### Title
A phase I trial of recombinant interferon-alpha and alpha-difluoromethylornithine in metastatic melanoma.




### Journal
Journal of biological response modifiers, 7(4)

### ISSN
0732-6580



### Authors
Croghan, MK
Booth, A
Meyskens, FL



### Publication Date
1988-08-01

*Journal of Biological Response Modifiers*
7:409–415 © 1988 Raven Press, Ltd., New York

# A Phase I Trial of Recombinant Interferon-α and α-Difluoromethylornithine in Metastatic Melanoma

Marilyn K. Croghan, Ann Booth, and Frank L. Meyskens, Jr.

*Division of Hematology and Oncology, Department of Medicine, University of Arizona Cancer Center, Tucson, Arizona, U.S.A.*

**Summary:** Both interferon-alpha (IFN-α) and α-difluoromethylornithine (DFMO) have shown modest activity as single-agent therapy in the treatment of malignant melanoma. Several investigators have demonstrated true synergism in vitro of the combination of DFMO and IFN-α against human tumor cells, including melanoma. We have investigated this combination in 17 patients with malignant melanoma in a Phase I trial. Patients were treated with 4 or 6 g/m$^2$/day of oral DFMO in 3 divided doses for 11 days, followed by a 3-day rest period. Concomitant administration of 1.5, 3.0, 6.0, or 9.0 × 10$^6$ U/m$^2$ IFN-α intramuscularly was given. The maximum tolerated dose was 4 g/m$^2$/day of DFMO plus 6 × 10$^6$ U/m$^2$/day of IFN-α. Dose-limiting toxicity occurred in 3 of 3 patients receiving 9 × 10$^6$ U/m$^2$ IFN-α and consisted of leukopenia, fatigue, and weight loss. Other toxicities were mild and included reversible hearing loss, diarrhea, nausea, and vomiting. Three responses were seen, including one partial response (PR) of soft tissue metastases, one PR of lung and liver, and one complete response of liver metastases without clearance of carcinomatous meningitis. A Phase II trial has been initiated based on these encouraging results. **Key Words:** α-Difluoromethylornithine—Interferon-α—Melanoma.

Polyamines are organic cations that have been used as intracellular and extracellular markers of cell growth (1,2). Disruption of polyamine biosynthesis can lead to alterations in cell growth, proliferation, and differentiation (3). α-Difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase, blocks the first step in the polyamine biosynthesis pathway and has been shown to have antiproliferative effects on B16 melanoma cells (4), as well as in vitro and in vivo activity against human melanoma tumors (5). Oral doses of up to 9 g/m$^2$/day of DFMO have been well tolerated in clinical Phase I–II trials (6,7). In a Phase II study at our institution (5), DFMO has been found to have some

Received December 28, 1987; accepted March 19, 1988.
Address correspondence and reprint requests to Dr. F. L. Meyskens, Jr. at University of Arizona Cancer Center, 1501 N. Campbell Avenue, Tucson, AZ 85724, U.S.A.

activity against malignant melanoma, with 1 of 21 evaluable patients having complete resolution of all known disease.

Interferon-alpha (IFN-α) is an immunomodulatory protein that has also been shown to have in vitro antitumor activity against many human malignancies, including malignant melanoma (8). Phase II clinical trials using intramuscular or subcutaneous injections of IFN-α in patients with metastatic melanoma have shown complete or partial response rates of 20–30% (9,10). Using a combination of DFMO and IFN-α, Sunkara et al. have demonstrated both in vitro and in vivo synergistic cytotoxicity against B16 melanoma cells (11). Synergistic antiproliferative effects have also been reported in both IFN-α–sensitive and IFN-α–resistant human melanoma cell lines (12). Talpaz et al. conducted a broad Phase I clinical trial using a combination of oral DFMO and intramuscular IFN-α in 25 patients with various tumor types, including 12 patients with malignant melanoma in whom 2 partial remissions were observed (13). In view of these data, we have conducted a Phase I study to further evaluate the role of combined DFMO and recombinant IFN-α therapy for the treatment of disseminated malignant melanoma.

## MATERIALS AND METHODS

Only patients with a histologically confirmed diagnosis of malignant melanoma were entered on study. All patients entered had stage III disease (SWOG criteria), measurable lesions, and a life expectancy greater than 12 weeks. Exclusion criteria included major organ dysfunction, unresected brain metastasis, history of prior malignancy other than noninvasive cutaneous carcinoma, Karnofsky performance status less than 50%, other therapy within 4 weeks of study entry, uncontrolled infection, or prior IFN-α therapy. A signed informed consent form, approved by the University of Arizona Institutional Review Board, was obtained from each patient prior to initiating therapy.

Recombinant interferon alpha-2a (Ro 22-8181/002) for sterile injection was provided by Hoffmann LaRoche Laboratories (Nutley, NJ, U.S.A.). DFMO, at a concentration of 200 mg/ml, was provided by Merrel Dow Research Institute (Cincinnati, OH, U.S.A.). Patient doses of DFMO and IFN-α are given in Table 1. DFMO was taken orally in 3 divided doses daily for 11 days consecutively, followed by a 3-day drug-free interval. IFN-α was administered as a single intramuscular injection on days 1–11 of each 14-day cycle. Medications were discon-

TABLE 1. *Study design*

| Patients | DFMO ($g/m^2$/day) | Roferon-A ($\times 10^6$ U/$m^2$/day) |
|---|---|---|
| 1–3 | 6.0 | 3.0 |
| 4–7 | 4.0 | 1.5 |
| 8–10 | 4.0 | 3.0 |
| 11–13 | 4.0 | 6.0 |
| 14–17 | 4.0 | 9.0 |

DFMO 4.0 or 6.0 $g/m^2$/day, divided into 3 doses; Roferon-A 1.5–9 $\times$ $10^6$ U/$m^2$/day.

tinued if grade III or IV toxicity persisted despite dose reductions or if progression of disease was documented following 4 cycles of treatment. Patients were monitored with weekly complete blood cell counts, white blood cell differentials, and platelet counts for the first 4 cycles of therapy, and monthly thereafter. Urinalysis, an SMA-20, and an audiogram were obtained every 4 weeks or sooner, as clinically indicated.

Patients were evaluated for response at the completion of 4 cycles of therapy and every 4 weeks thereafter. Bidimensional measurements of evaluable lesions were obtained from physical exam, chest roentgenogram, computerized tomography scans, and/or abdominal ultrasounds. Response was defined as follows: a complete response equaled total resolution of all known disease; a partial response (PR) equaled 50% or more decrease in the sum of the products of tumor diameters for lesions selected on baseline evaluations, with no increase in the size of any other lesions; stable disease was a less than 25% increase or 50% decrease in the sum of the products of the measured baseline lesions and no new disease. Progressive disease was defined as the appearance of a new metastatic lesion or a greater than 25% increase over baseline measurements. Duration of response was calculated from the time of maximal disease response to the time of documented disease progression.

## RESULTS

Seventeen patients were placed on study from November 1985 through June 1986. Using modified SWOG criteria, all patients were evaluated for evidence of drug toxicity. Patient characteristics are listed in Table 2. The majority of patients (65%) had metastatic disease to lung, with 5 patients having the lung as the only site of known metastasis. Ten patients (59%) had more than one site of metastatic disease.

Overall toxicities for all patients are shown in Table 3. In general, side effects were mild, with the most common (in order of frequency) being grade I or II fatigue, diarrhea, chills following the initial IFN-α administration, leukopenia, or

**TABLE 2.** *Characteristics of 17 eligible patients with stage III malignant melanoma*

| | |
|---|---|
| Mean age (range) | 51 (28–79) |
| Male:female | 12:5 |
| Performance status (Karnofsky) | |
| 90–100 | 12 |
| 70–89 | 4 |
| 50–69 | 1 |
| Prior treatment | 10 |
| Sites of metastatic disease (%) | |
| Skin/subcutaneous | 5 (29) |
| Lung | 11 (65) |
| Lymph nodes | 2 (12) |
| Liver | 4 (24) |
| Brain (resected) | 1 (6) |
| Bone | 2 (12) |
| Adrenal gland | 1 (6) |

TABLE 3. *Overall toxicity*

| Toxicity | Grade I | Grade II | Grade III | Total (%) |
|---|---|---|---|---|
| Fatigue | 2 | 5 | 5 | 13/17 (76)[a] |
| Fever | 2 | 3 | 0 | 5/17 (29) |
| Chills | 1 | 7 | 2 | 10/17 (59) |
| Nausea | 5 | 4 | 0 | 9/17 (53) |
| Vomiting | 0 | 2 | 0 | 2/17 (12) |
| Diarrhea | 8 | 3 | 0 | 11/17 (65) |
| Anorexia | 2 | 5 | 0 | 7/17 (41) |
| Weight loss | 0 | 1 | 3 | 4/17 (24) |
| Leukopenia | 2 | 8 | 0 | 10/17 (59) |
| Granulocytopenia | 1 | 3 | 1 | 5/15 (29) |
| Thrombocytopenia | 0 | 0 | 1 | 1/17 (6) |
| Anemia | 1 | 0 | 2 | 3/17 (18) |
| Tinnitus | 2 | 0 | 0 | 2/17 (12) |
| Hearing loss | 1 | 2 | 2 | 5/17 (29) |
| Local irritation at injection site | 1 | 0 | 0 | 1/17 (6) |
| ↑ SGOT | 2 | 1 | 0 | 3/17 (18) |
| Headache | 1 | 2 | 1 | 4/17 (24) |
| Myalgias | 0 | 3 | 0 | 3/17 (18) |
| Weakness | 1 | 2 | 0 | 3/17 (18) |
| Hair loss | 1 | 0 | 0 | 1/17 (6) |

[a] Includes 1 patient with grade IV fatigue.

nausea. Moderate granulocytopenia (750–999 granulocytes/$mm^3$) was observed in patients at all IFN-α dose levels and was rapidly reversible with IFN-α dose modifications.

Hearing loss appeared to be related to cumulative number of drug courses, with 4 of 17 patients developing grade II (10–20 db) or III (>20 db) ototoxicity. Three of the 7 patients who received 8 or more cycles of treatment developed a greater than 10-db hearing loss. In contrast, only 1 of the 10 patients who received less than 8 cycles of treatment developed grade II or III ototoxicity. The one exception was a patient with a documented hearing deficit prior to study entry who developed a 10–15-db loss after only 2 cycles of treatment. Audiometric testing returned to baseline levels in all four patients within 8 weeks of discontinuing therapy.

Dose-limiting toxicities are listed in Table 4. Three of 3 patients receiving 6 $g/m^2$/day DFMO developed overwhelming fatigue (in bed greater than 50% of the time). A reduction in dose to 4 $g/m^2$/day resulted in an acceptable decrease in this

TABLE 4. *Dose-limiting toxicity*

| DFMO ($g/m^2$/day) | IFN-α ($\times 10^6$ $U/m^2$/day) | No. of patients[a] | Toxicity |
|---|---|---|---|
| 6.0 | 3.0 | 3/3 | Fatigue |
| 6.0 | 3.0 | 1/3 | Thrombocytopenia |
| 4.0 | 9.0 | 2/4 | Fatigue |
| 4.0 | 9.0 | 2/4 | Weight loss |
| 4.0 | 9.0 | 1/4 | Granulocytopenia |

[a] Number of patients tested/number of patients with grade III/IV toxicity.

side effect, with patients spending less than 50% of the time in bed. Overwhelming fatigue was again noted at the combined dose of 4 g/m$^2$/day DFMO with $9 \times 10^6$ U/m$^2$/day IFN-α and was the major dose-limiting toxicity. Grade II (less than 5%) or III (5–10%) weight loss occurred in 3 of the 4 patients receiving $9 \times 10^6$ U/m$^2$/day IFN-α.

Fifteen patients received at least 4 cycles of therapy and were evaluated for response. Two patients received less than one complete cycle of therapy and were considered to be inevaluable. As shown in Table 5, 3 patients (20%) had an objective response. Patient 8 was a 39-year-old woman with multiple subcutaneous nodules who obtained a PR after 12 weeks of therapy. Her response lasted 12 weeks and was followed by the development of new subcutaneous nodules. Patient 10 was a 48-year-old man with multiple pulmonary metastasis who obtained a PR after 32 weeks of treatment for stable disease. Six weeks after obtaining a PR of the lung nodules, the patient developed a brain metastasis. Patient 13 was a 33-year-old man with multiple liver metastases that completely resolved after 8 weeks of therapy. This patient had had a prior resection of a single brain lesion. Twelve weeks into therapy, he developed central nervous system (CNS) symptoms and was found to have carcinomatous meningitis. His CNS disease persisted despite continued resolution of all known systemic disease for an additional 12 weeks. This patient died of CNS complications without documentable recurrence of the liver metastases.

## DISCUSSION

In this Phase I study, the maximum tolerated dose of combined therapy was 4.0 g/m$^2$/day DFMO plus $6 \times 10^6$ U/m$^2$/day IFN-α. Further increases in the dose of either IFN-α or DFMO resulted in overwhelming fatigue. These doses and dose-limiting toxicity differed from a previous report by a Talpaz et al. (13) in which 6.0 g/m$^2$/day DFMO plus $3.2 \times 10^6$ U/m$^2$/day IFN-α were reported as the maximal tolerated combined doses with gastrointestinal (GI) toxicities, including grade II or III nausea, vomiting, weight loss, and diarrhea, reported as dose limiting. GI toxicities were also noted in our study, but symptoms were generally less severe (grade I–II) and responded to symptomatic treatment. The higher dose of DFMO used by Talpaz et al. may account for the more severe GI toxicities observed. Clinical studies using 6.0–27.0 g/m$^2$/day DFMO as a single agent have found GI toxicities to be dose limiting (5,14). In agreement with our findings, Talpaz et al.

TABLE 5. *Results*

| No. of patients | Response | Duration (weeks) |
|---|---|---|
| 2 | CR | 12 |
| 1 | PR | 6,12 |
| 8 | S | 4–28 |
| 4 | P | — |
| 2 | Inevaluable | — |

CR, complete response; PR, partial response; P, progressive disease; S, stable disease.

noted an increase in the number of patients who developed grade II–III fatigue concomitant with escalation in the IFN-α dose. The higher dose of IFN-α in our study is therefore consistent with a higher incidence of fatigue. Some differences in the amount of fatigue reported by Talpaz et al. may have been due to the use of human leukocyte interferon (State Serum Institute, Helsinki, Finland) rather than recombinant IFN-α. In our clinical experience, DFMO and IFN-α used in combination produced more fatigue than equivalent doses of either DFMO or IFN-α used as single agents.

Ototoxicity has been reported in several clinical trials using DFMO as single-agent therapy (5,7,15). Using sequential audiometric testing, we documented a sensorineural defect in 5 (29%) patients. In all cases, the audiograms returned to baseline when therapy was discontinued.

Hematological toxicity when present was generally mild and rapidly reversed with dosage reductions or discontinuation of therapy. Other less frequently noted, reversible toxicities included headaches, myalgias, hair loss, weakness, and asymptomatic elevations in liver enzymes.

Overall, this combined modality therapy was well tolerated and could be given for prolonged periods of time on an outpatient basis. A greater than 50% decrease in the size of lung and subcutaneous metastases was seen in 2 patients. In a third patient, complete resolution of liver metastases were observed. We are currently pursuing these encouraging results with a clinical Phase II trial using 4.0 g/m$^2$/day DFMO plus $6 \times 10^6$ U/m$^2$/day IFN-α.

**Acknowledgment:** This study was supported in part by grants from the National Cancer Institute (CA27502, CA23074) and the Merrell Dow Pharmaceutical Company. The authors thank Cindy Ryan for secretarial assistance.

## REFERENCES

1. Russel DH, Durie BGM, eds. *Progress in cancer research and therapy: Polyamines as biochemical markers of normal and malignant growth, vol 8.* New York: Raven Press, 1978.
2. Raina A, Janne J. Physiology of the natural polyamines putrescine, spermidine and spermine. *Med Biol* 1975;53:121–47.
3. Pegg AE, McCann PP. Polyamine metabolism and function. *Am J Physiol* 1982;243:212–21.
4. Sunkara PS, Chang CC, Prakash NK. Effect of inhibition of polyamine biosynthesis by DL-alfa-difluoromethylornithine on the growth and melanogenesis of B16 melanoma in vitro and in vivo. *Cancer Res* 1985;45:4067–70.
5. Meyskens FL, Kingsley EM, Glattke T, Loescher L, Booth A. A phase II study of α-difluoromethylornithine (DFMO) for treatment of metastatic melanoma. *Invest New Drugs* 1986;4:257–62.
6. Abeloff MD, Slavik M, Luk GD, et al. Phase I trial and pharmacokinetic studies of α-difluoromethylornithine—an inhibitor of polyamine biosynthesis. *J Clin Oncol* 1984;2:124–30.
7. Abeloff MD, Rosen ST, Luk GD, Baylin SB, Zeltzman M, Sjoerdsma A. Phase II trials of α-difluoromethylornithine, an inhibitor of polyamine synthesis. *Cancer Treat Rep* 1986;70:843–5.
8. Salmon SE, Durie BGM, Young L, Liu RM, Trown PW, Stebbing N. Effects of cloned human leukocyte interferons in the human leukocyte tumor stem cell assay. *J Clin Oncol* 1983;1:217–25.
9. Creagan ET, Ahman DL, Green SJ, et al. Phase II study of recombinant leukocyte A interferon (rIFN-αA) in disseminated malignant melanoma. *Cancer* 1984;54:2844–9.
10. Dorval T, Palangie T, Jouve M, et al. Clinical phase II trial of recombinant DNA interferon (interferon alfa 2b) in patients with metastatic malignant melanoma. *Cancer* 1986;58:215–8.
11. Sunkara PS, Prakash NJ, Mayer GD, Sjoerdsma A. Tumor suppression with a combination of α-difluoromethylornithine and interferon. *Science* 1983;219:851–3.

12. Kovach JS, Svingen PA. Enhancement of the antiproliferative activity of human interferon by polyamine depletion. *Cancer Treat Rep* 1985;69:97–103.
13. Talpaz M, Plager C, Quesada J, Benjamin R, Kantarjian H, Gutterman J. Difluoromethylornithine and leukocyte interferon: a phase I study of cancer patients. *Eur J Cancer Clin Oncol* 1986;22: 685–9.
14. Natale RB, Meyer K, Forastiere A. Phase I trials of difluoromethylornithine alone and in combination with methylglyoxal bisguanylhydrazone. *Proc ASCO* 1985;4:C532.
15. Maddox AM, Keating MJ, McCredie KE, Ester E, Freireich EJ. Phase I evaluation of intravenous difluoromethylornithine—a polyamine inhibitor. *Invest New Drugs* 1985;3:287–92.