Bortezomib-Mediated Inhibition of Steroid Receptor Coactivator-3 Degradation Leads to Activated Akt

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Abstract

Purpose: To assess the safety of administering bortezomib to patients undergoing a radical prostatectomy, to assess pathologic changes induced by bortezomib in prostate cancer specimen, and to verify alterations by the drug in proteasome protein targets.

Experimental Design: Bortezomib is a proteasome inhibitor that has shown activity in vitro and in vivo in prostate cancer. We performed a neoadjuvant clinical trial of bortezomib in men with prostate cancer at high risk of recurrence. The primary endpoints were to evaluate safety and biological activity.

Results: Bortezomib is generally safe in the preoperative setting. Antitumor activity was manifested by tumor cytopathic effect, drops in serum prostate-specific antigen in some patients, and increases in tumor apoptosis. This was associated with cytoplasmic entrapment of nuclear factor-κB. We found an unexpected increase in proliferation in treated tissues and in vitro. Bortezomib also increased SRC-3 levels and phosphorylated Akt, both in vitro and in treated prostate cancer tissues. Knockdown of SRC-3 blocked the increase in activated Akt in vitro. Combined treatment with bortezomib and the Akt inhibitor perifosine was more effective than either agent alone in vitro.

Conclusion: These data suggest that combined therapies targeting the proteasome and the Akt pathway may have increased efficacy.

Radical prostatectomy is often successful in treating men with clinically localized disease; however, there is still a significant failure rate, particularly in men with adverse clinicopathologic parameters. New therapies for prostate cancer are necessary, as currently no effective adjuvant therapy is available for patients with high risk of recurrence after treatment of localized disease. The advent of new, targeted therapy drugs opens the door to currently no effective adjuvant therapy is available for patients with high risk of recurrence after treatment of localized disease. The primary endpoints were to evaluate safety and biological activity.

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Received 4/1/08; accepted 4/2/08.

Grants support: NIH Specialized Programs of Research Excellence CA58204.

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in men with aggressive clinically localized prostate cancer at high risk of recurrence.

Activation of the Akt pathway can limit the effectiveness of many cancer chemotherapies. Steroid receptor coactivator-3 (SRC-3) interacts with steroid receptors and is also overexpressed in prostate cancer patients. Overexpression of SRC-3 correlates well with the prostate cancer proliferation and cell survival (13). Knocking down of SRC-3 in prostate cancer cells leads to decreased cell proliferation, inhibition of cell cycle progression, and increased apoptosis. Most importantly, down-regulation of SRC-3 protein in prostate cancer cell lines results in decreased tumor growth in nude mice. Increased expression of SRC-3 results in up-regulation of the Akt pathway via activation of multiple genes in the Akt pathway. In this study, we have found that although bortezomib does have antitumor activity in prostate cancer, this activity is limited by bortezomib induction of SRC-3 and activation of Akt.

Materials and Methods

Study objectives. The primary objectives are to assess the safety of administering bortezomib to patients undergoing a radical prostatectomy, to assess pathologic changes induced by the drug in prostate cancer specimens, and to verify alterations in proteasome protein targets. This report emphasizes the results of these latter objectives. The secondary is long-term clinical follow-up to assess outcome in comparison with historical controls. We report here our initial observations on the histologic changes and alterations of protein targets in the pathologic specimens analyzed thus far and corresponding in vitro work that lends support to these observations. After completion of enrollment, we plan a complete analysis of these variables in all specimens and correlation with clinical response and, ultimately, clinical outcome.

Treatment plan. The study was designed for a total of 40 patients with locally advanced/high-grade prostate cancer. We currently have enrolled 40 patients; 37 patients completed both the protocol and the surgery, and 2 patients are currently undergoing treatment. The biological studies were done on 21 patients. Inclusion criteria were clinical stage T1, or T2a, with high-grade disease (Gleason 8-10), or clinical stage T3, with Gleason grade of 7 and PSA of >10 ng/mL or clinical stage T1, Patients received bortezomib by i.v. push at a dosage of 1.6 mg/m2 once weekly for 4 wk followed by radical prostatectomy within 72 h of the last dose. The study was approved by the Baylor Institutional Review Board (IRB H-11047).

Radical prostatectomy specimen processing and morphologic analysis. Whole-mount prostatectomy specimens were reviewed. We identified morphologic changes that were present in prostate cancer cells and that were not found in the pretherapy prostate biopsies of the same patients. The tumors were mapped on each whole-mount slide. The changes were mapped on superimposed whole-mount slides by the investigators (Fig. 1).

A tissue microarray (TMA) was built containing benign and cancer tissues from treated patients. Immunohistochemical stains of biomarkers were done on them and compared with a large TMA of radical prostatectomies described previously (13, 14). The biological studies were done on 21 patients. Only patients with a Gleason score of 7 or above were used from the control database. Whenever possible and necessary, they were also compared with the preoperative biopsies of the bortezomib-treated patients.

Immunohistochemistry. Immunohistochemistry was done with antibodies against P-Akt (S473), NF-κB, and Ki67 using a standard avidin-biotin peroxidase method as described previously (13). The detection of DNA fragmentation was determined in situ by the terminal deoxynucleotidyl transferase–mediated diUTP biotin nick-end labeling technique (13).

Assessment of immunostaining. All TMA-stained slides were digitized and quantified using a method previously described (13, 14). Each image was interpreted for immunoreactivity using a 0 to 3+...
Cell culture and reagents. Human prostate cancer cell lines DU145, PC3, and LNCaP were maintained in D-MEM/F-12 supplemented with 10% fetal bovine serum and penicillin (100 μg/mL), and streptomycin (100 μg/mL). Bortezomib and perifosine were dissolved in PBS and MG132 was dissolved in DMSO. PC3 prostate cancer cells were treated with 10 nmol/L bortezomib and/or 10 μmol/L perifosine. DU145 prostate cancer cells were treated with 1 μmol/L bortezomib and/or 10 μmol/L perifosine.

Proliferation, apoptosis, and growth assays. DU145, PC3, and LNCaP cells were seeded in 4-well chamber slide the day before treatment at a density of 3 × 10^4 cells per well. Bortezomib was added to cells for 48 h.

Cell proliferation was assessed by the BrdUrd incorporation assay. DU145 cells were seeded in 1-well chamber slide the day before bortezomib treatment. After addition of bortezomib for 48 h, cells were collected and suspended in 0.5 mL of PBS containing 0.1% (v/v) Triton-X100 for preparation of nuclei. The suspension was adjusted to a final concentration of 0.1% (w/v) RNase and 50 μg/mL propidium iodide. The DNA content was measured by flow cytometry, and apoptosis was assessed by the percentage of sub-G0 cells. Experiments were done in triplicate (13, 14).

Western blotting. Immunodetection was done using a primary antibody and peroxidase-conjugated secondary antibody. PC3 cells were lysed in radioimmunoprecipitation assay buffer with protease inhibitors (Roche Diagnostics) at indicated time points. The proteins were resolved by SDS-PAGE and transferred to nitrocellulose. The following antibodies were used for immunodetection: anti–SRC-3 (BD Biosciences), anti-FKHR1L (Thr32), anti-FKHR1L (Upstate), anti-actin, and anti–cyclin D1 (Sigma). Anti-Akt1, anti-pAkt (Ser472), anti-Akt (Thr308), anti-pGSK3/3 (Ser21/9), anti-GSK3, anti-S6 ribosomal protein (Ser240/244), and anti-S6 ribosomal protein antibodies are from Cell Signaling Technology, and anti–C-Myc are from Santa Cruz Biotechnologies. After peroxide-coupled secondary antiserum (Amer sham Pharmacia), bands were detected using an enhanced chemiluminescent detection system (Amer sham).

Statistics. All analyses were done with statistical software SPSS 11.0 (SPSS Inc.).

Results

Clinical and pathologic characteristics of patient cohorts. A total of 40 patients have been accrued to date. Patients ranged from 44 to 76 years (mean, 60.2 years); age of patients in the control group ranged from 37 to 80 years (mean, 62 years). To be enrolled, men had to have clinically localized cancers with high risk of recurrence after radical prostatectomy, and the clinical and postoperative pathologic variables are as expected for such men. In the treated group, most tumors were large, and all had established extraprostatic extension.

Side effects. In general, patients tolerated the treatment well, with grade 1 and 2 toxicity. One patient had grade 3 gastrointestinal toxicity and recovered before surgery, another patient developed atrial fibrillation soon after surgery; both cases were possibly related to the study medication. We did not observe any increased surgical morbidity attributable to the study drug.

PSA changes with therapy. Pretreatment PSA, obtained at least 6 weeks after biopsy (15), was available for 8 patients for comparison to preoperative PSA taken immediately before surgery. Two of 8 patients had a >50% decrease in serum PSA after bortezomib treatment, and 3 others had PSA declines of 14%, 25%, and 45%, respectively. Two patients had increases (7% and 14%), whereas 1 had no change. See Supplementary Fig. S1.

Cytopathic effect of bortezomib therapy. Comparison of pretreatment biopsies with their respective posttherapy prostatectomies showed cytopathic changes attributable to bortezomib therapy. Figure 1A shows unaffacted prostate cancer. The most characteristic changes identified were cytology and nuclear pyknosis (Fig. 1B). The nuclei shrank and became pyknotic, in contrast to the normal round/ovoid shape with large nucleoli (Fig. 1B). The cytoplasm of the affected cells has a frothy, foamy, microgranular appearance, with dissolution of the cell membranes. Cytopathic effects were confined to tumor cells, and no visible cytopathic change was noted in benign tissue. The percentage of tumor with visible cytopathic effects was variable. Some tumors had little or no morphologic effect, whereas in other cancer, 10% to 15% of the tumor showed cytopathic effect. Figure 1A shows unaffected prostate cancer. The most characteristic changes identified were cytology and nuclear pyknosis (Fig. 1B). The nuclei shrank and became pyknotic, in contrast to the normal round/ovoid shape with large nucleoli (Fig. 1B).

Small interference RNA treatment and transient transfection. For the small interference RNA (siRNA) treatment and transient transfection, the small interference RNA (siRNA) experiments, PC3 and DU145 cells were seeded the night before transfection at such a density that cells reach ~60% to 70% confluence by the time of transfection. siSRC-3 SMART pool (40 nmol/L; Dharmaco) was used for transfection using Lipofectamine 2000 (Invitrogen). SMART pool siRNA control was used as a negative control. Transfected cells were continued in culture for 2 to 3 d before harvesting for further analyses. Subsequently, PC3 and DU145 cells were treated with SRC-3 siRNA for 2 d, then treated with or without bortezomib for another 2 d. The concentration of bortezomib for PC3 cells is 10 nmol/L and 1 μmol/L for DU145. The viable cells were measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphe- nyltetrazolium bromide assay.
of NF-κB were as expected. The mean expression of nuclear NF-κB in prostate cancer was lower in patients treated with bortezomib than comparable patients without treatment from the master TMA database (median, 4 versus 1.5; \( P = 0.0020 \); Fig. 2A). In contrast, NF-κB cytoplasmic expression was higher in patients treated with bortezomib than controls (median, 0 versus 6; \( P < 0.0001 \); Fig. 2B).

**Effects on growth, apoptosis, and proliferation.** Our results on the effect of induction of apoptosis in prostate cancer *in vivo* are in concordance with previously published *in vitro* and preclinical data (16, 17). As seen in Fig. 2C, the apoptotic rate in tumors was significantly higher in patients treated with bortezomib compared with similar TMA controls (median, 1.97 vs 0; \( P = 0.0006 \)). We were also expecting a decrease or no effect on proliferation. Surprisingly, we identified a significantly higher proliferative rate in treated patients (median, 0.32 versus 2.5; \( P = 0.0033 \)). To corroborate these surprising results, we obtained the preoperative biopsies in these same patients (available for 21 patients). The median proliferative index (Ki67 expression) in preoperative biopsies was 0.00, in contrast to 3.35 in the same patient’s bortezomib-treated tissues (\( P = 0.0097 \), Wilcoxon signed-ranks test). The proliferative index in tumors of patients treated with bortezomib increased compared with their matched preoperative biopsies. Note that all patients, except two, have increase in the proliferation index. One had no change and one decreased (Fig. 2D).

**Effects of bortezomib on proliferation and apoptosis in vitro.** To determine if bortezomib had similar effects on proliferation and apoptosis *in vitro*, we examined the effect of varying doses of bortezomib on proliferation (BrdUrd incorporation) in the LNCaP, DU145, and PC3 prostate cancer cell lines. As shown in Fig. 3A, bortezomib treatment increases BrdUrd incorporation in DU145 cells, and increased effects were seen with increased dose (Fig. 3B). Similar results were

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**Fig. 2.** Biological effects of bortezomib in prostate cancer tissues. **A** and **B**, cytoplasmic and nuclear NF-κB were quantified using immunohistochemistry as described in Materials and Methods. NF-κB cytoplasmic expression is increased in patients treated with bortezomib (B), whereas nuclear expression is decreased compared with controls (A). Boxplot shows the median. **C**, apoptotic rate and proliferative index were determined as described in Materials and Methods. Results for patients treated with bortezomib and controls are shown. Boxplot shows the median. **D**, proliferative index in tumors of patients treated with bortezomib (1) compared with their matched preoperative biopsies (0). Note that all patients, except 2, have increase in the proliferation index. One had no change and one decreased.
Differences were significant on day 6. Time and greater with increasing doses. Growth are more noticeable with increasing mean (of trypan blue excluding cells). Points, as day 1) for 6 d by hemacytometer counts. Viable cell number was added at the final concentration of 0, 0.5, 1, and 2 nmol/L. Viability cell count was determined from the next day (as indicated as day 1) for 6 d by hemacytometer counts of trypan blue – excluding cells. Points, mean (n = 3); bars, SD. The differences in growth are more noticeable with increasing time and greater with increasing doses. Differences were significant on day 6.

![Image](http://www.aacrjournals.org/clincancerres2008;14(22)november15,20087515)

Fig. 3. In vitro effects of bortezomib on growth, proliferation, and apoptosis. DU145 cells were inoculate in a 4-well chamber slide (Lab-Tek II; Fisher) at a density of 3 × 10^4 per well and incubated overnight, and bortezomib was added at the indicated concentration for 48 h. BrdUrd labeling and detection was done as described in Materials and Methods. A, the percentage of BrdUrd-positive cells represents the average of four random microscopic fields from experiment 2. Columns, mean; bars, SD. 0 μmol/L, 19.5% ± 2.7%; 0.5 μmol/L, 29.6% ± 3.9%; 1 μmol/L, 28.2% ± 5%; 2 μmol/L, 29.8% ± 5.1%. B, DU145 cells were inoculated at 1-well chamber slide (Lab-Tek II; Fisher) overnight, and bortezomib was added at the indicated concentration for 48 h. Apoptosis was assessed by the percentage of sub-G1 cells though DNA content by flow cytometry, relative to untreated cells. Experiments were done in triplicate. DU145 (C) and PC-3 (D) cells were seeded at an initial density of 1.5 × 10^4 per well in the 2-well chamber slide. Twenty-four hours later, bortezomib was added at the final concentration of 0, 0.5, 1, and 2 μmol/L. Viable cell number was determined from the next day (as indicated as day 1) for 6 d by hemacytometer counts of trypan blue – excluding cells. Points, mean (n = 3); bars, SD. The differences in growth are more noticeable with increasing time and greater with increasing doses. Differences were significant on day 6.

seen in PC3 and LNCaP cells (data not shown). At the same time, apoptosis, as assessed by flow cytometry, increased with increasing doses of bortezomib (Fig. 3B). Thus, bortezomib can promote both proliferation and apoptosis. To determine the net effect of these processes, growth curves were obtained using DU145 and PC3 cells and analyzed on day 1 through 6. Cells numbers increased with increasing doses (Fig. 3C and D). The differences were significant on day 6 for both cell lines. Decreased proteasome activity at these doses was confirmed by cytoplasmic entrapment of NF-κB using immunofluorescence as previously described (Supplementary Fig. S2; ref. 18). Furthermore, we show that both C-Myc and Cyclin-D1 are up-regulated in cells treated with bortezomib (Supplementary Fig. S6). This further substantiates our findings of increased proliferation in bortezomib-treated cells.

**Effects on the Akt pathway.** To determine if the changes in proliferation were mediated by activation of the PI3-kinase/Akt pathway, we analyzed the phosphorylated Akt (P-Akt) content in treated tissues. As shown in Fig. 4A, there was a significant increase in P-Akt expression in PCa treated with bortezomib compared with the controls (median, 3 versus 6: P = 0.0001). Subsequently, we analyzed the preoperative biopsies for P-Akt expression. Four patients had similar levels, all of which had initial indices of 9 and could not be higher. All patients that had lower initial indices showed increases in their cytoplasmic expression of P-Akt (Fig. 4B) that were significant on matched pair analysis (P = 0.0455).

**Bortezomib activates the Akt pathway via increased SRC-3.** It is known that SRC-3 is degraded by the 20S proteasome (19) and that SRC-3 can induce the activation of Akt signaling in prostate cancer cell lines (13, 20). We therefore evaluated the effect of bortezomib and a second proteasome inhibitor, MG132, on SRC-3 levels in PC3 cells. SRC-3 was significantly induced after 8 hours of treatment with 1 μmol/L bortezomib, and even 10 nmol/L bortezomib was able to significantly increase SRC-3 (Fig. 5A). Similar effects were seen in DU145 cells (data not shown). To examine the activation of the Akt pathway by bortezomib, we carried out a more detailed study in PC3 cells. As shown in Fig. 5B, SRC-3 protein levels are increased by 2 hours of bortezomib treatment, and this is accompanied by increases in Ser473 and Thr308 P-Akt. This is expected because we have shown previously that SRC-3 can activate the Akt pathway (21). These changes in the Akt pathway were accompanied by increased phosphorylation of Akt targets such as GSK3α (Ser21), GSK3β (Ser9), S6 kinase (Ser240/244; Fig. 5C), and FKHRL (Thr32). Bortezomib is known to increase heat shock proteins such as Hsp90 (22), which can play an important role in cell signaling by promoting conformational integrity of key signaling molecules. Thus, bortezomib might be acting via Hsp90 to increase P-Akt. As shown in Fig. 5B, Hsp90 is increased by bortezomib after 8 hours of treatment. However, P-Akt (Ser473 and Thr308) is increased within 2 hours of treatment, as is SRC-3, arguing that induction of SRC-3 is the dominant mechanism for activation of SRC-3, although Hsp90 activation may play a lesser role in this process. To confirm that SRC-3 was responsible for the increase in P-Akt, we knocked down SRC-3 using siRNA in PC3 cells before treatment with bortezomib (Fig. 5D). Pretreatment with SRC-3 siRNA...
abolished the ability of bortezomib to increase P-Akt. More importantly, the depletion of SRC-3 in prostate cancer cells augments bortezomib-induced cell death (Supplementary Fig. S4).

To confirm that SRC-3 protein is increased in prostate cancer tissue in men treated with bortezomib, we compared SRC-3 protein levels in treated patients to untreated controls by immunohistochemistry. Both cytoplasmic and nuclear SRC-3 were significantly increased in patients treated with bortezomib as predicted from the known biology of SRC-3 and our \textit{in vitro} results (Supplementary Fig. S5).

\textbf{Inhibition of Akt activation increases the effectiveness of bortezomib treatment.} Our data are consistent with the idea that increased P-Akt, via increased SRC-3 protein levels, decreases the effectiveness of bortezomib therapy. To test this hypothesis, we used the Akt inhibitor perifosine (23), which has shown activity against prostate cancer (23). As shown in Fig. 6A, treatment with both agents is markedly more effective than either agent alone in both PC3 and DU145 cells. This is associated with a complete inhibition of the bortezomib-induced increase in P-Akt in both cell lines (Fig. 6B). We analyzed the data by two-way ANOVA for the experiments for studying the combination agents of bortezomib and perifosine. The cell numbers were logarithmically transformed for stabilizing the variability. For PC3, the cell numbers after treated with either bortezomib alone or perifosine alone were significantly lower than these of control group ($P < 0.0001$ and $P < 0.0001$, respectively). However, there was no statistically significant evidence suggesting that there is any interaction between...
Unfortunately, the low mitotic index of prostate cancer and the increase in cell number in cells treated with bortezomib. There is also a strong correlation of SRC-3 expression in prostate cancer (13, 28). Additionally, there is a strong correlation of SRC-3 expression in cancer progression by activating multiple antiapoptotic and proliferative pathways (26). Thus, activation of Akt by bortezomib will almost certainly tend to inhibit the proliferation-promoting proteins (26). Thus, activation of Akt by bortezomib will almost certainly tend to inhibit the proliferation-promoting proteins (26). Thus, activation of Akt by bortezomib will almost certainly tend to inhibit the proliferation-promoting proteins (26). Thus, activation of Akt by bortezomib will almost certainly tend to inhibit the proliferation-promoting proteins (26).

**Discussion**

A major goal in cancer therapy is to develop targeted agents that disrupt abnormally regulated pathways in the malignant cell. Bortezomib targets the proteasome pathway, which is upregulated in prostate cancer and other malignancies. In this neoadjuvant clinical trial of bortezomib in men with aggressive but clinically localized prostate cancer, we found evidence of antitumor activity, with only moderate side effects. This antitumor activity was manifested by histologic evidence of tumor-specific cytopathic effect, significant drops in serum PSA in some patients, and significant increases in tumor apoptosis in treated tumors. This was associated with the predicted cytoplasmic entrapment of NF-κB and loss of nuclear NF-κB. Given the antiproteolytic functions of NF-κB, it is likely that at least some of the increased apoptosis seen in vivo is mediated by loss of NF-κB signaling (24).

In addition to the predicted antitumor effects noted above, we identified a significant increase in proliferation as assessed by Ki67 immunohistochemistry in bortezomib-treated patients compared with the TMA database. This was further substantiated by comparing the proliferation rate in the pretreatment biopsies, which were also lower. Ki67 is a marker for cells in proliferation; hence, the most likely explanation is that proliferation is increased in patients treated with bortezomib. An alternative explanation is that the increase in Ki67 protein could be due to inhibition of its breakdown by the proteasome. However, in myeloma cells, treatment with several different proteasome inhibitors led to decreased Ki67 expression, so it seems unlikely that Ki67 is degraded predominantly via the proteasome, although degradation partially via the proteasome cannot be excluded (25). Our in vitro data showing increased cell proliferation, as measured by BrdUrd incorporation, argues that in prostate cancer cells, bortezomib can both increase proliferation and apoptosis and the increased Ki67 actually reflects increased proliferation in vivo, which is confirmed by the increase in cell number in cells treated with bortezomib. Unfortunately, the low mitotic index of prostate cancer and concerns regarding treating patients with Bortezomib make alternative methods of measuring proliferation in vivo problematic.

Activation of the Akt pathway plays a key role in prostate cancer progression by activating multiple antiapoptotic and proliferation-promoting proteins (26). Thus, activation of Akt by bortezomib will almost certainly tend to inhibit the effectiveness of this therapy. A previous study has already shown that bortezomib induces Akt phosphorylation/activation and the Akt inhibitor Perifosine augments bortezomib-induced cytotoxicity in multiple myeloma cells (27).

We previously showed that the expression of SRC-3 is increased in clinically localized human prostate cancers and that increased expression is associated with PSA recurrence (13, 28). Additionally, there is a strong correlation of SRC-3 expression in cancer tissue with proliferation and an inverse correlation with apoptosis (13). There is also a very strong correlation between SRC-3 and P-Akt levels in prostate cancer tumors. Finally, it was previously shown that SRC-3 actually increases the expression of Akt in prostate cancer cells (20). Thus, SRC-3 can increase activated Akt activity by increasing Akt protein levels and, based on its known biological activities, P-Akt can in turn increase proliferation and inhibit apoptosis. SRC-3 is degraded by the 20S proteasome by interaction with the nonubiquitin-mediated REGγ pathway and thus should be increased by bortezomib (19), as we showed here, both in vitro and in the tissues of treated patients. Thus, whereas proteasome inhibition can enhance apoptosis, it also activates antiapoptotic and proliferative pathways, which may reduce its overall effectiveness.

There are two implications to these findings for clinical use of proteasome inhibitors. First, it is possible that prostate cancers with or low basal SRC-3 expression may be more sensitive to bortezomib treatment. If true, it would allow for targeted therapy based on preoperative measurements of SRC-3 levels in tumor cells. Second, concomitant treatment with agents that inhibit activity or activation of Akt are likely to enhance the activity of proteasome inhibitors in cancers that express SRC-3, as indicated by the in vitro results reported here.

The ultimate clinical utility of neoadjuvant bortezomib in benefiting these patients cannot be assessed currently. Although
we are encouraged by the shown antitumor activity, additional follow-up to monitor PSA recurrence rates compared with historical controls is required. It should be noted that a bortezomib is not associated with increased rates of surgical complications such as bleeding, infection, or poor wound healing, so it is safe in the preoperative setting with manageable side effects similar to those reported in nonoperative patients. Further preclinical and clinical studies of bortezomib in a neoadjuvant setting, particularly in combination with Akt inhibitors, seems warranted.

Disclosure of Potential Conflicts of Interest

M.P. Mims received commercial research support from Keryx.

References

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