Pilot Study of Subcutaneous Recombinant Human Interleukin 12 in Metastatic Melanoma

Emilio Bajetta, Michele Del Vecchio, Roberta Mortarini, Rosemary Nadeau, Ashok Rakhit, Lorenza Rimassa, Camilla Fowst, Alessandra Borri, Andrea Anichini, and Giorgio Parmiani

Divisions of Medical Oncology B [E. B., M. D. V., L. R.] and Experimental Oncology D [R. M., A. B., A. A., G. P.], Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy, and from Hoffmann-La Roche, Milan, Italy [C. F.] and Nutley, New Jersey [R. N., A. R.]

ABSTRACT

The aim of this study was to evaluate the safety profile of s.c. administered recombinant human interleukin 12 (rHuIL-12). Pharmacokinetics and pharmacodynamics of rHuIL-12 and any evidence of antitumor effect were also considered. Ten pretreated patients with progressive metastatic melanoma were enrolled in this pilot study. Patients received a fixed dose of rHuIL-12 (0.5 μg/kg) for two identical 28-day cycles, with injections given on days 1, 8, and 15 of each cycle. In case of any evidence of response or disease stabilization, the treatment was continued for two further 28-day cycles. Toxicity mainly consisted of a flu-like syndrome. Transient increases in transaminasemia (6 of 10 patients) and triglyceridemia (8 of 10 patients) were observed. Peak serum IL-12 levels were reached 8–12 h after the first injection in all patients; no serum IL-12 was detectable in 6 of 9 evaluable patients after the last injection of the second cycle. No antibody response to rHuIL-12 could be detected in any of the patients. A marked, transient reduction in circulating CD8* and CD16* lymphocytes and neutrophils was observed after the first administration and high levels of serum IFN-γ and IL-10 were detected in all patients within 24–48 h. Tumor shrinkage, not reaching partial or complete remission, involved the regression of s.c. nodules in accordance with 18 U.S.C. Section 1734 solely to payment of page charges. This article must therefore be hereby marked by the costs of publication of this article were defrayed in part of 3 patients), superficial adenopathies (1 of 3 patients), and all levels of serum IFN-γ and IL-10 were detected in any of the patients. A marked, transient reduction in circulating CD8* and CD16* lymphocytes and neutrophils was observed after the first administration and high levels of serum IFN-γ and IL-10 were detected in all patients; no serum IL-12 was detectable in 6 of 9 evaluable patients after the last injection of the second cycle. No antibody response to rHuIL-12 could be detected in any of the patients. A marked, transient reduction in circulating CD8* and CD16* lymphocytes and neutrophils was observed after the first administration and high levels of serum IFN-γ and IL-10 were detected in all patients within 24–48 h. Tumor shrinkage, not reaching partial or complete remission, involved the regression of s.c. nodules (2 of 3 patients), superficial adenopathies (1 of 3 patients), and hepatic metastases (1 of 3 patients); regressions were detected after the first cycle of treatment and were maintained in spite of progression at different sites. s.c. rHuIL-12 treatment was well tolerated and had marked effects on immune parameters and potential antitumor activity.

INTRODUCTION

The incidence of melanoma is rapidly increasing in various countries (1, 2). When the disease recurs after the surgical excision of a primary lesion, a proportion of patients can benefit from adjuvant therapy with IFN-α2b (3), whereas chemotherapy or radiotherapy have little impact on most advanced metastatic patients. Dacarbazine [5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide] is the most effective monotherapy, with an overall response rate of ~20%, but durable responses are uncommon and cure is very rare (4). Available results suggest that no more than 25% of patients may transiently benefit from current biological therapies (Refs. 5–7; rHuIFN-α, IL-2 with or without lymphokine-activated killer cells), and although a 34% response rate has been reported in the case of tumor-infiltrating lymphocytes + IL-2, this treatment is highly toxic, costly, and difficult to use routinely (8).

These results highlight the need for new therapeutic approaches. There is evidence that human melanoma may be responsive to immunological therapies aimed at boosting the T cell-mediated response to tumor-associated antigens. In fact, the results of various studies indicate that human melanoma cells express a wide range of genes coding for tumor antigens recognized by T cells (9). Furthermore, analysis of the T-cell receptor repertoire in melanoma lesions has revealed selective expansions of T cell clonotypes with specificity for autologous tumor (10). These findings support the notion that at least some human melanomas may be immunogenic in vivo in the autologous host, which provides the rationale for attempting new therapeutic approaches that may have an impact on the activation, proliferation, and function of tumor-specific T cells and thus improve the host response to autologous tumors.

IL-12 is a relatively new cytokine with a number of biological functions that justify its potential use as a modulator of the immune response to neoplastic cells, including the stimulation of proliferation and activation of both NK cells and CTLs and the promotion of Th1-dependent responses (11). Furthermore, experimental models of immunotherapy have indicated that IL-12 has significant antineoplastic activity based on various mechanisms. IL-12 treatment in mice induces the infiltration of neoplastic lesions by NK cells, as well as by CD4+ and CD8+ T lymphocytes (12, 13). Leukocyte subset depletion studies have revealed that the NK and T cells infiltrating the
lesions are responsible for the antitumor activity and that IL-12 treatment can induce a long-lasting antitumor immunity (12–15) that is at least partially mediated by the promotion of Th1-dependent responses (16, 17). Furthermore, although the anti-neoplastic activity of IL-12 is not entirely dependent on IFN-γ (12), the therapeutic efficacy of IL-12 is abrogated by systemic treatment with anti-IFN-γ mAb (18) in some animal models, thus underlining the relevance of this cytokine in T cell and NK cell-mediated responses.

In addition to boosting T cell-mediated immunity to tumor antigens, new therapeutic approaches to melanoma aim at blocking tumor-dependent angiogenesis, a process that is affected by IL-12. Human melanomas produce angiogenic factors such as VEGF/vascular permeability factor that can increase tumor growth and metastasis (19), and it has recently been shown that the inhibition of melanoma angiogenesis leads to the impairment of growth and metastasis formation (20). Tumor-induced angiogenesis can be inhibited by IL-12 (21, 22). This occurs by means of an IFN-γ-dependent mechanism that is mediated by the chemokine IP-10, a potent antiangiogenic factor (23). Finally, a further antitumor effect induced by IL-12 (once again mediated by IFN-γ) is dependent on the synthesis of an inducible type of nitric oxide synthase, which in turn drives the synthesis of nitric oxide, a potential inhibitor of tumor growth (24).

On the basis of these experimental results, 10 patients with advanced melanoma were enrolled in a pilot study aimed at establishing the safety of a fixed s.c. rHuIL-12 dose of 0.5 μg/kg. Pharmacokinetics and pharmacodynamics of rHuIL-12 and any evidence of antitumor effect were also considered.

**PATIENTS AND METHODS**

**Patient Selection.** Italian Ministry of Health regulations allow a maximum of 10 patients to be treated with a drug that has never been used before. The eligibility criteria were as follows: measurable and/or evaluable, histologically confirmed, advanced and/or metastatic melanoma; Karnofsky performance status ≥80%; age, 18–75 years; a life expectancy of at least 4 months; no clinical or computerized tomography scan evidence of brain metastases; no clinical history of significant cardiovascular diseases or liver or metabolic/endocrine disorders; no significant extravascular fluid accumulations, such as ascites or pleural effusion; no systemic bacterial/fungal infections or Th1-mediated autoimmune diseases (such as rheumatoid arthritis and systemic lupus erythematosus); prior therapy limited to one chemotherapy regimen and/or one immunotherapy regimen for metastatic disease; creatinine, ≤1.5 mg/dL; bilirubin, ≤1.2 mg/dL; WBC count, ≥3,000/μL; platelet count, ≥75,000/μL; granulocytes, ≥2,000/μL; hemoglobin, ≥10 g/dL; aspartate aminotransferase/alanine aminotransferase, ≤2.5 × normal; alkaline phosphatase, ≤2.5 × normal; and normal CO diffusion capacity. Patients were considered ineligible for entry in the presence of any one of the following criteria: major surgery during the previous 4 weeks; chemotherapy, immunotherapy, hormonal treatment, or radiotherapy within the previous 3 weeks; depot systemic corticosteroid treatment within the previous 6 weeks; seropositivity for hepatitis B or C virus or HIV; or blood transfusions or growth factors administered within the previous 2 weeks. All of the enrolled patients had received only one line of biological treatment and/or chemotherapy for metastatic disease (Table 1). The treatment protocol was approved by the Ethical and Scientific Committees of our Institute, and written informed consent was obtained from each patient. rHuIL-12 was supplied by Hoffmann-La Roche.

**Study Design.** This study was an open-label, nonrandomized, single-center pilot study designed to evaluate the safety, tolerability, pharmacodynamics, and pharmacokinetics of s.c. administered rHuIL-12 in patients with metastatic melanoma. All of the patients received two identical 28-day cycles, with the s.c. injections given on days 1, 8, and 15 of each cycle. The injection sites were rotated (e.g., day +1 in the right upper arm, day +8 in the left upper arm, day +15 in the right shoulder, and so forth). A fixed dose of 0.5 μg/kg was used throughout the study, but if a patient experienced grade III or recurrent grade II toxicity, this was reduced to 0.3 μg/kg. The 0.5 μg/kg dosage was chosen on the basis of preliminary results of the United States Trial SO 14547. In that study, the maximum tolerated dose was reached at 1.0 μg/kg, given once weekly with two grade III toxicities (increase in transaminases and leukopenia). Therefore, 0.5 μg/kg was the only reasonable and acceptable maximum dosing scheme available. Tumor response was assessed at the end of the second cycle, after which, the responding patients and those with stable disease could receive a maximum of two additional cycles. After completing the last cycle, the patients were followed up for a further 28 days to check for any adverse event. Vital signs, including blood

---

4 A. Rakhit, personal communication.
pressure, pulse, and temperature, were measured at frequent and regular intervals on days 1, 2, 5, 8, 12, 15, and 19 during the first cycle and on days 1, 8, and 15 during subsequent cycles. On days 1, 8, and 15 of each cycle, the measurements were made before the injection and 8 and 16 h after the injection. Weight was measured prior to each rHuIL-12 administration. Electrocardiogram and CO diffusion capacity were recorded at baseline and on day 19 ± 3 of each cycle. In the case of grade II fever, 500 mg paracetamol p.o. every 8–12 h was provided; no other concomitant medications were routinely administered. Toxicity was assessed using the National Cancer Institute Common Toxicity Criteria (25). The patients experiencing grade I pulmonary toxicity (except cough), grade II hypotension, weight gain, or an increase in transaminases levels plus a decrease in the number of leukocytes were to be hospitalized, and the treatment was discontinued until recovery. For any patients experiencing grade III toxicity, drug administration was withheld and then, when the toxicity had recovered to grade 0–1, the therapy was continued at a dose of 0.3 µg/kg. If grade III toxicity occurred, the treatment was discontinued unless the investigator positively decided otherwise.

Patient Characteristics. Ten patients entered the study: five males and five females, with a median age of 50 years (range, 26–61). The Karnofsky performance status was 100% in eight patients and 80% in the remaining two patients. The initial dose given to all patients was 0.5 µg/kg; it was reduced to 0.3 µg/kg in one patient because of grade III neutropenia after the first and second administration of the first cycle, possibly as a result of a previous heavy treatment with dacarbazine and Fomustine. This patient received only one cycle of treatment. The remaining nine patients all received at least two cycles, and two follow-up cycles were given to two responsive patients. The main sites of metastatic disease were soft tissue (80%) and lung (40%), followed by superficial adenopathies (20%), abdominal nodes or organs, and liver (Table 1). All of the patients had undergone surgery of primary lesion. Patient 1 had received immunotherapy with IFN-α, initially in adjuvant setting and then for metastatic disease for 4 months. All of the other patients had received chemotherapy, and three had also received radiotherapy for superficial adenopathies; three had received hormonal therapy with tamoxifen; and seven had received immunotherapy with IL-2 and/or IFN-α.

rHuIL-12 Pharmacokinetics. Serial blood samples (2 ml) were collected immediately before the rHuIL-12 injection and 4, 8, 12, 24, 30, and 48 h after the first injection (day +1) of the first cycle. During the second cycle, the samples were taken immediately before the last injection (day +15) and then 4, 8, 12, 24, 30, and 48 h afterward. The serum levels of rHuIL-12 were measured using a two-step assay involving antibody capture followed by a functional bioassay; this method is specific for rHuIL-12 and has a sensitivity limit of about 50 pg/ml.

Pharmacodynamics. Blood (10 ml) and urine samples (10 ml of a 12-h collection) were taken at baseline and 24, 48, and 96 h after the first injection of the first cycle. During the second cycle, the samples were taken 24 h after the first injection, 24 h after the second injection, and before and 24, 48, and 96 h after the third injection. During the fourth week of the second cycle, one end-of-study sample was taken. Serum levels were determined using commercially available ELISA kits for the following factors: IL-2, IL-4, IL-6, IFN-γ, TNF-α, GM-CSF, TGF-B1, IL-10, (Genzyme Corporation, Cambridge, MA), IL-8 (Bender MedSystems, Vienna, Austria), and bFGF (Oncogene Science, Cambridge, MA). ELISA kits were also used to determine the levels of bFGF and VEGF (Genzyme Corporation) in urine samples. The leukocyte subsets were evaluated by means of direct immunofluorescence using phycoerythrin-conjugated antibodies (Becton Dickinson, Sunnyvale, CA) on whole blood, followed by flow cytometry analysis using a FACScan instrument (Becton Dickinson). For each sample, the proportion of lymphocytes, monocytes, and granulocytes was established by gating on forward versus side scatter plots. The following markers were analyzed: CD3, CD4, CD8, CD16, CD30, and CD45RO. The expression of each marker was evaluated on the whole leukocyte population or on lymphocytes only, after appropriate gating on forward versus side scatter plots.

rHuIL-12 Antibodies. The serum for anti-rHuIL-12 antibody determinations (2-ml blood samples) was obtained prior to treatment and at the beginning of each administration (with the exception of day 8 of the second cycle). A further sample was drawn during the off-study observation period (19–28 days after the last injection). The sensitivity limit of the antibody detection assay was 29.3 ng/ml.

RESULTS

rHuIL-12 Pharmacokinetics

IL-12 was detected in the serum of 9 of the 10 patients as early as 4 h after the first s.c. injection (Fig. 1); in the remaining patient (patient 8), it was found 8 h after the injection. Circulating IL-12 peaked 8–12 h after the first injection in all of the patients; the mean peak value was 250 ± 122 pg/ml (Fig. 1). The levels dropped below detectable values 24–30 h after drug administration in most patients. The same monitoring of IL-12 levels was repeated after the last injection (day +15 of the second cycle). At this time, there was no detectable IL-12 in the serum of 6 of 9 evaluable patients up to 48 h after the injection (patient 6 withdrew from trial at the end of the first cycle; Fig. 1). In the remaining three cases (patients 4, 5, and 10), the peak IL-12 values were much lower than after the first injection of the first cycle: the mean (± SD) peak concentration in these patients was 94 ± 35 pg/ml between 8 and 12 h after the injection. The estimated serum half-life of rHuIL-12 (Table 2) was 12 h after the first dose. The mean apparent systemic clearance was 1.22 ml/min/kg after single s.c. dose of 0.5 µg/kg. The pharmacokinetic parameters could not be evaluated at the end of the second cycle because of the nonmeasurable levels of IL-12 in serum.

These findings indicate that the initial s.c. rHuIL-12 administration led to significant serum levels of the cytokine; however, between the first and the last injection, an adaptive response occurred in all of the patients that progressively inhibited the diffusion of rHuIL-12 into the bloodstream or led to more rapid clearance of rHuIL-12 from the blood. The evaluation of the patients’ sera failed to reveal the presence of anti-rHuIL-12 antibodies in any of the patients up to 28 days after the completion of the two treatment cycles (data not shown), thus indicating that the progressive inhibition of IL-12 diffusion into
Table 2  Main pharmacokinetic parameters after the first IL-12 injection

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value, mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{max}}$ (h)$^a$</td>
<td>10 (2.8)</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (pg/ml)</td>
<td>277 (139)</td>
</tr>
<tr>
<td>$t_{1/2,\text{elim}}$ (h)</td>
<td>12 (4.1)</td>
</tr>
<tr>
<td>CL/F (ml/min/kg)</td>
<td>1.22 (0.31)</td>
</tr>
<tr>
<td>AUC 0–∞ (pg/h/ml)</td>
<td>7357 (2314)</td>
</tr>
</tbody>
</table>

$^a$ $T_{\text{max}}$, time at which maximum plasma concentration was reached; $C_{\text{max}}$, maximum concentration at $T_{\text{max}}$; $t_{1/2\,\text{elim}}$, elimination half-life; CL/F, clearance; AUC 0–∞, area under the concentration versus time curve extrapolated to infinity.

Fig. 1  Serum IL-12 levels in melanoma patients receiving s.c. rHuIL-12. Circulating IL-12 was measured by ELISA in serum samples obtained from each patient immediately before the first IL-12 injection (hour 0) and then at 4, 8, 12, and 24 h (day 1), as well as at 30 and 48 h (day +2) after the first IL-12 administration. The same schedule of serum sampling was repeated on days +43 and +44 after the last IL-12 administration (day +43). Patient 6 (O) withdrew from the trial at the end of the first cycle.

dehydrogenase, but no change in alkaline phosphatase or bilirubin was observed; eight patients experienced a transient increase in triglyceridemia, with an inversion of the cholesterol/triglyceridemia ratio that returned to normal in a median of 12 days in most cases. Evidence of pulmonary toxicity was observed in only two patients, as indicated by a grade I reduction in CO diffusion capacity; this side effect was not dose-related, and lung function subsequently improved even when rHuIL-12 treatment was continued at the same dosage. One case of hyperfibrinogenemia and two cases of anemia not requiring transfusion were also observed. Grade III neutropenia and grade I thrombocytopenia occurred in the same patient. There were no significant changes in serum creatinine levels, nor any signs of neurological toxicity or autoimmune phenomena. Only one patient experienced grade I stomatitis, which spontaneously resolved within a few days. No patient died because of treatment-related toxicity, nor was any case of infection observed.

Treatment-related Toxicity

A detailed list of the observed treatment-related toxic reactions is shown in Table 3. A flu-like syndrome affected 100% of patients; grade II fever was easily treated with paracetamol. The fever began within 12–24 h of administration and waned in a median of 3 days. The common side effects associated with fever included chills, fatigue, and arthromyalgias. No nausea or vomiting was observed. Transient increases in serum transaminases occurred in six patients, but the values returned to within the normal range at the end of the treatment. Additional abnormalities in liver function consisted of an increase in lactate dehydrogenase, but no change in alkaline phosphatase or bilirubin was observed; eight patients experienced a transient increase in triglyceridemia, with an inversion of the cholesterol/triglyceridemia ratio that returned to normal in a median of 12 days in most cases. Evidence of pulmonary toxicity was observed in only two patients, as indicated by a grade I reduction in CO diffusion capacity; this side effect was not dose-related, and lung function subsequently improved even when rHuIL-12 treatment was continued at the same dosage. One case of hyperfibrinogenemia and two cases of anemia not requiring transfusion were also observed. Grade III neutropenia and grade I thrombocytopenia occurred in the same patient. There were no significant changes in serum creatinine levels, nor any signs of neurological toxicity or autoimmune phenomena. Only one patient experienced grade I stomatitis, which spontaneously resolved within a few days. No patient died because of treatment-related toxicity, nor was any case of infection observed.

Pharmacodynamics

Effects on Leukocyte Subsets. One day after the first administration (day +2 of the cycle), a marked leukopenia was evident in all patients. The mean total number of circulating lymphocytes dropped from a pretreatment value of $1.49 \pm 0.45 \times 10^3$/mm$^3$ on day −4 to $0.53 \pm 0.22 \times 10^3$/mm$^3$; 7 days after the first injection, the number of lymphocytes returned to pretreatment values ($1.45 \pm 0.59 \times 10^3$/mm$^3$). Monocytes showed a similar pattern of decline and recovery but the initial
Table 3  Toxicity profile

<table>
<thead>
<tr>
<th>Category of toxicity</th>
<th>Toxicity</th>
<th>% of course</th>
<th>Grade*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic</td>
<td>Flu-like syndrome</td>
<td>100%</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>Fever</td>
<td>90%</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>Skin rash</td>
<td>20%</td>
<td>I</td>
</tr>
<tr>
<td>Local (site of injection)</td>
<td>Subcutaneous induration</td>
<td>10%</td>
<td>I</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>CO diffusion capacity</td>
<td>20%</td>
<td>I</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Stomatitis</td>
<td>10%</td>
<td>I</td>
</tr>
<tr>
<td>Hepatic</td>
<td>Transitory</td>
<td>60%</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Hypertransaminasemia</td>
<td>20%</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Increased levels of lactate dehydrogenase</td>
<td>20%</td>
<td>I</td>
</tr>
<tr>
<td>Hematological</td>
<td>Anemia</td>
<td>20%</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>Neutropenia</td>
<td>10%</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td>Thrombocytopenia</td>
<td>10%</td>
<td>I</td>
</tr>
<tr>
<td>Coagulation</td>
<td>Hyperfibrinogenemia</td>
<td>10%</td>
<td>I</td>
</tr>
<tr>
<td>Metabolic</td>
<td>Hypertriglyceridemia</td>
<td>80%</td>
<td>I</td>
</tr>
</tbody>
</table>

* National Cancer Institute Common Toxicity Criteria.

reduction was less pronounced (day-4: 0.41 ± 0.13 × 10^3/mm^3; day +2: 0.28 ± 0.11 × 10^3/mm^3; day +8: 0.46 ± 0.16 × 10^3/mm^3). On the contrary, neutrophil kinetic pattern was different, with a marked reduction becoming evident 4 days after the first injection (from 3.55 ± 1.33 × 10^3/mm^3 on day −4 to 1.74 ± 1.22 × 10^3/mm^3 on day +5), followed by an almost complete recovery on day +7 (2.83 ± 1.37 × 10^3/mm^3). Interestingly, all these effects were either absent (in the case of lymphocytes and neutrophils) or much weaker (in the case of monocytes) between 1 and 5 days after the last injection of the second cycle (data not shown).

The effect of rHuIL-12 treatment on the leukocyte subsets was also evaluated by means of flow cytometry on whole blood and confirmed a marked alteration in the relative proportions of circulating leukocytes in all of the patients within 24 h of the first injection of the first treatment cycle (Fig. 2). By gating on forward versus side scatter plots, a marked reduction in the proportion of lymphocytes associated with an apparent increase in granulocytes was observed. On day +5 of the cycle, flow cytometry analysis confirmed the reduction in granulocytes already indicated by the conventional neutrophil count: at the same time there was a transient increase in the proportion of monocytes that reflected the decrease in the number of granulocytes. A similar analysis made on day +44 (24 h after the last injection of the second cycle) revealed a much more limited change in the proportions of leukocytes than on day +2 (Fig. 2). Staining with anti-CD3, CD4, CD8, CD16, CD30 and CD45RO mAbs revealed a major shift in the CD4:CD8 ratio on day +2 of the first cycle (24 h after the first injection on day +1); whereas the proportion of CD3+ T cells remained constant (data not shown), there was a marked reduction in CD8+ cells and an increase in CD4+ cells leading to a transient inversion of the CD4:CD8 ratio in all patients on day +2 (Fig. 3). There was also a clear reduction in CD16+ cells (Fig. 3). None of these changes were observed on day +44, with the exception of a decrease in CD16+ cells in some of the patients. No significant changes were observed in the proportion of cells expressing the other markers CD30 and CD45RO.

Pharmacodynamics

Effects on Serum Cytokine Levels. The serum/urine levels of a number of cytokines/growth factors were measured by means of ELISA during the planned two cycles of treatment. The serum samples from each patient were tested for the presence of IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ, TNF-α, GM-CSF, TGF-β1, and bFGF. The serum IFN-γ levels in all of the patients markedly increased within 24 h of the first rHuIL-12 injection (Fig. 4), reaching a peak concentration in the ng/ml range in six patients; in most cases, these levels returned to their baseline values within 3–6 days. The increase in serum IFN-γ was also observed during the second cycle of treatment, but with the exception of patients 8 and 9, the peak levels were much lower than those observed after the first rHuIL-12 injection (Fig. 4). IL-10 was the only other cytokine the serum levels of which considerably increased in all of the patients. As shown in Fig. 5, the IL-10 modulation pattern was more complex than in the case...
of IFN-γ. In 5 of 9 evaluable cases (patients 4, 5, 8, 9, and 10), the highest IL-10 levels were observed during the second cycle of treatment rather than immediately after the first rHuIL-12 injection. However, as in the case of IFN-γ, the increase and decline in IL-10 levels appeared to be completed within 3–6 days after rHuIL-12 administration.

All of the other factors showed a less consistent pattern of change. Only 1–2 patients displayed moderate increases or decreases in serum IL-2, IL-4, TNF-α, and GM-CSF and urine VEGF levels in comparison to baseline (data not shown). IL-6, IL-8, TGF-β1, and bFGF levels were up- or down-regulated in 30–60% of the patients (data not shown), but no correlation could be established between these changes and the clinical response to rHuIL-12 treatment. In two of the three responding patients, we observed a reduction in urinary bFGF levels (a drop in patient 1 and a minor reduction in patient 5; data not shown); this could support a possible angiogenic activity of IL-12.

Clinical Responses

The details of the responding patients are shown in Table 4. Three of the 10 patients achieved regressions of some tumor lesions. One patient (patient 1), who had been pretreated with surgery and IFN-γ (first in an adjuvant setting and then for metastatic disease) and had three s.c. nodules (diameters, 1, 1, and 0.5 cm) and an abdominopelvic mass (global diameters, 8 × 3 cm), received a total of four cycles of rHuIL-12. After only two administrations (first cycle), the s.c. lesions had disappeared; at the end of the second cycle, the deep adenopathies showed no change, and the complete response of the s.c. lesions continued. This patient therefore underwent two additional cycles, during which two s.c. nodules reappeared in the same location, one of which subsequently disappeared, possibly as a result of a late response. After four cycles, computerized tomography revealed a progression in the abdominopelvic nodes.

The second responding patient (patient 5) had been pretreated with chemo/immuno/hormonal therapy consisting of a combination of dacarbazine, carboplatin, mytomycin-C, tamoxifen, and IFN-α. This patient had soft tissue and abdominopelvic node disease. The first cycle led to the complete remission of the soft tissue lesions (a superficial adenopathy with a diameter of 2 × 3 cm and a s.c. lesion with a diameter of 0.5 cm); this response continued, but at the end of the fourth cycle, ultrasonography revealed marked progression in the deep nodes.

The third patient (patient 10), who had been pretreated with hyperthermic L-phenylalanine mustard perfusion, had s.c. tissue and hepatic parenchyma disease. After the first two rHuIL-12 injections, a complete remission of hepatic lesions occurred (documented by ultrasonography and then by nuclear magnetic resonance), but progressive s.c. disease, with the appearance of a number of confluent and echo-documented nodules, was also observed (Table 4).

Another patient (patient 8) had s.c. disease (at least seven mammographically confirmed right breast nodules with a maximum diameter of 1 cm and a left arm nodule with a diameter of 2 cm) and a right adrenal lesion. Given that disease stabilization was observed at the end of the first two cycles, the treatment was continued; however, a new s.c. nodule appeared under the right eye after the first administration of the first follow-up cycle, and so the treatment was stopped.

DISCUSSION

IL-12 is a cytokine of great immunological interest that has been shown to have significant antitumor activity in a number of animal models. The present trial of rHuIL-12 in metastatic melanoma was designed to evaluate its side effects, pharmacokinetics, and immunological parameters and to document any evidence of clinically relevant activity. The results indicated that the once-weekly, fixed-dose s.c. administration of rHuIL-12 for two consecutive 28-day cycles is a well-tolerated regimen that has significant pharmacodynamic effects on circulating leukocytes and on serum levels of various cytokines. Furthermore, although there were no partial or complete remissions, tumor...
regression in the form of mixed responses was induced in 3 of 10 previously treated patients with metastatic melanoma. The treatment was well tolerated by most patients, and no pulmonary toxicity was observed in spite of the evidence obtained in experimental models (26). Many of the side effects observed after rHuIL-12 administration (in particular, liver function abnormalities, fever, fatigue, hematological changes, and hypertriglyceridemia) may reflect the induction of IFN-γ because similar effects have been observed in clinical trials using IFN-γ (27, 28). Fever (the most frequent side effect) was unusually delayed: it typically occurred 12 h after injection, rather than the 2–4 h observed with the use of other pyrogenic cytokines, such as IL-1, IL-2, and the IFNs. Taken together, these results indicate that s.c. administered rHuIL-12 has greater immunomodulatory effects and suggest that it may have therapeutic potential in advanced melanoma.

The pharmacokinetics of the fixed-dose regimen were associated with a striking difference between the first and last rHuIL-12 injection. IL-12 could be readily detected in the serum of all of the patients after the first injection, but not in 6 of 9 evaluable patients 48 h after the last injection of the second cycle; in the remaining three evaluable patients, peak serum levels after the last injection were much lower than after the first administration. This reduction in serum IL-12 levels at the end of the two cycles of treatment suggests that an adaptive response occurred in all of the patients and led either to greater removal of free IL-12 from peripheral blood or the inhibition of the access of the injected rHuIL-12 to the bloodstream. This adaptive response could not be explained on the basis of an antibody response to the injected cytokine because no anti-IL-12 antibodies could be detected in any of the patients. One possibility is that the initial rHuIL-12 injections led to an up-regulation of the high-affinity receptors for this cytokine: it is known that high-affinity IL-12 receptors are expressed on activated T and NK cells (29) and that such receptors are composed of two distinct β-type subunits (30). The initial rHuIL-12 injections may "prime" the host by leading to an enhanced expression of high-affinity IL-12 receptors, and this process may, subsequently, provide a mechanism for the enhanced suppression of serum IL-12 levels after later injections. In mice, the administration of IL-12 up-regulates the expression of the mRNA for both the β1 and β2 IL-12 receptor subunits, and an inverse
correlation has been found between the levels of IL-12-receptor mRNA and serum IL-12 levels, which supports the hypothesis that there is a positive feedback loop.

The contrast between the high initial serum levels of IL-12 after the first injection and the low or zero levels detected at the end of the two-cycle treatment was reflected by the pattern of changes in leukocyte subsets and IFN-γ levels. Interestingly, the progressive attenuation of serum IFN-γ levels at subsequent IL-12 injection has been observed even when IL-12 is i.v. administered (31). More importantly, all local clinical responses of superficial adenopathies and soft tissues appeared after the first two injections of the first cycle: only one late response, involving a s.c. lesion, occurred, during the follow-up cycles administered to patient 1. No responses occurred during the second cycle. The available evidence, therefore, suggests that a high initial serum level of IL-12 has both immunomodulatory and antitumor effects. Continued antitumor activity during or after the second cycle of treatment may be inhibited by the same adaptive mechanism(s) that blocks the presence of significant levels of IL-12 in peripheral blood. On the other hand, the kinetics of IL-10 serum levels in 5 of 9 patients indicate that peak levels were reached during the second cycle of rHuIL-12 injections, thus suggesting that the cytokine still had a significant, although greatly reduced, biological activity.

rHuIL-12 treatment led to a marked but transient reduction in circulating leukocyte subsets levels. The lowest lymphocyte and neutrophil counts were observed 24 h and 4 days after rHuIL-12 injection, respectively, which suggests that they were partially removed from the circulation by different mechanisms. The drop in the proportion of CD16+ cells 24 h after rHuIL-12 injection was not unexpected because of the known chemotactic activity of IL-12 in relation to NK cells and its ability to stimulate the adhesion of NK cells to the vascular endothelium (32). Furthermore, the induction of high IFN-γ levels after rHuIL-12 injection may lead to the synthesis of IP-10, a known chemoattractant and stimulator of adhesion of monocytes and T cells to endothelial cells (33, 34). Interestingly, IP-10 mRNA has recently been detected in the lymphocytes of a series of patients with renal cell carcinoma receiving s.c. IL-12 (35). Additional indirect mechanisms triggered by IL-12 may contribute to explaining the drop in neutrophil counts and may involve the induction of adhesion molecules, such as E-selectin (induced by IL-10 and IFN-γ; Refs. 36–38) or ICAM-1 (39), on endothelial cells.

In line with the known biological functions of IL-12 (40), IFN-γ and IL-10 were transiently induced in all treated patients. IL-12 can induce concomitant IL-10 and IFN-γ secretion by CD4+ and CD8+ T cells (41, 42), and therefore, it was not

---

5 Maurice Gately (Hoffmann-La Roche Inc., Nutley, NJ) personal communication.
unexpected to find both cytokines in the serum of the patients. However, the kinetics of the two cytokines were different: unlike IFN-γ, in 6 of 9 patients, peak serum IL-10 levels were observed during the second rather than the first cycle of treatment. IL-10 is a major inhibitor of IL-12 production by antigen presenting cells, and its production in response to IL-12 is regarded as a negative feedback mechanism (43). Therefore, it is possible that the increased IL-10 levels detected in the sera of many patients during the second cycle of treatment may reflect an ongoing negative feedback response aimed at contrasting the presence of IL-12.

The original hypothesis that IL-10 may be considered a down-regulator of cell-mediated immunity (44) has been partially contradicted by more recent results. In fact, the systemic administration of high IL-10 doses to mice bearing established tumors (including melanoma) led to tumor rejection, an effect that was abrogated by sublethal irradiation (45). Furthermore, the injection of IL-10 can suppress both experimental and spontaneous metastases in mice bearing murine or human melanomas, by means of a process dependent on NK cells (46). On the basis of this evidence, the induction of IL-10 in all patients of this study may be considered as a contribution to the antitumor activity of systemic rHuIL-12 (1 transient complete response out of 12 melanoma patients). This may depend on individual patient characteristics, but it is also possible that differences in the route (i.v. versus s.c.) and in the schedule of cytokine administration between the two studies may play a role in the antitumor efficacy of IL-12.

Finally, experimental models indicate that the therapeutic effects of IL-12 are dose dependent (14, 15); thus, it is possible that an increased antitumor activity in patients may be obtained by changing the dose and/or the administration schedule. In the present study, a dose higher than 0.5 μg/kg was not attempted. A possibility to be investigated in future trials is the safety and tolerability of a 0.75 μg/kg dose, which may improve the clinical efficacy of rHuIL-12, possibly without reaching the limiting liver toxicity and leukopenia observed at 1.0 μg/kg in the United States Trial SO 14547 (as mentioned in “Patients and Methods”). The problem of dose-finding is highly relevant because the possibility of increasing dosages without reaching limiting toxicity could lead to an improvement in the therapeutic index. A further and perhaps more significant challenge facing any improvement in the therapeutic efficacy of this cytokine is to devise protocols that can reverse or contrast the adaptive response that leads to the progressive inhibition of IL-12 serum levels. It is of note that the absence of a measurable antibody response to the injected cytokine suggests that the recombinant form of human IL-12 lacks immunogenicity in human hosts, thus providing a significant advantage for further clinical trials.

**ACKNOWLEDGMENTS**

We thank Dr. M. Gately (Hoffmann-La Roche Inc.) and Dr. H. Parmar (Roche Products Ltd., Welwyn Garden City, United Kingdom) for critically reading the manuscript. We also thank G. Pezzaglia, G. Mangiacorti, E. Di Dedda, A. Tamburo, and L. Cennamo for their nursing support.

**REFERENCES**


Pilot study of subcutaneous recombinant human interleukin 12 in metastatic melanoma.

E Bajetta, M Del Vecchio, R Mortarini, et al.

*Clin Cancer Res* 1998;4:75-85.

Updated version  Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/4/1/75