Treatment With Recombinant Interferon (α-2b) Early After Bone Marrow Transplantation in Patients at High Risk for Relapse


Relapse continues to be a problem after bone marrow transplantation (BMT) for hematologic malignancies, particularly in recipients of autologous or T-cell-depleted allogeneic grafts and in patients with advanced disease. Interferon (IFN) has shown antiproliferative activity in several malignant hematologic diseases and potentially may be of benefit when administered early after BMT when the number of residual cells is minimal. We tested in a phase I study the maximum tolerated daily dose of recombinant IFN α-2b in patients who had received a transplant for a disease at high risk for relapse (acute myeloid leukemia or non-Hodgkin’s lymphoma beyond first remission, advanced myelodysplastic syndrome, acute lymphoblastic leukemia at any stage, chronic myeloid leukemia in accelerated or blast phase). Recombinant IFN α-2b was started at a dose of 0.5 × 10⁴ IU/m² and escalated by 0.5 × 10⁴ IU/m² in groups of three or four patients. The intention was to administer IFN as soon as stable engraftment after BMT was achieved (defined as an absolute neutrophil count of > 2.0 × 10⁹/L and platelet count > 100 × 10⁹/L for 5 consecutive days) and continued for 2 months. A total of 14 patients were enrolled after autologous (n = 3) or allogeneic (n = 11) BMT. Dose-limiting toxicity was myelosuppression. Significant (grade 2 to 4) neutropenia and thrombocytopenia led to discontinuation or dose reduction in five of eight patients receiving 1.5 × 10⁴ or 2 × 10⁴ IU/m² IFN. Mild to moderate (grade 1 or 2) anorexia, weight loss, and fatigue occurred in the majority of patients independent of the IFN dose. De novo acute GVHD responsive to steroid treatment developed in 3 of 11 allograft recipients. Natural killer (NK) cell function was low before IFN treatment and was not improved with the cytokine. Conversely, interleukin-2–activated NK cells showed normal function even before starting IFN and no change was seen during IFN treatment. Clonogenic hematopoietic progenitor studies showed depression of all progenitor lines (colony-forming unit [CFU]-granulocyte, erythroid, monocyte, megakaryocyte, CFU-granulocyte-macrophage, burst-forming unit-erythroid) by IFN at all dose levels except at 0.5 × 10⁴ IU/m². Considering this result and the incidence and severity of marrow depression seen at doses greater than 1.0 × 10⁴ IU/m², we would consider this the maximum dose safely tolerated if IFN α-2b is administered in this setting for a prolonged course on a daily basis.

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were assayed in standard methylcellulose cultures optimized for 10 weeks. Hematopoietic progenitor assays were performed on peripheral blood before and during IFN treatment. Progenitors for acute GVHD consisted of cyclosporine and methotrexate for a normal neurologic examination, and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. To be eligible to receive IFN after allogeneic BMT, patients were not to have any active GVHD clinical grade II or higher and to live close enough to the study center to permit regular frequent out-patient visits. The protocol was approved by the University of British Columbia Ethics Committee and all patients gave informed consent to participate in this trial. BM for autologous BMT was harvested at the time of complete marrow remission and cryopreserved without purging. Conditioning regimens for autologous and allogeneic BMT were according to established protocols. Prophylaxis for acute GVHD consisted of cyclosporine and methotrexate as described before.\(^\text{11}\)

**IFN treatment.** Recombinant IFN α-2b (Intron A) was provided by Schering (Pointe Claire, Canada) and injected daily subcutaneously by the patient for a total of 2 months. Treatment had to be initiated within the first 100 days after marrow infusion. The first dose was always administered in the hospital because of the potential of acute hypersensitivity reactions to IFN. Toxicity was graded according to the World Health Organization (WHO) scale.\(^\text{12}\) Three of four patients were entered at each dose level starting at 0.5 \(\times\) 10\(^6\) IU/m\(^2\)/d and escalated by 0.5 \(\times\) 10\(^6\) IU/m\(^2\)/d. Grade 2 and 3 hematologic toxicity called for holding IFN for 1 day and then resumption at a reduced dose of 75% of the initial dose, if recovery occurred. IFN was discontinued in case of grade 4 toxicity.

**Biologic evaluation.** Routine hematologic counts and blood chemistry were performed before starting IFN and then weekly for 10 weeks. Hematopoietic progenor assays were performed on the peripheral blood before and during IFN treatment. Progenitors were assayed in standard methylcellulose cultures optimized for the support of large erythroid [burst-forming unit-erythroid [BFU-E]], granulocyte macrophage [CFU-GM]), and mixed erythroid/granulocyte-macrophage (CFU-granulocyte, erythroid, monocyte, megakaryocyte [CFU-GEMM]) colony formation.\(^\text{13}\) Natural killer (NK) and lymphokine-activated killer (LAK) cell function were studied before and during the first month of IFN therapy using a standard \(^{51}\text{Cr}\) release assay as described previously.\(^\text{14}\) Briefly, \(^{51}\text{Cr}\)-labeled target cells (2 \(\times\) 10\(^5\)) were added either fresh to peripheral blood mononuclear cells or to cells that had been cultured in 500 U/mL recombinant IL-2 (Cetus, Emeryville, CA) for 4 days. After 4 hours at 37°C, plates were centrifuged (150 mg for 5 minutes) and 150 \(\mu\)L of supernatant aspirated from each well. \(^{51}\text{Cr}\) content was determined using a gamma counter and percent specific \(^{51}\text{Cr}\) release was calculated according to the formula: (experimental cpm – spontaneous cpm)/(maximum cpm – spontaneous cpm) \(\times\) 100. Serum for IFN antibodies was collected before and after at least 4 weeks on IFN treatment and assayed at the laboratories of Schering-Plough (Bloomfield, NJ).

### RESULTS

During the study period 5/89 to 12/90, 44 patients at high risk of relapse after BMT were considered for early IFN treatment. Thirty of these patients were ineligible for the following reasons: 12 patients lived too far from the study center to permit regular evaluation but would otherwise have been eligible to enter the study; four patients relapsed before IFN could be started; five patients died early after BMT; and two patients had greater than grade II acute GVHD. In addition, seven patients, because of delayed engraftment, never recovered enough peripheral neutrophils or platelets to become eligible to receive IFN. This left a total of 14 patients who entered this study between 21 and

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age (yr)/ Sex</th>
<th>Diagnosis</th>
<th>Type of BMT</th>
<th>Starting Dose (x 10^6 IU/m^2)</th>
<th>Starting Day of IFN Post-BMT</th>
<th>Duration of IFN Treatment (d)</th>
<th>Dose Modification</th>
<th>GVHD Acute (grade)</th>
<th>GVHD Chronic</th>
<th>Disease Status (July 1, 1991)</th>
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<tr>
<td>382</td>
<td>14/M</td>
<td>T-ALL 1st CR</td>
<td>Allo</td>
<td>0.5</td>
<td>+42</td>
<td>27</td>
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<td>2</td>
<td>No</td>
<td>CR, d +883</td>
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<tr>
<td>403</td>
<td>51/M</td>
<td>RAEB</td>
<td>Allo</td>
<td>0.5</td>
<td>+35</td>
<td>14</td>
<td>Ceased due to AGVHD</td>
<td>2</td>
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<td>Relapse, d +188, Died, d +221</td>
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<tr>
<td>220</td>
<td>34/M</td>
<td>CML-BP*</td>
<td>Allo</td>
<td>0.5</td>
<td>+90</td>
<td>60</td>
<td>No</td>
<td>1</td>
<td>Extensive</td>
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<td>43/M</td>
<td>NHL 1st relapse†</td>
<td>Auto</td>
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<td>+21</td>
<td>60</td>
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<td>—</td>
<td>Relapse, d +193, Died d +490</td>
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<tr>
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<td>B-ALL 1st CR#</td>
<td>Allo</td>
<td>1.0</td>
<td>+40</td>
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<td>Modified due to cytopenia+</td>
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<td>60</td>
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<td>—</td>
<td>CR, d +461</td>
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<td>60</td>
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<td>0</td>
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<td>1.5</td>
<td>+34</td>
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<td>—</td>
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<td>Secondary AML (untreated)</td>
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<td>No</td>
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</table>

Abbreviations: T-ALL, T-cell ALL; RAEB, refractory anemia with excess blasts; CML-BP, CML in blast phase; NHL, non-Hodgkin’s lymphoma; IF, induction failure; AP, accelerated phase; RAEB-IT, RAEB in transformation; CR, complete remission.

*Relapse after 1st BMT.
‡Diffuse large cell.
§Morphologic but not cytogenetic remission.
$100\%$ dose for 40 days, ceased for 5 days, $75\%$ dose administered thereafter.
||Diffuse mixed small and large cell.
||Dose administered thereafter.

\(^{11}\text{Cr}\) release assay as described previously.

\(^{51}\text{Cr}\)-labeled target cells (2 \(\times\) 10\(^5\)) were added either fresh to peripheral blood mononuclear cells or to cells that had been cultured in 500 U/mL recombinant IL-2 (Cetus, Emeryville, CA) for 4 days. After 4 hours at 37°C, plates were centrifuged (150 mg for 5 minutes) and 150 \(\mu\)L of supernatant aspirated from each well. \(^{51}\text{Cr}\) content was determined using a gamma counter and percent specific \(^{51}\text{Cr}\) release was calculated according to the formula: (experimental cpm – spontaneous cpm)/(maximum cpm – spontaneous cpm) \(\times\) 100. Serum for IFN antibodies was collected before and after at least 4 weeks on IFN treatment and assayed at the laboratories of Schering-Plough (Bloomfield, NJ).
97 days after BMT. Eight patients finished the full treatment course and six patients had early discontinuation for the following reasons: acute GVHD (n = 3), severe cytopenia (n = 2), and relapse (n = 1).

Clinical toxicity. The patients' clinical toxicities are summarized in Table 2 according to WHO criteria. Constitutional symptoms (anorexia, weight loss, and fatigue) were seen in most patients independent of the IFN dose level. In only one patient (UPN 369) did it become necessary to reduce the dose of IFN by 25% because of grade 2 nausea, vomiting, and anorexia. No life-threatening complications were seen and all side effects reversed usually within 1 week after discontinuation of IFN. It is of note that none of these patients had fever, cardiac, or neurologic events during IFN treatment.

Acute GVHD developed in three patients. Because this side effect may have been induced by IFN, their cases are presented here briefly. UPN 403 commenced IFN (0.5 × 10⁶ IU/m²) 5 weeks after transplant. His early transplant course was complicated by persistent nausea and vomiting of uncertain etiology requiring upper gastrointestinal (GI) endoscopy. Nausea and vomiting recurred in the second week of IFN therapy, requiring hospital admission to correct volume depletion. Histology of the upper GI system showed mild histologic changes of GVHD. IFN was ceased without any improvement in symptoms. Subsequently, he was treated with 3 mg/kg/d methylprednisolone with substantial improvement in his GI symptoms. At no stage did he have cutaneous or oral evidence of GVHD or hepatic function abnormalities. Before commencing IFN in UPN 382, the patient had mild cutaneous evidence of GVHD confirmed by skin biopsy. One week after IFN (0.5 × 10⁶ IU/m²) was begun, he developed anorexia, vomiting, and diarrhea. Examinations showed marked oral mucosal changes compatible with GVHD, and a faint erythematous rash. A skin biopsy showed moderate GVHD and upper GI endoscopy showed moderately severe histologic evidence of GVHD. Alkaline phosphatase and aspartate transaminase (AST) were elevated 2 to 5 times normal. IFN was ceased and treatment begun with methylprednisolone 5 mg/kg/d, resulting in rapid improvement in his GI symptoms and hepatic abnormalities. Subsequent tapering of steroids did not result in a flare of GVHD. IFN (2.0 × 10⁶ IU/m²) was commenced in UPN 519 97 days after transplant, by which time cyclosporine had been discontinued. There had been no evidence of GVHD before this, although mild lichenoid change of his oral mucosa was noticed in the week before IFN therapy. Seven days after commencement, he developed severe erythema and ulceration of his mouth requiring hospital admission for treatment of dehydration. Biopsy confirmed moderately severe oral GVHD. He had no vomiting, diarrhea, rash, or hepatic function abnormalities. IFN was ceased and methylprednisolone 3 mg/kg commenced, and within 4 days his mouth changes improved significantly. Chronic GVHD in those patients who survived beyond day 100 post-BMT developed in 2 of 11 patients and therefore does not seem to be induced or augmented by IFN treatment.

Survival. Five of the 14 patients have relapsed (Table 1) and the overall probability of relapse free survival at two years is 60% (Fig 1).

Hematologic and immunologic changes. The pattern of peripheral blood count behavior during IFN is presented in Fig 2 and its classification according to WHO criteria summarized in Table 2. Three of four patients at the 1.5 × 10⁹ IU/m²/d dose level experienced grade 2 or 3 toxicity with significant depression of neutrophils (mean nadir, 1,133 × 10⁹/L) and platelets (range, 38% to 61% decrease after 4 weeks of IFN compared with prior IFN therapy). Hematologic toxicity grade 3 or 4 was seen in two of four patients (UPN 218 and 345) at 2 × 10⁹ IU/m² of IFN, requiring discontinuation of treatment. Leukocyte and platelet counts returned to the normal range in all patients within the first 2 weeks after discontinuation of IFN.

Hematopoietic progenitor cell levels (Table 3) indicated...
significant suppression of clonogenic activity (CFU-GEMM, CFU-GM, and BFU-E) starting at a dose level of $1.0 \times 10^6$ IU/m$^2$. No suppression was seen at $0.5 \times 10^6$ IU/m$^2$. NK activity, tested against cell line K562, was below normal before IFN in all patients and was not improved by IFN treatment (Fig 3A). Conversely, LAK cells activity was already normal before starting IFN and no change was seen in LAK activity while the patients were on treatment (Fig 3B and C). No neutralizing antibodies to IFN α-2b were detected in the serum of treated patients.

**DISCUSSION**

The mechanism by which IFNs inhibit tumor cell growth are largely unknown. Data suggest that they can induce increased expression of tumor-associated antigens and HLA antigens, have direct cytotoxic effects on tumor cells, and induce their differentiation and suppress expression of certain oncogenes.\(^5\) Antitumor effects of IFN have been documented for a variety of different hematopoietic malignancies, but the potential of recombinant IFN to influence relapse rate after BMT for leukemia or lymphoma has never been formally tested. The purpose of this phase I dose escalation study was to define a safe dose range for IFN α that can be administered to patients relatively early after transplantation without severe marrow-depressive effects or severe aggravation of symptoms that frequently occur early after the transplant, such as poor caloric intake, weight loss, and fatigue. Because relapse occurs most frequently within the first few months after BMT, the design of this study was to administer IFN as soon as stable engraftment had occurred. As expected, the major dose-limiting toxicity was marrow toxicity, which affected mainly platelet and neutrophil production leading to early discontinuation or dose modification in the majority of patients receiving $1.5 \times 10^6$ IU/m$^2$ or $2 \times 10^6$ IU/m$^2$. Hematopoietic clonogenic assays indicated that all three progenitor cell lines (CFU-GEMM, CFU-GM, and BFU-E) were suppressed beginning at an IFN dose of $1.0 \times 10^6$ IU/m$^2$/d or higher. Recovery of peripheral counts was prompt after IFN was discontinued, which supports the notion that IFN α preferentially inhibits late progenitor cells, leaving the early stem cell intact.\(^5\) These results suggest a daily dose of $1.0 \times 10^6$ IU/m$^2$ would be suitable in patients early after BMT particularly if the objective is to give a more prolonged treatment.

Although the dose tolerated by recipients early after marrow grafting appears to be relatively low, there are data from patients with hairy cell leukemia showing that such a "low" dose can be effective.\(^6\) Unresolved is whether this dose has immunomodulatory or antiproliferative effects. The activity of NK cells was not restored by IFN in these patients, which is in accordance\(^7,8\) and contradiction\(^9,10\) to other studies, respectively. However, NK cells represent just one facet of immunomodulation and other mechanisms, such as enhanced expression of tumor HLA-antigens
reported by Meyers et al for leukocyte IFN, there is some concern that IFN α may potentially induce GVHD because it can induce expression of class I and most likely also minor non-HLA antigens that are presumed to be involved in the induction of GVHD. Therefore, only patients who did not have active severe (grade II to IV) GVHD were eligible for this trial. Three of 11 patients after allogeneic BMT developed acute GVHD while receiving IFN. Although the number of patients is small, there does not appear to be a relationship to the dose of IFN because two patients had been administered $0.5 \times 10^6$ IU/m$^2$ and one patient $2.0 \times 10^6$ IU/m$^2$. In all three cases, IFN was discontinued and GVHD was responsive to treatment with steroids.

Although neurotoxicity is a known side effect of IFN treatment, it was not observed in any of our patients. This finding is in contrast to the report by Meyers et al, in which 6 of 39 patients developed leukoencephalopathy while on IFN. It is possible that the amount of pretransplant central nervous system (CNS) treatment (radiation and intrathecal chemotherapy) constitutes a predisposing factor for the development of this complication, as all six patients in the Seattle study had received extensive CNS prophylaxis compared with only one patient (UPN 457) in our series. In these patients, magnetic resonance imaging performed before IFN treatment might be useful to exclude those who could be at high risk for developing leukoencephalopathy.

Patient UPN 525 relapsed after being on IFN for about 3 weeks. She had a (9;11) (p21;q23) cytogenetic abnormality. This translocation positions the c-ets-1 oncogene next to the IFN α gene complex such that the expression of IFN α may also induce abundant transcription of c-ets-1 and mediate transformation. Although it is not known whether this oncogene is induced by IFN treatment, this possibility should at least be kept in mind when those patients are considered for IFN treatment.

For this phase I study, IFN was administered only over a period of 2 months, as patients usually return home around day 100 post-BMT, and for patients living any distance from the treatment center, the close follow-up required for a phase I study would not have been possible. This has also limited the accrual rate (only 32% of all patients initially considered for this study were actually enrolled). If those patients could be included in a phase II/III study, the expected accrual rate would almost double.

An alternative, albeit less pragmatic, approach to administration of IFN early after BMT would be to adjust the daily dose according to the actual peripheral neutrophil and platelet count. The availability of hematopoietic growth factors may, by supporting neutrophil and platelet production during IFN treatment, allow use of a higher dose and/or longer duration of treatment, especially because constitutional symptoms were not clearly dose related in this study.

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REFERENCES


Treatment with recombinant interferon (alpha-2b) early after bone marrow transplantation in patients at high risk for relapse [corrected] [published erratum appears in Blood 1992 Jun 15;79(12):3397]