JOURNAL OF INTERFERON AND CYTOKINE RESEARCH 21:257–263 (2001)
Mary Ann Liebert, Inc.

Randomized Multicenter Phase II Trial of Subcutaneous Recombinant Human Interleukin-12 Versus Interferon-α2a for Patients with Advanced Renal Cell Carcinoma

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ABSTRACT

Recombinant human interleukin-12 (rHuIL-12) is a pleiotropic cytokine with anticancer activity against renal cell carcinoma (RCC) in preclinical models and in a phase I trial. A randomized phase II study of rHuIL-12 compared with interferon-α (IFN-α) evaluated clinical response for patients with previously untreated, advanced RCC. Patients were randomly assigned 2:1 to receive either rHuIL-12 or IFN-α2a. rHuIL-12 was administered by subcutaneous (s.c.) injection on days 1, 8, and 15 of each 28-day cycle. The dose of IL-12 was escalated during cycle 1 to a maintenance dose of 1.25 μg/kg. IFN was administered at 9 million units by s.c. injection three times per week. Serum concentrations of IL-12, IFN-γ, IL-10, and neopterin were obtained in 10 patients treated with rHuIL-12 after the first full dose of 1.25 μg/kg given on day 15 (dose 3) of cycle 1 and again after multiple doses on day 15 (dose 6) of cycle 2. Thirty patients were treated with rHuIL-12, and 16 patients were treated with IFN-α. Two (7%) of 30 patients treated with rHuIL-12 achieved a partial response, and the trial was closed to accrual based on the low response proportion. IL-12 was absorbed rapidly after s.c. drug administration, with the peak serum concentration appearing at approximately 12 h in both cycles. Serum IL-12 concentrations remained stable on multiple dosing. Levels of IFN-γ, IL-10, and neopterin increased with rHuIL-12 and were maintained in cycle 2. rHuIL-12 is a novel cytokine with unique pharmacologic and pharmacodynamic features under study for the treatment of malignancy and other medical conditions. The low response proportion associated with rHuIL-12 single-agent therapy against metastatic RCC was disappointing, given the preclinical data. Further study of rHuIL-12 for other medical conditions is underway. For RCC, the study of new cytokines is of the highest priority.

INTRODUCTION

RECOMBINANT HUMAN INTERLEUKIN-12 (rHuIL-12) is a cytokine with a variety of immunomodulatory effects on T lymphocytes and natural killer (NK) cells including (1) enhancing the lytic activity of NK lymphokine-activated killer (LAK) cells, (2) facilitating specific cytolytic T lymphocyte responses, (3) inducing the secretion of interferon-γ (IFN-γ) by both T and NK cells, and (4) promoting the development of Th1 helper T cells, thereby contributing to the development of cell-mediated immune responses.[1–3] rHuIL-12 has also been demonstrated to inhibit angiogenesis, which may contribute to its antitumor activity.[4] rHuIL-12 has been shown to have striking therapeutic effects in a number of mouse tumor models, including the Renca renal cell model,[5] and mouse models of infectious disease and airway inflammation. On the basis of these studies, clinical trials investigating the potential therapeutic effects of rHuIL-12 in the treatment of cancer, human immuno-

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deficiency virus (HIV) infection, chronic viral hepatitis, and asthma were initiated.

IFN-α and IL-2 have a low level of antitumor effector against renal cell carcinoma (RCC).[6,7] As this malignancy is associated with a poor prognosis and is chemotherapy resistant,[8] the study of new cytokines is a priority. A phase I trial of rHuIL-12 was conducted in patients with advanced RCC using two schedules, as a fixed dose an an uptitration schedule, whereby the dose of rHuIL-12 was increased for each patient to a target dose.[9] The study showed antitumor activity against metastatic RCC and improved tolerability for the uptitration schedule and suggested a dose suitable for phase II study.[9] The pharmacokinetic and pharmacodynamic studies showed that serum levels of IL-12, IFN-γ, and IL-10 produced in response to IL-12 were all highest in the week after the first dose of rHuIL-12, were dose related, and decreased after long-term administration when rHuIL-12 was administered as a fixed dose.[10]

Clinical responses associated with rHuIL-12 treatment against RCC were reported in phase I trials conducted by others, as well.[11–14] To further define efficacy, toxicity, and pharmacology, we conducted a randomized phase II trial of rHuIL-12 in patients with RCC. Patients were randomized to receive rHuIL-12 by an uptitration schedule based on our phase I trial[9] or IFN-α2a (Roferon®-A) (Hoffman-La Roche, Inc., Nutley, NJ). A proportion of patients treated with rHuIL-12 had pharmacologic and pharmacokinetic studies performed. The results of this trial and these studies follow.

PATIENTS AND METHODS

Patients

Forty-six patients with advanced RCC were accrued from six centers to this Institutional Review Board-approved clinical trial between February and May 1997. All patients entered in the trial gave informed consent. They were required to have measurable disease, Karnofsky performance status $\geq$80%, white blood cell (WBC) count $\geq$3000 cells/mm^3, granulocytes $\geq$2000 cells/mm^3, platelet count $\geq$75,000/mm^3, hemoglobin $\geq$9 g/dl, serum bilirubin $\leq$1.5 times normal, transaminase levels and alkaline phosphatase $\geq$2.5 times normal, serum creatinine concentration $\leq$1.5 times normal, and PT/PTT within normal limits. Exclusion criteria included prior systemic treatment for RCC, active brain metastases, history of psychiatric disabilities or seizures, clinically significant comorbid conditions, active infection, and a history of any Th1-mediated autoimmune disease.

Trial design

Patients were prospectively randomized to receive treatment with either rHuIL-12 or IFN-α. The ratio of assignment for treatment with rHuIL-12 to IFN-α was 2:1. The target accrual was 80 patients treated with rHuIL-12 and evaluated for response. If an objective tumor response rate of 25% occurred in 80 patients, the 95% confidence interval (CI) would range from 16.0% to 35.9%. Thus, the lower limit of normal would be similar to the response rate induced by other therapies.[8] No formal interim analysis was planned, but an early stopping rule was implemented by the sponsor based on a low response pro-

rHuIL-12 therapy

rHuIL-12 was supplied by Hoffman-La Roche, Inc., as ready-to-use human serum albumin (HAS)-free solution in single-use glass vials in three concentrations: 10, 50, and 100 μg/ml purified rHuIL-12 in 1 ml sterile solution containing polysorbate 80 (0.2 mg/ml) and 67 mM phosphate-buffered saline (PBS) adjusted to pH 7.0. The vials were stored at 2–8°C and protected from light. rHuIL-12 was administered by subcutaneous (s.c.) injection using a 25G needle.

Patients were treated by s.c. injection on days 1, 8, and 15 of each 28-day cycle. The dose was escalated during the first cycle: day 1 dose = 0.1 μ/kg, day 8 dose = 0.5 μg/kg, and day 15 dose = 1.25 μg/kg, with a maximum dose of 100 μg. During subsequent cycles, all treatment was administered at a maintenance dose of 1.25 μg/kg. Treatment was delivered on an outpatient basis except when pharmacokinetic studies were being performed. Patients were treated until disease progression or unacceptable toxicity. The treatment dose was modified for toxicity according to a nomogram.

IFN-α therapy

IFN-α2a (Roferon®-A) was supplied by Hoffmann-La Roche, Inc., as ready-to-use, HSA-free solution in single-use glass vials. IFN-α2a was administered by s.c. injection at a dose of 9 million units three times each week. Dose modifications were based on toxicity grade.

Patient follow-up

Patients were seen and examined at periodic intervals, with laboratory evaluation that included hematologic, coagulation, and biochemistry panels. Tumor assessments were made after

Table 1. Results of Clinical Trial

Characteristic	*rHuIL-12 No.*	*IFN-α No.*
Patients	30	16
Males (%)	18 (60)	9 (56)
Females (%)	12 (40)	7 (44)
Median age (range)	55 (40–77)	60 (41–70)
Nephrectomy (%)	16 (53)	9 (56)
Total (%)	15 (50)	8 (50)
Partial (%)	1 (3)	1 (6)
Disease sites (%)		
One (%)	8 (27)	1 (6)
Two (%)	6 (20)	4 (25)
Three or more (%)	16 (53)	11 (69)
Response		
Evaluable	29	14
Favorable (partial) response (%)	2 (7)	0 (0)
Toxicity: grade $\geq$3		
Flu-like symptoms (%)	3 (10)	1 (7)
Liver function test elevation (%)	2 (7)	1 (7)
Neutropenia (%)	1 (3)	0 (0)
Ascites (%)	1 (3)	0 (0)
Stomatitis (%)	1 (3)	0 (0)

every two cycles. Responses were graded according to the World Health Organization (WHO) Criteria, and toxicity was graded by the National Cancer Institute (NCI) Common Toxicity Criteria.

Pharmacodynamic and pharmacokinetic methods

Serum concentrations of rHuIL-12 were measured at baseline, and 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, and 48 h after dosing on day 15 of the first two cycles in 10 patients treated at two of the centers. rHuIL-12 concentration was measured by a two-step method of antibody capture to insure specificity, followed by a cell proliferation assay. rHuIL-12 was isolated from serum by an affinity technique that involved incubation of samples in sterile tissue culture plates precoated with mouse anti-human IL-12 monoclonal antibody (mAb).[15] The method has been described previously.[9,10]

IFN-γ, neopterin, and IL-10 concentrations were measured in the serum of 10 patients at preselected times. Serum samples were obtained at baseline and 10, 24, 48, 72, 96, and 168 h after treatment on day 15 of the first two cycles of treatment. IFN-γ concentrations in serum were determined by a commercial assay (R&D Systems, Minneapolis, MN). Neopterin concentration in serum was measured using a radioimmunoassay that used ^{125}I-neopterin as a tracer (Incstar, Stillwater, MN). Serum IL-10 was measured by an ELISA with a commercial assay (Boehringer Mannheim, Mannheim, Germany). The methodologies have been described previously.[9,10]

Serum concentration compared with time data was analyzed for rHuIL-12 and other immunologic markers by a noncompartmental model.

RESULTS

Clinical trial

Forty-six patients were enrolled (Table 1). Thirty patients were randomized to treatment with rHuIL-12, and 16 were randomized to treatment with IFN-α2a. The median age was 55 years on the rHuIL-12 arm and 60 years on the IFN-α2a arm. Approximately one half of all patients had a prior nephrectomy. Two of 29 (7%, 95% C.I. 0-16%) evaluable patients treated with rHuIL-12 achieved a partial response. One patient who responded had prior nephrectomy with lung-only metastases, and the second patient had prior nephrectomy with three metastatic sites, lung, bone, and skin. No patient treated with IFN-α2a achieved a partial or complete response. Based on the low response to rHuIL-12, accrual to the trial was stopped.

The most common toxicity associated with rHuIL-12 was flu-like symptoms. Grade 3 or 4 elevation of hepatic transaminases occurred in 2 (7%) patients receiving rHuIL-12 and 1 (7%) receiving IFN-α2a. Other severe toxicities occurring on the rHuIL-12 arm were neutropenia, ascites, and stomatitis.

Pharmacokinetic studies

Serum concentrations of IL-12 were obtained in 10 patients treated with rHuIL-12 at two centers participating in this study. Because of gradual dose escalation in the first cycle of treatment, serum concentrations were measured after the first full dose of 1.25 μg/kg given on day 15 (dose 3) of cycle 1 and again after multiple doses on day 15 (dose 6) of cycle 2. IL-12 was absorbed rapidly after s.c. administration, with peak serum concentration noted at about 12 h in both cycles. We reported

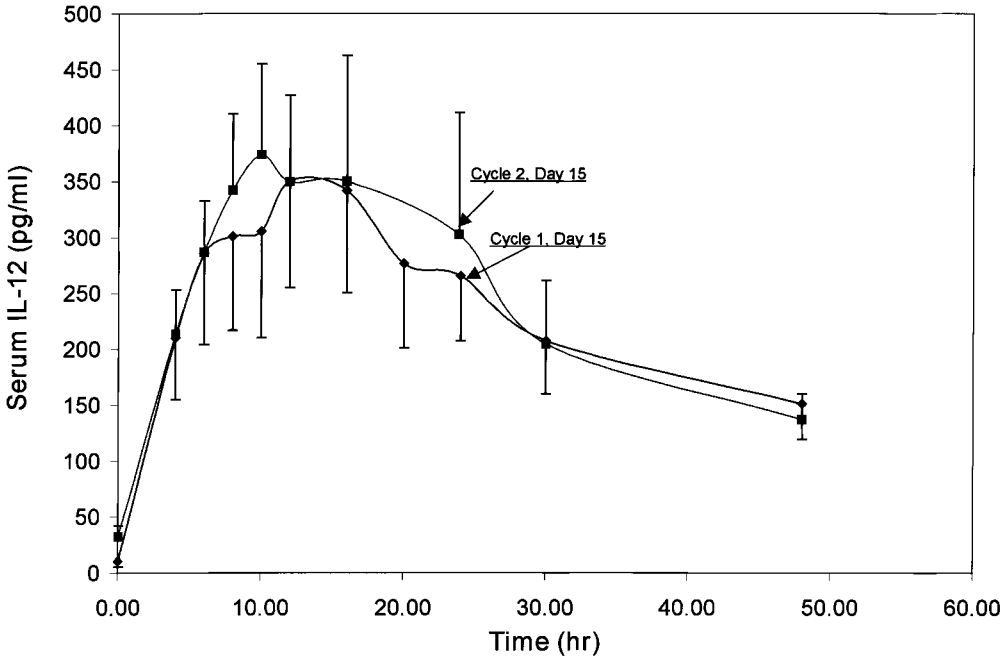


FIG. 1. Mean (±SE) serum concentrations of IL-12 in RCC patients after 1.25 μg/kg/week s.c. administration of rHuIL-12 us

Table 2. Comparison of Pharmacokinetic and Immunologic Surrogate Parameters in Cycle 1 vs. Cycle 2 After Administration of rHuIL-12 in RCC Patients

	C_{max}		T_{max} (h)		AUC	
Analyte	*Dose 3*	*Dose 6*	*Dose 3*	*Dose 6*	*Dose 3*	*Dose 6*
IL-12						
Mean	384	432	15	10	10,646 pg · h/ml	11,031
SE	88	112	4	1	2,583	2,886
IFN-γ (pg/ml)						
Mean	170	146	30	20	7,982 pg · h/ml	6,011
SE	39	33	5	5	2,185	1,525
IL-10 (pg/ml)						
Mean	65	96	36	25	6,483 pg · h/ml	9,345
SE	8	13	5	3	1,373	1,403
Neopterin (ng/ml)						
Mean	8.65	10.34	80	69	793 ng · h/ml	1,306
SE	1.37	1.67	14	6	154	336

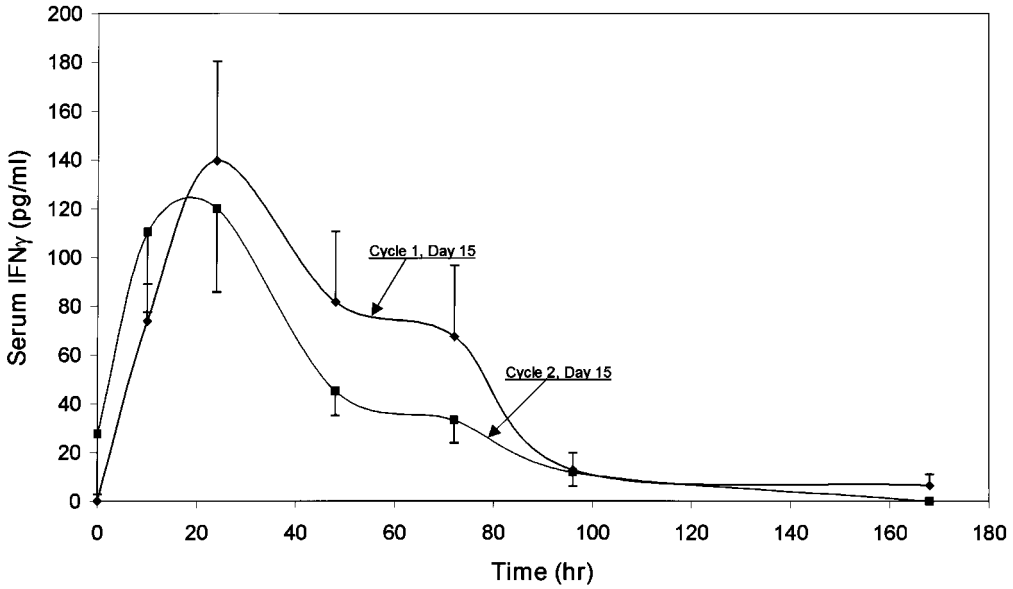
that serum concentrations of IL-12 decreased gradually after multiple doses in a fixed-dose scheme.[10] In contrast, the current study showed serum IL-12 concentrations were stable on multiple dosing when drug was administered in the slow dose-escalation pattern (Fig. 1). The maximum serum concentrations of IL-12 were maintained at comparable levels in cycles 1 and 2 (dose 3, 384 pg/ml, vs. dose 6, 432 pg/ml). Serum area under the curve (AUC) during the dosing interval was also maintained on multiple dosing (dose 3, 10,646 pg/ml, vs. dose 6, 11031 pg/ml). Individual pharmacokinetic parameters were compared for dose 3 (cycle 1) vs. dose 6 (cycle 2) (Table 2).

Pharmacodynamic studies

Three immunologic surrogate markers were monitored in the serum of the 10 patients treated with rHuIL-12 (Table 2). Serum IFN-γ increased after IL-12 dosing, with peak concentrations appearing at about 24 h into both cycles 1 and 2. Serum IFN-γ was not measurable at predose baseline in any of these patients. Similar to IL-12, the average peak serum concentration of IFN-γ was relatively unchanged (170 vs. 146 pg/ml) between the two cycles (Fig. 2). Five patients showed a small decrease, and 4 patients' peak concentration increased slightly. Serum samples could not be obtained in 1 patient in cycle 2.

The concentration of neopterin increased significantly from baseline as the dose was increased from 0.5 to 1.25 μg/kg/week in cycle 1, but induction was maintained at comparable levels as the 1.25 μ/kg dose was maintained on subsequent cycles (dose 3 vs. dose 6: C_{max} 8.65 vs. 10.34 ng/ml, and AUC 793 vs. 1306 ng · h/ml), demonstrating maintenance of the immunologic activity of rHuIL-12 in these patients at the end of 2 months treatment (Fig. 3).

Serum concentrationsof IL-10 increased after administration of rHuIL-12. Peak serum IL-10 concentrations appeared be-



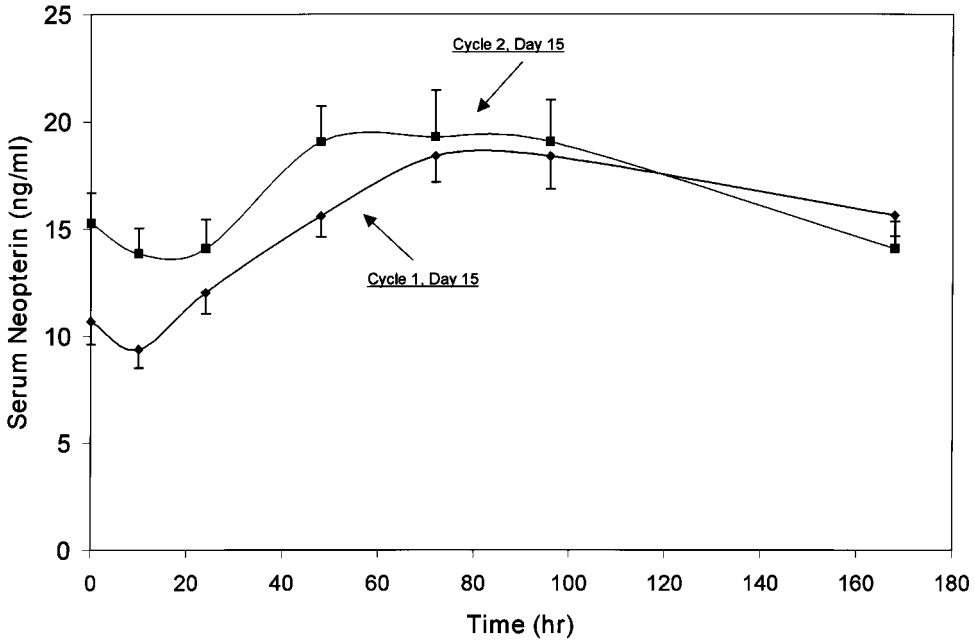
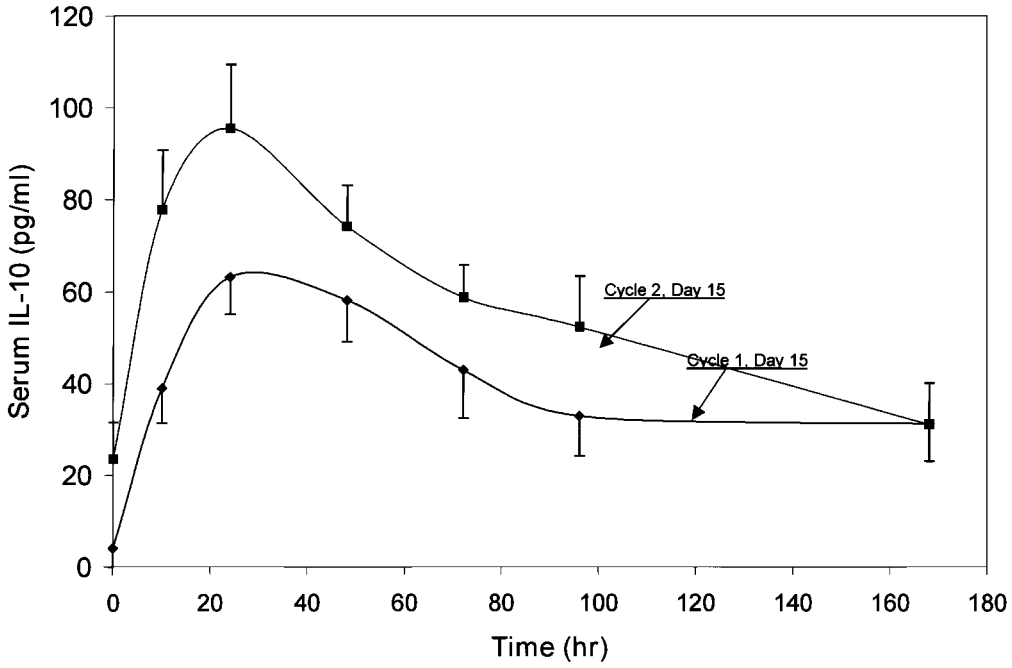


FIG. 3. Mean (±SE) serum concentrations of neopterin after s.c. administration of 1.25 μg/kg/week of rHuIL-12 in RCC patients given a slow dose escalation.

tween 24 and 48 h of IL-12 administration in both cycles. Similar to IL-12 and IFN-γ, IL-10 concentrations did not decrease on multiple dosing in this study. The mean C_{max} and AUC increased to a small but significant extent ($p < 0.05$ by paired t-test) (Fig. 4).

DISCUSSION

This randomized phase II trial stopped accrual after an interim analysis showed a low response proportion associated with rHuIL-12 treatment in previously untreated patients with



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