Monoclonal Antibody-Purged Bone Marrow Transplantation Therapy for Multiple Myeloma

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Forty patients with plasma cell dyscrasias underwent high-dose chemoradiotherapy and either anti-B-cell monoclonal antibody (MoAb)-treated autologous, anti-T-cell MoAb-treated HLA-matched sibling allogeneic or syngeneic bone marrow transplantation (BMT). The majority of patients had advanced Durie-Salmon stage myeloma at diagnosis, all were pretreated with chemotherapy, and 17 had received prior radiotherapy. At the time of BMT, all patients demonstrated good performance status with Karnofsky score of 80% or greater and had less than 10% marrow tumor cells; 34 patients had residual monoclonal marrow plasma cells and 38 patients had paraprotein. Following high-dose chemoradiotherapy, there were 18 complete responses (CR), 18 partial responses, one nonresponder, and three toxic deaths. Granulocytes greater than 500/µL and untransfused platelets greater than 20,000/µL were noted at a median of 23 (range, 12 to 46) and 25 (range, 10 to 175) days posttransplant (PT), respectively, in 24 of the 26 patients who underwent autografting. In the 14 patients who received allogeneic or syngeneic grafts, granulocytes greater than 500/µL and untransfused platelets greater than 20,000/µL were noted at a median of 19 (range, 12 to 24) and 16 (range, 5 to 32) days PT, respectively. With 24 months median follow-up for survival after autologous BMT, 16 of 26 patients are alive free from progression at 2+ to 55+ months PT; of these, 5 patients remain in CR at 8+ to 55+ months PT. With 24 months median follow-up for survival after allogeneic and syngeneic BMT, 8 of 14 patients are alive free from progression at 8+ to 34+ months PT; of these, 5 patients remain in CR at 8+ to 34+ months PT. This therapy has achieved high response rates and prolonged progression-free survival in some patients and proven to have acceptable toxicity. However, relapses post-BMT, coupled with slow engraftment post-BMT in heavily pretreated patients, suggest that such treatment strategies should be used earlier in the disease course. To define the role of BMT in the treatment of myeloma, its efficacy should be compared with that of conventional chemotherapy in a randomized trial.

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MULTIPLE MYELOMA remains an incurable malignancy with a median survival at best of only 48 months when conventional therapies are used. Such conventional treatments (ie, melphalan and prednisone or combination chemotherapy) have been associated with a 50% to 60% probability of transient disease regression. Although several reports have suggested improved survival after treatment with combination chemotherapy than after melphalan with or without prednisone,6,7 this remains controversial.6,7 α2b interferon (IFN) has recently been used to treat patients during initial and/or as a maintenance therapy4,14; although requiring confirmation, those patients with either near complete or complete response (CR) to chemotherapy in some studies appear to have prolonged survival with IFN maintenance treatment. Disease inevitably recurs, and although several effective salvage chemotherapy regimens achieve response rates of up to 50% to 75%,15-17 these responses are rarely complete and durable. To date, therefore, no therapies have achieved prolonged disease-free survival or cure.

A promising treatment approach for multiple myeloma stems from reports of CR after the administration of alkylating agents (melphalan, cyclophosphamide, busulfan) in higher than conventional doses, with or without total body irradiation, followed by transplantation of syngeneic, allogeneic, or autologous bone marrow (BM), or of autologous peripheral blood stem cells (PBSC).18-39 Reduction in tumor mass in some cases has been dramatic, with CR rates ranging up to 50% to 60%. Moreover, there are now relapse-free survivors as long as 14 years, 6 to 7 years, and 5 years posttransplantation, respectively. In the European Bone Marrow Transplant Group Study,32 median relapse-free survival for 37 of 90 (41%) patients who entered CR was 48 months; however, it remains unclear as to whether a plateau in the survival curve has yet been achieved in this and all other studies. Those patients who have sensitive disease and who are less heavily pretreated achieve the highest response rates and prolonged progression-free survival. More recent studies have therefore used high-dose alkylating agents, with or without radiotherapy, followed by transplantation of allogeneic BM or autologous BM and/or PBSC as initial therapy for patients with myeloma.18,29,37,38 Although response rates are high, their duration and the relative efficacy of these approaches compared with conventional initial therapies remains to be determined.

In the present study, we report the results of treatment with high-dose chemoradiotherapy and bone marrow transplantation (BMT) in 40 patients with responsive multiple myeloma. Thirteen patients less than 55 years of age with related histocompatible siblings received allografts, and a single patient underwent syngeneic BMT. Twenty-six pa-
tients less than 60 years of age, including those less than 55 years of age without related histocompatible siblings, received autografts. T cells were depleted from autografts and tumor cells from autografts, using in vitro lysis with monoclonal antibodies (MoAbs) and complement. The majority of patients were heavily pretreated and underwent BMT at the time of sensitive relapse. Although this therapy has achieved high response rates and prolonged progression-free survival in some patients and proven to have acceptable toxicity, relapses post-BMT support the view that such treatment strategies should be used earlier in the disease course and their efficacy compared with that of conventional chemotherapy.

MATERIALS AND METHODS

Selection of patients and treatment protocol. Patients were eligible for autologous BMT if they were less than 60 years of age; patients less than 55 years of age who had histocompatible sibling donors underwent allogeneic BMT. All patients had multiple myeloma, as well as reactivity of tumor cells with anti-CD10, CD19, and anti-PCA-1 MoAbs and rabbit complement. The majority of patients had achieved a minimal tumor burden, defined as less than 10% BM tumor cells. Pretransplant therapy was administered to achieve maximum cytoreduction either at Dana-Farber Cancer Institute (DFCI) or, under its supervision, administered at local institutions. Sites of bony disease received additional radiation therapy before BMT. Additional criteria for entry included the absence of comorbid disease of the heart, kidney, lung, and liver, and a Karnovsky score above 80%. Patients whose prior radiotherapy obviated the ability to deliver full-dose total body irradiation (TBI) received ablative therapy with chemotherapy only. In patients less than 55 years of age who had HLA identical mixed lymphocyte culture (MLC) nonreactive sibling donors, allogeneic BMT was performed in preference to autologous BMT; autologous BMT was done for patients less than 60 years of age, including those less than 55 years of age who did not have histocompatible sibling donors. Written informed consent was obtained from all patients for treatment protocols approved by the Human Protection Committee at DFCI.

Preparative therapy for patients undergoing autologous BMT included melphalan 70 mg/m² of body weight, infused on each of 2 consecutive days before 1,200 cGy TBI in 20 patients; and cyclophosphamide 60 mg/kg of body weight, infused on each of 2 consecutive days before TBI (1,200 cGy in one patient and 1,400 cGy in five patients). The initial 11 patients who received melphalan 70 mg/m² on 2 consecutive days followed by 1,200 cGy TBI were evaluated for toxicity in a phase I study. Ablative therapy included cyclophosphamide 60 mg/kg of body weight, infused on each of 2 consecutive days before 1,400 cGy TBI in 11 recipients of allografts and the sole recipient of syngeneic marrow; and busulphan 1 mg/kg of body weight orally every 6 hours for 16 doses over 4 consecutive days before cyclophosphamide 60 mg/kg of body weight infused on 2 consecutive days in two allotransplant patients who had received prior radiotherapy which precluded TBI. Within 18 hours of the completion of radiotherapy, either (1) cryopreserved autologous BM that had been previously treated in vitro with anti-CD10, anti-CD20, and anti-PCA-1 MoAbs and rabbit complement to deplete tumor cells was thawed rapidly and reinfused; or (2) allogeneic marrow that had been treated with anti-CD6 MoAb and rabbit complement to deplete T cells was reinfused fresh. Marrows were administered through a central venous catheter.

Collection, processing, and infusion of marrow. Bone marrow mononuclear cells (BMMCs) were obtained from the iliac crests, collected in RPMI-1640 medium with preservative-free heparin, filtered through stainless-steel mesh, and then washed and concentrated using a COBE 2991 cell washer (COBE Laboratories, Lake-wood, CO). A previously reported method developed at the Dana-Farber Cancer Institute that reproducibly deplete two to three logs of antigen (Ag) positive-cells without depleting hematopoietic stem cells. Specifically, anti-PCA-1 MoAb and complement in vitro can lyse two to three logs of the RPMI 8226 myeloma cell line. Mononuclear cells were isolated on Ficoll-Hypaque gradients and resuspended in RPMI-1640 at a concentration of 2 x 10⁷ cells/mL. These cells were subjected to three treatments, each consisting of a 30-minute incubation with MoAb at 20°C followed by incubation for 30 minutes at 37°C with rabbit complement (Pel Freeze, Brown Deer, WI). Marrow cells were treated with MoAbs directed at three Ags, including CALLA (CD10), which detects the common acute lymphoblastic leukemia Ag; B1 (CD20), a pan-B cell Ag; and PCA-1, which detects a plasma-cell-associated Ag. This MoAb cocktail was used to target cells in the malignant clone from the pre-B-cell to the plasma stage. Before in vitro marrow treatment, phenotypic analysis demonstrated less than 10% of cells bearing the CALLA, B1, or PCA-1 Ags; after treatment, repeat analysis showed absence of cells bearing these Ags. Cells were then cryopreserved in medium containing 10% dimethyl sulfoxide and 90% autologous serum at -196°C in the vapor phase of liquid nitrogen. Before infusion, the cryopreserved marrow cells were rapidly thawed and diluted in medium containing 25 IU DNase/mL to minimize clumping. The median number of reinforced marrow cells was 2.1 x 10⁸ (range, 1.4 to 5.2 x 10⁸), or a median of 3.1 x 10⁷/kg (range, 1.8 to 7.8 x 10⁷/kg), with 85% to 95% viability as measured by Trypan blue exclusion.

For allogeneic BMT, BM was harvested from histocompatible sibling donors under general anesthesia using standard techniques. Marrow was anticoagulated with preservative-free heparin in RPMI 1640. After BM harvest, marrow cells were washed free of the anticoagulant and plasma proteins and concentrated into a 50 to 100 mL buffy-coat fraction using a COBE 2991 blood-cell processor. Bone marrow mononuclear cells were isolated from the buffy-coat fraction on discontinuous gradients of Ficoll-Hypaque. Mononuclear cells were placed at a cell concentration of 2 x 10⁷/mL and were incubated with anti-T12 (CD6) MoAb (IgM isotype) for 15 minutes at 20°C followed by the addition of rabbit complement at a ratio of 1:8 or 1:10 final dilution for further incubation at 37°C for 45 minutes. Our method for in vitro treatment of marrow with anti-T12 MoAb to abrogate graft-versus-host disease (GVHD) was described previously. Incubation with anti-T12 and complement was repeated twice. After completion of treatment, marrow cells were combined and washed five times to remove both MoAb and complement, and then resuspended in 50 mL media that contained 10% human AB serum. After T12 treatment, patients received a median of 6.3 x 10⁸ (range, 2.5 to 10 x 10⁸) or a median of 7.4 x 10⁹ (range, 2.7 to 13 x 10⁹) cells/kg.

The single patient who underwent syngeneic BMT received non-purged marrow with 82.5 x 10³ total cells, or 101.2 x 10³ cells/kg.

Supportive care. Patients were treated in reverse-isolation rooms until they were discharged. Discharge was permitted if the absolute granulocyte count was stable at greater than 500/μL, and no fever had occurred in the absence of antibiotics for 24 to 48 hours. Trimethoprim-sulfamethoxazole or ciprofloxacin prophylaxis was begun in all patients when chemotherapy was initiated, but was discontinued if intravenous broad-spectrum antibiotics were required. Patients received prophylactic acyclovir (5 mg/kg or
400 mg orally every 8 hours) for Herpes simplex infections. Prophylaxis with trimethoprim-sulfamethoxazole or pentamidine inhaler was instituted at the time of discharge and continued for 12 months to prevent *Pneumocystis carinii* pneumonia. Patients were also treated with acyclovir to prevent Herpes zoster infection during the first 12 months posttransplant. Cytomegalovirus-negative blood products were used in all patients, regardless of prior exposure to the virus. Blood products were irradiated (2,500 cGy) to prevent transfusion-related GVHD.

**Clinical evaluation.** Before treatment, all patients were evaluated as follows: physical examination, blood chemistry profile, complete blood count, serum and urinary protein immunoelectrophoresis and immunofixation, human immunodeficiency virus serology, bone survey, bilateral BM biopsies and phenotypic analysis of BMMCs, pulmonary function tests, and ventriculogram. Criteria for CR included, for at least 3 months, both (1) the absence of serum paraprotein and Bence Jones proteinuria by immunoelectrophoresis and immunofixation; and (2) fewer than 5% polyclonal plasma cells, as demonstrated by immunoperoxidase staining of BM biopsy specimens for Ig heavy and light chains. A 50% decrease in measurable protein sustained for at least 1 month constituted a partial response. Follow-up evaluations included serum protein studies monthly and BM aspiration and biopsy at 3-month intervals for the first year PT; protein studies at 3-month intervals and BM aspiration and biopsy at 6-month intervals for the second year PT; and protein studies at 3-month intervals thereafter. Radiographic bone surveys were performed at 12 month intervals.

**a 2b IFN maintenance therapy.** For patients treated since 1990, a 2b IFN therapy, 3 × 10⁸ U subcutaneously 3 times weekly, has been used beginning at 3 months PT and continued for at least 9 months PT in an attempt to prolong the duration of responses achieved. IFN was not used in patients who did not achieve transfusion independence with granulocytes ≥1,500/μL and PLTs ≥100,000/μL by 3 months PT.

**Statistical methods.** Survival and failure-free survival (FFS) were calculated from date of BMT. Cases were censored for FFS if alive and disease free at analysis. Three patients who underwent autologous BMT died free from progression at 2, 6, and 19 months, and are considered failures for this analysis. This underestimates FFS, but perhaps better reflects the experience of the patients.

**RESULTS**

**Patient characteristics.** Twenty-five patients with myeloma and 1 patient with recurrent extramedullary plasma cytomas underwent autologous BMT between February 1987 and November 1992 (Table 1). There were 16 men and 10 women with a median age of 47 (35 to 59) years, including 2, 4, 13, and 6 patients with Durie-Salmon stages IA, IIA, IIIA, and IIIB disease, respectively, at time of diagnosis. Serum β₂ microglobulin and BM labeling indices were not routinely performed. Patients received a median of three (two to four) treatment regimens over a period of 23 (8 to 52) months before BMT. Fourteen patients had received radiotherapy before BMT. At the time of autologous BMT, all patients had sensitive disease in that they had achieved minimal disease status, defined as less than 10% BM plasma cells. Three patients had polyclonal plasma cells evident on bilateral bone marrow biopsy; 1 of these also had no serum and/or urine monoclonal protein and was therefore the only patient in complete remission. The remaining patients demonstrated both monoclonal marrow plasma cells and monoclonal paraprotein at the time of autologous BMT.

Thirteen patients with multiple myeloma underwent allogeneic BMT between February 1990 and November 1992 (Table 1). There were 9 men and 4 women with a median age of 43 (37 to 52) years, including 2, 3, 6, and 2 patients with Durie-Salmon stages IA, IIA, IIIA, and IIIB disease, respectively, at the time of diagnosis. Serum β₂ microglobulin and BM-labeling indices were not routinely performed. Patients received a median of two (one to four) treatment regimens over a period of a median of 25 (9 to 48) months before BMT. Three patients had received radiotherapy before BMT. At the time of allogeneic BMT, all patients again had sensitive disease and achieved minimal disease status, defined as less than 10% BM plasma cells. Three patients had polyclonal plasma cells evident on bilateral bone marrow biopsy; 1 of these also had no serum and/or urine monoclonal protein and was therefore the only patient in complete remission. The remaining patients demonstrated both monoclonal marrow plasma cells and monoclonal paraprotein at the time of allogeneic BMT.

A 44-year-old man with stage IIIB myeloma received chemotherapy without radiotherapy and achieved less than 10% monoclonal marrow cells, with persistent serum paraprotein (Table 1). After ablative chemoradiotherapy, he received nonpurged syngeneic marrow.

**Hematologic engraftment.** Engraftment post-autologous BMT with granulocytes greater than 500/μL was noted.

<table>
<thead>
<tr>
<th>Source of Cells</th>
<th>No. of Patients</th>
<th>Median Age (yr) (range)</th>
<th>Sex</th>
<th>No. of Patients: Stage at Diagnosis</th>
<th>No. of Patients: Isotype</th>
<th>No. of ChemoRx Regimens Before Transplant</th>
<th>No. of Patients: With (+) or Without (-) RadioRx Before Transplant</th>
<th>No. of Patients: Median Interval Diagnosis to Transplant (mo) (range)</th>
<th>No. of Patients: With (+) or Without (-) Monoclonal Plasma Cells at Transplant</th>
<th>No. of Patients: With (+) or Without (-) Monoclonal Protein at Transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purged autologous marrow</td>
<td>26</td>
<td>47 (35-59)</td>
<td>16 M</td>
<td>2:IA</td>
<td>16:lgG</td>
<td>7:2</td>
<td>14+</td>
<td>23 (8-52)</td>
<td>23+</td>
<td>25+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 F</td>
<td>4:IA</td>
<td>7:lgA</td>
<td>11:3</td>
<td>12−</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>13:III</td>
<td>3:K or λ</td>
<td>8:4</td>
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<td>6:III</td>
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<tr>
<td>T depleted allogeneic marrow</td>
<td>13</td>
<td>43 (37-52)</td>
<td>9 M</td>
<td>2:IA</td>
<td>7:lgG</td>
<td>1:1</td>
<td>3+</td>
<td>25 (9-48)</td>
<td>10−</td>
<td>12−</td>
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<td></td>
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<td></td>
<td>4 F</td>
<td>3:IA</td>
<td>2:lgA</td>
<td>7:2</td>
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<td>6:III</td>
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<td>2:III</td>
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<tr>
<td>Syngeneic marrow</td>
<td>1</td>
<td>44</td>
<td>1 M</td>
<td>III</td>
<td>lgG</td>
<td>1:1</td>
<td>−</td>
<td>8</td>
<td>−</td>
<td>+</td>
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</tbody>
</table>
at a median of 23 (range, 12 to 46) days PT in 25 patients and untransfused platelets greater than 20,000/μL at a median of 25 (range, 10 to 175) days PT in 24 of the 26 patients (Table 2). A median of 9 U (range, 4 to 30 U) of red blood cells (RBCs) and 56 U (range, 4 to 329 U) of platelets were transfused. Patients were discharged from the hospital at a median of 31 (range, 24 to 67) days PT. A single patient who was heavily pretreated with chemotherapy including nitrousorea developed pneumonia, typhlitis, and refractory thrombocytopenia and succumbed of a central nervous system hemorrhage at day 67 PT. She did not engraft and required 29 U of RBCs and 329 U of platelet transfusion support during her 67-day hospitalization. Another patient remained platelet transfusion dependent until his death at 6 months PT.

Engraftment post-allogeneic BMT with granulocytes greater than 500/μL was noted at a median of 19 (range, 12 to 24) days PT and untransfused platelets greater than 20,000/μL at a median of 16 (range, 5 to 32) days PT (Table 2). A median of 8 U (range, 4 to 23 U) of RBCs and 45 U (17 to 307 U) of platelets were transfused. A single patient required 307 units of platelet transfusion support in the setting of venocclusive disease (VOD). Patients were discharged from the hospital at a median of 31 (27 to 52) days PT.

The single patient who underwent syngeneic BMT achieved granulocytes >500/μL and untransfused platelets greater than 20,000/μL at 19 and 25 days PT, respectively (Table 2). He required 6 U of RBCs and 76 U of platelets during a 33-day hospitalization.

Acute and chronic toxicity. Of the 26 patients who underwent autologous grafting, all developed fevers (>101°F) while leukopenic, but only 3 patients had positive blood cultures: Staphylococcus epidermidis in 2 patients and α-hemolytic streptococcus in a single patient (Table 2). Eighteen patients developed mucositis. Herpes zoster and venous thrombosis occurred in 4 and 1 patient, respectively. Three patients developed hypothyroidism and a single patient hypoparathyroidism PT.

As noted above, there was 1 acute in-hospital treatment-related death. This death occurred in a 50-year-old woman who presented with plasmacytoma of the proximal left femur necessitating total hip replacement, associated with Durie-Salmon stage IIIB multiple myeloma. After the next 2 years she received therapy with four combination chemotherapy regimens (total dose of 90 mg carmustine). After melphalan and TBI-ablative therapy, she received 2.9 × 10^7 cells/kg body weight marrow infusion. Fever without source was noted on day 5 PT and leukocytes reappeared as expected on day 10 PT. Her hospital course was complicated by pneumonia (day 11 PT), a diffuse rash thought to be drug-related (day 30 PT), and clinical and radiographic findings consistent with typhlitis (day 50 PT). BM aspirate at day 41 PT was hypocellular, but without evidence of myeloma, and leukocyte count peaked on day 48 PT at 800/μL with 40% granulocytes. Despite aggressive antibiotic and transfusion support, the patient became alloimmunized and refractory even to HLA-matched platelet transfusions and succumbed with a central nervous system hemorrhage on day 67 PT. Postmortem examination showed numerous petechial and ecchymotic lesions of the skin and serosal surfaces of the peritoneum, pleura, and pericardium, as well as hemorrhage of the respiratory, gastrointestinal, and urothelial mucosa. The BM was hypocellular but all cell lines were recovering; immunoperoxidase studies of marrow indicated that the scattered plasma cells were of a polyclonal nature.

Of the 13 patients who underwent allogeneic grafting, 11 developed fever (>101°F) while leukopenic (Table 2). Blood cultures were positive in 3 patients: Staphylococcus

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**Table 2. Hematologic Engraftment and Toxicity After BMT for Myeloma**

<table>
<thead>
<tr>
<th>Source of Stem Cells</th>
<th>No. of Patients</th>
<th>Median Days PT with Granulocytes &gt;500/μL (range)</th>
<th>Median Days PT to Platelets &gt;20,000/μL (range)</th>
<th>Median Units of RBCs Transfused (range)</th>
<th>Median Units of Platelets Transfused (range)</th>
<th>Median Days in Hospital (range)</th>
<th>No. of Patients: Complication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purged autologous marrow</td>
<td>26</td>
<td>23 (12-46)*</td>
<td>25 (10-175)*</td>
<td>9 (4-30)</td>
<td>56 (4-329)</td>
<td>31 (24-67)</td>
<td>26: Fever</td>
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<td>3: Bacteremia</td>
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<td>16: Mucositis</td>
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<td>4: Herpes zoster</td>
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<td>1: Venous thrombosis</td>
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<td>3: Hypothyroidism</td>
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<td>1: Hypoparathyroidism</td>
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<td>6: GVH disease (4 grade I, 1 grade II, 1 grade III)</td>
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<td>1: Chronic GVH disease</td>
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<td>2: Venocclusive disease</td>
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<td></td>
<td></td>
<td>1: Graft failure</td>
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<tr>
<td>T-depleted allogeneic marrow</td>
<td>13</td>
<td>19 (12-24)</td>
<td>16 (5-32)</td>
<td>8 (4-23)</td>
<td>45 (17-307)</td>
<td>31 (27-52)</td>
<td>11: Fever</td>
</tr>
<tr>
<td>Syngeneic marrow</td>
<td>1</td>
<td>19</td>
<td>25</td>
<td>6</td>
<td>76</td>
<td>33</td>
<td>1: Fever</td>
</tr>
</tbody>
</table>

Abbreviation: PT, posttransplant.

* One patient did not achieve greater than 500 granulocytes/μL and 2 patients did not achieve untransfused platelets greater than 20,000/μL.
epidermidis, *Escherichia coli*, and enterobacter in 1 patient each. Six of the 13 patients developed acute GVHD: grade I in 4 patients, grade II in 1 patient, and grade III in 1 patient. Acute GVHD resolved in 3 patients with topical and/or oral corticosteroid therapy, and in 2 patients without treatment. A single patient developed chronic GVHD and was treated with corticosteroids and cyclosporine for 4 months, and has required no therapy thereafter.

Two patients developed VOD characterized by weight gain, ascites, and liver function test abnormalities in the first month PT. In the first patient, total bilirubin peaked at 4.3 mg/dL on day 15 PT; treatment with pentoxifylline led to resolution of VOD and discharge at day 33 PT. The other patient developed clinical VOD and rising total bilirubin on day 11 PT; despite treatment with tissue plasminogen activator, heparin, pentoxifylline, and prostatlandin E1, he expired on day 40 PT with total bilirubin of 40 mg/dL. At postmortem examination, the liver showed signs of profound venous congestion and biliary stasis and ascites was present. Characteristic signs of GVHD in the gastrointestinal tract, skin, and liver were not present, nor was there any evidence of residual myeloma in marrow.

A third patient engrafted promptly, with granulocytes greater than 500/µL and platelets greater than 20,000/µL at days 17 and 19 PT, respectively. The marrow donor was her brother who was both HLA and ABO compatible. On day 21 PT, the patient was begun on Bactrim therapy for a urinary tract infection; by day 49 PT she had developed profound pancytopenia, with fewer than 100 lymphocytes/µL, all of which were of her own type. No evidence of viral or other infection was documented, and a trial of granulocyte colony stimulating factor did not increase her leukocyte counts. She therefore underwent a second allograft with her brother's non-T-cell depleted marrow, after preparation with cyclophosphamide 50 mg/kg on 4 consecutive days and antithymocyte globulin. Although she engrafted promptly with greater than 500 granulocytes/µL and untransfused platelets greater than 20,000/µL on days 11 and 14 PT, respectively, she died in the setting of GVHD, which was refractory to immunosuppressive therapy including corticosteroids and cyclosporine as well as IL-1 receptor antagonist.

**Therapeutic results.** Among the patients who underwent autografting, there were 11 complete and 14 partial responders, and 1 toxic death. All responding patients achieved pathologically normal marrows with less than 5% marrow plasma cells; in complete responders, monoclonal proteins were also absent in serum and urine, and monoclonal plasma cells were polyclonal on immunoperoxidase staining. On staging marrow biopsies performed routinely during the first 24 months PT, the presence of less than 5% polyclonal plasma cells was confirmed in 11 patients (7 complete and 4 partial responders) at a median of 4 (2 to 8) months PT. In 2 of the 7 complete responders, monoclonal plasma cells have returned at 6 and 7 months PT. Monoclonal proteins disappeared in the 7 patients with CR at a median of 3 (1 to 4) months PT; in 2 of the 7 complete responders, monoclonal protein also returned at 6 and 7 months PT. Of the 7 complete responders to allogeneic BMT, normal levels of IgG, IgA, and IgM were noted in 5, 6, and 7 patients at (range, 2 to 12), 5 (range, 2 to 14), and 5 (range, 1 to 6) months PT, respectively. Of the 4 partial responders, normal levels of the uninvolved IgG, IgA, and IgM were noted in 4, 1, and 1 patient at 5, 2, and 3 (range, 2 to 5) months PT, respectively. Polyclonal elevation of IgG and monoclonal IgG protein have each been noted in a single patient at 14 and 9 months PT, respectively.

As of November 1, 1992 with 24 months median follow-up, 9 of the 14 patients who received either allogeneic or syngeneic grafts were alive at 2+ to 68+ months PT, and 16 patients remain alive free from progression at 2+ to 55+ months PT (Fig 1); of these, five patients remain in CR at 6+ to 55+ months PT. Three patients died free from progression at 2, 6, and 19 months PT. Seven patients relapsed at 3 to 36 months PT; of these, 5 patients remain alive at 22+ to 68+ months PT, and 2 patients died at 14 and 27 months PT. The median progression-free survival is 36 months and median overall survival has not yet been reached.

Among the 14 patients who received either allogeneic or syngeneic grafts, there were 7 complete responders, 4 partial responders, 1 non-responder, and 2 toxic deaths. Again, all responding patients achieved pathologically normal marrows with less than 5% marrow plasma cells; in complete responders, monoclonal proteins were also absent in serum and urine, and monoclonal plasma cells were polyclonal on immunoperoxidase staining. On staging marrow biopsies performed routinely during the first 24 months PT, the presence of less than 5% polyclonal plasma cells was confirmed in 11 patients (7 complete and 4 partial responders) at a median of 4 (2 to 8) months PT. In 2 of the 7 complete responders, monoclonal plasma cells have returned at 6 and 7 months PT. Monoclonal proteins disappeared in the 7 patients with CR at a median of 3 (1 to 4) months PT; in 2 of the 7 complete responders, monoclonal protein also returned at 6 and 7 months PT. Of the 7 complete responders to allogeneic BMT, normal levels of IgG, IgA, and IgM were noted in 5, 6, and 7 patients at (range, 2 to 12), 5 (range, 2 to 14), and 5 (range, 1 to 6) months PT, respectively. Of the 4 partial responders, normal levels of the uninvolved IgG, IgA, and IgM were noted in 4, 1, and 1 patient at 5, 2, and 3 (range, 2 to 5) months PT, respectively. Polyclonal elevation of IgG and monoclonal IgG protein have each been noted in a single patient at 14 and 9 months PT, respectively.
as maintenance therapy post-BMT, and 1 patient both before BMT and as maintenance therapy post-BMT. Five patients did not receive maintenance IFN post-BMT because of low counts; of those receiving maintenance therapy, dose reduction (1 patient) or discontinuation (1 patient) was required due to pancytopenia; IFN was discontinued in an additional patient due to the development of pulmonary infiltrates. One of the 6 patients on maintenance IFN therapy has progressed.

Of the 14 patients who underwent allogeneic or syngeneic BMT, 10 patients received IFN therapy: 1 patient pre-BMT, 2 patients at the time of relapse post-BMT, and 7 patients as maintenance therapy post-BMT. Two patients did not receive IFN post-BMT because of low counts; discontinua-
tion of maintenance IFN post-BMT was required in 3 patients due to the development of thrombocytopenia and fatigue. One of the 7 patients on maintenance IFN post-BMT has progressed.

**DISCUSSION**

In this study, we report the results of 40 patients with multiple myeloma who underwent high-dose chemoradiotherapy and either anti-B-cell MoAb-treated autologous BMT or anti-T-cell MoAb-treated allogeneic BMT. The majority of patients had advanced Durie-Salmon stage myeloma, had sensitive relapsed myeloma and were heavily pretreated with chemotherapy; 17 had received prior radiotherapy. At the time of BMT, all patients demonstrated good performance status with Karnofsky score of 80% or greater and had less than 10% marrow tumor cells; 34 patients had residual monoclonal marrow plasma cells and 38 patients had paraprotein. There were 18 CRs, 18 partial responses, 1 nonresponse, and 3 toxic deaths. Less than 5% polyclonal marrow plasma cells were noted in all responders after BMT. As of November 1, 1992 with 24 months median follow-up for survival among the allogeneic BMT cases, 16 of 26 patients are alive free from progression at 2+ to 55+ months PT; of these, 5 patients remain in CR at 6+ to 55+ months PT. With 24 months median follow-up for survival among the allogeneic and syngeneic BMT cases, 8 of 14 patients are alive free from progression at 8+ to 34+ months PT; of these, 5 patients remain in CR at 8+ to 34+ months PT. Although this therapy has achieved high response rates and prolonged progression-free survival in some patients and proven to have acceptable toxicity, relapses post-BMT suggest that such treatment strategies should be used earlier in the disease course and their efficacy compared with that of conventional chemotherapy in a randomized trial.

Melphalan and prednisone therapy can result in response rates of 50% to 60% and a median survival of 48 months; and to date it remains controversial as to whether combination chemotherapy programs have improved on this outcome.1,6,7 Once patients become refractory to initial therapies, salvage therapies such as vincristine, doxorubicin, and dexamethasone6 can achieve response rates of 30% to 75%; however, these responses are uncommonly either complete or durable. High-dose melphalan with or without total body irradiation and hematopoietic stem cell support was initially used by McElwain et al.27,28 This strategy has achieved responses even in the setting of patients refractory to initial therapy.1,6,7 However, in most studies, significant relapse-free survival was noted only in a minority of patients with sensitive disease, and those patients with resistant relapse or with a combination of risk factors (ie, advanced tumor burden, absence of IgG isotype)30 did particularly poorly. Nonetheless, as is true in B-cell non-Hodgkin’s lymphomas (NHL), high-dose therapy followed by autologous BMT is the only strategy to have achieved significant relapse-free survival. For example, at our institute, MoAb-purged autologous BMT for patients with relapsed B- and T-cell NHL sensitive to chemotherapy has resulted in 40% to 50% relapse-free survival at 2 to 3 years.37-40 Although follow-up is short and long-term outcome remains to be determined, the current study of either MoAb-purged autologous or allogeneic BMT in myeloma sensitive to therapy has achieved significant overall and relapse-free survivals (Figs 1 and 2). However, only 5 of 26 patients undergoing autologous BMT and 5 of 14 patients undergoing allogeneic BMT remain in CR at 6+ to 55+ and 8+ to 34+ months PT, respectively. In the largest reported series to date of high-dose therapy followed by allogeneic BMT in myeloma, median relapse-free survival for 37 of 90 (41%) patients who entered complete remission was 48 months.23 In this study, those patients with sensitive disease and who were less heavily pretreated achieved the highest response rates and prolonged progression-free survival. These studies suggest, as has occurred in leukemias and lymphomas, that high-dose approaches should be evaluated earlier in the disease, before the emergence of drug resistance and refractory disease.

An additional impetus for using high-dose approaches earlier in the disease course, now that toxicities of such approaches have proven to be tolerable, relates to the quality of autologous hematopoietic stem cells and related speed and completeness of hematologic recovery after high-dose therapies. For example, Jagannath et al have attempted PBSC collection in 75 previously treated patients with myeloma after the administration of high-dose cyclophosphamide with or without granulocyte-macrophage colony-stimulating factor (GM-CSF).39 Among 72 patients undergoing PBSC apheresis, good mobilization (>50 colony-forming units GM per 10^5 mononuclear cells) was achieved when prior chemotherapy did not exceed 1 year and when GM-CSF was used post-high-dose cyclophosphamide; similarly, rapid platelet recovery (to 50,000/μL) was associated with good PBSC mobilization. In the present study of high-dose chemoradiotherapy followed by autologous BMT in heavily pretreated patients with myeloma, delayed engraftment was also noted; greater than 500 granulocytes/μL and greater than 20,000/μL platelets were noted as long as 46 days and 175 days PT, respectively, and 2 patients did not achieve platelet transfusion independence at the time of their deaths at 2 and 6 months PT. Moreover, it was not possible to treat all patients with IFN post-BMT due to related myelosuppression. Treatment of patients with high-dose approaches earlier in their disease course may therefore be useful not only for the avoidance of drug resistance, but also to assure preservation of sufficient hematopoietic stem cells to permit rapid hematologic recovery and low treatment-related morbidity and mortality.

Several investigators have used intensive treatment of multiple myeloma supported by autologous BM and/or PBSC transplantation as initial therapy in myeloma.18,28,37,38 For example, Gore et al treated 50 patients with myeloma with repeated cycles of 4-day infusions with vincristine, doxorubicin, and methylprednisolone followed by high-dose melphalan, with autologous BMT where possible.28 The overall response rate was 74%, with 50% complete hematologic and biochemical remissions. More recently, these investigators have demonstrated that patients who receive maintenance IFN post-autologous BMT have prolonged median progression-free survival (39 months) compared...
with those patients who are not treated with IFN (27 months) \((P < .025)\).\textsuperscript{50} Attal et al recently treated 35 patients with myeloma with repeated cycles of either vincristine, doxorubicin, and dexamethasone, or vincristine, melphalan, cyclophosphamide, and prednisone until plateau phase was achieved.\textsuperscript{34} High-dose melphalan, and TBI followed by autologous BMT and maintenance IFN resulted in 43% and 40% complete and partial responses, respectively. The 33-month post-BMT probability of progression-free survival was 85% for patients in complete and 24% for patients in partial response. The 42-month post-diagnosis probability of survival was 81%. These and other similar studies\textsuperscript{37} suggest that high-dose approaches early in the disease course can achieve high response rates and that therapeutic interventions post-BMT, such as IFN maintenance, may be better tolerated by such patients than in more heavily pre-treated patients. They provide the basis on which a randomized trial comparing high-dose versus conventional-dose chemoradiotherapy for patients with untreated myeloma can be based.

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Monoclonal antibody-purged bone marrow transplantation therapy for multiple myeloma

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