Proc. Am. Soc. Clin. Oncol., *6:* 209, 1987.
33. Talpaz, M., Plager, C., Quesada, J., Benjamin, R., Kantarjian, H., 288and Gutterman, J. Difluoromethylornithine and leukocyte interferon: a phase I study in cancer patients. Eur. J. Cancer Clin. Oncol., *22:* 685–689, 1986.

Phase I Study of Difluoromethylornithine in Combination with Recombinant α2a-Interferon

John H. Edmonson, John S. Kovach, Jan C. Buckner, et al.

Cancer Res 1988;48:6584-6586.