Interleukin 12 Immunotherapy after Autologous Stem Cell Transplantation for Hematological Malignancies

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ABSTRACT

Purpose: To determine the safety, maximum tolerated dose, and biological effects of recombinant human IL-12 after autologous stem cell transplantation for cancer.

Experimental Design: Twelve patients with hematological malignancies (8 non-Hodgkin’s lymphoma, 2 Hodgkin’s disease, and 2 plasma cell myeloma) began interleukin (IL)-12 therapy at a median of 66 days after transplantation. Recombinant human IL-12 was given by bolus i.v. injection in doses of 30, 100, or 250 ng/kg once as an inpatient and then, after a 2-week hiatus, once daily for 5 consecutive days every 3 weeks on an outpatient basis.

Results: Common side effects included fever, chills, fatigue, nausea or vomiting, and asymptomatic elevation in serum liver function tests. Transient neutropenia and thrombocytopenia were also common, but no patient required platelet transfusion or had a neutropenic fever. Dose-limiting toxicities (diarrhea and elevated liver function tests) occurred in 2 of 3 patients treated in the 250 ng/kg cohort. Biological effects, including increases in serum IFN-γ levels and transient lymphopenia involving CD4 T cells, CD8 T cells, B cells, and NK cells, were seen at all three dose levels.

Conclusions: Biologically active doses of IL-12 can be given safely to patients after autologous stem cell transplantation for high-risk hematological malignancies. Further studies are indicated to assess the efficacy of IL-12 in this setting.

INTRODUCTION

Autologous stem cell transplantation is being increasingly used for the treatment of hematological malignancies. Prospective randomized clinical trials have shown that autologous stem cell transplantation is superior to conventional chemotherapy for patients with plasma cell myeloma (1) and relapsed aggressive non-Hodgkin’s lymphoma (2, 3). It is also generally accepted that autologous stem cell transplantation is the treatment of choice for eligible patients with relapsed or refractory Hodgkin’s disease (4–6). Nevertheless, only about 30–40% of patients with relapsed or refractory lymphoma enjoy prolonged disease-free survival after autologous transplantation (2, 4, 5, 7–10), and virtually all patients with plasma cell myeloma will ultimately experience progressive disease (1, 11, 12). Improvement in the outcome of autologous transplantation for cancer will require novel approaches to reduce the risk of relapse after transplantation.

One strategy is to attempt to stimulate effective antitumor immune responses after transplantation. The rationale for post-transplant immunotherapy is straightforward: (a) preclinical models indicate that the immune system is most effective at eradicating malignant cells when the tumor burden is relatively small (13–16); (b) many patients with hematological malignancies achieve a state of minimal residual disease after autologous stem cell transplantation (17, 18); and (c) malignant tumor cells that are resistant to high-dose chemotherapy and/or radiotherapy can be eliminated by human allogeneic immune effector cells in vivo (19–21). Thus, it is reasonable to hypothesize that the human immune system can be stimulated to destroy residual chemotherapy-resistant tumor cells in the posttransplant setting.

IL-12 is a 70,000 heterodimeric cytokine that stimulates both innate and adaptive immune responses (22, 23). IL-12 supports the proliferation of activated T cells and promotes the differentiation of CD4 T cells into Th1 helper effector cells and of CD8 T cells into cytotoxic T lymphocytes, respectively (24–27). Moreover, IL-12 augments the cytolytic activity of NK cells (24, 28–31) and stimulates IFN-γ production by both NK cells and T cells (32, 33). By virtue of its combined effects on CD4 T cells, CD8 T cells, and NK cells, IL-12 can promote effective cell-mediated immune responses to malignant tumors. In several preclinical tumor models, IL-12 therapy induces regression of established primary tumors, inhibits the formation of tumor metastases, and prolongs the survival of tumor-bearing mice (13, 14, 34, 35). Furthermore, objective complete and...
PATIENTS AND METHODS

Patient Selection. Patients at least 18 years of age who had undergone high-dose chemotherapy or chemoradiotherapy with autologous hematopoietic stem cell transplantation for cancer were eligible for participation in this study. Achievement of complete response after transplantation was not required. Patients were required to have an Eastern Cooperative Oncology Group performance status $<$2, absolute neutrophil count of at least 1000/$\mu$L (not supported by growth factors), platelet count of at least 50,000/$\mu$L (not supported by platelet transfusions), and hemoglobin of at least 9 g/dl (could be after red cell transfusion). Patients were also required to have a serum total bilirubin $\leq$2 mg/dl and AST $\leq$ twice the upper limits of normal, and calculated creatinine clearance $\geq$60 ml/min. Patients had to be seronegative for the human immunodeficiency virus and hepatitis B surface antigen. Patients were not eligible if they were pregnant, had serious concurrent infection, or had uncontrolled, clinically significant cardiac, pulmonary, endocrine, or autoimmune disease.

Study Design. The study was a single-center, Phase I dose-escalation clinical trial. The clinical research protocol was approved by Cancer Therapy Evaluation Program at the NCI (protocol T97-0027) and by the Institutional Review Board at the Indiana University Medical Center. Written informed consent was obtained from each patient before registration in the study. rhIL-12 was supplied by Genetics Institute (Cambridge, MA) and was obtained through Cancer Therapy Evaluation Program (IND 6798). Data were submitted on a regular basis to the Clinical Trials Monitoring Service and onsite auditing of the study; this was designated cycle 1. It was intended that all patients should receive cycles 0 and 1 of therapy; continuation of treatment beyond cycle 1 was optional. In the absence of disease progression or unacceptable toxicity, patients who wished to continue in the study could receive a maximum of six 5-day, multiple-dose treatment cycles (cycles 1 to 6) administered at 21-day intervals. Patients were treated in the General Clinical Research Center at Indiana University Medical Center. Patients were admitted overnight after the first injection of IL-12 so that serial blood samples could be obtained for pharmacokinetic and pharmacodynamic studies. All of the remaining doses of rhIL-12 were given on an outpatient basis.

To ameliorate the anticipated fever and chills during rhIL-12 therapy, patients were routinely treated with 650 mg of acetaminophen every 4 h p.o. and 25–50 mg of indomethacin every 6 h p.o. on the days that they received rhIL-12 injections. A histamine type 2 receptor blocker was prescribed while patients were taking indomethacin. No other concomitant medications were prescribed routinely.

rhIL-12 doses ranging between 30 and 250 ng/kg of body weight were given to successive cohorts of patients. The ideal weight was used to calculate rhIL-12 doses for patients whose true weight exceeded their ideal weight by at least 20%. At least three patients were treated at each dose level. If any patient in a dose cohort experienced unacceptable toxicity (as defined below), then an additional two patients were enrolled at that dose level. Dose escalation was halted when two or more patients in a particular dose cohort experienced unacceptable toxicity, and the preceding dose level was designated the MTD.

Toxicity was assessed using the NCI Common Toxicity Criteria. Grade 2 or greater motor, sensory, or cerebellar neurotoxicity or hemorrhage was considered unacceptable. Any grade 3 or 4 toxicity was considered unacceptable, with the following exceptions: grade 3 hyperglycemia, fever, nausea, vomiting; grade 3 elevations of total bilirubin, AST, ALT, or alkaline phosphatase that were not associated with clinical signs of hepatic dysfunction and returned to pretreatment baseline levels within 1 week of discontinuing rhIL-12; grade 4 anemia, leukopenia, neutropenia, or thrombocytopenia that was not associated with clinically significant bleeding or neutropenic fever and that returned to baseline levels within 1 week; and grade 4 lymphopenia were deemed to be acceptable toxicities.

Escalation of the rhIL-12 dose for an individual patient was not permitted. Dose reductions were allowed for transient grade 3 elevations of liver function tests and transient grade 4 cytopenias. Use of hematopoietic growth factor support was not permitted in this study. Treatment with rhIL-12 was permanently discontinued for patients who required more than two dose reductions for toxicity or who experienced any unacceptable toxicity.

Pharmacokinetic and Pharmacodynamic Analysis. Blood samples were obtained before and 2, 6, 12, and 24 h after the first injection of rhIL-12 and at the rest period visit during cycle 0. Routine complete blood counts with WBC differentials were obtained at the same time as research blood samples. PBMCs were isolated on a Ficoll-diatrizoate gradient from venous blood samples. Control PBMCs were obtained from
healthy volunteer donors. Freshly isolated PBMCs were used for immunofluorescence studies. Aliquots of PBMCs were cryopreserved in liquid nitrogen for subsequent in vitro studies. Serum samples, obtained by centrifugation after allowing whole blood to clot at room temperature for ~10 min, were stored in aliquots at ~70°C. Serum IL-12 levels were measured using an ELISA kit specific for the human IL-12 p70 heterodimer (R&D Systems, Minneapolis, MN). The lower limit of detection for this assay is 5 pg/ml. Serum IFN-γ levels were measured using an ELISA kit specific for human IFN-γ (R&D Systems, Minneapolis, MN). The lower limit of detection for this assay is 8 pg/ml, and the upper limit of linearity is 1000 pg/ml. For data analysis, samples with levels <8 pg/ml were assigned a value of 8 pg/ml, and samples with levels >1000 pg/ml were assigned a value of 1000 pg/ml.

PBMCs were stained directly with fluorochrome-conjugated monoclonal antibodies, washed, fixed in 1% formaldehyde, and analyzed by flow cytometry as described previously (39) using a FACScan or FACSCalibur instrument from Becton Dickinson (San Diego, CA). Fluorochrome-conjugated CD3, CD4, CD8, CD20, CD45RA, and CD56 were purchased from Beckman-Coulter (Brea, CA), and CD45RO was from Pharmingen (San Diego, CA). During analysis, forward and side scattering properties were used to create a lymphocyte gate. Thresholds for discriminating levels of staining above background were established by analysis of PBMCs stained with FITC- and phycoerythrin-conjugated control monoclonal antibodies. The absolute number of various lymphocyte subsets was calculated by multiplying the total lymphocyte count (derived from a routine complete blood count performed at the same time a blood sample was obtained for flow cytometry studies) by the percentage of cells in a sample expressing the relevant phenotype (derived from flow cytometric analysis).

**In Vitro Stimulation of PBMCs.** IL-2 (specific activity, 1.7 × 10^7 units/mg) was obtained from Chiron (Emeryville, CA), and IL-12 (specific activity, 1.7 × 10^7 units/mg) from Genetics Institute. For the preparation of IL-12, 1 unit/ml was equivalent to 59 pg/ml or 0.84 fmol of the cytokine. Cryopreserved patient and control PBMCs were thawed, washed in medium, and plated at 50,000–60,000 cells/well in 96-well, U-bottomed microtiter plates in medium alone or medium containing various concentrations of IL-2 and/or IL-12 as indicated. After 4–5 days of incubation at 37°C, cell-free supernatants were collected, and IFN-γ levels were measured by ELISA as described above.

**Response Criteria and Statistical Analysis.** Histopathological diagnoses were classified using the Revised European-American Lymphoma classification (40). Response to treatment for patients with lymphoma was assessed using the criteria of the Non-Hodgkin’s Lymphoma International Working Group (41). Patients who failed to achieve at least an initial partial response to an anthracycline-containing regimen or who experienced disease progression within 3 months of completing treatment with such a regimen were deemed to have primary refractory disease. Recurrence of disease between 3 and 12 months after completion of primary chemotherapy was termed early relapse, and recurrence >12 months after completion of primary chemotherapy was termed late relapse.

Patients who did not receive any conventional dose therapy before stem cell transplantation were said to have had untreated disease. Patients who achieved a complete or partial response to the last conventional dose chemotherapy regimen given before high-dose therapy were deemed to have chemosensitive disease at the time of stem cell transplantation. All other patients were said to have chemorefractory disease. Chemosensitive disease for patients with plasma cell myeloma was defined as at least a 50% reduction in the serum paraprotein level with no clinical evidence of progressive disease. Patient follow-up were analyzed as of April 5, 2002. Means, SD, and SE were calculated using STATview software (Abacus Concepts, Piscataway, NJ).

**RESULTS**

**Patient Characteristics.** Twelve patients were enrolled, including 10 men and 2 women (Table 1). The median age was 46 years (range, 34–66 years). All patients had undergone autologous stem cell transplantation for hematological malignancies and were at high risk for relapse or progression after transplantation. At the time of stem cell transplantation, 1 patient had primary refractory Hodgkin’s disease, one patient had Hodgkin’s disease in untreated first relapse, 2 patients had plasma cell myeloma, and 8 patients had relapsed or refractory non-Hodgkin’s lymphoma (Table 1).

None of the patients received total body irradiation before transplantation. High-dose chemotherapy consisted of 200 mg/m^2^ melphalan i.v. for the 2 patients with myeloma. Patient 09 received rhIL-12 therapy after the second of two sequential autologous transplants. The other 11 patients underwent one transplant procedure. High-dose chemotherapy consisted of 1800 mg/m^2^ cyclophosphamide i.v. daily for 4 days, 200 mg/m^2^ carmustine i.v. daily for 2 days, and 800 mg/m^2^ etoposide i.v. daily for 3 days for 9 of the 10 patients with lymphoma. Patient 10 received the BEAM (carmustine, etoposide, cytarabine, and melphalan) regimen (8). All patients were infused with unpurged and unselected autologous peripheral blood stem cell grafts that had been mobilized by either G-CSF alone (3 patients) or chemotherapy plus G-CSF (9 patients). All patients received G-CSF support after transplantation, but they had to have maintained stable neutrophil engraftment off G-CSF therapy before being enrolled in the study.

Only three patients (01, 02, and 06) had achieved objective complete responses by the time they began rhIL-12 therapy. Patient 10 had developed new lesions on positron emission tomography scanning after stem cell transplantation that were felt to be compatible with progressive disease. The remaining patients were assessed as having achieved partial responses at the time they began therapy with rhIL-12.

**Treatment Characteristics.** rhIL-12 therapy was initiated at a median of 66 days (range, 26–210 days) after autologous stem cell transplantation. The initial dose of rhIL-12 was 30 ng/kg for 3 patients, 100 ng/kg for 6 patients, and 250 ng/kg for 3 patients. As described below, some patients had one or more subsequent rhIL-12 doses reduced or held because of toxicity. All patients received the single test dose (cycle 0) and at least one multiple-dose treatment cycle; continuation of treatment beyond cycle 1 was optional. Six patients received one multiple-dose treatment cycle, 5 patients received two multiple-dose cycles, and one patient received six multiple-dose cycles.
Toxicity and Determination of MTD. All but 1 of the patients experienced fever, chills, and/or myalgias during treatment with rhIL-12, despite routine premedication with acetaminophen and indomethacin. These symptoms were mild (grade 1 or 2) and were delayed in onset, occurring 6–12 h after injections of rhIL-12. During multiple-dose treatment cycles, these symptoms tended to be most prominent after the first and/or second doses of the cycle and were often less pronounced or absent after the third through fifth dose injections. Mild anorexia, nausea, or vomiting was also common and was not clearly dose dependent: 7 patients had grade 1 and 2 patients had grade 2 nausea and/or vomiting. Four patients experienced grade 1 or 2 stomatitis. Stomatitis occurred after 3–5 days of rhIL-12 therapy during multiple-dose treatment cycles and resolved after 2–4 days without treatment.

Common, dose-dependent laboratory abnormalities included transient depression of the blood counts and transient elevations in serum liver function tests (Table 2). Neutropenia and thrombocytopenia were asymptomatic and usually resolved within 3–5 days. No patient required a platelet transfusion or experienced fever during neutropenia requiring antibiotic therapy. Liver function test abnormalities usually resolved within 7–10 days and were not associated with any symptoms or signs of liver dysfunction. rhIL-12 doses had to be held or reduced because of liver function test abnormalities for none of the 9 patients treated in the 30 or 100 ng/kg cohorts as compared with 3 of 3 patients treated in the 250 ng/kg cohort (Table 3).

Three patients experienced unacceptable toxicity as defined by the study protocol. Patient 04 developed a Staphylococcus aureus central venous catheter tunnel infection that progressed to bacteremia with presumed septic pulmonary emboli. This was deemed to be grade 3 infection; it resolved with appropriate antibiotic therapy after removal of the central line. Although central line infections are not uncommon in this patient population, the possibility that rhIL-12 therapy had contributed to the risk of infection in this patient could not be excluded. Therefore, rhIL-12 treatment was stopped for this patient, and 2 additional patients were added to the 100 ng/kg cohort. None of the other 4 patients treated in the 100 ng/kg cohort subsequently experienced unacceptable toxicity, and dose escalation to 250 ng/kg proceeded as planned.

Two of 3 patients treated in the 250 ng/kg cohort experienced unacceptable toxicity. After receiving a single injection of 250 ng/kg rhIL-12 in cycle 0, patient 09 experienced asymptomatic grade 3 elevation of the serum ALT, which did not return to baseline within 1 week. The ALT did return to baseline within 3 weeks; therefore, this patient continued treatment in the study at a reduced dose of 100 ng/kg for cycle 1. Patient 11 developed grade 3 elevation in the serum bilirubin after receiving three doses of rhIL-12 250 ng/kg during his second multiple-dose cycle. Two days later, he developed grade 3 diarrhea (11 loose stools/24 h), rectal urgency, and pain on defecation. None

Table 1  Characteristics of patients receiving IL-12 therapy after transplantation

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Disease*</th>
<th>Disease status at transplant</th>
<th>Response to conventional dose therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>62</td>
<td>Female</td>
<td>DLBCL</td>
<td>PRD</td>
<td>Untested</td>
</tr>
<tr>
<td>02</td>
<td>35</td>
<td>Male</td>
<td>DLBCL-PM</td>
<td>PRD</td>
<td>Unassessable*</td>
</tr>
<tr>
<td>03</td>
<td>39</td>
<td>Male</td>
<td>PTCL</td>
<td>PRD</td>
<td>Chemosensitive</td>
</tr>
<tr>
<td>04</td>
<td>60</td>
<td>Male</td>
<td>DLBCL-HP</td>
<td>Late relapse</td>
<td>Chemorefractory</td>
</tr>
<tr>
<td>05</td>
<td>63</td>
<td>Male</td>
<td>DLBCL</td>
<td>Late relapse</td>
<td>Chemosensitive</td>
</tr>
<tr>
<td>06</td>
<td>39</td>
<td>Male</td>
<td>DLBCL</td>
<td>PRD</td>
<td>Unassessable*</td>
</tr>
<tr>
<td>07</td>
<td>43</td>
<td>Male</td>
<td>PCM</td>
<td>First relapse</td>
<td>Chemosensitive</td>
</tr>
<tr>
<td>08</td>
<td>34</td>
<td>Male</td>
<td>HD</td>
<td>Early relapse</td>
<td>Untested</td>
</tr>
<tr>
<td>09</td>
<td>49</td>
<td>Male</td>
<td>PCM</td>
<td>First relapse</td>
<td>Untested</td>
</tr>
<tr>
<td>10</td>
<td>38</td>
<td>Male</td>
<td>HD</td>
<td>PRD</td>
<td>Untested</td>
</tr>
<tr>
<td>11</td>
<td>66</td>
<td>Male</td>
<td>ALCML</td>
<td>PRD</td>
<td>Chemosensitive</td>
</tr>
<tr>
<td>12</td>
<td>49</td>
<td>Female</td>
<td>ALCML</td>
<td>PRD</td>
<td>Chemosensitive</td>
</tr>
</tbody>
</table>

* Diagnosis according to the Revised European-American Lymphoma Group classification: DLBCL, diffuse large B-cell lymphoma; DLBCL-PM, primary mediastinal DLBCL; PTCL, peripheral T-cell lymphoma, unspecified; DLBCL-HP, DLBCL with histological progression from low-grade lymphoma; PCM, plasma cell myeloma; HD, relapsed or refractory Hodgkin’s disease; ALCML, anaplastic large cell lymphoma.

Table 2  Selected toxicity associated with rhIL-12 therapy after autologous stem cell transplantation

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Dose level of rhIL-12, ng/kg (no. of patients in cohort)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 (3)</td>
</tr>
<tr>
<td>Hematological</td>
<td></td>
</tr>
<tr>
<td>Leukopenia</td>
<td>(3, 3, 0, 0)*</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>(1, 2, 2, 0)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>(2, 1, 1, 0)</td>
</tr>
<tr>
<td>Anemia</td>
<td>(0, 0, 0, 0)</td>
</tr>
<tr>
<td>Hepatic</td>
<td></td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>(0, 1, 0, 0)</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>(0, 0, 0, 0)</td>
</tr>
<tr>
<td>AST</td>
<td>(2, 0, 0, 0)</td>
</tr>
<tr>
<td>ALT</td>
<td>(3, 0, 0, 0)</td>
</tr>
</tbody>
</table>

* Values in parentheses are the numbers of patients in the cohort experiencing grade 1, 2, 3, or 4 toxicity, respectively, in all cycles of treatment.

* Elevations in serum levels.
Table 3  Actual doses of rhIL-12 received by patients during each cycle of therapy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose of rhIL-12 administered (ng/kg) in cyclea</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>30 30 30 NA NA NA NA</td>
</tr>
<tr>
<td>02</td>
<td>30 30 30 NA NA NA NA</td>
</tr>
<tr>
<td>03</td>
<td>30 30 NA NA NA NA NA</td>
</tr>
<tr>
<td>04</td>
<td>100 100b NA NA NA NA NA</td>
</tr>
<tr>
<td>05</td>
<td>100 30 NA NA NA NA NA</td>
</tr>
<tr>
<td>06</td>
<td>100 30c NA NA NA NA NA</td>
</tr>
<tr>
<td>07</td>
<td>100 100 30a NA NA NA NA</td>
</tr>
<tr>
<td>08</td>
<td>100 100 NA NA NA NA NA</td>
</tr>
<tr>
<td>09</td>
<td>250 100d NA NA NA NA NA</td>
</tr>
<tr>
<td>10</td>
<td>250 250d 250e 100f 30g 30 30</td>
</tr>
<tr>
<td>11</td>
<td>250 250 250f NA NA NA NA</td>
</tr>
<tr>
<td>12</td>
<td>100 100 100 NA NA NA NA</td>
</tr>
</tbody>
</table>

a For cycles 1 to 6, all five planned doses were given except as specifically noted below. NA, not applicable.
b Only three of five doses given because of dose-limiting toxicity (grade 3 infection).
c Only four of five doses given because of grade 4 neutropenia.
d Only three of five doses given because of grade 4 neutropenia.
e Only four of five doses given because of grade 3 elevated ALT.
f Only three of five doses given because of grade 3 elevated total bilirubin.
g Only three of five doses given because of grade 3 elevated total bilirubin and grade 4 leukopenia.
h Only four of five doses given because of grade 3 elevated total bilirubin.
i Only three of five doses given because of grade 3 elevated total bilirubin.

Table 4  Lymphocyte subsets in the peripheral blood of patients and control subjects

<table>
<thead>
<tr>
<th>Lymphocyte subseta</th>
<th>Control subjects (n = 12)</th>
<th>IL-12 patients (n = 12)</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total T cells</td>
<td>76 ± 6</td>
<td>55 ± 24</td>
<td>≤0.005</td>
</tr>
<tr>
<td>CD4 T cells</td>
<td>Total 51 ± 6</td>
<td>11 ± 7</td>
<td>≤0.0005</td>
</tr>
<tr>
<td>CD45RA+</td>
<td>24 ± 6</td>
<td>2 ± 1</td>
<td>≤0.005</td>
</tr>
<tr>
<td>CD45RO+</td>
<td>27 ± 7</td>
<td>8 ± 6</td>
<td>≤0.0005</td>
</tr>
<tr>
<td>CD8 T cells</td>
<td>14 ± 5</td>
<td>38 ± 22</td>
<td>≤0.0005</td>
</tr>
<tr>
<td>B cells</td>
<td>8 ± 3</td>
<td>8 ± 10</td>
<td>&gt;0.4</td>
</tr>
<tr>
<td>NK cells</td>
<td>12 ± 4</td>
<td>18 ± 14</td>
<td>&gt;0.1</td>
</tr>
</tbody>
</table>

a Values are mean ± SD of percentages of peripheral blood lymphocytes expressing CD3 (total T cells), CD4 (total CD4 T cells), both CD4 and CD45RA (CD45RA+), both CD4 and CD45RO (CD45RO+), CD8 (CD8 T cells), CD20 (B cells), or CD56 in the absence of CD3 (NK cells). Blood samples were collected from patients before their first injections of rhIL-12.
b P (from Student’s t test) for comparison of patients versus control subjects.

of the other patients treated in this study developed significant diarrhea or symptoms of proctitis.

After patient 11 experienced unacceptable toxicity, accrual to the 250 ng/kg cohort was stopped, and the 100 ng/kg was defined as the MTD for this study. Patient 12 had already been invited to participate in the study and was found to be eligible. She was treated in the expanded 100 ng/kg cohort as allowed by the original study design.

Dose Reductions for Hematological Toxicity. No grade 4 hematological toxicity was observed in patients treated with 30 ng/kg rhIL-12, and these patients received all planned rhIL-12 doses. Only 1 of 6 patients treated in the 100 ng/kg cohort experienced unacceptable toxicity as defined by the study. Nevertheless, protocol-mandated dose reductions for hematological toxicity were required for some patients (Table 3). Of the 6 patients treated in the 100 ng/kg cohort, 2 developed transient grade 4 neutropenia or leukopenia after receiving a single injection of rhIL-12 in cycle 0. Their rhIL-12 doses was thus reduced to 30 ng/kg for their first multiple-dose treatment cycles. Three patients (patients 07, 08, and 12) received all five planned doses of rhIL-12 at 100 ng/kg in a total of 4 multiple-dose treatment cycles. One of these patients developed grade 4 neutropenia during cycle 1 that required his rhIL-12 dose to be reduced to 30 ng/kg for cycle 2. No patient had to have rhIL-12 doses held or reduced because of thrombocytopenia or anemia. Use of G-CSF or other growth factor support was not permitted in this study.

Serum IL-12 Levels during rhIL-12 Therapy. Serum IL-12 levels 2 h after the first bolus i.v. injection of rhIL-12 were 264 ± 170 pg/ml (mean ± SD) for the 30 ng/kg cohort, 866 ± 176 pg/ml for the 100 ng/kg cohort, and 2059 ± 482 pg/ml for the 250 ng/kg cohort. Serum IL-12 levels generally became undetectable by 12–24 h postbolus for patients in the 30 and 100 ng/kg cohort. However, serum IL-12 levels 24 h after injection of 250 ng/kg rhIL-12 were 333, 437, and 70 pg/ml, respectively, in patients 09, 10, and 11; IL-12 levels became undetectable in the serum of these 3 patients by the rest period visit (3–4 days after injection).

Effect of rhIL-12 Therapy on Peripheral Blood Lymphocyte Subsets. The mean absolute lymphocyte count of all study patients at the time rhIL-12 therapy was initiated was 1233 ± 623 (mean ± SD, n = 12) cells/µl, which is within the normal range (800–4800 cells/µl). However, 3 patients (patients 06, 09, and 10) had subnormal lymphocyte counts of 300, 500, and 300 cells/µl, respectively, at the time they commenced rhIL-12 treatment. These 3 patients received their first rhIL-12 injections on days 69, 81, and 210 after transplantation, respectively. Because the median time to initiation of rhIL-12 therapy on this study was 66 days after transplantation, the baseline lymphopenia in these patients was not attributable to their commencement of treatment earlier in the posttransplant phase as compared with the other patients.

Although most patients had a normal total lymphocyte count at the time they initiated rhIL-12 therapy, the distribution of lymphocyte subsets in the blood of these patients was substantially different from that of healthy control subjects (Table 4). As would be expected for patients evaluated 1–6 months after autologous stem cell transplantation, the CD4 T-cell counts were very low, whereas the proportion of CD8 T cells was higher than normal (42–44). Moreover, 80 ± 12% (mean ± SD) of patient CD4 T cells expressed CD45RO, which was substantially higher than the 54 ± 13% CD45RO+ CD4 T cells observed in the peripheral blood of control subjects (P ≤ 0.0005). In contrast, the number of B cells and NK cells did not differ significantly between the patients and control subjects.

As expected (45), all patients developed transient lymphopenia during rhIL-12 therapy. The peripheral blood lympho-
cyte counts during cycle 0 for the 6 patients treated at the MTD are shown in Fig. 2. Profound lymphopenia occurred 6–12 h after rhIL-12 injection and persisted at 24 h after injection. The absolute lymphocyte count returned to baseline within 3–8 days after injection (Fig. 2). A comparable degree of lymphopenia was seen in the 3 patients treated in the 30 ng/kg cohort (data not shown).

Lymphopenia induced by rhIL-12 injections was attributable to decreases in all of the major lymphocyte subsets (Table 5). Statistically significant reductions in the absolute number of peripheral blood CD4 T cells, CD8 T cells, B cells, and NK cells were seen 24 h after single i.v. bolus injections of rhIL-12. By the rest period visit, all of the lymphocyte subsets had returned to baseline values except for CD8 T cells, which remained somewhat below pretreatment levels.

**Serum IFN-γ Levels during rhIL-12 Therapy.** Serum IFN-γ levels were undetectable in the serum of patients before rhIL-12 therapy (Fig. 3). By 6 h after a single bolus i.v. injection of rhIL-12, IFN-γ was detected in the serum of all patients except 1 patient (03) treated in the 30 ng/kg cohort. Serum IFN-γ reached peak levels by 12 h after injection in the 30 and 100 ng/kg cohorts; peak IFN-γ levels were sustained at ~250 pg/ml for 12–24 h after injection in the 250 ng/kg cohort. IFN-γ was no longer detectable in the serum by the rest period visit (3–8 days after injection), except in a single patient (08) treated in the 100 ng/kg cohort.

**Production of IFN-γ in Vitro by PBMCs.** The serum IFN-γ levels observed during rhIL-12 therapy in this study appear to be considerably lower than those seen in patients with advanced solid tumors treated with comparable doses of rhIL-12 (Table 6). To determine whether this was attributable to an intrinsic deficiency of posttransplant patient lymphocytes, PBMCs obtained from patients before rhIL-12 treatment were stimulated in vitro with IL-12, and their production of IFN-γ was measured. IFN-γ was not detectable in the supernatants of posttransplant patient PBMCs after stimulation with IL-12 in concentrations as high as 100 units/ml (Fig. 4). By contrast, IFN-γ was readily detectable in the supernatants of control PBMCs stimulated with IL-12 in concentrations as low as 1 unit/ml. Patient PBMCs were not completely unable to produce IFN-γ, because they secreted 43 ± 19 pg/ml of IFN-γ in response to costimulation with IL-2 and IL-12. However, this was markedly inferior to the level (708 ± 173 pg/ml) produced by control PBMCs under identical conditions (Fig. 4).

**Status of Patients at Most Recent Evaluation.** Six patients have died with disease progression, including 5 patients...
with non-Hodgkin’s lymphoma and 1 with Hodgkin’s disease. Six patients are alive; 1 patient with myeloma has experienced progressive disease, and 5 patients (patients 01, 06, 09, 10, and 12) have been free of disease progression at a median of 35 months after transplantation (range, 14.6–54.2 months) and a median of 32.4 months after initiating rhIL-12 therapy (range, 10.5–50.8 months).

**DISCUSSION**

IL-12 stimulates the regression of established tumors and the development of durable antitumor immunity in preclinical animal models (13, 14, 34, 35). IL-12 also augments graft-versus-host dis- 

tumor activity without promoting graft-versus-host dis-

ease in bone marrow transplantation models (46, 47) and stimulates the antitumor activity of peripheral blood lymphocytes obtained from patients who have undergone bone marrow transplantation (48). Moreover, objective tumor responses have been seen in patients with advanced solid tumors treated with rhIL-12 (36– 

38, 49). These objective responses, albeit uncommon, indicate that systemic administration of rhIL-12 can stimulate effective antitumor immune responses even in patients with advanced, chemotherapy-refractory cancers. Patients with hematological malignancies can often achieve a state of minimal residual disease after autologous stem cell transplantation, which would appear to be a particularly favorable setting in which to test the antitumor efficacy of rhIL-12. Before this study, however, it was not certain that rhIL-12 therapy would be feasible in the post-transplant setting. Our results clearly demonstrate that rhIL-12 can be administered safely in biologically active doses to patients with hematological malignancies who have undergone autologous stem cell transplantation.

The MTD of rhIL-12 administered as bolus i.v. injections by the schedule used in this study was 100 ng/kg. Dose-limiting toxicities, seen in 2 of 3 patients treated with 250 ng/kg rhIL-12, included abnormalities in serum liver function tests and grade 3 diarrhea that was accompanied by symptoms of proctitis. Transient elevation in the serum liver function tests occurs commonly in advanced cancer patients treated with rhIL-12 (36–38, 49). These laboratory abnormalities have generally been rapidly reversible and not associated with any symptoms. Diarrhea is a less common adverse effect, having been reported in approximately 30–40% of treated patients (Investigator’s Brochure for rhIL-12; Genetics Institute). Symptoms of proctitis (urgency and pain on defecation) were prominent in our patient with grade 3 diarrhea but appear to be reported infrequently by patients treated with rhIL-12. Nevertheless, similar symptoms have been described by a patient with renal cell carcinoma treated with 250 ng/kg rhIL-12 by the same schedule used in this study.4 It is possible that these symptoms are attributable to transient inflammation and/or ulcer formation in the rectal mucosa, similar to the aphthous stomatitis observed in this and previous (36–38, 49) rhIL-12 clinical trials. Of interest, IL-12 has been implicated in the pathogenesis of inflammatory bowel disease (50).

Transient cytopenias, including grades 3 and 4 neutropenia, have been observed after rhIL-12 treatment in cancer patients who had not undergone antecedent stem cell transplantation (36–38, 49). Therefore, the common occurrence of hematological toxicity in patients treated with rhIL-12 after transplantation was to be expected. Although 100 ng/kg was the MTD of rhIL-12 as defined by the original study design, rhIL-12 doses had to be held or reduced because of grade 4 leukopenia or neutropenia for 3 of 6 patients treated in the 100 ng/kg cohort. However, rhIL-12-induced neutropenia was of brief duration, resolving within 3–4 days after the last dose of rhIL-12 was given. No patient treated on this study experienced a neutropenic fever. The only significant infection that occurred was the central venous catheter infection in patient 04. This patient did

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**Table 6** Serum IFN-γ levels during rhIL-12 therapy: comparison of autologous stem cell transplantation patients versus advanced solid tumor patients

<table>
<thead>
<tr>
<th>rhIL-12 dose cohort</th>
<th>Stem cell transplant patients (n = 12)</th>
<th>Advanced cancer patients (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 ng/kg</td>
<td>8 ± 0</td>
<td>338 ± 480</td>
</tr>
<tr>
<td>100 ng/kg</td>
<td>104 ± 171</td>
<td>679 ± 388</td>
</tr>
<tr>
<td>250 ng/kg</td>
<td>267 ± 119</td>
<td>2096 ± 645</td>
</tr>
</tbody>
</table>

* Values are mean ± SD of the IFN-γ levels in serum 24 h after single i.v. bolus injections of rhIL-12 at the indicated dose levels.
  * Data taken from Table 3 in Ref. (45). Patients had advanced solid tumors (renal cell carcinoma, melanoma, or colon cancer) and were treated with rhIL-12 by rapid bolus i.v. injections in a schedule identical to that used for this study.

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4 M. J. Robertson, personal observation.
not experience any grade 1 or greater neutropenia while in the study, and his infection may well have been unrelated to rhIL-12 therapy.

There are several approaches that could be taken to address the hematological toxicity of rhIL-12 after transplantation. Because dose reductions were required in this study for neutropenia but not thrombocytopenia or anemia, G-CSF or granulocyte/macrophage-CSF could be used to support the neutrophil count during repetitive daily dosing with 100 ng/kg of rhIL-12. Consideration could also be given to continuing planned rhIL-12 dosing during a cycle despite the occurrence of asymptomatic grade 4 neutropenia, with the expectation that the latter will rapidly resolve after treatment. Alternatively, a less dose-intensive schedule of rhIL-12 could be planned; administration of rhIL-12 once a week by s.c. injection or twice a week by bolus i.v. injection has proven feasible and biologically active in patients with advanced cancer (37, 38, 49). The optimal schedule and dose of rhIL-12 has not yet been defined for any clinical indication.

Moreover, the best time to initiate cytokine-based immunotherapy after stem cell transplantation has not been determined. It can be argued that cytokine administration should be started soon after high-dose chemotherapy, when the tumor burden should be at its nadir (51). However, the effector cells that are presumably required to mediate an antitumor immune response are not present in significant numbers until hematological engraftment has occurred. Moreover, administration of immunostimulatory cytokines immediately after stem cell infusion has been associated with severe toxicity (51). rhIL-12 therapy in our study was intended to be started as soon as patients had engrafted and recovered from the acute toxicities of the stem cell transplant procedure. Patients began rhIL-12 treatment at a median of 2 months after transplantation, when ongoing recovery of the various lymphocyte subsets is occurring (42, 44). Most patients had normal absolute lymphocyte counts, and the proportions of B cells and NK cells in their peripheral blood were similar to those of healthy control subjects. However, these patients had very low numbers of CD4 T cells in the peripheral blood at the time they began treatment with rhIL-12 and exhibited a great preponderance of CD4 T cells with a memory (CD45RO+) as opposed to naive (CD45RA+) phenotype. In contrast, CD8 T cells were present in normal or increased numbers. These abnormalities in T-cell subsets were not unexpected, based on well-described differences in the kinetics of lymphocyte subset recovery after autologous stem cell transplantation (42–44). It is currently not known whether such perturbations in the relative or absolute numbers of peripheral blood lymphocyte subsets have any effect on the likelihood of successful immunotherapy after autologous stem cell transplantation. Theoretically, the paucity of CD4 T cells in the early posttransplant setting could be deleterious for outcome of IL-12-based immunotherapy. On the other hand, the presence of relatively higher numbers of CD8 T cells and NK cells could prove beneficial. In preclinical animal studies, the relative contributions of CD4 T cells, CD8 T cells, and NK cells to the antitumor effects of IL-12 therapy vary considerably, depending on the specific tumor model system that is used (52).

All patients treated in this study experienced transient, profound lymphopenia after receiving rhIL-12 injections. Lymphocyte counts began to decline by 2 h after an injection of rhIL-12, and nadir levels occurred by 6 h after injection and persisted for 12–24 h. rhIL-12-induced lymphopenia involved all of the major lymphocyte subsets, including CD4 T cells, CD8 T cells, B cells, and NK cells. Lymphocyte counts generally recovered to baseline within 3–4 days. The lymphopenia that occurs after rhIL-12 administration is believed to result from in vivo activation of lymphocytes, with their subsequent extravasation into tissues (45). Consistent with this hypothesis, increased numbers of lymphocytes are found in the spleen, liver, and lungs of normal mice given IL-12 systemically (25). The kinetics and nature of rhIL-12-induced lymphopenia in posttransplant patients treated in this study were very similar to those observed in advanced cancer patients who had not undergone autologous stem cell transplantation before rhIL-12 therapy (45). Together with the similar toxicity profiles seen in this and previous rhIL-12 clinical studies, our results suggest that several major biological effects of rhIL-12 are not substantially altered by the differences in peripheral blood lymphocyte subsets that are present in the posttransplant versus nontransplant patient populations.

Nevertheless, one striking disparity was observed in this study; the serum levels of IFN-γ in posttransplant patients after rhIL-12 injections appeared to be much lower than those of nontransplant cancer patients receiving the same doses of rhIL-12 (45). Because different immunoassays were used to measure IFN-γ in the two studies, the serum levels cannot be directly compared. Nevertheless, it is very unlikely that methodological factors alone can account for the ~10-fold differences in serum IFN-γ levels seen 24 h after single i.v. bolus injections of rhIL-12 in the two patient populations (Table 6). The nontransplant patients were treated for advanced solid tumors, whereas the transplant patients had hematological malignancies. It cannot be excluded that this difference in underlying tumor types was responsible in part for the disparate IFN-γ responses observed after rhIL-12 therapy. However, the marked similarity in rhIL-12 toxicity in these two patient populations argues against the hypothesis that many of the toxicities observed during rhIL-12 therapy are actually attributable to high levels of IFN-γ production in vivo (53).

The mechanisms responsible for the low serum IFN-γ levels seen in posttransplant patients after rhIL-12 therapy have not been defined. Our laboratory studies indicate that posttransplant patient PBMCs are intrinsically defective in their ability to produce IFN-γ upon direct stimulation with IL-12 in vitro. Thus, it is unlikely that the low serum IFN-γ levels seen during rhIL-12 therapy after transplantation are predominantly attributable to indirect effects on lymphocytes in vivo (e.g., defective secondary cytokine production by other cell types). The paucity of CD4 T cells in the peripheral blood of posttransplant patients could be responsible, at least in part, for the low levels of IFN-γ observed after rhIL-12 therapy. Moreover, all of our patients received G-CSF for mobilization of their peripheral blood stem cell autografts, and G-CSF administration can affect dendritic cell subsets in the peripheral blood (54). Because we did not assess dendritic cell subsets in this study, we do not know whether the presence of increased numbers of DC2-type cells may have contributed to the relatively low IFN-γ responses we observed. Nevertheless, Gailloume et al. (55) have described
defective production of IFN-γ and other cytokines after in vitro stimulation of PBMCs obtained from patients after autologous transplantation, regardless of the stem cell source. Further investigation will be required to elucidate the mechanisms underlying defective cytokine production in the posttransplant period.

Whatever its mechanism, the relatively low serum IFN-γ levels in posttransplant patients after rhIL-12 therapy may raise concerns regarding the antitumor efficacy of rhIL-12 in this clinical setting. In several preclinical tumor models, production of IFN-γ is required for the antitumor effect of systemic IL-12 therapy (14, 34, 56). However, high serum IFN-γ levels alone are not associated with substantial antitumor activity (56). Rather, local production of IFN-γ in vivo in the vicinity of malignant tumor cells appears to be important for the efficacy of IL-12 (34). Therefore, it cannot be assumed that the magnitude of IFN-γ levels in the serum will correlate with a successful outcome of rhIL-12 therapy. Nevertheless, it is possible that optimal posttransplant rhIL-12-based immunotherapy will require administration of additional cytokines to augment in vivo production of IFN-γ. IL-18 would be worthy of consideration in this regard, given its potent synergy with IL-12 for IFN-γ production (57).

The patients enrolled on this clinical trial were at exceptionally high risk for disease progression. Seven patients were referred for transplantation because of primary refractory lymphoma, 1 patient had chemorefractory relapsed lymphoma at the time of transplant, 1 patient had evidence of persistent Hodgkin’s disease after transplantation, and 2 patients had plasma cell myeloma. It is therefore gratifying to note that 5 of 12 patients remain free of disease progression with a median follow-up of almost 3 years after transplantation. However, only prospective comparative clinical trials can determine whether rhIL-12 therapy reduces the risk of relapse after autologous stem cell transplantation. The results of this Phase I study appear sufficiently promising to justify further clinical studies of rhIL-12 after transplantation.

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REFERENCES


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