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# A phase I trial of recombinant gamma interferon in patients with cancer*

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**Summary.** A total of 11 patients were treated on an escalating, single dose trial of recombinant gamma interferon (rIFN-γ), 6 patients by the i.m. and 5 patients by the i.v. route of administration. Dose ranges within each individual were from 0.05 mg/m$^2$ of IFN (1 mg $\geqslant 10 \times 10^6$ units of IFN) escalating to 10 mg/m$^2$. All dosages were delivered twice weekly and the i.v. dose was infused over 5 min. The most common toxicities encountered included fever, chils, fatigue, anorexia, and granulocytopenia. The influenza-like symptoms were very similar to those encountered with IFN-α but were generally less severe. The granulocytopenia was dose-related and transient with recovery generally seen within 48–72 h following administration of rIFN-γ. Absolute granulocyte counts only rarely dropped below 1000 mm$^3$. Hepatotoxicity was not observed. IFN levels were determined by both a bioassay and an enzyme-linked immunosorbent assay. By the i.v. route, the peak level of IFN activity could usually be seen at completion of the infusion with a serum half-life of 30 min. By the i.m. route, the peak level of serum activity was generally detected between 4–8 h with a serum half-life of 4.5 h after the initial elimination phase. Peak IFN levels appeared to correlate with maximum toxicity. One patient with melanoma had a 25% reduction in a cutaneous lesion, but there were no other minimal, partial, or complete responses.

## Introduction

Interferons (IFNs) clearly have a wide variety of antiproliferative and immunomodulatory effects, which have led to extensive clinical trials of various IFN preparations in the treatment of patients with advanced cancer. Most of the trials have been phase I and phase II trials with IFN-α. Trials with IFN-α have demonstrated substantial antitumor activity in favorable histology non-Hodgkin's lymphoma [9, 12, 17, 19, 26], cutaneous T-cell lymphoma [3], hairy cell leukemia [23], kaposi's sarcoma [11, 16], and chronic myelogenous leukemia [30]. Activity has been reported for myeloma [12, 20], breast cancer [15, 27], renal cell carcinoma [22], and melanoma [4], but considerably less activity than that described for the hematopoietic malignancies described above. Recently, recombinant IFN-β and natural and recombinant IFN-γ (rIFN-γ) have been brought into clinical trials.

IFN-γ has distinct antiproliferative and immunomodulatory properties from IFN-α and IFN-β, which might result in a different array of clinical effects. In early studies, IFN-γ was shown to have more potent antiproliferative effects than the other IFN preparations [25]. However, many of these studies were performed with only partially purified IFN-γ preparations that may have contained other biologically active molecules. Recent evidence suggests, however, that IFN-γ has antiproliferative activity and in certain instances, the spectrum of activity differs from IFN-α and IFN-β depending on the tumor cell type being tested. IFN-γ has been shown to synergize with IFN-α and IFN-β in in vitro antiproliferative assays using various human tumor cell lines [5, 8]. IFN-γ interacts with a different cell surface receptor than IFN-α and IFN-β which explains the rationale for possible synergy between IFN-γ and either IFN-α or IFN-β [2]. IFN-γ has also been shown to have potent macrophage activating effects, including induction of tumoricidal and microbicidal activity [15]. IFN-γ is thought to be the same molecule as macrophage activating factor by some investigators [29]. Augmentation of antibody-dependent cellular cytotoxicity [14], stimulation of peroxide generation [21], and enhancement of expression of Fc receptors, HLA-DR antigens, and class I histocompatibility antigens on the cell surface have been described [1]. IFN-α and IFN-β are not as effective as IFN-γ in enhancing class I and class II histocompatibility antigens [6, 7, 18]. These activities might be expected to make IFN-γ a potent biological response modifier in cancer patients and thus warrant the clinical evaluation of IFN-γ in the treatment of patients with cancer.

The gene for IFN-γ has been successfully cloned and expressed in *Escherichia coli* [10]. The nucleotide and amino acid sequences of rIFN-γ were determined to be ident-

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ical to native IFN-γ, except for the presence of a methionine residue at the N-terminal [24]. In this paper, we report the results of a phase I trial with a rIFN-γ in patients with disseminated cancer. Patients received escalating single doses on a twice weekly schedule by i.m. or i.v. routes of administration and were monitored for evidence of clinical effects, levels of circulating IFN, and immunomodulatory effects. We report here the clinical and pharmacokinetic results.

## Materials and methods

*rIFN-γ preparation.* rIFN-γ was prepared using recombinant DNA technology (Genentech, Inc., South San Francisco, Calif.). The final product was >98% pure as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Sterility, purity, pyrogenicity, and general safety met the standards of the Office of Biologics. Tests for endotoxin in the limulus assay routinely revealed <0.5 ng/mg protein. Acute and subacute toxicology studies were carried out in two species (the rat and the squirrel monkey), and no significant toxicity was observed. The specific activity of rIFN-γ was $\geqslant 1 \times 10^7$ units/mg protein based on an antiviral bioassay with encephalomyocarditis virus and human A549 cells (standardized with NIH IFN-γ standard Gg 23-901-530).

*Patient selection.* A total of 11 patients with disseminated malignancy refractory to standard therapy were entered onto this trial (Table 1). All patients were required to have an ambulatory performance (Karnofsky) score of 60%, no intervening anticancer therapy for a minimum of 4 weeks, and adequate hematologic (white blood cell count >4000/mm$^3$, platelet count <100.000 mm$^3$), hepatic (bilirubin $\leqslant$1.5 mg/dl), and renal (creatinine <2 mg/dl) function. Informed consent was obtained from all patients prior to the initiation of therapy.

*Study design.* Patients received escalating single doses of rIFN-γ by either i.m. or i.v. administration on a twice weekly schedule for seven or eight doses, respectively. One injection was given at each of the following dose levels: 0.05, 0.1., 0.25, 0.5, 1, 2.5, 5, and 10 mg/m$^2$ (10 mg/m$^2$ was given i.v. only). All i.v. doses of rIFN-γ were administered by infusion over a period of 5 min. Dosage escalation continued in the absence of unacceptable systemic toxicity or tumor progression. The 10 mg/m$^2$ dose was not given i.m. because an excessive injection volume of the available formulation would have been required.

The half-life for the i.v. route was calculated using nonlinear least square fit of a one compartment model, and for the i.m. route using a linear least square fit of the terminal portion of individual patient log concentration time curves.

*Patient monitoring.* Prior to the initiation of therapy, all patients underwent a history and physical examination, complete blood counts, serum chemistry profiles, chest x-ray, electrocardiogram, and radiologic studies as appropriate for documentation of the extent of their disease. Following each injection of rIFN-γ, patients were carefully monitored for toxicity by repeated physical examinations, complete blood counts, and serum chemistry profiles. Reassessment of the extent of the disease to determine antitumor activity was performed as clinically indicated, during the course of the study or at its conclusion. All patients were monitored for levels of serum IFN, using an antiviral biological assay with encephalomyocarditis virus and human A549 lung carcinoma cells and by an enzyme-linked immunosorbent assay (ELISA) using a polyclonal antiserum to rIFN-γ with limits of detectability to 0.01 ng/ml.

**Table 1.** Patients entered on rIFN-γ trial

| | Intramuscular | Intravenous |
|---|---|---|
| Number | 6 | 5 |
| Age (median) | 45–64 | 18–71 |
| Sex (M/F) | 5/1 | 2/3 |
| Diagnosis: | | |
| Melanoma | 2 | 2 |
| Adenocarcinoma lung | 1 | |
| Multiple myeloma | 2 | |
| Renal cell carcinoma | | 2 |
| Giant cell sarcoma | | 1 |
| Hairy cell leukemia | 1 | |

## Results

### *Clinical summary*

Of the patients who entered the study, 6 received i.m. and 5 received i.v. administrations. One patient with hairy cell leukemia was removed from the study after only one i.m. dose of rIFN-γ as it was determined that his granulocyte count was too low to be followed adequately for potential IFN-related toxicity. Another patient treated with i.m. rIFN-γ was removed after one dose due to abnormal liver function tests not related to the IFN therapy. Another patient with multiple myeloma was removed from the i.m. dose after three doses due to hypercalcemia. One patient with melanoma did not complete the i.v. therapy (three doses) due to abdominal pain that was subsequently determined to be secondary to an ovarian cyst.

The most notable toxicity was fatigue, which was clearly dose-related, with the degree of fatigue increasing with increasing doses of rIFN-γ, and it was more prolonged by the i.m. route of administration. The degree of fatigue appeared to be dose-limiting at 5 mg/m$^2$ by both routes of administrations with patients essentially bedridden for 12 h following this dose. Body temperature was $\geqslant$39° C in only one patient at the 5 mg/m$^2$ i.m. dose. Onset of fevers were demonstrated from 2–4 h (peak at 6–8 h) following the i.m. dose and usually occurred within 1–2 h (peak at 4–6 h) following the i.v. dose. Hypotension with a systolic blood pressure <90 mm Hg was seen in only one patient treated with i.v. rIFN-γ at 0.25 mg/m$^2$ and another at 5 mg/m$^2$. A depression of granulocytes was common, but only two patients experienced granulocytopenia with between 500 and 1000 granulocytes/mm$^3$ (1 at 0.25/m$^2$ i.v. and another at 5 mg/m$^2$ i.m.). There was no depression of any other blood components including lymphocytes, platelets, and eryhtrocytes. There were no changes in serum chemistry profiles (except for those mentioned above) or physical findings.

**Table 2.** Toxicity of rIFN-γ

| Route i. m. ($mg/m^2$) | No. patients | Temperature (°F) | | | Chills | Fatigue | Nausea | Vomiting | Hypotension (systolic < 90 mm Hg) | Granulocytopenia ($mm^3$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 99–100 | 101–102.9 | ⩾ 103 | | | | | | 500–1000 | < 500 |
| 0.05 | 6 | 3 | 2 | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 0 |
| 0.1 | 4 | 4 | 0 | 0 | 2 | 3 | 0 | 0 | 0 | 0 | 0 |
| 0.25 | 4 | 3 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 3 | 2 | 1 | 0 | 2 | 3 | 0 | 1 | 0 | 0 | 0 |
| 1.0 | 3 | 1 | 2 | 0 | 2 | 3 | 0 | 1 | 0 | 0 | 0 |
| 2.5 | 3 | 0 | 3 | 0 | 2 | 3 | 0 | 1 | 0 | 0 | 0 |
| 5.0 | 3 | 0 | 2 | 1 | 3 | 3 | 1 | 1 | 0 | 1 | 0 |
| i. V. | | | | | | | | | | | |
| 0.05 | 5 | 3 | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| 0.1 | 5 | 2 | 3 | 0 | 3 | 2 | 0 | 0 | 0 | 0 | 0 |
| 0.25 | 5 | 1 | 3 | 0 | 4 | 2 | 0 | 0 | 1 | 1 | 0 |
| 0.5 | 4 | 1 | 3 | 0 | 4 | 2 | 1 | 0 | 0 | 0 | 0 |
| 1.0 | 4 | 2 | 2 | 0 | 3 | 3 | 1 | 0 | 0 | 0 | 0 |
| 2.5 | 4 | 2 | 2 | 0 | 4 | 3 | 1 | 1 | 0 | 0 | 0 |
| 5.0 | 4 | 1 | 3 | 0 | 3 | 3 | 2 | 3 | 1 | 0 | 0 |
| 10.0 | 4 | 1 | 3 | 0 | 4 | 3 | 3 | 1 | 0 | 0 | 0 |

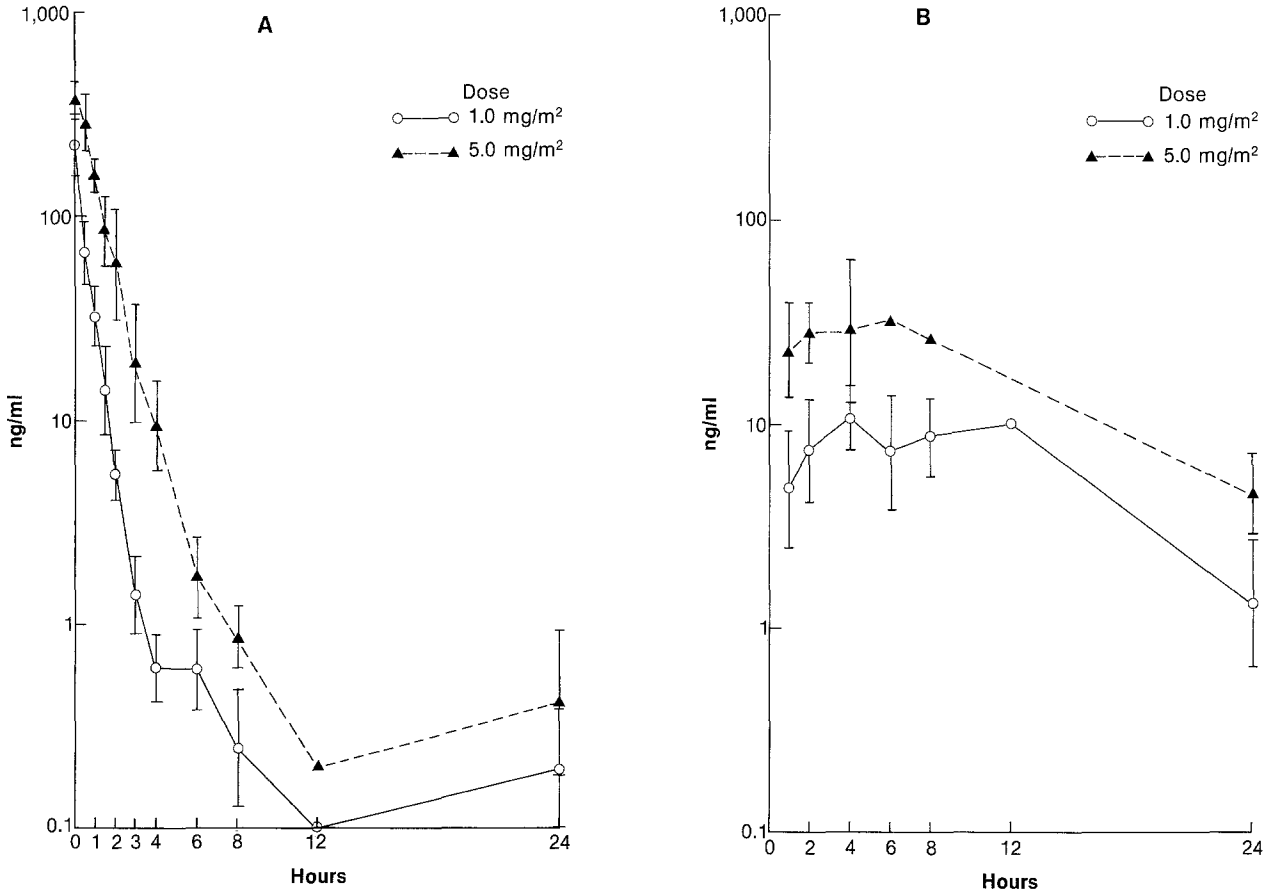


**Fig. 1.** Pharmacokinetics of serum rIFN-γ after **(A)** i.v. or **(B)** i.m. administration of rIFN-γ measured by an ELISA with standard deviations

*Antitumor activity*

No partial or complete responses were seen in any of the patients treated with rIFN-γ in this study. One patient with melanoma treated by the i.m. route had a minor regression of her only measurable disease which was a cutaneous lesion and was treated for 1 additional month with 0.25 mg/$m^2$ without further change. One patient with multiple myeloma demonstrated likely progressive disease growth following the third dose of rIFN-γ, as evidenced by increasing hypercalcemia, which required discontinuation of rIFN-γ therapy. One patient with renal cell carcinoma

completed the study but demonstrated minimal disease progression, and all of the other patients remained stable.

*Serum IFN levels*

Serum IFN activity was measured by both a biological antiviral assay and by an ELISA. Serum rIFN-γ levels were routinely found by both the i.v. and i.m. routes of administration (Fig. 1). Detectable levels of IFN by the ELISA in ng/ml were consistently found by both the i.m. and i.v. routes at the lowest dose of IFN (0.05 mg/m$^2$) administered. The patterns of the curves of the bioassay generally paralleled the results of the ELISA although the bioassay did not detect activity by the i.m. route at doses below 1 mg/m$^2$, whereas, detectable levels could be measured in the bioassay as well as ELISA at all doses by the i.v. route. These data clearly demonstrate a dose-dependent peak level of serum rIFN-γ by both the i.m. and i.v. routes. The serum half-life was approximately 30 min by the iv.v. route and 4.5 h for the elimination phase following the i.m. injection. Antibodies ro rIFN-γ (by both a neutralizing antibody detection assay and an immunoassay for antibody) were tested for in 8 of 11 patients on this trial, and none were detected.

## Discussion

In this paper, we report the results of a rIFN-γ study for patients with disseminated cancer. Our results indicate that rIFN-γ can be given by both the i.m. and i.v. routes with serum levels measured in an ELISA at all doses (0.05–10 mg/m$^2$) and in a biological assay at all doses by the i.v. route and at $\geqslant$1 mg/m$^2$ by the i.m. route. Previous studies with nonrecombinant IFN-γ at comparable doses to those used in the current study reported no detectable serum levels following the i.m. route of administration [13, 28]. In both of these other studies measurements were carried out by a bioassay rather than an ELISA, with the latter having a sensitivity of 0.01 ng/ml. Although we did detect serum IFN by the i.m. route using our bioassay, it was only at i.m. doses greater than those reported in these other studies. The serum half-life following the i.v. dose of rIFN-γ of 30 min was very similar to previous reports for i.v. bolus natural IFN-γ [13, 28]. Toxicities were very similar to those reported for natural IFN-γ as well as IFN-α. The precise maximum tolerated dose for single dose administration could not be accurately assessed due to the potential cumulative toxicity of rIFN-γ by the single dose escalation design of this study. Toxicities included fatigue, fever, chills, anorexia, occasional nausea and vomiting, headaches, mild hypotension, and granulocytopenia.

Pharmacokinetic data revealed a relatively rapid clearance for the i.v. administered rIFN-γ and a more prolonged serum level by the i.m. route, with activities still demonstrable at all dose levels 24 h after the injection. As might be expected, the toxicities of the i.m. route were later in onset and more prolonged.

Immunologic studies will be reported separately. An optimum biological response modifying dose was not determined in this study. Future trials with rIFN-γ should focus on determining an optimum biological response modifying dose. Possibly, the optimum biological response modifying dose is at a very low dose and we may have been unable to identify it because of our rapid dose escalation.

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