A Phase I/II Trial Combining High-Dose Melphalan and Autologous Transplant with Bortezomib for Multiple Myeloma: A Dose- and Schedule-Finding Study

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Abstract

Purpose: We did a randomized phase I/II trial designed to evaluate the safety and efficacy of combining the proteasome inhibitor bortezomib with high-dose melphalan as the conditioning for high-dose therapy and autologous transplant for myeloma.

Experimental Design: Enrolled patients were limited to those who did not achieve a very good partial remission (VGPR) following one or more induction regimens, and were randomized to receive a single escalating dose of bortezomib (1.0, 1.3, or 1.6 mg/m2) either 24 hours before or 24 hours after high-dose melphalan. Dose escalation was based on the escalation with overdose control (EWOC), a Bayesian statistical model. Bone marrow aspirates were collected before initiation of therapy and at the time of transplant to evaluate which sequence resulted in maximal plasma cell apoptosis, and response to transplant was assessed by the International Myeloma Working Group criteria.

Results: Among 39 randomized patients, 20 received bortezomib after melphalan and 19 received bortezomib before melphalan. Toxicities and posttransplant hematopoietic recovery rates were similar between arms. The overall response rate for all patients was 87%, with 51% achieving a VGPR or better. Pharmacodynamic studies showed greater plasma cell apoptosis among patients who received bortezomib following melphalan.

Conclusions: The use of bortezomib in conjunction with high-dose melphalan is safe, with data suggesting improved efficacy. A single dose of bortezomib administered after high-dose melphalan is the recommended dose and schedule for future clinical investigation.

Multiple myeloma (MM) is a clonal plasma cell disorder characterized by lytic bone disease, renal dysfunction, abnormal hematopoietic function, and the presence of a monoclonal paraprotein in the blood and/or urine. Historical induction regimens rarely achieved major responses [very good partial remission (VGPR) or complete remission (CR)], and before the use of high-dose therapy (HDT) and autologous transplant, few therapeutic options showed significant improvements in overall survival (OS) for newly diagnosed myeloma patients (1, 2). Led first by the Intergroupe Francophone du Myelome (IFM), several groups have now shown improvements in overall response rate, progression-free survival (PFS), and OS for patients randomized to receive HDT when compared with conventional dose chemotherapy (3), rendering HDT a standard treatment approach for younger patients with newly diagnosed MM. In addition, the IFM has shown that achievement of a VGPR is a surrogate for improvement in PFS and OS (4), adding it as a new category in the revised International Myeloma Working Group response criteria (5). Despite these improvements, patients are rarely, if ever, cured of MM through the use of HDT. To improve the efficacy of the HDT maneuver itself, groups have explored the use of multiple cycles of HDT (tandem transplant; refs. 6–8), the use of combination chemotherapy conditioning regimens using agents in addition to or replacing melphalan in HDT conditioning (9–12), and the addition of radiation therapy using targeted antibodies (13, 14) or external beam radiation (15). Unfortunately, none of these approaches has been shown to be superior to the use of 200 mg/m2 of melphalan. Thus, if we are to improve on the efficacy of HDT, alternative combination approaches are needed.

Bortezomib is a boronated peptide inhibitor of the chymotryptic site of the proteasome and has shown efficacy in newly diagnosed, relapsed, and refractory MM (16–18).
Preclinical data using bortezomib show pleiotropic effects on plasma cell homeostasis and suggest that the combination of an alkylating agent and bortezomib induces synergistic myeloma cell apoptosis (19, 20). Mechanistically, this is thought to occur because bortezomib induces cleavage of DNA-dependent protein kinase catalytic subunit (DNA-PKCs), enzymes used to facilitate repair of double-stranded DNA breaks (21). Clinical trials such as VISTA (MPV versus MP; ref. 22) and MMY-3001 (bortezomib + liposomal doxorubicin versus bortezomib; ref. 23) confirm the clinical benefit of combination therapy; however, the role of sequence of administration is unclear, with some data suggesting that the DNA-damaging agent should be given before bortezomib to maximize the synergistic effect (21, 24). Based on preclinical and clinical data, we designed a randomized phase I study combining escalating doses of bortezomib with standard high-dose melphalan with the intent of asking two major questions: First, what is the maximum tolerated dose (MTD) of bortezomib that can be safely administered with high-dose melphalan? Second, does the sequence of administration affect the efficacy or toxicity of the combination? We choose to give a single dose of bortezomib to address the synergy interaction between bortezomib and melphalan rather than a multiple dose approach where one could argue that the benefit for the combination was related to independent efficacy of the two agents. Our study indicates that the addition of bortezomib to high-dose melphalan is safe and that the biologically optimal sequence is to deliver the proteasome inhibitor following exposure to melphalan.

Materials and Methods

Eligibility criteria
Eligible subjects for this trial were selected from the population of MM patients referred to the Winship Cancer Institute of Emory University for myeloma therapy. Eligible patients were required to have measurable disease in the bone marrow (an identifiable clone of malignant plasma cells seen by flow cytometry or morphology) and a response of less than a VGPR following induction therapy. Induction therapy for the trial was not specified, and there was no limit on the number of prior lines before HDT and autologous transplant. Suitable patients had to meet eligibility criteria for HDT based on standard institutional guidelines for autologous transplant, including performance status, organ function, and comorbidities (25). All patients signed an informed consent for participation in an Institutional Review Board–approved investigational protocol (National Cancer Institute NCT00793650).

Stem cell collection and conditioning regimen
Peripheral blood hematopoietic progenitor cell grafts were collected by apheresis following chemo-mobilization in conjunction with 10 to 15 μg/kg/d hematopoietic growth factors [granulocyte colony-stimulating factor (G-CSF) or G-CSF + granulocyte macrophage CSF] or cytokines alone. The method of collection (chemo-mobilization or growth factors) was not specified in the protocol. All patients received melphalan (100 mg/m²/d × 2; days −3 and −2), for a total dose of 200 mg/m². Patients were randomized to receive either bortezomib 24 hours before administration of high-dose melphalan (arm A) or 24 hours after melphalan (arm B) in escalating dose cohorts of 1.0, 1.3, and 1.6 mg/m² (Fig. 1). Doses >1.6 mg/m² were not part used or planned for this study based on our hypothesis that DNA repair inhibition could be achieved without escalation beyond 1.6 mg/m². Dose escalation for each arm was done using the escalation with overdose control (EWOC) methodology described below. Individual patients only received one dose of bortezomib.

Transplant patients were housed in private rooms outfitted with high-efficiency particulate air–filtered air during their hospital stay, and they received standard antibacterial, antiviral, and antifungal prophylaxis (25). Blood products were administered according to standard transfusion parameters for the unit. G-CSF was administered beginning on day +7 and continued until neutrophil recovery.

Toxicity and response assessment
Toxicity assessment was done using the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. Dose-limiting toxicity (DLT) was defined as failure to engraft within 30 days of HDT, grade 4 mucositis occurring in >10% of patients, or a treatment-related mortality of >10% for the trial overall. The response assessment occurred at day 100 following HDT using the modified European Group for Blood and Bone Marrow Transplant criteria (5).

Correlative studies
All patients underwent bone marrow aspirate and biopsy on days −4 and 0 (before stem cell infusion) for correlative...
studies. An aliquot of bone marrow aspirate on each day was obtained for flow cytometry analysis of Annexin V and propidium iodide (PI) staining on the CD138+ gate of the bone marrow aspirate.

Statistics
Patients were randomly allocated to receive bortezomib before melphalan (arm A) or bortezomib after melphalan (arm B). Dose escalation started at 1.0 mg/m² in both arms and escalated independently based on observed toxicities for each arm. For each arm, the MTD was defined to be the dose level at which the probability of grade 3 non-hematologic toxicity or grade 4 hematologic toxicity (θ) resulted was equal to 0.33. Dose escalation for both arms was carried out using the EWOC algorithm (26–28). The dose for each subsequent patient was determined so that, on the basis of all available data, the posterior probability that this dose exceeded the MTD was equal to a prespecified value α, where α = 0.3 at the start of the trial and was increased in small increments of 0.05 until α = 0.5. The dose of bortezomib selected for each patient in the trial was to be between the minimum dose of 0.4 mg/m² and the maximum allowable dose of 1.6 mg/m². In each arm, prior distributions for the MTD and the probability of DLT at the initial dose were taken and uniformly distributed over their respective intervals.

Calculation of response was based on greatest disease burden before transplant. OS and PFS curves were estimated using the nonparametric Kaplan-Meier estimator. Comparison of OS and PFS was carried out using the log-rank test. The two-sample t test was used to compare the mean Annexin V and PI staining between the two arms after applying a log transformation to the data.

Results

Demographics
Thirty-nine patients were enrolled into this trial: 19 randomized to arm A, and 20 randomized to arm B (Fig. 1). Eight patients were allocated to dose cohort 1 (1.0 mg/m²; 5 arm A, 3 arm B), 6 to dose cohort 2 (1.3 mg/m²; 3 in both arms), and 25 to dose cohort 3 (1.6 mg/m²; 11 arm A, 14 arm B). The median age was 60, and the median percentage of plasma cells in the marrow before HDT was 10% (see Table 1). The median M-protein for patients at the time of transplant was 1.25 g/dL (range, 0.19-4.0 g/dL), with three patients having elevations in the free light chain as the measure of disease (Fig. 2).

Transplant and toxicity
Peripheral blood progenitor cells were collected with either chemo-mobilization (27 of 39, 69%) or growth factor mobilization (12 of 39, 31%). Patients received an average of 9.0 × 10⁶/kg CD34⁰ cells (range, 2.3-65) as their transplant graft. The median time to neutrophil recovery was 12 days (range, 9-35), and the median time to platelet recovery was 16 days (range, 8-58; Table 2). The two outliers with regard to count recovery had received more than three lines of therapy before stem cell mobilization, or had significant disease burden and had received a prior autologous transplant. Transplant-related toxicities included gastrointestinal and mucosal toxicity (Table 3). Median grade of diarrhea and mucositis was grade 1 (range, 0-3). One patient in arm A had a DLT of grade 5 metabolic toxicity with the development of severe ileus possibly related to enteritis and mucositis. A second patient, from arm B, died following engraftment as a result of infection with parainfluenza. Both deaths occurred with the lowest doses of bortezomib. The DLT seen in the patient with grade 5 metabolic toxicity and ileus resulted in an expansion of the initial cohort per EWOC rules, and subsequent DLTs were not seen. The second death occurred following count

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Table 1. Patient demographics

<table>
<thead>
<tr>
<th></th>
<th>Arm A (n = 19)</th>
<th>Arm B (n = 20)</th>
<th>Total (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range)</td>
<td>60 (45-68)</td>
<td>58.5 (44-74)</td>
<td>60 (44-74)</td>
</tr>
<tr>
<td>Male: female</td>
<td>10:9</td>
<td>13:7</td>
<td>23:16</td>
</tr>
<tr>
<td>Prior autologous transplantation</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>No. prior lines</td>
<td>1 (1-3)</td>
<td>2 (1-5)</td>
<td>1 (1-5)</td>
</tr>
<tr>
<td>Type IgG</td>
<td>12</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Iga</td>
<td>4</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Light chain</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Prior bortezomib</td>
<td>11</td>
<td>13</td>
<td>24</td>
</tr>
</tbody>
</table>
recovery and was not considered study related but rather related to HDT and viral infection. Neither metabolic toxicity nor viral infections were issues with the remaining enrolled patients.

**Response**

The overall response rate for the trial was 87% (PR or better) as assessed at day +100 after transplant. Among patients treated on arm A, 47% achieved ≥VGPR with 11% achieving a CR. Among patients treated on arm B, 55% achieved ≥VGPR with 30% achieving a CR (Table 4). Two patients developed progressive disease and two patients achieved stable disease. With a median follow-up of 17.3 months, PFS and OS were 15.3 and 36.7 months, respectively. There were no differences in PFS and OS between the two arms \( (P = 0.48 \text{ and } P = 0.60, \text{ respectively}) \). When responses were evaluated among the fraction of patients that had received prior bortezomib, there was no diminution in the overall fraction of patients that achieved ≥VGPR, with arm B containing a greater number of CRs than was seen in arm A.

**Correlative studies**

Seventeen patients in arm A and 14 patients in arm B had paired samples of bone marrow, where the fraction of Annexin V and PI staining on the malignant plasma cell population could be successfully done. To account for variable amounts of apoptosis at baseline (the day –4 marrow), the change in Annexin V and PI staining was expressed as a fold change representing either an increase (0.5% on day –4 to 2.5% on day 0; represents a 5-fold increase) or a decrease (2.0% on day –4 to 1.0% on day 0; represents a 0.5-fold increase) when the samples were compared from day –4 to day 0.

All patients had detectable populations of CD138⁺ cells at baseline (range, 0.18-98%). In arm A, 17 of 19 patients had detectable CD138⁺ cells on day 0 and 8 of 17 patients had a >1.5-fold increase in apoptosis by Annexin V (mean Annexin 0.21-fold increase on the log scale). In arm B, 14 of 20 patients had detectable CD138⁺ cells on day 0 and 7 of 14 patients had a >1.5-fold increase in apoptosis by Annexin V (mean Annexin 0.30-fold increase; \( P = 0.91 \)). By PI staining, 10 of 18 of patients had a >1.5-fold increase in apoptosis (mean, 0.05) in arm A compared with 11 of 14 patients with a >1.5-fold increase in apoptosis (mean, 1.16) in arm B \( (P = 0.057) \). The mean fold change in Annexin V staining in arm A was 0.21 compared with 0.30 in arm B. This is not statistically significant \( (P = 0.91) \); however, the mean fold change in PI staining in arm A was 0.05 compared with 1.16 in arm B \( (P = 0.057) \), a value that shows a trend toward significance.

**Discussion**

The use of HDT and autologous transplant was the first major advance in the therapy of patients with newly
diagnosed myeloma and is able to improve the median PFS and OS, yet HDT does not offer benefit for all patients. The proteasome inhibitor bortezomib has shown activity in myeloma patients and has proven to have synergistic activity when combined with alkylators and anthracyclines (22, 23). Based on this observation, we designed the current trial to test the safety, efficacy, and optimal sequence for combining a single dose of

Table 3. Adverse events

<table>
<thead>
<tr>
<th></th>
<th>All adverse events, n (%)</th>
<th>Grade 1-2, n (%)</th>
<th>Grade 3, n (%)</th>
<th>Grade 4, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arm A</td>
<td>Arm B</td>
<td>Arm A</td>
<td>Arm B</td>
</tr>
<tr>
<td>Mucositis (%)</td>
<td>18 (95)</td>
<td>16 (80)</td>
<td>11 (58)</td>
<td>11 (55)</td>
</tr>
<tr>
<td>Diarrhea (%)</td>
<td>16 (84)</td>
<td>18 (90)</td>
<td>13 (68)</td>
<td>16 (80)</td>
</tr>
<tr>
<td>Nausea (%)</td>
<td>19 (100)</td>
<td>18 (95)</td>
<td>15 (79)</td>
<td>16 (80)</td>
</tr>
<tr>
<td>Vomiting (%)</td>
<td>11 (58)</td>
<td>11 (55)</td>
<td>10 (53)</td>
<td>11 (55)</td>
</tr>
<tr>
<td>Febrile neutropenia (%)</td>
<td>11 (58)</td>
<td>13 (65)</td>
<td>2 (11)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Sepsis (%)</td>
<td>3 (16)</td>
<td>2 (10)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
<td>Liver toxicities (%)</td>
<td>1 (26)</td>
<td>4 (20)</td>
<td>5 (26)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Renal toxicities (%)</td>
<td>4 (21)</td>
<td>7 (35)</td>
<td>3 (16)</td>
<td>6 (30)</td>
</tr>
<tr>
<td>Clostridium difficile (%)</td>
<td>3 (16)</td>
<td>1 (5)</td>
<td>2 (11)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Rash (%)</td>
<td>2 (11)</td>
<td>5 (25)</td>
<td>2 (11)</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Cognitive disturbance (%)</td>
<td>2 (11)</td>
<td>1 (5)</td>
<td>1 (5)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Seizures (%)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>DVT/PE (%)</td>
<td>1 (5)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Esophagitis (%)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>2 (10)</td>
<td>1 (5)</td>
<td>1 (5)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Headaches (%)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Bone pain (%)</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Pulmonary toxicities (%)</td>
<td>2 (11)</td>
<td>5 (25)</td>
<td>2 (11)</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Incontinence (%)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Gastrointestinal bleed (%)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Neutropenic colitis (%)</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Blistering on feet (%)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Urinary retention (%)</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Hypotension (%)</td>
<td>4 (21)</td>
<td>2 (10)</td>
<td>1 (5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>A.fib (%)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
<td>Sinus bradycardia (%)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (5)</td>
</tr>
</tbody>
</table>

Abbreviation: DVT/PE, deep vein thrombosis/pulmonary embolism.

Table 4. Response rates between treatment arms

<table>
<thead>
<tr>
<th></th>
<th>Arm A</th>
<th>Arm B</th>
<th>Total (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bortezomib → melphalan (n = 19)</td>
<td>Melphalan → bortezomib (n = 20)</td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>2 (11%)</td>
<td>6 (30%)</td>
<td>8 (21%)</td>
</tr>
<tr>
<td>≥VGPR</td>
<td>9 (47%)</td>
<td>11 (55%)</td>
<td>20 (51%)</td>
</tr>
<tr>
<td>PR</td>
<td>6 (32%)</td>
<td>7 (40%)</td>
<td>13 (33%)</td>
</tr>
<tr>
<td>MR/SD</td>
<td>2 (10%)</td>
<td>0</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>PD</td>
<td>1 (5%)</td>
<td>1 (5%)</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Among bortezomib-exposed patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;VGPR bortezomib prior exposed</td>
<td>6/11 (55%; 2 CR)</td>
<td>7/12 (58%; 5 CR)</td>
<td>13/23 (7 CR)</td>
</tr>
</tbody>
</table>

NOTE: Two patients died and were not assessable for response.
bortezomib with high-dose melphalan and autologous transplant.

This trial, via its unique design and correlative studies, provides four major features that separate it from other HDT trials done in MM to date: (a) the novel dose escalation scheme, (b) the patient population studied, (c) the addition of a novel agent to a standard conditioning regimen, and (d) the evaluation of drug sequencing using correlative measures of myeloma apoptosis.

The EWOC statistical method is one that uses individual patient experiences and allows more rapid dose escalation while minimizing the number of patients that are underdosed in a phase I trial (29). The basic premise behind this Bayesian method for dose escalation is that each patient provides real-time assessment of toxicity and allows for dose escalation if no DLT is encountered and dose de-escalation if the patient exhibits DLT. The operating characteristics of this design were studied using extensive simulations (26), and it was shown that, relative to the continuous reassessment method, EWOC overdosed a smaller proportion of patients and estimated the MTD with comparable accuracy. When compared with different up-and-down schemes, including "3 + 3" designs, EWOC assigned fewer patients to either subtherapeutic or toxic dose levels, treated more patients at optimal dose levels, and estimated the MTD with smaller average bias and mean squared error.

The patients enrolled in this trial were a relatively resistant cohort, who had not achieved ≥VGPR following at least one induction regimen, and had plasma cells detectable in their marrow using flow cytometry or routine microscopic assessment at the time of transplant. This poor response to induction therapy occurred despite 62% of patients having received bortezomib-based induction, and another 18% having received lenalidomide-based induction. Among patients randomized to arm B, the median number of induction regimens was 2 (range, 1-5), whereas patients randomized to arm A received a median of one induction regimen (range, 1-3). The overall response rate as measured by ≥VGPR at day +100 after transplant is 51% for all patients, and in arm B the ≥VGPR rate was 55%. The overall response rate and ≥VGPR rate were independent of prior bortezomib exposure. However, because many of these patients did not receive their initial induction or diagnostic marrow at our center, more recent risk stratification using β2-microglobulin and cytogenetics was not available on a large number of patients, and thus, differences in response between the groups could be confounded by unbalanced prognostic group differences.

In a contemporary retrospective review reported by Kumar et al. (30) from the Mayo clinic, MM patients similar to those enrolled on this trial with measurable disease at the time of transplant (M-protein of >1 g/dL or urine proteins of >200 mg/d) achieved ≥VGPR rate of 30%. This suggests that the inclusion of a single dose of bortezomib in our trial enhances the VGPR rate for a group of patients in whom we know that responses are suboptimal. In a trial with a similar concept of adding bortezomib to high-dose melphalan from the IFM, a similarly high overall and ≥VGPR rate (70%) was observed, although 30% of patients in the IFM trial had already achieved ≥VGPR before HDT. Thus, although the IFM study is further corroborative evidence that the overall response rate following HDT can be improved with the addition of bortezomib, the patient population (superior induction response in IFM), dosing schema of bortezomib (two doses before and two doses after high-dose melphalan), and absence of correlative studies clearly differentiate the two trials and render each experience important for different reasons (31). Additionally, the fact that prior bortezomib exposure did not affect the overall response rate or the fraction of patients who achieved a VGPR suggests that the combination is greater than simply the two individual agents acting independently, but confirmation that the preclinical hypothesis supporting the combination is at work and results in clinical benefit. Furthermore, by delivering a single dose of bortezomib, we were able to test the clinical synergy of the combination and in fact showed comparable clinical efficacy with other trials that used multiple doses of bortezomib with high-dose melphalan (31).

Other investigators have attempted to enhance the response rate and efficacy of HDT by adding bortezomib to the conditioning. The Arkansas group has reported that delivering hyperfractionated melphalan with escalating doses of bortezomib was safe and effective in a small cohort of patients (32). The M.D. Anderson Cancer Center group has also presented preliminary data on a combination of melphalan, arsenic trioxide, ascorbic acid, and bortezomib in a phase II trial but showed no benefit for the addition of bortezomib (33). However, the concomitant administration of ascorbic acid may have compromised the efficacy of the combination, as our group and others have reported that ascorbic acid can inhibit the effects of bortezomib (34–36).

Finally, our comparison of apoptosis in CD138+ cells across treatment arms was designed to be a method by which we could functionally determine the biologically optimal sequence of administration for bortezomib. Despite our small sample, there was a significant difference between the fraction of PI-positive malignant plasma cells in the marrow on day 0 favoring the sequence of bortezomib following melphalan (arm B), and this corresponds to a trend toward a higher CR rate among patients treated in that arm as well (6 of 20 for arm B versus 2 of 19 for arm A). Interestingly, among all tested patients, we were clearly able to assess the fraction of Annexin V– and PI-positive plasma cells at baseline, but the day 0 sample was particularly challenging for patients enrolled in arm B (6 of 20 in evaluable versus 1 of 19 in evaluable in arm A). It is likely that the enhanced efficacy of arm B may have hindered our ability to fully show the superiority of arm B by our correlative studies, as there were too few remaining plasma cells in the marrow on day 0. Although the predictive value of Annexin V and PI staining does not have clear clinical relevance, the fact that its positivity correlated with clinical outcomes (improved efficacy in arm B versus arm A) suggests
that this approach may be useful in future studies. However, due to the small sample size and intrinsic difficulties in collecting tumor samples on all patients, we cannot definitively confirm that our correlative studies confirm sequence specificity.

In conclusion, the efficacy of adding bortezomib to conventional chemotherapy is one that has been tested and confirmed in the context of standard dose therapy (22, 23, 37), but is not one that has been fully evaluated with high-dose melphalan and autologous transplant. Based on laboratory rationale for the use of proteasome inhibitors in conjunction with melphalan, this combination represents a novel way to improve efficacy, yet maintains a similar toxicity profile to high-dose melphalan alone, which has not been easily accomplished by adding radiation, additional chemotherapy agents, or radioimmunotherapy to the conditioning regimen for myeloma transplants. Our pilot trial supports the idea that bortezomib can be safely added to high-dose melphalan, that the fraction of apoptotic plasma cells in the marrow is increased when bortezomib is given following melphalan, and that the VGPR or better rate is superior to that seen for a similar group of patients with poor responses to induction therapy from historical data. Although this trial seems promising, the true efficacy of this combination needs to be further explored and validated in the context of a randomized clinical trial to ultimately show the superiority of this approach to high-dose melphalan alone.

Disclosure of Potential Conflicts of Interest

S. Lonial has received research support from Millennium Pharmaceuticals. S. Lonial has received consulting fees from Millennium and Celgene; C.R. Flowers: consultant and research support, Millennium. In addition, Millennium provided funding for the conduct of the trial. The writing and interpretation and editorial control of the data from the trial and the enclosed manuscript were fully under the control of the authors.

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