Bortezomib, doxorubicin and dexamethasone in advanced multiple myeloma

A. Palumbo1*, F. Gay1, S. Bringhen1, A. Falcone2, N. Pescosta3, V. Callea4, T. Caravita5, F. Morabito6, V. Magarotto1, M. Ruggeri1, I. Avonto1, P. Musto7, N. Cascavilla2, B. Bruno1 & M. Boccadoro1

1Divisione di Ematologia dell’Università di Torino, Azienda Ospedaliera S. Giovanni Battista, Torino; 2U.O. di Ematologia e Trapianto di Cellule Staminali, IRCCS Casa Sollievo della Sofferenza, S. Giovanni Rotondo; 3Divisione di Ematologia, Ospedale Centrale, Bolzano; 4Divisione di Ematologia, Azienda Ospedaliera Bianchi-Melacrino-Morelli, Reggio Calabria; 5Cattedra e Divisione di Ematologia, Università Tor Vergata, Ospedale San Eugenio, Roma; 6U.O.C. di Ematologia, Azienda Ospedaliera di Cosenza, Cosenza; 7U.O. di Ematologia e Trapianto di Cellule Staminali, CROB—Centro di Riferimento Oncologico della Basilicata, Rionero in Vulture (PZ), Italy

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Background: Bortezomib has shown significant activity in myeloma. In this multicenter trial, we assessed for the first time the combination of bortezomib, doxorubicin and low-dose dexamethasone (PAd) in the treatment of relapsed/refractory myeloma.

Patients and methods: Sixty-four patients were treated for a median of four 28-day cycles (1–6). Bortezomib was given at 1.3 mg/m² (days 1, 4, 8, 11) and dexamethasone at 40 mg (days 1–4); 34 patients receive doxorubicin at 20 mg/m² (days 1, 4) while 30 patients pegylated liposomal doxorubicin at 30 mg/m² (day 1).

Results: Fifty-eight percent of patients had undergone prior autologous transplantation, 70% prior anthracycline and 27% prior bortezomib-based regimens. Forty-three patients (67%) achieved at least a partial response including 16 (25%) with at least a very good partial response. One-year event-free survival was 34% after PAd and 31% after the previous line of therapy (hazard ratio 1.20, 95% confidence interval 0.76–1.90, \(P = 0.43\)). One-year overall survival from the start of PAd was 66%. Grade 3–4 toxic effects included thrombocytopenia (48%), neutropenia (36%), infections (15%), anemia (13%), gastrointestinal disturbances (11%) and peripheral neuropathy (10%). Two patients had grade 3–4 cardiac heart failure.

Conclusions: PAd is an active salvage therapy with manageable toxicity in patients with relapsed/refractory myeloma.

Key words: bortezomib, myeloma, relapse

Introduction

Multiple myeloma (MM) is a malignant plasma cell disorder. In 2004, its incidence was estimated for >13 700 new diagnoses in men and 15 000 in women in the European Union [1]. It is considered incurable and conventional salvage therapy in relapsed and refractory disease has traditionally been disappointing with progression-free survival (PFS) usually not exceeding 6 months and median overall survival (OS) of 1 year after relapse [2, 3]. The recent introduction of novel agents, including thalidomide, lenalidomide and bortezomib, has transformed the treatment paradigm for MM. Bortezomib, a specific inhibitor of the 26S proteasome, is associated with profound antimyeloma activity against both MM cells and their microenvironment [4]. Bortezomib has also shown in vitro synergistic activity with several agents with enhanced sensitivity to doxorubicin or melphalan shown in drug-sensitive and chemo resistant MM cell lines as well as primary MM cells derived from patients [5].

Clinical trials employing bortezomib in combination with other agents have shown additive or synergistic activity. Partial response (PR) rates have ranged from 27% to 50% in relapsed/refractory MM [2, 6–8]. The combination of bortezomib and pegylated liposomal doxorubicin (PLD) in patients with advanced disease has further increased the PR rate up to 48%–73% [9, 10]. In a large study, 646 patients with advanced myeloma were randomized to receive bortezomib either alone or with PLD and a final analysis showed a significant benefit in terms of time to progression in the bortezomib/PLD arm (\(P < 0.0001\)) [10]. In newly diagnosed MM patients, the combination of bortezomib, doxorubicin and dexamethasone has been encouraging. The intensity of dexamethasone and the use of 21-day regimen have, however, been associated with a high incidence of infections and peripheral neuropathy [11]. These observations provide the...
patients and methods

patients

Between March 2005 and November 2006, 64 consecutive MM patients were treated at seven Italian centers. Inclusion criteria were as follows: patients in first relapse or higher or refractory to salvage treatment defined as progression during treatment or within 60 days after its completion; age >18 years; adequate cardiac, hepatic, renal and pulmonary functions; measurable disease [12]. The Durie and Salmon staging system was used [13]. Previous treatment with bortezomib or anthracycline was permitted. Exclusion criteria included grade 3–4 peripheral neuropathy, the presence of another cancer or uncontrolled medical problems or conditions at the discretion of the attending physicians, psychiatric disease and hypersensitivity to bortezomib, boron or mannitol. Pre-existing grade 1–2 peripheral neuropathy was not an exclusion criteria. Patients agreed to use effective contraception and women of childbearing age had a pregnancy test before enrollment. All patients gave written informed consent to participation in the study in accordance with the Declaration of Helsinki.

treatment plan

Bortezomib, at a dose of 1.3 mg/m², was given as an i.v. bolus injection on days 1, 4, 8 and 11. Intravenous doxorubicin was administered at a dose of 20 mg/m² on days 1 and 4 while i.v. PLD was administered at a dose of 30 mg/m² on day 1. Thirty-four patients received doxorubicin and 30 patients received PLD. The use of doxorubicin or PLD depended on drug availability at each participating center. Oral dexamethasone was given at a dose of 40 mg/day on days 1 through 4. Each cycle was repeated every 28 days for up to six cycles. Treatment was withheld if drug-related grade 4 hematological toxic effects or grade 3–4 non-hematological toxic effects occurred. After their resolution, bortezomib could be resumed with a 25% dose reduction (from 1.3 to 1.0 mg/m²), and, if needed, from 1.0 to 0.7 mg/m²). If two or more doses of bortezomib were skipped because of hematological toxicity, a dose reduction of 25% was performed during the following cycle. A 25% dose reduction was required for symptomatic grade 2 neuropathy, whereas bortezomib was interrupted for symptomatic grade 3 neuropathy and resumed with a 50% dose reduction upon complete resolution or reduction of severity to grade 1 neuropathy. Oral antibiotic prophylaxis (ciprofloxacin 250 mg b.i.d.), acyclovir (400 mg b.i.d) and gastroprotection were recommended. No prophylaxis for thromboembolism was instituted. The use of bisphosphonates, erythropoietic and myeloid growth factors was allowed as accepted standard of care.

efficacy and safety assessments

Treatment response was evaluated according to the recently published International uniform response criteria. Briefly, complete response (CR) was defined as undetectable serum and urine monoclonal paraproteins (M-protein) by immunofixation, disappearance of any soft tissue plasmacytomas and marrow plasma cells ≤5%. Very good partial response (VGPR) required serum and urine M-proteins detectable by immunofixation but not by standard electrophoresis or a ≥90% reduction in serum M-protein with urinary M-protein excretion <100 mg/24 h. Partial response (PR) was defined as a ≥50% reduction of serum M-protein, reduction in 24-h urinary M-protein by ≥90% or to <200 mg/24 h, a ≥50% reduction in bone marrow plasma cells. Progressive disease was defined as a ≥25% increase of serum and/or urinary M-protein or bone marrow plasma cells (absolute amount ≥10%), an increase in the size or development of new bone lesions or soft tissue plasmacytomas or development of hypercalcemia. The disease was defined as stable if the criteria for CR, VGPR, PR or progressive disease were not met [12]. All adverse events were graded according to the National Cancer Institute Common Terminology Criteria (version 3) [14].

FISH analyses were performed on bone marrow plasma cells that were purified using anti-CD138-coated magnetic beads (Miltenyi Biotech GmbH, Germany). FISH was performed on fixed plasma cells, as previously described [15, 16].

results

patients’ characteristics

The patients’ characteristics are reported in Table 1. At study entry, the median time from diagnosis was 31 months (range 2–181 months) and the median number of prior therapy lines was 2 (range 1–7). Fifteen patients (23%) received PAD as second-line therapy, 24 patients (38%) as third line and 25 patients (39%) beyond third line. Thirty-seven patients (58%) had previously undergone autologous stem-cell transplantation, 17 (27%) had received prior bortezomib-based regimens, 45 (70%) prior anthracycline, 48 (75%) prior thalidomide and 6 (9%) prior conventional chemotherapy. The median number of PAD cycles administered was 4 (range 1–6). The assigned treatment was discontinued in 27 patients: 6 patients stopped treatment because of toxic effects (one case each of grade 3 neurological toxicity, grade 3 acute heart failure, grade 4 acute heart failure and grade 3 infection and two cases of grade 4 infections) and 21 patients because of disease progression.

efficacy

Disease response is illustrated in Table 2. CR or VGPR was achieved in 16 of the 64 patients (25%) and at least PR in 43 (67%). The median time to the best response was 2 months (range 1–6 months). The best response occurred within the first three cycles (3 months) in 76% of responding patients. Response was higher or equal to that induced by the previous therapy line in 69% of patients. In those patients who received PAD as second-line therapy, 4 (27%) achieved a CR or VGPR and 12 (80%) had at least a PR.

Response rates were similar in both patients who had received previous bortezomib-based regimens and those who had not (the CR or VGPR rates were 18% versus 28% and the PR rates 41% versus 38%, respectively). Response rates were higher or equal to those induced by the previous
Table 1. Patient and disease characteristics

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at PAd, median (range), years</td>
<td>65 (41–85)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>34 (53)</td>
</tr>
<tr>
<td>Female</td>
<td>30 (47)</td>
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</table>

<table>
<thead>
<tr>
<th>Prior lines of treatment</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>15 (23)</td>
</tr>
<tr>
<td>2</td>
<td>24 (38)</td>
</tr>
<tr>
<td>3–7</td>
<td>25 (39)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Prior treatments</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>High-dose therapy with PBSC rescue</td>
<td>35 (55)</td>
</tr>
<tr>
<td>Conventional chemotherapy</td>
<td>21 (33)</td>
</tr>
<tr>
<td>Thalidomide-based regimen</td>
<td>8 (12)</td>
</tr>
<tr>
<td>Median time from diagnosis of PAd (range), months</td>
<td>31 (2–181)</td>
</tr>
<tr>
<td>Bortezomib-based regimen</td>
<td>17 (27)</td>
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</table>

*Bortezomib-based regimen in 65%. In the 12 patients who achieved at least a PR in response to previous bortezomib, one CR, one VGPR, six PR were reported in response to retreatment with PAd. In the five patients who did not reach PR in response to previous bortezomib, one CR and one PR were achieved after PAd. In the 34 patients who received doxorubicin, 10 (29%) had a CR or VGPR and 17 (50%) had a PR, while in the 30 patients who received PLD, CR or VGPR was achieved in 6 (20%) and PR in 10 (33%). The PR rate was significantly higher in patients who received doxorubicin (P = 0.03). The median follow-up from study entry was 8 months (range 1–21 months) for survivors. Progression, relapse or death occurred in 29 patients (45%). Overall, the 1-year EFS was 34% which was similar to that reported after the previous line of therapy (HR 1.20, 95% CI 0.76–1.90, P = 0.43; Figure 1A). At the time of this analysis, 16 deaths had been reported, 13 due to disease progression and 3 due to treatment-related causes (two infections and one case of acute heart failure). Overall, the 1-year OS from the start of therapy was 66% (Figure 1B).

A subgroup analysis showed a 1-year EFS of 57% in patients who received PAd as second-line therapy as compared with 30% in those who received PAd beyond the second line (HR 1.91, 95% CI 0.67–5.49, P = 0.23). One-year EFS was 39% in those who had never received bortezomib and 16% in those who had received prior bortezomib-based regimens (HR 1.79, 95% CI 0.83–3.88, P = 0.14). No differences in EFS were reported between patients who received doxorubicin and patients who received PLD (HR 1.30, 95% CI 0.62–2.74, P = 0.49). One-year EFS in patients who achieved VGPR or CR was 83% and 16% in those who achieved only a PR (HR 4.90, 95% CI 1.31–16.34, P = 0.02). The landmark analysis at 6 months showed a similar trend (HR 2.85, 95% CI 0.68–11.97, P = 0.12). One-year OS from the start of therapy was 90% in patients who achieved VGPR or CR and 63% in those who achieved only a PR (HR 7.09, 95% CI 0.88–56.91, P = 0.06). The landmark analysis at 6 months also showed a similar trend (HR 3.50, 95% CI 0.49–24.96, P = 0.24). No differences in EFS were reported between patients with chromosome 13 deletion and without such abnormality (HR 1.32, 95% CI 0.13–13.0, P = 0.8). One-year EFS among patients with β2-microglobulin levels ≤4.0 mg/dl was 32% and 41% in those with higher β2-microglobulin levels (HR 1.07, 95% CI 0.51–2.20, P = 0.86). Similarly, OS from the start of therapy was not different between these two groups (HR 1.79, 95% CI 0.56–3.81, P = 0.43).

**safety**

Adverse events are shown in Table 3. The most common grade 1–2 adverse events were anemia (42%), thrombocytopenia (30%), peripheral neuropathy (27%), nausea (14%), diarrhea (11%) and fever of unknown origin (11%). Three patients, who were not on acyclovir, developed grade 2 herpes zoster infections; no patient treated with prophylactic acyclovir developed viral infections. The most common grade 3 adverse events were thrombocytopenia (23%), neutropenia (20%), anemia (11%), peripheral neuropathy (10%) and pneumonia (9%). The incidence of all other grade 3 adverse events was <5%. Deep vein thrombosis was reported in only two patients (3%). Grade 4 adverse events included hematological toxic effects [thrombocytopenia (25%), neutropenia (16%) and anemia (2%)], one episode of acute heart failure (2%) and two cases of pneumonia (3%). No patient experienced grade 4 peripheral neuropathy. Fifteen patients received support with granulocyte colony-stimulating factor.

Among the 50 patients who had no evidence of neuropathy before study entry, grade 1 sensory neuropathy developed in 11 patients, grade 2 in 5 patients (three sensory and two painful neuropathy) and grade 3 sensory neuropathy in 3 patients. Sensory neuropathy remained stable in eight patients and worsened to grade 2 in one patient and to grade 3 in another, among the 10 patients with pre-existing grade 1 neuropathy, whereas it remained stable in two patients and worsened to grade 3 in two of the four patients with pre-existing grade 2 neuropathy.

The dose of bortezomib was reduced from 1.3 to 1.0 mg/m² because of hematological toxicity (five patients), grade 2...
Among these patients, the dose of bortezomib was further reduced to 0.7 mg/m² because of worsening neuropathy (n = 2) and grade 3 gastrointestinal toxicity (n = 2). Two patients with pre-existing grade 2 neuropathy reduced from 1.3 to 0.7 mg/m² because of grade 3 neuropathy. Both hematological and non-hematological toxic effects were similar between patients younger and older than 65 years. Patients treated with doxorubicin showed a higher incidence of grade 3–4 hematological toxicity (85%) than patients treated with PLD (23%) (P < 0.001); non-hematological toxicity was not significantly different in the two groups (30% and 27%, respectively, P < 0.1). The patient who experienced grade 3 acute heart failure was treated with doxorubicin, whereas the patient who died from acute heart failure received PLD.

**discussion**

In this study, we evaluated the efficacy and safety profile of PAd combination in patients with relapsed or refractory myeloma. The median EFS of 9 months after PAd was similar to that observed after the previous line of therapy, suggesting an efficacious combination with tangible clinical benefit. Importantly, the achievement of VGPR appears to be associated with a significantly longer survival than that of patients who attained only PR. Severe non-hematologic adverse events included infections (15%), gastrointestinal disturbances (11%) and peripheral neuropathy (10%).

In relapsed myeloma, bortezomib as a single agent induced at least a PR in 43% of patients including CR in 6% [6, 8]. The addition of dexamethasone increased response rates in patients with suboptimal response to bortezomib alone [18]. In a large study, 646 patients with advanced myeloma were randomized to receive bortezomib either alone or with PLD: in the bortezomib/PLD group the PR rate was 44% including 9% near CRs [10]. In our study, PAd induced at least a PR in 67% of patients, including a CR rate of 9% and a VGPR rate of 16%. CR or VGPR are considered important surrogates for remission duration and survival [19–22]. Despite the relatively small number of patients, the achievement of at least VGPR or CR improved both EFS and OS. These findings further suggest that effective cytoreduction should be considered a primary treatment goal.

During the first 2 years of follow-up, in each individual patient the time to disease progression after PAd was similar to that observed after the previous line of chemotherapy. This provides evidence of the efficacy of PAd, as the remission duration usually becomes progressively shorter after each line of therapy.

**Table 2.** Response of patients with relapsed/refractory multiple myeloma receiving bortezomib, doxorubicin and dexamethasone (PAd)

<table>
<thead>
<tr>
<th>Response</th>
<th>All patients, N = 64 (%)</th>
<th>PAd as second-line therapy, N = 15 (%)</th>
<th>PAd as third-line therapy, N = 24 (%)</th>
<th>PAd as fourth- to eighth-line therapy, N = 25 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR or VGPR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;90% protein reduction</td>
<td>16 (25)</td>
<td>4 (27)</td>
<td>6 (25)</td>
<td>6 (24)</td>
</tr>
<tr>
<td>CR</td>
<td>6 (9)</td>
<td>2 (13.5)</td>
<td>1 (4)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>VGPR</td>
<td>10 (16)</td>
<td>2 (13.5)</td>
<td>5 (21)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>PR</td>
<td>27 (42)</td>
<td>8 (53)</td>
<td>9 (38)</td>
<td>10 (40)</td>
</tr>
<tr>
<td>50%–89% myeloma protein reduction</td>
<td>15 (24)</td>
<td>3 (20)</td>
<td>6 (25)</td>
<td>6 (24)</td>
</tr>
<tr>
<td>Stable disease</td>
<td>6 (9)</td>
<td>0</td>
<td>3 (12)</td>
<td>3 (12)</td>
</tr>
</tbody>
</table>

CR, complete response; VGPR, very good partial response; PR, partial response.
of salvage therapy. These results have been achieved despite 58% of patients had previously undergone autologous transplant and 75% of them had already received thalidomide. Moreover, PAd activity was similar in both patients treated with PAd as second-line treatment and those treated as fourth-line treatment. EFS and OS were not affected by serum β2-microglobulin levels; this finding is consistent with observations on the use of thalidomide as maintenance therapy after autologous stem-cell transplantation or in combination with dexamethasone as salvage treatment [23, 24]. Similarly, EFS was not modified by the presence of chromosome 13 deletion. In a previous report, elevated β2-microglobulin levels and presence of chromosome 13 deletion, generally considered poor prognostic factors for conventional chemotherapy, were not predictive of poor outcome in patients treated with bortezomib [25]. As shown in other studies [25–28], in our trial, bortezomib also overcomes the adverse prognostic effects of both β2-microglobulin and chromosome 13 deletion. Moreover, patients who were retreated with bortezomib after prior exposure still showed an EFS similar to that of patients who had never received the drug, encouraging its use in multiple lines of salvage therapy.

Most adverse events reported were rather expected and were managed with standard supportive care. Doxorubicin was delivered on days 1 and 4 and not by continuous infusion. This was a very convenient outpatient infusion regimen that allowed the simultaneous administration of doxorubicin with bortezomib. The incidences of grade 3–4 neutropenia and thrombocytopenia were 36% and 48%, respectively. In relapsed patients treated with bortezomib and dexamethasone, the rates of both neutropenia (13%) and thrombocytopenia (33%) were lower [18] as compared with regimens containing bortezomib and d oxorubicin [9, 10]. Despite this hematological toxicity, a higher incidence of bleeding or infections was not reported.

Grade 3–4 infections occurred in 15% of patients after PAd, in 15% after bortezomib as a single agent [7], in 24% after bortezomib and PLD [9] and in 33% after bortezomib doxorubicin and high-dose dexamethasone [11]. Low-dose dexamethasone markedly reduced the risk of infections in comparison with a regimen including both doxorubicin and high-dose dexamethasone. Severe grade 3 peripheral neuropathy has been reported in 8%–15% of patients who received bortezomib alone [2, 6, 7] and in 17% of those who received the combination of bortezomib, melphalan and prednisone [26]. In relapsed patients treated with bortezomib, melphalan, prednisone and thalidomide, the incidence of grade 3–4 peripheral neuropathy was 7% only, despite the concomitant administration of thalidomide [29]; the weekly administration of bortezomib at a dose of 1.3 mg/m² may have contributed to the reduced rate of peripheral neuropathy. In our PAd regimen, a 28-day cycle instead of 21-day cycle may have contributed to the reduced neurotoxicity of 10% as suggested by Berenson et al. [30]. Only two episodes of deep vein thrombosis and no pulmonary embolism were recorded, whereas these complications were frequently reported in patients treated with drug combinations including doxorubicin [31, 32].

No differences in non-hematological toxic effects between patients treated with doxorubicin or PLD were reported. The incidence of cardiac toxicity was inferior to that recently reported [10] and was equally distributed between the two cohorts of patients. When the study was designed, the use of doxorubicin or PLD was on the basis of the Institutional guidelines of each participating center as data comparing the two drugs were not available. Our data show that there are no significant differences in terms of toxicity profile and clinical efficacy. However, it should be noted that the recent approval of the combination bortezomib/PLD by the Food and Drug Administration and the European Medicines Evaluation Agency has provided an important treatment option for relapsed/refractory MM patients.

In conclusion, PAd induces clinically significant responses and prolonged remission duration in patients with relapsed and refractory myeloma. Adverse events are reduced by the administration of low-dose dexamethasone and the 28-day schedule of bortezomib.

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references