

# High-Dose Melphalan With Autologous Bone Marrow Transplantation for Multiple Myeloma

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A large dose of melphalan was given to 23 patients with advanced multiple myeloma that was refractory to multiple prior treatments. Sixteen patients received a dose of 80 to 100 mg/m<sup>2</sup>, and seven were given 140 mg/m<sup>2</sup> followed by autologous bone marrow infusion. Tumor mass was reduced by more than 75% in 14 patients, including four who died of bone marrow aplasia. Serious infections were prevented in six of seven patients who received autologous bone marrow. The marked cytoreduction in patients with

previously refractory disease indicated that apparent drug resistance could be overcome by dose escalation. However, short remission times in most responding patients were consistent with rapid regrowth of primordial tumor cells with high proliferative activity. Although high-dose melphalan was of limited benefit to patients with refractory myeloma, further studies are necessary to clarify its role during earlier phases of disease.

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UNTIL RECENTLY, few treatment programs have been effective in patients with multiple myeloma resistant to alkylating agent-prednisone combinations. Using frequent courses of glucocorticoids with vincristine and doxorubicin by continuous infusion (VAD), about one half of our patients achieved a marked tumor reduction.<sup>1</sup> These results indicated that both dose and schedule modification of standard drugs would enhance tumor cell kill, a finding that was supported by the marked cytoreduction achieved in most patients with very high doses of melphalan.<sup>2</sup> To improve the results from salvage therapies, we evaluated high doses of melphalan in 23 patients with advanced multiple myeloma that was resistant to both standard melphalan and the VAD regimen. Sixty percent of our patients showed rapid and marked tumor reductions. Severe and prolonged bone marrow depression caused the death of about one third of our patients, a complication usually prevented by autologous bone marrow infusion.

## MATERIALS AND METHODS

A large dose of melphalan was given intravenously to 23 patients with advanced refractory myeloma (Table 1). Lower doses of 80 mg/m<sup>2</sup> (one 74-year-old patient) and 100 mg/m<sup>2</sup> (15 patients) were given as a single 30-minute infusion. When the limited efficacy and severe toxicity of this program were appreciated, a higher dose of 140 mg/m<sup>2</sup> was delivered in two daily fractions followed by the infusion of 12 to 16 × 10<sup>9</sup> autologous marrow cells one day later (seven patients). Autologous bone marrow had been stored during VAD-induced second remissions in six patients and following ineffective VAD treatment in one patient with only extramedullary disease. Bone marrow was collected under general anesthesia, frozen at 1 °C/min and kept at -196 °C for 4 to 27 months. CFU-GM of the stored marrows ranged from eight to 92 colonies per 10<sup>5</sup> nucleated cells (median, 21). Plasma cell counts ranged from 2% to 7% (median, 3%). All patients were admitted to the hospital, and seven were treated in a protected environment unit under strict gnotobiotic conditions. Antibiotic prophylaxis was performed with trimethoprim-sulfamethoxazole and ketoconazole. Patients were

advised of procedures and attendant risks in accordance with institutional guidelines and gave informed consent.

The median age of patients treated with melphalan alone was 44 years, whereas the age of those who also received autologous marrow was 63 years. All patients were resistant to multiple prior chemotherapy regimens, and all but two were refractory to salvage VAD therapy.<sup>1</sup> Ten patients had never responded to any prior treatment, and 13 were relapsing to usually two prior regimens. All but three had a high or intermediate tumor mass by standard staging criteria. Flow cytometric (FCM) analyses of bone marrow samples for DNA and RNA content of plasma cells were conducted as described previously.<sup>3</sup> Unfavorable cytometric features included the presence of hypodiploid and biclonal DNA stemlines and/or a RNA content of <4 times that of normal lymphocytes.<sup>4</sup> With any of these features, only 20% of previously untreated patients and less than 10% of patients with resistant myeloma had responded to VAD.<sup>1,5</sup> Ten of our patients had such unfavorable FCM features, previously recognized by multivariate regression analysis to represent the single most important variable both for initial remission<sup>6</sup> and to response to salvage treatment.<sup>1</sup>

Serial studies were conducted to monitor antitumor effect and toxicity. A significant response was defined by a reduction in tumor mass (serum myeloma protein) exceeding 75% with the disappearance of Bence Jones protein excretion for at least 2 months.<sup>7</sup> Five patients died within 6 weeks (early deaths), four of them with a marked antitumor effect comparable to that observed in responding patients. Relapse-free and overall survival times were calculated by life table analyses from the onset of treatment.

## RESULTS

Ten of 23 patients who received high-dose melphalan responded for at least 2 months; four other patients responded, but were rated as failures because they died between 4 and 6 weeks from infection due to neutropenia (Table 2). Tumor-halving times calculated from changes in serum myeloma protein levels were very short in all responding patients (median, 0.3 months), with a tumor mass reduction in four patients to levels lower than those observed after any prior therapy. All patients considered responsive showed clearing of bone marrow plasma cells (<7%). Patients with or without response had similar features in terms of tumor mass and immunoglobulin type. Likewise, the incidence of response was not affected by treatment conditions such as the protective environment or autologous bone marrow transplantation. Although only one of 13 patients with unfavorable FCM features had responded to VAD previously, seven of ten patients with such abnormalities achieved a marked cytoreduction with high-dose melphalan ( $P < .01$ , chi-square test) (Table 2). Similarly, five of ten previously unresponsive

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Table 1. High-Dose Melphalan in Refractory Myeloma

| Characteristic                   | Autologous Bone Marrow Support |       | Total |
|----------------------------------|--------------------------------|-------|-------|
|                                  | No                             | Yes   |       |
| N                                | 16                             | 7     | 23    |
| Age (yr)                         |                                |       |       |
| Range                            | 24-74                          | 35-66 | 24-74 |
| Median                           | 44                             | 63    | 54    |
| Treated in protected environment | 4                              | 3     | 7     |
| No. prior treatment regimens     |                                |       |       |
| Range                            | 1-7                            | 3-6   | 1-7   |
| Median                           | 3                              | 3     | 3     |
| Prior VAD salvage treatment      | 14                             | 7     | 21    |
| No prior response                | 8                              | 2     | 10    |
| Unfavorable FCM features         | 9                              | 2     | 11    |
| Intermediate and high tumor mass | 14                             | 6     | 20    |
| Treatment outcome                |                                |       |       |
| Response                         | 6                              | 4     | 10    |
| Early death*                     | 4                              | 1     | 5     |
| Failure†                         | 6                              | 2     | 8     |

Abbreviations: FCM, flow cytometric.

\*At  $\leq 6$  weeks from high dose melphalan.†Survived  $>6$  weeks from high-dose melphalan and achieved  $<75\%$  tumor mass reduction.

patients experienced a marked antitumor effect with high-dose melphalan in contrast with a 20% response rate for similar patients receiving VAD. The median survival of all patients was 4 months (Fig 1), and the median relapse-free survival of our ten responders was 4 months (range, 2.5 to 18+ months). Four patients are still alive (3, 6, 13, 20 months), including two patients who received bone marrow support.

The major complication was marked and prolonged agranulocytosis and severe thrombocytopenia to less than  $10,000/\mu\text{L}$  for a median of 4 weeks that required a median of 40 units of transfused platelets per patient. With bone marrow transplantation and despite a higher melphalan dose, minimal leukocyte (but not granulocyte or platelet) recovery to at least  $200/\mu\text{L}$  proceeded significantly faster and more uniformly than in the other 16 patients not receiving bone marrow support ( $P < .05$ , Wilcoxon test) (Fig 2). Further hematologic recovery with granulocytes greater than  $1,500/\mu\text{L}$  and platelets greater than  $150,000/\mu\text{L}$  occurred in 11 and seven of 16 patients without marrow support and in six and three of seven patients who received autologous marrow.

As a result of severe and persistent neutropenia in all 16

Table 2. Response to High-Dose Melphalan According to Pretreatment Features

| Prior Response | N   | Nucleic Acid Features |             |           | Percentage |
|----------------|-----|-----------------------|-------------|-----------|------------|
|                |     | Favorable             | Unfavorable | Total     |            |
| Yes            | 13  | 5/8                   | 1(4)/5      | 6(9)/13   | 46(69)     |
| No             | 10  | 1(2)/4                | 3/6         | 4(5)/10   | 40(50)     |
| Total          | 23  | 6(7)/12               | 4(7)/11     | 10(14)/23 |            |
| Percentage     | 100 | 50(58)                | 36(64)      |           | 43(61)     |

Parentheses include patients who died with  $>75\%$  tumor cytereuction.

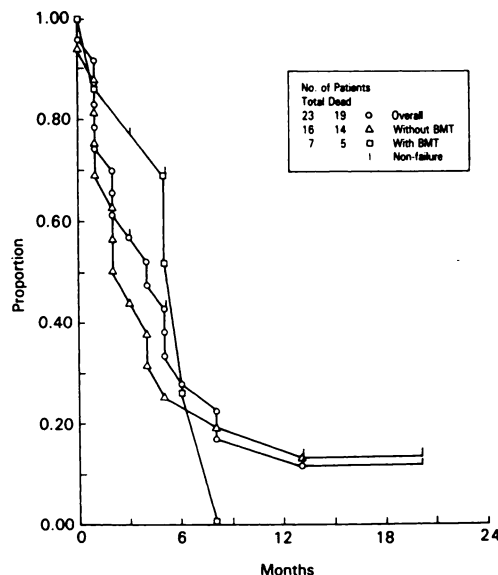


Fig 1. Kaplan-Meier survival plots in patients with refractory myeloma receiving high-dose melphalan with ( $\square$ ) or without ( $\Delta$ ) autologous bone marrow. The melphalan dose was higher in the marrow support group ( $140 \text{ mg}/\text{m}^2$ ) compared with patients not receiving autografts. There was only one of seven drug-related deaths in the marrow transplant group compared with six of 16 among the remaining patients (see also Table 3). Four patients remain alive at 3, 6, 13, and 20 months.

patients without marrow support, four patients with a marked antitumor effect and one patient with resistant myeloma died of disseminated fungal infection (Table 3). An additional patient, still in complete remission of his myeloma, died of *Escherichia coli* sepsis resulting from secondary marrow aplasia after previous complete hematologic recovery. The high incidence of suspected or proven fungal infection led to amphotericin B treatment in 11 of 16 patients. In contrast, the only death among the seven patients

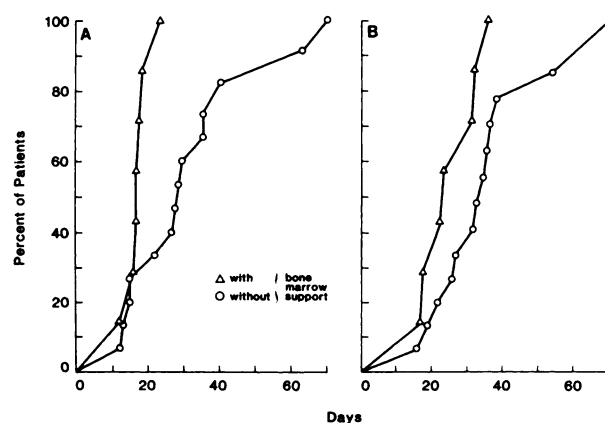


Fig 2. Peripheral blood leukocyte recovery with and without bone marrow transplantation following high-dose melphalan treatment for refractory myeloma (for further details, see text and Fig 1). Even though the time course to complete hematologic recovery was similar in both groups, patients receiving marrow support demonstrated a faster and more uniform leukocyte (but not granulocyte or platelet) recovery to 200 and  $500/\mu\text{L}$ , preventing death from neutropenic sepsis in all but one transplant recipient.

Table 3. High-Dose Melphalan in Refractory Myeloma

| Marrow Support | Antitumor Effect | N  | Alive | Causes of Death   |                     |
|----------------|------------------|----|-------|-------------------|---------------------|
|                |                  |    |       | Melphalan-Related | Progressive Disease |
| No             | Yes              | 10 | 1     | 5                 | 4                   |
|                | No               | 6  | 1     | 1                 | 4                   |
|                | Total            | 16 | 2     | 6                 | 8                   |
| Yes            | Yes              | 4  | 1     | 0                 | 3                   |
|                | No               | 3  | 1     | 1                 | 1                   |
|                | Total            | 7  | 2     | 1                 | 4                   |

receiving marrow infusion occurred in a patient with agranulocytosis persisting beyond day 35 after treatment who died of *Pseudomonas* sepsis. This patient had received prior high-dose cyclophosphamide on two occasions prior to marrow storage.

Thus, although their median age was 20 years less, six of seven treatment-related deaths occurred among the patients who did not receive bone marrow support (Table 3). Other toxicities included reversible hemorrhagic episodes in eight, diarrhea in 13, and pancreatitis in two patients.

#### DISCUSSION

The median survival of patients with multiple myeloma is approximately 30 months, with both the pretreatment tumor load and the sensitivity to chemotherapy representing the major prognostic factors.<sup>8</sup> Until recently, the likelihood of controlling myeloma resistant to melphalan-prednisone has been less than 20%.<sup>9</sup> Although about 50% of the patients responded to the VAD regimen,<sup>1</sup> those who remain unresponsive or relapse to this program present a difficult problem. The observation by McElwain and Powles that a high dose of melphalan induced a marked degree of remission in all nine patients including five who were resistant to standard melphalan seemed promising.<sup>2</sup> We evaluated this program in a group of patients who were older, had received extensive prior chemotherapy including doxorubicin, and had cytometric features associated with resistance to treatment.<sup>1,5</sup>

Considering the adverse prognostic features, the rapid and marked tumor cytorreduction in 61% of 23 patients confirmed remarkable antitumor activity from a single course of melphalan at a dose only three to four times higher than that given with standard therapy. Responses occurred even in patients who had been refractory to all prior therapies and with plasma cell nucleic acid features associated with drug resistance.<sup>1,5</sup> Thus, as with the VAD regimen, the activity of high-dose melphalan confirmed that primary drug resistance could be overcome by dose escalation.<sup>2</sup>

Although myeloma protein reductions were rapid and marked, the remission durations were short. If one considers tumor-halving time as an index of drug sensitivity, shorter halving times should be associated with longer remissions. However, we observed the paradox of progressive shortening of both tumor-halving time (medians of 1.5, 0.5, and 0.3 months) and remission duration (medians of 22, 12 and 4 months) from successive treatments (initial chemotherapy, first salvage VAD, and high-dose melphalan, respectively). These findings were compatible with the progressive expansion of more proliferative primordial tumor cells during the disease course.

The high-dose melphalan program was more toxic than any treatment evaluated previously in patients with multiple myeloma. The 31% mortality rate in patients who did not receive bone marrow support was explained by the high incidence of infection during marked and prolonged neutropenia. This complication was alleviated by autologous bone marrow support in older patients who received even higher doses of melphalan. The comparable degree and speed of response after high-dose melphalan with or without marrow support suggested that marrow contamination by well-differentiated tumor cells did not compromise tumor cytorreduction. Persistent thrombocytopenia of 50 to 75,000/ $\mu$ L has been observed in other autologous marrow transplantation programs, but usually in conjunction with considerably higher doses of cytotoxic drugs. Our patients had extensive prior therapy and marrow involvement by tumor cells, either of which might have contributed to the reduced functional capacity of transplanted normal cells.

The short remission duration should discourage further use of this highly toxic treatment in patients with resistant myeloma. The observation by McElwain and Powles of durable remissions in previously untreated patients, however, deserves further exploration.<sup>2,10</sup> We favor such high-dose therapy approach, perhaps in conjunction with total-body irradiation, for consolidation in remission. The availability of autologous marrow stored early in remission would facilitate host support, and selection of patients with chemotherapy-sensitive myeloma should enhance the probability of attaining marked tumor cytorreduction and prolonged disease control. The recent demonstration of common acute lymphoblastic leukemia antigen-positive plasma cell precursors and of plasma cell-specific antigens offers the possibility of in vitro monoclonal antibody treatment for preferential tumor cell removal prior to marrow infusion.<sup>11-13</sup>

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