Phase I Trial of Twice-Weekly Intravenous Interleukin 12 in Patients with Metastatic Renal Cell Cancer or Malignant Melanoma: Ability to Maintain IFN-γ Induction Is Associated with Clinical Response[1]

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ABSTRACT

The aim of this study was to examine the tolerability, antitumor activity, and biological effects of a new schedule of i.v. recombinant human interleukin 12 (rhIL-12). Twenty-eight patients were enrolled in a Phase I trial in which rhIL-12 was administered twice weekly as an i.v. bolus for 6 weeks. Stable or responding patients were eligible to receive additional 6-week cycles until there was no evidence of disease or until tumor progression. Patient cohorts were treated with escalating doses of rhIL-12 (30–700 ng/kg). The maximum tolerated dose (MTD) was 500 ng/kg, with dose-limiting toxicities consisting of elevated hepatic transaminases and cytopenias. At the MTD ($n = 14$), there was one partial response occurring after 6 cycles of rhIL-12 in a patient with renal cell cancer. Two additional renal cell cancer patients treated at the MTD had prolonged disease stabilization, with one of these exhibiting tumor regression after 8 cycles of rhIL-12. IFN-γ, IL-15, and IL-18 were induced in patients treated with rhIL-12. Whereas IFN-γ and IL-15 induction were attenuated midway through the first cycle in patients with disease progression, those patients with tumor regression or prolonged disease stabilization were able to maintain IFN-γ, IL-15, and IL-18 induction. The down-modulation of IFN-γ induction during rhIL-12 treatment did not relate to IL-10 production or alterations in rhIL-12 bioavailability but was associated with an acquired defect in lymphocyte IFN-γ production in response to IL-12, IL-2, or IL-15. This defect could be partially overcome *in vitro* through combined stimulation with IL-12 plus IL-2. These findings show that the chronic administration of twice-weekly i.v. rhIL-12 is well-tolerated, stimulates the production of IL-12 costimulatory cytokines and IFN-γ, and can induce delayed tumor regression. Strategies aimed at maintaining IFN-γ induction, such as the addition of IL-2, may further augment the response rate to this schedule of rhIL-12.

INTRODUCTION

IL[3]-12 is a cytokine with considerable promise for the treatment of human malignancies because of its pleiotropic immunostimulatory effects on lymphocytes (1–5), dendritic cells (6), and neutrophils (7–8), as well as its potent antitumor activity in murine tumor models (9–10). Whereas immune activation by IL-12 in mice has resulted in both tumor necrosis factor and NO production, the antitumor effect of IL-12 has been more dependent on IFN-γ production (10) and the activation of either CD8+ T cells (9–11) or NKT cells (12). There seem to be a number of mechanisms through which IL-12 can induce tumor regression, including the direct killing of tumor cells by activated lymphocytes, the antiangiogenic effects of IL-12-induced IFN-γ (13), and injury both to the tumor microcirculation and to the tumor itself by activated neutrophils (11).

The immunomodulatory activity of IL-12 is considerably dependent on costimulatory cytokines. When the ability of IL-12 to activate unmanipulated peripheral blood NK cells and CD8+ T cells in humans was examined, it was found that these lymphocyte subsets responded to IL-12 only when stimulated together with IL-2 (14). Both IL-15 and IL-18 are also key costimulatory cytokines, which, when combined with IL-12, induce strong IFN-γ production by T and NK cells (15, 16). In mice treated with IL-12, the neutralization of endogenous IL-18 significantly blunts IFN-γ production (17), further emphasizing the fact that the biological activity of IL-12 *in vivo* is likely dependent on the presence and/or induction of endogenous costimulatory cytokines.

The promising preclinical data showing IL-12 to be highly effective against murine melanoma, renal cell cancer, and sarcoma led to its testing in clinical trials in cancer patients starting in 1994. In the first published trial, rhIL-12 was administered i.v. daily for 5 days, with a 2-week break between cycles. In

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[3] The abbreviations used are: IL, interleukin; rh, recombinant human; NO, nitric oxide; DLT, dose-limiting toxicity; MTD, maximal tolerated dose; PR, partial response; PBMC, peripheral blood mononuclear cell

addition, a single test dose was given 2 weeks before the first cycle. With that dosing schedule, the MTD was 500 ng/kg, with DLTs consisting of liver function test abnormalities and stomatitis (18). Although signs of immune activation were observed, including dose-dependent IFN-γ production and reversible decreases in CD8+ T cell and NK cell numbers (19), only two responses were seen among 40 patients (one PR in a patient with renal cell cancer and one transient complete response (CR) in a patient with melanoma). Similarly low response rates were observed in two subsequent trials of weekly s.c. rhIL-12 in melanoma (20) and renal cell cancer (21), as well as in a trial testing a thrice weekly schedule of s.c. rhIL-12 (22).

In patients treated with either i.v. or s.c. rhIL-12, IFN-γ production induced *in vivo* by rhIL-12 has attenuated rapidly with consecutive cycles (18, 20–22), which indicates that the biological response to rhIL-12 is down-modulated during therapy. Even a single test dose administered 2 weeks before the first cycle of rhIL-12 seemed to attenuate IL-12-induced IFN-γ production (23). This down-modulation of IFN-γ production has been shown to result in diminished IL-12-induced tumor regression in mice (24). In addition, multiple doses of IL-12 have also been shown in animals to induce a temporary state of immunosuppression (25–26), perhaps analogous to the down-modulation of IFN-γ production in patients receiving multiple doses of rhIL-12. This paradoxical immunosuppression after a relatively brief period of immune activation by rhIL-12 may explain the limited antitumor activity observed to date in rhIL-12 clinical trials. Although the mechanism of this IL-12-induced down-modulation of subsequent IFN-γ induction remains undefined, data from animal models have suggested that IL-12-induced NO may be operative (26), whereas observations from clinical trials have also implicated changes in rhIL-12 pharmacokinetics (20, 22).

In June of 1998, we initiated a Phase I dose escalation trial of i.v. rhIL-12 in patients with renal cell cancer and melanoma, using a new dosing schedule. To try to prevent or delay the dampening of IFN-γ induction, we eliminated the test dose. In addition, we implemented a twice-weekly dosing schedule to determine whether moderate and sustained IFN-γ production could be stimulated without prohibitive toxicity. Although important aims of this trial included determining the safety and tolerability as well as the antitumor activity of this regimen, this study was also undertaken to further explore the mechanism through which rhIL-12 activates the immune system *in vivo* and to examine how IFN-γ induction by rhIL-12 is modulated with chronic dosing.

PATIENTS AND METHODS

Patient Selection. All of the patients were adults with histologically proven advanced malignancy that was metastatic or unresectable and for which standard curative or palliative measures did not exist or were no longer effective. All of the patients had measurable or evaluable disease that was clearly progressive. Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 and adequate organ function defined by WBC >4000/μl, platelet count >100,000/μl, creatinine <1.5 mg/dl, bilirubin <1.5 mg/dl, and electrocardiogram and chest X-ray without clinically significant nonmalignant abnormalities. Patients with brain metastases, seizure disorders, organ allografts, concurrent requirement for corticosteroids, more than two prior chemotherapy regimens, more than two prior immunotherapy regimens, or prior IL-12 therapy were ineligible.

Study Design. The study was an open-label, nonrandomized, single-center Phase I dose escalation trial. The treatment protocol was approved by the Cancer Therapy Evaluation Program (CTEP) of the National Cancer Institute (protocol T97-0053) and by the Human Institutional Review Board at the Beth Israel Deaconess Medical Center (protocol 97-1083), and written informed consent was obtained from each patient. rhIL-12, produced by Genetics Institute, Inc. (Cambridge, MA), was supplied by the National Cancer Institute (IND 6798). The rhIL-12 was administered by i.v. bolus injection.

The treatment schedule is shown in Fig. 1. Patients were treated in the General Clinical Research Center at the Beth Israel Deaconess Medical Center, and received i.v. bolus injections of rhIL-12 twice weekly, with doses given 3–4 days apart. A cycle of therapy lasted 6 weeks, with patients receiving a total of 12 doses during that period. During the first cycle only, patients were admitted overnight after the first, second, and seventh doses of rhIL-12 for observation and serial blood draws. All of the remaining doses were administered on an outpatient basis, with patients observed for 1 h after each dose. Patients were evaluated for tumor response at the end of each 6-week cycle, and patients with stable or regressing disease could continue receiving additional cycles until there was no evidence of disease or until there was disease progression. Patients were allowed up to a 2-week break between cycles for the resolution of any significant rhIL-12-induced toxicity.

The rhIL-12 dose was increased from 30 to 700 ng/kg in successive cohorts of patients. No intrapatient dose escalation was permitted. A minimum of three patients were enrolled at each dose level, and all of the patients had to have completed the first 3 weeks of cycle 1 before initiating enrollment to the next dose level. Toxicity was assessed using the National Cancer Institute Common Toxicity Criteria. In general, grade 3 or greater toxicities were considered dose-limiting. However, liver function test abnormalities were not classified as dose-limiting until the total bilirubin was >3 times normal or the hepatic transaminases or alkaline phosphatase were >10 times normal. In addition, the WBC count and neutrophil count were not considered dose-limiting until criteria for grade 4 toxicity were met, and no degree of lymphopenia was dose-limiting. Grade 2 cardiovascular toxicity (except for hypotension) and neurological toxicity were considered dose-limiting. The IL-12 dose was escalated when 0 of 3 patients at a dose level had a DLT. If 1 of 3 experienced a DLT, three more patients were enrolled at that dose level, and the dose was escalated if no more than 1 of 6 patients had a DLT. Patients experiencing a DLT could resume the IL-12 at the next lowest dose level if the toxicity resolved within 2 weeks. When two or more DLTs were experienced at a dose level, the MTD was determined to be the next previous dose level.

All of the patients received ranitidine for the duration of their IL-12 treatment. Acetaminophen was administered prophy-

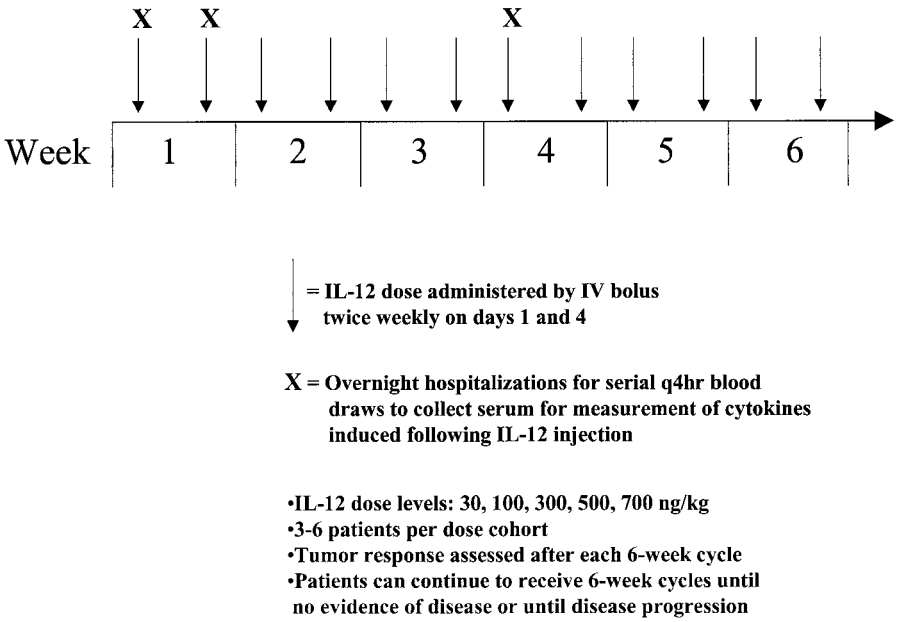

Fig. 1 Schema for clinical trial of twice-weekly i.v. rhIL-12 without a test dose.

needed thereafter. Indomethacin was used to control fever that was not responsive to acetaminophen, and demerol was used to treat rigors.

Assessment of Tumor Response. Tumor measurements were obtained by CT scan at the end of each 6-week cycle of IL-12.

Measurement of IL-12- and rhIL-12-Induced Cytokines. Serial blood specimens were collected in heparinized tubes immediately before and 4, 8, 12, 16, 20, and 24 h after the first, second, and seventh rhIL-12 doses during the first cycle. The tubes were centrifuged immediately after collection, and the plasma was then removed and stored at −20°C. Plasma IL-12 levels were measured using an ELISA that detects only the p70 IL-12 heterodimer (Endogen, Cambridge, MA, sensitivity <3 pg/ml). ELISA kits were also used to measure plasma IFN-γ (Endogen, sensitivity <2 pg/ml), IL-10 (Endogen, sensitivity <3 pg/ml), IL-15 (R&D, Minneapolis, MN, sensitivity <1 pg/ml), and IL-18 (R&D, sensitivity <15 pg/ml).

***In Vitro* Assays of Lymphocyte Cytokine Responsiveness.** Blood specimens were collected in heparinized tubes immediately before the first and seventh doses of rhIL-12 during cycle 1. PBMCs were isolated from blood samples through density gradient centrifugation using Histopaque-1077 (Sigma, St. Louis, MO). PBMCs were incubated in 96-well U-bottomed plates at 5×10^4 cells/well with medium alone (RPMI 1640 plus 15% FCS, 2% L-glutamine, 1% sodium pyruvate, 1% gentamicin, and 1% penicillin-streptomycin) or with medium plus one of the following: (*a*) 50 ng/ml IL-2 (Chiron Corporation, Emeryville, CA, specific activity 18×10^6 units/mg); (*b*) 1 nM IL-12 (Genetics Institute, Cambridge, MA, specific activity 1.7×10^7 units/mg); (*c*) 10 ng/ml IL-15 (Endogen, specific activity $\geq 2 \times 10^6$ units/mg); (*d*) IL-2 + IL-12; or (*e*) IL-15 + IL-12. Conditions were plated in triplicate, and after a 72-h incubation at immediately before pulsing each well with 1 μCi [^{3}H]thymidine (DuPont-New England Nuclear, Boston, MA). The IFN-γ concentration in the harvested supernatants was assayed using an IFN-γ ELISA (Endogen). Cell proliferation was determined by measuring [^{3}H]thymidine incorporation 8 h after pulsing, as described previously (27).

Measurement of NO in Expired Air. The concentration of NO in expired air was measured in five patients receiving IL-12 at either the 500-ng/kg or the 700-ng/kg dose levels. Measurements were made immediately before and 24 h after the first and second IL-12 doses. Expired air was collected in self-sealing balloons after first clearing the upper airway of ambient air NO by having patients take four deep inspirations through a tube fitted with a charcoal filter (Omega Engineering Co.). The NO concentration in the air expired after the fourth breath was measured using a high-sensitivity NO detector based on a gas-phase chemiluminescent reaction between NO and ozone (Model 280 Nitric Oxide Analyzer, Sievers Instruments, Inc., Boulder, CO). Patients receiving high-dose IL-2 (600,000 IU/kg i.v. every 8 h) were used as positive controls. IL-2 patients had NO samples obtained before the start of the 1st week of IL-2 and then daily for the 1st 3 days of IL-2 treatment.

RESULTS

Patient Characteristics

Between June 1998 and June 1999, 28 patients were enrolled in this study. Patient characteristics are shown in Table 1. The majority of patients had metastases to two or more sites (including 15 of 28 with liver, adrenal, and/or kidney involvement and 10 of 28 with bone metastases), and 23 of 28 had received one or more prior immunotherapy regimens (primarily IL-2-based regimens). Only 3 of 23 patients had responded to

Table 1 Patient characteristics

	No. of patients
Total patients	28
Median age (range)	56 yr (32–72 yr)
Gender (male/female)	20/8
Performance status	
ECOG[a] 0	15
ECOG 1	13
Tumor types	
Renal cell cancer	20
Melanoma	8
Prior therapy	
Chemo/Immunotherapy	24
High-dose IL-2	14
Low-dose IL-2	6
IFN α-2b	12
Biochemotherapy	3
Chemotherapy	1
Surgery	20
Radiotherapy	13
None	1
Prior systemic treatment regimens	
0	4
1	12
2	10
3	2
Prior response to chemo/immunotherapy	3
Disease sites	
Lung	21
Lymph nodes	13
Skin or soft tissue	5
Liver	9
Bone	10
Adrenal/kidney	6
Number of disease sites	
1	6
2	9
≥3	13

[a] ECOG, Eastern Cooperative Oncology Group.

Treatment Administered and Toxicity

Dose Escalation Phase. Three patients were treated at each of the first three rhIL-12 dose levels (30, 100, and 300 ng/kg), with no DLTs. Common side effects included self-limited fever and chills, occurring 6–10 h after the dose, and mild malaise. These side effects were observed even at the 30-ng/kg dose level, and were greatly attenuated by the start of the 3rd week of therapy. One patient, treated at 100 ng/kg, developed a supraventricular tachycardia (Table 2) in the setting of fever after the second rhIL-12 dose, which resolved spontaneously. Small, minimally symptomatic, transient oral aphthous ulcers developed during the first cycle of therapy in one patient at the 300-ng/kg dose level. No significant (Grade 2 or greater) cytopenias or liver-function test abnormalities were noted at the first three dose levels.

At the 500-ng/kg dose level, the fever and chills were more severe with previously untreated patients or with patients for whom more than 1 year had passed since prior therapy. Fevers were highest after the second dose and were minimal-to-absent by the 3rd week of therapy in the majority of patients. Indomethacin was added to acetaminophen for the control of fevers and chills in only 3 of 14 patients and was never required were observed, but no diarrhea or gastrointestinal bleeding. Stomatitis was uncommon and was never greater than grade 2. Grade 1–2 elevations of serum transaminases were common, usually peaking after the second dose and normalizing by the start of week 3 (Table 2). Orthostatic hypotension 24 h after the second rhIL-12 dose occurred in one patient and constituted the one DLT among the six patients treated at the 500-ng/kg dose level during the escalation phase. No fluid retention or evidence of capillary leak syndrome was observed at either the 500-ng/kg dose level or any other rhIL-12 dose level, nor was there any renal or pulmonary toxicity.

A total of five patients were treated at the 700-ng/kg dose level. Two patients (one with melanoma and one with renal cell cancer) who had received high-dose IL-2 <6 months before the rhIL-12 had either no fever or low-grade fevers and minimal-to-no liver function test abnormalities during rhIL-12 treatment. In contrast, the other three patients who received either high-dose IL-2 therapy >1 year previously or low-dose IL-2 >6 months previously experienced higher and more sustained fevers (requiring both acetaminophen and indomethacin during the first 2 weeks of therapy) as well as more protracted constitutional symptoms (including malaise and anorexia). Two DLTs were observed among these three patients, including grade-3 hemolytic anemia (occurring during week 5 of cycle 1) in one patient and a grade-3 elevation of serum hepatic transaminases (occurring after the second dose of rhIL-12) in another (Table 2). The hemolytic anemia was Coombs negative and required both the discontinuation of the rhIL-12 and a 1-week course of prednisone to resolve. IL-12-induced hypersplenism leading to extravascular hemolysis was suspected because CT scans showed the development of splenomegaly after the first cycle of rhIL-12 (not shown). The grade 3 transaminase elevation resolved within 1 week of stopping the rhIL-12.

Safety Phase. On the basis of the two DLTs observed at the 700-ng/kg dose level, the MTD for the twice-weekly schedule of i.v. rhIL-12 administered without a test dose was determined to be 500 ng/kg. To better assess the safety of the MTD, an additional eight patients were treated at 500 ng/kg. As shown in Table 3, 7 of 8 patients tolerated the rhIL-12 well without any DLTs. One patient tolerated cycle 1 without difficulty but then developed grade-4 neutropenia after the first 2 weeks of cycle 2. Bone marrow biopsy revealed agranulocytosis, which resolved after discontinuation of the IL-12 and treatment with prednisone plus low-dose oral cyclophosphamide.

With the exception of the case of agranulocytosis, no unusual or severe toxicities occurred among patients receiving two or more uninterrupted 6-week cycles of rhIL-12, including two patients who had been on rhIL-12 for 36 and 48 weeks, respectively (Table 3). Several patients, including the one on rhIL-12 for 36 weeks, experienced grade 1–2 arthralgias (Table 2), involving primarily the shoulders and fingers, beginning with the second cycle of therapy. The arthralgias were episodic, unaccompanied by joint swelling or tenderness, and responsive to therapy with nonsteroidal anti-inflammatory drugs.

Biological Effects of Twice-Weekly i.v. rhIL-12

***In Vivo* IFN-γ Induction.** IFN-γ levels were obtained in eight patients treated at the 500-ng/kg dose level as well as

Table 2 Number of patients experiencing select toxicities during treatment with IL-12 (grades 2, 3, 4)

	Dose level, ng/kg (no. of patients)				
Toxicity	30 (3)	100 (3)	300 (3)	500 (14)	700 (5)
Hepatic					
AST[a]				(4, 0, 0)	(0, 1, 0)
Bilirubin		(1, 0, 0)		(1, 0, 0)	
Alk phosphatase				(1, 0, 0)	(1, 0, 0)
Hematologic					
Neutropenia				(2, 1, 1)	(2, 0, 0)
Anemia				(2, 0, 0)	
Hemolytic anemia					(0, 1, 0)
Thrombocytopenia				(1, 0, 0)	(1, 0, 0)
Oral mucositis				(2, 0, 0)	(1, 0, 0)
Cardiovascular (hypotension)				(1, 0, 0)	
Cardiovascular (arrhythmia)		(1, 0, 0)			
Fever		(1, 0, 0)		(2, 1, 0)	(2, 0, 0)
Arthralgia				(2, 0, 0)	

[a] AST, aspartate aminotransferase; Alk, alkaline.

Table 3 Summary of tolerability of IL-12 among patients treated at the MTD of 500 ng/kg

	Patient no.	No. of cycles completed	Results
Dose escalation phase	10	8	No DLT
	11	3	No DLT
	12	1	Grade 2 orthostatic hypotension; IL-12 dose reduced to 300 ng/kg
	13	6	No DLT
	14	1	No DLT
	15	1	No DLT
Safety phase	21	1	No DLT
	22	2[a]	Grade 4 neutropenia (agranulocytosis); IL-12 discontinued
	23	1	No DLT
	24	2	No DLT
	25	1[b]	No DLT
	26	1	No DLT
	27	3	No DLT
	28	2	No DLT

[a] Received four doses in cycle 2.
[b] Received only two doses in cycle 1 because of rapid disease progression.

in Fig. 2 and Table 4, we were able to discern three patterns of IFN-γ induction among these 10 patients. In all of the patterns, the first significant rise in plasma IFN-γ occurred between 4 and 8 h after the rhIL-12 dose, corresponding to the onset of fevers/chills. In the type-I pattern (Table 4 and Fig. 2*A*, *top*), the IFN-γ level peaked at a modest 450-1600 pg/ml (with peaks occurring between 8 and 24 h for individual patients) after the first rhIL-12 dose (week 1/day 1). After the second dose (week 1/day 4), peak levels were 2–3-fold higher than those induced by the first dose. However, after the seventh dose (week 4/day 1), peak IFN-γ levels were comparable with those after the first dose. Patients with this type-I pattern tended to have modest fever/chills after each rhIL-12 dose during cycle 1, with the most prominent symptoms occurring after the second dose. However, whereas IFN-γ could be detected in the plasma 24 h after an IL-12 dose, it always dropped to undetectable levels by the time of the next

type-I pattern of IFN-γ induction had all been treated previously with an IL-2-based regimen and were either >6 months past a low-dose IL-2 regimen or >1 year past a high-dose IL-2 regimen.

In the type-II pattern, peak IFN-γ levels after the first dose were, on the average, 2-fold higher than those measured in patients with the type-I pattern (Table 4 and Fig. 2*B*, *top*). The augmentation in peak IFN-γ levels after the second dose was also higher in the type-II pattern compared with the type-I pattern, increasing 2- to 4-fold over the peak levels after dose 1. This difference in the magnitude of IFN-γ production was associated with higher fevers and more pronounced chills/rigors in these patients after the first two doses of rhIL-12 compared with patients exhibiting the type-I pattern of IFN-γ induction. However, despite this larger surge of IFN-γ production after the second dose, IFN-γ induction after the seventh dose of rhIL-12

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