

# Eastern Cooperative Group Trial of Interferon Gamma in Metastatic Melanoma: An Innovative Study Design

Joan H. Schiller,<sup>1</sup> Marian Pugh,  
John M. Kirkwood, Daniel Karp,  
Marilyn Larson, and Ernest Borden

University of Wisconsin Comprehensive Cancer Center, Madison, Wisconsin 53792 [J. H. S., M. L.]; Emmes Corporation, Potomac, Maryland 22102 [M. P.]; University of Pittsburgh, Pittsburgh, Pennsylvania 15213 [J. M. K.]; Tufts University, New England Medical Center, Boston, Massachusetts 02111 [D. K.]; and University of Maryland, Baltimore, Maryland 21201 [E. B.].

## ABSTRACT

**IFN- $\gamma$  is a potent immunomodulator, which has activity against melanoma *in vitro* and in murine models. However, preclinical data suggests that the optimal therapeutic and immunomodulatory dose may not be the maximally tolerated clinical dose. We conducted a Phase II/III trial in good prognosis patients with metastatic melanoma to determine whether a therapeutic and immunomodulatory dose-response curve of IFN- $\gamma$  could be identified, and whether the two could be correlated.**

**Ninety-eight patients with metastatic melanoma were randomized to one of seven dose levels of IFN- $\gamma$  ranging from 0.01 to 0.90 mg/m<sup>2</sup>. All patients were required to have s.c., skin, soft tissue, or nodal disease, although visceral disease was also allowed, and no more than one prior chemotherapy regimen. Patients received IFN- $\gamma$  as a 1-h i.v. infusion three times per week for at least 8 weeks or until progressive disease.**

**Ninety-five patients were eligible for toxicity evaluation; 81 were eligible for tumor response. Four patients responded to therapy (response rate, 5%) at three dose levels: two patients at 0.01 mg/m<sup>2</sup> and one each at 0.5 and 0.9 mg/m<sup>2</sup>. The duration of response ranged from 5 to 58 weeks. Toxicities were typical of IFNs and included flu-like constitutional symptoms. No dose-response relationship was identified for efficacy. A dose-response relationship for toxicity was observed only for fever and chills ( $p = 0.035$ ) and hepatic toxicity ( $p = 0.034$ ).**

**IFN- $\gamma$  has minimal activity in metastatic melanoma, and a therapeutic dose-response curve could not be identified. Although potent dose-dependent effects on immunomodulation were identified (J. M. Kirkwood, J. Bryant, J. H. Schiller, M. M. Oken, E. C. Borden, and T. L. Whiteside. Immunomodulatory function of interferon gamma in patients with metastatic melanoma: results of a phase IIB trial in sub-**

**jects with metastatic melanoma: ECOG Study E4987, submitted for publication), this biological activity does not translate into therapeutic activity in the metastatic disease setting in this trial.**

## INTRODUCTION

(IFNs) are a family of proteins with antiviral, immunomodulatory, and antiproliferative effects. IFN- $\alpha$ , the first of these proteins to be identified, has been shown to have antitumor activity against a number of human neoplasms, including hairy cell leukemia, Kaposi's sarcoma, chronic myelocytic leukemia, indolent lymphomas, renal cell carcinoma, melanoma, and multiple myeloma (1, 2). Despite this activity, however, the majority of responses are partial, and the overall impact of these molecules on the treatment of human cancers is small.

One possible explanation for these disappointing results is that the therapeutic doses and schedules for IFN have not been optimized for inducing immunomodulation. Although *in vitro* antiproliferative studies indicate that the growth inhibitory properties of IFN- $\alpha$  are dose dependent, other preclinical studies suggest that high doses of biologicals may not be optimal for immune stimulation (3, 4).

IFN- $\gamma$  is a cytokine, produced by T lymphocytes, which has broad potent immunoregulatory effects that distinguish it from IFN- $\alpha$  and - $\beta$ . In addition to a unique spectrum of antiproliferative properties, IFN- $\gamma$  is a potent monocyte activator. It has been reported to augment monocyte tumoricidal activity (4), hydrogen peroxide generation (5), monocyte antibody-dependent cellular cytotoxicity, Fc receptor expression, and both class I and class II MHC (6). However, studies suggest that these effects may not be greatest at the maximally tolerated clinical doses. For example, peak effects on natural killer cell activation, T-cell subset distribution, and enzyme oligo-2'5'-adenylate synthetase were found to occur at doses from 300 to 1000  $\mu\text{g}/\text{m}^2/\text{day}$ , intermediate between the lowest and highest dosages tested and well below the maximally tolerated dose identified (7). In another Phase I trial, daily i.m. administrations of 0.25-0.5 mg/m<sup>2</sup>/day produced significant activation of tumoricidal activity in peripheral monocytes, whereas the daily administration of 1.0 mg/m<sup>2</sup> did not (4). A third Phase I trial evaluated biological effects in patients receiving daily IFN- $\gamma$  by i.m. injection, at doses ranging from 0.0001 to 0.25 mg/m<sup>2</sup> and concluded that the optimal dose for inducing immune modulation was 0.1 mg/m<sup>2</sup> (8).

Preclinical studies with recombinant murine IFN- $\gamma$  also suggest that the therapeutic activity depends on the dose, schedule, and route of administration (9). Optimal antitumor activity in spontaneous and experimental B16-BL6 metastases was demonstrated when IFN- $\gamma$  was administered i.v. three times per week at 50,000 units/animal but not at 10,000 or 100,000 units/animal, suggesting a bell-shaped dose-response curve. No therapeutic activity was detected with twice weekly i.v. or daily i.m. administration compared with i.v. administration three times per week.

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<sup>1</sup> Partially supported by the Veterans Administration. To whom requests for reprints should be addressed, at University of Wisconsin Comprehensive Cancer Center, 600 Highland Avenue, K4/666, Madison, WI 53792. Phone: (608) 263-5343; Fax: (608) 263-8613.

Table 1 Dose levels of IFN- $\gamma$ 

Dose level	IFN- $\gamma$ dose (mg/m <sup>2</sup> )	No. of patients	No. evaluable
1	0.01mg/m <sup>2</sup>	16	14
2	0.03mg/m <sup>2</sup>	15	13
3	0.10mg/m <sup>2</sup>	14	11
4	0.30mg/m <sup>2</sup>	14	11
5	0.50mg/m <sup>2</sup>	12	8
6	0.70mg/m <sup>2</sup>	11	8
7	0.90mg/m <sup>2</sup>	13	12

Based on these preclinical and clinical results, the Eastern Cooperative Oncology Group conducted a Phase II/III dose-seeking trial of IFN- $\gamma$  in patients with metastatic melanoma with nonbulky s.c., skin, or nodal disease. Using an innovative trial design, the objectives of this trial were, primarily, to define a therapeutic dose-response curve to IFN- $\gamma$ , secondarily, to delineate the immunomodulatory dose response curve further, and, finally, to correlate the two. We report here the clinical results of this trial.

## MATERIALS AND METHODS

All patients were required to have measurable metastatic malignant melanoma with s.c., soft tissue, or nodal disease. Patients had to have adequate bone marrow (WBC,  $\geq 4,000/\text{mm}^3$ ; granulocytes,  $\geq 2,000/\text{mm}^3$ ; and platelets,  $\geq 100,000/\text{mm}^3$ ), renal (creatinine,  $\leq 2.0$  mg/dl; protein,  $\leq 2+$ ), and hepatic function (bilirubin,  $< 1.5$  mg/dl; aspartate aminotransferase  $\leq 3.0$  times the upper limit of normal) and an European Cooperative Oncology Group performance status of 0 or 1 with an estimated life expectancy of at least 3 months. Patients may have received one prior cytotoxic chemotherapy regimen but no prior immunotherapy. Patients with visceral disease only, central nervous system metastases, history of seizures, cardiac disease, active infection, or second primary cancer or requiring therapy with steroidal or nonsteroidal anti-inflammatory drugs, antihistamines, barbiturates, palliative chemotherapy, radiotherapy, hormone therapy, immunotherapy, or any drugs required for the treatment of active cardiac disease were excluded from the study. All patients gave informed consent before enrolling in the study. For the first year of accrual, eligibility was limited to patients with "nonbulky" disease. Nonbulky disease was defined as no single tumor mass  $> 3$  cm in largest diameter and the liver  $< 30\%$  involved with tumor on computed tomography. Twenty-nine patients were entered with the nonbulky disease requirement. This entry criterion was dropped when it proved to be too restrictive for accrual purposes.

Patients were randomized to one of seven dose levels of IFN- $\gamma$  and were stratified by predominant site of disease (s.c., soft tissue, skin, or nodal disease *versus* other) and prior chemotherapy (none *versus* one regimen). The dose levels of IFN- $\gamma$  are detailed in Table 1. Doses ranged over a two-log range, from 0.01 to 1.0 mg/m<sup>2</sup>, the MTD<sup>2</sup> of IFN- $\gamma$  as a 1-h i.v. infusion (10).

<sup>2</sup> The abbreviations used are: MTD, maximum tolerated dose; RMSE, root mean squared error; BRR, best response ratio; HLA, human leukocyte antigen; NK, natural killer.

IFN- $\gamma$  was provided by Genentech (South San Francisco, CA). The drug was administered by 1-h i.v. infusion in 100 ml 5% dextrose in water containing 200 mg human serum albumin three times a week, every week, with each treatment separated by at least 47 h. The length of each cycle was 28 days.

Toxicity was assessed on days 1 and 2 for all patients during the first 4 weeks of treatment. Patients entered at the two highest dose levels had additional assessments on days 3, 4, and 8. In subsequent courses, all patients were assessed by their physician every 28 days. The hematology survey, differential, platelet, aspartate aminotransferase, and creatinine laboratory tests were performed once a week for 4 weeks, then on days 1 and 15. Chemistry surveys were obtained every 28 days.

Toxicities were graded according to the Common Toxicity Criteria. The acute flu-like syndrome, consisting of anorexia, fatigue or malaise, chills or rigors, myalgias, arthralgias, and other flu-like symptoms, which often occurs after the first several IFN treatments, was graded according to Table 2.

Treatment was interrupted for all grade 3 toxicities until the parameter returned to grade 2 toxicity or less, and then treatment was resumed with the dose of IFN- $\gamma$  reduced by one dose level (or 50% for patients at dose level one). If the grade 3 toxicity recurred at the reduced dose, the patient was removed from the study. Patients were removed from study for grade 4 toxicity.

Patients were treated for a minimum of 8 weeks unless serious toxicity or complications occurred; otherwise, treatment continued until progression of their disease. Patients who progressed during weeks 1–4 were continued on treatment weeks 5–8 unless there was documentation of continued deterioration. Patients were evaluated for response following 8 weeks of therapy and every 4 weeks thereafter. A complete response to therapy was defined as a complete disappearance of all clinically detectable malignant disease for at least 4 weeks. A 50% or greater decrease in tumor size for at least 4 weeks, without an increase in size of any known malignant disease or appearance of new lesions, constituted a partial response. Stable disease was defined as no significant change in measurable disease for at least 4 weeks, and progressive disease was defined as the appearance of new lesions or a 25% or greater increase in the area of tumor size.

**Statistical Design.** The primary purpose of the study was to determine the dose of IFN- $\gamma$  that produces the greatest objective response rate. The essential features of this study design involve randomization into a broad range of dose levels, followed by nonlinear statistical modeling to establish the optimal biological and therapeutic dose, with a two-stage stopping rule to permit early termination of a nonresponsive agent. In the first stage of accrual, 84 patients would be allocated among each of seven dose groups ranging from 0.01 mg/m<sup>2</sup> to the MTD, using permuted blocks of size 7. The dose-response curve would be estimated from the data using methods described later in this section. If the estimated dose-response curve was below 20% for all doses up to the MTD, the study would be terminated. Otherwise, the study would proceed to accrue an additional 84 patients, who would be allocated randomly among four doses equally spaced in the range of doses with at least a 20% estimated response rate. The data from both stages of the study would be used to estimate the final dose-response curve and to determine the dose producing the peak response.

Table 2 Signs and symptoms associated with acute, flu-like syndrome associated with IFN

Grade	Mild 1	Moderate 2	Severe 3	Life-Threatening 4
Lack of appetite after treatment	Miss one meal	Miss two meals	Continue to miss two or more meals on the day of therapy despite repeated IFN treatments (2 wk)	
Acute fatigue and malaise after treatment	In bed for <6 h after treatment	In bed for >6 h after treatment	In bed for >6 h on the day of therapy despite repeated IFN treatments (2 wk)	
Chills	Rigors that do not require parental medications for relief	Rigors requiring parental medications for relief	Rigors unrelieved by parental medications despite repeated IFN treatments (2 wk)	
Myalgias/arthralgias	Subjectively mild	Subjectively moderate	Subjectively severe or with significant disability and unrelieved with medications	

In the first stage of the study, it was assumed that little or nothing was known about the dose-response relationship. To have the best chance of finding a range in which the therapy was effective, the decision was made to use equally spaced doses between zero and the MTD. The best metric for the dose level was unknown but was assumed to be either log or linear. The choice of dose levels, 0.01, 0.03, 0.10, 0.30, 0.50, 0.70, and 0.90 mg/m<sup>2</sup>, is a compromise between equal spacing on the log dose scale, 0.01, 0.03, 0.10, 0.33, and 1.0 mg/m<sup>2</sup>, and equal spacing on the linear dose scale, 0.10, 0.30, 0.50, 0.70, and 0.90 mg/m<sup>2</sup>. The use of seven compared with five dose levels provided a hedge against improper scaling of the dose axis. If the first stage of the study was successful in detecting a therapeutic range, this range would determine the dose levels for the second stage of the study. Specifically, let *A* be the minimum dose having at least a 20% response rate and *B* be the maximum dose, and let  $C = (B - A)/3$ . The four dose levels in the second stage would be spaced equally across the therapeutic range at *A*, *A* + *C*, *A* + 2*C*, and *B*.

At the completion of follow-up at each stage of the study, the dose-response curve would be estimated from the data. Usually in a dose-response or dose-ranging study, the dose-response function is often assumed to be nondecreasing over the therapeutic range and is modeled using a logistic model, which relates the logit or log odds of the response probability to a linear function of the dose, *i.e.*,  $\text{logit}[p(d)] = \alpha + \beta d$ . However, previous studies have suggested that the effect of IFN- $\gamma$  may peak at a dose below the MTD. It was proposed that the dose-response curve be estimated using a four-parameter logistic model:  $p(d) = p_1(d) p_2(d)$ , where  $\text{logit}[p_i(d)] = \alpha_i + \beta_i d$  (11). Here,  $\alpha_i$  and  $\beta_i$  are parameters, and *d* is the dose level of IFN- $\gamma$  expressed as a percentage of the MTD. If the data suggested that this model was inappropriate (*e.g.*, a bimodal response curve was observed), then other techniques, which do not assume a parametric model, would be used (12). The final estimate of the dose-response curve would incorporate response data from both stages of the study.

Simulation studies were used to assess the properties of our statistical design for five different hypothetical underlying dose-

response curves, each representing a potential true situation against which we would want our design to perform well (Fig. 1). These dose-response curves are described by the four-parameter logistic model, with parameters selected to fix the response levels at doses 5, 25, 50, and 75% of the MTD to preselected values. The first curve is unimodal, with probability of response of 0.05, 0.15, 0.35, and 0.15 at these dose levels. The second curve is flat, with constant response probability of 0.15. The third dose-response curve is gently rising, with probability of response of 0.05, 0.15, 0.30, and 0.35 at dose levels 5, 25, 50, and 75%, respectively, of the MTD. The fourth dose-response curve rises more steeply and then reaches a plateau, with response probabilities of 0.05, 0.30, 0.35, and 0.35 at these dose levels. The fifth curve reaches a peak response probability of 0.35 at 5% of the MTD quickly and then falls to zero at dose levels 25, 50, and 75% of the MTD.

Monte Carlo simulations were used to assess the performance of the design described above and to compare it with that of other candidate designs. In the simulation, it was assumed that roughly 10% of the patients would be discovered to be ineligible due to pathology review after enrollment, leaving 75 eligible for evaluation of responses after the first stage and 150 after the second stage. Each replicate of the simulation corresponds to conducting the clinical trial once from start to finish, using random-number generators to allocate patients to dose levels and to create response data with a probability of response given by one of the four parameter logistic models described above. In all, the conduct of the trial was replicated 100 times to ascertain the probability of events such as early stopping for each of the proposed designs.

The results of the simulation for each of the proposed designs were summarized by averaging the mean squared prediction error, the best response ratio, and the probability of early stopping across the (*n* = 100) replicate trials according to the formula given below. The mean squared prediction error for the *i*th replicate was obtained as follows: the squared differences in response rate between the true dose-response curve, *p*(*d*), and estimated dose-response curves, *p<sub>i</sub>*(*d*), were averaged over 100 dose levels, *d*, ranging from 1 to 100% of the MTD. The best

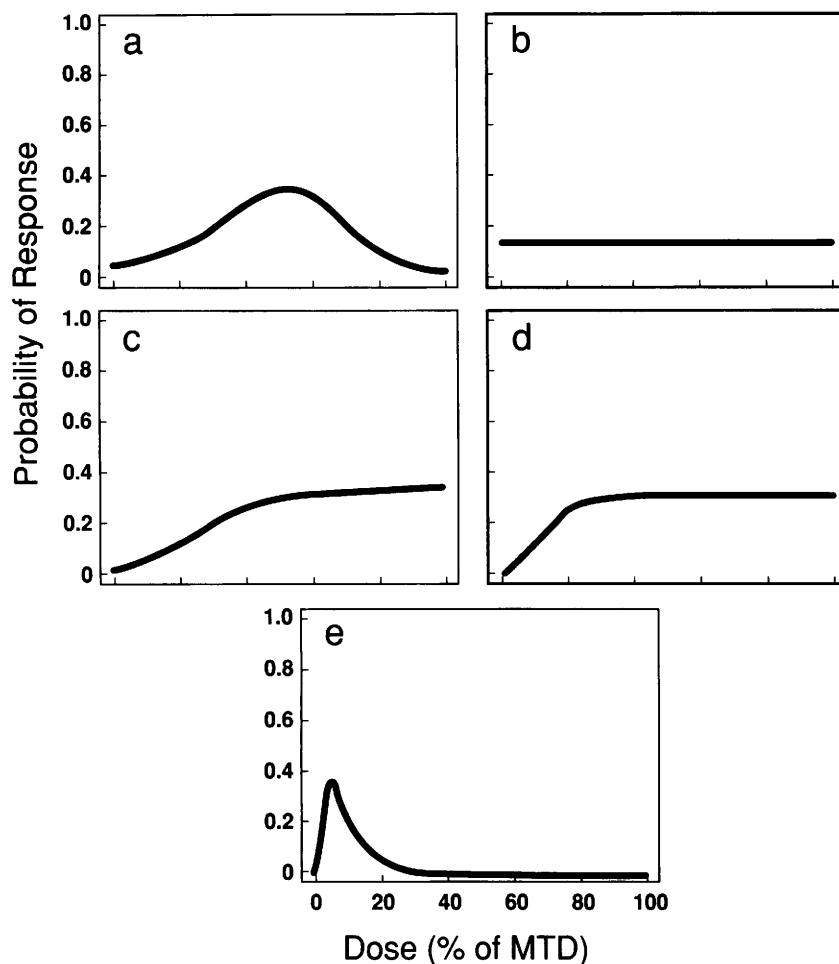


Fig. 1 Five dose-response (a-e) against which the trial design was simulated.

response ratio was computed by taking the ratio of the true response probability,  $p(d)$ , evaluated at two different optimal doses, that obtained from the final dose-response curve fitted to the simulated data,  $\hat{d}_i$ , and that obtained from the true dose-response curve,  $d$ .

$$MSE = N^{-1} \sum_{i=1}^N \sum_{d=1}^{100} [p(d) - \hat{p}_i(d)]^2 / 100$$

$$BRR = N^{-1} \sum_{i=1}^N p(\hat{d}_i) / p(d^*).$$

Table 3 Design with dose levels at 0.01, 0.03, 0.1, 0.3, 0.5, 0.7, and 0.9 mg/m<sup>2</sup>

Dose-response curve	RMSE	PES <sup>a</sup>	BRR
a	0.076	0.04	0.87
b	0.056	0.40	1.00
c	0.076	0.02	0.91
d	0.081	0.00	0.95
e	0.043	0.04	0.87

<sup>a</sup> PES, probability of early stopping.

Table 3 shows the RMSE, BRR, and probability of early stopping for the design used in the study. This seven-dose design has a minimum BRR of 87% against dose curves *a* and *e*. Designs with only five dose levels had lower minimum BRRs. A design with linearly spaced doses at 20, 40, 60, 80, and 100% of the MTD has a BRR of 64% against curve *e*, and a design with log-spaced doses at 1, 3, 10, 33, and 100% of the MTD has a BRR of 74% against response curve *a*. Twelve different trial designs with between 5 and 7 doses spaced on either pure log, pure linear, or mixed log/linear scales were compared. The design selected for the study was reviewed and approved by the National Cancer Institute prior to the activation of the study.

## RESULTS

Ninety-eight patients were entered in the study. Three patients were canceled: one was ineligible; one refused treatment; and one died before initiation of treatment. The demographics of the remaining 95 patients are shown in Table 4. Fourteen patients were considered ineligible for tumor response (13 secondary to absence of s.c., nodal, or soft tissue metastases; one due to the presence of bulky disease) but were considered eligible for toxicity evaluation. Patient characteristics were similar across dose levels (Table 5).

Table 4 Demographics

Sex	
Male	56
Female	39
Age	
Median	61
Range	20–85
Performance status	
0	50
1	45
Prior therapy	
No prior therapy	73
Prior chemotherapy	12
Prior radiotherapy	5
Prior radiotherapy and chemotherapy	4
Unknown	1
Sites of metastatic disease	
Lymph node	20
Skin	12
s.c.	28
Gastrointestinal	5
Liver	24
Lung	35
Pleura	2
Bone	8
Other	18

**Response Data.** Four patients responded to therapy: one complete response and three partial responses, for an overall response rate of 5%. The responses occurred at three different dose levels: two patients at level A (0.01 mg/m<sup>2</sup>) and one each at dose levels E (0.5 mg/m<sup>2</sup>) and G (0.9 mg/m<sup>2</sup>). Three patients had nodal disease only, and one patient had a soft tissue mass. The duration of response ranged from 5 to 58 (mean, 36.5)

weeks. One of the partial responders had his remaining disease resected after 1 year and continues to be disease free.

Because the estimated dose-response curve was less than 20%, the study was terminated after the first stage of accrual. In the simulations characterizing the properties of the study design, the probability of early stopping was 40% for the second dose-response curve, with a constant response probability of 15%, and negligible for the other four hypothetical dose-response curves. The observed data are consistent with a flat dose-response curve.

One patient with nodal disease who had only a partial response about 6 months after initiating therapy with 0.01 mg/m<sup>2</sup> became a complete responder about 3 months later. This patient developed progressive disease approximately 5 months after that. Median survival for the entire group was 43 months.

**Toxicity.** The most common toxicity observed was constitutional, including myalgias, arthralgias, headaches, anorexia, fever, chills, and weakness (Fig. 2). Low-grade leukopenia was observed at all but the highest dose level. However, neutropenia was rare (Fig. 2). No thrombocytopenia was observed. Grade 2 nephrotoxicity was observed at all dose levels but was not clinically significant except in one patient at the lowest dose level who developed a grade 4 toxicity (Fig. 2). Three episodes of moderate to severe hepatic toxicity were observed. Other rare grade 3 and 4 toxicities include anemia and proteinuria. One patient had grade 3 diarrhea at the 0.3-mg/m<sup>2</sup> dose level.

Even though a significant clinical response to IFN- $\gamma$  was not observed in this trial, it is possible that toxicity reflecting biological activity of this agent might be associated with dose level. Fig. 2 shows the proportion of patients assigned to each dose level of IFN- $\gamma$  who experienced constitutional symptoms

Table 5 Patient characteristics by dose level of IFN- $\gamma$ 

Characteristic	Dose (mg/m <sup>2</sup> )							All
	0.01	0.03	0.10	0.30	0.50	0.70	0.90	
Sex								
Male	11	9	6	7	7	8	8	56
Female	5	6	8	7	5	3	5	39
Age								
Median	64	61	67	50	56	61	62	61
Range	29–82	39–81	33–85	29–74	25–83	36–71	37–83	25–85
Performance status								
0	6	12	9	5	6	6	6	50
1	10	3	5	9	6	5	7	45
Prior therapy								
No prior therapy	14	11	11	10	10	7	10	73
Prior chemotherapy	2	1	2	2	1	2	2	12
Prior radiotherapy	0	1	1	1	0	1	1	5
Prior radiotherapy and chemotherapy	0	2	0	1	1	0	0	4
Unknown	0	0	0	0	0	1	0	1
Sites of metastatic disease								
Lymph node	3	6	3	1	4	4	0	21
Skin	2	3	2	1	0	2	2	12
s.c.	2	3	6	7	2	3	5	28
Gastrointestinal	1	1	0	1	1	0	1	5
Liver	3	5	3	5	1	3	4	24
Lung	3	10	4	7	5	4	2	35
Pleura	1	0	0	1	0	0	0	2
Bone	2	1	2	2	1	0	0	8
Other	5	2	2	3	3	2	1	18

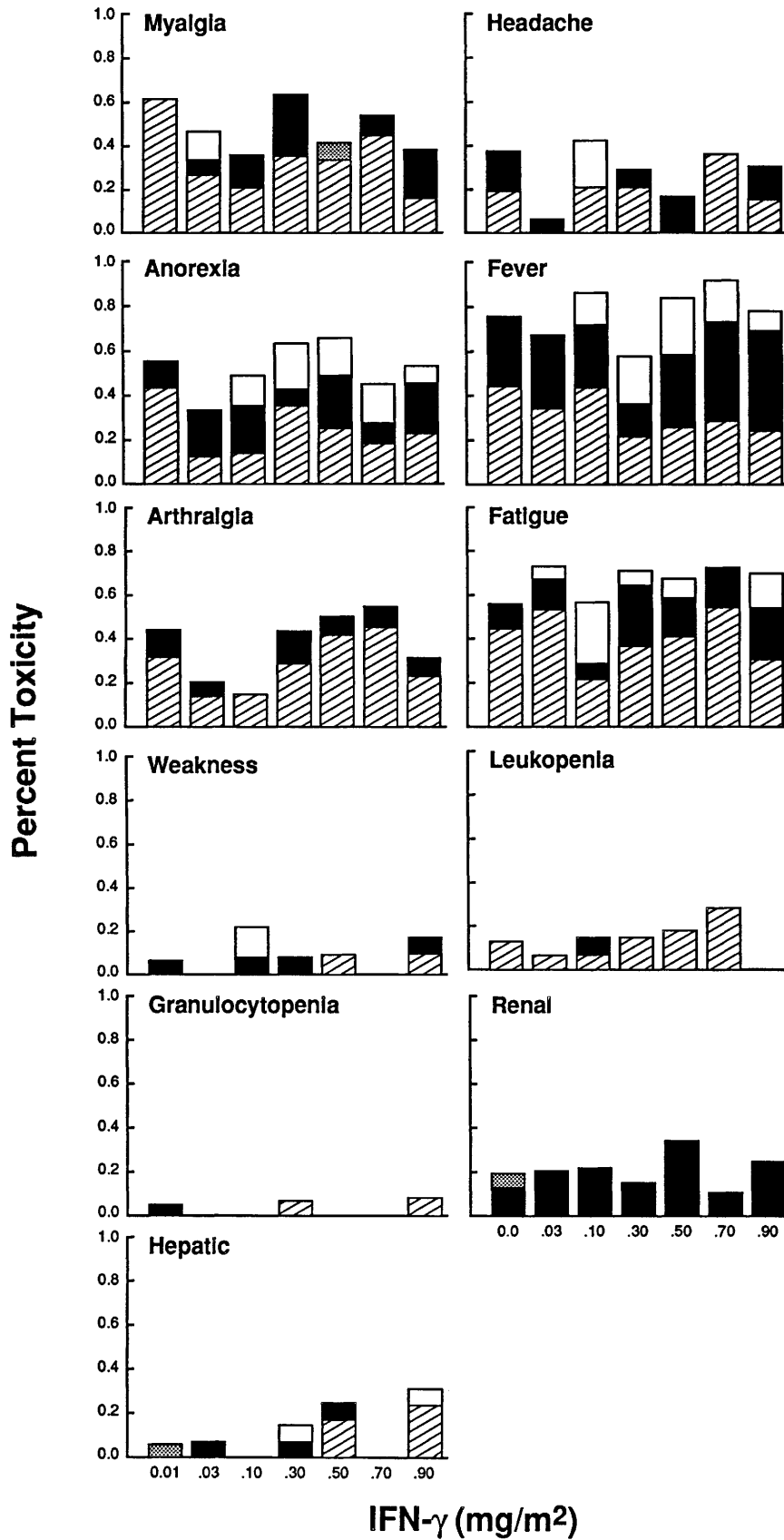


Fig. 2 Toxicities of IFN- $\gamma$  per dose level. ▨, grade 4; □, grade 3; ■, grade 2; ▨, grade 1.

or organ toxicity. There is an increase in fever and chills with dose (Jonckheere-Terpstra test statistic, 1.8; one-sided  $P = 0.04$ ) and an increase in hepatic toxicity with dose (Jonckheere-Terpstra test statistic, 1.8; one-sided  $P = 0.04$ ).

Three grade 3 or 4 cardiac episodes were observed. These included one myocardial infarction on day 1 and two episodes of arrhythmia requiring therapy. One patient developed a pulmonary embolism. The relationship of these events to IFN- $\gamma$  was unclear.

Neurotoxicity included grade 1 or 2 light-headedness, confusion, dizziness, anxiety, vertigo, insomnia, decrease in libido, and twitching in the hands and feet. Two patients developed severe depression.

## DISCUSSION

Despite the extensive study of these proteins since their production with recombinant DNA technology in the early 1980s, many crucial questions regarding the optimal use of IFNs remain to be answered. One of these questions centers around dose. *In vitro* antiproliferative studies indicate that the growth-inhibitory properties of IFN- $\alpha$  are dose dependent (3). These observations are supported by clinical trials suggesting a dose-response effect in Kaposi's sarcoma, lymphoma, and renal cell carcinoma (9, 13–15). In hairy cell leukemia, however, high response rates are observed with low doses of IFN (16), and in malignant melanoma, clinical trials suggest comparable results with both high- and low-dose IFN (17, 18). However, the latter were not randomized trials, and the dose-response relationship of IFN to melanoma has not been established.

A number of preclinical and clinical studies have suggested that the optimal immunostimulatory dose of IFN may not be the clinical MTD. In a Phase I study of s.c. IFN- $\gamma$  reported by Thompson *et al.* (19), monocyte activation, as detected by cell surface HLA-DR expression, was seen at the three lowest dose levels but decreased in patients treated at higher dose levels. No dose-response effect was observed for NK activity, although only a small number of patients were treated at each dose level. Another study, reported by Maluish *et al.* (8), demonstrated that  $H_2O_2$  production was enhanced longer following 0.1 mg/m<sup>2</sup> i.m. IFN- $\gamma$  compared with 0.25 mg/m<sup>2</sup>; no dose-response effects were observed for HLA-DR expression on monocytes, whereas one was observed for Fc receptor expression. Kirkwood *et al.* (7) found the induction of 2'5'A synthetase, NK activity, and increased helper and suppressor T-cell ratios occurred at dosages of 300 to 1000  $\mu$ g/m<sup>2</sup>, whereas mononuclear cell HLA-DR and DQ antigen expression showed no consistent dose-related effect. Kleinerman *et al.* (4) observed effects dependent on dose, schedule, and route of administration; activation of monocyte tumoricidal activity was dose dependent when patients were receiving IFN- $\gamma$  as a 6-h i.v. infusion, whereas daily i.m. injections produced significant activation at doses of 0.25 to 0.5 mg/m<sup>2</sup>/day, whereas at the dose of 1.0 mg/m<sup>2</sup>/day, monocytes could not be activated. In a Phase I trial reported by Kopp *et al.* (20), IFN- $\gamma$  doses of 0.1 or 0.25 mg/m<sup>2</sup> induced significantly greater immunomodulation of monocyte-associated immune parameters than 0.01 mg/m<sup>2</sup>.

IFN- $\gamma$  has been reported to have some activity in disseminated melanoma. A randomized Phase I/II study of IFN- $\gamma$  in

patients with metastatic melanoma, evaluating a range of dose levels and two different schedules, observed two responses in patients receiving 3 and 300  $\mu$ g/mg<sup>2</sup>/day (21). Another Phase II study reported an 11% response rate in patients receiving 0.25 mg/m<sup>2</sup> (22). It has been postulated that these relatively low response rates were due to use of doses and schedules that were not optimal for inducing immune stimulation (9).

Given the potent immunostimulatory activity of IFN- $\gamma$ , the preclinical data suggesting that the maximally tolerated dose may exceed the optimal immunomodulatory dose, and the lack of adequate studies to define a clinical dose-response relationship, we conducted a clinical trial the major aim of which was to determine whether the optimal therapeutic dose was dose related.

We elected to pursue administration of IFN- $\gamma$  by the venous route, based on data by Talmadge *et al.* (9), who demonstrated a bell-shaped therapeutic curve and optimal antitumor activity when IFN- $\gamma$  was administered i.v. three times per week. Other routes and schedules in preclinical models also have demonstrated optimal biological activity at doses below the maximally tolerated dose. Kleinerman *et al.* (4) observed potent activation of monocyte tumoricidal activity at doses below the maximally tolerated dose when IFN- $\gamma$  was administered i.m. daily. Maluish *et al.* (8) reported that three-times-per-week dosing by the s.c. route achieved comparable biological results as the i.m. route.

In our study, no bell-shaped therapeutic curve was identified, and minimal antitumor activity was observed, despite the fact that entry criteria for this study were chosen deliberately to optimize chances of identification of therapeutic activity. Initially, patients were required to have nonbulky disease; this eligibility criteria proved too limiting for accrual purposes and was dropped subsequently. Patients were required, however, to have dominant soft tissue, s.c., or nodal disease in an effort to limit poorer prognosis patients with visceral dominant disease.

These findings are consistent with observations made in a smaller trial of IFN- $\gamma$  in metastatic melanoma (20). In that trial, no therapeutic bell-shaped curve was observed over three dose levels (0.01, 0.1, and 0.25 mg/m<sup>2</sup>), although only five patients per dose level were studied. A similar dose-response study of IFN- $\gamma$  in patients with advanced non-small cell lung cancer also failed to confirm a bell-shaped therapeutic curve (23).

Despite the fact that no therapeutic bell-shaped curve was identified, dose-dependent effects on immunomodulatory stimulation were observed and are discussed in more detail elsewhere.<sup>3</sup> IFN- $\gamma$  induced significant rises in the CD4:CD8 ratio early in treatment, which were sustained in only the lowest dosage tier; suppression of helper T cells was observed with repeated treatments of higher doses of IFN- $\gamma$  (0.01 mg). NK cell activation was dose dependent and durable over 29 days (23, 24). Therefore, we conclude that, although IFN- $\gamma$  is a potent immunomodulator in terms of a number of immune parameters studied in this trial, this biological activity on NK, monocyte,

<sup>3</sup> J. M. Kirkwood, J. Bryant, J. H. Schiller, M. M. Oken, E. C. Borden, and T. L. Whiteside, submitted for publication.

and nontumor-restricted, T-cell parameters does not correlate with therapeutic activity in metastatic melanoma.

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