

## ORIGINAL ARTICLE

## Replacement of bortezomib with carfilzomib for multiple myeloma patients progressing from bortezomib combination therapy

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In this open-label, intra-patient phase I/II trial, bortezomib was replaced with carfilzomib (escalated from 20 to 45 mg/m<sup>2</sup> on days 1, 2, 8, 9, 15 and 16 of a 28-day cycle) for multiple myeloma (MM) patients who progressed while on or within 12 weeks of receiving a bortezomib-containing combination regimen. Study objectives included determination of the maximum tolerated dose (MTD), overall response rate (ORR), clinical benefit rate (CBR), time to progression, time to response, duration of response, progression-free survival and overall survival (OS). Of 38 registered patients, 37 were treated and evaluable for efficacy and safety. Thirty-one carfilzomib-based regimens using 14 different drug combinations were tested. One regimen (carfilzomib (45 mg/m<sup>2</sup>), ascorbic acid (1000 mg) and cyclophosphamide (2.2 mg/kg)) reached MTD. ORR and CBR were 43.2 and 62.2%, respectively. Median progression-free survival, time to progression and OS were 8.3, 9.9 and 15.8 months, respectively. Hematologic adverse events (AEs; ≥grade 3) included lymphopenia (35.1%), thrombocytopenia (24.3%), anemia (10.8%) and neutropenia (10.8%). Nonhematologic AEs (≥grade 3) included fever (5.4%) and hypokalemia (5.4%). These results demonstrate that replacing bortezomib with carfilzomib is safe and can be effective for MM patients failing bortezomib-containing combination regimens. This trial was registered at <http://www.clinicaltrials.gov> (#NCT01365559).

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## INTRODUCTION

In 2013, ~22 500 individuals in the United States were diagnosed with multiple myeloma (MM), and nearly 11 000 died from the disease.<sup>1</sup> Although the use of novel agents and high-dose therapy has improved overall survival (OS),<sup>2</sup> MM remains incurable; nearly all patients relapse or become refractory to treatment. Thus, more treatment combinations are needed, especially for those patients who have exhausted all available treatment options.<sup>3</sup>

In July 2012, the proteasome inhibitor (PI) carfilzomib was approved as a single agent in the United States for the treatment of relapsed and refractory MM based on the findings from the pivotal PX-171-003-A1 (#NCT00511238) trial.<sup>4,5</sup> In this study, nearly all patients who received carfilzomib had received prior therapy with bortezomib, a first-generation PI.<sup>4</sup> Among patients who were refractory or intolerant to bortezomib and lenalidomide, the ORR was 20.1% (95% confidence interval (CI): 14.9–26.1). Similarly, many relapsed and refractory MM patients who received single-agent carfilzomib in the PX-171-004 study (#NCT00530816) had also received bortezomib and had an ORR of 17.1%.<sup>6</sup> These results suggest the potential for this newer PI to overcome

resistance to other PIs such as bortezomib. This type of strategy has proven to be successful, as MM patients failing regimens that include steroids and an immunomodulatory drug (IMiD) often respond to a different IMiD.<sup>7</sup>

The success achieved following retreatment of patients with bortezomib has occurred in specific clinical settings. Bortezomib retreatment has been successful for some patients that were previously responsive to this PI,<sup>8–10</sup> and for relapsed and refractory MM patients treated with it in combination with other anti-MM agents.<sup>11,12</sup> The ability to overcome bortezomib resistance following retreatment with a different PI has been demonstrated in preclinical studies with bortezomib, carfilzomib, oprozomib (ONX 0912), ixazomib (MLN 9708), delanzomib (CEP-18770) and MLN 2238. For instance, MLN 2238 was shown to induce apoptosis in bortezomib-resistant MM cells.<sup>13</sup> Carfilzomib in combination with marizomib (NPI-0052) was also found to induce cell death in MM cells that were derived from bortezomib refractory patients.<sup>14,15</sup> In a separate study, the PI delanzomib had anti-MM effects in bortezomib-resistant MM, and the combination of delanzomib and bortezomib was found to have greater anti-MM activity compared with either agent alone.<sup>16</sup> These preclinical

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studies provided the rationale for evaluating whether replacing a PI for one that is ineffective in a combination treatment can achieve clinical efficacy for patients with MM. In this context, carfilzomib treatment for patients that failed bortezomib therapy in an otherwise novel treatment combination has shown clinical activity,<sup>4,6</sup> yet the concept of substituting PI with another into an otherwise unchanged combination treatment in an attempt to overcome PI resistance has not been investigated in the clinic. Given that single-agent carfilzomib can produce responses in some bortezomib refractory patients<sup>4</sup> and shows high response rates when combined with other agents,<sup>17,18</sup> we hypothesized that carfilzomib would be an effective and well-tolerated replacement for bortezomib among MM patients who had failed bortezomib-containing combination regimens. In this study, we investigated the feasibility of replacing bortezomib with carfilzomib in an otherwise identical combination regimen for MM patients who had progressed on or within 12 weeks of receiving their most recent bortezomib-based combination regimen.

## PATIENTS AND METHODS

This was a multicenter, open-label, nonrandomized, intra-patient dose-escalating phase I/II clinical study that involved seven sites in the United States. This study was conducted in accordance with US Food and Drug Administration regulations, the International Conference on Harmonisation Guidelines for Good Clinical Practice and the Declaration of Helsinki. The study was compliant with all local health authority and Institutional Review Board requirements. All patients provided written informed consent in accordance with federal, local and institutional guidelines. The investigators designed the study and collected and analyzed data from the study. All participating institutions received financial assistance from Onyx Pharmaceuticals Inc. (South San Francisco, CA, USA).

### Patients

MM patients  $\geq 18$  years old with measurable and progressive disease and who had progressed while on or within 12 weeks of receiving the last dose of their most recent bortezomib-containing regimen were eligible. All patients must have received four doses of bortezomib ( $\geq 1.0$  mg/m<sup>2</sup>) in 21 or 28 days per cycle for at least one cycle. In addition to bortezomib, a patient's most recent bortezomib-containing regimen must have contained an alkylating agent (for example, melphalan, cyclophosphamide or bendamustine), anthracycline (for example, doxorubicin or pegylated liposomal doxorubicin), IMiD (thalidomide and/or lenalidomide) and/or glucocorticosteroid (prednisone, dexamethasone or methylprednisolone). Patients were required to have not received any anti-MM therapy for  $\geq 3$  weeks before enrollment to ensure that there was adequate washout of the prior anti-myeloma regimen (see below).

Patients must have had a life expectancy of  $\geq 3$  months and an Eastern Cooperative Oncology Group performance status of 0–2. Laboratory entry criteria included an absolute neutrophil count of  $\geq 1.5 \times 10^9/l$  ( $\geq 1.0 \times 10^9/l$  if the bone marrow was extensively infiltrated); hemoglobin count of  $\geq 8$  g/dl; platelet count of  $\geq 75 \times 10^9/l$  ( $\geq 50 \times 10^9/l$  if the bone marrow was extensively infiltrated); creatinine clearance  $\geq 30$  ml/min; aspartate transaminase (serum glutamic oxaloacetic transaminase) and ALT (serum glutamic-pyruvic transaminase)  $\leq 3 \times$  upper limit of normal or  $\leq 5 \times$  upper limit of normal if hepatic metastases were present; serum total bilirubin  $\leq 1.5 \times$  upper limit of normal and serum potassium  $\geq 3$  and  $\leq 5$ . Men were required to use contraception; women of childbearing potential were also required to use contraception and receive pregnancy tests throughout the study.

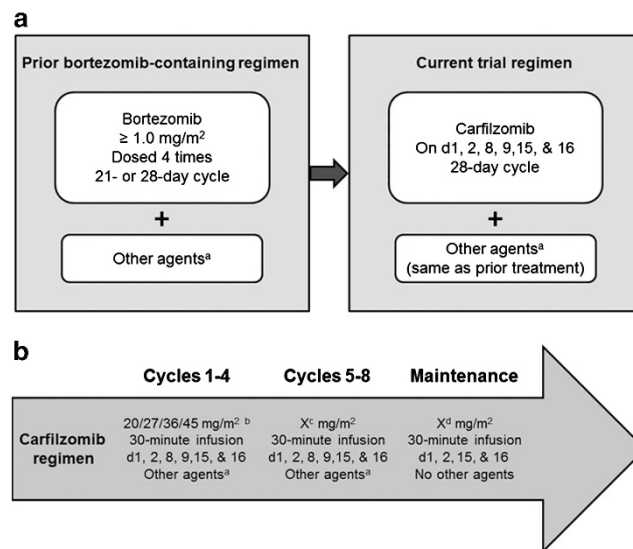
Patients were excluded if they had peripheral neuropathy (grades 3 or 4 or grade 2 with pain) within 14 days before enrollment; impaired cardiac function or clinically significant cardiac disease (for example, unstable angina or myocardial infarction within 4 months before enrollment; New York Heart Association Class III or IV congestive heart failure (CHF); uncontrolled angina; clinically significant pericardial disease or arrhythmias); gastrointestinal disease or impaired gastrointestinal function; plasma cell dyscrasia with polyneuropathy, organomegaly, endocrinopathy, M-protein and skin changes syndrome; plasma cell leukemia; a prior malignancy within 5 years except non-melanomatous skin cancer; major surgery within 28 days before enrollment or severe hypercalcemia (corrected serum calcium  $\geq 12$  mg/dl). Patients were also excluded if they

received prior therapy with carfilzomib or chemotherapy, corticosteroids ( $>10$  mg per day prednisone or equivalent), immunotherapy, antibody therapy, thalidomide, lenalidomide, arsenic trioxide, bortezomib or radiation therapy within 21 days of enrollment; other experimental drug or therapy within 28 days of enrollment or concurrent use of any other anticancer agents or treatments. In addition, patients were excluded if they had experienced an acute active infection that required treatment, known human immunodeficiency virus infection or active hepatitis B, or C infection. Pregnant or lactating females were also excluded.

### Study design and drug administration

The study design is shown in Figure 1. The primary objectives of the study were to determine the maximum tolerated dose (MTD) for each combination regimen and the ORR for all regimens. The regimen, dose and schedule of the prior bortezomib-containing regimen remained unchanged except that bortezomib was replaced with carfilzomib. For three patients, cycle length was extended from 21 to 28 days by adding a week without additional treatment in order to conform to the carfilzomib schedule. During cycle 1, a starting dose of carfilzomib (20 mg/m<sup>2</sup>) was given by intravenous administration over 30 min on days 1, 2, 8, 9, 15 and 16 of a 28-day cycle. During cycles 2, 3 and 4, carfilzomib was escalated to 27, 36 and 45 mg/m<sup>2</sup>, respectively; the same infusion time and dosing schedule that was used during cycle 1 was also used during cycles 2–4.

During cycle 1, all patients received oral or intravenous fluids (250–500 ml) before each dose of carfilzomib. Patients also received prophylactic acyclovir (or another similar anti-varicella agent). Bisphosphonates and erythropoietic agents were permitted during the study. Colony-stimulating factors were permitted if grade 4 neutropenia occurred but could not be used prophylactically. During cycle 1, oral or intravenous dexamethasone (4 mg) was given before each carfilzomib dose unless the



**Figure 1.** Trial design and dosing schema. **(a)** The trial replicates patients' last bortezomib-containing combination regimen except that bortezomib is replaced with carfilzomib. The other agent(s), dose and administration schedule in the regimen remain the same as the prior bortezomib-containing regimen. **(b)** Carfilzomib dosing and schedule for the study treatment and maintenance periods. <sup>a</sup>Alkylating agents with or without steroids; glucocorticoids; IMiDs with or without other agents; and PLD with or without steroids. <sup>b</sup>Patients underwent intra-patient dose escalation during cycles 1–4, unless a DLT occurred. Following the protocol amendment, if a DLT occurred, the patient continued dosing at the dose administered in the previous cycle. <sup>c</sup>The carfilzomib dose in cycles 5–8 was the highest tolerated dose received in cycles 1–4 without DLTs, or significant AEs. <sup>d</sup>For maintenance, carfilzomib was administered as a single agent at the last dose that the patient was receiving when he or she completed the combination study treatment. AE, adverse event; DLT, dose-limiting toxicity; IMiD, immunomodulatory drug; PLD, pegylated liposomal doxorubicin.

patient was already receiving glucocorticosteroids on the same day as a part of their anti-MM treatment regimen. Patients who were believed to be at risk for tumor lysis syndrome (TLS) were allowed to receive allopurinol prophylaxis.

During cycles 1–4, patients were required to complete a full cycle without experiencing a dose-limiting toxicity (DLT) before they could begin a new cycle. During cycles 5–8, the maximum dose that the patient tolerated during the first four cycles was given using the same dosing and administration schedule. Each of the first 19 patients continued to receive treatment until one of the following occurred: a DLT, a maximum response plus one additional treatment cycle was completed, a maximum of eight treatment cycles was received, disease progression (PD), or unacceptable toxicity. After enrolling the first 19 patients, the study protocol was amended to allow the following: (1) up to two carfilzomib dose reductions ( $9\text{ mg/m}^2$  per dose reduction) for patients that experienced a DLT (previous to approval of this amendment, patients experiencing DLTs were taken off the trial); and (2) maintenance therapy with single-agent carfilzomib for patients that completed eight cycles of treatment without PD. For maintenance treatment, carfilzomib was administered as a single agent on days 1, 2, 15 and 16 of each 28-day cycle at the last dose that the patient was receiving when he or she completed the combination study treatment. Once a patient stopped receiving study drug (carfilzomib), follow-up for PD and OS was completed every 3 and 6 months, respectively.

#### Dose-limiting toxicities

DLTs were defined as the occurrence of any of the following during the first four cycles of dose escalation treatment: grade 3 or 4 hematologic toxicity (excluding lymphopenia); grade 3 thrombocytopenia with grade 3 or 4 hemorrhage; grade 3 or 4 nausea or vomiting refractory to anti-emetic therapy; grade 3 or 4 diarrhea or constipation refractory to antidiarrheal or constipation therapy; any other study treatment-related  $\geq$  grade 2 nonhematologic toxicity (except alopecia); or any treatment-related death.

#### Determination of the MTD

MTD declaration required a minimum of three patients for each specific combination treatment. If all three patients showed no DLTs during the first four cycles of dose escalation to  $45\text{ mg/m}^2$ , the MTD was declared at this maximally administered dose (MAD). If a DLT occurred in one subject at any dose level during any of the first four dose escalation cycles, additional subjects were enrolled at this dose level until either another DLT was observed or a total of six patients were treated without an additional DLT. If no additional DLTs occurred among six patients treated, then the MTD was considered to be the MAD. However, if more than one patient experienced a DLT, then the MTD was declared at one dose level lower than that at which the second DLT occurred as long as three patients had been treated at the lower level without a DLT or no more than one DLT occurred among six patients.

#### Safety assessments

Adverse events (AEs) were assessed for any patient receiving study drug at each visit and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.0. Serious AEs included the following: any death occurring within 28 days of receiving study drug regardless of discontinuation from the study; a life-threatening experience that the investigator believed placed the participant at immediate risk of death; an event requiring inpatient hospitalization or prolongation of an existing hospitalization (excluding scheduled hospitalizations for nonacute medical conditions or elective surgery) or a condition that resulted in persistent or significant disability/incapacity.

#### Response criteria

Response and PD were determined according to a modified version of the European Blood and Marrow Transplantation criteria for evaluating disease response and progression among MM patients and the updated International Myeloma Working Group criteria.<sup>19,20</sup> The ORR (complete response + very good partial response + partial response) and clinical benefit rate (CBR;  $\geq$  minimal response (MR)) were determined. Disease assessment occurred during the last week of the cycle. If a patient experienced  $\geq$ MR, assessment of disease response was performed 4 weeks later to confirm the patient's response to treatment.

#### Statistical methods

The intent-to-treat population included all patients who registered and met eligibility criteria. The safety and efficacy population included all patients who received  $\geq 1$  dose of study drug. ORR estimates were accompanied by a two-sided 95% exact CI that used a binomial distribution. PD had to be confirmed in all cases but was recorded when laboratory tests first showed PD. Patients who did not meet protocol-defined PD but were taken off study owing to worsening disease were treated as if they were taken off study for PD. Median time to progression, duration of response, progression-free survival and OS were estimated using Kaplan–Meier methods. Median follow-up time was estimated using the reverse Kaplan–Meier method.<sup>21</sup> *P*-values for patient response were calculated using Fisher's test (two tails). Statistical analyses were performed using SPSS version 21 (IBM, Armonk, NY, USA) or GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA).

## RESULTS

### Patient disposition

From 2 May 2011 to 14 January 2013, 38 patients entered the intent-to-treat population, but 1 patient chose not to receive study treatment following registration. Thus, the efficacy and safety populations both consisted of 37 patients. The cutoff date for this data analysis was 25 April 2013. At data cutoff, 13 patients were still active on the trial, 5 patients were receiving active combination treatment and 8 were receiving maintenance with single-agent carfilzomib.

Baseline demographics and clinical characteristics are summarized in Table 1. Median time from diagnosis of MM to study enrollment was 4.2 years (range, 5 months–10.5 years), and patients had received a median of five (range, 1–23) prior lines of anti-MM therapy. Patients had received a median of two (range, 1–13) different prior bortezomib-containing regimens. Dosage and schedule of the last bortezomib-containing regimens are outlined in Supplementary Table S1. Only 26.3 and 15.8% of patients had achieved  $\geq$ MR or  $\geq$ PR, respectively, on the bortezomib-containing regimen that they eventually failed. Patients had IgG (55.3%), IgA (21.1%) or light-chain-only (21.1%) MM. At baseline, 15.8% of patients had peripheral neuropathy (any grade).

### Treatment exposure and treatment discontinuations

Patients received 31 unique treatment regimens throughout the trial (Table 1 and Supplementary Table S1). One patient who withdrew consent before receiving study treatment had previously been treated with bortezomib and lenalidomide. The 31 specific treatment regimens stemmed from 14 different drug combinations across all four active anti-MM drug classes (Table 2 and Supplementary Table S2). Drug combinations included carfilzomib and the following agents: dexamethasone alone (31.6% of patients); the alkylating agents melphalan, cyclophosphamide or bendamustine (28.9%) with or without steroids; pegylated liposomal doxorubicin (18.4%) with or without steroids; and/or the IMiDs thalidomide and/or lenalidomide (21.1%) with or without other agents. More than half of patients (59.5%) received the highest planned carfilzomib dose ( $45\text{ mg/m}^2$ ; Supplementary Table S2). The median duration of carfilzomib treatment was four cycles (range, <1–8 cycles). Eighteen patients completed eight cycles of treatment and chose to continue receiving single-agent carfilzomib maintenance therapy.

Twenty-five patients discontinued study treatment for the following reasons: nine because of PD; one because of death; one was removed because of the worsening of MM-related symptoms thought to be related to PD; five owing to withdrawal of informed consent because of personal reasons ( $n=3$ ) or AEs that interfered with quality of life ( $n=2$ ); seven owing to DLTs (thrombocytopenia ( $n=2$ ); abdominal pain ( $n=1$ ); CHF ( $n=1$ ); TLS ( $n=1$ ); neutropenia ( $n=1$ ); and febrile neutropenia ( $n=1$ ));

**Table 1.** Baseline demographics and clinical characteristics (ITT population:  $n = 38$  patients)

Characteristic	Value
Median age, years (range)	67 (47–79)
Male, $n$ (%)	25 (65.8)
Female, $n$ (%)	13 (34.2)
<b>Ethnicity, <math>n</math> (%)</b>	
White	29 (76.3)
Asian/Pacific Islander	3 (7.9)
Black	2 (5.3)
Hispanic	1 (2.6)
Other	1 (2.6)
Unknown	2 (5.3)
Median time from diagnosis (range)	4.2 years (0.4–10.5 years)
<b>Ig type, <math>n</math> (%)</b>	
IgG	21 (55.3)
IgA	8 (21.1)
Light chain only or not specified	8 (21.1)
Unknown	1 (2.6)
<b>International staging system, <math>n</math> (%)<sup>a</sup></b>	
I	15 (40.6)
II	9 (24.3)
III	13 (35.1)
<b>ECOG performance status, <math>n</math> (%)</b>	
0	17 (45)
1	17 (45)
2	4 (10)
<b>PN, <math>n</math> (%)<sup>b</sup></b>	
0	32 (84.2)
1	6 (15.8)
2 (without pain)	0
Median creatinine, mg/dl (range)	1.0 (0.6–2.3)
Median serum $\beta$ 2-microglobulin, mg/l (range)	4.1 (1.7–11.9)
Prior lines of therapy, median (range)	5 (1–23)
Prior bortezomib-containing regimens <sup>c</sup> , median (range)	2 (1–13)
<b>Bortezomib dose of prior bortezomib-containing regimen</b>	
1.0 mg/m <sup>2</sup>	23 (60.5)
$\geq 1.3$ mg/m <sup>2</sup>	15 (39.4)
<b>Cycle length of prior bortezomib-containing regimen</b>	
28 days	31 (81.5)
21 days	7 (18.4)
<b>PD on prior bortezomib treatment, <math>n</math> (%)</b>	
Progressed while receiving prior bortezomib treatment	6 (15.8)
Progressed $\leq 60$ days from end of prior bortezomib treatment	26 (68.4)
Progressed $\geq 60$ and $\leq 84$ days from end of prior bortezomib treatment	4 (10.5)
Unknown ( $\leq 84$ days from end of prior bortezomib treatment)	2 (5.3)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; ITT, intent-to-treat; PD, progressive disease; PN, peripheral neuropathy. <sup>a</sup>International Staging System stage as determined at study enrollment for patients who received treatment ( $n = 37$ ). <sup>b</sup>Patients with  $\geq$  grade 3 PN or grade 2 with pain were not allowed to enroll in the study. <sup>c</sup>Bortezomib-containing regimens are listed in Supplementary Table S1.

and two owing to AEs (intercurrent illness ( $n = 1$ ) and sepsis ( $n = 1$ )). Twenty-five patients progressed, including 10 patients who progressed after showing an initial response.

**Table 2.** Listing of anti-MM agents that were administered in combination with carfilzomib in patients with RRMM (enrolled population:  $n = 37$  patients)

Highest carfilzomib dose administered ( $n = 37$ )	$n$ (%)
20 mg/m <sup>2</sup>	5 (13.5)
27 mg/m <sup>2</sup>	5 (13.5)
36 mg/m <sup>2</sup>	5 (13.5)
45 mg/m <sup>2</sup>	22 (59.5)
<b>Carfilzomib combination regimen (<math>n = 38</math>)</b>	
Dexamethasone	11 (31.6)
PLD and dexamethasone	6 (15.8)
Cyclophosphamide and ascorbic acid	4 (10.5)
Lenalidomide and dexamethasone	4 (10.5)
Bendamustine	3 (7.9)
Lenalidomide, melphalan, ascorbic acid, clarithromycin and dexamethasone	1 (2.6)
Melphalan	1 (2.6)
PLD	1 (2.6)
Melphalan and ascorbic acid	1 (2.6)
Bendamustine and methylprednisolone	1 (2.6)
Thalidomide and dexamethasone	1 (2.6)
Cyclophosphamide, ascorbic acid and dexamethasone	1 (2.6)
Lenalidomide, dexamethasone and PLD	1 (2.6)
Thalidomide, lenalidomide, bendamustine, methylprednisolone and clarithromycin	1 (2.6)

Abbreviations: MM, multiple myeloma; PLD, pegylated liposomal doxorubicin; RR, relapsed and refractory.

## Safety

**DLTs and determination of the MTD.** Eight carfilzomib-based drug combinations led to the occurrence of 10 DLTs across a variety of combination treatments (Supplementary Table S2), including grade 4 thrombocytopenia ( $n = 4$ ); grade 3 neutropenia ( $n = 2$ ); grade 3 sepsis ( $n = 1$ ); grade 3 CHF ( $n = 1$ ); grade 3 TLS ( $n = 1$ ); and grade 2 abdominal pain ( $n = 1$ ). Seven patients were taken off the study owing to DLTs and three were removed before the study protocol amendment allowing carfilzomib dose reduction was approved (Supplementary Table S2). After the amendment, four patients were removed after completion of one ( $n = 2$ ), two ( $n = 1$ ) or three cycles ( $n = 1$ ) of treatment. Of the 31 treatment regimens tested, the MTD was reached at MAD for the regimen that contained carfilzomib (45 mg/m<sup>2</sup>), ascorbic acid (1000 mg) and cyclophosphamide (2.2 mg/kg; Supplementary Table S2).

**Treatment-emergent AEs.** The most common hematologic and nonhematologic treatment-emergent AEs across all patients are shown in Table 3. Hematologic AEs  $\geq$  grade 3 included lymphopenia (35.1%), thrombocytopenia (24.3%), anemia (10.8%), neutropenia (10.8%), leukopenia (5.4%) and decreased red blood cell count (2.7%). Lymphopenia ( $\geq$  grade 3) occurred in all five patients who received bendamustine. Overall, five patients (13.5%) experienced grade 4 hematologic AEs, including thrombocytopenia (10.8%) and lymphopenia (5.4%). One patient experienced both of these grade 4 AEs. Only one patient experienced a hematologic serious AE (febrile neutropenia).

Nonhematologic AEs  $\geq$  grade 3 included fever (5.4%), hypokalemia (5.4%), increased creatinine (2.7%), chills (2.7%), hypertension (2.7%), migraine (2.7%), dehydration (2.7%), pneumonia (2.7%), urinary tract infection (2.7%), hypoechoic liver lesions (2.7%), sepsis (2.7%), steel plate owing to osteonecrosis of the jaw (2.7%), TLS (2.7%) and hyponatremia (2.7%). Peripheral neuropathy was infrequent (16%) and all cases were  $\leq$  grade 2 (grade 1 and grade 2 ( $n = 3$  each)).

There were 12 (32.4%) nonhematologic serious AEs, including the following: rectal bleeding (grade 2); cellulitis, dehydration,

fever with chills, generalized weakness, influenza, pruritus, renal dysfunction/failure, TLS and urinary tract infection with fever (all grade 3); tachyarrhythmia (grade 4); and pneumonia (grade 5). There was one grade 4 nonhematologic AE (sepsis).

Of the 21 patients that were not removed from the trial because of DLTs, AEs or informed consent withdrawal, treatment was held in three patients (14.3%), two (9.5%) of whom experienced a subsequent dose reduction.

### Efficacy

Among the 37 treated patients, the ORR was 43.2% and the CBR was 62.2% with responses observed across all drug classes (Table 4). Specifically, among patients ( $n=12$ ) who received carfilzomib with dexamethasone alone, the ORR was 33.3% and

the CBR was 58.3%. The ORR was 54.5% and the CBR was 63.6% for patients ( $n=11$ ) who were given carfilzomib and an alkylating agent with or without steroids. For those patients who received carfilzomib and an IMiD with or without other agents ( $n=7$ ), the ORR was 42.9% and the CBR was 57.1%. For patients ( $n=7$ ) who received carfilzomib and pegylated liposomal doxorubicin with or without steroids, the ORR was 42.9% and the CBR was 71.4%. Five patients (13.5%) who were not evaluable for response (two patients had no confirmed response; three patients suffered DLTs in cycle 1 and were either hospitalized or never completed a full cycle) were considered as not responsive to treatment.

There was a trend, albeit not significant for patients who had achieved  $\geq$ MR on the bortezomib-containing version of the regimen (before developing PD) to respond better to treatment with the carfilzomib-containing version ( $\geq$ PR, 60.0%) than patients who did not ( $\geq$ PR, 36.8%;  $P=0.5097$ ). The proportion of patients achieving  $\geq$ MR with the carfilzomib-containing regimen who had previously been treated with 1.0 mg/m<sup>2</sup> bortezomib (63.6%) was similar to that of patients with  $\geq$ MR who had received  $\geq$ 1.3 mg/m<sup>2</sup> bortezomib (53.3%;  $P=0.7893$ ).

Progression-free survival, OS and duration of response are shown in Figure 2. Median follow-up time was 13.7 months (range, 1.2–19.4; Table 4). Median progression-free survival was 8.3 months (95% CI: 4.6–12.1), and median OS was 15.8 months (95% CI: 13.5–18.3). Median time to progression was 9.9 months (95% CI: 6.7–13.1). Median duration of response among patients who achieved  $\geq$ PR (43.2%) and  $\geq$ MR (62.2%) was 9.9 months (95% CI: 7.6–12.2) and 9.2 months (95% CI: 6.7–11.7), respectively. A summary of time-to-event outcomes is shown in Table 5. Median time to first response ( $\geq$ MR) was 1.6 months (95% CI: 0.55–2.68), and the median time to best response was 3.4 months (95% CI: 2.7–4.1).

### DISCUSSION

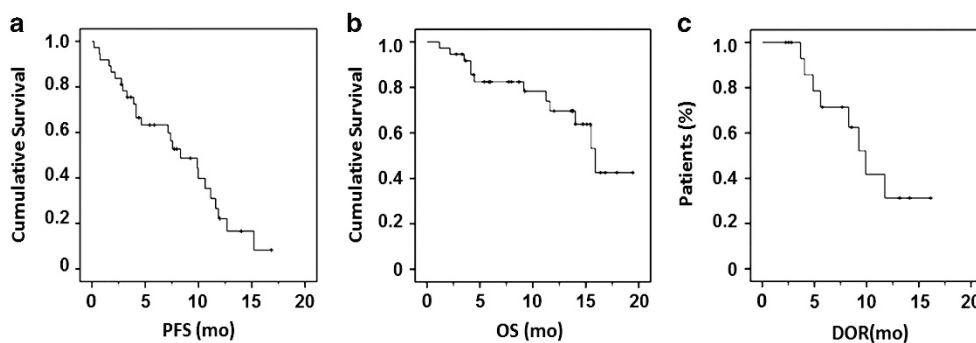
Our study demonstrates that carfilzomib can be used safely and effectively in place of bortezomib when combined with many other classes of drugs including alkylators, anthracyclines, IMiDs and glucocorticosteroids. In addition, this trial shows that carfilzomib is often an effective replacement for bortezomib for patients with MM who progressed from the same combination treatment containing bortezomib. Concerns about safety and tolerability and drug–drug interactions often arise whenever new agents are introduced into combination regimens, particularly in heavily pretreated patients who are more likely to be unfit or frail compared with patients with newly diagnosed MM. The breadth of different regimens ( $n=31$ ) that were evaluated in our study suggests that carfilzomib can be combined with the other classes of drugs that have shown efficacy for treating MM.

In our study, the AE profile of the carfilzomib-containing regimens was similar to that observed in 526 patients who were

**Table 3.** Incidence and severity of treatment-emergent AEs occurring in  $\geq$ 13% of patients (safety analysis population:  $n=37$  patients)

AEs	All grades	$\geq$ Grade 3
<i>Hematologic</i>		
	n (%)	n (%)
Thrombocytopenia	30 (81.1)	9 (24.3)
Lymphopenia	26 (70.3)	13 (35.1)
Anemia	22 (59.5)	4 (10.8)
Decreased total protein	17 (45.9)	0
Leukopenia	16 (43.2)	2 (5.4)
Neutropenia (SAE was grade 3 febrile)	15 (40.5)	4 (10.8)
Hematocrit (decreased)	10 (27.0)	0
<i>Nonhematologic</i>		
Hyperglycemia	18 (48.6)	0
Nausea	18 (48.6)	0
Blood urea nitrogen (increased)	17 (45.9)	0
Hypocalcemia	17 (45.9)	0
Hyponatremia	16 (43.2)	1 (2.7)
Insomnia	16 (43.2)	0
Upper respiratory infection	14 (37.8)	0
Hypoalbuminemia	13 (35.1)	0
Fatigue	13 (35.1)	0
ALT (increased)	12 (32.4)	0
Fever	12 (32.4)	2 (5.4)
ALT (decreased)	11 (29.7)	0
Creatinine (increased)	11 (29.7)	1 (2.7)
Hypokalemia	11 (29.7)	2 (5.4)
AST (increased)	10 (27.0)	0
Diarrhea	10 (27.0)	0
Headache	10 (27.0)	0
Vomiting	9 (24.3)	0
Peripheral neuropathy	6 (16.2)	0

Abbreviations: AEs, adverse events; AST, aspartate transaminase; SAE, serious adverse event.



**Figure 2.** The Kaplan–Meier curve for time-to-event data for all evaluable patients. (a) PFS in months. (b) OS in months. (c) DOR in months. DOR, duration of response; OS, overall survival; PFS, progression-free survival.

**Table 4.** Response rates for all evaluable patients (efficacy population:  $n = 37$  patients)

Best overall responses	Evaluable patients ( $n = 37$ )
<i>Response category</i>	<i>n (%)</i>
Complete response	3 (8.1)
Very good PR	6 (16.2)
PR	7 (18.9)
MR	7 (18.9)
Stable disease	6 (16.2)
Progressive disease	3 (8.1)
Not evaluable	5 (13.5)
Overall response rate ( $\geq$ PR), $n$ (%)	16 (43.2)
95% confidence interval	(29–63)
Clinical benefit rate ( $\geq$ MR)	23 (62.2)
95% confidence interval	(42–75)
Median treatment cycles completed, $n$ (range)	4 (<1–8)
Median maintenance therapy cycles completed <sup>a</sup> , $n$ (range)	3 (1–13 +)
Median follow-up time, months (range)	13.73 (1.2–19.4)

Abbreviations: MR, minimal response; PR, partial response. <sup>a</sup>Applicable to the 14 patients who received maintenance therapy with single-agent carfilzomib.

**Table 5.** Summary of time-to-event outcomes for the trial (efficacy population:  $n = 37$  patients)

Time-to-event	Time (months)
<i>TTP</i>	
Median (Kaplan–Meier)	9.9
95% CI	(6.7–13.1)
<i>PFS</i>	
Median (Kaplan–Meier)	8.3
95% CI	(4.6–12.1)
<i>DOR</i>	
Median (Kaplan–Meier)	9.2
95% CI	(6.7–11.7)
<i>OS</i>	
Median (Kaplan–Meier)	15.8
95% CI	(13.5–18.3)
<i>Time to first response (<math>\geq</math>MR)</i>	
Median (Kaplan–Meier)	1.6
95% CI	(0.6–2.7)
<i>Time to best response (<math>\geq</math>MR)</i>	
Median (Kaplan–Meier)	3.4
95% CI	(2.7–4.1)

Abbreviations: CI, confidence interval; DOR, duration of response; MR, minimal response; OS, overall survival; PFS, progression-free survival; TTP, time to progression.

previously enrolled in four phase II clinical studies (PX-171-003-A0, 003-A1, PX-171-004 and PX-171-005) and received single-agent carfilzomib.<sup>22</sup> In our study, lymphopenia, thrombocytopenia, anemia and neutropenia were the most common  $\geq$ grade 3 hematologic AEs, which is similar to those observed with single-agent carfilzomib.<sup>22</sup> Fever and hypokalemia were the most common  $\geq$ grade 3 nonhematologic AEs in our study, whereas pneumonia, fatigue and dyspnea were the most common  $\geq$ grade 3 nonhematologic AEs with single-agent carfilzomib.<sup>22</sup> One patient (2.7%) had CHF, which compares favorably with the

four phase II studies that investigated single-agent carfilzomib, in which 30 patients (5.7%) had  $\geq$ grade 3 CHF. Dyspnea was not observed in our study.

The trial involved intra-patient dose escalation of carfilzomib (20, 27, 36 and 45 mg/m<sup>2</sup>) during the first four cycles of treatment. More than half (59.5%) of the treated patients received the MAD of carfilzomib (45 mg/m<sup>2</sup>). Because of the large number ( $n = 31$ ) of different carfilzomib-containing combination treatments among the 37 enrolled patients, the MTD was only established for one regimen (ascorbic acid (1000 mg) and cyclophosphamide (2.2 mg/kg)) at the MAD of carfilzomib.

Overall, 14 different combinations of drugs using agents from four therapeutic classes were tested. At least one patient in 11 (78.8%) of these 14 drug combinations achieved  $\geq$ MR. Similar CBRs were observed among the four therapeutic classes combined with carfilzomib. The observation of clinical benefit across all effective anti-MM agent classes is promising and is consistent with recent studies showing that carfilzomib is safe and effective when used in combination with a variety of drugs, including lenalidomide and dexamethasone,<sup>17,18,23–25</sup> pomalidomide and dexamethasone,<sup>26</sup> melphalan and prednisone,<sup>27</sup> cyclophosphamide and dexamethasone,<sup>28</sup> thalidomide and dexamethasone,<sup>29</sup> and cyclophosphamide, thalidomide and dexamethasone.<sup>30</sup> Results from those studies also compare favorably with bortezomib-based regimens, including bortezomib in combination with lenalidomide and dexamethasone.<sup>31,32</sup> Although the latter combination yielded a high response rate, 64% of patients developed grade 1 or grade 2 peripheral neuropathy, and 62% of patients required a dose reduction.<sup>32</sup> A recent clinical trial evaluating carfilzomib administered at 45 and 56 mg/m<sup>2</sup> in combination with dexamethasone showed an ORR of 55% and good tolerability for relapsed and refractory MM patients without bortezomib resistance.<sup>25</sup> Our study extends these findings and demonstrates that carfilzomib at doses up to 45 mg/m<sup>2</sup> and combined with dexamethasone is efficacious and has an acceptable safety profile for MM patients who are refractory to bortezomib.

The mechanisms by which carfilzomib produces responses for patients who have failed bortezomib-based combination treatments is unclear, but it may partially depend on differences in the chemical properties and mode of action of these two PIs. For example, bortezomib is a reversible PI, whereas carfilzomib binds selectively and irreversibly to the proteasome.<sup>14,33,34</sup> In addition, there may be differences in the synergistic effects between bortezomib and other agents relative to carfilzomib. The specific mechanisms that cause bortezomib resistance<sup>35</sup> will need to be more fully elucidated to determine the underlying basis for carfilzomib's ability to overcome bortezomib resistance.

Although it has been clearly shown that patients resistant to a bortezomib-containing combination regimen often respond to other agents combined with bortezomib, this is the first trial to demonstrate that a different PI (carfilzomib) can overcome resistance to bortezomib when replacing it in the same combination treatment. Findings from our trial suggest that carfilzomib can be safely and effectively used as a replacement for bortezomib across a wide variety of anti-MM regimens to treat patients that have failed prior bortezomib-containing regimens. However, this study was not designed to determine which specific carfilzomib-containing treatment combinations have better safety or efficacy profiles in the setting of bortezomib resistance, and further phase 1 and 2 studies testing the most promising combinations will be required.

Although we succeeded in establishing that replacement of bortezomib with carfilzomib can be effective across many different combination regimens, we only determined the MTD for one regimen. Nevertheless, more than half of all treated patients received the MAD for carfilzomib (45 mg/m<sup>2</sup>). The dose and schedule of bortezomib used in the different combination treatments that patients had failed varied, which could have

potentially affected the efficacy of subsequent treatment with carfilzomib. However, our data did not reveal any bias toward responses being more frequently observed with a specific prior bortezomib dose or schedule. Along with others, our group has shown that different doses (1 or 1.3 mg/m<sup>2</sup>) and schedules (3 or 4 weeks per cycle; Supplementary Table S1) of bortezomib produce similar results when used either as a single-agent or in combination with other agents.<sup>11,12,36</sup> Moreover, although patients may initially be treated at a dose of 1.3 mg/m<sup>2</sup>, many patients require reductions in their dose to 1.0 mg/m<sup>2</sup>, weekly dosing or longer cycles because of toxicity.<sup>37</sup> Overall, the results demonstrate that carfilzomib can safely and effectively replace bortezomib for the treatment of MM patients who progress while on or within 12 weeks of failing a wide range of bortezomib-containing combination regimens. Expanded phase 1 and 2 trials will help characterize the activity and tolerability of several of the most promising treatment combinations revealed in this study. By partnering with new agents, including those that are from the same therapeutic class as the agent that is being replaced, patients may be able to overcome treatment resistance and expand their therapeutic options. Similar results have been observed among MM patients who were previously treated with IMiDs.<sup>7</sup> Thus, the findings from this trial suggest the same phenomenon occurs with PIs and should provide clinicians with a new therapeutic option: replacement of bortezomib with carfilzomib for patients who fail a bortezomib-containing combination regimen.

## CONFLICT OF INTEREST

JRB and RAS are consultants and on the speakers bureau for Onyx Pharmaceuticals Inc., and receive research funding from Onyx Pharmaceuticals Inc. RVB is on the speakers bureau for Onyx Pharmaceuticals Inc. The remaining authors declare no conflict of interest.

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## AUTHOR CONTRIBUTIONS

JRB designed and performed research, analyzed data and wrote the paper; JDH designed research, analyzed data and wrote the paper. OY designed research. RD, DP-D, RVB, AB, LS, DG, SE, YN, RAS and RAV performed research.

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