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A Phase 1 Dose Escalation Study of the Safety and Pharmacokinetics of the Novel Proteasome Inhibitor Carfilzomib (PR-171) in Patients with Hematologic Malignancies

Owen A. O'Connor,[1,2] A. Keith Stewart,[4] Marcy Vallone,[5] Christopher J. Molineaux,[5] Lori A. Kunkel,[5] John F. Gerecitano,[3] and Robert Z. Orlowski[6]

Abstract

Purpose: Carfilzomib (formerly PR-171) is a novel proteasome inhibitor of the epoxyketone class that is selective and structurally distinct from bortezomib. Proteasome inhibition by carfilzomib is mechanistically irreversible. Consequently, proteasome inhibition is more sustained with carfilzomib than with bortezomib.

Experimental Design: In a phase 1 trial evaluating the safety and efficacy of carfilzomib in relapsed or refractory hematologic malignancies, eight dose groups of three to six patients received 5 consecutive days of carfilzomib i.v. push at doses of 1.2, 2.4, 4, 6, 8.4, 11, 15, and 20 mg/m^2 within 14-day cycles.

Results: Twenty-nine patients enrolled that were relapsed or refractory after at least two prior therapies. Nonhematologic toxicities included fatigue, nausea, and diarrhea in more than one third of patients—mostly grade 1 or 2 in severity. At 20 mg/m^2, grade 3 febrile neutropenia and grade 4 thrombocytopenia were reported, establishing 15 mg/m^2 as the maximum tolerated dose. No grade 3 or 4 peripheral neuropathies were reported. Antitumor activity was observed at doses ≥11 mg/m^2: one unconfirmed complete response (mantle cell), one partial response (multiple myeloma), and two minimal responses (multiple myeloma and Waldenström's macroglobulinemia).

Conclusion: This is the first clinical use of carfilzomib that shows tolerability and clinical activity in multiple hematologic malignancies using consecutive-day dosing. (Clin Cancer Res 2009;15(22):7085–91)

The 20S proteasome plays a critical role in cellular homeostasis through its function in ubiquitin-dependent protein turnover of target proteins that regulate signal transduction, cell cycle progression, apoptosis, survival, and stress response pathways. Importantly, cancer cells appear to be particularly dependent on these proteasome-regulated homeostatic pathways (1–4). The three proteolytic activities of the 20S core of the proteasome are the chymotrypsin-like, trypsin-like, and caspase-like activities associated with the β5, β2, and β1 subunits, respectively (5). Of these activities, the one that makes the greatest contribution to the degradation of model substrates *in vitro* and to overall cellular protein turnover *in vivo* is the chymotrypsin-like activity (6). The chymotrypsin-like activity is also most sensitive to inactivation (7).

The rationale for the proteasome as a target for cancer therapy was validated with the approval of bortezomib (Velcade), a slowly reversible dipeptide boronate proteasome inhibitor that targets the chymotrypsin-like and, to a lesser extent, the caspase-like activities. Bortezomib has shown activity as a single agent in the treatment of multiple myeloma (MM), mantle cell lymphoma (MCL), and other select subtypes of non–Hodgkin's lymphoma (NHL). Despite this, many patients do not respond to bortezomib, whereas others develop resistance to this compound or experience dose-limiting toxicity (DLT). These toxicities have included painful peripheral neuropathy, orthostatic hypotension, cardiac and pulmonary disorders, gastrointestinal disorders, myelosuppression, and fatigue (8, 9). Collectively, these findings suggest the need for an active proteasome inhibitor with improved efficacy that is noncross-resistant and has an improved safety profile.

Carfilzomib (formerly PR-171) is a novel proteasome inhibitor of the epoxyketone class that is structurally and mechanistically

Authors' Affiliations: [1]NYU Cancer Institute, NYU Langone Medical Center, [2]Department of Medicine, College of Physicians and Surgeons, and [3]Memorial Sloan-Kettering Cancer Center, New York, New York; [4]Mayo Clinic, Scottsdale, Arizona; [5]Proteolix, Inc., South San Francisco, California; and [6]M.D. Anderson Cancer Center, University of Texas, Houston, Texas

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Requests for reprints: Owen A. O'Connor, NYU Cancer Institute, NYU Langone Medical Center, 522 First Ave., Smilow 1101, New York, NY. Phone: 212-263-9884; Fax: 212-263-9210; E-mail: owen.o'connor@nyumc.org.

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Translational Relevance

The experience with bortezomib in multiple myeloma and MCL has recently validated the therapeutic merits of targeting the ubiquitin-proteasome pathway. Although bortezomib has helped change the natural history of these diseases, it carries with it a significant risk of neuropathy and other toxicities. The value of reversible versus irreversible, or pan-protease versus very selective protease inhibitors remains a matter of significant debate. Carfilzomib belongs to the epoxyketone class, is an irreversible inhibitor, and seems more selective for the chymotrypsin-like protease, with less affinity for other proteasome proteases. This first phase 1 study of carfilzomib shows that the drug is well tolerated, and produces signals of activity in patients with multiple myeloma. These data show that carfilzomib did not produce any grade 3 or 4 neuropathy. These data support the further development of carfilzomib in patients with hematologic malignancies.

distinct from bortezomib (10). Proteasome inhibition by carfilzomib is mechanistically irreversible (10–12) and requires new protein synthesis for recovery of cellular proteasome activity. Consequently, proteasome inhibition is more sustained with carfilzomib than with the slowly reversible inhibitor bortezomib (10). The epoxyketone pharmacophore of carfilzomib exhibits a high level of specificity for the NH_2-terminal threonine residue that catalyzes enzymatic activity in each of the proteolytic active sites within the proteasome (11). Carfilzomib shows minimal activity against off-target enzymes, including serine proteases (13). In contrast, the boronate pharmacophore of bortezomib reacts with active-site serine residues found in serine proteases including chymotrypsin, cathepsins A and G, elastase, and chymase (14, 15), albeit at concentrations higher than required to inhibit the proteasome.

Carfilzomib inhibited autocrine- and cytokine-dependent proliferation of primary acute myeloid leukemia blasts when tested at nanomolar levels (0.1-100 nmol/L; ref. 16). In addition, proteasome inhibition by carfilzomib promoted apoptosis in a variety of tumor cell lines. Daily dosing schedules that induced high levels of proteasome inhibition resulted in antitumor activity in several xenograft models (10).

Preclinical studies in rats and monkeys have been done administering carfilzomib i.v. for 5 consecutive days followed by 9 days of rest for two 14-day cycles. Proteasome inhibition of >80% was well tolerated (10) and supported initiation of a phase 1 clinical investigation using daily dosing with carfilzomib as an opportunity to achieve improved activity in hematologic malignancies. Consecutive-day dosing with bortezomib has been found to result in excessive toxicity in animals and has not been pursued in clinical trials (8).

This initial phase 1 investigation was designed to evaluate the tolerability and maximum tolerated dose (MTD), pharmacokinetic profile, pharmacodynamics, and preliminary evidence of antitumor activity of carfilzomib in patients with relapsed or refractory hematologic malignancies. Carfilzomib was administered on 5 consecutive days followed by 9 days of rest (14-day cycle).

Patients and Methods

Patient selection. Patients at least ages 18 y with histologically confirmed MM, NHL, Waldenström's macroglobulinemia, or Hodgkin's lymphoma that were treatment-refractory or had relapsed after at least two standard therapies were eligible for the trial. Other key eligibility criteria were an Eastern Cooperative Oncology Group performance status of 0 to 2; expected survival of >6 wk; no prior radiation, chemotherapy, or immunotherapy within 4 wk of screening; and no active infection. Baseline whole blood count had to be $>2.0 \times 10^9$/L, absolute neutrophil count of $>1.0 \times 10^9$/L, hemoglobin of >80 g/L, and platelet count of $>50.0 \times 10^9$/L. Patients with prior bortezomib treatment were eligible, as were patients with a prior history of neuropathy provided the neurotoxicity was less than grade 2. Other eligibility criteria were standard (adequate cardiovascular, hepatic, and renal function).

The protocol, informed consent, and other relevant study documentation were approved by the appropriate Institutional Review Board before any patients were enrolled in the study. All participants provided written informed consent in accordance with federal and institutional guidelines.

Drug administration. Patients were enrolled in cohorts of three to receive carfilzomib in a dose-escalating fashion until the MTD was established. Dose escalation occurred after three patients completed their first two 14-d cycles without DLT. If one DLT was observed within a dose cohort, additional three patients were accrued, and further escalation could occur only if no additional DLTs were seen. Intrapatient dose escalations were permitted for individual patients only after the intended dose was shown to be safe in that corresponding cohort.

Carfilzomib for injection was provided as a sterile, frozen liquid formulation containing 2 mg/mL of drug (Proteolix). Dose levels were 1.2, 2.4, 4, 6, 8.4, 11, 15, and 20 mg/m^2. The initial dose level was one tenth of the dose that was severely toxic in 10% in rats (17). Carfilzomib was given as an i.v. push over 1 to 2 min on days 1 through 5, followed by 9 d of rest in each 14-d cycle. Treatment was continued until unacceptable toxicity or progressive disease.

Response criteria. The European Group for Blood and Marrow Transplantation response criteria were used for patients with MM (18) and the International Working Group Response were used Criteria for patients with NHL and Hodgkin's lymphoma (19). For patients with Waldenström's macroglobulinemia, a complete response was defined as total resolution of measurable disease parameters and a partial response was defined as >50% resolution with no new evidence of disease.

Pharmacokinetics and pharmacodynamics. Blood samples for pharmacokinetic analysis were collected in cycle 1 before carfilzomib dosing and at 5, 15, and 30 min, and at 1, 2, and 4 h after carfilzomib administration. Additional samples were collected predose and 30 min postdose on day 5 of cycle 1 and on day 1 of cycles 2 and 3. Plasma samples for pharmacokinetic studies were assayed using validated liquid chromatography/mass spectrometry with a lower limit of detection of 0.10 μg/mL.

In preclinical studies, carfilzomib rapidly exited the intravascular compartment and was widely distributed, and serum concentrations approached the limit of assay detection within 1 h (10). Because pharmacokinetic parameters were not likely to guide dosing, a pharmacodynamic assay measuring the inhibition of proteasome chymotrypsin-like activity in whole blood and peripheral blood mononuclear cells (PBMC) was used (10). During day 1 of the first cycle, samples were drawn predose, and at 1 and 24 h postdose. Additional samples were collected predose and 1 h postdose on day 5 of cycle 1 and on day 1 of cycles 2 and 3.

Data analysis. Adverse events were defined using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. DLT was defined as any of the following occurring in the first 28 d of study participation: (*a*) greater than grade 3 nausea, vomiting, or diarrhea that was uncontrolled by maximal antiemetic/antidiarrheal therapy; (*b*) greater than grade 2 neuropathy with pain; (*c*) other nonhematologic toxicity greater than grade 3; (4) grade 4

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Table 1. Patient demographics

	***N* = 29**
Median age (y)	62.4
Range	21.9-85.1
Male, *n* (%)	20 (69)
Female, *n* (%)	9 (31)
White, *n* (%)	23 (79)
Black, *n* (%)	4 (14)
Hispanic, *n* (%)	2 (7)
Diagnosis, *n* (%)	
MM	10 (34)
Secretory	9 (31)
Nonsecretory	1 (3)
Waldenström's macroglobulinemia	1 (3)
NHL	15 (52)
Mantle cell	5 (33)
T-cell	5 (33)
SLL/CLL	4 (27)
Diffuse large cell	1 (7)
Hodgkin's lymphoma	3 (10)
Median years since diagnosis	4.3
Range	0.0-28.0

Abbreviations: NHL, non–Hodgkin's lymphoma; SLL, small lymphocytic lymphoma; CLL, chronic lymphocytic leukemia.

neutropenia (absolute neutrophil count of $<0.5 \times 10^9$/L) lasting >14 d without growth factor support; (*d*) febrile neutropenia (absolute neutrophil count of $<1.0 \times 10^9$/L with fever of ≥38.3°C); or (*e*) grade 4 thrombocytopenia ($<25.0 \times 10^9$/L) or thrombocytopenia associated with bleeding.

Pharmacokinetic data were analyzed and modeled using WinNonLin Professional software, Version 5.2 (Pharsight Corp).

Results

Patients. Twenty-nine patients at three centers were enrolled between September 2005 and June 2007: 10 had MM, 15 had NHL, 3 had Hodgkin's lymphoma, and 1 had Waldenström's macroglobulinemia. The median age was 62 years (Table 1). There were more men than women and nearly 80% were Caucasian. The median time from diagnosis was 4.3 years.

Dose escalation. Carfilzomib doses administered ranged from 1.2 to 20 mg/m^2 (Table 2). Three patients were allowed intrasubject dose escalation by one or more dose levels but are reported in their starting dose level (one patient each in the 2.4, 6, and 11 mg/m^2 dose cohorts). The median number of 14-day cycles per patient was 4.8 (range, <1-16). The mean cumulative carfilzomib exposure was 233 mg/m^2 (range, 22.8-885).

No DLTs were observed in any of the initial seven dose cohorts (carfilzomib 1.2-15 mg/m^2). At the 20 mg/m^2 level, one of three patients experienced DLT; one of two additional patients enrolled in that cohort also experienced DLT (see below). Per protocol, three additional patients were enrolled in the preceding cohort (15 mg/m^2) without occurrence of DLT. Hence, the 15 mg/m^2 dose level was determined to be the MTD.

Safety. Adverse events were reported in all 29 patients and at all carfilzomib dose levels. Adverse events with an incidence of 20% or higher are summarized in Table 3. Fatigue and nausea were the most common events reported.

Among nonhematologic toxicities, grade 1/2 gastrointestinal symptoms were common; these included, across all cohorts, mild to moderate nausea (48%), diarrhea (35%), and constipation (21%). Respiratory symptoms were also common and included cough, dyspnea (both 28%), and exertional dyspnea (21%). Neurologic symptoms included hypoesthesia (28%), headache (24%), and paresthesia (21%). Additional adverse events seen in 20% or more of patients were fatigue (48%), pyrexia (28%), and peripheral edema (24%); all other events occurred in fewer than five patients each. Events of increase in blood creatinine were reported as adverse events in three of 29 subjects (10.3%); two of these were grade 1, and one was grade 2. One was deemed possibly related, in a subject treated at 1.2 mg/m^2 carfilzomib, whereas the others were classified as unlikely related to treatment with study drug. All three subjects entered the study with elevated or increasing serum creatinine.

Hematologic toxicities were less common. Across all carfilzomib cohorts, anemia was reported in five patients (17%)—three patients with grade 2 and two patients with grade 3. No dose relationship was apparent; anemia occurred at doses ranging from 1.2 to 15 mg/m^2. A total of four patients (14%) experienced thrombocytopenia—two patients with grade 1 and two patients with grade 4, all at dose levels of 15 mg/m^2 or higher.

Across all dose cohorts, 14 patients (48%) had one or more adverse events that were grade 3 or 4 (Table 4). With the exception of two patients with grade 4 thrombocytopenia and one patient each with grade 3 or 4 dyspnea, all other severe events occurred in only one patient each. The grade 3 and 4 dyspnea

Table 2. Diagnosis and dosing information by cohort

	1.2 mg/m^2 *n* = 3	**2.4 mg/m^2 *n* = 3**	**4.0 mg/m^2 *n* = 3**	**6.0 mg/m^2 *n* = 3**	**8.4 mg/m^2 *n* = 3**	**11.0 mg/m^2 *n* = 3**	**15.0 mg/m^2 *n* = 6**	**20.0 mg/m^2 *n* = 5**
Diagnosis, *n*								
NHL	3	1	3	0	1	2	3	3
Hodgkin's lymphoma	0	2	0	1	0	0	0	0
MM	0	0	0	2	2	1	3	2
Cycles per patient								
Mean	5.7	7.3	2.7	5.7	3.0	7.7	3.8	4.0
Range	4-8	2-16*	2-4	4-8*	2-4	4-13*	2-4	<1-8
Cumulative dose, mg/m^2								
Mean	34	142	55	172	132	475	285	404
Range	23-48	24-336	40-80	120-252	101-168	209-885	150-450	60-780

*Includes one patient with multiple dose escalations to higher dose levels.

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Table 3. Treatment-emergent adverse events reported in at least 20% of patients

Adverse event	All patients (*N* = 29)				
	Grade 1 *n* (%)	Grade 2 *n* (%)	Grade 3 *n* (%)	Grade 4 *n* (%)	All Grades *n* (%)
Any	4 (14)	11 (38)	11 (38)	3 (10)	29 (100)
Fatigue	10 (35)	3 (10)	1 (3)	0 (0)	14 (48)
Nausea	11 (38)	3 (10)	0 (0)	0 (0)	14 (48)
Diarrhea	7 (24)	3 (10)	0 (0)	0 (0)	10 (35)
Cough	8 (28)	0 (0)	0 (0)	0 (0)	8 (28)
Dyspnea	6 (21)	0 (0)	1 (3)	1 (3)	8 (28)
Hypoesthesia	5 (17)	3 (10)	0 (0)	0 (0)	8 (28)
Pyrexia	5 (17)	3 (10)	0 (0)	0 (0)	8 (28)
Headache	7 (24)	0 (0)	0 (0)	0 (0)	7 (24)
Peripheral edema	7 (24)	0 (0)	0 (0)	0 (0)	7 (24)
Constipation	5 (17)	1 (3)	0 (0)	0 (0)	6 (21)
Exertional dyspnea	6 (21)	0 (0)	0 (0)	0 (0)	6 (21)
Paresthesia	4 (14)	2 (7)	0 (0)	0 (0)	6 (21)

were considered not related or unlikely to be related to study drug; the grade 4 dyspnea occurred in a subject on cycle 4, day 4, and resolved in 1 day.

No increasing trends in incidence or severity of adverse events were apparent in the first five carfilzomib dose cohorts (1.2-8.4 mg/m^2) and no grade 4 toxicities were reported, although the cohort sizes were small. In the upper dose cohorts (15 and 20 mg/m^2), more toxicities overall and higher severities were reported; grade 3 and 4 events at these dose levels consisted of two patients with thrombocytopenia and one patient each with anemia, pain unspecified, aspartate aminotransferase increase, febrile neutropenia, neutropenia, chills, and dyspnea; grade 3 syncope occurred in one subject during the fourth cycle of dosing with carfilzomib at 15 mg/m^2.

DLTs (reported in the 20 mg/m^2 cohort) consisted of grade 3 febrile neutropenia requiring hospitalization and prolonged grade 4 thrombocytopenia together with rapidly progressing myeloma in the central nervous system.

Four of the 29 patients (14%) discontinued study treatment due to adverse events. Three of these events were considered unlikely to be related to drug; a subject with small lymphocytic lymphoma experienced grade 3 abdominal pain (4 mg/m^2 cohort); a grade 3 skin infection (4 mg/m^2 cohort) was seen in a subject with cutaneous anaplastic large cell NHL who had preexisting skin lesions; and grade 3 gastrointestinal hemorrhage (11 mg/m^2 cohort) was found in a subject with MCL. Grade 4 thrombocytopenia with grade 2 hematemesis (20 mg/m^2 cohort) occurred in a subject with MM during rapid tumor progression before death; these events were considered not related to study drug administration.

Pharmacokinetics. Due to the limited number of samples with detectable carfilzomib, pharmacokinetic parameter estimates are reported only for the 11, 15, and 20 mg/m^2 dose levels (Table 5).

High interpatient and intrapatient variability in the plasma concentration of carfilzomib was observed. Clearance was rapid, with an elimination half-life of <30 minutes and a clearance higher than liver blood flow that suggests there are multiple clearance pathways. Although C_{max} and area under the curve increased with dose, the increase was not dose-proportional. The volume of distribution at steady-state suggested wide tissue distribution of carfilzomib, consistent with studies in rats and monkeys that showed broad distribution of proteasome inhibition (10).

Pharmacodynamics. Carfilzomib induced a dose-dependent inhibition of the 20S chymotrypsin-like proteasome activity in whole blood and PBMCs 1 hour after dosing on day 1 (Fig. 1). Proteasome inhibition of >75% was seen in whole blood and PBMCs after a single dose of carfilzomib at dose levels of 15 mg/m^2 and higher. With repeat dosing, cumulative proteasome inhibition was observed in complete response and PBMCs, such that >90% inhibition was observed after the fifth consecutive day of dosing in the highest dose groups. Activity generally returned to baseline in PBMCs during the 9-day rest period, but only partially recovered in whole blood due to the inability of erythrocytes to synthesize new proteasomal protein.

Antitumor activity. Twenty-eight of 29 patients were evaluable for response. Four subjects—all treated at carfilzomib doses of 11 mg/m^2 and higher-achieved objective tumor responses as described below. Three of six subjects with plasma cell dyscrasias treated at or above 11 mg/m^2 had objective responses, including two with MM and one with Waldenström's macroglobulinemia.

Of seven patients with NHL treated at or above 11 mg/m^2, one subject with MCL had an unconfirmed complete response based on colonic biopsy as has previously been used by others (20). This patient was an 85-year-old female with MCL involving the colon but no nodal disease who had two positive colon biopsies during treatment followed by negative biopsy after eight cycles of carfilzomib 20 mg/m^2; because the patient refused additional biopsies, this response could not be confirmed. A 65-year-old male with Waldenström's macroglobulinemia had a 25% reduction in serum M-protein after two cycles of carfilzomib 15 mg/m^2.

Table 4. Grade 3 and 4 adverse events

Adverse event	All patients (*N* = 29)		
	Grade 3 n (%)	Grade 4 n (%)	Grades 3-4 n (%)
Any	11 (38)	3 (10)	14 (48)
Dyspnea	1 (3)	1 (3)	2 (7)
Thrombocytopenia	0 (0)	2 (7)	2 (7)
Abdominal pain	1 (3)	0 (0)	1 (3)
Acute bronchitis	1 (3)	0 (0)	1 (3)
Alkaline phosphatase increased	1 (3)	0 (0)	1 (3)
ALT increased	1 (3)	0 (0)	1 (3)
Anemia	1 (3)	0 (0)	1 (3)
AST increased	1 (3)	0 (0)	1 (3)
Chills	1 (3)	0 (0)	1 (3)
Disease progression	1 (3)	0 (0)	1 (3)
Fatigue	1 (3)	0 (0)	1 (3)
Febrile neutropenia	1 (3)	0 (0)	1 (3)
Gastrointestinal hemorrhage	1 (3)	0 (0)	1 (3)
Hemoglobin decreased	1 (3)	0 (0)	1 (3)
Hyperglycemia	1 (3)	0 (0)	1 (3)
Neutropenia	1 (3)	0 (0)	1 (3)
Pain	1 (3)	0 (0)	1 (3)
Skin infection	1 (3)	0 (0)	1 (3)
Syncope	1 (3)	0 (0)	1 (3)

Abbreviations: ALT; alanine aminotransferase; AST, aspartate aminotransferase.

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Table 5. Pharmacokinetic parameters for carfilzomib (11, 15, and 20 mg/m^2 dose levels)

	11 mg/m^2 *n* = 2	15 mg/m^2 *n* = 5	20 mg/m^2 *n* = 4
C_{max}, ng/mL			
Mean	90.2	325.9	683.0
SD	84.9	217.8	598.5
Min, max	28.0, 187.0	83.7, 620.0	151.0, 1,450.0
T_{max}, minutes			
Mean	9.0	5.8	6.0
SD	3.4	1.1	1.1
Min, max	7.0, 13.0	5.0, 7.0	5.0, 7.0
C_0, ng/mL			
Mean	762	2,123	5,454
SD	623	2,602	4,432
Min, max	182, 1,422	0.39, 6,521	594, 10,606
AUC_{last}, ng·mL/h			
Mean	4,457	9,728	23,123
SD	2,520	10,897	17,323
Min, Max	1,757, 6,750	1,616, 28,426	3,659, 40,264
AUC_{inf}, g·mL/h			
Mean	4,463	9,760	23,660
SD	2,519	10,893	18,011
Min, max	1,767, 6,756	1,621, 28,433	3,663, 42,164
$t_{1/2}$ minutes			
Mean	25.3	28.9	17.1
SD	14.1	37.4	7.7
Min, Max	11.4, 39.6	6.8, 95.5	11.2, 28.3
CL, mL/min			
Mean	5,062	7,054	4,127
SD	3,958	7,177	4,981
Min, max	2,486, 9,620	950, 18,511	854, 11,465
Vz, L			
Mean	234	192	84.7
SD	276	178	90.0
Min, max	41.1, 550	16.0, 392	19.3, 216
Vss, L			
Mean	42.5	942	140
SD	65.2	2046	231
Min, max	4.02, 117	1.47, 4603	4.25, 485

Abbreviations: C_{max}, maximum concentration; T_{max}, time of maximum concentration; C_0, concentration at t = 0 (extrapolated); AUC_{last}, area under the curve to last measurable time point (includes C_0); AUC_{inf}, area under the curve to infinity (included C_0); Lambda z, elimination rate constant; $t_{1/2}$, elimination half-life; CL, systemic clearance; MRT_{last}, mean residence time to last measurable concentrations; Vz, initial volume of distribution; Vss, volume of distribution to steady state.

Two patients with MM achieved objective responses. An 81-year-old male with IgA MM had a serum M-protein that could not be assessed by serum protein electrophoresis (SPEP) due to interference from other proteins. This patient had received bortezomib as the immediately preceding therapy, obtained a partial response as best response, but was taken off therapy due to the development of peripheral neuropathy; the patient experienced a rapid increase in M-protein immediately after bortezomib was discontinued. Total IgA increased more that 3-fold in the next 3 months at which time carfilzomib therapy was initiated. IgA level decreased within two cycles (10 doses) at 11 mg/m^2 from 709 to 297 mg/dL, less than half the total IgA. By the end of the fifth cycle, the IgA level reached 235 mg/dL, the lowest value for the entire previous year. A second patient, a 43-year-old female with IgG MM, had a minimal response after one cycle of carfilzomib at 15 mg/m^2. This patient had had a partial response to bortezomib as an earlier therapy.

Across all dose cohorts and diagnoses, nine subjects displayed stable disease with an average duration on therapy of eight cycles (116 days); of these nine patients, only three were taken off for progressive disease, with the remainder withdrawing consent or discontinuing due to the Investigator's decision. A total of 15 patients had progressive disease while on study.

Discussion

Preclinical and clinical studies with bortezomib have established the proteasome as a therapeutic target in patients with select hematologic malignancies. Carfilzomib, a novel proteasome inhibitor, inhibited proliferation and induced apoptosis in bortezomib-resistant MM cell lines and samples from patients with clinically established resistance to bortezomib and other conventional agents. Carfilzomib also acted synergistically with dexamethasone to enhance cell death *in vitro* (21).

Carfilzomib was well tolerated in animals and showed clear evidence of schedule-dependent proteasome inhibition and antitumor activity. Carfilzomib administered for 5 consecutive days in rats and monkeys resulted in >80% inhibition of the chymotrypsin-like activity of the proteasome in multiple tissues. Dosing for two consecutive days was superior in antitumor efficacy in xenograft studies when compared with carfilzomib or bortezomib given in a split dosing schedule (day 1, day 4) or with carfilzomib given once weekly at twice the dose (10).

Based on the *in vitro* and *in vivo* activity of carfilzomib observed in preclinical studies, we sought to determine the MTD of carfilzomib using a 14-day cycle—five consecutive days of dosing followed by 9 days of rest-in patients with hematologic malignancies. A carfilzomib dose of 15 mg/m^2 was found to be well tolerated. Premedication with antiemetics and antidiarrheals was generally not required. DLTs at least possibly related to carfilzomib were hematologic and included thrombocytopenia and febrile neutropenia in patients with preexisting bone marrow compromise. There was a trend for the frequency and severity of the hematologic toxicities to be dose related.

The most common reason for treatment discontinuation in this study was progressive disease. However, among patients who completed six cycles (84 days) of treatment, patients were discontinued primarily for inability to maintain the scheduled dosing. Consequently, twice-weekly therapy for 3 weeks with the 4th week off has been chosen for subsequent phase 2 studies, as it seems well tolerated by patients at carfilzomib doses of 20 mg/m^2 (17, 22).

Grade 1 and 2 hypoesthesias and paresthesias were reported; however, grade 3 and 4 peripheral neuropathy was not—despite the fact that patients with symptomatic neuropathy or a history of neuropathy were enrolled in this study and treated with carfilzomib on an intensive schedule. Although interpretation of these data is limited by the small numbers, these findings suggest a possible advantage of carfilzomib over other proteasome inhibitors.

Other studies of carfilzomib using a twice-weekly consecutive-day schedule reported a first-dose effect of increased creatinine at doses of 20 mg/m^2 and higher (23). In the present study, carfilzomib was nevertheless well tolerated in patients

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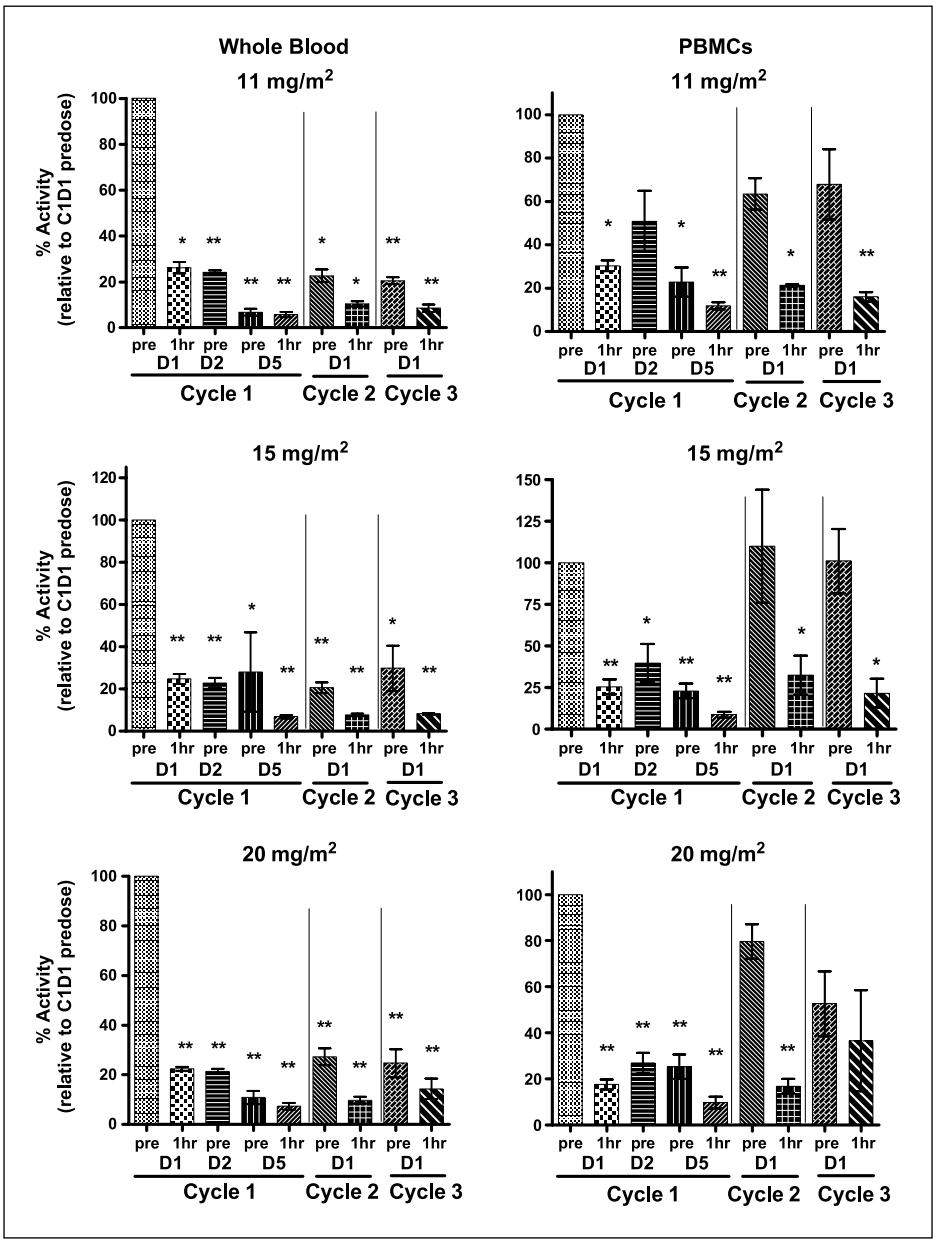

Fig. 1. Proteasome chymotrypsin-like specific activity was measured in whole blood and PBMCs at the indicated time points during the first three cycles of the study using a fluorogenic substrate. The percent proteasome activity was calculated by comparing the activity in the sample taken before the first dose (cycle 1 day 1 predose) with the specific activity measured in samples taken at subsequent time points. Columns, mean; bars, SEM. **, $P < 0.001$; *, $P < 0.01$.

with compromised renal function, with only three reports of grade 1 or 2 creatinine increase.

Pharmacodynamic modeling of the 20S proteasome activity data showed dose-dependent proteasome inhibition that was related both to the carfilzomib dose level and to the cumulative dose of carfilzomib. More than 75% inhibition of chymotrypsin-like activity was achieved with the first dose of carfilzomib at or above 11 mg/m^2. Despite the cumulative inhibition of the proteasome over 5 days of dosing, such that >90% inhibition in whole blood and PBMCs was recorded 1 h after the fifth dose of carfilzomib (Fig. 1), this regimen seems to be well tolerated. Although direct comparison with the less intensive dosing regimen used in other studies (23) is not possible due to the small numbers of patients examined, there were no unexpected adverse events that seemed to be specifically associated with extended, 5-day dosing with carfilzomib. Additional pharmacodynamic data are being collected in conjunction with other ongoing clinical trials with carfilzomib.

Antitumor activity was seen in heavily pretreated MM, WM, and MCL patients. The lowest dose at which responses were seen was 11 mg/m^2. Carfilzomib treatment resulted in sustained inhibition of the chymotrypsin-like subunit of the proteasome by virtue of the consecutive-day dosing and the irreversible mechanism of action. Because one characteristic of bortezomib-resistant cells is the overexpression of proteasome subunits (24), the longer-lasting inhibition of carfilzomib (10) might overcome the cell's attempt at resistance by overproduction of proteasomal protein. The ability of carfilzomib to overcome bortezomib resistance does not seem to be linked to cellular transport mechanisms, as the rate of onset of proteasome inhibition does not differ greatly in a variety of cell types exposed to carfilzomib and bortezomib (data not shown) or in

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human pharmacodynamic testing. Further studies will be needed to elucidate the contribution of these and other possible mechanisms of action.

Exposure to carfilzomib results in potent (>75%) inhibition of the chymotrypsin-like activity of the proteasome at tolerable doses in humans. Up to five consecutive days of carfilzomib dosing for multiple cycles were well tolerated in this phase 1 study in chronic hematologic malignancies. Drug discontinuation was considered unlikely related or not related to administration of carfilzomib and no patients discontinued therapy due to peripheral neuropathy. The overall incidence of treatment-emergent adverse events as well as the number and type of grade 3 and 4 adverse events occurring at active doses yielding potent proteasome inhibition support that carfilzomib is well tolerated with intensive, daily dosing.

Taken together, the above data provide a strong rationale that carfilzomib, as a new chemical entity, offers a novel mechanism of action with an acceptable risk profile for further investigation in hematologic malignancies.

Disclosure of Potential Conflicts of Interest

O. O'Connor and J. Gericitano have received research funding from Proteolix; A. Stewart is a consultant for Proteolix; M. Vallone, C. Molineaux, and L. Kunkel are employed by and own stock in Proteolix; R. Orlowski is a consultant with Proteolex.

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Owen A. O'Connor, A. Keith Stewart, Marcy Vallone, et al.

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