Cancer Chemother Pharmacol (2008) 63:109–115
DOI 10.1007/s00280-008-0719-5

ORIGINAL ARTICLE

A phase I pharmacodynamic trial of bortezomib in combination with doxorubicin in patients with advanced cancer

Noelle K. LoConte · James P. Thomas · Dona Alberti · Jennifer Heideman · Kimberly Binger · Rebecca Marnocha · Kyle Utecht · Peter Geiger · Jens Eickhoff · George Wilding · Jill Kolesar

Received: 3 November 2007 / Accepted: 21 February 2008 / Published online: 6 March 2008

Abstract
Purpose This phase I trial sought to define the toxicity, maximally tolerated dose (MTD) and pharmacodynamics of a combination of bortezomib and doxorubicin in patients with advanced malignancies.
Patients and methods Twenty-six patients were treated with bortezomib intravenously on days 1, 4, 8 and 11, with doxorubicin also administered intravenously on days 1 and 8, both in a 21-day cycle. Dosing ranged from 1.0 mg/m^2 of bortezomib with 15 mg/m^2 of doxorubicin to 1.5 mg/m^2 of bortezomib with 20 mg/m^2 of doxorubicin. Pharmacodynamic studies performed included assessment of levels of 20S proteasome activity and ubiquitin-protein conjugates.
Results The combination of bortezomib and doxorubicin was generally well tolerated. There were two dose limiting toxicities (DLT) at dose cohort 3 (1.3 mg/m^2 bortezomib, 20 mg/m^2 doxorubicin) and 2 DLT at dose cohort 3a (1.5 mg/m^2 bortezomib, 15 mg/m^2 doxorubicin). DLT seen included neutropenia, thrombocytopenia, and neuropathy. In addition, one patient developed grade 3 central nervous system toxicity in cycle 2 (not a DLT). One patient with hormone refractory prostate cancer had a partial response. Proteasome inhibition in whole blood was demonstrated and an increase in ubiquitin-protein conjugates was observed in peripheral blood mononuclear cells of most patients.
Conclusions Bortezomib and doxorubicin can be administered safely. The recommended phase II dose for this 21-day cycle is bortezomib 1.3 mg/m^2 intravenously on days 1, 4, 8 and 11, and doxorubicin 20 mg/m^2 intravenously on days 1 and 8. This combination may be of special interest in multiple myeloma, given the activity of both drugs in that disease.

Keywords Bortezomib · Doxorubicin · Ubiquitination · Proteasome inhibition

Supported by grant: U01 CA062491 "Early Clinical Trials of Anti-Cancer Agents With Phase I Emphasis, NCI" and M01 RR03186 "General Clinical Research Center Program of The National Center for Research Resources, NIH".

N. K. LoConte (✉) · D. Alberti · J. Heideman · K. Binger · R. Marnocha · P. Geiger · J. Eickhoff · G. Wilding · J. Kolesar
University of Wisconsin Paul P. Carbone Comprehensive Cancer Center, K4/548 CSC, 600 Highland Avenue, Madison, WI 53792, USA
e-mail: ns3@medicine.wisc.edu

J. P. Thomas
The Ohio State University Comprehensive Cancer Center, Columbus, OH 43210, USA

K. Utecht · J. Kolesar
University of Wisconsin School of Pharmacy, Madison, WI 53792, USA

Introduction

Bortezomib (PS-341, VELCADE®), a specific proteasome inhibitor, is approved for the treatment of patients with multiple myeloma [2, 13, 20–22] who have received at least one prior therapy and for the treatment of patients with mantle cell lymphoma [9] who have received at least one prior therapy. Common moderate to severe toxicities seen with bortezomib include neutropenia, anemia, and thrombocytopenia.

Doxorubicin (Adriamycin) is an anthracycline-type of chemotherapy, which induces DNA strand breakage by multiple mechanisms. It is indicated in several malignancies, including leukemia, sarcoma, breast and ovarian cancer, among others. Common toxicities of doxorubicin

include myelosuppression, nausea and vomiting, mucocutaneous effects and cardiomyopathy.

There have been several pre-clinical studies, which suggest synergy for the combination of bortezomib and doxorubicin. For example, Mitsiades et al. [16] have examined the combination of doxorubicin and bortezomib in multiple myeloma cells in vitro. A large increase in doxorubicin-mediated cytotoxicity in cells treated with bortezomib was seen, significantly more than that seen with other multiple myeloma agents such as dexamethasone or thalidomide. Using microarray techniques, changes in cellular transcripts of apoptosis, cell growth, proteasome function and heat shock response were seen after bortezomib treatment, suggesting that these cells would then be more sensitive to doxorubicin mediated cell death. Similarly, Ma et al. [15] saw a marked potentiation of doxorubicin-induced cytotoxicity of multiple myeloma cells when given in combination with bortezomib. This effect was much more pronounced in doxorubicin resistant cells; a phenomenon also noted by Hideshima et al. [12]. Finally, recent evidence suggests that doxorubicin binds to and inhibits both 26S and 20S proteasome chymotrypsin-like function in a dose-dependent fashion [14] and that doxorubicin utilizes proteasomes for transport to the nucleus [8], suggesting that the synergy observed with the combination of doxorubicin and bortezomib may be related to targeting of the proteasome. Despite this promising pre-clinical data, there has been no clinical evaluation of proteosome inhibition by doxorubicin.

The chosen schedule for chemotherapy combinations with bortezomib may be important. An et al. determined that bortezomib and chemotherapy (doxorubicin and paclitaxel) interactions were schedule dependent [3]. Synergistic interactions were identified when PEL (primary effusion lymphoma) cells were pre-treated with bortezomib prior to chemotherapy. Chemotherapy pre-treatment or concurrent treatment with bortezomib and chemotherapy lead to additive or antagonistic interactions [3]. Conversly, Gummerlock et al. [10] found that when they treated a p53-null NSCLC cell line with docetaxel and bortezomib, greatest cytotoxicty occurred when the docetaxel preceded bortezomib.

Proteasome inhibition by bortezomib occurs preferentially in transformed cells, most likely due to upregulation of ubiquitin functions. Proteasome inhibition in whole blood after treatment with bortezomib demonstrates a linear dose-response curve for doses up to 1.3 mg/m2, after which it plateaus at approximately 65–70% inhibition [1, 18]. Maximal percent inhibition of proteasome activity is observed by 1 h after dosing, followed by a return toward baseline within 72 h. Papandreou et al. [18] evaluated proteasome inhibition in human tumor samples concurrently with its activity in blood, and identified that proteasome inhibition in prostate and lymph node samples was similar to that observed in blood while inhibition of activity in the bone marrow was approximately one half that observed in blood.

Ubiquitination is a reversible posttranslational modification of cellular proteins, in which ubiquitin is primarily attached to the ε-amino group of lysines in target proteins [17]. Ubiquitination serves as a signal to the proteasome to initiate the degradation of these cellular proteins, and degradation of proteins via this mechanism controls the majority of cellular processes, including transcription, apoptosis and cell cycle regulation [4, 6]. Theoretically, a proteasome inhibitor should increase the total cellular quantity of ubiquitin-protein complexes by inhibiting protein breakdown.

The objective of this study was to determine the maximum tolerated dose (MTD) of bortezomib in combination with doxorubicin. Secondary objectives included a preliminary evaluation of antitumor activity and exploring the pharmacodynamics of proteasome inhibition and ubiquitination in patients receiving this combination.

Methods

Patient selection

Patients with histologically confirmed, advanced malignancies, refractory to standard therapy or for whom no standard therapy existed, were eligible for enrollment. Inclusion criteria included Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, age greater than or equal to 18 years and presence of measurable or evaluable disease by the response evaluation criteria in solid tumors (RECIST) criteria [23]. Patients had no signs of brain metastases, preexisting grade 2 or higher peripheral neuropathy, serious active infection, or prior cumulative doxorubicin exceeding 280 mg/m^2. Patients had not received any chemotherapy or radiation therapy within 4 weeks prior to entering this study. Patients had adequate organ function defined as an absolute neutrophil count of at least 1,500/mm^2, platelets of at least 100,000/mm^3, total serum bilirubin within normal limits, serum creatinine less than 1.5 mg/dl or creatinine clearance greater than 60 ml/min and left ventricular ejection fraction (LVEF) greater than or equal to 45%. All patients had to practice effective birth control and gave written informed consent in accordance with federal and institutional guidelines. This protocol was approved by the University of Wisconsin Institutional Review Board prior to opening.

Study design

This was a phase I trial designed to determine the MTD and dose limiting toxicities (DLT) of bortezomib in combina-

tion with doxorubicin and to study the pharmacodynamics of this combination. It was planned to treat three patients at each dosing cohort until MTD was reached. Patients with a treatment delay during cycle 1 that was not from DLT were replaced. If no DLT was observed within a cohort, the next dosing level was opened. If one of the initial three patients in a cohort experienced a DLT, the cohort was expanded to six patients. DLT was defined using the National Cancer Institute Common Toxicity Criterion version 2.0, included any toxicity thought to be probably or definitely related to treatment, and was grade 3 or higher. Exceptions to this included neutrophils, which must have exceeded 5 days duration or be accompanied by fever; hemoglobin, which must have been grade 4; or platelets, which must have been 25,000/mm^3 or less for any duration. Nausea and vomiting or diarrhea of grade 3 or higher despite maximal medical therapy was also considered a DLT. MTD was defined as the highest dose level for which the incidence of DLT was 33% of patients treated at that level or higher. For MTD determination, only toxicities that occurred within the first cycle of therapy were used.

A previously completed phase I study of single agent bortezomib found that the MTD was 1.3–1.5 mg/m^2 when given in a 2 weeks on and 1 week off schedule [7]. Accordingly, in the present study, the first cohort (level 1) received bortezomib at a dose of 1.0 mg/m^2 and doxorubicin at 15 mg/m^2, the second (level 2) at 1.3 mg/m^2 and 15 mg/m^2, the third (level 3) at 1.3 mg/m^2 and 20 mg/m^2, the fourth (level 4) at 1.5 mg/m^2 and 20 mg/m^2. To further define the DLT observed at level 4, an intermediate dose level of 1.5 mg/m^2 and 15 mg/m^2 (cohort 3a) was opened. Bortezomib was administered intravenously on days 1, 4, 8 and 11 of a 21 day cycle. Doxorubicin was given on days 1 and 8 as an intravenous bolus 1 h after bortezomib administration.

Bortezomib was supplied as a sterile lyophilized powder containing bortezomib and mannitol. Each vial was reconstituted with normal saline to a final concentration of 1 mg/ml and administered as an intravenous push over 3–5 s. Doxorubicin was also supplied as a sterile lyophilized powder, and was reconstituted with normal saline, and given intravenously as a bolus over 3–5 min.

Response criteria

RECIST was utilized for determining tumor response and progression [23]. Computed tomography or magnetic resonance imaging with slice thicknesses of 10 mm or less were the preferred methods of tumor assessment and measurement, but chest X-ray was allowed if the measurable lesions were clearly defined. Tumor measurements were assessed every two cycles and patients who experienced disease progression were withdrawn from study.

Pharmacodynamics—determination of proteasome inhibition and quantity of ubiquitinated proteins

Proteasome activity of whole blood was determined by Millennium Pharmaceuticals Inc. for whole blood samples obtained at the following times: pretreatment, day 1 and 8 of cycle 1 at 1, 6, and 24 h post-bortezomib administration as described previously [1, 19]. Briefly, in this assay, the chymotryptic activity of the 20S proteasome is measured by release of the fluorophore amidyl methylcoumarin from the peptidyl substrate Bz-Val-Gly-Arg-7-amido-4-methylcoumarin.

To determine whether the level of proteasome inhibition was sufficient to cause an accumulation of ubiquitinated proteins, blood samples were collected and peripheral blood mononuclear cells (PBMC) were isolated with cell preparation tubes by standard procedures. PBMC samples pre-study and 6 h following bortezomib administration on day 1 of cycle 1 were analyzed for protein-ubiquitin conjugates after separation by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in 25 patients with evaluable samples. The 6-h timepoint was chosen based on previous pre-clinical data demonstrating that accumulation of ubiquitinated proteins occurred after 6 h [25]. Samples were diluted in protein buffer solution and protein concentration was determined using Pierce's bicinchoninic acid protein assay kit. A measure of 15 μg aliquots of protein from each sample were loaded into 8% SDS PAGE gels in a Laemmli buffer system before being run at 100 mV for 1 h. Upon completion, proteins were electrophoretically transferred from the gel to a polyvinylidene fluoride membrane at 40 mV for 90 min. The resulting membrane was then blocked with a solution of 5% non-fat dry milk in Tris Buffered Saline Tween (TBST) buffer for 1 h with constant agitation. The membrane was then bathed in a 1:1,000 dilution of the primary antibody (anti-rabbit ubiquitin conjugate) for 12 h with constant agitation. To eliminate excess primary antibody, the membrane was washed in TBST five times, with each wash including 5 min of agitation. The membrane was then bathed in a 1:3,000 dilution of the secondary antibody (goat-anti-rabbit horseradish peroxidase) for 1 h with constant agitation. The membrane was washed in a similar manner before being developed with Pierce's Super-Signal® West Pico chemiluminescent substrate for 5 min.

Images of each gel were captured directly after development using Fugifilm® Medical X-Ray film in a dark room with exposure times appropriate for the degree of chemiluminescence on each membrane. The film of each gel was developed and scanned from 300 to 25 kDa for densitometry analysis using the program NIH Image as previously described [19].

Results

Demographics

A total of 26 patients were enrolled from 30 July 2001 to 24 July 2003 (Table 1) and all patients were assessed for toxicity. No patients had received prior central nervous system radiation therapy. In total, 79 complete cycles were administered to 26 patients (median 2 cycles, range 1–12 cycles).

Table 1 Patient characteristics

Characteristic	No
No. of patients	26
Median age, years (range)	66.5 (38–81)
Median # prior chemotherapy regimens (range)	4 (0–6)
% Of male (*n*)	73 (19)
% Of Caucasian (*n*)	92.3 (24)
ECOG performance status (%)	
0	5 (19.2)
1	20 (76.9)
2	1 (3.8)
Cancer diagnoses (n)	
Pancreas	2
Prostate	9
Lung	3
Colon	3
Thyroid	1
Renal	1
Gallbladder	1
Stomach	1
Sarcoma	1
GIST	1
Head and neck	1
Lymphoma	1
Mesothelioma	1

Dose escalation, DLT, and MTD

DLT was seen at two different dosing levels (Table 2). Two DLT were observed at cohort level 3 (1.3 mg/m^2 bortezomib and 20 mg/m^2 doxorubicin) out of a total of ten patients. Two DLT were also seen at cohort level 3a (1.5 mg/m^2 bortezomib and 15 mg/m^2 doxorubicin) out of a total of five patients. Level 3a was opened because of a neurological adverse event (but not DLT) observed in a patient during his second course of treatment at level 4 (1.5 mg/m^2 bortezomib and 20 mg/m^2 doxorubicin) out of a total of four patients (see Adverse Events, below). There were no DLT seen at level 4. The MTD was determined to be dose cohort 3 (1.3 mg/m^2 bortezomib and 20 mg/m^2 doxorubicin).

Adverse events

The combination regimen was generally well tolerated (Table 2). Most events were mild to moderate in intensity. The most common grade 3/4 adverse events were thrombocytopenia, neutropenia and neuropathy. Also seen in more than one patient were moderate fatigue, nausea and anemia. The hematologic toxicities seen were generally mild and did not require intervention. No patient had a decrease in LVEF of greater than 15%.

One prostate cancer patient enrolled at level 3, experienced grade 3 confusion and grade 3 depressed level of consciousness during cycle 2 of treatment. The patient became progressively obtunded over a 3-week period. An MRI scan revealed no evidence of intracranial metastases, a lumbar puncture was unremarkable and no metabolic explanation could be found for his altered level of consciousness. His condition improved somewhat 1 month after his last dose of bortezomib, but the patient never returned to his prior baseline. He died from progressive disease 3 months after starting on study and approximately

Table 2 Grade 3 and 4 toxicities, which were thought to be possibly or definitely related to treatment

Cohort level	Dose level (mg/m^2)	Total number of patients treated (range of number of cycles received)	Description of grade 3/4 toxicities
1	1.0 mg/m^2 bortezomib/ 15 mg/m^2 doxorubicin	4 (1–12)	
2	1.3 mg/m^2 bortezomib/ 15 mg/m^2 doxorubicin	3 (1–5)	Thrombocytopenia
3	1.3 mg/m^2 bortezomib/ 20 mg/m^2 doxorubicin	10 (1–8)	Febrile Neutropenia (DLT) Leukopenia Neutropenia without fever Thrombocytopenia (DLT)
3a	1.5 mg/m^2 bortezomib/ 15 mg/m^2 doxorubicin	5 (1–4)	Neuropathy (DLT) Thrombocytopenia (DLT)
4	1.5 mg/m^2 bortezomib/ 20 mg/m^2 doxorubicin	4 (2–4)	Thrombocytopenia Diarrhea

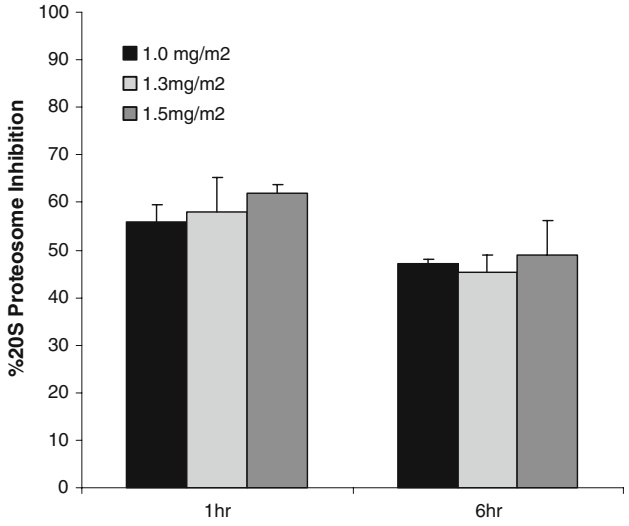

Fig. 1 Mean percent of proteasome inhibition after administration of bortezomib and doxorubicin. 20S proteasome inhibition was measured in whole blood. Substantial proteasome inhibition was evident on day 1 at both the 1- and 6-h time points ($n = 26$), but did not vary by dose level. *Error bars* represent the standard deviation (P = NS across all comparisons)

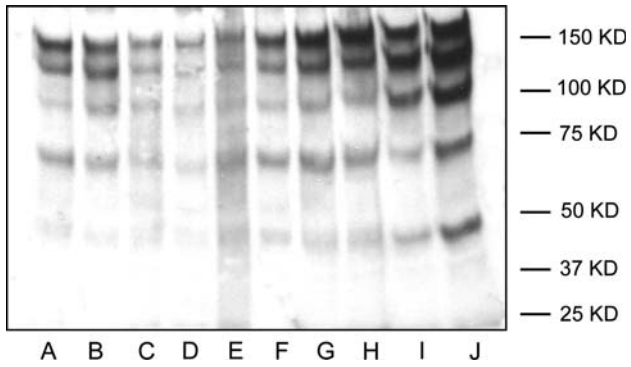

Fig. 2 Immunoblot of ubiquitinated proteins. Proteins from isolated peripheral blood mononuclear cells were separated by SDS-PAGE and then probed with an anti-ubiquitin-protein conjugate antibody. Day 1 pre-therapy and 6 h post-therapy samples are shown for the four patients at dose level 1 and one patient at dose level 2 (*lanes A/B*, *C/D*, *E/F*, *G/H*, *I/J*, respectively). An increase in total amount of ubiquitinated proteins is reflected by an increase in the total amount of shading in the respective column. All patients had either consistent or increased ubiquitination after treatment, although this did not reach statistical significance for individual dose levels (P = NS across all comparisons)

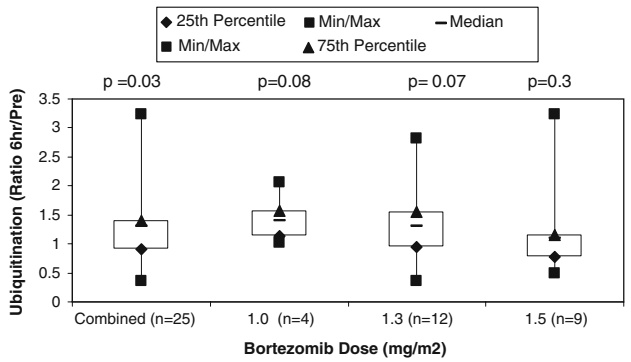

Fig. 3 Ubiquitination in peripheral blood mononuclear cells. Data is expressed as a ratio of 6 h after bortezomib administration to pre-administration on day 1 of cycle 1 and shown as *box plots*. Total ubiquitination was analyzed by Western blot over the range of 300–25 kDa. A statistically significant increase ($P = 0.03$, one-sided Wilcoxon Signed Rank Test) in total ubiquitination was observed after administration of bortezomib when all dose levels were combined, while individual dose level showed non-significant increases in total ubiquitination

6 weeks after last receiving study medications. His symptoms were considered possibly related to both study medications since no other cause could be identified.

Pharmacodynamics and changes in protein-ubiquitin conjugates

Proteasome inhibition approached 60% at 1 h after bortezomib administration and 45% 6 h after bortezomib administration. There was no statistical difference in levels of proteasome inhibition among the different doses of bortezomib administered (Fig. 1). Our data are consistent with other reports that demonstrated a 65–75% proteasome inhibition after administration of similar doses of bortezomib [1, 19].

Total level of ubiquitinated proteins increased after administration of bortezomib and doxorubicin (Figs. 2, 3). Data is represented as the ratio of the total amount of ubiquitination at 6 h after administration of bortezomib to pre-bortezomib amount of ubiquitination. Total ubiquitination between the pre-administration sample and 6 h post-bortezomib administration sample increased for all dose levels but did not reach statistical significance, however, when data from all dose levels was combined, there was a statistically significant increase (mean ratio 1.26 with a standard deviation of 0.67) in ubiquitination ($P = 0.03$, one-sided Wilcoxon signed rank test, Fig. 3). There was no difference in ubiquitination observed between the dose levels.

Antitumor activity

One patient with prostate cancer (PSA negative, hormone refractory, three prior therapies) demonstrated a partial response on dose level-1. This patient exhibited a marked response to therapy including a dramatic reduction in pulmonary symptoms and improvement in his chest X-ray (Fig. 4). This patient remained on study for 10 months before having progressive disease. A total of eight other patients with prostate cancer were enrolled on this study with no other patients demonstrating a complete or partial response. There were no other responses seen in any other tumor types.

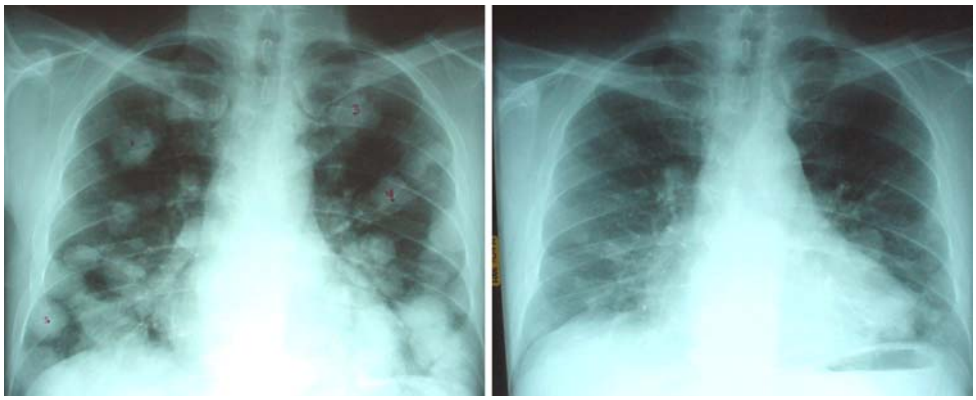

Fig. 4 Chest X-ray of patient with metastatic prostate cancer prior to cycle 1 (*left*) and after four cycles of study treatment

Discussion

Bortezomib significantly improves the efficacy of doxorubicin in pre-clinical models, including a marked sensitization of doxorubicin resistant myeloma cells [15, 16]. Therefore the combination of doxorubicin and bortezomib was evaluated in this phase I trial. This combination is also of interest given the activity of each agent in multiple myeloma. We sought to determine the MTD of bortezomib given on days 1, 4, 8 and 11 in combination with doxorubicin given on days 1 and 8 with a 21-day cycle. A weekly schedule of doxorubicin was chosen to increase the potential for interaction between the two agents [3].

In general this combination was well tolerated and the doses employed were close to those used for each compound as a single agent on a comparable schedule. Hematologic toxicities were as expected with this combination. Two patients demonstrated neurologic toxicity, one central and one peripheral; notably, peripheral neuropathy is a known side effect of bortezomib.

Although doxorubicin as a single agent has shown only modest activity in prostate cancer [5, 11, 24], one patient in this clinical trial with hormone refractory prostate cancer did demonstrate an improvement in symptoms and objective partial response of lung metastases to the combination of bortezomib and doxorubicin (Fig. 4). Of note, no patients with multiple myeloma were treated in this trial.

The pharmacodynamic effect of bortezomib on blood proteasome activity was similar to results reported in previous single agent studies [1]. The dose of doxorubicin did not seem to influence the degree of proteasome inhibition, despite in vitro evidence demonstrating that doxorubicin also inhibits proteasomes. This suggests that proteasome inhibition of approximately 60% is the maximal inhibition, which can be achieved by these agents clinically.

The degree of proteasome inhibition seen was examined to determine if it was of sufficient magnitude to cause accumulation of ubiquitinated proteins. Western blots showed an increased amount of total ubiquitinated-protein conjugates although the identity of these ubiquitinated proteins was not determined. Over the limited dose range of bortezomib and small sample size evaluated in this study, no consistent dose-dependent pattern was observed. When all dosing levels were combined, however, there was a significant increase in the amount of ubiquitination. These results indicate that the level of proteasome inhibition achieved with bortezomib may be sufficient to increase the amount of ubiquitinated proteins.

In summary, the combination of bortezomib and doxorubicin was well tolerated and is currently being studied in a multiple myeloma phase II clinical trial. The recommended phase II doses are bortezomib 1.3 mg/m^2 given intravenously on days 1, 4, 8 and 11 with doxorubicin 20 mg/m^2 given intravenously on days 1 and 8 in a 21-day cycle.

Acknowledgments The authors are grateful to Dr. Arthur Haas (Louisiana State University) and Mr. Richard Bohnsack (Medical College of Wisconsin) for providing the anti-ubiquitin conjugate antibody.

References

1. Aghajanian C, Soignet S, Dizon DS et al (2002) A phase 1 trial of the novel proteosome inhibitor PS314 in advanced solid tumor malignancies. Clin Cancer Res 8:2505–11
2. Adams J (2002) Preclinical and clinical evaluation of proteasome inhibitor bortezomib for the treatment of cancer. Curr Opin Chem Biol 6:493–500
3. An J, Sun Y, Fisher M et al (2004) Antitumor effects of bortezomib (PS-341) on primary effusion lymphomas. Leukemia 18:1699–704
4. Devoy A, Soane T, Welchman R et al (2005) The ubiquitin-proteasome system and cancer. Essays Biochem 41:187–203
5. DeWys WD, Bauer M, Colsky J et al (1977) Comparitive trial of adriamycin and 5-fluorouracil in advanced prostatic cancer-progress report, Cancer Treat Rep 61:325–328
6. Dhananjayan SC, Ismail A, Nawaz Z (2005) Ubiquitin and control of transcription. Essays Biochem 41:69–80
7. Dy GK, Thomas JP, Wilding G et al (2005) A phase I and pharmacologic trial of two schedules of the proteasome inhibitor, PS-341 (bortezomib, velcade), in patients with advanced cancer. Clin Cancer Res 11(9):3410–6
8. Fekete MR, McBride WH, Pajonk F (2005) Anthracyclines, proteasome activity and multi-drug-resistance. BMJ Cancer 5:114

9. Fisher RI, Bernstein SH, Kahl BS et al (2006) Multicenter phase II study of bortezomib in patients with relapsed or refractory mantle cell lymphoma. Jl Clin Oncol 24(30):4867–4874
10. Gumerlock PH, Kawaguchi T, Moisan LP et al (2002) Mechanisms of enhanced cytotoxicity from docetaxel—PS-341 combination in non-small cell lung carcinoma (NSCLC). Proc Am Soc Clin Oncol 21:304a (abstract 1214)
11. Hass NB, Manola J, Hudes G et al (2000) Phase II pilot study of combined chemohormonal therapy with doxorubicin and estramustine in metastatic prostate cancer. Am J Clin Oncol 23:589–592
12. Hideshima T, Richardson P, Chauhan D et al (2001) The proteasome inhibitor bortezomib inhibits growth, induces apoptosis and overcomes drug resistance in human multiple myeloma cells. Cancer Res 61:3071–3076
13. Jagannath S, Barlogie B, Berenson J et al (2004) A phase 2 study of two doses of bortezomib in relapsed or refractory myeloma. Br J Haematol 127(2):165–172
14. Kiyomiya J, Hiroshi H, Masaru K et al (2002) Correlation between nuclear action of anthracycline anticancer agents and their binding affinity to the proteasome. Int J of Oncol 21:1081–1085
15. Ma MN, Yang HH, Parker K et al (2003) The proteasome inhibitor bortezomib markedly enhances sensitivity of multiple myeloma tumor cells to chemotherapeutic agents. Clin Cancer Res 9:1136–1144
16. Mitsiades N, Mitsiades CS, Richardson PG et al (2003) The proteasome inhibitor bortezomib potentiates sensitivity of multiple myeloma cells to conventional chemotherapeutic agents: therapeutic applications. Blood 101:2377–2380
17. Nandi D, Tahiliani P, Kumar A et al (2006) The ubiquitin-proteasome system. J Biosci 31(1):137–155
18. Papandreou CN, Daliani DD, Nix D et al (2004) Phase I trial of the proteasome inhibitor bortezomib in patients with advanced solid tumors with observations in androgen-independent prostate cancer. J Clin Oncol 22(11):2108–2121
19. Pereira ME, Yu B, Wilk S (1992) Enzymatic changes of the bovine pituitary multicatalytic proteinase complex induced by magnesium ions. Arch Biochem Biophys 294:1–8
20. Richardson PG, Barlogie B, Berenson J et al (2003) A phase 2 study of bortezomib in relapsed, refractory myeloma. N Engl J Med 348(26):2609–2617
21. Richardson PG, Barlogie B, Berenson J et al (2004) Survival, duration of response, and time to progression with bortezomib in patients with relapsed and refractory myeloma: an update of the SUMMIT trial with additional follow-up. Hematol Jl 5(Suppl 2):S103–S104
22. Richardson P, Sonneveld P, Schuster MW et al (2005) Bortezomib or high dose dexamethasone for relapsed multiple myeloma. N Engl J Med 352(24):2487–2498
23. Therasse P, Arbuck SG, Eisenhauer EA et al (2000) New guidelines to evaluate the response to treatment in solid tumors. J Natl Canc Inst 92:205–216
24. Torti FM, Aston D, Lum BL et al (1983) Weekly doxorubicin in endocrine-refractory carcinoma of the prostate. J Clin Oncol 1:477–482
25. Xu Q, Farah M, Webster JM, Wojcikiewicz RJ (2004) Bortezomib rapidly suppresses ubiquitin thiolesterification to ubiquitin-conjugating enzymes and inhibits ubiquitination of histones and type I inositol 1,4,5-trisphosphate receptor. Mol Cancer Ther 3(10):1263–9