Phase I Study of Subcutaneously Administered Recombinant Human Interleukin 12 in Patients with Advanced Renal Cell Cancer

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ABSTRACT

A phase I study was conducted to characterize the maximum tolerated dose (MTD), dose-limiting toxicity (DLT), and pharmacokinetics of a single dose followed by three times weekly s.c. injections of recombinant human interleukin 12 (rHuIL-12). The study encompassed 28 patients with advanced renal cell carcinoma. rHuIL-12 was administered on day 1, followed by an observation period of 7 days. Starting on day 8, repeated s.c. injections were administered 3 times a week for 2 weeks. The MTD of the initial injection was evaluated at dose levels of 0.1, 0.5, and 1.0 μg/kg. DLT was observed at 1.0 μg/kg and consisted of fever, perivasculitis of the skin, and leukopenia. The MTD of the subsequent repeated injections after 1 week of rest was studied at dose levels 0.5, 1.0, and 1.25 μg/kg. DLT at 1.25 μg/kg comprised deterioration of performance status, fever, vomiting, mental depression, and leukopenia. Other notable toxicities were oral mucositis and elevation of hepatic enzymes. Fever, leukopenia, and elevation of hepatic enzymes were more severe after the initial injection than after repeated injections at the same dose level. At dose level 0.5 μg/kg, the mean area under the plasma concentration-time curve decreased from 7.4 ng/h/ml after the first injection to 3.3 ng·h/ml ($P = 0.034$) after repeated administrations, and at dose level 1.0 μg/kg, it ranged from 31.8 ng/h/ml to 6.0 ng·h/ml ($P = 0.041$). One patient had a partial response and seven had stable disease. The MTD of a single s.c. injection of rHuIL-12 was 0.5 μg/kg, and the MTD of three subsequent administrations per week was 1.0 μg/kg. In comparison with a single administration, the three times weekly administrations at the same dose level was accompanied with a milder pattern of side effects and a reduction of the area under the plasma concentration-time curve.

INTRODUCTION

IL-12[2] is a heterodimeric cytokine with immunoregulatory functions. It stimulates the proliferation and activation of T lymphocytes and natural killer cells (1, 2) and induces the production of IFN-γ by these cells (1, 3). IL-12 promotes T-helper type 1 responses (4, 5), including the commitment of naive helper T cells to the T-helper type 1 developmental pathway, while inhibiting T-helper type 2 development and function, thereby promoting cellular immunity (6). Additionally, IL-12 can inhibit tumor-associated angiogenesis (7, 8). The antitumor effects of IL-12 have been evaluated in a large number of murine tumor models and more recently in nonhuman primate tumor models (9–13). Cures and long term survival were seen in murine renal cell carcinoma (RCC) at doses that resulted in mild toxicities. These results show that IL-12 has important immunomodulatory and antitumor effects in animal models. Clinical experience with IL-12 in humans is limited. Metastatic RCC appears to be more responsive to immunomodulatory treatment than to conventional cytotoxic chemotherapy. However, despite progress in treatment, overall prognosis remains poor, and effective immunotherapies are needed (14). Therefore, we performed a Phase I study of s.c. administration of rHuIL-12 in patients with RCC.

The choice of schedule and route of administration were based on experiments in cynomolgus monkeys (Hoffmann La Roche, personal communication). IL-12 in s.c. doses of 0.1 to 1.0 μg/kg/day, three times a week, were shown to modulate immune activity without provoking substantial toxicity in these animals. The observation of a nonlinear relationship between dose and drug exposure formed the rationale to study the effects of a single and multiple doses of IL-12. The purpose of the study was to investigate the toxicity and pharmacokinetics of a single s.c. administration of rHuIL-12 and of a schedule of three s.c. administrations per week for 2 weeks, started 1 week after this single s.c. injection.

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[2] The abbreviations used are: IL-12, interleukin 12; RCC, renal cell carcinoma; rHuIL-12, recombinant human interleukin 12; ALT, serum alanine aminotransferase; DLT, dose-limiting toxicity; MTD, maximum tolerated dose; AUC, area under the plasma concentration-time curve.

PATIENTS AND METHODS

Patient Selection. Patients had histological proof of RCC with measurable locally advanced or metastatic disease. Patients were between 18 and 75 years of age, had a WHO performance score of 0 to 1, a life expectancy >4 months, adequate renal function (serum creatinine, <1.5 times normal), adequate hepatic function (normal serum bilirubin, ALT, and/or aspartate aminotransferase, <2.5 times normal; serum alkaline phosphatase, <2.5 times normal), normal serum calcium, serum hemoglobin >10 g/dl, WBC $>3 \times 10^9$/liter, granulocytes $>2 \times 10^9$/liter, platelets $>75 \times 10^9$/liter, and normal pulmonary function. They had not received more than one previous immunotherapy. All former therapies were ended at least 6 weeks before start of treatment with rHuIL-12. Patients did not use systemic corticosteroids. Patients with brain or leptomeningeal metastases or major fluid effusions (*e.g.*, ascites, pleural effusions) were excluded. Patients with major concurrent systemic disease, an organ graft, or a prior history of other malignancy were excluded as were patients known to be seropositive for HIV or hepatitis B surface antigen. All patients gave written informed consent.

rHuIL-12. rHuIL-12 (Ro 24-7472) was supplied by Hoffmann La Roche, Nutley, NJ, and administered by s.c. injection. The first injection was an in-hospital treatment. Subsequent injections were given on an outpatient basis.

Study Design. The study was an open label nonrandomized Phase I dose escalation trial carried out in two European cancer centers to evaluate the safety and tolerability of an initial single injection of rHuIL-12 as well as the safety and tolerability of repeated s.c. injections administered in treatment cycles of 2 weeks with three injections per week. Pharmacokinetics and pharmacodynamics were studied simultaneously. The treatment protocol was approved by the ethics board of the participating institutions.

Treatment Schedule and Follow-up. On day 1, a single s.c. injection of rHuIL-12 was given, followed by an observation period of 7 days. Subsequently, on day 8 repeated injections were started, with rHuIL-12 s.c., three times a week, for 2 weeks. After a 2-week rest period, repeated injections were resumed with an identical schedule. Tumor volume was assessed after 2 months of treatment. Patients who did not experience tumor progression or DLT could be treated with additional cycles. The dose of rHuIL-12 was calculated per kilogram (kg) body-weight, where 80 kg was taken as the maximum multiplication factor.

The MTD was defined as one dose level below the dose that causes DLT, *i.e.*, the dose that causes drug-related grade 3 or 4 toxicity, with the exception of lymphopenia, in one-third of patients. Dose escalation for the initial single injection was decided upon the toxicity encountered during the week of observation that followed, until repeated dosing was started from day 8 onwards. Toxicity was assessed using the National Cancer Institute Common Toxicity Criteria. To define the MTD, cohorts of three patients were entered at each dose level until a grade 3 or 4 toxicity occurred; for these and subsequent dose escalation levels, three more patients were entered. If more than one patient experienced drug-related grade 3 or 4 toxicity, three more patients were entered at the previous dose level. As long as the MTD for the initial single injection had not been reached, the dose of rHuIL-12 for repetitive administrations was identical with the initial dose.

Once the MTD was reached for the initial single dose, this dose was fixed in all subsequent patients. To define the MTD for repeated injections, the toxicity encountered during the first two cycles (8th until 64th day) was evaluated. Further dose escalation steps were performed as previously described.

Before start of the study, all considered patients underwent a complete medical history and physical examination; electrocardiography, hematology, and blood chemistry tests; dipstick urinalysis; pulmonary function test with carbon monoxide (CO) diffusion capacity; and measurements of study parameters by chest X-ray and computerized tomography scan.

After the single initial injection, patients had physical examination and complete blood counts daily, and serum chemistry and urinalysis were repeated on days 1 and 2. During the repetitive injections, vital signs and complete blood counts were assessed after all drug administrations, and serum chemistry and urinalysis were repeated twice a week. Pulmonary function tests were performed on days 12, 19, and 26. Study parameters were measured by chest X-ray and computerized tomography scan after each treatment period of 2 months.

Pharmacokinetic Sampling and Data Analysis. Pharmacokinetic parameters were calculated from serum concentrations of rHuIL-12 in blood samples taken before and 4, 6, 8, 10, 12, 18, 24, 30, 36, 48, 72, and 96 h after the single initial injection. During the repetitive injections, blood samples were taken on days 15 and 17 before drug administration; on day 19 before and 4, 6, 8, 10, 12, 18, 24, and 36 h after drug administration; and on day 26.

Serum concentrations of rHuIL-12 were measured by a method of antibody capture followed by a cell proliferation assay (15). This assay has a lower limit of detection of 50 pg/ml.

Individual patients' plasma concentration-time data were analyzed using the Siphar software package (version 4.0; SIMED, Créteil, France) by noncompartmental analysis. The area under the plasma concentration-time curve (AUC) for rHuIL-12 was calculated by the linear trapezoidal rule up to the last sampling point with detectable levels (C), with extrapolation to infinity ($AUC_{0\rightarrow indefinite}$) by the equation $AUC + C/k_{el}$, where k_{el} represents the terminal disposition rate constant. The latter term was calculated from the slope of the data points in the final log-linear part of the concentration-time curve by weighed ($1/y$) least squares linear regression analysis. Maximum plasma concentrations (C_{max}) and the time to maximum concentration (T_{max}) were estimated by visual inspection of the semilogarithmic plot of the concentration-time curve. The terminal disposition half-life ($t_{1/2}$ was calculated by dividing ln2 by k_{el}.

To test parameter differences for statistical significance among treatment courses, a two-tailed paired Student's t test was performed. Probability values of less than 0.05 were regarded as statistically significant. All statistical calculations were performed with the Number Cruncher Statistical System (version 5.X; Dr. Jerry Hintze, Kayesville, UT) and STATGRAPHICS Plus (version 2; Manugistics, Inc., Rockville, MD).

Table 1 Patient characteristics

No. of patients	28
Median age (yr)	56 (41–70)[a]
Gender (male/female)	20/8
Performance status	
Karnofsky 100%	15
Karnofsky 90%	9
Karnofsky 80%	4
Previous therapy	
Surgical only	13
Immunotherapy	9
Chemotherapy	2
Chemo- and immunotherapy	4
Median duration of disease (mo)	38 (1–264)
Disease status	
Locally advanced	1
Metastatic	27

[a] Results are given in numbers; a range is shown in parentheses.

RESULTS

Patient Population. The characteristics of the 28 patients who participated in the study are given in Table 1. Twenty patients were male. The median age was 56 years. Twenty-seven patients had undergone nephrectomy, and 13 of them received surgical treatment only. There were nine patients pretreated with immunotherapy (IFN-γ, IL-2, or a combination of IFN-α and IL-2 with or without lymphokine-activated killer cells), two patients with chemotherapy, and four patients with immunotherapy and chemotherapy. The results of three patients were excluded from analysis. One patient erroneously received an overdose at the first injection without major sequelae. In two patients, both with a history of atrial fibrillation, this arrhythmia recurred after the first single injection. The patients were removed from the study. Twelve patients received one treatment cycle, nine patients received two cycles, and four patients had more than two cycles with a maximum of six.

Side Effects and Laboratory Abnormalities. Common side effects and laboratory abnormalities observed after the first, as well as after repeated injections of rHuIL-12, were fever and flu-like symptoms (chills, sweating, headache, myalgia), anorexia, nausea, vomiting, fatigue, leukopenia, lymphopenia, granulopenia, anemia, thrombopenia, hypocalcemia, and elevation of hepatic enzymes. Oral mucositis, reduction of pulmonary CO diffusion capacity, and hyponatremia mainly occurred after repeated injections. Fever sometimes persisted for several days after discontinuation of rHuIL-12.

Determination of the MTD for the Single Initial Dose. The dose levels studied for the initial single dose were 0.1, 0.5, and 1.0 μg/kg. Tables 2–4 present the side effects and their grading at these dose levels.

Three patients received a single initial rHuIL-12 dose of 0.1 μg/kg. No grade 3 toxicities were observed. On dose level 0.5 μg/kg, six patients were treated and one developed a grade 3 leukopenia. Of the four patients who received 1.0 μg/kg, three developed DLT. One patient had a grade 3 leukopenia with grade 3 fever, and one experienced a grade 2 leukopenia that lasted 10 days, necessitating delay of the repetitive injection cycle. The third patient developed an erythema of the skin, which persisted for 8 days. Histology of the lesions showed a perivasculitis. Consequently, 0.5 μg/kg rHuIL-12 was regarded as MTD for the initial single dose. Therefore, the 12 patients, who were subsequently enrolled to define the MTD of repetitive injections, had their first injections fixed at a dose of 0.5 μg/kg. The side effects in these 12 patients are displayed in the bottom lines of Table 2. Of note, 3 of 12 patients experienced grade 3 leuko- and granulopenia.

Determination of the MTD for the Repetitive Administration Cycles. The dose levels studied for the repetitive administration cycles were 0.5, 1.0, and 1.25 μg/kg. In Tables 5–7, the toxicities according to these dose levels are shown. The worst observed toxicities observed in the first two treatment cycles were analyzed.

Six patients, who had received a single initial dose of rHuIL-12 at 0.5 μg/kg received the same dose from day 8 onwards, three times a week. Because 3 of the 12 additional patients at the initial single dose of 0.5 μg/kg experienced grade 3 leuko- and granulopenia, we decided to extend our experience at this dose level with repeated injections. Hence, nine patients received repeated injections, three times per week, at 0.5 μg/kg. Only one developed a grade 3 granulopenia during repeated administrations. These 9 patients had a reduction of certain side effects and laboratory abnormalities (fever, leukopenia, and elevation of hepatic enzymes) when the single initial injection was compared with the repeated injections (Tables 5–7). This effect was also observed at consecutive higher dose levels. The next three patients received repeated injections at 1.0 μg/kg without grade 3 or 4 toxicities. However, at a dose of 1.25 μg/kg, four of four patients exhibited DLT. All had grade 3 progressive fatigue with deterioration of performance status. One patient had grade 3 leukopenia and grade 4 fever, one patient had grade 3 vomiting and nausea, and one patient developed grade 3 psychoneurotoxicity (a mental depression persisting for weeks after discontinuation of rHuIL-12). As indicated in the protocol, more patients were subsequently entered at the previous dose level of 1.0 μg/kg. In two additional patients, no grade 3 or 4 toxicity was observed. Added to the three patients earlier enrolled on this dose level, a total of five patients were treated with repeated injections at 1.0 μg/kg. Because none suffered grade 3 or 4 adverse event, we declined from entering a sixth patient. Consequently, 1.0 μg/kg of rHuIL-12 was regarded as MTD for the repeated injections.

Unrelated Side Effects. Five patients developed complications unrelated to the study medication. In a patient who died of ventricular fibrillation 11 days after the last rHuIL-12 administration, autopsy showed extensive coronary atherosclerosis. A patient with a history of myocardial infarction had congestive heart failure. Of two patients with retroperitoneal lymph node metastasis, one developed hydronephrosis and renal insufficiency and the other developed extensive deep venous thrombosis. Finally, a patient had gastrointestinal bleeding, due to duodenal tumor invasion.

Tumor Response. Tumor response could be evaluated in 22 patients. A partial response, which lasted for 4 months, was observed in a patient treated with repeated injections at dose level 0.5 μg/kg. At study entry, the patient had local tumor recurrence and bone metastasis, 5 months after diagnosis of RCC and nephrectomy. He had not received prior systemic treatment. Seven patients had stable disease that lasted between

Table 2 Toxicity profile initial single injection (part 1)

Dose level (μg/kg)	No. of patients	Hemoglobin				Leukopenia				Granulopenia				Lymphopenia				Thrombopenia			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0.1	3	0	0	0	0	1	1	0	0	1	0	0	0	0	2	1	0	0	0	0	0
0.5	6	3	0	0	0	0	1	1	0	0	1	0	0	1	5	0	0	0	0	0	0
1.0	4	3	0	0	0	0	3	1	0	1	2	0	0	1	2	0	1	4	0	0	0
0.5	12[a]	2	0	0	0	3	2	3	0	3	2	3	0	3	5	2	1	2	0	0	0

[a] After defining MTD for the initial injection, the additional patients enrolled to define MTD for repeated injections received their initial injection at a dose of 0.5 μg/kg.

Table 3 Toxicity profile initial single injection (part 2)

Dose level (μg/kg)	No. of patients	Fever				Fatigue				Headache				Nausea				Vomiting			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0.1	3	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0.5	6	1	5	0	0	2	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
1.0	4	0	3	1	0	1	3	0	0	2	0	0	0	3	0	0	0	2	0	0	0
0.5	12[a]	3	9	0	0	1	1	0	0	6	0	0	0	4	0	0	0	2	1	0	0

[a] After defining MTD for the initial injection, the additional patients enrolled to define MTD for repeated injections received their initial injection at a dose of 0.5 μg/kg.

Table 4 Toxicity profile initial single injection (part 3)

Dose level (μg/kg)	No. of patients	Mucositis			Skin			Diarrhea			Pulmonary			Liver enzymes			Hyponatremia		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0.1	3	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
0.5	6	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	2	0	0
1.0	4	0	1	0	0	0	1	0	0	0	0	0	0	2	2	0	0	0	0
0.5	12[a]	0	0	0	0	0	0	0	1	0	0	0	0	2	1	0	0	2	0

[a] After defining MTD for the initial injection, the additional patients enrolled to define MTD for repeated injections received their initial injection at a dose of 0.5 μg/kg.

Table 5 Toxicity profile repeated injections, worst of cycles 1 and 2 (part 1)

Dose level (μg/kg)	No. of patients	Hemoglobin				Leukopenia				Granulopenia				Lymphopenia				Thrombopenia			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0.5	9	3	0	0	0	5	3	0	0	3	2	1	0	4	3	1	0	0	0	0	0
1.0	5	2	0	0	0	2	1	0	0	3	0	0	0	0	5	0	0	0	0	0	0
1.25	4	4	0	0	0	1	1	1	0	1	2	0	0	0	2	2	0	0	1	0	0

2 and 6 months. All other patients had progressive disease. Sudden death and cardiac failure impeded evaluation of response in two patients and in another patient who received only a first dose of IL-12, evaluation was omitted.

Pharmacokinetics. Results of the pharmacokinetic studies are shown in Table 8. Samples for pharmacokinetic analysis, after the initial injection and subsequent injections, could be obtained from all 13 patients who were enrolled to define the MTD for the initial injection. From six additional patients, entered to define the MTD of repeated injections, samples after the initial injection at 0.5 μg/kg could be analyzed. At the 0.1 μg/kg dose level, serum concentrations of rHuIL-12 were below the detection limit of the assay, after the initial single injection as well as after repeated injections. After an initial injection of 0.5 μg/kg, serum rHuIL-12 increased to a mean level of 362 pg/liter (C_{max}). Peak levels of rHuIL-12 were reached at a mean of 9.67 h (T_{max}) after administration. The mean half-life ($t_{1/2}$) of rHuIL-12 was 9.36 h. Mean C_{max}, T_{max}, and $t_{1/2}$ for repeated administrations at the 0.5 μg/kg dose level did not differ significantly from the results after the initial injection. However, after repeated dosing of rHuIL-12, a considerable decrease of 55% in serum AUC was observed. The mean AUC of 7.43 ng·h/ml after the initial injection dropped to a mean AUC of 3.33 ng·h/ml after the last injection of the first cycle of repeated administration. This difference was highly significant ($P = 0.034$). After an initial injection of 1.0 μg/kg, mean C_{max} was 1131 pg/ml, with a mean T_{max} of 17 h and a mean $t_{1/2}$ of 12.1 h. These values did not differ significantly when measured after

Table 6 Toxicity profile repeated injections, worst of cycles 1 and 2 (part 2)

Dose level (μg/kg)	No. of patients	Fever				Fatigue				Headache				Nausea				Vomiting			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0.5	9	3	4	0	0	5	0	0	0	3	1	0	0	4	0	0	0	4	0	0	0
1.0	5	0	3	0	0	2	3	0	0	1	0	0	0	1	1	0	0	1	1	0	0
1.25	4	0	2	0	1	0	0	4	0	1	1	0	0	1	1	1	0	1	1	1	0

Table 7 Toxicity profile repeated injections, worst of cycles 1 and 2 (part 3)

Dose level (μg/kg)	No. of patients	Mucositis				Pulmonary				Liver enzymes				Hyponatremia				Hypocalcemia			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0.5	9	2	1	0	0	1	0	0	0	2	0	0	0	4	1	0	0	1	0	0	0
1.0	5	2	1	0	0	2	1	0	0	4	0	0	0	2	1	0	0	1	1	0	0
1.25	4	2	2	0	0	1	1	0	0	2	1	0	0	2	2	0	0	1	0	0	0

Table 8 Pharmacokinetic data: mean (± SD) pharmacokinetic parameters at dose levels 0.5 and 1.0 μg/kg on days 1 and 19

	Unit	0.5/0.5		1.0/1.0	
		Day 1 (n = 12)	Day 19 (n = 4)	Day 1 (n = 4)	Day 19 (n = 2)
T_{max}	h	9.7 (5)	9.5 (2.2)	17 (7)	4 (0)
$t_{1/2}$	h	9.4 (3.5)	7.9 (1.6)	12.1 (3.6)	4.9 (—)[a]
C_{max}	pg/ml	362 (214)	255 (200)	1131 (1051)	376 (49)
AUC	ng × t/ml	7.4 (5.4)[b]	3.3 (1.6)[b]	31.8 (22.3)[c]	6 (2.4)[c]

[a] n = 1.
[b] P = 0.034.
[c] P = 0.041.

repeated injections. However, at this dose level, a significant decrease of 80% in serum AUC could be demonstrated as well, when comparing the AUC after repeated injections with the AUC after the initial injection (P = 0.041). In none of the patients could the development of antibodies to rHu-IL12 be detected during treatment.

DISCUSSION

The primary objective of this Phase I study was to define the MTD of an initial single s.c. administration of rHuIL12 and of subsequent repeated doses and to study pharmacokinetics. The MTD of the initial single injection was 0.5 μg/kg, whereas the MTD of the repeated administrations was 1.0 μg/kg. DLTs of the single injection consisted of fever, perivasculitis of the skin, and leukopenia. DLTs of repeated injections were progressive fatigue and deterioration of performance status, mental depression, nausea, vomiting, and leukopenia.

The MTDs are comparable with those reported in other Phase I studies (15, 16). In a trial that compared a fixed, s.c., once a week dose scheme with an up-titration schedule of IL-12, the MTDs were 1.0 and 1.5 μg/kg, respectively, and DLTs were elevation of transaminase concentration, pulmonary toxicity, and leukopenia (15). In another trial, in which IL-12 was given by i.v. injection 5 days a week, after an initial injection 2 weeks earlier, DLT consisted of liver function abnormalities and oral mucositis, and MTD was 0.5 μg/kg (16). In contrast, we did not encounter hepatotoxicity as a dose-limiting adverse event.

The toxicity profile that we observed resembles that encountered in other studies (15–17), but two patients suffered side effects not earlier described in association with IL-12 therapy. One patient, who had no psychiatric history, experienced mental depression, necessitating antidepressive medication. Although similar complaints have not been attributed to treatment with IL-12 thus far, the use of other cytokines, such as IL-2 (18) and IFN-α (19), has induced mental depression and a variety of other neuropsychiatric complaints. Another patient developed erythema of the skin, and histological examination showed a perivasculitis. In a pilot study at 0.5 μg/kg, a skin rash was observed in two patients, for whom no biopsies were available (17). Extreme progressive fatigue with severe deterioration of performance status was dose limiting for all patients treated at 1.25 μg/kg. Fatigue was not described as dose limiting in other Phase I studies. It was, however, a consistent observation in murine and primate models (13, 20). Furthermore, in an early terminated Phase II study, one-third of patients suffered grade 3 to 4 fatigue (21).

We observed a reduction of 55–80% of the mean AUC when repeated injections of IL-12 were compared with the initial injection at the same dose level. This was accompanied by reduction of side effects and resultant increase of the MTD. Motzer *et al.* (15) observed an AUC after up-titration dose escalation that was lower than the AUC of the first injection at the same dose. We could not attribute the decrease of the AUC with repeated injections to inhibited resorption or greater clear-

ance from the peripheral blood of free IL-12, because T_{max} and $T_{1/2}$ did not change significantly. Neither did we observe the development of IL-12 antibodies. However, a possible explanation, which we have not investigated, would be that concentrations of soluble IL-12 receptors increase in the course of IL-12 treatment. IL-12 has been shown to up-regulate its own receptors in peripheral blood CD56+ NK cells (22). Also, IL-12 enhances the expression of mRNA transcripts of one of the subunits of the IL-12 receptor in naive T cells (23). For other cytokines, a negative feedback mechanism operates at persistent high cytokine levels, by the increased release of soluble cytokine receptor fragments that inhibit the effects of the cytokines. The eventual existence of such a mechanism in IL-12 requires additional research.

Recently, it was shown in humans that the insertion of a treatment-free period of 1 week after the first administration of IL-12 reduces the toxicity of subsequent injections (21, 24). In a study that used a dose of IL-12 that was previously well tolerated in a schedule that was identical except for the omission of a treatment-free period after the first dose, severe toxicity and deaths occurred and necessitated early cessation of the study (16, 21). Subsequently, it was shown in murine and primate models that a single injection of IL-12 before consecutive daily dosing protected the animals from toxicity and mortality and was accompanied by reduced IFN-γ levels (20, 21, 25). Reduced IFN-γ production seems an important feature of the down-regulation of the toxic effects of IL-12 in the course of treatment (25). Many of the side effects that accompany IL-12 therapy are considered to be IFN-γ-dependent because they are also encountered in studies with IFN-γ (26, 27). In a pilot study of s.c. IL-12, 0.5 μg/kg/week, a decrease of IFN-γ peak levels was demonstrated with subsequent injections (17). Although Atkins *et al.* (16) report higher IFN-γ peak levels during the first 5-day treatment course than after the initial dose, peak levels thereafter declined during subsequent cycles. IL-10 may be another cytokine that contributes to the counterregulation of the biological effects of IL-12. We have observed a decrease of IFN-γ peak levels together with increased levels of IL-10 in the course of treatment (Ref. 28; definitive results will be published separately).

Our results raise the question whether the antitumor activity of IL-12 is down-regulated in the course of treatment as well. An unfortunate correlation may exist between IL-12-induced toxicity and antitumor efficacy.

Antibody neutralization of endogenous IFN-γ was shown to suppress antitumor effectiveness of IL-12 in murine models. Therefore, part of the antitumor effects of IL-12 seems due to induction of IFN-γ. However, exogenous administration of IFN-γ is not as effective as IL-12 (10, 11, 29). Additionally, it was shown in an animal model that giving a single dose of IL-12, a week prior to daily administration, did diminish IFN-γ induction and toxic effects but left the antitumor activity largely unaffected (20).

In conclusion, the s.c. administration of rHuIL-12 as an initial injection at a dose of 0,5 μg/kg, followed after a week rest by repeated injections at a dose of 1.0 μg/kg, 3 times a week, was well tolerated. A decrease of toxicities and a reduction in the AUC of IL-12 was observed with repeated injections.

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