Monoclonal Antibody-Purged Autologous Bone Marrow Transplantation Therapy for Multiple Myeloma

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Eleven patients with plasma cell dyscrasias underwent high-dose chemoradiotherapy and anti-B-cell monoclonal antibody (MoAb)-treated autologous bone marrow transplantation (ABMT). The majority of patients had advanced Durie-Salmon stage myeloma at diagnosis, all were pretreated with chemotherapy, and six had received prior radiotherapy.

At the time of ABMT, all patients demonstrated good performance status with Karnofsky score of 80% or greater and had less than 10% marrow tumor cells. Eight patients had residual monoclonal marrow plasma cells and 10 patients had paraprotein. Following high-dose melphalan and total body irradiation (TBI) there were seven complete responses, three partial responses, and one toxic death. Granulocytes greater than 500/mm³ were noted at a median of 21 (range 12 to 46) days posttransplant (PT) and untransfused platelets greater than 20,000/mm³ were noted at a median of 23 (12 to 53) days PT in 10 of the 11 patients. Natural killer cells and cytotoxic/suppressor T cells predominated early PT, with return of B cells at 3 months PT and normalization of T4:T8 ratio at 1 year PT. Less than 5% polyclonal marrow plasma cells were noted in all patients after transplant. Three of the seven complete responders have had return of paraprotein, two with myeloma, and have subsequently responded to α-2 interferon therapy. Eight patients are alive at 18.9 (8.9 to 43.1) months PT and four remain disease-free at 12.3, 17.5, 18.9, and 29 months PT. This preliminary study confirms that high-dose melphalan and TBI can achieve high response rates without unexpected toxicity in patients who have sensitive disease, and that MoAb-based purging techniques do not inhibit engraftment. Although the follow-up is short and long-term outcome to be determined, relapses post-ABMT in these heavily pretreated patients suggest that ABMT or alternative treatment strategies should be evaluated earlier in the disease course.

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MUltiple studies have documented the sensitivity of myeloma cells to radiotherapy and chemotherapy, but durable complete responses are rare and this disease remains uniformly fatal. A most encouraging lead for new treatment approaches for myeloma stems from reports of complete clinical remissions after the administration of melphalan in a higher-than-conventional dose with or without total body irradiation (TBI), followed by transplantation of syngeneic, allogeneic, and autologous marrow, or of autologous peripheral blood (PB) stem cells. Although reduction in tumor mass in some cases has been dramatic, short remission times in most responding patients are consistent with rapid regrowth of primordial tumor cells with high proliferative activity.

In the present study, we report the results of treatment of 11 patients with multiple myeloma with high-dose chemoradiotherapy and anti-B-cell monoclonal antibody (MoAb)-purged autologous bone marrow transplantation (ABMT). The benefits of ABMT for patients with lymphoproliferative diseases are limited to those with responsive diseases; therefore, we used ABMT only for patients who had disease sensitive to therapy. Because myeloma is a disease intrinsic to the BM, MoAb and complement-mediated in vitro lysis methods were used to deplete residual tumor cells from the graft before ABMT. This study demonstrates that (1) high-dose chemoradiotherapy can achieve high response rates in pretreated patients with sensitive disease; and (2) MoAb-based purging techniques do not inhibit engraftment. However, relapses post-ABMT and partial responses suggest that this treatment strategy should be evaluated earlier in the disease course, as a primary therapy for selected patients with multiple myeloma.

MATERIALS AND METHODS

Selection of patients and treatment protocol. Patients were eligible for study if they were less than 60 years of age and had multiple myeloma, as well as reactivity of tumor cells with anti-plasma cell-associated-1 (PCA-1) MoAb. All original laboratory parameters were reviewed to establish a proper Durie-Salmon stage at diagnosis. Patients must have achieved a minimal tumor burden defined as less than 10% BM tumor cells, regardless of serum paraprotein, before ABMT. Pretransplant therapy was administered to achieve maximum cytoreduction either at Dana-Farber Cancer Institute or, under its supervision, administered at local institutions. Sites of bony disease received additional radiation therapy before ABMT. Additional criteria for entry included the absence of co-morbid disease of the heart, kidney, lung, and liver, and a Karnofsky score above 80%. Any circumstance that would obviate the ability to deliver full-dose (1200 cGy) TBI excluded patients from the protocol. Informed consent was obtained for all patients.

Preparative therapy consisted of melphalan 70 mg/m² of body weight, infused on each of 2 consecutive days before radiotherapy. Because of the unavailability of melphalan, a single patient (patient 3, Table 1) received cyclophosphamide 60 mg/kg of body weight on 2 consecutive days before TBI. TBI, 5 to 10 cGy per minute, was then administered in fractionated doses (200 cGy) twice daily on 3 consecutive days (total of 1,200 cGy). Within 18
hours of the completion of radiotherapy, cryopreserved ABM that had been previously treated in vitro with MoAbs and rabbit complement was thawed rapidly and reinfused through a central venous catheter.

**Collection, processing, and infusion of marrow.** BM cells 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, Lakewood, CO). Ex vivo treatment of BM was based on previously reported methods developed at the Dana-Farber Cancer Institute that reproducibly deplete 2 to 3 logs of antigen (Ag) positive cells without depleting hematopoietic stem cells.\(^3\) Specifically, anti-PCA-1 MoAb and complement in vitro can lyse 2 to 3 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 $2 \times 10^9$ cells/mL. These cells were subjected to three treatments, each consisting of a 30-minute incubation with MoAb at $20^\circ C$ followed by incubation for 30 minutes at $37^\circ C$ with rabbit complement. Marrow cells were treated with MoAbs directed at 3 Ags, including CALLA (CD10), which detects the common acute lymphoblastic leukemia Ag\(^1\); B1 (CD20), a pan-B cell Ag\(^6\); and PCA-1, which detects a plasma-cell-associated Ag.\(^1\) This MoAb cocktail was used to target cells in the malignant clone from the pre-B-cell to plasma-cell 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 (DMSO) and 90% autologous serum at $-196^\circ C$ 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. A median of

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**Table 1. Characteristics of Patients Undergoing ABMT Therapy for Myeloma**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Stage at Diagnosis</th>
<th>Type</th>
<th>Therapy Before Transplant*</th>
<th>Interval Diagnosis to Transplant (mo)</th>
<th>Interval Last Therapy to Transplant (d)</th>
<th>Monoclonal Marrow Plasma Cells at Transplant</th>
<th>Monoclonal Paraprotein at Transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>F</td>
<td>IIIA</td>
<td>IgG</td>
<td>ABCVP $\times$ 3 (S)</td>
<td>9</td>
<td>44</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ACVP $\times$ 1 (S)</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>M</td>
<td>IIA</td>
<td>IgG</td>
<td>AP $\times$ 18 (S)</td>
<td>33</td>
<td>35</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>D $\times$ 3 (R)</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>VaD $\times$ 2 (S)</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>F</td>
<td>IIIA</td>
<td>IgA</td>
<td>AP $\times$ 4 (S)</td>
<td>36</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>M</td>
<td>IIIA</td>
<td>IgA</td>
<td>AP $\times$ 2 (R)</td>
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<td>8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>41</td>
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<td>IA</td>
<td>IgA</td>
<td>AP $\times$ 4 (S)</td>
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<td>59</td>
<td>+</td>
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</tr>
<tr>
<td>6</td>
<td>52</td>
<td>M</td>
<td>-</td>
<td>IgG</td>
<td>MP $\times$ 6 (S)</td>
<td>52</td>
<td>22</td>
<td>-</td>
<td>-</td>
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<td>57</td>
<td>F</td>
<td>IIIA</td>
<td>IgA</td>
<td>MPV $\times$ 9 (S)</td>
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<td>7</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>F</td>
<td>IIIA</td>
<td>IgA</td>
<td>AP $\times$ 3 (R)</td>
<td>13</td>
<td>32</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>44</td>
<td>M</td>
<td>IIIA</td>
<td>IgG</td>
<td>MP $\times$ 2 (S)</td>
<td>40</td>
<td>8</td>
<td>+</td>
<td>+</td>
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<tr>
<td>10</td>
<td>52</td>
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<td>IIA</td>
<td>IgA</td>
<td>CAVP $\times$ 9 (S)</td>
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<td>54</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>50</td>
<td>F</td>
<td>IIIB</td>
<td>IgG</td>
<td>BCAP $\times$ 3 (S)</td>
<td>24</td>
<td>36</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Abbreviations: A, alkeran; B, BCNU; C, cyclophosphamide; V, vincristine; a, adriamycin; P, prednisone; D, decadron; M, melphalan; XRT, radiation therapy.

*Patients either had sensitive disease (S) and achieved partial response, or resistant disease (R) and achieved no response.
4.35 × 10⁸ (range 2.3 to 9.0 × 10⁸) cells was harvested; the median number of reinfused cells was 3.03 × 10⁹/kg (range 2.04 to 6.71 × 10⁹/kg), with 85% to 95% viability as measured by Trypan blue exclusion.

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/mm³, 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 was reinstituted at the time of discharge and continued for 6 to 12 months to prevent Pneumocystis carinii pneumonia. Cytomegalovirus-negative blood products were used in all patients regardless of prior exposure to the virus (frozen deglycerolized red cells [RBCs] and screened platelet donors). Blood products were irradiated (2000 cGy) to obviate problems of alloreactivity.

Phenotypic analysis. A panel of MoAbs directed at B and T cells, monocytes, and natural killer (NK) cells was used in an indirect immunofluorescence assay to monitor the phenotype of PB mononuclear cells (PBMCs) as BM engraftment and reconstitution of PB leukocytes proceeded, as previously described. MoAbs were directed at the T4 (CD4), T8 (CD8), T11 (CD2), B1 (CD20), Mo2 (CD14), and NKH1 (CD56) Ags. Cells were analyzed by weeks 1 through 5, 6 through 10, 11 through 15, 15 through 30, 31 through 45, and 45 through 52 posttransplant (PT). Normal values are between 60% and 80% for the CD11 Ag; 40% to 60% for the CD4 Ag; 15% to 30% for the CD8 Ag; 5% to 10% for the CD20 Ag; 15% to 30% for the CD14 Ag; and 10% to 15% for CD56 Ag.

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 complete response 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, BM aspiration and biopsy at 3-month intervals, and radiographic bone surveys at 6-month intervals.

RESULTS

Patient characteristics. Ten patients with multiple myeloma and one patient with recurrent extramedullary plasmacytomas underwent ABMT between February 1987 and December 1989 (Table 1). There were six men and five women with a median age of 46 (40 to 57) years, including 1, 2, 6, and one patient with Durie-Salmon stages II, IIIA, IIIB, and IIIB disease, respectively, at time of diagnosis. Serum B₂ microglobulin and BM-labeling indices were not routinely performed. Patients received a median of three (two to four) treatment regimens over a period of a median of 13 (6 to 33) months. The interval from diagnosis to ABMT was a median of 25 (9 to 52) months (Table 1). The interval from last treatment to ABMT was 32 (5 to 59) days.

Sensitivity or resistance to individual pretransplant treatments is shown in Table 1. Patients 2 and 3 had stable responses lasting 6 months and patients 4, 6, and 9 stable responses for 12 months before ABMT. Six patients received radiotherapy to sites of bone disease before ABMT: 2,800 cGy to ilium bilaterally (patient 3); 1,600 to 2,000 cGy to right humerus as well as ischium, femur, and clavicle on left (patient 4); 4,600 cGy to nasopharynx (patient 6); 4,100 cGy to left humerus and 2,800 cGy to right femur and thoracolumbar spine (patient 7); 3,000 cGy to right femur (patient 8); and 3,000 cGy to left femur (patient 9). At the time of ABMT, all patients had sensitive disease in that they had achieved minimal disease status, defined as less than 10% BM plasma cells. Three patients (Table 1, patients 1, 3, and 6) had polyclonal plasma cells evident on bilateral BM biopsy; one of these (patient 3) 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 ABMT.

Hematologic engraftment. Engraftment post-ABMT was prompt with granulocytes greater than 500/mm³ at a median of 21 (12 to 46) days PT and untransfused platelets greater than 20,000/mm³ at a median of 23 (12 to 53) days PT in 10 of the 11 patients (Table 2). Complete hematologic engraftment, defined as granulocytes greater than 1500/mm³ and platelets greater than 150,000/mm³, occurred at a median of 49 (18 to 82) days PT and by a median of 87 (24 to 365) days PT, respectively. A median of 10 (4 to 30) units of RBCs and 41 (4 to 199) units of platelets were transfused. Patients were discharged from the hospital at a median of 27 (24 to 40) days PT. A single patient (patient 11), who was heavily pretreated with chemotherapy, developed pneumonia, typhlitis, and refractory thrombocytopenia and succumbed of a central nervous system (CNS) hemorrhage at day 67 PT. She did not engraft and required 29 units of RBCs and 329 units of platelet-transfusion support.

Acute and chronic toxicity. Ten patients developed fever without documented infectious source while leukopenic; eight patients developed mucositis (Table 2). Four patients developed dermatomal Herpes zoster at a median of 3.2 (2 to 12) months PT. Two patients developed hypothyroidism and a single patient hypoparathyroidism. As noted above, there was one acute in-hospital treatment-related death (patient 11, Tables 1 and 2). 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 IIIIB multiple myeloma. Over 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 × 10⁸ 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.
Table 2. Hematologic Engraftment and Toxicity Post-ABMT Therapy for Myeloma

<table>
<thead>
<tr>
<th>Patient</th>
<th>Granulocytes &gt; 500/mm³ (d PT)</th>
<th>Platelets &gt; 20,000/mm³ (d PT)</th>
<th>Units of RBCs Transfused</th>
<th>Units of Platelets Transfused*</th>
<th>Duration of Hospitalization (d)</th>
<th>Complications (mo PT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39</td>
<td>40</td>
<td>12</td>
<td>83</td>
<td>40</td>
<td>Fever (1)</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
<td>53</td>
<td>14</td>
<td>87</td>
<td>39</td>
<td>Mucositis (1) Mucositis (1) Herpes zoster (3.2) Fever (1)</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>35</td>
<td>8</td>
<td>60</td>
<td>25</td>
<td>Herpes zoster (2)</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>12</td>
<td>4</td>
<td>27</td>
<td>27</td>
<td>Fever (1)</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>23</td>
<td>8</td>
<td>40</td>
<td>26</td>
<td>Mucositis (1)</td>
</tr>
<tr>
<td>6</td>
<td>26</td>
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<td>35</td>
<td>Fever (1)</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
<td>21</td>
<td>18</td>
<td>163</td>
<td>25</td>
<td>Mucositis (1)</td>
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<td>10</td>
<td>41</td>
<td>32</td>
<td>Fever (1)</td>
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<td>Venous thrombosis (1)</td>
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<td>17</td>
<td>20</td>
<td>30</td>
<td>4</td>
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<td>Fever (1)</td>
</tr>
<tr>
<td>11</td>
<td>NA</td>
<td>NA</td>
<td>29</td>
<td>329</td>
<td>67</td>
<td>Fever (1) Pneumonia (1) Typhlitis (1.6)</td>
</tr>
</tbody>
</table>

*Each unit contains 5.5 x 10¹⁵ platelets. Each transfusion contains 6 to 8 units.

on day 48 PT at 800/mm³ 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 CNS 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.

Phenotypic analysis of reconstituting PBMCs. The recovery of T, B, and NK cells, as well as monocytes, during the first year after ABMT is shown in Table 3. Anti-CD2 MoAb, which stains both T and NK cells, reacted with a median of 67% (31% to 92%) of PBMCs in the first 5 weeks PT. During this interval, CD8 Ag-positive cells were twice as common as CD4 Ag-staining cells (median 25% v 13% PBMCs). CD20 Ag-positive B cells were largely absent; CD14-bearing monocytes and CD56-staining NK cells represented 19% and 16% of PBMCs, respectively.

CD2-bearing cells remained predominant (≥ 50% PBMCs) during the first year PT. The CD4:CD8 ratio approached 1:1 at 1 year PT, due to both an increase in CD4 and decrease in CD8-positive cells. CD20-positive cells reappeared in the third month PT; at 1 year, increased numbers (median 15% of PBMCs) of polyclonal B cells were present. Both CD14-bearing monocytes and CD56-positive NK cells represented 10% to 20% of PBMCs during the first year PT.

Therapeutic results. There were seven complete and
Table 4. Clinical Outcome of ABMT Therapy for Multiple Myeloma

<table>
<thead>
<tr>
<th>Patient</th>
<th>Response</th>
<th>Status*</th>
<th>Marrow Status*</th>
<th>Monoclonal Paraprotein*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Complete</td>
<td>Alive (37.1), IFN (22)</td>
<td>Pathologically normal, polyclonal (6.2) and monoclonal plasma cells (16)</td>
<td>Absent (3.5) Reappeared (16)</td>
</tr>
<tr>
<td>2</td>
<td>Partial</td>
<td>Died (19.4)</td>
<td>Pathologically normal monoclonal (2.9), polyclonal (5.9), myelofibrotic (8.9), polyclonal (19.4)</td>
<td>Decreasing (8.9) Stable (18.3) Stable (19.4)</td>
</tr>
<tr>
<td>3</td>
<td>Complete</td>
<td>Alive (23.6)</td>
<td>Pathologically normal, polyclonal (24.6)</td>
<td>Absent (29)</td>
</tr>
<tr>
<td>4</td>
<td>Complete</td>
<td>Alive (17.8), IFN (12.2)</td>
<td>Pathologically normal, polyclonal (6.1), monoclonal (10)</td>
<td>Absent (4.1) Reappeared (5.0) Stable (23.8)</td>
</tr>
<tr>
<td>5</td>
<td>Complete</td>
<td>Alive (18.9)</td>
<td>Pathologically normal, polyclonal (13.1)</td>
<td>Absent (18.9)</td>
</tr>
<tr>
<td>6</td>
<td>Complete</td>
<td>Alive (11.5)</td>
<td>Pathologically normal, polyclonal, normal, Gallium scan (6.7)</td>
<td>Absent (17.5)</td>
</tr>
<tr>
<td>7</td>
<td>Complete</td>
<td>Alive (9.4), 1 IFN (4.5)</td>
<td>Pathologically normal, polyclonal (2.8), monoclonal (4.5)</td>
<td>Absent (1.9) Reappeared (3.3) Stable (9.4)</td>
</tr>
<tr>
<td>8</td>
<td>Complete</td>
<td>Alive (6.9)</td>
<td>Pathologically normal, polyclonal (12.3)</td>
<td>Absent (12.3)</td>
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<tr>
<td>9</td>
<td>Partial</td>
<td>Alive (8.1)</td>
<td>Pathologically normal (12.0)</td>
<td>Decreasing (8.1) Stable (15.0)</td>
</tr>
<tr>
<td>10</td>
<td>Partial</td>
<td>Alive (3.2)</td>
<td>Pathologically normal, polyclonal (8.9)</td>
<td>Decreasing (3.2) Stable (8.9)</td>
</tr>
<tr>
<td>11</td>
<td>Not evaluable</td>
<td>Died (2.2)</td>
<td>Hypocellular, polyclonal (2.2)</td>
<td>Decreasing (2.0)</td>
</tr>
</tbody>
</table>

*In months PT as of September 15, 1990.

three partial responses (Table 4, Fig 1). All responding patients (patients 1 through 10) achieved pathologically normal marrows with less than 5% marrow plasma cells remaining monoclonal on immunoperoxidase staining of BM biopsy specimens at a median of 8.9 (2.8 to 24.6) months PT. Serum Ig (IgG, IgA, and IgM) returned to normal levels in seven patients at a median of 6 (3 to 9) months PT. In three of the seven complete responders, monoclonal plasma cells have returned at 4.5, 10, and 16 months PT. Monoclonal proteins disappeared in the seven patients with complete response and remain absent in three patients (Table 4, patients 8, 6, 5, and 3 at 12.3, 17.5, 18.9, and 29 months PT, respectively); they have reappeared in three patients at 3.3, 5, and 16 months PT. Patient 1 had an IgG λ paraprotein pretreatment and also recurred with IgG λ paraprotein. However, two patients had return of monoclonal protein patterns distinct from that present before BMT: patient 4 had free λ light chains in serum and urine before transplant and recurred with IgG λ after transplant; and patient 7 had IgA λ myeloma before transplant and recurred PT with IgA λ as well as IgG λ monoclonal proteins.

A greater than 50% decrease of abnormal proteins associated with less than 5% polyclonal plasma cells was

![Percentage of Monoclonal Protein](https://example.com/monoclonal-protein-graph.png)

**Fig 1.** Effect of therapy before transplant and of transplant on monoclonal protein levels.
MoAbs-PURGED ABMT IN MYELOMA

noted in the three partial responders (Table 4, Figure 1). In two of these patients, the monoclonal protein either remains stable or continues to decrease at 8.9 and 15.0 months PT; the other patient died of suspected sepsis at 19.4 months PT, with a stable greater than 50% decrease in paraprotein and no monoclonal cells evident on BM biopsy. A greater than 50% decrease in paraprotein was noted without evidence of myeloma on BM biopsy at the time of the only toxic death (patient 11).

As of September 15, 1990, eight patients are alive at 18.9 (8.9 to 43.1) months PT. Four of the seven complete responders remain disease-free at 12.3, 17.5, 18.9, and 29 months PT. Three patients have had return of paraprotein, two with myeloma, and all have responded to a 2 interferon (IFN); two remain alive at 23.8 and 43.1 months PT. Two of the three partial responders are alive off therapy at 8.9 and 15.0 months PT. Three patients have died at 2.2, 13.6, and 19.4 months PT. Actuarial overall and relapse-free survival are shown in Fig 2.

DISCUSSION

In this study, we report the results of 11 patients with multiple myeloma who underwent high-dose chemoradiotherapy and anti-B-cell MoAb-treated ABMT. The majority of patients had advanced Durie-Salmon stage myeloma, all were pretreated with chemotherapy, and six had received prior radiotherapy. At the time of transplant, all patients demonstrated good performance status with Karnofsky score of 80% or greater and had less than 10% marrow plasma cells; eight patients had residual monoclonal marrow plasma cells and 10 patients had paraprotein. There were seven complete responses, three partial responses, and one toxic death. Less than 5% polyclonal marrow plasma cells were noted in all responders after transplant. Three of the seven complete responders have had return of paraprotein, two with myeloma, and have subsequently responded to IFN therapy. This preliminary study suggests that high-dose melphalan and TBI can achieve high response rates without unexpected toxicity in patients who have sensitive disease, and that MoAb-based purging techniques do not inhibit engraftment. Although the follow-up is short and long-term outcome needs to be determined, relapses post-ABMT in these pretreated patients suggest that ABMT or alternative treatment strategies should be evaluated earlier in the disease course.

The complete responses noted after high-dose melphalan with or without TBI in this study confirm the results of other series of high-dose chemoradiotherapy and ABMT in similar patients. Gore et al have treated patients with vincristine, doxorubicin, and methyl prednisolone therapy followed by high-dose melphalan and, in some cases, with ABMT. The overall response rate was 74%, with 50% of patients achieving complete hematologic and biochemical remission. Barlogie et al have treated 73 patients with refractory myeloma and found that the addition of TBI to melphalan doubled the incidence of marked (>75%) tumor regression as well as median relapse-free and overall survival time to 8 and 16 months, respectively. When applied to drug-sensitive myeloma, only about 20% of patients achieved a median relapse-free survival time of 14 months. The complete response rate achieved in our series may primarily reflect the selection of patients with sensitive disease who could achieve less than 10% marrow plasma cell status. It is of particular note that the three of the four patients who have remained disease free the longest (patients 6, 1, and 3 at 17.5+, 16, and 29+ months PT, respectively) lacked monoclonal marrow plasma cells at the time of ABMT. These three studies using ABMT, as well as those using high-dose chemoradiotherapy followed by either allogeneic BMT or PB stem cell transplantation, all confirm the significant-response rates to high-dose melphalan with or without TBI and suggest that ABMT can be more effective when used in patients with sensitive disease, analogous to the responses to ABMT noted in patients with lymphoma. This finding may be particularly evident if these approaches are used earlier in the disease course, before heavy pretreatment and/or the emergence of drug resistance.

This study does not define the need, if any, for marrow purging, because this cannot be adequately assessed until more effective ablative strategies are developed. However, it does demonstrate no adverse effects of marrow purging. To date, several Ags have been described on the surface of normal and neoplastic plasma cells and are candidates for use in depleting these cells from autologous marrow before BMT for myeloma. Although several investigators have developed various MoAb-based techniques for depletion of marrow myeloma cells, clinical studies using these techniques have not yet been reported. In this study, we have used anti-PCA-1, a MoAb directed to a 26-Kd protein on the surface of myeloma cells that is of the IgG2a isotype and therefore capable of complement-mediated lysis of Ag-bearing tumor cells in vitro. Most importantly, it is not expressed on BM stem cells and would not be
expected to inhibit hematologic reconstitution after ABMT. Our study also uses MoAbs directed at pre-B (anti-CALLA) and B cells (anti-B1) to deplete these cells within the abnormal myeloma clone. A variety of phenotypic and functional studies document the presence of pre-B- and B-cell Ags, and even of non-B-cell (ie, myelomonocytic) Ags on myeloma cells, suggesting that an early marrow progenitor cell may be part of the myeloma clone.36-46,55-57

Recent studies using flow cytometric techniques have provided evidence for the expression of genes specific for different hematopoietic lineages by aneuploid myeloma cells, also suggesting that an early hematopoietic stem cell is involved in the myeloma clone.47 Moreover, growth-factor-responsive myeloma cells may also bear early B-cell Ags.58 Although our purging approach depletes CALLA+B1+PCA-1+ cells and has not inhibited engraftment, it is not yet clear whether the “clonogenic” myeloma cell has been removed because the phenotype of this cell is undefined. Therefore, it is not possible at present to assess the independent effect of marrow-depletion techniques on either response or relapse rates.

In all series of BMT approaches for myeloma, the majority of responders have relapsed and 2-year disease-free survival is very rare. Thus, complete remissions can be achieved, but these remissions cannot be maintained. improved systemic therapies and/or treatment of patients earlier in the disease at a time when they have less tumor burden and sensitive cells may result in higher complete response rates. Most encouraging are reports of maintenance IFN therapy, which has been shown to increase median response duration, decrease death rate, and increase median survival duration of patients with myeloma who have responded to either alkeran and prednisone or combination chemotherapy.59,60 Moreover, it is of interest in the present study that myeloma that has recurred post-ABMT remains sensitive to IFN. Future studies will define whether systemic therapies can be improved and whether maintenance IFN therapy may prolong duration of complete remissions achieved using ABMT approaches.

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Monoclonal antibody-purged autologous bone marrow transplantation therapy for multiple myeloma

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