Phase I Trial of Interleukin-2 After Unmodified HLA-Matched Sibling Bone Marrow Transplantation for Children With Acute Leukemia

By Nanette Robinson, Jean E. Sanders, Mark C. Benyunes, Kathy Beach, Catherine Lingdren, John A. Thompson, Frederick R. Appelbaum, and Alexander Fefer

ALLOGENEIC BONE MARROW transplantation (BMT) for acute leukemia beyond first remission is associated with a high risk of relapse. Efforts to intensify conditioning regimens have been limited by an increase in nonrelapse mortality.3,4 There is considerable evidence that a graft-versus-leukemia (GVL) effect mediated by donor cells contributes to the benefit of BMT.5,8 The GVL effect is associated with, but not dependent on, the development of clinical graft-versus-host disease (GVHD).7 The potential effector mechanisms for GVL include cytotoxic T and non-T lymphocytes and cytokine cascades.6,8 Because some or all of these mechanisms may be enhanced by pharmacologic doses of interleukin-2 (IL-2); it is possible that IL-2 therapy after BMT, a mechanism for GVL might be shared by GVHD, IL-2 therapy could induce or exacerbate GVHD.12-17 T-cell depletion of allogeneic marrow reduces the incidence of GVHD.18-20 IL-2 therapy after T-depleted BMT has been reported to decrease relapses without increasing GVHD, as compared with historical non-T-depleted controls.21 Non-T-depleted BMT is associated with a lower relapse rate than T-depleted BMT.18-20 IL-2 therapy after unmodified, non-T-depleted BMT could potentially induce a greater GVL effect by stimulating the proliferation and reactivity of T cells in the marrow and, thereby, further reduce the relapse rate. However, it could also potentially induce or exacerbate GVHD.

To identify a regimen of IL-2 that could be used in future studies to test the hypothesis that IL-2 can reduce relapses, a phase I dose-escalation trial of IL-2 after unmodified allogeneic BMT was performed in children in complete remission (CR) without active GVHD off immunosuppressive agents. This trial shows that IL-2 in this clinical setting does not cause a significant incidence of acute GVHD and identifies a tolerable IL-2 regimen whose clinical activity will be tested in a phase II trial.

MATERIALS AND METHODS

Patient characteristics. Between April 1990 and May 1995, 17 patients (1 to 16 years of age; median age, 4 years) in CR after undergoing allogeneic BMT from an HLA-matched sibling for acute myelogenous leukemia (AML; n = 6) or acute lymphoblastic leukemia (ALL; n = 11) were treated with IL-2 (Table 1). As preparative regimens for BMT, 14 patients received total body irradiation and cyclophosphamide and 3 patients received three other regimens. For GVHD prophylaxis, methotrexate (MTX) alone was used in all patients except unique patient number (UPN) 9046 who received MTX plus cyclosporine (CSP). Six patients receiving MTX developed grade II or III acute GVHD that was successfully treated before protocol entry.

Study design. Patients were evaluated for IL-2 protocol eligibility after receiving their day 32 (post-BMT) dose of intravenous (IV) MTX. Initially, patients who had experienced any acute GVHD while on GVHD prophylaxis were excluded from the IL-2 protocol. The eligibility criteria were modified in July 1992 to include patients who had developed GVHD while on prophylaxis but whose GVHD had resolved. Other eligibility requirements included trilineage engraftment with adequate bone marrow function (neutrophils >500/µL and platelets ≈20,000/µL with transfusion support), adequate hepatic function (SGOT <150 mg/dL, bilirubin <3 mg/dL) and adequate renal function (creatinine <2X normal). Patients were ineligible if they had World Health Organization (WHO) grade III central nervous system (CNS), cardiac, pulmonary, hepatic, or renal toxicity; were on corticosteroids; or had an active infection. GVHD prophylaxis was stopped, and all patients who did not develop acute GVHD during the subsequent 8 to 14 days became eligible for IL-2 therapy. Written informed consent was obtained from all parents or responsible guardians. The treatment protocol was approved by the Fred Hutchinson Cancer Research Center and the Division of Medical Oncology, Department of Medicine, University of Washington, Seattle, WA.

Address reprint requests to Alexander Fefer, MD, University of Washington, Division of Medical Oncology, Box 356527, Seattle, WA 98195-6527.

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GVHD was graded according to Glucksberg criteria. The side effects of IL-2 in patients with solid tumors and recipients of allogeneic marrow, such as skin rash, diarrhea, and cholestasis, may be clinically and histologically similar to GVHD. but are quickly reversible on discontinuation of IL-2 therapy. The diagnosis of acute GVHD was, therefore, based on clinical parameters such as persistence or worsening of signs or symptoms despite cessation of IL-2 therapy. IL-2 was stopped once a diagnosis of acute GVHD was made.

The maximum tolerated dose (MTD) was defined as the dose level below that at which 3 of a maximum of 6 patients developed grade III toxicity or grade III GVHD. Transient hyperbilirubinemia without other symptoms or signs of GVHD or liver failure was not considered dose-limiting because it is a well-described benign and reversible side effect of IL-2 therapy. (Supportive care. Acetaminophen, indomethacin, diphenhydramine, meperidine, prochlorperazine, furosemide, and/or human albumin were administered as needed. Beginning in 1993, patients received IV vancomycin and ceftazidime during induction IL-2 and amoxicillin/clavulanic acid or cephalexin for the remainder of the treatment period.

**Immunologic studies.** Immunophenotyping of peripheral blood mononuclear cells (PBMC) of 1 patient at level I (UPN 5780) and all patients at levels II and III was determined by standard methods. Lymphokine-activated killer precursor (LAKp) activity was assessed by the ability of PBMC, incubated with 1,000 U/mL IL-2 for 5 days, to lyse the natural killer (NK)-resistant Daudi target in a 4-hour ^51Cr-release assay. LAK effector (LAKe) activity was similarly assayed, but without exposing PBMC to IL-2 in vitro.

**RESULTS**

**Clinical toxicity.** All 17 patients treated with IL-2 were evaluable for toxicity. The full prescribed doses of induction IL-2 were received by all patients at levels I and II and 3
of 6 patients at level III. The toxicities of induction IL-2 are summarized in Table 2. During induction, fever, nausea, vomiting, diarrhea, weight gain, and mild rash were common, reversible, and not dose-limiting. Induction levels I and II were not associated with any toxicities >grade II. However, level III was too toxic. One patient developed acute hypotension requiring a 50% dose reduction, 1 patient developed capillary leak syndrome requiring supplemental oxygen, 2 patients developed transient hyperbilirubinemia, and 1 patient developed fatal streptococcal sepsis and acute respiratory disease syndrome (ARDS).

After level I induction, 2 of 3 patients received the full dose of maintenance IL-2 without any toxicity. One patient (UPN 5658) received no maintenance IL-2 because of the presence of acute GVHD (described below). After level II induction, 5 of 8 patients received the full maintenance dose of IL-2, while 3 patients received 60% to 90% of the dose because of the following ≥grade II toxicities: Clostridium difficile diarrhea (1), transient pulmonary crackles (1), and urticaria secondary to cephalaxin (1). After level III induction, only 1 of 6 patients received the full dose of maintenance IL-2. Because of induction IL-2 toxicities, 2 patients received no maintenance, 2 patients had maintenance therapy delayed for 5 days, and 1 patient had maintenance IL-2 stopped after 1 day due to the development of acute GVHD (described below).

Most patients exhibited a mild, reversible thrombocytopenia that reached a nadir during the rest phase of the IL-2 protocol (median, day 7), but 3 patients at level III, with pre-IL-2 baseline platelet counts of 113,000 to 138,000/µL, developed transient nadir counts of 12,000 to 50,000/µL. Neutrophil counts did not change significantly, whereas eosinophil counts increased at all levels and peaked at the end of maintenance therapy.

GVHD. Sixteen patients were evaluable for acute and chronic GVHD (Table 1). Acute GVHD during IL-2 therapy was observed in 2 patients (12%), neither of whom had a prior history of acute GVHD after BMT. The similarity between the clinical manifestations of GVHD and IL-2 toxicity complicated the assessment of both. One patient at level I had grade II acute GVHD on the basis of nausea, vomiting, skin erythema, and hyperbilirubinemia—all of which developed on day 6 of induction IL-2. The diagnosis of GVHD was confirmed by gut biopsy. One patient at level III developed nausea, vomiting, and rash after 1 day of maintenance IL-2 and was considered to have grade II acute GVHD because, although these symptoms are commonly seen with induction IL-2, they are not seen with maintenance IL-2. In both patients, symptoms of acute GVHD resolved within 1 week of discontinuing IL-2. No additional therapy was required.

Five of the 16 evaluable patients (31%) developed extensive chronic GVHD a median of 27 days (range, 16 to 250 days) after IL-2 therapy, representing a median of 107 days (range, 88 to 320 days) after BMT. This group included only 1 of the 6 patients who had had acute GVHD before IL-2 therapy. Chronic GVHD occurred in 1 patient at level I, none at level II, and 4 at level III. The clinical severity of chronic GVHD varied, with 2 patients at level III having easily controlled skin and mucosal involvement, whereas 1 patient at level I and 2 at level III developed scleroderma and involvement of liver or lung.

Immuno modulatory effects. Rebound lymphocytosis was observed 24 to 48 hours after completion of induction IL-2 with maximal median total lymphocyte counts of 2,300/µL, 3,800/µL, and 9,300/µL after levels I, II, and III, respectively. Mild lymphocytosis persisted at the end of maintenance IL-2. Phenotypic analysis of the lymphocytes (Fig 1) showed significant increases in the percentage of CD56+ cells after induction IL-2 (P = .001). The values remained significantly elevated after maintenance IL-2 (P = .008). The percentage of CD8+ cells also increased after induction IL-2 (P = .001) and then returned to baseline.

Cells from each of 15 patients tested had significant LAKp activity against the Daudi cell line before IL-2 therapy, with a median lysis of 54% at an effector to target (E:T) ratio of 50:1. The percentage did not change significantly after IL-2 therapy (data not shown). LAKp activity against Daudi was observed in cells from 3 of 13 patients tested before IL-2 therapy.
2 therapy and 3 additional patients after induction IL-2. LAKe lysis ranged from 13% to 47% (data not shown).

Patient outcome. Six patients have relapsed, 5 within 1 year of BMT and 1 at 31 months (Table 1). Ten patients remain in CR, 5+ to 67+ months (median, 18+ months) after BMT. Patients surviving in CR more than 2 years include 1 patient transplanted for ALL in second CR, 1 transplanted for AML in third CR after relapsing after autologous BMT (ABMT), and 1 transplanted for AML in first relapse.

DISCUSSION

BMT for advanced hematologic malignancies is limited largely by posttransplantation relapses. IL-2 therapy has induced clinical responses in some patients with refractory AML.29-32 IL-2 administration, like other forms of immunotherapy, is likely to be more effective against minimal residual disease than against a large tumor burden. Preliminary data suggest that IL-2 may decrease relapses when used as consolidation after autologous BMT for AML.26,32,33 With allogeneic BMT, additional benefit has been ascribed to a GVL effect.5,7,8 It is postulated that IL-2 might induce or augment such a GVL effect through its effect on T and non-T lymphocytes or other cytokines.6 However, the use of IL-2 in the allogeneic setting has been limited by the concern that it could also induce or exacerbate acute GVHD through some or all of these same mechanisms.15,16

In animal models, IL-2 has been reported to induce a GVL effect and to induce, exacerbate, or protect against GVHD depending on the model, the IL-2 regimen, and the time of administration.10-14 In humans, T-cell depletion of marrow reduces the incidence of GVHD after allogeneic BMT. Soiffer et al21 recently reported that prolonged infusion of low-dose IL-2 did not increase the incidence of GVHD after T-depleted allogeneic BMT and was associated with fewer relapses than were seen in non-IL-2—treated historical controls.

Unmodified allogeneic BMT is associated with a lower relapse rate than T-depleted BMT.18-20 IL-2 therapy after unmodified BMT could possibly further decrease relapses by stimulating proliferation and antitumor reactivity of T cells as well as non-T cells. To date, reports of IL-2 therapy after unmodified allogeneic BMT have been limited to 1 patient who received IL-2 for relapsed neuroblastoma 90 days after BMT and developed GVHD, and 1 patient who received IL-2 for relapsed leukemia 3 months after BMT and did not develop GVHD.34,35 This trial represents the first series of patients treated with IL-2 as consolidative immunotherapy after unmodified allogeneic BMT.

The regimen used in this study was based on our experience with solid tumor and ABMT patients.24,26,36-38 IL-2 therapy was begun as early as possible after recovery from the toxicities of BMT and at a time of presumed minimal residual disease. Because IL-2 could conceivably exacerbate GVHD and because the immunosuppressive agents used for GVHD prophylaxis could conceivably interfere with the immunologically mediated antileukemic effect of IL-2, IL-2 was administered only to children who showed no GVHD after withdrawal of GVHD prophylaxis. High doses of IL-2 were administered to enhance the expansion of both T and non-T lymphocytes.37 Induction IL-2 was followed by a rest period to allow recovery from IL-2 toxicities and then by lower doses of IL-2 to maintain the immunomodulatory effects. This trial showed that induction levels I and II were quite tolerable, with relatively minor reversible toxicities, whereas level III was associated with serious toxicities, including 1 death due to complications of sepsis.

Only 2 patients developed acute GVHD after IL-2 therapy; neither required treatment. Acute GVHD occurring on MTX before IL-2 did not predict for GVHD after IL-2. The incidence of chronic GVHD seen in this study was similar to that reported by others in children who underwent unmodified allogeneic BMT without IL-2 therapy.39-41 There was a significant incidence of chronic GVHD at level III as compared with level II. Although it is possible that the higher dose of IL-2 caused or contributed to the development and severity of chronic GVHD, the patients are too small in number and clinically too heterogeneous (eg, level III included 3 female donors to male recipients v none at level II) to permit such a conclusion. Moreover, although for analysis of phase I data the acute and chronic GVHD temporally related to IL-2 therapy is being attributed to IL-2, the incidence of acute and chronic GVHD in patients taken off GVHD prophylaxis early after BMT and not receiving IL-2 is unknown.

The immunomodulatory effects of IL-2 on both NK and T cells observed in this trial are similar to those reported after ABMT.27,28 LAKp activity was observed after BMT and before IL-2 therapy, indicating the availability of a cell population capable of responding to IL-2, as previously reported.42-44 The clinical significance of these immunomodulatory effects is not known.

Although the optimal regimen of IL-2 after unmodified allogeneic BMT has not been determined, a regimen of IL-2 has been identified that can be administered without a
significant incidence of GVHD or other toxicity. The MTD of this regimen was identified as 3.0 × 10^6 IU/m² of induction IL-2. Although the clinical toxicities were similar to those seen for IL-2 therapy after ABMT, the MTD was one-third that of ABMT patients given a similar regimen. The toxicity profile, immunomodulatory effects and clinical results of the present trial are sufficiently encouraging to justify efforts to assess the clinical activity of this regimen in a phase II trial.

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