ORIGINAL ARTICLE

# Alkaline phosphatase variation during carfilzomib treatment is associated with best response in multiple myeloma patients

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## Abstract

The ubiquitin–proteasome pathway regulates bone formation through osteoblast differentiation. We analyzed variation alkaline phosphatase (ALP) during carfilzomib treatment. Data from 38 patients enrolled in the PX-171-003 and 29 patients in PX-171-004 studies, for patients with relapsed/refractory myeloma, were analyzed. All patients received 20 mg/m$^2$ of carfilzomib on Days 1, 2, 8, 9, 15, and 16 of a 28-day cycle. Sixty-seven patients from ALP data were evaluable. In PX-171-003, the ORR (>PR) was 18% and the clinical benefit response (CBR; >MR) was 26%, while in PX-171-004, the ORR was 35.5% overall and 57% in bortezomib-naive patients. ALP increment from baseline was statistically different in patients who achieved ≥VGPR compared with all others on Days 1 ($P$ = 0.0049) and 8 ($P$ = 0.006) of Cycle 2. In patients achieving a VGPR or better, ALP increased more than 15 units per liter at Cycle 2 Day 1 over baseline. An ALP increase over the same period of time was seen in 26%, 13% and 11% of patients achieving PR, MR, and SD, respectively. This retrospective analysis of patients with relapsed or refractory myeloma treated with single-agent carfilzomib indicates that early elevation in ALP is associated with subsequent myeloma response.

**Key words** multiple myeloma; alkaline phosphatase; carfilzomib

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Multiple myeloma (MM) is a plasma cell malignancy with impaired bone formation. In myeloma, RANKL expression is markedly increased while its decoy receptor, osteoprotegerin (OPG), is decreased (1). The Wnt signaling inhibitor DKK1 expression is significantly increased and correlates with the extent of bone disease (2, 3). The ubiquitin–proteasome pathway, an essential cellular degradative system in myeloma cells, can also regulate bone formation via effects on osteoblast differentiation (4, 5). Bortezomib, the first proteasome inhibitor, has been shown to have inhibitory effect on myeloma SCID-hu model *in vivo*. Primary human myeloma cells are grown in a human fetal bone, engrafted in the flank of SCID mice. In this model, the use of bortezomib (0.5 mg/kg

twice a week) was associated with disease control and increase in bone mineral density (BMD) (20 ± 14%), while myeloma growth induced a decrease in BMD (13 ± 12%) and progressive tumor in untreated animals. Exposure of non-myeloma-bearing control mice to bortezomib also resulted in a significant increase in BMD (6). While osteoprogenitor differentiation is not affected by immunomodulatory analogues (lenalidomide, CC-4047, CC-6032), both bortezomib and immunomodulatory compounds retain inhibitory effect on osteoclast differentiation (7, 8). Retrospective analysis of the bone alkaline phosphatase (ALP) variation during treatment of myeloma patients with bortezomib has indicated a close correlation between myeloma response and drug activity (9). An analysis of the APEX data showed that a 25% increase in ALP after 6 wks' treatment was the best predictor of myeloma response and was associated with time to progression (10).

The phase 3 Vista trial compared the use of melphalan and prednisone with or without bortezomib in previously untreated patients with MM who were ineligible for a high-dose therapy. Six hundred and eighty-two patients were randomly assigned to receive nine 6-week cycles of melphalan and prednisone either alone or with bortezomib (11). A retrospective analysis showed that rates of bisphosphonates use and bone disease events were significantly lower in patients enrolled in the VMP arm compared with the MP control arm (12).

Carfilzomib is a novel inhibitor that binds selectively and irreversibly to the proteasome resulting in greater and more sustained inhibition compared with bortezomib, and *in vitro* studies have shown to be able to overcome bortezomib resistance (13). Carfilzomib selectively inhibits the N-terminal threonine protease activity of the proteasome with minimal cross-reactivity with serine and other proteases. This analysis is the first to focus on the variations of ALP and myeloma response in carfilzomib-treated patients with myeloma.

## Material and methods

Retrospective analysis of the relationship between serum ALP and tumor response was performed on patients with relapsed or refractory myeloma.

Analysis of serum ALP variation was completed in patients with myeloma enrolled on two phase 2 studies (PX-171-003 and PX- 171-004) evaluating the safety and efficacy of single-agent carfilzomib dosed at 20 mg/m$^2$. We analyzed data from 38 patients in the PX-171-003-A0 study, a relapsed and refractory myeloma trial for patients who have received ≥3 prior therapies including bortezomib and an immunomodulatory drug (IMiD), along with 29 patients in PX-171-004, a relapsed or refractory myeloma trial that included bortezomib-naive patients. All patients received 20 mg/m$^2$ of carfilzomib on Days 1, 2, 8, 9, 15, and 16 of a 28-day cycle and remained on study until progression, intolerable adverse events, or withdrawal of consents.

Serum ALP measurements were taken prior to the first dose of carfilzomib (baseline) and periodically during the trial. Change in ALP levels from baseline to Cycle 2 Day 1 was measured in a group of 117 patients but only 67 patients with ALP data were evaluable for response. ALP data were aligned with clinical response data and (as per international myeloma working group (IMWG) response criteria) with ALP variations associated with response groups. All patients enrolled in this study have signed informed consent approved by the IRB.

## Results

Sixty-seven patients were included in this analysis. The median age was 63 yrs with a median time since diagnosis of 4.6 yrs, 52% were men, 84% had relapsed after Autologous stem cell transplantations, 82% were previously exposed to bortezomib, and 92% were previously exposed to an IMiD (Table 1). In PX-171-003, the overall response rate (ORR) (≥PR) was 18% and the clinical

**Table 1** Patients characteristics

| | Number of (%) of subjects | | | |
|---|---|---|---|---|
| | | 004 | | |
| | 003-A0 | Bortezomib treated | Bortezomib naive | Total |
| Characteristic | (*N* = 38) | (*N* = 29) | (*N* = 51) | (*N* = 117) |
| Median age (years) | 64 | 63 | 64 | 64 |
| Median years since diagnosis | 5.9 | 3.6 | 3.5 | 4.3 |
| Gender: male | 19 (51.4) | 14 (48.3) | 28 (54.9) | 61 (52.1) |
| Mean number of prior therapies | 6.1 | 2.9 | 2.0 | 3.5 |
| Received bortezomib | 37 (100.0) | 29 (100.0) | 0 (0.0) | 66 (54.5) |
| Received IMiD | 37 (100.0) | 22 (75.9) | 47 (92.2) | 106 (90.6) |
| Received transplant | 30 (81.1) | 25 (86.2) | 41 (80.4) | 96 (82.1) |
| Refractory to most recent therapy | 37 (100) | 9 (31) | 20 (39) | 66 (56) |

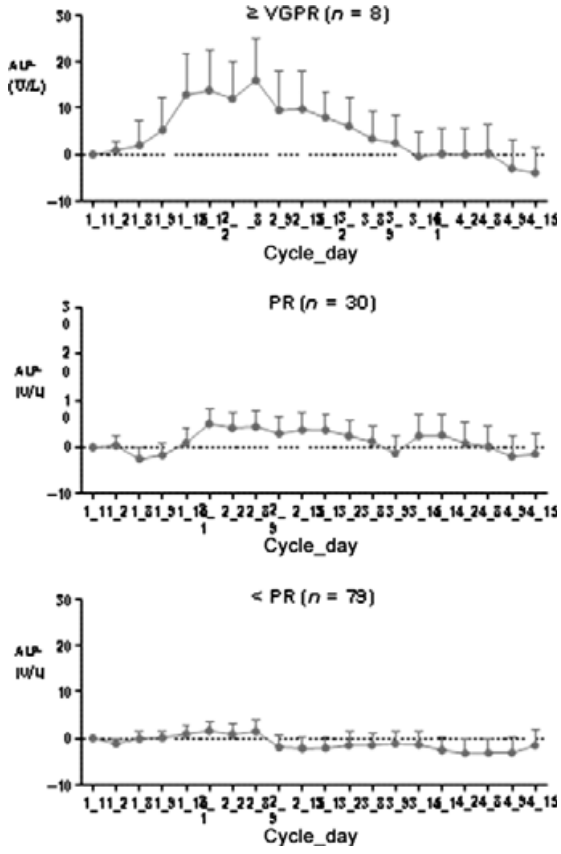


**Figure 1** Average alkaline phosphatase change from baseline – increase associated with response.

benefit response (CBR; ≥MR) was 26%, while in PX-171-004, the ORR was 35.5% overall and 57% in bortezomib-naive patients. Average ALP change from baseline appeared to be associated with responses. Figure 1 shows ALP variation during treatment in patients according to clinical response.

ALP increment from baseline, which was most evident during the second cycle of treatment, was statistically different in patients who achieved ≥ very good partial remission (VGPR) compared with all others on Days 1 ($P = 0.0049$) and 8 ($P = 0.006$) of Cycle 2. In all patients achieving a VGPR or better, ALP increased more than 15 units per liter at Cycle 2 Day 1 over baseline. An ALP increase over the same period of time was seen in 26%, 13% and 11% of patients achieving PR, MR, and SD, respectively.

None of the patients with progressive disease exhibited a similar increase. Our study indicates that response first assessed on Day 15 of Cycle 1 parallels the ALP elevation that returned to baseline levels at the end of Cycle 3. Twenty-nine percent (11/38) of subjects achieving at least a PR had an increase in ALP of >15 U/L from baseline, while only 7.6% (6/79) of subjects failing to achieve at least a PR showed a similar increase. Of the 17 patients that had increases of >15 U/L, 11 patients (65%) responded (>PR) to carfilzomib treatment. An increase in serum ALP levels on Cycle 2 Day 1 from baseline is associated with best response.

## Discussion

Several *in vivo* studies have shown that proteasome inhibition is associated with an increase in bone formation. Pennisi *et al.* (6) reported a significant ($P < 0.04$) increase in BMD in SCID-rab mice engrafted with MM cells treated with bortezomib; increases in bone volume, trabecular thickness, and bone formation were also observed in bortezomib-treated mice, both in myelomatous and non-myelomatous bones. Similarly, a decrease in osteolytic lesions and increases in trabecular number and bone volume have been reported following bortezomib treatment of 5T2MM mice (14).

In a murine model of MM, bortezomib-treated mice showed a 400% increase in osteoblast numbers compared with vehicle mice. Furthermore, the bortezomib-treated mice had around 50% more osteoblasts than tumor-naive mice, suggesting a more direct effect of bortezomib on osteoblasts than was seen on osteoclasts in the same study (14).

This is supported by a previous *in vitro* study that demonstrated a direct effect of bortezomib in enhancing differentiation of fetal human MSCs into osteoblasts (6).

A study by De Matteo *et al.* (15) showed that osterix, a key transcription factor required for osteoblast differentiation, is down-regulated in MM but can be up-regulated by bortezomib.

In a prospective phase 2 study, bortezomib was associated with a statistically significant increase from baseline in bone volume/total volume (as assessed via comparative histomorphometric microCT analysis) in six of seven patients with relapsed/refractory MM. This study has also indicated that response to bortezomib was associated with changes in serum parathyroid hormone (PTH) concentrations (16).

This retrospective analysis on a subset of patients in these ongoing phase 2 studies of single-agent carfilzomib in relapsed or refractory MM has shown for the first time that elevation in serum ALP during carfilzomib is associated with best tumor responses. Sixty-five percent (11/17) of subjects with an increase in ALP of >15 U/L above baseline achieved a PR or better. Initial tumor response paralleled the ALP elevation, with ALP values generally returning to baseline levels at the end of Cycle 3 even if the response seems to continue. PTH levels were not measured in these phase 2 studies of carfilzomib.

Taken with previous publications describing bortezomib treatment, these results suggest that this specific ana-

 

bolic bone phenomenon could be a class effect of proteasome inhibitors. Additional phase 2 studies with a higher dose of carfilzomib (i.e. 27 mg/m$^2$) are ongoing. The data from this small subset analysis suggest that further exploration of this relationship between bone-derived markers and myeloma response is warranted.

## Author contributions

Conception and design: Maurizio Zangari, Guido Tricot, Monette Aujay; Provision of study materials or patients: Ravi Vij, Sundar Jagannath, David Siegel, A. Keith Stewart, Luhua Wang, Robert Z. Orlowski, Andrew Belch, Andrzej Jakubowiak, George Somlo, Suzanne Trudel, Nizar Bahlis, Sagar Lonial, Seema Singhal, Vishal Kukreti; Collection and assembly of data: Monette Aujay, Tamara Berno; Data analysis and interpretation: Maurizio Zangari, Monette Aujay; Manuscript writing: Maurizio Zangari, Guido Tricot, Kristina L. Hetherington; Final approval of manuscript: Maurizio Zangari, Guido Tricot.

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