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

Jared A. Gollob,^2 James W. Mier, Korina Veenstra, David F. McDermott, Daniel Clancy, Marguerite Clancy, and Michael B. Atkins
Beth Israel Deaconess Medical Center, Division of Hematology/Oncology, Boston, Massachusetts 02215

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. Cancer patients treated at the MTD had prolonged disease stabilization, with one of these exhibiting tumor regression. Two additional renal cell cancer patients treated at the MTD had prolonged disease stabilization, with one of these exhibiting tumor regression.

INTRODUCTION
IL-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
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 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, aspartate aminotransferase <2 times the upper limit of normal, 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 neurologic 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 prophylactically for 24 h after each IL-12 dose and could be taken as
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 $-20^\circ\text{C}$. 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 $5 \times 10^5$ 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 $18 \times 10^9$ units/mg); (b) 1 mIU IL-12 (Genetics Institute, Cambridge, MA, specific activity $1.7 \times 10^6$ units/mg); (c) 10 ng/ml IL-15 (Endogen, specific activity $2 \times 10^6$ 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 $37^\circ\text{C}$, aliquots of supernatants from each well were harvested immediately before pulsing each well with 1 μCi [$^3\text{H}$]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 [$^3\text{H}$]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 their prior immunotherapy.
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 in two patients enrolled at the 700-ng/kg dose level. As shown
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. 2A, 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 dose 2–3 days later (Fig. 2, A-C, top). Patients exhibiting the type-I pattern of IFN-γ induction had all been treated previously with an IL-2-based regimen and were either.

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. 2B, 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 was markedly curtailed compared with IFN-γ levels after the

---

### Table 2

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Dose level, ng/kg (no. of patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 (3)</td>
</tr>
<tr>
<td>Hepatic</td>
<td></td>
</tr>
<tr>
<td>AST*</td>
<td></td>
</tr>
<tr>
<td>Bilirubin</td>
<td>(1, 0, 0)</td>
</tr>
<tr>
<td>Alk phosphatase</td>
<td>(1, 0, 0)</td>
</tr>
<tr>
<td>Hematologic</td>
<td></td>
</tr>
<tr>
<td>Neutropenia</td>
<td>(2, 1, 1)</td>
</tr>
<tr>
<td>Anemia</td>
<td>(2, 0, 0)</td>
</tr>
<tr>
<td>Hemolytic anemia</td>
<td>(1, 0, 0)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>(2, 0, 0)</td>
</tr>
<tr>
<td>Oral mucositis</td>
<td>(1, 0, 0)</td>
</tr>
<tr>
<td>Cardiovascular (hypotension)</td>
<td>(1, 0, 0)</td>
</tr>
<tr>
<td>Cardiovascular (arrhythmia)</td>
<td>(1, 0, 0)</td>
</tr>
<tr>
<td>Fever</td>
<td>(1, 0, 0)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>(2, 0, 0)</td>
</tr>
</tbody>
</table>

* AST, aspartate aminotransferase; Alk, alkaline.

### Table 3

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>No. of cycles completed</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose escalation phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>No DLT</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>No DLT</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>Grade 2 orthostatic hypotension; IL-12 dose reduced to 300 ng/kg</td>
</tr>
<tr>
<td>13</td>
<td>6</td>
<td>No DLT</td>
</tr>
<tr>
<td>14</td>
<td>1</td>
<td>No DLT</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>No DLT</td>
</tr>
<tr>
<td>Safety phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>1</td>
<td>No DLT</td>
</tr>
<tr>
<td>22</td>
<td>2*</td>
<td>Grade 4 neutropenia (agranulocytosis); IL-12 discontinued</td>
</tr>
<tr>
<td>23</td>
<td>1</td>
<td>No DLT</td>
</tr>
<tr>
<td>24</td>
<td>2</td>
<td>No DLT</td>
</tr>
<tr>
<td>25</td>
<td>1*</td>
<td>No DLT</td>
</tr>
<tr>
<td>26</td>
<td>1</td>
<td>No DLT</td>
</tr>
<tr>
<td>27</td>
<td>3</td>
<td>No DLT</td>
</tr>
<tr>
<td>28</td>
<td>2</td>
<td>No DLT</td>
</tr>
</tbody>
</table>

* Received four doses in cycle 2.
* Received only two doses in cycle 1 because of rapid disease progression.
first dose. This was associated with a greatly diminished-to-absent febrile response to rhIL-12 by the third week of cycle 1. All of the three patients with this pattern of IFN-γ induction had not received any prior immunotherapy for their metastatic disease.

The type-III pattern was characterized by modest peak IFN-γ levels in response to the first dose of rhIL-12, followed by the rapid attenuation of IFN-γ production (Table 4 and Fig. 2C, top). Whereas peak IFN-γ levels reached their maximum after the second rhIL-12 dose in the type-I and type-II patterns, in the type-III pattern, peak IFN-γ levels were lower after dose 2 compared with those after dose 1 (Table 4), and they continued to decline when measured again after the seventh dose. This type-III pattern was associated with a weak-to-absent febrile response to IL-12, and, of the three patients exhibiting this pattern, all had received one course or multiple courses of high-dose IL-2 <6 months before starting the rhIL-12.

**In Vivo Induction of IL-15 and IL-18.** To determine whether IL-15 and IL-18 were induced by rhIL-12 in cancer patients and to examine whether there was an association between IL-15/IL-18 induction and IFN-γ induction by rhIL-12, we measured the plasma levels of IL-15 and IL-18 at the same time points used to measure IFN-γ levels. As shown in Fig. 2, A-C (middle), IL-15 was not detectable in the plasma before starting rhIL-12 but was detectable at low levels 4 h after the first injection. With each pattern of IFN-γ induction, plasma IL-15 levels reached their maximum either before, or at the same time as, peak IFN-γ levels. However, the magnitude of IL-15 induction did not correlate with the magnitude of IFN-γ induction, because peak IFN-γ levels after the second rhIL-12 dose in the type-I and type-II patterns were not associated with similar increases in peak IL-15 induction. Nonetheless, there was an association between the ability to sustain comparable levels of IL-15 induction during a cycle of rhIL-12 and the
Table 4  Relation between pattern of IFN-γ induction by IL-12 during cycle 1, clinical response, and cytopenias

<table>
<thead>
<tr>
<th>IFN-γ induction pattern</th>
<th>Cycle 1 Peak IFN-γ level (pg/ml)</th>
<th>IL-12 dose level (ng/kg)</th>
<th>Response</th>
<th>Cytopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disease</td>
<td>Wk1D1*</td>
<td>Wk1D4</td>
<td>Wk4D1</td>
</tr>
<tr>
<td>Type I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 10</td>
<td>RCC</td>
<td>1600</td>
<td>2560</td>
<td>1200</td>
</tr>
<tr>
<td>13</td>
<td>RCC</td>
<td>1100</td>
<td>2235</td>
<td>1340</td>
</tr>
<tr>
<td>18</td>
<td>RCC</td>
<td>450</td>
<td>1290</td>
<td>400</td>
</tr>
<tr>
<td>22</td>
<td>RCC</td>
<td>1000</td>
<td>1840</td>
<td>850</td>
</tr>
<tr>
<td>Type II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 24</td>
<td>RCC</td>
<td>2365</td>
<td>4960</td>
<td>220</td>
</tr>
<tr>
<td>26</td>
<td>RCC</td>
<td>2040</td>
<td>9060</td>
<td>248</td>
</tr>
<tr>
<td>28</td>
<td>Mel</td>
<td>1560</td>
<td>5918</td>
<td>77</td>
</tr>
<tr>
<td>Type III</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 21</td>
<td>Mel</td>
<td>2229</td>
<td>1476</td>
<td>159</td>
</tr>
<tr>
<td>23</td>
<td>Mel</td>
<td>1256</td>
<td>951</td>
<td>90</td>
</tr>
<tr>
<td>19</td>
<td>RCC</td>
<td>1800</td>
<td>890</td>
<td>125</td>
</tr>
</tbody>
</table>

*Wk1D1, week 1 day 1; Wk1D4, week 1 day 4; Wk4D1, week 4 day 1; RCC, renal cell cancer; Mel, melanoma; SD, stable disease; PD, progressive disease.

ability to sustain IFN-γ induction. As shown in Fig. 2A (middle), only small differences in the peak and plateau levels of IL-15 after the first, second, and seventh rhIL-12 doses were evident in patients with the type-I IFN-γ pattern. In contrast, there was a 50–70% drop in the peak and plateau IL-15 levels by week 4 in patients with the type-II and type-III IFN-γ patterns (Fig. 2, B-C, middle), and no IL-15 could be detected in the plasma before the seventh rhIL-12 dose in these patients.

In all of the patients tested, small amounts of IL-18 were detected in the plasma before starting rhIL-12 (Fig. 2, A-C, bottom), with higher levels present before the second and seventh rhIL-12 doses. However, plasma IL-18 levels after an rhIL-12 injection usually peaked later than IFN-γ. In addition, the loss of IFN-γ production after the seventh rhIL-12 dose among patients with the type-II and type-III IFN-γ patterns occurred despite continued IL-12 production at levels comparable with those measured immediately before and after the first rhIL-12 dose.

IL-10 Induction and rhIL-12 Pharmacokinetics. As shown in Fig. 3A, IL-10 was induced after the first dose of rhIL-12 in a similar manner in all of the patients, returning to undetectable levels before the next rhIL-12 dose. A stronger increase in IL-10 production was observed after the second dose in all of the patients, with the highest peak levels detected in patients with the type-II IFN-γ pattern (Fig. 3A, middle). However, IL-10 induction continued after the seventh dose in all of the patients, including those with the type-I IFN-γ pattern (Fig. 3A, top), as well as those with the type-III pattern exhibiting a significant suppression of IFN-γ (Fig. 3A, bottom).

When the pharmacokinetics of rhIL-12 was examined, we found that the levels of IL-12 in the plasma fell more quickly after the second and subsequent rhIL-12 doses compared with after the first dose (Fig. 3B). However, these changes were not necessarily associated with changes in IFN-γ induction. For example, for patients with the type-I IFN-γ induction pattern, plasma IFN-γ levels were comparable after the first and seventh IL-12 doses despite the more rapid drop in IL-12 levels after the seventh dose. Furthermore, as shown in Fig. 3B, after the second and seventh doses of IL-12, the molar concentrations of IL-12 measured in the plasma 4–24 h after the IL-12 injection were lower for a patient with the type-I IFN-γ pattern (Fig. 3B, top) compared with a patient with the type-III IFN-γ pattern (Fig. 3B, bottom).

Clinical Response to rhIL-12: Association with IFN-γ Induction Pattern

Among nine patients treated at rhIL-12 dose levels that were below the MTD of 500 ng/kg, there were no responses, nor did any of these patients achieve disease stabilization for more than two cycles. At the MTD (n = 14), one patient with renal cell cancer had a PR, and two others with renal cell cancer have had long-term disease stabilization for 6+ and 12+ months (Table 4). Among the 7 of 14 patients who received more than one cycle of IL-12 (Table 3), none required a break between cycles. The patient with the PR had multiple, large, pleural-based, and parenchymal lung metastases (Fig. 4A) as well as a solitary liver metastasis, and had disease progression after a course of low-dose IL-2 before starting the IL-12. After the first three cycles of rhIL-12, the patient had stable disease but, after the fourth cycle, showed the first signs of tumor regression in the lung. After six cycles, he had achieved a PR, with complete resolution of most of the parenchymal and pleural-based tumors and a 70% reduction in the size of the largest pleural-based mass (Fig. 4B). The single liver lesion remained unchanged. The rhIL-12 was stopped during the seventh cycle because of the development of a small cerebellar metastasis, which was excised. Follow-up CT scans 4 months after stopping the rhIL-12 revealed a continued regression of the remaining lung tumors (Fig. 4C).

One of the patients (Table 4, patient 10) with long-term disease stabilization had multiple lung, liver, bone, and lymph node metastases, and a history of disease progression while on high-dose IL-2. She remains on rhIL-12 therapy after having completed 8 cycles. CT scans after cycle 8 showed the first signs of tumor regression, with shrinkage of lesions in the liver and abdominal lymph nodes (not shown). The other patient with long-term disease stabilization (Table 4, patient 22) had lymph node, bone, and lung metastases and also had progressive dis-
ease before beginning rhIL-12 despite having received high-dose IL-2 1 year earlier. The rhIL-12 was discontinued in this patient because of the development of agranulocytosis, but he continues to have stable disease 4 months after the cessation of the rhIL-12.

At the 700-ng/kg dose level, one renal cell cancer patient with extensive lung, bone, and lymph node metastases, who had failed prior treatment with low-dose IL-2, received 5 weeks of rhIL-12 before it was stopped because of the development of a Coombs’ negative hemolytic anemia (Table 4, patient 18). This patient continues to have stable disease 4 months after stopping the rhIL-12. Among the remaining 15 patients treated at the 500-ng/kg and 700-ng/kg dose levels, all had disease progression during the first 1–3 cycles of rhIL-12, with the majority progressing during the first cycle.

When we examined the responses among the 10 patients selected for IFN-γ/IL-15/IL-18 analysis (Table 4), we found that, of the patients who were unable to sustain IFN-γ induction through the first cycle (type-II and type-III patterns), all had disease progression. In contrast, of the four patients who had either a PR or prolonged disease stabilization, all exhibited the type-I pattern of sustained IFN-γ induction. In addition, the two

Fig. 3 A, IL-10 induction in patients treated with rhIL-12. Plasma IL-10 levels were measured before and during a 24-h period after the first, second, and seventh rhIL-12 doses in patients with the type-I (top), type-II (middle), and type-III (bottom) IFN-γ induction patterns. Each panel shows the data from one patient in each group (similar results were obtained from two patients in each group). B, plasma IL-12 levels in patients after injections of i.v. rhIL-12. The p70 IL-12 heterodimer was measured in the plasma at regular intervals over a 20-h period starting 4 h after a rhIL-12 injection. This analysis was performed after the first (Wk1D1), second (Wk1D4), and seventh (Wk4D1) IL-12 injections during cycle 1. The results from two patients treated at the 500-ng/kg dose level are shown, including one patient with the type-I IFN-γ induction pattern (top) and the other with the type-III pattern (bottom). These are representative of what was observed in four patients treated with 500 ng/kg rhIL-12. The concentration of IL-12 represented on the Y axis is converted from pg/ml to molar concentration at several points, as indicated next to the horizontal dotted lines on each graph.
Fig. 4 Antitumor response in a patient treated with twice-weekly i.v. rhIL-12. CT scans of the chest in a patient with renal cell cancer metastatic to the lung, performed before starting rhIL-12 (A), after six cycles of rhIL-12 (B), and 4 months after the completion of six cycles of rhIL-12 (C).
episodes of cytopenias, including the Coombs negative hemolytic anemia that was responsive to steroids and the agranulocytosis that was responsive to steroids plus low-dose cyclophosphamide, occurred among patients with the type-I IFN-γ induction pattern.

Although patients with the type-II or type-III IFN-γ induction pattern all had progressive disease on rhIL-12, two patients with metastatic melanoma developed ecchymoses that appeared only over their s.c. metastases (Fig. 5). These ecchymoses developed during the first 2 weeks of rhIL-12 therapy but were not associated with any change in the size or consistency of the metastases. Neither patient had any measurable coagulopathy or significant thrombocytopenia during IL-12 therapy (data not shown), and there were no other cutaneous or mucosal changes. By the 6th week of therapy, the ecchymoses over the s.c. metastases had faded considerably in both patients, and there was no measurable tumor regression. Excisional biopsy of one of these s.c. lesions at the end of cycle 1 (at which time the overlying bruising had resolved) revealed viable tumor with no lymphocytic infiltration and no ischemic necrosis (data not shown).

Fig. 5  Skin changes in melanoma patients treated with rhIL-12. Two patients (A and B) with melanoma developed extensive ecchymoses limited to the sites of s.c. metastases during the first several weeks of rhIL-12 therapy.
Effect of rhIL-12 Therapy on Lymphocyte Cytokine Responsiveness

Among patients exhibiting the type-II and type-III IFN-γ induction patterns, we found that the proliferative response to IL-12, IL-2, and IL-15 was preserved at the midway point of cycle 1 (Fig. 6A, top). In contrast, the ability of IL-2, IL-12, and IL-15 to induce IFN-γ production was completely abrogated by the start of week 4 (Fig. 6A, bottom). The induction of IFN-γ by the combination of IL-12 plus IL-15 was also greatly diminished at the start of week 4 compared with the start of week 1. This change in IFN-γ production was not associated with any change in the number of circulating CD4+ and CD8+ T cells or NK cells, nor was it associated with any change in lymphocyte IL-12 or IL-2 receptor expression (data not shown). Although IFN-γ production in response to IL-12 plus IL-2 decreased as well during rhIL-12 therapy, the combination of IL-12 plus IL-2 still induced higher levels of IFN-γ in vitro at week 4 than those induced by IL-12 plus IL-15 at week 1 (Fig. 6A, bottom). When we examined the in vitro cytokine response of PBMC isolated from patients exhibiting the type-I pattern of IFN-γ induction, we found that proliferation and, to a large extent, IFN-γ production were preserved from weeks 1 to 4 (Fig. 6B).

NO Production in Patients Receiving rhIL-12 or IL-2

For patients treated with i.v. rhIL-12 at the 500-ng/kg and 700-ng/kg dose levels, expired air was collected before starting rhIL-12 and on the morning after the first two doses. Before rhIL-12, the mean NO concentration was 21 ± 9.5 ppb. The mean peak NO level after the first two doses of rhIL-12 was only 2.5 ± 7.8 ppb, with patients, on average, exhibiting a

Fig. 6 In vitro response of PBMCs to cytokines before and midway through the first cycle of rhIL-12. PBMCs were isolated from the blood before the start of rhIL-12 therapy (Wk1D1) and before the start of the 4th week of cycle 1 (Wk4D1). Cells were then cultured in medium alone (neg), IL-2, IL-12, IL-15, IL-2 + IL-12, or IL-15 + IL-12 for 72 h, after which both proliferation (A and B, top) and IFN-γ production (A and B, bottom) were measured. A shows the data from a patient with the type-II IFN-γ pattern (representative of three type-II patients examined), whereas B shows the data from a patient with the type-I IFN-γ pattern (representative of three type-I patients examined).
measured during IL-2 or rhIL-12 therapy. rhIL-12 (n = 5) in the expired air of patients before starting either IL-2 (n = 5) or rhIL-12 (n = 5); □, the mean peak NO concentration in expired air measured during IL-2 or rhIL-12 therapy.

1.5-fold increase (range, 1- to 2-fold) over the baseline value (Fig. 7). The one patient with the modest 2-fold increase in NO production was treated at the 700-ng/kg dose level and had a peak plasma IFN-γ level of 40,000 pg/ml after the second dose of rhIL-12. Among patients with renal cell cancer and melanoma who were receiving the standard i.v. bolus high-dose IL-2 regimen (600,000 IU/kg every 8 h × 14 doses; Ref. 28), the mean NO concentration was 14.7 ± 5.6 ppb before starting IL-2 and 53 ± 33 ppb during IL-2 therapy. Patients, on average, exhibited a 3.5-fold increase (range, 2- to 6-fold) in exhaled air NO concentration compared with the baseline value (Fig. 7).

DISCUSSION

In this report, we have shown that a new schedule of i.v. rhIL-12, consisting of twice-weekly injections administered without a test dose for 6 weeks, has acceptable toxicity at the MTD of 500 ng/kg and is capable of inducing antitumor responses. We observed one response (ongoing PR) among 14 patients treated at the MTD, with no responses at doses <500 ng/kg. The kinetics of this patient’s response is of particular interest, because regression did not begin until he had been continuously on the rhIL-12 for 6 months. This pattern of prolonged disease stabilization followed by tumor regression could be indicative of an antiangiogenic effect of rhIL-12 (29). A similar pattern of delayed response to therapy in renal cell cancer patients has been reported with thalidomide (30), another agent shown to have antiangiogenic effects (31). Among the other three patients with renal cell cancer who were treated at, or above, the MTD and who have had long-term disease stabilization, only one has remained on IL-12 for more than two cycles. After 8 cycles, this patient is starting to exhibit the first signs of regression of multiple liver and abdominal lymph node metastases. Therefore, for patients treated with twice-weekly i.v. rhIL-12, disease stabilization that is maintained for a prolonged period of time may have a significant likelihood of translating into tumor regression. This finding provides a compelling reason for dosing rhIL-12 chronically, a strategy that may also prove to be necessary for antitumor responses to agents that are more “pure” antiangiogenic drugs.

Although the majority of patients enrolled in the trial of twice-weekly i.v. rhIL-12 had received prior immunotherapy, their response to prior therapy was not predictive of a response to rhIL-12. In fact, the patients with renal cell cancer who exhibited disease regression or prolonged disease stabilization in response to rhIL-12 had all failed prior therapy with IL-2. This finding suggests that IL-12 and IL-2 may mediate tumor regression through distinct mechanisms and is an indication that rhIL-12 may be a viable treatment option in renal cell cancer patients who have not responded to IL-2.

One of the goals of measuring in vivo cytokine induction in our patients who were receiving rhIL-12 was to determine whether there was any association between patterns of IFN-γ induction and antitumor responses. Importantly, it was not the magnitude of IFN-γ production but rather the ability to sustain IFN-γ induction by rhIL-12 during the first cycle that was associated with outcome. Plasma IFN-γ levels that were induced by the twice-weekly schedule at the MTD of 500 ng/kg were comparable with the levels induced by the daily-for-5-days schedule with a test dose at 500 ng/kg (18, 19). However, consistent with what has been demonstrated in previous trials of rhIL-12, the majority of patients treated with twice-weekly i.v. rhIL-12 lost the ability to produce IFN-γ relatively soon after the start of dosing. Importantly, those few patients with the type-I IFN-γ induction pattern, who produced only modest amounts of IFN-γ but could sustain that induction over the course of the first cycle, exhibited signs of antitumor immunity such as tumor regression or disease stabilization. This is the first demonstration in cancer patients that sustained IFN-γ induction by rhIL-12 may be necessary for antitumor responses, and it is consistent with the murine data which have shown that rhIL-12-induced tumor regression is IFN-γ-dependent (10). The association of high plasma IFN-γ levels (5,000–10,000 pg/ml) after the second rhIL-12 dose with subsequent down-modulation of IFN-γ induction (type-II IFN-γ induction pattern) suggests that overactive immune stimulation may lead to the early curtailment of IL-12 responsiveness. If antiangiogenic and cytolytic antitumor responses to rhIL-12 require chronic immune stimulation, then strong levels of immune activation that can be maintained for only brief periods of time are likely to be counterproductive in patients treated with rhIL-12.

Hemolytic anemia or agranulocytosis, requiring cessation of the rhIL-12 and treatment with a brief course of low-dose Cytoxan and/or prednisone, occurred in 2 of 19 patients treated at the 500-ng/kg and 700-ng/kg dose levels on the twice-weekly schedule. These toxicities have never been reported among patients treated with i.v. or s.c. rhIL-12 on other dosing schedules (18, 20–22, 32), nor have they been observed in patients treated with IL-2 (33). The finding that these rhIL-12-induced cytopenias persisted for 1 week after stopping the rhIL-12 and were resolved only after treatment with immunosuppressive drugs such as prednisone and Cytoxan suggests they may have been autoimmune phenomena and raises the possibility that this may be a unique toxicity of chronic stimulation with rhIL-12 in certain susceptible patients. However, it is also notable that these cytopenias were restricted to two of the four patients who were able to maintain IFN-γ induction during cycle 1 (Table 4).
In mice, IL-12 has been shown to induce bone marrow suppression as well as splenomegaly, both of which are dependent on IL-12-induced IFN-γ (34). Therefore, the agranulocytosis and Coombs negative hemolytic anemia may not have been the result of specific immune responses directed against erythroid or myeloid antigens. Instead, direct toxic effects of rhIL-12-induced IFN-γ on myeloid precursors, as well as stimulatory effects of IFN-γ on NK cells (34), monocytes, and other reticuloendothelial elements in the spleen, may have led to the observed cytopenias.

The bruising that developed over the s.c. metastases in two patients with melanoma was provocative, for it appeared during the first several weeks of cycle 1 when IFN-γ was being induced by rhIL-12 and then resolved when IFN-γ induction was shut down. Whereas it is possible that these ecchymoses represented the early phase of an antiangiogenic process that was aborted when IFN-γ induction ceased, there was no tumor regression or biopsy evidence of tumor necrosis/microvessel damage to support this hypothesis.

IFN-γ production in vivo indicates that the elements required for the elicitation of a T helper type-1-like immune response by IL-12 are present and intact. As IL-12 by itself is only a weak inducer of IFN-γ production by PBMCs in vitro, the induction of endogenous costimulatory cytokines such as IL-15 and IL-18 may be necessary to induce IFN-γ production in vivo. Our results show for the first time that rhIL-12 does induce both IL-15 and IL-18 production in vivo in cancer patients. Because IL-15 and IL-18 are synthesized by activated monocytes and dendritic cells (35, 36), it is possible that the direct activation of these antigen-presenting cells by rhIL-12 (6) in vivo was responsible for the induction of these costimulatory cytokines. However, as IFN-γ was often induced concurrently with IL-15 and IL-18, it is not possible to deduce whether the IL-15 and IL-18 augmented IFN-γ production by IL-12 (36, 37), or whether rhIL-12-derived IFN-γ may have augmented antigen-presenting cell production of IL-15 and IL-18. It is possible that both mechanisms were operative. It is notable that IL-18 could be detected in the plasma even before the first dose of rhIL-12, which suggests that low-level constitutive production of IL-18 may allow for the immediate synergistic activation of lymphocytes early during an immune response when IL-12 is first being synthesized. Whereas IL-15 was not detectable in the plasma before starting IL-12, small amounts (5–10 pg/ml) of IL-15 were induced by rhIL-12 within 4 h after an injection. However, it is not clear whether the small amount of IL-15 detected in plasma with an ELISA represents the true amount available in vivo for lymphocyte activation, or whether such a low effective concentration would be capable of influencing IFN-γ production together with IL-12 and IL-18. It is notable that monocytes have been shown to express a membrane-bound form of IL-15 that is capable of stimulating lymphocytes (38), because this suggests that the amount of IL-15 induced by rhIL-12 in vivo and available to activate lymphocytes in conjunction with rhIL-12 may be greater than the amount detected in the plasma by ELISA.

Because IL-15 and IL-18 are induced by rhIL-12 and may be involved in rhIL-12-induced IFN-γ induction, it is reasonable to speculate that changes in the production of these costimulatory cytokines could underlie the attenuation of IFN-γ produc-

tion during rhIL-12 therapy. In patients with sustained IFN-γ induction during cycle 1, the magnitude of IL-15 induction remained constant, whereas peak and plateau levels of IL-15 diminished 50–70% by mid-cycle in patients unable to sustain IFN-γ induction at week 4. This may be an indication that IL-15 production is operative in IFN-γ production by rhIL-12 in vivo, or it may simply reflect the fact that the production of smaller amounts of IFN-γ leads to weaker IFN-γ-induced IL-15 production. Unlike IL-15, IL-18 induction remained fairly intact by mid-cycle, even in patients whose IFN-γ induction had greatly attenuated. If IL-18 is necessary for IFN-γ induction by rhIL-12, this observation may be an indication that lymphocyte responsiveness to IL-18 was diminished midway through the first cycle of rhIL-12. Alternatively, because an IL-18 binding protein (IL-18BP) that neutralizes the activity of IL-18 has recently been identified (39), it is possible that the down-modulation of IFN-γ production may have involved the induction of IL-18BP by rhIL-12.

The attenuation of IFN-γ production at week 4 of cycle 1 in patients with the type-II and type-III IFN-γ induction patterns was associated with an acquired defect in in vitro IFN-γ production stimulated by IL-12, IL-2, or IL-15. This defect was selective, inasmuch as proliferation induced by these cytokines was unaffected. Furthermore, the defect was not observed in patients with the type-I IFN-γ induction pattern. Because IL-12 did not diminish the number of circulating T or NK cells and did not down-regulate IL-12 or IL-2/IL-15 receptor expression, it is likely that the diminished IFN-γ response to these cytokines represents either an acquired defect in lymphocyte cyto-

keine signaling and/or a defect in monocyte/dendritic cell function. It remains to be determined whether this change in IL-2/IL-15- and IL-12-induced IFN-γ production is attributable to alterations in the Jak/Stat (40), mitogen-activated protein kinase (41), or NF-κB (6) signaling pathways used by these cytokines, or whether it might involve changes in the IFN-γ gene itself, such as methylation of the promoter (42). Although IL-10 was induced in vivo in patients with the type-II and type-III IFN-γ patterns, it was also strongly induced in patients with the type-I pattern. It seems unlikely, therefore, that the observed defect in IFN-γ production in vivo is simply the result of an inhibitory effect of IL-10 on lymphocytes or monocytes/dendritic cells. In addition to its potential inhibitory effects on IFN-γ production, IL-10 can also stimulate IFN-γ production by NK cells in combination with IL-18 (43). This dual effect of IL-10 may have contributed to our inability to detect an association between in vivo IL-10 and IFN-γ production in patients receiving rhIL-12.

Our analysis of plasma IL-12 levels after bolus i.v. injections of rhIL-12 showed that rhIL-12 was cleared more rapidly from the blood after the second and seventh doses compared with after the first dose. However, this accelerated clearance by itself did not seem to be responsible for the attenuation of IFN-γ production midway through the first cycle, because the same pharmacokinetics were observed in patients with the type-I and type-II/type-III IFN-γ induction patterns. Although diminished IFN-γ production has correlated with the down-regulation of

4 J. A. Gollob, K. Veenstra, and J. W. Mier, unpublished observations.
serum IL-12 levels (44) in patients and mice receiving multiple doses of s.c. IL-12, the mechanisms underlying the decrease in IFN-γ and IL-12 levels have not yet been elucidated. As peak levels of rhIL-12 in the plasma are considerably higher after i.v. bolus injection than after s.c. injection (18, 20), accelerated clearance occurring with repeated injections may have a more detrimental impact on the effective in vivo concentration of rhIL-12 in patients treated via the s.c. route.

Whereas NO has been implicated in mice as a cause of IL-12-induced immune suppression (26), we detected little NO production in patients treated with twice-weekly i.v. rhIL-12. This was in contrast to patients treated with high-dose IL-2, in whom we observed 3- to 5-fold increases in NO production during therapy. Although it is possible that expired air NO did not reflect systemic NO production in our patients, this is unlikely because, in animals treated with lipopolysaccharide (LPS), the augmentation of systemic NO production correlates well with changes in the NO concentration in expired air (45, 46). Furthermore, because in vitro cytokine-induced IFN-γ production was largely unaffected in patients treated with high-dose IL-2 (whereas it was inhibited in many patients treated with rhIL-12), it seems unlikely that NO production is an important cause of diminished IFN-γ production in patients receiving rhIL-12.

If, as our results suggest, chronic T helper type-1-like immune activation involving IFN-γ production is necessary for rhIL-12-induced antitumor effects, it is possible to prevent or delay the down-modulation of IFN-γ induction in patients treated with rhIL-12? Whereas genetic factors, tumor burden, or prior treatment history may be determinants of the type of IFN-γ induction pattern that a patient will exhibit during rhIL-12 therapy, it is clear that the majority of patients will fail to sustain IFN-γ induction and will not respond to rhIL-12. However, our findings indicate that the down-modulation of IFN-γ induction by i.v. rhIL-12 may not be an insurmountable problem, for it is not attributable to irreversible factors such as the loss of key lymphocyte subsets, the down-regulation of cytokine receptor expression, or greatly diminished bioavailability of the administered rhIL-12. Whereas our data indicate that changes in in vivo costimulatory cytokine production and lymphocyte responsiveness to these cytokines probably contribute to the attenuation of IFN-γ induction, we have also shown that lymphocyte IFN-γ production by IL-12 can be revived in vitro if IL-2 is added. It is possible, therefore, that strategies involving the addition of IL-2 to i.v. rhIL-12 may be able to lengthen the duration of immune stimulation by rhIL-12 in vivo, thereby augmenting its antitumor activity.

ACKNOWLEDGMENTS

We thank Donna Martin, Nancy Brown, Christine Moan, JoAnne Swenson, Julie Carpenter, Nancy Salonpuro, Debora Weed, and the rest of the staff in the Clinical Research Center of the Beth Israel Deaconess Hospital for their nursing support.

REFERENCES


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

Jared A. Gollob, James W. Mier, Korina Veenstra, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/6/5/1678

Cited articles
This article cites 45 articles, 30 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/6/5/1678.full#ref-list-1

Citing articles
This article has been cited by 54 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/6/5/1678.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.