Phase I Evaluation of Intravenous Recombinant Human Interleukin 12 in Patients with Advanced Malignancies

Michael B. Atkins, Michael J. Robertson, Michael Gordon, Michael T. Lotze, Michelle DeCoste, Jon S. DuBois, Jerome Ritz, Alan B. Sandler, Howard D. Edington, Pamela D. Garzone, James W. Mier, Christine M. Canning, Linda Battiato, Hideaki Tahara, and Matthew L. Sherman

Tupper Research Institute and the Division of Hematology/Oncology, Tufts-New England Medical Center, Boston, Massachusetts 02111 [M. B. A., J. S. D., J. W. M.]; Division of Hematology/Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts 02115 [M. J. R., J. R., C. M. C.]; Division of Hematology/Oncology, Indiana University School of Medicine, Indianapolis, Indiana 46202 [M. G., A. B. S., L. B.]; Department of Surgical Oncology, Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania 15261 [M. T. L., H. D. E., H. T.]; and Genetics Institute, Inc., Cambridge, Massachusetts 02140 [M. D., P. D. G., M. L. S.]

ABSTRACT

A Phase I dose escalation trial of i.v. administered recombinant human interleukin 12 (rhIL-12) was performed to determine its toxicity, maximum tolerated dose (MTD), pharmacokinetics, and biological and potential antineoplastic effects. Cohorts of four to six patients with advanced cancer, Karmofsky performance ≥70%, and normal organ function received escalating doses (3–1000 ng/kg/day) of rhIL-12 (Genetics Institute, Inc.) by bolus i.v. injection once as an inpatient and then, after a 2-week rest period, once daily for five days every 3 weeks as an outpatient. Therapy was withheld for grade 3 toxicity (grade 4 hyperbilirubinemia or neutropenia), and dose escalation was halted if three of six patients experienced a dose-limiting toxicity (DLT). After establishment of the MTD, eight more patients were enrolled to further assess the safety, pharmacokinetics, and immunobiology of this dose.

Forty patients were enrolled, including 20 with renal cancer, 12 with melanoma, and 5 with colon cancer; 25 patients had received prior systemic therapy. Common toxicities included fever/chills, fatigue, nausea, vomiting, and headache. Fever was first observed at the 3 ng/kg dose level, typically occurring 8–12 h after rhIL-12 administration, and was incompletely suppressed with nonsteroidal anti-inflammatory drugs. Routine laboratory changes included anemia, neutropenia, lymphopenia, hyperglycemia, thrombocytopenia, and hypoalbuminemia. DLTs included oral stomatitis and liver function test abnormalities, predominantly elevated transaminases, which occurred in three of four patients at the 1000 ng/kg dose level. The 500 ng/kg dose level was determined to be the MTD. This dose, administered by this schedule, was associated with asymptomatic hepatic function test abnormalities in three patients and an on-study death due to Clostridia perfringens septicemia but was otherwise well tolerated by the 14 patients treated in the dose escalation and safety phases. The T1/2 elimination of rhIL-12 was calculated to be 5.3–9.6 h. Biological effects included dose-dependent increases in circulating IFN-γ, which exhibited attenuation with subsequent cycles. Serum neopterin rose in a reproducible fashion regardless of dose or cycle. Tumor necrosis factor α was not detected by ELISA. One of 40 patients developed a low titer antibody to rhIL-12. Lymphopenia was observed at all dose levels, with recovery occurring within several days of completing treatment without rebound lymphocytosis. There was one partial response (renal cell cancer) and one transient complete response (melanoma), both in previously untreated patients. Four additional patients received all proposed treatment without disease progression. rhIL-12 administered according to this schedule is biologically and clinically active at doses tolerable by most patients in an outpatient setting. Nonetheless, additional Phase I studies examining different schedules and the mechanisms of the specific DLTs are indicated before proceeding to Phase II testing.

INTRODUCTION

IL-12 is a heterodimeric cytokine with potent immunoregulatory activity. It was initially given the names natural killer cell stimulatory factor and cytotoxic lymphocyte maturation factor (1, 2) based on its stimulatory effects on these cytolytic lymphokine populations. The cDNAs encoding the two IL-12 subunits are predominant in activated PBMC suspensions (7). Although EBV-transformed B cells appear to produce IL-12, significant

Received 8/20/96; revised 11/21/96; accepted 12/2/96.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported in part by Grants M01-RR-00054 and M01-RR750 from the Division of Research Resources, NIH, and a stipend from Genetics Institute, Inc.

2 To whom requests for reprints should be addressed, at Division of Adult Hematology/Oncology, Department of Medicine, New England Medical Center, 750 Washington Street, Box 542, Boston, MA 02111.

3 The abbreviations used are: IL, interleukin; PBMC, peripheral blood mononuclear cell; NK, natural killer; TNF, tumor necrosis factor; AST, aspartate aminotransferase; DLT, dose-limiting toxicity; MTD, maximum tolerated dose; ANC, absolute neutrophil count.

Downloaded from clincancerres.aacrjournals.org on May 2, 2017. © 1997 American Association for Cancer Research.
icant production by T cells or NK cells has not been demonstrated. Two low-affinity rhIL-12 receptor subunits have been cloned, which together constitute a high-affinity IL-12 receptor (8). High-affinity IL-12 receptors have been identified on phytohemagglutinin-activated T cells and IL-2-activated NK cells (9, 10). In addition, specific binding sites (possibly low-affinity) have recently been detected on the B1 subset of B lymphocytes (11).

IL-12 has been shown to regulate a number of activities central to both cellular and humoral immune responses. For example, in vitro studies have demonstrated that IL-12 can enhance the non-MHC-restricted cytolytic activity of NK cells (12, 13) and facilitate specific allogeneic human cytolytic T lymphocyte responses against allogeneic irradiated melanoma cells (14). IL-12 has been shown to be mitogenic, allowing the continued proliferation of both activated T lymphocytes and NK cells (15–17). IL-12 has also been shown to be a potent inducer of IFN-γ release from both T lymphocytes and NK cells (1, 18) while inducing much lower amounts of inflammatory cytokines, such as TNF-α, especially when compared with IL-2 (13, 19). Furthermore, IL-12 promotes T helper 1 cell development while inhibiting T helper 2 cell development and characteristic cytokine expression (20–22), favoring the development of cellular immunity and immunological memory over humoral immunity. In this regard, exposure to IL-12 enhanced survival in murine models of toxoplasmosis and leishmaniasis, two intracellular infections that require host T helper 1 cell responses (23–25).

IL-12 has also shown potent antitumor activity in a number of in vivo murine tumor models, including the B16F10 melanoma, RENCA renal cell adenocarcinoma, and M5076 reticulum cell sarcoma (26, 27). i.p. administration of IL-12 suppressed the growth of both pulmonary and s.c. B16F10 melanoma deposits and induced regression of RENCA. In the RENCA model, long-term survival and resistance to rechallenge were observed in mice injected peritumorally with IL-12. Antitumor effects were demonstrated both in microscopic disease models and in animals bearing large established tumors in which IL-12 was initiated 7, 14, or even 28 days after tumor inoculation (26, 27). The antitumor activity observed in these model systems was more potent than that observed with IL-2 (26).

Antitumor activity was slightly reduced in cytotoxic granule-deficient beige mice, but was nearly eliminated in T cell-deficient nude mice (26). Depletion of the CD8+ (but not CD4+) T-cell subset by systemic administration of monoclonal antibodies diminished the efficacy of IL-12 in the RENCA system (26), whereas depletion of either CD8+ or CD4+ T cells reduced antitumor activity in other models (27). In addition, most studies have shown that administration of antibodies to IFN-γ significantly reduces IL-12 antitumor activity (4). These experiments suggest that the antitumor effect of IL-12 is largely mediated by CD4+ and CD8+ T cells through the release of IFN-γ. However, the antitumor effect of IL-12 in most tumor systems is superior to that of IFN-γ, and furthermore, efficacy is absent in nude mice despite dramatically enhanced IFN-γ production (26), suggesting that other factors besides IFN-γ must contribute to the antitumor effects of IL-12. These preclinical data suggest that IL-12 may have potent immunoregulatory and antitumor activities that might be useful in the treatment of human infections and malignancies and encouraged us to proceed with this Phase I clinical trial of rhIL-12 in cancer patients.

PATIENTS AND METHODS

Patient Selection. All patients were adults with histologically proven advanced malignancy that was measurable, clearly progressive, and refractory to conventional therapy. Patients were required to have a Karnofsky performance status of ≥70% and adequate organ function defined by white blood count > 4000/μL, hemoglobin > 10 g/dL, platelet count > 100,000/μL, creatinine < 2 mg/dL, creatinine clearance > 60 mL/min, bilirubin < 2.0 mg/dL, AST < 2 times the upper limit of normal, electrocardiogram and chest X-ray without clinically significant nonmalignant abnormalities, and an FEV1 > 2.0 liters or 75% of predicted. Patients with history of peripheral neuropathy, brain metastases, or other central nervous system disease, prior exposure to nephrotoxic or neurotoxic chemotherapy agents, more than two prior systemic therapies, history of autoimmune disease, concurrent requirement for corticosteroids, seropositivity for human immunodeficiency virus or hepatitis BAg, serious concurrent infection or other major active illness, or who had received other chemotherapy, biological therapy, or any investigational drug within the preceding 3 weeks were ineligible.

Study Design. The study was an open-label, nonrandomized, multicenter Phase I dose escalation trial. The treatment protocol was approved by the Human Investigation Review Board at the participating institutions, and written informed consent was obtained from each patient. rhIL-12 was supplied by Genetics Institute, Inc. (Cambridge, MA) and administered by rapid i.v. injection into a rapidly flowing i.v. line.

The treatment schedule is displayed in Fig. 1. A test dose was administered on day 1, and patients were observed overnight as inpatients and were seen in follow-up on days 5 and 8. Patients who tolerated the test dose without DLT received rhIL-12 by rapid i.v. injection daily for 5 days beginning on day 15 in an outpatient setting. Treatment cycles were repeated every 21 days. Vital signs, including supine blood pressure, pulse, respiratory rate, and temperature, were measured at frequent regular intervals for up to 6 h after rhIL-12 administration on each day of the first 5-day course and for 1 h after IL-12 administration on all subsequent cycles. Weight was measured before each rhIL-12 administration, and rhIL-12 dosages were recalculated before each treatment course. Patients were evaluated for tumor response at the end of every second 21-day treatment cycle, and patients with stable or regressing disease could receive a maximum of six 5-day treatment courses.

rhIL-12 doses were increased from 3 to 1000 ng/kg in successive cohorts of patients. There was no intrapatient dose escalation. A minimum of four patients were enrolled at each dose level, and all patients had to have completed day 35 (end of first 21-day cycle) before initiating enrollment to the next dose level. If any patient developed grade 3 or greater toxicity at a particular dose level, then an additional two patients were enrolled at that dose level. Dose escalation was halted when three patients at a particular dose level experienced a grade 3 or greater toxicity and the preceding dose level was designated the MTD.

Patients experiencing a fever after the test dose could receive prophylactic acetaminophen (up to 2600 mg/day) for subsequent cycles. If fever recurred then indomethacin (25 mg, p.o., every 8 h) was provided. No other concomitant medications were routinely administered.
Toxicity was assessed using the National Cancer Institute Common Toxicity Criteria with slight modifications to address issues of fever, neutropenia, lymphopenia, elevated bilirubin, and capillary leak syndrome. Grade 3 elevations in bilirubin or decreases in neutrophil count and grade 4 lymphopenia were not considered DLTs. Grade 3 capillary leak syndrome was defined as fluid retention associated with hypotension, or symptomatic or laboratory evidence of organ dysfunction. Patients experiencing grade 3 or grade 4 toxicity were taken off the study, except for those patients treated at the highest dose level administered (beyond the MTD), who were allowed to continue therapy at the preceding dose level. Otherwise, no intrapatient dose modifications were permitted.

Pharmacokinetics. Serial blood specimens were collected for determination of circulating rhIL-12 levels before the administration of rhIL-12 on day 1 and 10, 20, and 30 min, and 1, 2, 4, 6, 10, 12, and 24 h post-drug administration. Additional blood specimens were also collected 10 min after rhIL-12 administration in subsequent treatment cycles. Serum was isolated and analyzed by an ELISA (28) with a lower limit of detection of 40 pg/ml.

Immunobiology. Serum for neopterin, IFN-γ, and TNF-α determinations was obtained before each rhIL-12 administration, at all interim visits, and at the final study visit. Sera for quantitative immunoglobulin measurements and PBMC for FACS analysis were obtained before the test dose and immediately before each rhIL-12 5-day treatment course, and at the final visit. PBMCs were shipped overnight to Nichols Laboratory (San Juan Capistrano, CA) and analyzed for the CD3, CD4, CD8, CD14, CD16, CD20, and CD56 expression using standard techniques.

rhIL-12 Antibodies. Serum for anti-rhIL-12 antibody determinations was obtained pretreatment, at the beginning of each treatment cycle and at the final visit.

Assessment of Tumor Response. Tumor measurements were obtained after the second 21-day cycle and then every other cycle. Responses were determined according to previously developed and published criteria (29).

RESULTS

Patient Population. Thirty-two patients participated in the dose escalation phase of this study at seven different dose levels. After determination of the MTD, an additional eight patients were enrolled at that level to further assess its safety. Table 1 depicts the relevant demographic and previous treatment information for all 40 study patients. As can be seen, the majority of patients had either renal cell cancer or melanoma and had received prior therapy. In particular, 9 of the 20 patients with renal cell carcinoma and 5 of the 12 patients with metastatic melanoma had received prior IL-2 therapy.

Treatment. Three patients received only the test dose of rhIL-12 due to either the clinical appearance of previously unsuspected brain metastases (two patients with melanoma treated at the 250 and 500 ng/kg dose levels) or transient grade 3 hepatic transaminase elevations (one patient at the 500 ng/kg dose level).

Six additional patients were not treated beyond the first 5-day treatment course due to disease progression (3 patients), asymptomatic premature ventricular contractions (1 patient), and transient liver function test abnormalities (2 patients). Five of the remaining 31 patients received the test dose and all six of the intended 21-day treatment cycles.

Clinical and Laboratory Toxicity. Common side effects associated with rhIL-12 included fever, chills, fatigue, headache, and, less commonly, nausea and vomiting. The fever was first observed at the 3 ng/kg dose level and occurred in the majority of patients receiving 10 ng/kg rhIL-12. Fever was delayed at onset, typically occurring 8–12 h after the rhIL-12 injection, and was incompletely suppressed with nonsteroidal anti-inflammatory drugs. Significant laboratory toxicities according to dose level are displayed in Table 2. Frequent laboratory changes included anemia, neutropenia, lymphocytopenia, hyperglycemia, thrombocytopenia, and hypoalbuminemia. Most of these abnormalities appeared independent of dose, peaked at day 5 of a treatment cycle, and resolved quickly. Grade 4 lymphocytopenia occurred in the majority of patients at all dose levels and resolved without rebound lymphocytosis. No consistent changes in serum creatinine or neurological toxicity were observed.

Dose-dependent abnormalities included stomatitis, which first became apparent during the sixth 5-day treatment cycle in one patient at the 100 ng/kg dose level and was evident in three of four patients at the 1000 ng/kg dose level, and liver function test abnormalities, which first became evident in one patient at the 250 ng/kg dose level and were universal by the 1000 ng/kg dose level. Liver function test abnormalities consisted predominantly of elevations in hepatic transaminases and lactate dehy-
dysgenase, but alkaline phosphatase and bilirubin were occasion-
ally elevated as well. Stomatitis usually resolved over 3
days, whereas liver function test abnormalities resolved over
7–10 days. Mucosal biopsies were not performed, but herpes
simplex virus cultures from the oral lesions were negative on
multiple occasions in several patients.

Determination of MTD. During the dose escalation
phase, one patient each at the 250 and 500 ng/kg dose levels had
DLT (grade 3 nausea and vomiting secondary to previously
undiagnosed brain metastases and grade 3 elevations of AST, respec-
tively). Consequently, two additional patients were added
to each dose level and tolerated therapy without difficulty.

Three of four patients treated at the 1000 ng/kg dose level
experienced DLT. One patient developed grade 3 elevation of
hepatic transaminases, grade 3 stomatitis, and a mild capillary
leak syndrome after three doses. Another patient developed
grade 3 elevation of hepatic transaminases and grade 2–3 sto-
matitis after four doses, and the third patient developed grade 3
elevation of alkaline phosphatase after completing all five doses.
The fourth patient at this dose level experienced both grade 2
stomatitis and grade 2 elevations in hepatic transaminases.
On the basis of this experience, the DLTs were defined as liver
function test abnormalities and stomatitis, and the MTD for this
schedule was determined to be 500 ng/kg.

Safety Experience. After determination of the MTD, an
additional eight patients were treated at this dose level to verify
its safety. The results of all 14 patients treated at the 500 ng/kg
dose level are displayed in Table 3. One patient went off study
after only receiving the test dose for early disease progression
and was considered invaluable for toxicity. Nine patients re-
ceived all of the first 5-day treatment course without significant
toxicity. Two of these nine patients experienced potential DLT
during later cycles: an upper gastrointestinal bleed in one patient
after three cycles and an admission for dehydration and ortho-
stasis in another patient after the second 5-day treatment course.
Both of these toxicities were believed not to be directly related
to rhIL-12. Three of the remaining four patients had doses held
for hepatotoxicity: grade 3 elevations in transaminases or alkali-
ne phosphatase that were largely transient and asymptomatic.
One of these patients was able to continue to receive therapy
without additional grade 3 hepatic toxicity. The final patient
developed grade 3 elevation of alkaline phosphatase after four
doses in the first 5-day treatment course and had his treatment
stopped. Subsequently, he developed abdominal pain and ane-
mia and was admitted for evaluation of potential gastrointestinal
bleeding. No mucositis was documented. Within 24 h he devel-
oped fever, followed by rapid onset of irreversible shock and
death. Blood cultures grew Clostridia perfringens from an un-
known source. No autopsy was performed. The death was felt to
be secondary to sepsis, presumably from necrotic intra-abdom-
nal tumor, and was classified as “possibly related to rhIL-12.”

Toxicity was fairly consistent from one cycle to the next
within individual patients without evidence of either cumulative
effect or attenuation corresponding to the changes observed in
some biological parameters (see below). Fig. 2, A and B, dis-
plays hepatic transaminase levels and neutrophil counts follow-
ing the test dose and subsequent cycles for four patients who
received at least two 5-day courses of therapy at the 500 ng/kg
dose level. AST levels were maximal at day 5 of the first 5-day
treatment course, with possible attenuation with subsequent
cycles. ANC nadirs occurred around day 5 of each cycle without
apparent cumulative toxicity. ANC recovery occurred by day 15
of each cycle without rebound neutrophilia.

Tumor Response. Objective tumor responses were ob-
served in two patients. One patient with metastatic renal cell
carcinoma with small pulmonary nodules and hilar adenopathy
experienced a partial response that is still ongoing at 22+ months.
The second patient, a 57-year-old woman with melanoma, expe-
rienced a complete regression of small pleural-based nodules and
cervical adenopathy lasting approximately 4 weeks. Four additional
patients completed six 21-day treatment cycles with stable disease.
Of the 14 patients treated at the MTD (6 renal, 4 melanoma; 3
without previous therapy), no responses were seen.

Biological Parameters. Mean circulating IFN-γ concen-
trations for the top three cohorts are displayed in Fig. 3. IFN-γ
appeared to be induced in a dose-dependent manner with peak
concentrations exceeding 1000 pg/ml observed 24 h after a test
dose of 1000 ng/kg rhIL-12, with return to baseline by day 8.
During the 5-day treatment courses, IFN-γ concentrations
were highest at 48–72 h after the first rhIL-12 injection and returned
to baseline by 72 h after the last dose. Peak IFN-γ concentra-
tions in general were higher during the first 5-day treatment
course than after the test dose. Peak IFN-γ concentrations dur-
ing subsequent cycles tended to progressively decline.

Mean serum neopterin concentrations are displayed in Fig.
4. Neopterin tended to rise in a non-dose-dependent manner,

---

Table 2  Number of patients experiencing selected laboratory toxicity in all cycles (grades 2, 3)

<table>
<thead>
<tr>
<th>Dose level (ng/kg)</th>
<th>3</th>
<th>10</th>
<th>30</th>
<th>100</th>
<th>250</th>
<th>500</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>(1, 0)</td>
<td>(1, 0)</td>
<td>(1, 0)</td>
<td>(7, 2)</td>
<td>(1, 1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>(1, 0)</td>
<td>(1, 0)</td>
<td>(2, 0)</td>
<td>(2, 1)</td>
<td>(3, 1)</td>
<td>(1, 0)</td>
<td></td>
</tr>
<tr>
<td>Bilirubin</td>
<td>(1, 0)</td>
<td>(1, 0)</td>
<td>(2, 0)</td>
<td>(2, 1)</td>
<td>(3, 1)</td>
<td>(1, 0)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>(3, 0)</td>
<td>(1, 0)</td>
<td>(2, 0)</td>
<td>(2, 1)</td>
<td>(3, 1)</td>
<td>(1, 0)</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>(1, 0)</td>
<td>(1, 0)</td>
<td>(2, 0)</td>
<td>(2, 1)</td>
<td>(3, 1)</td>
<td>(1, 0)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
reaching a maximum at day 5 and declining slowly thereafter. In contrast to IFN-γ, no attenuation in peak concentrations was observed with successive rhIL-12 cycles. TNF-α was not detected in any of the plasma specimens at any dose level.

**Immunophenotyping.** Mean lymphocyte and monocyte counts fell promptly during therapy and recovered quickly after treatment without significant rebound or dose dependence. Pheno-typic analyses showed reduction in all lymphocyte subsets without significant rebound or dose dependence. The neutrophil and lymphocyte subset values are shown in Table 4. Both maximum concentration and area under the concentration-time curve were proportional to the dose administered, indicating that over the dose range of 30–1000 ng/kg, the pharmacokinetic characteristics of rhIL-12 were linear and not dependent on dose. The elimination half-life was calculated to be between 5.3 and 10.3 h. Fig. 5 displays the concentration-time profiles of patients receiving 500 ng/kg, the MTD in this study. All patient profiles fell well within the range around the mean expected for this limited number of samples, and there was no correlation observed between rhIL-12 elimination half-life and toxicity.

**DISCUSSION**

IL-12 has potent immunomodulatory and antitumor effects in preclinical studies. This report describes the results of the first clinical trial of this promising cytokine. In this study of rhIL-12 administered by bolus i.v. injection initially as a single test dose, then daily for 5 days every 3 weeks, we defined the MTD for rhIL-12 to be 500 ng/kg. DLT, transient reversible stomatitis and/or hepatic dysfunction, occurred in three of four patients treated at the 1000 ng/kg dose level. One patient treated at the 500 ng/kg dose level died of *C. perfringens* sepsis. It is possible that some effect of rhIL-12 on intestinal mucosa or bacterial surveillance may have contributed to this event; however, because mucositis was not a prominent toxicity at this dose level, and because this dose and schedule were well tolerated by most other patients in an outpatient setting, we felt this dose did not exceed the MTD. Other prominent clinical abnormalities included fever and chills, fatigue, and, less commonly, nausea, vomiting, and headache. Commonly observed laboratory abnormalities included anemia, neutropenia, lymphopenia, thrombocytopenia, hyperglycemia, and hypoalbuminemia. The fever was unusually delayed, typically occurring 8–12 h after an injection rather than at 2–4 h as is typically seen with other pyrogenic cytokines such as IL-1, IL-2, IL-4, IL-6, and the interferons (30–34). The half-life of rhIL-12 was also unusually prolonged relative to these other cytokines (5–10 h versus 20 min) perhaps explaining the delay in onset of the fever.

Similar to preclinical observations, rhIL-12 was found to be a potent inducer of IFN-γ. IFN-γ was observed to be induced in a dose-dependent manner, but to a progressively lesser degree with each successive 5-day treatment course. Many of the side effects observed with rhIL-12 administration, in particular the liver function test abnormalities, fever, flu-like symptoms, fatigue, hematological changes, and hyperglycemia, could be directly or indirectly attributable to IFN-γ production because they have been observed in clinical trials with this cytokine (35).

The etiology of the stomatitis is less clear. It was a surprising DLT because it had not been observed in preclinical toxicology studies. Potential mechanisms include: decrease in mucosal IgA immunity, an autoimmune phenomenon, viral activation, or inhibition of constitutive mucosal epithelial proliferation. Although in the absence of histological confirmation it is difficult to distinguish between these possibilities, no unusual bacterial, fungal, or viral organisms were obtained from any cultures, and no other evidence of autoimmune phenomena was observed, making the first three possibilities unlikely. IL-12 has been observed to enhance the mucosal toxicity associated with radiation therapy, presumably through an IFN-γ-mediated mechanism (36), and IFN-γ has been shown to inhibit TGF-β production (37), a factor purported to be important in maintaining mucosal integrity, perhaps implicating IFN-γ in this toxicity as well. However, IFN-γ did not cause mucositis in Phase I trials (35), arguing against it having a sole causative role in the stomatitis associated with rhIL-12. Perhaps the more sustained release of IFN-γ in response to prolonged presence of IL-12 or the concomitant effect of other factors may have enabled this new toxic effect to become manifest. This side effect limited further escalation of rhIL-12 on this schedule. Whether a better understanding of the underlying mechanism for this toxicity or

**Table 3** Results of patients treated at the 500 ng/kg dose level of rhIL-12

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>No. of cycles completed</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>023</td>
<td>2</td>
<td>No DLT</td>
</tr>
<tr>
<td>024</td>
<td>2</td>
<td>No DLT</td>
</tr>
<tr>
<td>025</td>
<td>2</td>
<td>No DLT</td>
</tr>
<tr>
<td>026</td>
<td>Test dose</td>
<td>Grade 3 AST, discontinued</td>
</tr>
<tr>
<td>027</td>
<td>Test dose</td>
<td>Brain metastases, discontinued</td>
</tr>
<tr>
<td>028</td>
<td>2</td>
<td>No DLT</td>
</tr>
<tr>
<td>033</td>
<td>1∗</td>
<td>Grade 3 alkaline phosphatase, sepsis/death</td>
</tr>
<tr>
<td>034</td>
<td>2∗</td>
<td>No DLT</td>
</tr>
<tr>
<td>035</td>
<td>2∗</td>
<td>Grade 3 orthostasis, discontinued</td>
</tr>
<tr>
<td>036</td>
<td>3</td>
<td>Gastrointestinal bleed, discontinued</td>
</tr>
<tr>
<td>037</td>
<td>4∗</td>
<td>Grade 3 liver function test, dose held in cycle 2, continued on therapy without subsequent DLT</td>
</tr>
<tr>
<td>038</td>
<td>2</td>
<td>No DLT</td>
</tr>
<tr>
<td>039</td>
<td>2</td>
<td>No DLT</td>
</tr>
<tr>
<td>040</td>
<td>1∗</td>
<td>Grade 3 alkaline phosphatase, discontinued</td>
</tr>
</tbody>
</table>

∗Received four doses in cycle 1.
∗Received two doses in cycle 2.
∗Received four doses in cycle 1.
∗Received three doses in cycle 1.
∗Possibly related to rhIL-12.
∗Not directly related to rhIL-12.
aggressive preventative treatment will enable higher doses of rhIL-12 to be safely administered remains to be determined.

Other cytokines have been shown to be potent inducers of TNF-α (19), but despite the ability of IL-12 to induce some TNF in vitro and the appearance of some side effects potentially attributable to TNF, no TNF was detectable in the plasma of patients receiving rhIL-12. Therefore, in contrast to mechanisms for toxicity proposed for other cytokines, toxicities observed in response to rhIL-12 administration are unlikely to be related to secondary TNF production.

IL-12 was shown in preclinical studies to allow the continued proliferation of preactivated NK and T cells, to enhance
cytotoxic activity of T cells and NK cells, and to induce T helper 1 cell and inhibit T helper 2 cell differentiation (20–22). Special studies to determine potential effects of rhIL-12 on TH cell distribution were not performed and, therefore, must await future rhIL-12 studies. PBMC phenotypic analyses showed reduction in all lymphocyte subsets.

Tumor responses were observed in one patient each with melanoma and renal cell carcinoma treated near the MTD. Because this study involved few patients per dose level and many of these patients had previously received other immunotherapy agents (most commonly IL-2), it is impossible to determine the cancer immunotherapeutic potential of rhIL-12 or the most appropriate dose for Phase II testing. However, based on this study, we feel that Phase II testing eventually will be.
416 Phase I Trial of i.v. rhIL-12 in Advanced Malignancies

**Table 4** Mean pharmacokinetic parameter estimates in patients receiving rhIL-12 as single i.v. dose on day 1

All values are mean ± SD. C<sub>max</sub>, maximum concentration; V<sub>ss</sub>, the volume of distribution at steady state; T<sub>1/2</sub>, half-life; CL, clearance; and AUC<sub>0→</sub>, area-under-concentration versus time curve extrapolated to infinity.

<table>
<thead>
<tr>
<th>Dose (ng/kg)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (pg/ml)</th>
<th>V&lt;sub&gt;ss&lt;/sub&gt; (ml/kg)</th>
<th>T&lt;sub&gt;1/2&lt;/sub&gt; (hours)</th>
<th>CL (ml/hr/kg)</th>
<th>AUC&lt;sub&gt;0→&lt;/sub&gt; (pg × hr/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>744.3 ± 313.1</td>
<td>2877.8 ± 904.7</td>
<td>5.3 ± 0.69</td>
<td>6.8 ± 5.3</td>
<td>6119.2 ± 3232.9</td>
</tr>
<tr>
<td>100</td>
<td>1022.1 ± 502.8</td>
<td>3491.5 ± 1290.7</td>
<td>6.2 ± 1.1</td>
<td>9.8 ± 4.5</td>
<td>9821.0 ± 5162.9</td>
</tr>
<tr>
<td>250</td>
<td>1385.3 ± 507.8</td>
<td>4343.7 ± 1452.8</td>
<td>7.2 ± 2.5</td>
<td>13.3 ± 5.6</td>
<td>19821.0 ± 9762.9</td>
</tr>
<tr>
<td>500</td>
<td>1768.5 ± 557.8</td>
<td>5269.0 ± 1708.3</td>
<td>8.2 ± 3.5</td>
<td>19.2 ± 7.8</td>
<td>29621.0 ± 1362.9</td>
</tr>
<tr>
<td>1000</td>
<td>2151.7 ± 597.8</td>
<td>6192.8 ± 2152.8</td>
<td>9.2 ± 4.5</td>
<td>25.2 ± 9.8</td>
<td>39421.0 ± 1952.9</td>
</tr>
</tbody>
</table>

![Fig. 5](image) Concentration versus time profiles of all patients receiving 500 ng/kg IL-12 as a single i.v. dose on day 1. ——— individual patients; —— mean of all patients.

warranted, and if this schedule is to be used, that doses in the range of 500 ng/kg should be tolerated by most patients.

Of note, a Phase II trial of rhIL-12 in metastatic renal cell carcinoma using this schedule (without the test dose) at the 500 ng/kg dose level was initiated, but had to be closed because of unexpected severe toxicity (38). Although the reason for the severe toxicity in this Phase II trial is still being explored, initial results indicate that the omission of the test dose was a major contributing factor. The observation from this study that IFN-γ levels progressively declined with each successive cycle of rhIL-12 supports the contention that prior rhIL-12 administration may in some way exert a protective effect against subsequent toxicity and that this effect may be sustained over at least a 10–17-day interval. If this hypothesis is confirmed in ongoing clinical and subsequent clinical testing, then this would have a major impact on the design of future rhIL-12 studies. Because of the severe toxicity associated with minor schedule changes, additional Phase I studies are necessary to examine alternative schedules and further evaluate the significance and mechanism underlying this “desensitization effect.”

**ACKNOWLEDGMENTS**

We thank Kerry Kappler, R. N., M. S., N. P., Sandy Dunstan, R. N., Lynn Colicchio, R. N., Lisa O’Connor, R. N., and Donna Kinzler, R. N., for nursing support; Deborah Rovner, Steven Chartier, and Dr. Beth Harrison-Mann for data management; Christine Cameron, Dr. Zhiling Yu, and Kyle McCarthy for laboratory assistance; and Jacqueline L. Myers for assistance in preparing the manuscript.

**REFERENCES**


---


Phase I evaluation of intravenous recombinant human interleukin 12 in patients with advanced malignancies.

M B Atkins, M J Robertson, M Gordon, et al.


Updated version  Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/3/3/409

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.