Three Consecutive Phase II Studies of Recombinant Interferon Alfa–2a in Advanced Malignant Melanoma

Updated Analyses

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In three consecutive Phase II trials of recombinant interferon alfa–2a (rIFN alfa–2a; Roferon®–A Hoffmann-La Roche, Nutley, NJ) involving 96 patients with advanced malignant melanoma, an overall response rate of 22% was observed. For all study participants, the median time to disease progression was 1.7 months, and the median survival was six months. Most regressions occurred within one month of commencing therapy, were usually limited to soft tissue metastases, and were transient. However, responses in three patients were long term, lasting 32+, 36+, and 41+ months. A thrice weekly intramuscular dose of 50 × 10^6 U/m^2 produced an intolerable flulike illness concomitant with a median weight loss of 5.6 kg. The addition of cimetidine to the same dose in 35 patients was of no therapeutic value. A dose of 12 × 10^6 U/m^2 produced clinically acceptable toxicities, and a median weight loss of 2.1 kg. There was no apparent dose response relationship, nor were there any obvious sequelae from antibody formation to interferon alfa–2a. As single agent therapy in malignant melanoma, interferon alfa–2a was only marginally useful in most patients. Nevertheless, combination regimens of this agent with cytotoxic agents, alternative molecular species of interferon, and lymphokines, notably tumor necrosis factor, offer a conceptually intriguing dimension in the design of future clinical trials.

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DISSEMINATED MALIGNANT MELANOMA usually follows a relentless course, despite a spectrum of systemic interventions with single agent or multidrug chemotherapeutic programs using investigational or conventional regimens.[1–4] "Standard" therapy with decarbazine was assessed in one randomized trial which compared it with polychemotherapy using vinblastine, bleomycin, and cisplatin.[5] The response rate with decarbazine was only 14%. Similarly, additive hormonal therapies for disseminated malignant melanoma have not provided any meaningful palliation.[6] Alternative systemic programs are obviously warranted for patients with this neoplasm.

Experimental studies using interferon preparations in malignant melanoma models indicated some possible therapeutic benefit.[7] Subsequent early clinical trials, which used relatively crude preparations of leukocyte-derived human interferon, showed some objective regressions in limited numbers of patients.[8–10] The biological impurities in the interferon preparations used in these initial studies, and the limited quantities of available human interferon, significantly hampered large scale Phase II trials of these immunomodulatory proteins in the treatment of disseminated malignant melanoma. However, enormous strides in recombinant DNA technology culminated in a 1982 Phase I study that clearly defined the pharmacokinetics and anticipated toxicities from interferon alfa–2a.[11] The study provided a rational foundation for launching extensive Phase II studies in malignant melanoma.

In this study, we report our experience in three consecutive Phase II studies employing interferon alfa–2a. The initial study commenced in July 1982, and updated analyses are provided through May 1986.[12–14]

Materials and Methods

Each patient in these three studies had biopsy-confirmed, bidimensionally measurable disease, and were treated on an outpatient basis. Each had an expected survival time of at least 12 weeks, was at least 18 years old, and had no acute intercurrent illness or active viral disease, and no history of cardiac impairment. None of the study participants received cytotoxic, radiotherapeutic, or im-

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munotherapeutic modalities during the month preceding the trial. Each patient had adequate renal function (serum creatinine < 1.9 mg/dl); hepatic function (no elevation of direct-reacting serum bilirubin); and hematologic functions (leukocyte count > 3000/μl, granulocyte count >1000/μl, and platelet count >100,000/μl). All patients provided written informed consent.

Each study was designed so that patients would be assigned to "risk categories." We defined a "good risk" patient as having a performance score of 0 or 1, and no previous chemotherapy; whereas a "poor risk" patient had a performance score of 2 or 3, or any previous chemotherapy, or both. These categories were deemed to be important because of the well recognized relationship between performance score or earlier chemotherapy, and response rate to investigational or conventional systemic therapies in patients with malignant melanoma.

Interferon alfa-2a was injected intramuscularly into either the deltoid muscle of one arm, or one of the gluteus muscles on a rotational basis. The injected dose was either 12×10^6 U/m^2 or 50×10^6 U/m^2 three times weekly for an anticipated treatment duration of approximately three months. Injections were given on alternate days. Any patient demonstrating an objective regression of disease was eligible to receive an additional month of therapy. We typically premedicated patients on the first visit only with oral acetaminophen, and a single intravenous dose of hydrocortisone. During subsequent visits, oral acetaminophen was used for symptomatic toxicity. Patients were advised not to use aspirin or other nonsteroidal antiinflammatory agents.

Before commencing therapy, and at two weeks, four weeks, and monthly intervals thereafter, each patient underwent a thorough medical and neurologic evaluation. Measurements were taken of indicator lesions, performance score, and weight; along with a chest x-ray, electrocardiogram, radionuclide liver scan, routine hematologic and chemical indices, and urinalysis. Serum levels of interferon alfa-2a and antibodies to interferon alfa-2a were also determined.

Objective responses were defined by the following criteria: a complete response (CR) indicated the absence of all measurable disease; a partial response (PR) indicated a ≥50% decrease in the product of the longest perpendicular diameters of the most clearly measurable indicator lesion, without disease-related symptomatic deterioration, or the appearance of new lesions. Progressive disease indicated a >25% increase in the product of any indicator lesion, or the development of new lesions. We calculated the time to progression from the date of registration to the date on which we first detected evidence of progressive disease.

We used the technique of logistic regression to analyze the association of treatment and patient characteristics with response. The time to progression and survival distributions were calculated by the method of Kaplan and Meier. We used the log rank tests, and proportional hazards modeling method to analyze time to disease progression and death. The techniques of log hazard ratios and standard error were used to calculate the confidence intervals on the hazard ratios.

TABLE 1. Patient Characteristics in Three Phase II Trials

	Initial trial*	Second trial†	Third trial‡
Number of patients	31§	30‖	35#
Median age (yr)	56	53	57
Sex			
Male/Female	23/8	17/13	20/15
Previous chemotherapy			
Yes/No	9/22	13/17	10/25
ECOG PS			
0	12	12	10
1	14	15	18
2, 3	5	3	7
Dominant metastases			
Visceral	19	22	23
Nonvisceral	12	8	12

ECOG PS: Eastern Cooperative Oncology Group (US) performance scores; IM: intramuscular; t.i.w.: three times a week.

* Interferon alfa-2a; 50×10^6 U/m^2 IM t.i.w.

† Interferon alfa-2a, 12×10^6 U/m^2 IM t.i.w.

‡ Interferon alfa-2a, 50×10^6 U/m^2 IM t.i.w.; plus cimetidine, 300 mg orally, four times a day.

§ 21 good risk patients: ECOG PS 0 or 1 and any previous chemotherapy; 10 poor risk patients: ECOG PS 2 or 3 or no previous chemotherapy.

‖ 15 good risk patients; 15 poor risk patients.

21 good risk patients; 14 poor risk patients.

Results

Trial I

A total of 31 patients received 50×10^6 U/m^2 of interferon alfa-2a, administered intramuscularly three times weekly for approximately three months (Table 1).[12] It should be noted that 22 patients had no previous chemotherapy, and that most individuals had excellent performance scores (Eastern Cooperative Oncology Group performance scores [ECOG PS] 0, 1). We observed an overall response rate of 23% with median times to progression and survival of two and six months, respectively (Table 2). However, we were particularly concerned about significant constitutional sequelae in virtually all patients (Table 3). Only six of 13 patients who remained in the study for 12 weeks could tolerate the full dose regimen. The median weight loss of 5.6 kg per patient clearly indicated the toxicity of this program.

Trial II

It appeared from our initial study that a clinically tolerable dose would be 12×10^6 U/m^2 administered thrice

TABLE 2. Objective Regressions in Initial Trial

	Metastatic sites	Duration (mo)
Among good risk patients (PS 0, 1; no previous chemotherapy) 5/21 (24%)	Skin	CR 32+
	Skin	CR 6
	Lung	PR 7
	Skin	PR 5
	Prostate	PR 3
Among poor risk patients (PS 2, 3; or previous chemotherapy) 2/10 (20%)	Nodes, liver	CR 41+
	Skin	PR 3

PS: performance score; CR: complete response; PR: partial response.

TABLE 4. Objective Regressions in Second Trial

	Metastatic site	Duration (mo)
Among good risk patients (PS 0, 1; no previous chemotherapy) 3/15 (20%)	Soft tissue and lung	CR 35+
	Soft tissue	PR 2
	Soft tissue	PR 10
Among poor risk patients (PS 2, 3; or previous chemotherapy) 3/15 (20%)	Soft tissue	PR 2
	Soft tissue	PR 3
	Soft tissue	PR 11

PS: performance score; CR: complete response; PR: partial response.

weekly. Therefore, we embarked on a second Phase II study involving 30 patients (Table 1).[13] As in the first study, the majority of patients had no previous chemotherapy, excellent performance scores, and visceral metastases. The overall response rate was 20%, with no differences between good risk, and poor risk patients (Table 4). The survival and time to progression distributions were similar to the results of our initial study. However, clinical toxicities were far more manageable with the lower dose regimen, than with the 50×10^6 U/m^2 thrice weekly regimen (Table 5). Particularly noteworthy was a median weight loss of only 2.1 kg compared with 5.6 kg in the earlier study.

Trial III

Our two trials involving 61 patients documented some objective regressions in patients with disseminated malignant melanoma. Yet it did not appear that interferon alfa-2a as a single agent would induce durable responses in such patients. Then a report by European investigators indicated that cimetidine, an H_2-receptor antagonist with provocative immunomodulatory properties, may enhance the antitumor characteristics of interferon in malignant melanoma.[15] We embarked on a third Phase II study using an interferon alfa-2a dose of 50×10^6 U/m^2 intramuscularly thrice weekly, plus cimetidine, 300 mg orally, four times a day.[14] As noted in Table 1, the study population was similar to those in our two initial studies: most patients had no previous chemotherapy, excellent performance scores, and visceral involvement. Seven of 21 good risk patients (33%), and one of 14 poor risk patients (7%), manifested transient objective regressions of disease, predominantly in soft tissue lesions (Table 6). In general, the clinical toxicities were of intermittent severity between our high dose and low dose regimens (Table 7). The time to progression and survival distributions were not dissimilar to the distributions in our initial studies. Therefore, it did not appear from our data that cimetidine offered a substantial therapeutic advantage when used concomitantly with interferon alfa-2a.

Sequelae and Antibody Formation

Most participants of these three trials manifested leukopenia, thrombocytopenia, and elevations in hepatic transaminase levels (Table 8). However, these findings were rarely clinically significant, and we did not detect any meaningful complications from these developments.

Serum titers of interferon alfa-2a were assayed by en-

TABLE 3. Clinical Toxicities of Interferon Alfa-2a (50×10^6 U/m^2 t.i.w.)

Toxic effect	% Of patients
Fever (>38.9°C)	100
Fatigue	87
Anorexia	58
Confusion	23
Myalgias	19
Median weight loss (kg)	5.6

t.i.w.: three times a week.

TABLE 5. Clinical Toxicities of Interferon Alfa-2a (12×10^6 U/m^2 t.i.w.)

Toxic effect	% Of patients
Fever (>38.9°C)	80
Fatigue	50
Anorexia	47
Nausea	33
Myalgias	27
Confusion	3
Median weight loss (kg)	2.1

t.i.w.: three times a week.

TABLE 6. Objective Regressions in Third Trial

	Metastatic site	Duration (mo)
Among good risk patients (PS 0, 1; no previous chemotherapy) 7/21 (33%)	Soft tissue (5 patients)	PR 2–4
	Lung	PR 2
	Lung	PR 4
Among poor risk patients (PS 2, 3; or previous chemotherapy) 1/14 (7%)	Soft tissue	PR 4

PS: performance score; PR: partial response.

TABLE 7. Clinical Toxicities of Interferon Alfa-2a (50×10^6 U/m^2 t.i.w.) With Cimetidine (300 mg Orally q.i.d.)

Toxic effect	% Of patients
Fever (>38.3°C)	89
Fatigue	80
Anorexia	63
Nausea	34
Confusion	11
Median weight loss (kg)	3.6

t.i.w.: three times a week; q.i.d.: four times a day.

zyme linked monoclonal antibody based immunoassay performed at Hoffmann-La Roche (Nutley, NJ). Of 22 initial patients, 21 had levels < 15 pg/ml. Serum titers were also determined in six of seven responding patients. There were no meaningful differences in titers between responding and nonresponding patients.

No pretreatment antibodies to interferon alfa-2a were detected in 23 patients. However, three patients did show antibody levels three to four months after commencing therapy. Interestingly, one of these patients was the 56-year-old responding woman who remains without any evidence of malignant melanoma 41+ months following the commencement of her interferon program. Thirty-seven months have elapsed since the patient's last interferon injection without any detectable adverse effects. Among these small numbers of patients, we could not discern any firm correlation between serum interferon levels, antibody formation, and clinical tolerance to treatment.

Long Term Responding Patients

Three individuals have demonstrated durable objective regressions following treatment with interferon (Table 9). Among the 21 responding patients in these three clinical trials, there were no consistently predictable characteristics that may have portended an objective regression with interferon therapy.

Statistical Analyses

We assessed the correlation between objective response and the following prognostic discriminants: performance score (ECOG PS 0 *versus* other); previous chemotherapy or radiation therapy (yes/no); visceral metastases (yes/no); age (<56 *versus* >56); sex; interferon therapy (low dose *versus* high dose regimen); and cimetidine therapy (yes/no). Viscerally dominant disease was the only discriminant with a statistically significant negative impact on response (two-sided $P = 0.003$). Treatment with cimetidine did not influence response probabilities. We also performed Cox covariate analysis using the same group of discriminants; none was significantly associated with time to progression. The estimated time to progression hazard ratios (high dose/low dose; [high dose + cimetidine]/low dose) were 0.91 and 1.55 with 95% confidence intervals of 0.54 to 1.55, and 0.93 to 2.58 respectively. We were unable to demonstrate any substantial advantage as reflected in time to progression among any of the three treatment regimens. For all 96 patients, the median time to progression was 1.7 months (Fig. 1). For the 21 responding patients, the median time to progression was 3.9 months (Fig. 2).

We then analyzed survival data relative to the impact of the aforementioned covariants. The only statistically significant covariant impacting on survival was the ECOG performance score (two-sided, $P = 0.013$). As anticipated, patients with an ECOG PS 0 survived longer than patients with clinical disability from malignant melanoma. As with

TABLE 8. Hematologic and Hepatic Toxicities of Interferon Alfa-2a (Three Times a Week)

	50×10^6 U/m^2	12×10^6 U/m^2	50×10^6 U/m^2 plus cimetidine
Leukocytes ($\times 10^3$ cells/μl)/Nadir median (range)	3.1 (1.6–7.3)	3.9 (1.9–8.8)	3.1 (1.3–8.4)
Platelets ($\times 10^3$ cells/μl)/ Nadir median (range)	158 (56–296)	186 (76–472)	197 (100–385)
SGOT above normal range (12–31 μg/l)	86%	97%	97%

SGOT: serum glutamic-oxaloacetic transaminase.

TABLE 9. Characteristics of Long Term Responding Patients to Interferon Alfa-2a

Patient	Age/sex (yr)	Starting dose (U/m^2 IM t.i.w.)	Previous therapy	Indicator lesion	Response	Duration (mo)	Interval from last injection (mo)	Interval from onset of therapy to first regression (mo)
1	41/M	50×10^6	None	Diffuse subcutaneous nodules	Complete	32+	27	1
2	56/F	50×10^6	Chemotherapy	Left inguinal mass	Complete	41+	37	1
				Liver scan without tissue confirmation	Complete			
3	54/F	12×10^6	None	Extremity soft tissue mass	Complete	36+	32	1
				Chest x-ray	Partial			

IM: intramuscularly; t.i.w.: three times a week.

time to progression, the impact of treatment on survival was not significant. The estimated death hazard ratios as described above were 0.89 and 1.14, with 95% confidence intervals of 0.50 to 1.56, and 0.68 to 1.91 respectively. We could not document any advantage to the high dose program over the other treatment alternatives as reflected in survival.

For all three trials, the median survival was six months from the onset of therapy (Fig. 3). Responding patients had a median survival of 11.3 months (Fig. 4).

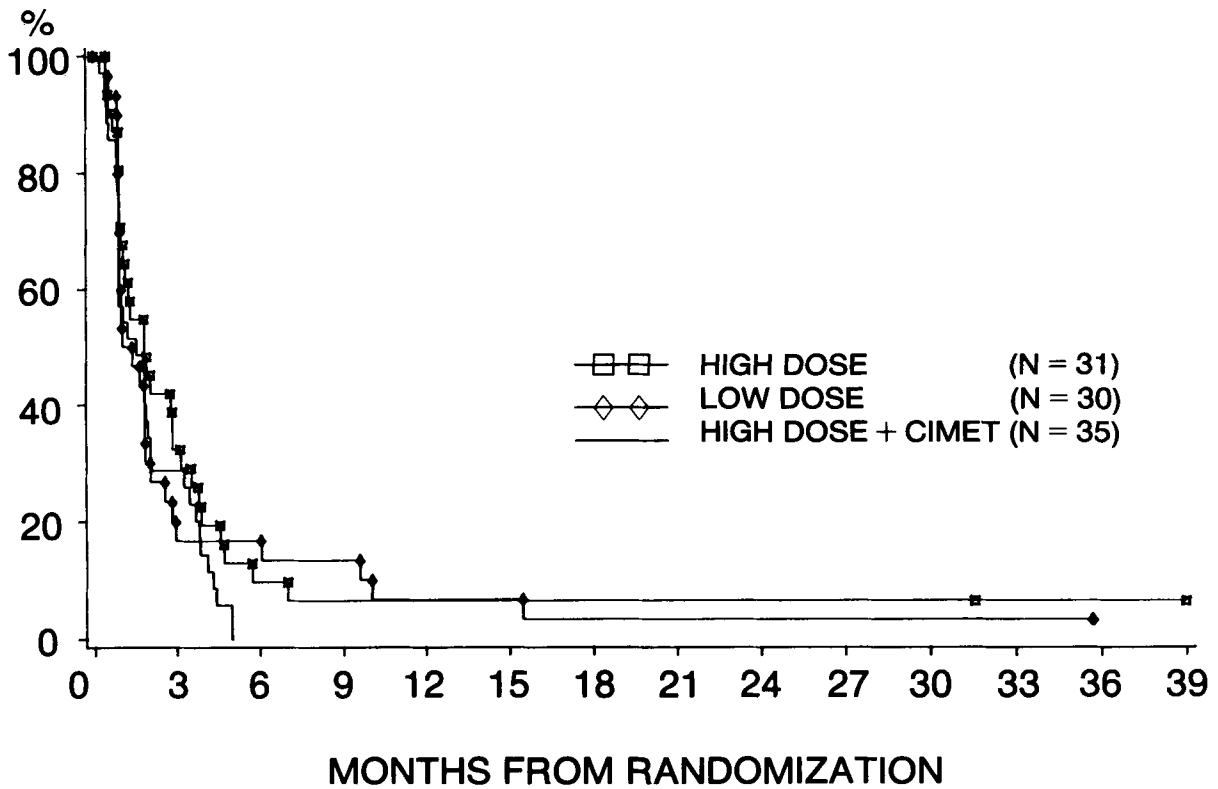

FIG. 1. Interferon alfa-2a in malignant melanoma, percentage of patients without progression. Time to progression for all study participants after start of interferon alfa-2a. Cimet: high dose plus cimetidine.

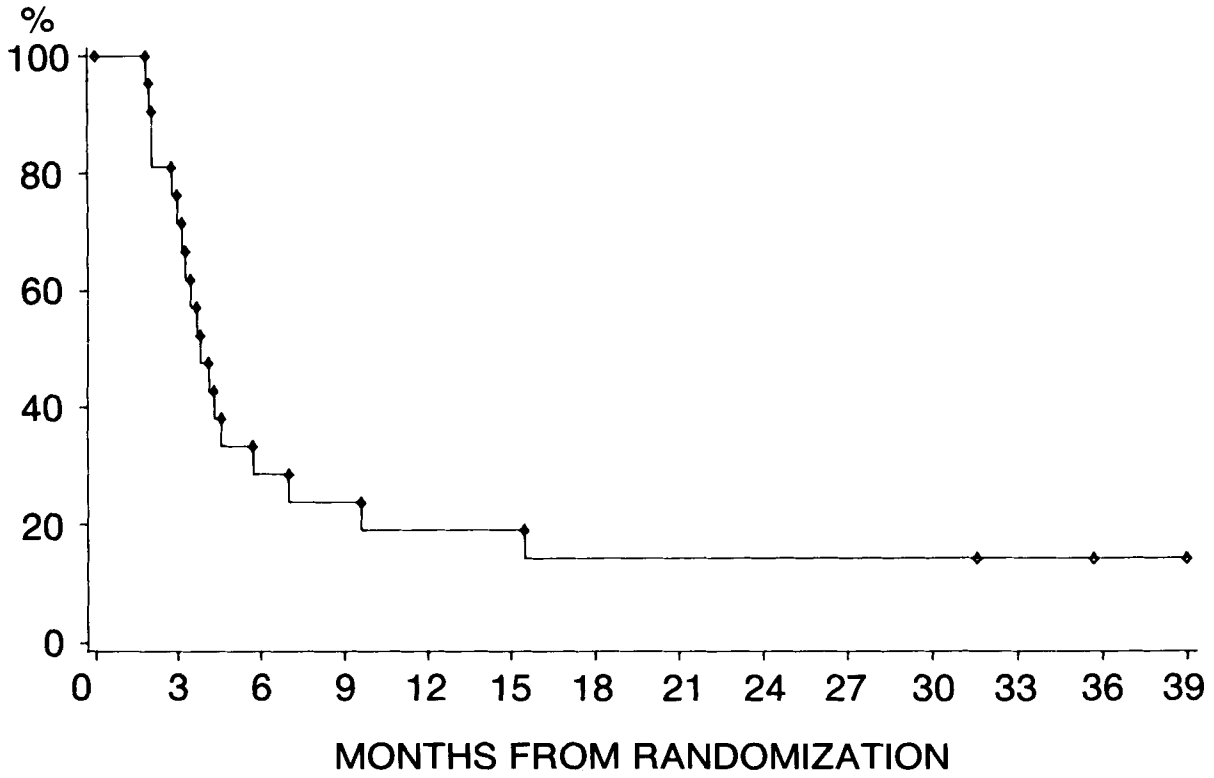

FIG. 2. Interferon alfa-2a in malignant melanoma. Time to progression for the 21 responding patients.

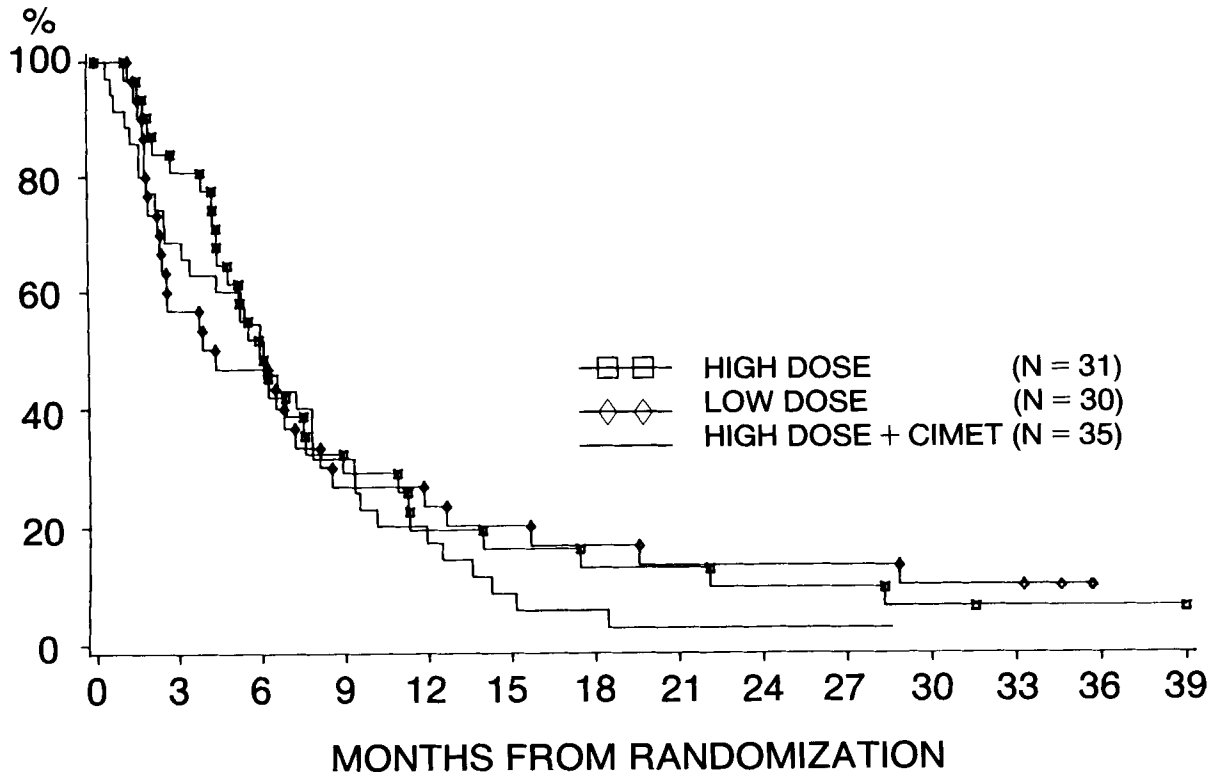

FIG. 3. Interferon alfa-2a in malignant melanoma. Survival of all patients after start of interferon alfa-2a.

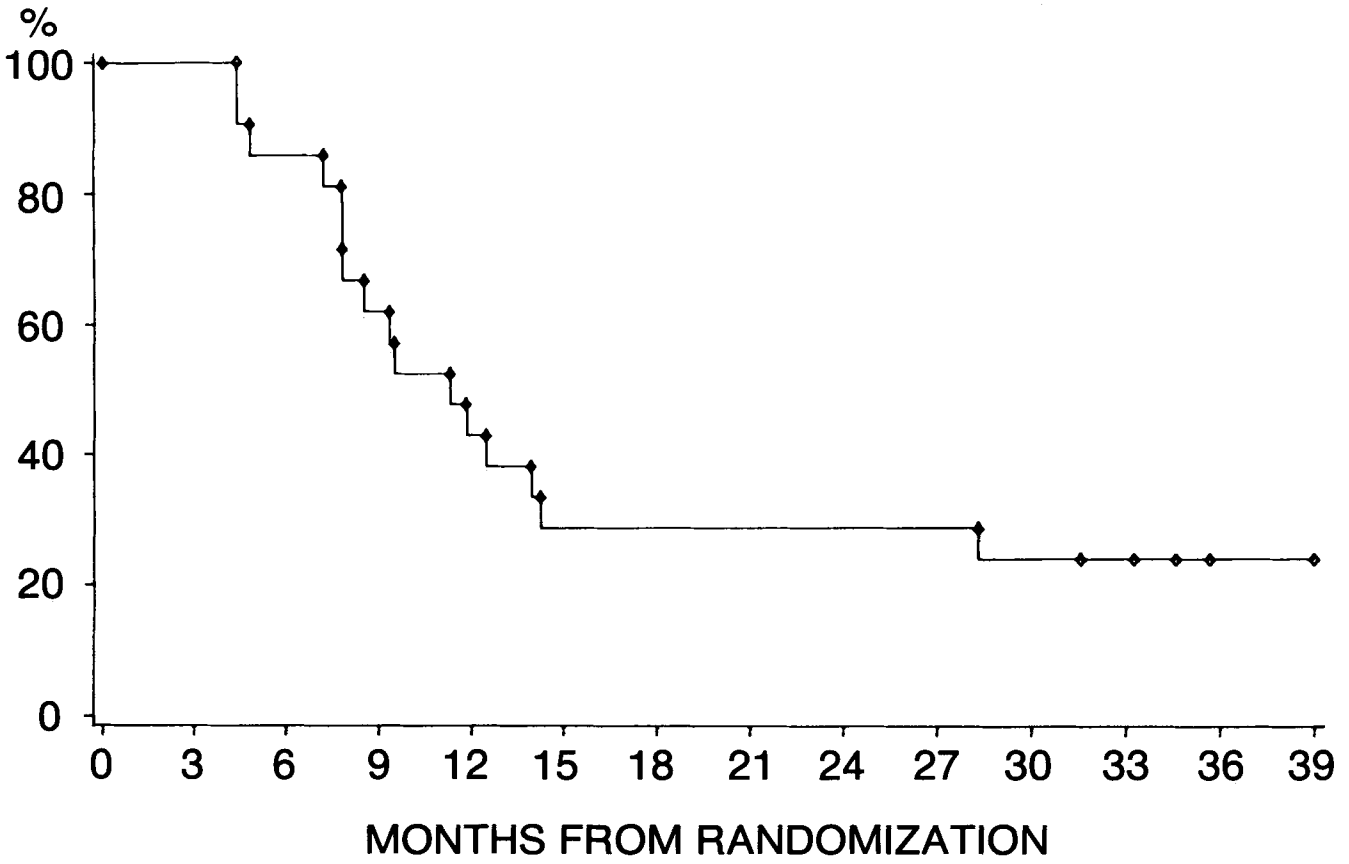

FIG. 4. Interferon alfa-2a in malignant melanoma. Survival of the 21 responding patients.

Discussion

Our experience with 96 patients with disseminated malignant melanoma receiving interferon alfa-2a is generally consistent with the observations of other investigators.[16-21] Interferon alfa-2a is clearly capable of inducing objective regressions in patients with disseminated malignant melanoma. However, most responses are limited to soft tissue sites, are relatively transient, and do not appear to have a meaningful impact on survival. The toxicities from a dose of 50×10^6 U/m^2 thrice weekly were not easily manageable, and most patients required dose reductions. However, the regimen of 12×10^6 U/m^2 was eminently tolerable, with transient short term complications and no long term sequelae as yet.[19,20] We emphasize and recognize that our three trials were not randomized assessments but sequential Phase II studies. Therefore, comparative analyses may be tenuous and should be interpreted cautiously. We could not document any definitive dose response relationship between objective regressions, and the specific interferon program used. On the contrary, studies utilizing recombinant interferon in renal adenocarcinoma[22] strongly suggest that the most durable and sustained regressions are achieved at a daily dose of approximately 10×10^6 U/m^2; a clinically manageable level in our experience.

From currently available interferon preparations used in tolerable dosages, it appears that these cytokines will have limited efficacy as single agents in the management of patients with advanced malignant melanoma. However, increasingly sophisticated experimental studies with human and murine cell lines have documented therapeutic synergism between cytotoxic agents and interferons. Murine, recombinant, human lymphoblastoid, and virally-induced rat interferons have been combined with a variety of antineoplastic agents including: doxorubicin, cyclophosphamide, methotrexate, nitrosoureas, cis-diamine-dichloroplatinum, and 5-fluorouracil, with well documented, synergistically enhanced antiproliferative characteristics.[23-28] We have recently completed a Phase I study which documents that carmustine (BCNU), can be safely administered with interferon alfa-2a with clinically acceptable hematologic and gastrointestinal toxicities.[29] Another interesting combination is interferon with α-difluoromethylornithine (DFMO), an inhibitor of ornithine carboxylase which thus depletes polyamines, rendering

cells more susceptible to cytotoxic interventions. Polyamine depletion in cell culture has unequivocally enhanced the antiproliferative activity of human interferon.[30] These findings have profound implications in the design of future clinical trials employing interferons.

In addition to concomitant regimens of cytotoxic agent and interferon, an area with direct clinical application is combination regimens of interferon with other biologic response modifiers. Experimental studies assessing combinations of diverse interferon species clearly show enhanced antiproliferative effects from gamma interferon plus interferon alfa-2a; enhanced antiproliferative effects from combinations of recombinant gamma interferon with either alpha or beta interferon; as well as synergistic antiproliferative response to the combination of gamma and beta interferons in human melanoma cell lines assessed in the tumor colony-forming assay.[31-34] Another cytotoxic protein undergoing intense investigation is tumor necrosis factor (TNF). This substance has been isolated in the sera from endotoxin treated rodents that have been previously sensitized with Bacillus Calmette-Guérin (BCG). TNF demonstrates discriminatory cytotoxic and cytocidal characteristics against several transformed cell lines, but does not affect normal cell cultures. A unique experimental study has indicated that combinations of gamma interferon plus TNF are therapeutically synergistic *in vitro* in soft agar clonogenic assays against human tumor cell lines. In one experimental model, single agent therapy reduced cell survival to 35% to 40% of control. Combination therapy reduced survival to only 4% of control.[35] More recent studies have similarly documented antiproliferative potentiation from combinations of TNF and interferon.[36,37] Such observations, while obviously in their preclinical evolution, may provide some meaningful directions for future studies using biologic response modifiers against malignant melanoma and other selected neoplasms.

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QUESTIONS AND ANSWERS

Question: Regarding the three patients who had longterm complete responses in your Phase II trials, were they in any way different immunologically from the other patients? For example, in terms of T-cell subsets, or natural killer cell function?

Answer: The answer is a distressing "no."

Question: There's a suggestion from your data that interferon has no dose response effect in malignant melanoma. Certainly there's no dose response relationship in hairy cell leukemia, but it has been maintained that one exists in Kaposi's sarcoma. Would you comment whether that is telling us something?

Answer: A short while ago, Dr. John Kirkwood published a very elegant paper in *Cancer Research* in which he clearly showed that there was a dose response relationship in renal adenocarcinoma, and that daily doses of 10×10^6 U/m^2 seemed to be the most consistently effective. Patients with renal carcinoma who received doses of 1×10^6 U/m^2 apparently had far less predictable responses.[38]

Question: What are the results of your recent trial using interferon in combination with chemotherapy or adjuvant therapy for melanoma?

Answer: I am not aware of any persuasive data that would indicate that an immunomodulator with a cytotoxic agent would be more efficacious than either modality by itself. Now we will be performing a Phase II study with alpha plus gamma interferon, which I think has enormous conceptual appeal in the light of our understanding of interferon receptors. But, in answer to your question, I am not aware of data showing that interferon with chemotherapy for melanoma is better than either approach alone.