Autologous and Allogeneic Bone Marrow Transplantation for Poor Prognosis Patients With B-Cell Chronic Lymphocytic Leukemia


Twenty patients with poor prognosis B-cell chronic lymphocytic leukemia (B-CLL) underwent uniform high-dose chemoradiotherapy followed by rescue with multiple monoclonal antibody-purged autologous bone marrow (BM) (12 patients) or T-cell–depleted allogeneic BM from HLA-identical siblings (8 patients) in a pilot study to assess the feasibility of BM transplantation (BMT) in this disease. All had poor prognosis disease by either staging, BM pattern, tumor doubling time criteria, or cytogenetics. All patients achieved remission criteria (defined as ≤2 cm adenopathy, absence of splenomegaly, ≤20% of the inter trabecular space involved on BM biopsy) before BMT. Despite the use of fludarabine, a median of three treatment regimens were required to achieve BMT eligibility. After BMT, all patients achieved complete hematologic engraftment. Toxicities were not significantly different between autologous versus allogeneic BMT. Two toxic deaths were observed. Of 19 evaluable patients, 17 clinical complete clinical remissions (89%) were observed, with 2 patients (1 allogeneic and 1 autologous) exhibiting persistent BM disease. Complete clinical remissions were documented at the phenotypic and molecular level for the majority of patients in whom dual fluorescence for CD5 and CD20 (15 of 15; 100%) and Ig gene rearrangements (11 of 14; 79%) were performed. Although long-term follow-up is needed to assess any potential impact on the disease-free and overall survival of these patients, this study shows the feasibility of using high-dose chemoradiotherapy and BMT in patients with poor prognosis B-CLL.

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donor underwent auto-BMT using marrow purged with multiple anti–B-cell monoclonal antibodies (MoAbs). All patients were treated with the identical ablative regimen. After BMT, 17 of 19 (89%) evaluable patients achieved a clinical CR, 15 of 15 (100%) tested achieved a phenotypic CR, and 11 of 14 (79%) tested achieved a molecular CR, with acceptable morbidity and mortality. This study shows the feasibility of BMT as a therapeutic approach in suitable poor prognosis patients with B-CLL.

MATERIALS AND METHODS

Selection of patients and treatment protocol. Patients with a histologic diagnosis of CLL were eligible for study if (1) they had B-CLL documented by tumor cell reactivity with anti-B1 (CD20) MoAb; (2) they were less than or equal to 60 years of age; (3) their disease was International Workshop on CLL stage B (II), C (III), or C (IV); (4) there was absence of comorbid disease of the lungs, liver, or lymphadenopathy within a 6- to 12-month period), these were not formal eligibility requirements for the study. Patients with T-CLL or prolymphocytic leukemia were not eligible. In addition, any circumstance that precluded the ability to deliver full-dose total body irradiation (TBI) was a reason for exclusion from the study. Patients with HLA-matched siblings underwent allo-BMT, whereas all other patients underwent auto-BMT. The preparative regimen was identical for all patients and consisted of cyclophosphamide (CTX) at 60 mg/kg of body weight infused on each of 2 consecutive days followed by 1,400 cGy TBI administered in fractionated doses (200 cGy twice daily on 3.5 consecutive days). Within 18 hours of the completion of radiotherapy, patients received either cryopreserved autologous BM that had been previously treated in vitro with multiple anti–B-cell MoAbs and rabbit complement or newly harvested BM from an HLA-identical sibling donor that had been treated in vitro with anti-T12 (CD6) MoAb and complement. No other GVHD prophylaxis was given. Informed consent was obtained from all patients and the protocol was approved by the Human Protection Committee at Dana-Farber Cancer Institute.

Collection, processing, and infusion of marrow. Within 4 weeks before admission for BMT, autologous BM was harvested from the iliac crests, treated in vitro as previously described for patients with non-Hodgkin’s lymphoma with anti-B1 (CD20), B5, and J5 (CD10), and cryopreserved. Anti-T1 (CD5) was not used because of a previous report documenting an increased incidence of Epstein-Barr virus-associated lymphoproliferative syndrome after purging of the BM with anti-T1 for patients undergoing autologous BMT for T-cell lymphoma. After completion of radiotherapy, the cryopreserved marrow cells were rapidly thawed and diluted in medium containing DNAase. The median number of cells infused was 2.87 × 10^7/kg (range, 2.60 to 8.01) with 85% to 95% viability, as measured by trypan blue dye exclusion. For patients undergoing allo-BMT, BM was harvested from the iliac crests of normal HLA-matched sibling donors and incubated in vitro with anti-T12 (CD6) MoAb and rabbit complement, as previously described. The median number of allogeneic cells infused was 4.97 × 10^7/kg (range, 4.4 to 8.0).

Supportive care. Patients were treated in reverse isolation rooms. All patients received oral prophylactic antibiotics, with either ciprofloxacin or trimethoprim-sulfamethoxazole when chemotherapy was begun. These were discontinued when intravenous antibiotics were instituted for fever. In addition, all patients received prophylactic acyclovir (5 mg/kg intravenously [IV] or 400 mg orally every 8 hours) for herpes simplex infections. Cytomegalovirus-negative or leuco-filtered blood products were used in all patients regardless of prior exposure to cytomegalovirus. All blood products were irradiated (2,000 Gy) to prevent transfusion-related GVHD.

Clinical evaluation. Before admission for BMT, all patients were evaluated by physical examination, blood-chemistry profile, complete blood count (CBC), differential, serum protein immunoelectrophoresis, chest x-ray, abdominal-pelvic computer tomography (CT) scanning, as well as bilateral BM aspirates and biopsies. Chest CT scans were performed in patients with evidence of abnormalities on chest x-ray evaluation. Splenomegaly was defined by CT scan evaluation: mild, 12 to 14 cm; moderate, greater than 14 to 17 cm; massive, greater than 17 cm. Clinical CR was defined as the absence of any detectable disease. The criteria for achievement of clinical CR were identical after BMT, with all evaluations performed within 3 months of BM infusion. Follow-up restaging for assessment of the durability of remissions was performed every 3 to 6 months thereafter or more frequently if clinically indicated.

Pathologic review. BM aspirates were reviewed at the Dana-Farber Cancer Institute and the Hematopathology Department at Brigham and Women’s Hospital (Boston, MA). BM biopsies, lymph node biopsies (when available), and all splenectomy specimens were reviewed by the Hematopathology Department at Brigham and Women’s Hospital. BM biopsies were examined for cellularity, lymphocyte percentages of the overall cellularity, and intertrabeclular space and pattern of BM infiltration. Five different patterns on biopsy were reported: (1) normal, with no evidence of lymphoproliferation in the BM; (2) nodular, with nodules of small mature lymphocytes lacking clear germinal centers; (3) interstitial, with replacement of normal hematopoietic tissue by small mature lymphocytes infiltrating between and without disruption of the marrow architecture; (4) mixed, with both nodular and interstitial involvement; and (5) diffuse, with effacement of the marrow architecture by small mature lymphocytes.

Hematologic engraftment. Neutrophil engraftment was defined as the achievement of an absolute neutrophil count (ANC) of greater than or equal to 500/μL on 2 consecutive days, with the time to neutrophil engraftment calculated using day 0 as the day of BM infusion and measuring to the first of 2 consecutive days. Platelet engraftment was defined as the first of 2 consecutive days with platelets greater than or equal to 20,000/μL, and followed by 7 days without platelet transfusion.

Dual fluorescence analysis. To further assess remission status immediately before and after BMT, dual fluorescence of BM mononuclear cells for CD5 and CD20 was performed for the majority of patients. Cells were analyzed at the time of initial evaluation, immediately before BMT, and at varying intervals between 3 and 6 months after BMT. Immunophenotypic analysis was performed on an EPICS ELITE flow cytometer (Coulter Cytometry, Hialeah, FL). Negative isotypic controls as well as single color (fluorescein isothiocyanate [FITC] and phycoerythrin [PE]) stained samples were analyzed to insure that the flow cytometer was adjusted for maximum sensitivity as well as proper fluorescence compensation. For dual-labeled samples, the degree of reactivity was performed on 1 × 10^4 scatter gated lymphocytes and percent of coexpression was determined by using a Quad-stat statistics program (Coulter Electronics, Hialeah, FL). The presence of more than 10% of the total
lymphocyte population coexpressing CD5 and CD20 was considered positive for residual disease.

Ig gene rearrangements. Assessment of Ig gene rearrangements were performed on BM and peripheral blood (PB) samples for the majority of patients. High molecular weight DNA was extracted from cells using standard protocols and 10 μg of individual DNA samples digested using the restriction enzymes EcoRI, BamHI, and HindIII (New England Biolabs, Beverly, MA). The digested samples were analyzed by electrophoresis in 0.8% agarose gels and Southern blotted onto Nylon membranes (BioRad, Richmond, CA). Hybridization of the Southern blots was performed using oligolabeled probes to the JH region of the Ig heavy chain region. After hybridization, the blots were exposed for 3 days at -70°C. On dilution assays, the detection of Ig gene rearrangements was sensitive to the 1% level.

Statistical methods. Descriptive statistics based on ranks (such as the median, minimum, and maximum) are used to present time to engraftment, disease-free survival, and overall survival. Survival variables are assessed by the method of Kaplan and Meier, with confidence intervals calculated using Greenwood’s formula.

RESULTS

Patient characteristics. Eight patients (7 males and 1 female) with B-CLL underwent allo-BMT and 12 patients (10 males and 2 females) underwent auto-BMT between January 1990 and August 1992. The characteristics of these patients are shown in Table 1. The median age of the cohort that underwent allo-BMT was 40 years (range, 31 to 54), whereas that of the group that underwent auto-BMT was 45 years (range, 27 to 54). Five patients had a positive family history for CLL. Of the entire group, 13 patients had International Working Group stage B (II), 3 had stage C (III), 3 had stage C (IV), and 1 had Stage B (I) with a history of Richter’s transformation. At the time of initial evaluation for BMT, the majority of patients had a diffuse BM pattern on biopsy, whereas the remainder exhibited a variety of patterns (3, mixed; 1, interstitial; 1, nodular; and 1, histologically normal) (Table 1). Of 18 patients evaluated, 5 had cytogenetic abnormalities and 13 had normal analyses. Ten of the 20 patients had evidence for a rapid tumor growth rate, with doubling of either lymphocyte count and/or lymphadenopathy within a 6- to 12-month period. Before BMT, 7 patients were invaluable for tumor doubling time because of extensive disease at presentation requiring immediate therapy and were also considered to have rapid tumor growth. In addition, 2 patients (nos. 10 and 20) had 20% to 50% prolymphocytes on peripheral blood smear, which was assessed to be a poor prognostic feature. Eight patients had a history of massive splenomegaly (patient no. 18 had a postsplenectomy status), whereas 4 had moderate splenomegaly and 6 had mild splenomegaly as documented on CT scan evaluation (Table 1).

Treatment before BMT for B-CLL. The therapy received before BMT is shown in Table 2. Nine patients had

<table>
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<th>Table 1. Characteristics of Patients Undergoing BMT for B-CLL</th>
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<td>20</td>
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</tbody>
</table>

Abbreviations: D, diffuse; N, nodular; I, interstitial; M, mixed; NI, normal; ND, not done; mild, spleen 12 to 14 cm on CT scan; moderate, spleen >14 cm on CT scan; massive, spleen >17 cm on CT scan.

* Dry BM aspirate.
† Twenty percent to 50% prolymphocytes on peripheral smear.
‡ Status postsplenectomy.
**Table 2. Treatment Before BMT for B-CLL**

<table>
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<tr>
<th>Patient No.</th>
<th>Therapy Pre-BMT Evaluation</th>
<th>Therapy to Achieve Minimal Disease</th>
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<th>Splenectomy</th>
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<td>+</td>
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</table>

**Abbreviations:** CHL/P, chlorambucil/prednisone; CVP, cyclophosphamide, vincristine, prednisone; M-BACOD, methotrexate, bleomycin, adriamycin, cyclophosphamide, oncovin, dexamethasone; PROMACE-MOPP, prednisone, methotrexate, adriamycin, cyclophosphamide, etoposide, nitrogen mustard, vincristine, procarbazine; COP-BLAM, cyclophosphamide, vincristine, prednisone, bleomycin, doxorubicin, procarbazine; ESAP, etoposide, methylprednisolone, cytosine arabinoside, cis platinum; FLU, fludarabine; CEP, cyclophosphamide, etoposide, cis platinum; XRT, radiotherapy.

received chemotherapy before the initial BMT evaluation. With the exception of 1 patient (no. 19), a variety of induction regimens were subsequently required to achieve the minimal disease criteria requisite for BMT, including CVP (cyclophosphamide, vincristine, prednisone) (n = 3), CHOP (cyclophosphamide, adriamycin, vincristine, prednisone) (n = 14), fludarabine (n = 15), CEP (cyclophosphamide, etoposide, cis platinum) (n = 1), 60 mg/kg CTX (n =
between one and six treatment regimens before BMT (median of 3). In addition, 6 patients underwent splenectomy before BMT because of persistent splenic enlargement, with 5 of 6 having evidence of persistent disease on pathologic examination (Table 2). The median interval from the time of diagnosis to BMT was 28 months (range, 12 to 115 months) (Table 2).

**Disease status at BMT.** Despite aggressive attempts to induce CR, only 3 patients were in clinical CR before BMT (patients no. 3, 15, and 19), whereas 1 patient achieved a CR in the BM but still had residual adenopathy (patient no. 6) (Table 3). Although having achieved the minimal disease state requisite for BMT, 8 patients continued to have evidence of both lymph node and BM involvement, whereas 8 patients had residual BM involvement alone. The degree of residual BM involvement as assessed by percentage of intertrabecular space infiltrated varied from less than 5% (6 patients), to 5% to 10% (9 patients), to 10% to 20% (1 patient).

### Table 3. Disease Status at BMT

<table>
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<th>Patient No.</th>
<th>Status at BMT</th>
<th>Site at BMT</th>
<th>BM Bx Status at Harvest (%) (IT)</th>
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### Table 4. Type of BMT and Hematologic Engraftment After BMT for B-CLL

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Date of BMT</th>
<th>Conditioning Regimen (CTX 120 mg/kg, 1,400 cGy TBI)</th>
<th>ANC (≥500/μL)</th>
<th>PLT (≥20 × 10³/μL)</th>
<th>Duration of Hospitalization (d)</th>
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<td>CTX/TBI</td>
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</table>

### Abbreviation: PLT, platelets.

The predominant pattern of BM involvement was nodular (10 patients), but 6 patients exhibited a mixed pattern (Table 3). Ten patients were hypogammaglobulinemic, whereas 3 patients had monoclonal serum proteins before BMT (patient no. 13, IgM 564 mg/dL; patient no. 18, IgG 2,360 mg/dL; patient no. 20, IgM 666 mg/dL).

**Type of BMT and hematologic engraftment.** Eight patients underwent allo-BMT with anti-T12-depleted BM from normal HLA-identical siblings and 12 patients underwent auto-BMT (Table 4). All patients achieved neutrophil engraftment. After auto-BMT, 3 patients (nos. 14, 17, and 20) received recombinant human granulocyte colony-stimulating factor (rhG-CSF) on day 29 (patient no. 14) and day 20 (patients no. 17 and 20), respectively, with achievement of an ANC ≥500/μL on days 30, 25, and 21, respectively. For the remaining 17 patients who did not receive rhG-CSF, the median time to an ANC ≥500/μL was 22.5 days (range, 14 to 29) for 8 patients after allo-BMT and 20 days (range, 18 to 27) for 9 patients after auto-BMT (Table 4). All patients achieved platelet engraftment. The median time to platelet recovery (≥20,000/μL) was 20.5 days (range, 13 to 34) after allo-BMT and 29 days after auto-BMT (range, 16 to 69). Patients were discharged from the hospital at a median of 33 days (range, 26 to 91) after allo-BMT and 31 days (range, 24 to 122) after auto-BMT (Table 4).

**Acute and chronic toxicity post-BMT.** Eighteen patients developed fever (≥101°F) (6 after allo-BMT; all 12 after auto-BMT) and all patients experienced mucositis in association with neutropenia (Table 5). Other acute infectious toxicities included Hickman line infections with coagulase-negative *Staphylococcus,* which led to septic thrombophlebitis in 1 patient and bacteremia in another, one transient culture negative pneumonitis, and 1 life-threatening case of
Table 5. Acute Toxicity Post-BMT for B-CLL

<table>
<thead>
<tr>
<th>Type of BMT</th>
<th>No. of Patients</th>
<th>Infectious</th>
<th>Noninfectious</th>
<th>Toxic Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fever</td>
<td>Line Sepsis</td>
<td>CHF</td>
</tr>
<tr>
<td>Allo-BMT</td>
<td>8</td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Auto-BMT</td>
<td>12</td>
<td>12</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviations: VOD, venocclusive disease; DAHS, diffuse alveolar hemorrhage syndrome; CHF, congestive heart failure.
* CNS toxoplasmosis (transient pneumonitis).

central nervous system (CNS) toxoplasmosis (Table 5). Acute noninfectious complications included one congestive heart failure and one transient pericarditis, as well as GVHD (2 with grade I and 1 with grade IV) (Table 5). Venocclusive disease was not observed. One acute in-hospital death (day 62; patient no. 12) occurred after auto-BMT secondary to diffuse alveolar hemorrhage syndrome. There was no significant increase in toxicities observed for those patients who had received fludarabine before BMT.

Late infectious complications (after discharge) included one Staph epidermidis bacteremia from a Hickman line infection, one bacterial sinusitis, and three Herpes zoster (Table 6). Of interest, all 3 patients who developed bacterial sinusitis had undergone splenectomy before BMT. Late noninfectious complications included three cases of hemolytic-uremic syndrome and one chronic GVHD (Table 6). The patient with grade IV chronic GVHD of the skin and gastrointestinal tract (patient no. 1) required immunosuppressive therapy and ultimately succumbed to pneumocystis pneumonia. No significant differences in toxicities were observed between those patients who underwent allo-BMT versus those who underwent auto-BMT.

Treatment outcome. Nineteen patients survived for greater than 3 months and were evaluable for response to BMT. Sixteen patients were documented to achieve clinical CR within the first 3 months after BMT and remain in unmaintained CR at last restaging (6 for allo-BMT; 10 for auto-BMT) (median follow-up, 11.7 months for allo-BMT, 5.0 months for auto-BMT; range, 2 to 31 months) (Table 7). Two patients (nos. 8 and 14) had evidence of persistent BM infiltration on BM biopsy after allo- and auto-BMT, respectively. Both patients remain alive and well untreated 15 months (no. 14) and 6 months (no. 8), respectively, post-BMT and continue to have the BM as their only site of disease. One patient (no. 1) was not restaged until 12 months post-BMT, when she was found to be in clinical CR. Patient no. 12 was never restaged to assess whether a clinical CR had been achieved. Of the 5 patients with known cytogenetic abnormalities before BMT, 4 evaluated after BMT reverted to normal. Ten of 16 patients who were documented to be in clinical CR ≥3 months post-BMT have been restaged at various intervals after BMT and continue in clinical CR (Table 7). Of the 10 patients who were hypogammaglobulinemic before BMT, 5 of 7 who were evaluable and in clinical CR were examined at ≥6 months after BMT and found to have a normal serum protein electrophoresis (SPEP). Patient no. 13 has reverted to a normal SPEP greater than 1 year post-BMT (from a monoclonal serum IgM), whereas patient no. 18 continues to show a monoclonal serum protein (2,210 mg/dL) within the first 3 months post-BMT. Follow-up is short for this patient population and, therefore, the long-term impact of achievement of clinical CR after BMT for B-CLL is unknown. The predicted disease-free survival of the combined patient group who have undergone allo-BMT and auto-BMT is shown in Fig 1. At 12 months, the predicted disease-free survival is 82% (95% CI, 64% to 100%).

Assessment of CR by phenotypic and molecular biologic analysis. In an attempt to assess more accurately the remission status of these patients, samples were assessed for the presence of CLL cells by phenotypic analysis and for the presence of detectable Ig gene rearrangements by restriction fragment analysis and Southern blotting. At the time of initial evaluation, the CLL cells of all 20 patients were documented to coexpress CD5 and CD20 by dual-color immunofluorescence of BM, PB, or lymph node samples. Similarly, all 20 patients had evidence of monoclonal Ig gene rearrangements at the time of initial evaluation. At the time of BM harvest, all 20 patients had persistent evidence of detectable Ig gene rearrangements, including the 3 patients who

Table 6. Late Toxicity Post-BMT for B-CLL

<table>
<thead>
<tr>
<th>Type of BMT</th>
<th>No. of Patients</th>
<th>Infectious</th>
<th>Noninfectious</th>
<th>Toxic Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Line Sepsis</td>
<td>Bacterial Gastrointestinal</td>
<td>CHF</td>
</tr>
<tr>
<td>Allo-BMT</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Auto-BMT</td>
<td>11</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Abbreviation: HUS, hemolytic uremic syndrome.
* Chronic-skin, GI tract.
Table 7. Treatment Outcome After BMT for B-CLL

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Clinical Response</th>
<th>Cytogenetic Response</th>
<th>Months Post BMT</th>
<th>Clinical Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allo-BMT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>12</td>
<td>Complete</td>
</tr>
<tr>
<td>2</td>
<td>Complete</td>
<td>Normal</td>
<td>18</td>
<td>Complete</td>
</tr>
<tr>
<td>3</td>
<td>Complete</td>
<td>Normal</td>
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<td>Complete</td>
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<tr>
<td>4</td>
<td>Complete</td>
<td>Normal</td>
<td>12</td>
<td>Complete</td>
</tr>
<tr>
<td>5</td>
<td>Complete</td>
<td>Normal</td>
<td>11</td>
<td>Complete</td>
</tr>
<tr>
<td>6</td>
<td>Complete</td>
<td>Normal</td>
<td>12</td>
<td>Complete</td>
</tr>
<tr>
<td>7</td>
<td>Complete</td>
<td>Normal</td>
<td>12</td>
<td>Complete</td>
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<tr>
<td>8</td>
<td>PD</td>
<td>Normal</td>
<td>6</td>
<td>PD</td>
</tr>
<tr>
<td>Auto-BMT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Complete</td>
<td>Normal</td>
<td>31</td>
<td>Complete</td>
</tr>
<tr>
<td>10</td>
<td>Complete</td>
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<td>25</td>
<td>Complete</td>
</tr>
<tr>
<td>12</td>
<td>NE (died 62 d)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Complete</td>
<td>NA</td>
<td>13</td>
<td>Complete*</td>
</tr>
<tr>
<td>14</td>
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<td>Complete</td>
<td>Normal</td>
<td>6</td>
<td>Complete</td>
</tr>
<tr>
<td>16</td>
<td>Complete</td>
<td>Normal</td>
<td>—</td>
<td>Too Early</td>
</tr>
<tr>
<td>17</td>
<td>Complete</td>
<td>NA</td>
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</tr>
<tr>
<td>19</td>
<td>Complete</td>
<td>Normal</td>
<td>—</td>
<td>Too Early</td>
</tr>
<tr>
<td>20</td>
<td>Complete</td>
<td>NA</td>
<td>—</td>
<td>Too Early</td>
</tr>
</tbody>
</table>

Complete response by clinical staging: CBC, differential, blood-chemistry profile, serum protein immunoelectrophoresis, physical examination, x-rays, CT scans, bilateral BM aspirates and biopsies.

Abbreviations: NA, not assessed; PD, persistent disease; NE, not evaluable.

* Refused BM.

had achieved a clinical CR. However, only 4 of 13 patients assessed had evidence of detectable cells expressing both CD5 and CD20 (data not shown).

The results of both dual-color immunofluorescence and Southern blot analysis of BM samples obtained at intervals after allogeneic BMT are shown in Fig 2. All samples analyzed were obtained when the patients were assessed to be in clinical CR. As can be seen, 5 of these 6 patients had detectable residual CLL cells within 6 months of receiving the BMT. After 6 months, no evidence of these residual CLL cells could be detected by dual-color immunofluorescence. Of interest, 4 patients had complete resolution of detectable CLL cells at further follow-up despite the fact that they received no additional therapy. One patient (no. 7) who remains in clinical CR at 12 months post-BMT has persistent residual CLL cells, as assessed by the presence of detectable Ig gene rearrangements. Five patients had no evidence of residual CLL cells by phenotypic or molecular biologic assessment of their most recent BM samples.

The results of dual-color immunofluorescence and Southern blot analysis of BM samples obtained at intervals after auto-BMT are shown in Fig 3. Again, all samples analyzed were obtained when the patients were in clinical CR. Six of these patients (nos. 15 through 20) have been observed for only a very short period and in 3 cases residual CLL cells were detected within 3 months after BMT. In 1 of these cases (no. 15), subsequent analysis documented a return to germline configuration. In the 3 cases in whom longer-term follow-up samples are available for analysis, no residual lymphoma cells were detected by either phenotypic or molecular biologic analysis. The results of dual-color immunofluorescence analysis for CD5 and CD20 of samples obtained from patient no. 9, who underwent auto-BMT, are shown in Fig 4. As can be seen, at the time of initial evaluation (Fig 4A) the majority of cells are CLL cells and coexpress CD5 and CD20. At the time of BM harvest (Fig 4B), a

Fig 1. Predicted disease-free survival (DFS) of 20 patients undergoing auto-BMT or allo-BMT for B-CLL.
small but distinct residual population of CLL cells are observed. By 24 months after auto-BMT (Fig 4C), CLL cells coexpressing CD5 and CD20 are no longer detectable.

**DISCUSSION**

CLL is one of the few hematologic malignancies for which high-dose myeloablative therapy and BMT has played a limited therapeutic role. We report the results of a pilot study in which patients with aggressive poor prognosis B-CLL underwent either allo-BMT or auto-BMT to examine the safety and efficacy of this treatment approach. Twenty patients with poor prognosis B-CLL were treated to a minimal disease state using conventional chemotherapy. Only 3 attained a clinical CR, whereas the remainder attained a good partial remission before BMT. After BMT, all patients fully engrafted and transplant-related mortality was relatively low (10%). After BMT, of the 19 patients evaluable for response, 17 (89%) achieved a clinical CR. Although follow-up is too short to evaluate the impact of BMT on long-term disease-free survival (median follow-up, 11.7 months), assessment for residual B-CLL cells in the BM by CD5/CD20 dual-color flow cytometry as well as Ig gene rearrangements shows that most patients evaluated have achieved both phenotypic (100%) and molecular CRs (79%) with this approach.

Although B-CLL is traditionally considered to be sensitive to conventional therapy, this was not our experience in the subgroup of poor prognosis patients treated on this study. Most patients were initially treated with CHOP, which usually resulted in cytoreduction of nodal disease but did not result in dramatic marrow responses.\(^{27,28}\) In fact, no clinical CRs were achieved for those 15 patients who were treated with CHOP induction therapy. Of only 3 patients who attained a clinical CR before BMT, 2 received fludarabine. The CR rates for CHOP and fludarabine in this patient population are certainly lower than has been reported previously.\(^{1,3,27,28}\) These lower response rates are likely due both to the poor prognostic features of our patient cohort as well as our aggressive staging, which included CT scan evaluations. In addition, higher response rates might have been achieved if fludarabine had been used more frequently as first-line therapy.

Both the time to engraftment and toxicity observed in this study appeared to be comparable to those previously reported for auto-BMT and allo-BMT in patients with other hematologic malignancies treated at our institution.\(^{22,23}\) However, 1 patient who underwent auto-BMT developed CNS toxoplasmosis and survived. CNS toxoplasmosis has not been observed in nearly 500 patients undergoing auto-BMT for other hematologic malignancies at Dana-Farber Cancer Institute. Whether the immunosuppressive effects of fludarabine contributed to this event is unknown but certainly is of concern. The majority of complications after allo-BMT were comparable to those seen after auto-BMT. The similar toxicities were due to the fact that only 1 of the 8
patients who underwent allo-BMT developed clinically significant GVHD (grade 4). We conclude that these toxicities are acceptable and should not preclude further use of this approach for suitable patients with B-CLL.

Because it will require several more years of follow-up and significantly larger numbers of patients transplanted before any statement of long-term efficacy can be made, we attempted to determine whether both phenotypic and molecular remissions were achieved in the marrow after allo-BMT and auto-BMT. Recently, Robertson et al. have examined the response rates of patients with progressive or symptomatic CLL treated with fludarabine and prednisone. Of note, CT scans were not routinely performed to determine remission status. Response was defined by clinical criteria as well as assessment of residual disease by dual-color flow cytometry for CD5/CD20-positive cells and Ig gene rearrangements. Although most patients in clinical CR (89%) had no residual disease detected by flow cytometry, only 51% of those with a nodular marrow CR and 19% of the partial responders had phenotypic remissions. A small subgroup of patients in clinical CR were also examined for Ig gene rearrangements, with a return to the germline configuration observed in the majority (5 of 7 CRs and 2 of 8 nodular CRs). In addition, all patients who reverted to a germline DNA pattern after treatment remained free of disease. Of the 19 evaluable patients post-BMT in this study, 89% attained a clinical CR. Of the 15 patients in clinical CR who were evaluated for phenotypic remission, all lacked evidence of residual disease at follow-up 3 to 24 months post-BMT. Of the 14 patients in clinical CR who were evaluated for molecular remission, 79% had returned to the germline configuration from 3 to 24 months post-BMT. It is important to note that of the 9 patients who underwent auto-BMT and were assessed for phenotypic and/or molecular CR, only 4 have been observed for more than a 3-month period (range, 6 to 24 months). Nevertheless, these results are encouraging and suggest that both allo-BMT and auto-BMT for B-CLL can result in phenotypic and molecular CRs. Clearly, much longer follow-up will be essential to determine whether the attainment of such a CR will lead to prolonged disease-free survival.

To date, our results extend the encouraging results of the previously reported European multi-center study. Transplant-related toxicity was greater in their study, with 5 toxic deaths observed (29%) and all 15 evaluable patients developing GVHD (5 cases being chronic). Fourteen patients received non-T-cell–depleted BM, which probably explains the high incidence of GVHD. Nevertheless, 9 patients remained disease free with a median follow-up of 25.6 months. Our study differed in several respects. Only patients who achieved a minimal disease state after a variety of chemotherapy regimens were eligible for BMT and T-cell depletion of the donor BM was the only method of GVHD prophylaxis. In addition, auto-BMT was performed if an HLA-identical donor was not available. Finally, all patients in this study received a uniform myeloablative regimen. In addition, we have documented both phenotypic and molecular CRs in the majority of patients after both allo-BMT and auto-BMT.

Long-term follow-up will be essential to determine whether the CRs that we have observed after BMT for B-CLL will translate into long-term disease-free survival and overall survival. Our observation that several patients in whom BMs initially positive for Ig gene rearrangements subsequently returned to the germline configuration suggests that CLL cells may die slowly over time and that there may be a potential role for immune modulation after BMT. It seems clear that CRs can be achieved after both
allo-BMT and auto-BMT for B-CLL, and thus far we have observed no significant differences in either toxicity or treatment outcome between these two approaches. Because we are a tertiary referral center, and therefore evaluate a relatively small cohort of patients B-CLL, it is difficult to assess how applicable this technique will be to the entire population of patients with poor prognosis disease. However, our experience of patients who were referred to this institution for consideration of this approach has been that virtually every patient who presents with International Workshop stage II could be rendered into a protocol eligible minimal disease state and proceed to transplant. In contrast, of those patients with International Workshop stage III and IV, less than one-third of patients responded to conventional dose therapy to achieve a protocol eligible minimal disease state and proceed to transplant. Because the nonresponders probably represent the worst prognostic group, it is difficult to determine whether BMT will have any impact on overall survival. Although the present study shows that allo-BMT and auto-BMT are feasible treatment options for selected younger patients with CLL, future studies will be required to delineate the role of BMT in the management of this disease.

ACKNOWLEDGMENT
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To the Editor:

Brugnara et al have recently shown that the well-established effect of recombinant human erythropoietin in chronic renal failure also occurs in normal subjects. Therapeutic erythropoietin can stimulate erythropoiesis to the extent that the demand for iron by the proliferating marrow exceeds the rate at which it can be released from intracellular pools. The result of this is that the supply of iron to the developing erythron is limited. This functional iron deficiency occurs even when there is ample iron in the stores, as reflected by the serum ferritin concentration.

Functional iron deficiency has been associated with reduced transferrin saturation, but this parameter may be comprised by many factors. It is most closely reflected by the production of hypochromic red blood cells. The emergence of these cells in the circulation may be uniquely quantified by the Technicon H* System (Miles Diagnostics Inc, Tarrytown, NY). Although it is not clear from the study of Brugnara et al what proportion of the cells were hypochromic, we have found that transferrin saturation is markedly and consistently reduced when more than 20% of the cells in the circulation are hypochromic.

Because this condition is the result of a failure of the rate of delivery of iron through the plasma transferrin pool to the proliferating erythroblast, the remedy should concentrate on this area. It is pointless to address the level of iron stores when these are already adequate. We have shown that iron dextran, which is rapidly metabolized to release iron to the transferrin pool, will allow sufficient iron to reach the proliferating erythroblast to abrogate production of hypochromic red blood cells.

The occurrence of functional iron deficiency in patients treated with recombinant human erythropoietin is an important limiting factor in the effectiveness of this expensive therapy. We suggest that it is more widespread than measurements of serum ferritin or mean corpuscular volume might indicate and that its presence should be sought by the quantitation of hypochromia whenever there is an inadequate response to recombinant human erythropoietin.

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REFERENCES
To the Editor:

We have read with interest the paper of Landolfi et al.1 “Increased Thromboxane Biosynthesis in Patients With Polycythemia Vera: Evidence for Aspirin-Suppressible Platelet Activation In Vivo.” In a recent publication2 on 11 patients with chronic myeloproliferative disorders (CMPD), 7 with essential thrombocytosis and 4 with polycythemia vera, we have reached a similar conclusion (with respect to enhanced thromboxane generation) in our patients. Yet, the mechanism of this abnormality seems to be different from that suggested by Landolfi et al and, consequently, might have important clinical implications. It seems unlikely that in CMPD patients there is a “biochemical selective alteration in cyclooxygenase/thromboxane synthetase pathway” as stated by Landolfi et al, but rather an increase in platelet aggregation inducer (possibly thrombin) that stimulates platelet thromboxane A₂ (TXA₂) synthesis. Pertinent is our observation3 on normal TXB₂ generation (corrected to a normal platelet concentration: 2.5 × 10⁵/mL) in platelet-rich plasma of our patients after stimulation by ADP, collagen, or epinephrine (described also by Landolfi et al), despite a reduced extent of platelet aggregation by these inducers.3 However, this finding could not explain a pronounced increase in serum TXB₂ generation (measured in suboptimal conditions, 22°C, and corrected to a normal platelet concentration) 2.9 to 7.1 times higher than that of controls2 and the presence of a potent agonist, possibly thrombin, stimulating platelet TXB₂ generation was indicated.2 The latter was also supported by the reported elevated plasma fibrinopeptide A level,3 a marker of thrombin genera-
tion,\(^4\) in CMPD patients.\(^5\) Moreover, other investigators have observed even a defective signal transduction through the TXA\(_2\) receptor in a CMPD patient,\(^6\) contrary to the conclusion of Landolfi et al.

We agree with Landolfi et al that increased TXA\(_2\) synthesis in CMPD patients may represent an enhanced platelet activation for two reasons. We have obtained a markedly elevated plasma \(\beta\)-thromboglobulin level (corrected to a normal platelet concentration), a marker of platelet activation,\(^6\) in our CMPD patients. In addition, picotamide (a thromboxane synthetase/receptor antagonist) was beneficial in the management of thromboembolic complications and in the reduction of elevated plasma fibrinopeptide A level in CMPD patients.\(^5\)

Finally, and in accordance with Landolfi et al, aspirin might be effective in the suppression of enhanced TXA\(_2\) biosynthesis, but it was ineffective in the management of thrombotic complications (arterial and venous) present in 8 of our 11 CMPD patients. It was therefore substituted with success by heparin-coumadin, compatible with our above-mentioned mechanism.

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REFERENCES


RESPONSE

We are grateful to Drs M. Zahavi and J. Zahavi for their comments. The mechanisms underlying enhanced thromboxane biosynthesis in polycythemia vera\(^1\) and essential thrombocythemia\(^2\) remain elusive. As we reported, it seems likely that thromboxane is produced in response to in vivo stimuli and that the platelet cyclooxygenase/Tx-synthase pathway is involved in their transduction. Unfortunately, the findings of Drs Zahavi do not provide clues for the identification of these stimuli mainly because triggers to platelet activation operating in vivo are unlikely to be detected by ex vivo capacity measurements. As for the role of aspirin in preventing thrombotic complications in this setting, this requires a randomized clinical trial of adequate sample size. One such trial has just started (GISP: Gruppo Italiano di Studio della Policitemia) with the aim of assessing the long-term efficacy and safety of low-dose aspirin (40 mg/d) in patients with polycythemia vera.

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REFERENCES

REMITISSION AFTER ERYTHROPOIETIN ADMINISTRATION FOR ERYTHROLEUKEMIA—A CASE STUDY

To the Editor:

Erythropoietin (EPO) has proved useful for the recovery of anemia under the condition of renal insufficiency or myeloma. In vitro colony assay has shown that EPO stimulates the growth and differentiation of burst-forming unit-erythroid and colony-forming unit-erythroid colonies of bone marrow progenitor cells.1 It has been noted that marrow erythroblasts in erythroleukemia were decreased after transfusion, suggesting that a humoral factor such as EPO in serum may contribute to the regulation of erythroleukemia cells.2 However, clinicians have withheld application of EPO in erythroleukemia because this agent may stimulate the proliferation of leukemia cells; on the other hand, it has not yet been shown that EPO is capable in vitro to force differentiation of erythroleukemia cells, as all-trans retinoic acid has been observed to do for promyelocytic leukemia cells.3 We present here a case of erythroleukemia who
achieved complete remission after it was confirmed in vitro that EPO had brought about differentiation of leukemia cells.

A 17-year-old male was admitted to our hospital for anemia. His red blood cell (RBC) count was $266 \times 10^6/\mu L$; hemoglobin (Hb) 9.5 g/dL; reticulocyte count 2.7%; white blood cell (WBC) count 1,700/µL with 42% neutrophils, 54% lymphocytes, and 4% monocytes; and platelet count $7.5 \times 10^9/\mu L$. Nuclear RBCs were found in 36 of 100 RBCs. The bone marrow aspirate disclosed an erythroid dominant marrow with a nuclear cell count of 206,000/µL, with 68% erythroid cells. The percentage of blasts among nonerythroid mononuclear cells was 37.8%. The erythroid cells showed a high nuclear/cytoplasmic ratio and were positive for Periodic acid Schiff stain. A diagnosis of erythroleukemia (M6 in the French-American-British classification) was made and he was subsequently treated with several chemotherapies, including daunomycin, 6-mercaptopurine, prednisolone, and behenoyl cytosine arabinoside, etc; however, these were not effective.

We then investigated the potential of differentiation therapy. The patient’s bone marrow aspirates were subjected to Ficoll-Hypaque discontinuous centrifugation and mononuclear cells were obtained. The cells were incubated in 10% fetal calf serum supplemented with Iscove’s modified Dulbecco’s Medium for 7 days and cultured for another 7 days in the presence of EPO. After EPO addition, the total cell number $(0.5 \times 10^6/mL)$ started to increase, reached a plateau $(3.5 \times 10^6/mL)$ at day 5, and thereafter rapidly decreased. It was noteworthy that the leukemic blasts in culture had almost disappeared and that the erythroblasts and mature RBCs increased by day 7, as shown in the photomicrograph (Fig 1). These in vitro studies showed that differentiation can be stimulated in erythroleukemia cells by EPO.

As the patient was refractory to conventional chemotherapies, we explained his situation and the results of the in vitro study to his family and obtained informed consent to use EPO. EPO was administered subcutaneously at a dose of 12,000 U/d. The patient’s clinical course is shown in Fig 2. After 6 days of EPO administration, the Hb value started to increase and the nuclear RBCs had disappeared in the peripheral blood at day 14. Bone marrow aspirate showed a remarkable decrease in megaloblasts and an increase in polychromatic and orthochromatic erythroblasts. By 2 weeks of administration of EPO, the leukemia had gone into remission without any other cytotoxic drugs. We decided to continue daily administration of EPO because it appeared to be the decisive factor in the patient’s general improvement as well as his increase in the Hb, WBC, and platelet count. No donor for a bone marrow transplant could be found with matching HLA. However, 3 months after his first treatment with EPO, the patient developed pancytopenia and showed an increase of unclassified blasts both in peripheral blood and bone marrow. Conventional chemotherapies including cytosine arabinoside and other drugs were ineffective. These blasts were also refractory to EPO addition in vitro. The patient died from cerebral hemorrhage.

Erythroleukemia is still one of the leukemias with a poor prognosis, because it has a high tendency to deteriorate into acute nonlymphocytic leukemia. Our patient’s clinical course strongly suggests that EPO has potential for differentiation therapy of erythroleukemia. As far as we known, this is the first reported case in which EPO
has stimulated differentiation of erythroleukemia cells in vitro as well as substantively improving a patient’s condition. Further clinical studies of this agent seem to be warranted; however, users must take the potential for tumor growth stimulation into account and interrupt the treatment promptly if rapid progression occurs.

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REFERENCES


Autologous and allogeneic bone marrow transplantation for poor prognosis patients with B-cell chronic lymphocytic leukemia