Treatement of Aggressive Multiple Myeloma by High-Dose Chemotherapy and Total Body Irradiation Followed by Blood Stem Cells Autologous Graft

By Jean-Paul Fermand, Yves Levy, Jean Gerota, Marc Benbunan, Jean Marc Cosset, Sylvie Castaigne, Maxime Seligmann, and Jean-Claude Brouet

Eight patients with stage III aggressive multiple myeloma, refractory to current chemotherapy in six cases, were treated by high-dose chemotherapy (nitrosourea, etoposide, and melphalan) (HDC) and total body irradiation (TBI), followed by autografting with blood stem cells. These cells were previously collected by leukapheresis performed during hematologic recovery following cytotoxic drug-induced bone marrow aplasia. Seven patients were alive 9 to 17 months after HDC-TBI and graft. One died at day 40 from cerebral bleeding. All living patients achieved a 90% or greater reduction in tumor mass. In two cases, a complete remission (CR) has persisted at a follow-up of 15 and 16 months. Three patients have been well off therapy with stable minimal residual disease (RD) since 10, 11, and 17 months, respectively. A patient in apparent CR and another with RD have relapsed 9 to 12 months posttreatment. Autologous blood-derived hematopoietic stem cells induced successful and sustained engraftment in all living patients. These results, although still preliminary, indicate that HDC and TBI, followed by blood stem cells autograft, which has both practical and theoretical interest over allogeneic or autologous bone marrow transplantation, deserve consideration in selected patients with multiple myeloma.

IN THE PAST 20 years, little progress has been made in the treatment of patients with multiple myeloma (MM), which leads to an overall survival of only 2 to 4 years. Results are even more disappointing in patients with high tumor mass (stage III patients). Current therapeutic regimens yield only partial remissions in less than two thirds of the patients, and, when relapse occurs, a sustained second remission is seldom achieved. Intensification of doses of cytotoxic drugs, and especially high-dose single intravenous (IV) injection of melphalan, can provide high response rates and apparent complete remission in previously untreated patients with MM. However, this treatment is of limited benefit in patients with refractory disease and carries a high incidence of morbidity and mortality.

Several reports outline the potential interest of high-dose chemotherapy (HDC) and total body irradiation (TBI) to obtain a marked tumor mass reduction in patients with solid tumors or hematologic malignancies. This treatment has been applied to a few patients with MM and followed by allogeneic, syngeneic, or autologous bone marrow transplantation (BMT). Allogeneic graft, however, can be applied only to patients with HLA-identical siblings and carries risks due to graft-versus-host disease, which increase with the patient’s age. In view of the present lack of effective tumor cell purging, autologous BMT implies the reinfusion of malignant cells, which is potentially detrimental although it has been performed without any evident deleterious effect in a few patients. To minimize this hazard, we have chosen to support HDC and TBI by autologous graft with circulating blood stem cells. We report here our experience of such a therapeutic strategy in eight patients with stage III MM.

PATIENTS AND METHODS

Patients. Eight patients (four men and four women) received a treatment combining HDC and TBI followed by autologous graft using previously collected blood stem cells. Their median age was 41 years (range, 29 to 55). The iso type of the monoclonal Ig (M Ig) was κ-type in three cases and IgG or IgA in two cases each. The last patient was affected with myeloma MM.

Initially, all patients had a high tumor mass. Before blood cell collection (see below), five patients had been treated: two had achieved a partial remission (defined by a 50% or greater decrease of tumor cell mass) under alkylating agents-steroid treatment and had relapsed after 6 (case no. 2) and 12 (case no. 4) months off therapy; one (case no. 7) had progressive disease in spite of repeated courses of steroids with vincristine and doxorubicin administered by continuous infusion (VAD regimen). Two other patients (case nos. 5 and 8) had an overt MM complicated by paraplegia regressive after laminec tomy and 20 Gy local irradiation; in patient 8, spinal cord compression developed during treatment with steroids and mel phalan of a nonsecretory MM revealed by a fracture of iliac bone and hypercalcemia.

Between the time of blood stem cell collection and that of autograft, the patients usually received 3 to 4 monthly courses of therapy with steroids, cyclophosphamide, vincristine, and, during the first two cycles, adriamycin. At the time of HDC and TBI, patients 3 and 6 had achieved a partial remission, whereas the six others still had a high mass MM and progressive disease (with multiple skull plasma cell tumors in patient 7, plasmablastic cervical lymph nodes in patient 1, and hypercalcemia in patient 8) (Table 1).

All patients were advised of HDC-TBI and autograft procedures and attendant risks and gave informed consent.

Collection of blood stem cells. Since the number of circulating blood stem cells increased during recovery of a short period of therapeutic aplasia,16,11 patients received a chemotherapy regimen including cyclophosphamide (1,500 mg/m²), adriamycin (90 mg/ m²), vincristine (1.4 mg/m²), and steroids that led to a short period of cytopenia (WBC count under 1 × 10⁹/L for three to seven days). At hematologic recovery, when neutrophil and platelet counts reached 500/mcL and 20,000/mcL, respectively, autopheresis was performed without any evident deleterious effect in a previous patient.
BLOOD STEM CELL AUTOGRAFT IN MYELOMA

Seven cases, the total number of CFU-GM/kg ranged between 2.2 and 4.2 x 10⁸/kg. Precryopreserved samples were analyzed for the presence of three or four leukapheresis performed on consecutive days were harvested using a cell separator (COBE 2997) by four-hour (12 L) continuous flow leukapheresis. The cells obtained by a single series were frozen at 2°C/min and kept at -196°C. The mean total number of mononuclear cells collected per patient was 2.6 x 10⁹/kg (range, 1 to 4.2 x 10⁸/kg). Precryopreserved samples were analyzed for the presence of granulocyte-macrophage progenitors (CFU-GM). In seven cases, the total number of CFU-GM/kg ranged between 2.2 and 13 x 10⁹ (mean, 5.5 x 10⁹); in the eighth patient, it reached 50 x 10⁹. Recovery after thawing was above 70% in all cases.

Hybridization to BamHI digested genomic DNA samples extracted from collected mononuclear cells of most patients was performed with a procedure using an Ig heavy chain (JH) probe that detects around 1% clonal cells. A monoclonal rearrangement was extracted from collected mononuclear cells of most patients was performed with a procedure using an Ig heavy chain (JH) probe that detects around 1% clonal cells. A monoclonal rearrangement was detected in two of them it has remained stable after 10 and 11 months. Patient 6 displays a serum M Ig level of 5 g/L and patient 5 shows a urinary Bence Jones protein excretion below 0.1 g/d. Likewise, 17 months after HDC, TBI, and graft, the bone marrow aspirate of the third patient (case 1) has remained normal. His urinary Bence Jones amount is oscillating between 0.1 and 1 g/d.

A patient initially in apparent complete remission (patient 7) and another one (patient 4) with a minimal residual disease relapsed 9 and 12 months after treatment, respectively. In both cases the M Ig level increased dramatically whereas bone lesions (case no. 4) or soft tissue plasmacytomas (case no. 7) rapidly developed.

In all cases, the decrease in the serum level of the monoclonal component was roughly exponential, in accordance with the physiologic half-life of these molecules; for instance, in patient 7 the level of serum monoclonal IgG became undetectable after 3 months. Of note, polyclonal Ig increased in all cases and returned to normal values in three patients. Skeletal X-rays performed at 6 months did not reveal appreciable improvement of bone lesions. Tomodensitometric examination, however, showed a partial bone lesion healing in three of the four cases studied. The five patients in remission are off therapy and well.

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Hematologic recovery and toxicity. A granulocyte count of 0.5 x 10⁹/L was reached within 10 to 25 days (median, 16 days). Platelet recovery was similarly rapid in four patients but delayed in four others. For the seven living patients, the median recovery time of platelets to 25 x 10⁹/L was 34 days (range, ten to 90 days). RBC transfusions were

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**Table 1. Clinical and Hematologic Features of the Eight Patients**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex/Age (yr)</th>
<th>Time From Diagnosis (mo)</th>
<th>Status</th>
<th>HDC-TBI and Graft</th>
<th>Survival (mo Post HDC-TBI)</th>
<th>Bone Marrow Plasma Cells (%)</th>
<th>M Ig</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 B.C.</td>
<td>M/39</td>
<td>10</td>
<td>Progressive disease</td>
<td>BJ K: 100 g/d</td>
<td>17+</td>
<td>1</td>
<td>0.1-1 g/d</td>
</tr>
<tr>
<td>2 P.P.D.</td>
<td>M/55</td>
<td>39</td>
<td>Progressive disease</td>
<td>IgA K: 69 g/L</td>
<td>1</td>
<td>4</td>
<td>25 g/L</td>
</tr>
<tr>
<td>3 M.N.</td>
<td>F/29</td>
<td>8</td>
<td>PR</td>
<td>BJ K: 1 g/d</td>
<td>15+</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>4 M.S.</td>
<td>F/48</td>
<td>48</td>
<td>Progressive disease</td>
<td>IgA K: 50 g/L</td>
<td>12+</td>
<td>PR</td>
<td>Relapse at 12 mo</td>
</tr>
<tr>
<td>5 J.L.</td>
<td>F/48</td>
<td>5</td>
<td>Progressive disease</td>
<td>BJ K: 1 g/d</td>
<td>11+</td>
<td>1</td>
<td>&lt;0.1 g/d</td>
</tr>
<tr>
<td>6 R.G.</td>
<td>M/41</td>
<td>5</td>
<td>PR</td>
<td>IgG K: 18 g/L</td>
<td>10+</td>
<td>2</td>
<td>&lt;5 g/L</td>
</tr>
<tr>
<td>7 C.C.</td>
<td>F/31</td>
<td>11</td>
<td>Progressive disease</td>
<td>IgG K: 50 g/L</td>
<td>9+</td>
<td>CR</td>
<td>Relapse at 9 mo</td>
</tr>
<tr>
<td>8 S.S.</td>
<td>M/39</td>
<td>5</td>
<td>Progressive disease, nonsecretory</td>
<td></td>
<td>16+</td>
<td>1</td>
<td>None</td>
</tr>
</tbody>
</table>

Abbreviations: PR, partial remission; CR, complete remission; BJK, Bence Jones protein kappa.

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RESULTS

Tumor mass reduction following HDC and TBI. Seven patients are alive 9 to 17 months (median, 13 months) following HDC-TBI and autograft (Table 1). One patient (case no. 2) died at day 40 from cerebral bleeding. At the time, he was in partial remission though thrombocytopenic. All living patients achieved a 90% to 100% reduction in M Ig level. In two cases (patients 3 and 8), a complete remission was obtained and has persisted with a follow-up of 15 and 16 months: M Ig is no longer detectable by immunochemical analysis (immunoelectrophoresis and immunofixation) of serum and/or 100-fold concentrated urine; no tumoral plasma cell is detectable in bone marrow aspirates and biopsies. Three patients have a minimal residual disease; in two of them it has remained stable after 10 and 11 months. Patient 6 displays a serum M Ig level of 5 g/L and patient 5 shows a urinary Bence Jones protein excretion below 0.1 g/d. Likewise, 17 months after HDC, TBI, and graft, the bone marrow aspirate of the third patient (case 1) has remained normal. His urinary Bence Jones amount is oscillating between 0.1 and 1 g/d.

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required beyond day 30 in two patients. Bone marrow biopsies and aspirates performed in all surviving patients at 6 months showed in all instances a normal or slightly decreased cellularity.

Of note, the kinetics of hematopoietic recovery was roughly related to the amount of infused CFU-GM. For example, when a low number of CFU-GM (2.2 x 10^4/kg) was reinfused, the recovery of granulocyte count over 0.5 x 10^9/L and of platelets over 25 x 10^9/L occurred after 25 and 60 days, respectively, whereas the corresponding values for patient 5, who received 13 x 10^9 CFU-GM/kg, were 15 and 12 days, respectively.

All patients had fever without documented infection during the five to ten days of agranulocytosis, prompting empirical administration of antibiotics; no severe infection or interstitial pneumonitis was recorded. The seven living patients were discharged from the hospital within 16 to 50 days (median, 32 days).

**DISCUSSION**

We report the achievement of impressive tumor mass reduction, with two apparent complete remissions, in seven of eight patients with stage III aggressive myeloma treated by HDC and TBI followed by blood stem cells autograft. Three patients presently have a stable minimal residual disease, and two have relapsed 9 and 12 months after the graft. Five of the seven surviving patients had been heavily treated and were refractory to current therapy. Although high-dose cytotoxic drugs were shown to overcome resistance to conventional therapeutic regimens in a number of hematologic or solid tumors, such a strategy has mostly led only to transient remission in refractory MM. One aim of this study was therefore to test the association of HDC with TBI, since plasma cell tumors have been shown to be radiosensitive. Some patients with progressive MM were indeed improved after sequential half-body irradiation. Although our chemotherapeutic regimen with high-dose melphalan, etoposide, and nitrosourea had not been tested alone, we believe that its association with TBI is responsible for the striking initial tumor mass reduction. This is consistent with the recently reported occurrence of remission in patients with refractory MM treated by a combination of various HDC regimens and irradiation, followed by BMT.

Our therapeutic approach led to apparent complete remission in two patients and to stable minimal residual disease in three others, with a present follow-up of 9 to 17 months. Such minimal disease has also been observed after allogeneic or syngeneic BMT. Whereas some of these patients may relapse, as observed in two of our cases, it is of interest that residual disease has remained stable for 7 years in one patient who had received a syngeneic BMT. Such observations suggest the occurrence of a control of tumor growth induced by HDC, irradiation, or autologous transplantation, which is obviously different from the so-called graft-versus-tumor reaction described, for example, in acute leukemias. The study of this minimal residual disease state may provide new insights into the physiopathology of indolent MM, "benign" monoclonal Ig, and of the plateau phase usually observed after initial chemotherapy for MM. Residual disease lasting for months or years has not yet been described (but is likely to occur) in other patients treated similarly for lymphomas or solid tumors, probably because of the lack of a sensitive tumor marker such as the Mlg in MM.

It has been recently acknowledged that HDC and TBI followed by autologous bone marrow graft must be restricted to a small group of patients with hematologic neoplasias whose tumor is responsive to second-line chemotherapy and who therefore have a low tumor burden at the time of HDC and graft. Whether or not this holds true for MM remains open to question. In this unselected series, relapses were observed in two patients with aggressive and refractory disease. It is therefore too early to speculate on the optimal time for such intensive treatment in MM.

Our choice of hematologic reconstitution with blood stem cells relied both on theoretic and practical grounds. Blood stem cells have been recently shown to allow a complete and sustained hematopoietic reconstitution in patients treated for acute leukemias, lymphomas, or solid tumors. We confirm here in patients with MM the feasibility of this approach and show that the period preceding hematopoietic recovery, with its attendant risks, is relatively short. We also hypothesized that the chance to collect circulating malignant myeloma cells would be minimal after recovery from myelo-suppressive chemotherapy. Indeed, the study of cells collected by leukapheresis for clonal Ig gene rearrangements indicative of circulating tumor cells or clonal B cell precursors yielded negative results. Although the interest of hematologic reconstitution with stem cells devoid of malignant plasma cells is obvious, it is worth noting that reconstitution with autologous bone marrow containing such cells has led to some sustained remissions. However, purging of blood or marrow cells remains clearly a desirable goal, since autologous reconstitution in patients with MM avoids the morbidity and mortality of allogeneic transplantation due to graft-versus-host disease, which increases with the patient's age.

The prognosis of patients with high risk or relapsing MM is still very poor despite recent advances in chemotherapy. Our results obtained by HDC and TBI, although preliminary, seem encouraging. Hematologic reconstitution with blood stem cells has both practical and theoretic advantages over allogeneic or autologous bone marrow grafts and deserves further studies in selected patients.

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