Drug Interactions between the Proteasome Inhibitor Bortezomib and Cytotoxic Chemotherapy, Tumor Necrosis Factor (TNF) \(\alpha\), and TNF-Related Apoptosis-Inducing Ligand in Prostate Cancer

Jiabin An, Yi-Ping Sun, Julian Adams, Myrna Fisher, Arie Beldegrun, and Matthew B. Rettig

Veterans Administration Greater Los Angeles Healthcare System, Los Angeles, California 90073 [J. An, Y.-P. S., M. F., M. B. R.]; Millennium Pharmaceuticals, Inc., Cambridge, Massachusetts 02139 [J. Ad.]; and University of California at Los Angeles School of Medicine, Los Angeles, CA 90095 [A. B., M. B. R.]

ABSTRACT

**Purpose:** Proteasome inhibition has been shown to be an effective anticancer therapy in many tumor models, including prostate cancer. We sought to identify drug interactions between the proteasome inhibitor bortezomib and other apoptotic stimuli, including cytotoxic chemotherapy and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). In addition, we wanted to gain insight into the role of nuclear factor \(\kappa B\) inhibition as a mediator of bortezomib cytotoxic effects.

**Experimental Design:** Prostate cancer cell lines (LNCaP, LAPC4, CL1, and DU145) were treated with bortezomib and apoptotic stimuli (TRAIL, chemotherapy, and tumor necrosis factor \(\alpha\)), alone or in combination. Apoptosis and cell viability were measured, and median effect/combination index analyses were used to quantitate drug interactions. Nuclear factor \(\kappa B\) activity at baseline and in response to drug treatment was determined by gel shift and reporter gene assays.

**Results:** Bortezomib induced cell death of androgen-dependent (LNCaP and LAPC4) and androgen-independent (CL1 and DU145) prostate cancer cell lines, although androgen-dependent cells were more sensitive to proteasome inhibition. Bortezomib synergized with TRAIL and tumor necrosis factor \(\alpha\) to induce death in both androgen-dependent and androgen-independent cells.

Conclusions: Bortezomib and TRAIL represent a synergistic drug combination that warrants further evaluation in *in vivo* models of prostate cancer.

INTRODUCTION

The ubiquitin-proteasome pathway represents the principal mechanism whereby cytosolic proteins are degraded. Proteins degraded by the proteasome are targeted by ubiquitination, which subsequently results in protein degradation by the proteasome, a large complex of proteins that are the executioners of the degradation process. The degradation of proteins is crucial for maintenance of cellular homeostasis. The proteasome plays a critical role in modulating intracellular levels of proteins that are involved in cell cycle regulation, including cyclins and cyclin-dependent kinase inhibitors (1, 2). The proteasome also regulates the activity of signal transduction pathways, such as the NF-\(\kappa B\) pathway, in that the degradation of the inhibitor of nuclear factor-\(\kappa B\), the NF-\(\kappa B\) inhibitory protein, is also dependent on the ubiquitin-proteasome pathway (3). Degradation of tumor suppressor genes, such as p53, and oncogenes, including \(c-jun\) and \(c-myc\), is also modulated by the proteasome (4–6).

Proteasome inhibitors have been actively studied for their antitumor effects and have been shown to induce cytotoxicity of many tumor models both *in vitro* and *in vivo*. In prostate cancer, the proteasome inhibitor bortezomib is active against AD prostate cancer cells (LNCaP cell line) as well as AI lines (PC3 and DU-145; Refs. 7 and 8). In addition, bortezomib reduces prostate cancer tumor growth in murine tumor models (8). Importantly, the activity of proteasome inhibitors does not seem to be influenced by the low growth fractions of tumors, including prostate cancer, which is in contradistinction to cytotoxic chemotherapy, which is more often cell cycle dependent (8–10). In addition, proteasome inhibitors seem to induce cytotoxicity of prostate cancer cells independent of p53 status and *bcl-2* expression (11). Thus, proteasome inhibition represents a suitable approach to treatment of prostate cancer.

The interactions between proteasome inhibitors and other apoptotic stimuli in prostate cancer have not been studied. In other tumor models, such as colon cancer, proteasome inhibition sensitizes cancer cells both *in vitro* and *in vivo* to cytotoxic chemotherapy (12). TNF\(_\alpha\), TRAIL, and chemotheraphy consistently induce apoptosis of prostate cancer cells *in vitro*, although
the activity of these cytokines can vary depending on the cell line or hormone-dependency status (13–17). Thus, we investigated the potential of the proteasome inhibitor bortezomib to sensitize AD and AI prostate cancer cells to these apoptosis-inducing agents. In these studies, we specifically tested chemotherapeutic agents that are in clinical use for prostate cancer patients. In addition, because TNFα, TRAIL, and chemotherapy can activate NF-κB (13, 16, 18–22) and inhibition of NF-κB is thought to play a critical role in the mechanism of action of proteasome inhibitors, we also studied the relationship between activation of NF-κB and sensitization to bortezomib-induced death.

MATERIALS AND METHODS

Cell Culture and Prostate Cancer Cell Lines. AD LNCaP cells (American Type Culture Collection, Manassas, VA) and LAPC4 cells (a gift from Dr. Charles Sawyers, University of California, Los Angeles, CA) were maintained in RPMI 1640 supplemented with 10% fetal bovine serum and penicillin (100 µg/ml) and streptomycin (100 µg/ml). CL1 cells represent an AI subclone of LNCaP that was generated by culturing LNCaP in charcoal-stripped, androgen-depleted serum, as described (23). CL1 cells were maintained as for LNCaP cells, but continuously in charcoal-stripped serum. AI DU145 cells (American Type Culture Collection) were maintained in DMEM containing 10% fetal bovine serum and antibiotics. All culture media were purchased from Omega Scientific (Thousand Oaks, CA).

Reagents. The proteasome inhibitor bortezomib was provided by Millennium, Inc. (Cambridge, MA) and dissolved in DMSO. Recombinant human TNFα (R&D Systems, Minneapolis, MN) and recombinant human TRAIL (Calbiochem, San Diego, CA) were dissolved in PBS. Methyltrienolone (R1881) was purchased from New England Nuclear Life Science Products (Boston, MA) and dissolved in ethanol. For subsequent experiments, the final concentration of all solvents was maintained at 0.1%. A κB-responsive plasmid (p4x-κB-luc), in which four copies of the κB-response element drives expression of firefly luciferase, was purchased from Invitrogen (Carlsbad, CA). The pRL-SV40 plasmid, in which Renilla luciferase is constitutively expressed under the regulation of the SV40 promoter/enhancer, was purchased from Promega Corp. (Madison, WI) and was used for normalization of firefly luciferase activity.

Transient Transfections and Reporter Gene Assays. Cells were plated at 10^5 cells/well in 24-well plates the day before transfection. The plasmids were transfected with Lipofectamine Plus (Life Technologies, Inc., Gaithersburg, MD) according to the manufacturer’s instructions. Protein was extracted 48 h after transfection, and firefly and Renilla luciferase were measured on a TD20/20 tube luminometer (Turner Designs, Sunnyvale, CA) using a Dual Luciferase Assay kit (Promega Corp.), according to the manufacturer’s instructions. Firefly luciferase activity was normalized to Renilla luciferase expression.

EMSA. Wild-type and mutant κB oligonucleotide probes were purchased from Santa Cruz Biotechnology. Fifteen micrograms of nuclear protein were combined with end-labeled, double-stranded κB oligonucleotide probe, 1 µg of poly-dIdC (Amersham Pharmacia Biotech, Piscataway, NJ), 1 µg of BSA, and 5 mM spermidine in a final reaction volume of 20 µl for 20 min at room temperature. The DNA protein complex was run on a 4% nondenaturing polyacrylamide gel with 0.4 Tris-borate EDTA running buffer before subsequent autoradiography. Cold-competition experiments were performed with a 100-fold molar excess of cold wild-type or cold mutant κB oligonucleotides. For supershift assays, nuclear protein was preincubated with specific or control antibodies (6 µg) for 20 min at room temperature.

Assessment of Cytotoxicity. LNCaP, LAPC4, CL1, and DU145 cells were seeded in 96-well plates the day before chemical treatment at concentrations of 4 × 10^4, 4 × 10^3, 1 × 10^4, and 2 × 10^4 cells per well, respectively. Various combinations of bortezomib and apoptotic stimuli were added to cells, and 48 h later, cell viability was assessed by the MTT assay. All experiments were performed in quadruplicate.

Assessment of Apoptosis. Cells were plated in 10-cm dishes (2 × 10^5 cells/dish). The next day, cells were treated with various concentrations of bortezomib or vehicle control for 48 h. Cells were harvested and then stained with an annexin V-FITC kit (BD Biosciences, Palo Alto, CA) according to the manufacturer’s instructions. Cells were analyzed on a Becton Dickinson FACS Caliber flow cytometer with CellQuest software (BD Biosciences). Median Effect/CI Isobologram Method for Multiple Drug Effect Analysis. The effect of drug combinations on cytotoxicity was performed by the median effect method using Calcsyn software, version 1.1.1 (Biosoft, Ferguson, MO; Ref. 24). CI values were calculated using the most conservative assumption of mutually nonexclusive drug interactions. CI val-

Fig. 1 Cytotoxicity of bortezomib in AD and AI prostate cancer cell lines. Prostate cancer cell lines were plated in 96-well plates. Bortezomib or vehicle control was added the next morning. After 48 h, MTT assays were performed. Results are means of four experiments +/− SD and are normalized to that of vehicle control.

Table 1 IC_{50} values (µM) for bortezomib

<table>
<thead>
<tr>
<th></th>
<th>IC_{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAPC4</td>
<td>0.054</td>
</tr>
<tr>
<td>LNCaP</td>
<td>0.0070</td>
</tr>
<tr>
<td>CL1</td>
<td>0.42</td>
</tr>
<tr>
<td>DU145</td>
<td>1.7</td>
</tr>
</tbody>
</table>
ues were calculated from median results of the cytotoxicity (MTT) assays, which were performed in quadruplicate. CI values significantly greater than 1 indicate drug antagonism, CI values significantly less than 1 are indicative of synergy, and CI values not significantly different than 1 indicate an additive drug effect. Linear regression correlation coefficients of the median effects plots were required to be >0.90 to demonstrate that the effects of the drugs follow the law of mass action, which is required for a median effect analysis.

RESULTS
Cytotoxicity of Bortezomib. We confirmed the ability of bortezomib to induce cytotoxicity as measured by a MTT assay in AD (LNCaP and LAPC4) and AI (CL1 and DU145) prostate cancer cell lines. After 48 h of treatment, bortezomib potently induced cytotoxicity of all cell lines tested (Fig. 1). The IC_{50} values were substantially lower for the AD cell lines compared with the AI cell lines (Table 1). Induction of apoptosis was confirmed in all four cell lines by annexin V staining. Fig. 2 shows a representative experiment in CL1 cells treated with various concentrations of bortezomib. As shown in Fig. 2, a concentration-dependent increase in apoptosis was identified, which correlated with the effective concentrations of bortezomib in the MTT assays (Fig. 1).

Androgen Does Not Protect AD Prostate Cancer Cells from Bortezomib. Androgens have been shown to protect androgen-responsive prostate cancer cells from apoptotic stimuli, including Fas activation and TNFα (25). Consequently, we tested whether androgen could block bortezomib-induced apoptosis in the AD cell lines LAPC4 and LNCaP. Pilot studies demonstrated that the optimal concentration of the synthetic androgen R1881 to induce reporter gene expression driven by the androgen-response element was 1.0 nM for LAPC4 and 0.1 nM for LNCaP cells. Pretreatment of LAPC4 and LNCaP cells
with R1881 had no effect on protecting cells from bortezomib-induced apoptosis/death (data not shown). In these experiments, cells were stained with annexin V to detect cells in the early stages of apoptosis and with propidium iodide to identify dead cells.

**Proteasome Inhibition Sensitizes AD and AI Prostate Cancer Cells to TRAIL-induced Cytotoxicity.** TRAIL is a member of the TNF superfamily that induces apoptosis in tumor cell lines by engaging and activating death receptors (26–28). We studied the ability of bortezomib to sensitize AD and AI prostate cancer cells to TRAIL-induced death. TRAIL (0–200 ng/ml) alone had virtually no cytotoxic effect on LNCaP and CL1 cells and only modest cytotoxic effects on LAPC4 and DU145 cells, as measured in a MTT assay (Fig. 3). However, when bortezomib and TRAIL were used in combination, median effect analysis demonstrated synergistic interactions across all concentrations of both bortezomib and TRAIL in both AD (LNCaP and LAPC4) and AI (DU145) cell lines (Fig. 4). Up to 90% cell death was observed at concentrations well below those that induced death by either agent alone in both AD and AI cells (Fig. 3). Bortezomib and TRAIL were antagonistic in CL1 cells (Fig. 4).

Like TRAIL, TNFα can induce apoptosis in a wide variety of cells by activating signaling pathways through death receptors (16, 29). Thus, we also tested the drug interactions between TNFα and bortezomib. By itself, TNFα (20 ng/ml) had modest cytotoxic effects on AD LNCaP and LAPC4 cells, as measured by the MTT assay, but no significant effect on AI CL1 and DU145 cell lines (data not shown). Combinations of TNFα and bortezomib demonstrated similar interactions by median effect analysis as TRAIL and bortezomib, although the synergy between TNFα and bortezomib was observed in CL1 cells rather than DU145 cells (Fig. 4). The synergistic interaction between TNFα and bortezomib in CL1 cells was of borderline statistical significance (P = 0.08).

Because the effects of drug interactions may be schedule dependent, we studied the effects of varying the duration of preincubation with bortezomib. In the experiments described above, cells were exposed to bortezomib for 90 min before the addition TRAIL or TNFα. When we used CL1 cells to study the effects of extending the preincubation period to 24 h, we did not observe any difference in the drug interaction profiles between bortezomib and either TRAIL or TNFα (data not shown). In addition, reversing the sequence of drugs by adding TRAIL or TNFα for 24 h before bortezomib did not affect the drug interaction data (data not shown).

**Bortezomib Does Not Sensitize Prostate Cancer Cells to Cytotoxic Chemotherapy.** We studied the interactions between bortezomib and cytotoxic chemotherapeutic agents (doxorubicin, paclitaxel, and vinblastine) used in the treatment of prostate cancer patients and their potential interaction with bortezomib. Paclitaxel and vinblastine as single agents were significantly more cytotoxic than doxorubicin (Table 2 and Fig. 5). When we assessed the effects of drug combinations by median effect/CI analysis, there were no consistent interactions for any of the chemotherapeutic agents and bortezomib (Fig. 6). None of the drugs displayed synergy across the many concentrations tested. As described for TRAIL and TNFα, neither a more prolonged preincubation with bortezomib (24 h versus 90

---

Fig. 3 Cytotoxicity of combinations of TRAIL or TNFα and bortezomib in AD and AI prostate cancer cells. Prostate cancer cells were plated in the 96-well format and incubated overnight. TRAIL or TNFα alone or in combination with bortezomib or appropriate vehicle controls were added at the indicated concentrations for 48 h, and cell viability was measured by the MTT assay. For drug combinations, bortezomib was added 90 min before TRAIL. Experiments were performed in quadruplicate and are reported as means +/- SD. A, LNCaP; B, LAPC4; C, CL1; D, DU145.
min) before chemotherapy treatment nor reversing the order with which we added the chemotherapeutic agents and bortezomib influenced the cytotoxicity or median effect analysis of drug interactions (data not shown).

The Relationship between NF-κB Activation and Effects of Bortezomib Alone or in Combination with other Cytotoxic Agents. Inhibition of NF-κB has been postulated as a mechanism of action of bortezomib. We assayed NF-κB activation by EMSA in CL1 cells and found that a concentration of 10 μM bortezomib was required to inhibit basal NF-κB activation (Fig. 7A). Modest reduction in TNFα-induced NF-κB activation was observed at 0.25 μM, and further, but not complete, inhibition was observed at a concentration of up to 10 μM.

**Table 2** IC₅₀ values (nM) for chemotherapy

<table>
<thead>
<tr>
<th></th>
<th>CL1</th>
<th>DU145</th>
<th>LNCaP</th>
<th>LAPC4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>1.2 × 10⁴</td>
<td>1.5 × 10⁴</td>
<td>9.1 × 10³</td>
<td>2.9 × 10⁴</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>32</td>
<td>5.6 × 10³</td>
<td>1.5 × 10²</td>
<td>1.5 × 10²</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>22</td>
<td>0.08</td>
<td>93</td>
<td>58</td>
</tr>
</tbody>
</table>

**Fig. 4** Drug interactions between TRAIL or TNFα and bortezomib. Data were analyzed by the median effect/CI method (see “Materials and Methods” for details). CI values >1 indicate antagonistic effect, CI values <1 indicate synergy, and CI values =1 indicate an additive effect. Note the logarithmic scales. Ps are two tailed and represent differences between all CI values and 1.

**Fig. 5** Cytotoxicity of combinations of chemotherapy and bortezomib in AD and AI prostate cancer cells. Prostate cancer cells were plated in the 96-well format and incubated overnight. Chemotherapy (doxor, doxorubicin; pacl, paclitaxel; vinb, vinblastine), bortezomib, or appropriate vehicle controls were added at the indicated concentrations for 48 h, and cell viability was measured by the MTT assay. For drug combinations, bortezomib was added 90 min before the indicated chemotherapeutic agent. Experiments were performed in quadruplicate and are reported as means ± SD. Units of concentration are nanomoles for paclitaxel and vinblastine and micromolars for doxorubicin. A, LNCaP; B, LAPC4; C, CL1; D, DU145.
bortezomib (Fig. 7A). Similar results were obtained for all other cell lines (data not shown). The bandshift pattern was characterized with the addition of various antibodies to the EMSA reaction and demonstrated that the NF-κB protein complexes consisted of a p65/p50 heterodimer and a p50/p50 homodimer (Fig. 7B). We also used a reporter gene assay to assess bortezomib-mediated inhibition of basal NF-κB activity. Bortezomib induced partial blockade of basal κB-driven reporter gene expression at 0.1 μM and complete inhibition at 10 μM (Fig. 7C). Given that the IC₅₀ of bortezomib is 0.42 μM in CL1 cells, there is a good correlation between the concentrations of bortezomib required to induce cytotoxicity and inhibit NF-κB.

We next investigated the importance of heightened NF-κB activation induced by apoptotic stimuli as a determinant of sensitivity to bortezomib. Fig. 8 shows the effects of various apoptotic stimuli on activation of NF-κB in DU145 cells. We demonstrated that neither chemotherapy nor TRAIL activated NF-κB, whereas TNFα did augment NF-κB activity. Similar results were obtained for the other cell lines (data not shown). Thus, given the aforementioned drug interactions with bortezomib, there does not seem to be a correlation between the ability of an apoptotic stimulus to activate NF-κB and to synergize with bortezomib.

DISCUSSION

Proteasome inhibition has been demonstrated to be an effective antitumor agent for prostate cancer both in vitro and in mouse models (7, 12, 30, 31). In this study, we evaluated the potential synergistic effects of the proteasome inhibitor bortezomib alone and in combination with various apoptotic stimuli. Our results demonstrate that bortezomib was synergistic with TRAIL and TNFα, but chemotherapeutic agents had no consistent effect. We have shown that bortezomib is a potent apoptotic stimulus in LNCaP and DU145 cells, as described previously (7, 32), as well as the LAPC4 and CL1 prostate cancer cell lines. Interestingly, the synthetic androgen R1881, which blocks apoptosis induced by Fas activation and TNFα (25), did not prevent bortezomib-mediated apoptosis. This suggests that bortezomib may not require androgen deprivation to achieve its maximal effect in patients with prostate cancer.

The concentrations of bortezomib required to inhibit NF-κB correlated with those required to induce cytotoxicity. In addition, the IC₅₀ values for bortezomib were substantially higher for AI, which manifest significantly greater basal NF-κB activity compared with their AD counterparts (19, 20, 33, 34). These latter results indicate that NF-κB activation status may represent one factor that predicts for sensitivity to bortezomib. However, many other factors are likely to be involved, especially given the understanding that there are a multitude of differences in the biochemical and gene expression profiles of AD and AI prostate cancer cells and that bortezomib affects the degradation of a multitude of proteins that regulate proliferation and apoptosis.

TRAIL is a death receptor ligand that effectively induces apoptosis in a wide variety of tumor types, although the studies in prostate cancer have yielded variable results (13, 35–37). TRAIL seems to spare normal tissues from its apoptotic effects, although hepatotoxicity caused by TRAIL has been noted when TRAIL is synthesized with an epitope tag for the purpose of
performed in triplicate and are reported as means ± SD.

Fig. 7  Bortezomib inhibits both basal and TNFα-induced NF-κB activation. A, EMSA for NF-κB activity. CL1 cells (5 × 10⁵ cells) were plated in 150-mm dishes and incubated overnight. Cells were then pretreated with bortezomib or vehicle control for 90 min before the addition of TNFα (20 ng/ml) for 30 min. Nuclear protein was then harvested for EMSA. Cold competition experiments were performed with a 100-fold molar excess of cold wild-type or mutant κB probes. B, EMSA on CL1 cells. Cells were plated as in A but treated with TNFα or vehicle control only. EMSA was performed as in A, but nuclear extracts were preincubated with the indicated antibodies. Note that the p65 and p50 antibodies bind to the DNA-binding domains of the p65 and p50, respectively, and consequently cause abrogation of the shifted band rather than a supershift. C, NF-κB reporter gene assay. CL1 cells were transfected with the p4x-κB-luc reporter construct (and pRL-SV40 for normalization of transfection efficiency). After 24 h, cells were treated with bortezomib at the indicated concentrations. After an additional 24 h, Dual Luciferase assays were performed. Experiments were performed in triplicate and are reported as means ±/− SD.

purification (38, 39). Recombinant TRAIL produced without an epitope tag has been successfully administered to animals, including mice and primates, without significant systemic toxicity (38, 39). Because of the promise of both TRAIL and bortezomib as single antitumor agents, we tested drug interactions between these compounds. We found TRAIL and bortezomib synergize to induce cytotoxicity in LNCaP, LAPC4, and DU145 cells across many concentrations. The synergistic interaction was quite significant for LAPC4, LNCaP, and DU145, as exemplified by the fact that the combination of TRAIL and bortezomib induced >90% cytotoxicity at concentrations that induced minimal cytotoxicity with either drug alone. Moreover, because synergy was observed in AD (LNCaP and LAPC4) and AI (DU145) cell lines, hormone-dependency status does not seem to be a predictor for synergistic interactions between TRAIL and bortezomib. However, given that TRAIL and bortezomib may be antagonistic in some cell prostate cancer cells (i.e., CL1 cells), there must be molecular and/or biochemical predictors for synergy between TRAIL and bortezomib.

Similar to TRAIL, synergistic interactions between bortezomib and TNFα were observed for AD cell lines. In addition, the combination of bortezomib and TNFα demonstrated modest synergy in CL1 cells, whereas bortezomib and TRAIL potently synergized in DU145 cells. The discrepancy in synergy between bortezomib and these two death ligands in DU145 and CL1 cells cannot be attributed to differences in NF-κB activation, because both cell lines manifested similar baseline and induced NF-κB activation. It is possible that the ability of TNFα and TRAIL to activate the extrinsic caspase cascade via binding to their respective cellular receptors varies among different cell lines.

When bortezomib was used in combination with cytotoxic chemotherapeutic agents (i.e., doxorubicin, paclitaxel, and vinblastine) that are commonly used in the treatment of patients with prostate cancer, we did not observe any consistent synergistic interactions. We rigorously assessed the effects of these drug interactions with the use of median effect/CI analysis. These drug interactions observed in prostate cancer are in contradistinction to the synergistic interaction between bortezomib and cytotoxic chemotherapy in colon cancer (12). Interestingly, a synergistic interaction between bortezomib and chemotherapy in colon cancer was observed for SN38, the active metabolite of the topoisomerase I inhibitor irinotecan, which activates NF-κB in colon cancer cells (21). However, none of the chemotherapeutic agents we tested augmented NF-κB activity in prostate cancer cells. Given that NF-κB inhibition represents a putative mechanism of action of bortezomib, the discrepancy in the drug interactions between bortezomib and chemotherapy in colon and prostate cancer may, thus, reflect the difference in the ability of chemotherapy to induce NF-κB in these tumor models and/or that resistance to chemotherapy in prostate cancer is mediated by biochemical pathways other than NF-κB. In addition, we excluded the possibility that the outcome of our drug interaction studies was the result of the scheduling of drug exposure. In particular, neither a more prolonged preincubation with bortezomib (24 h versus 90 min) nor reversing the order with which we added the bortezomib and the other agents influenced the cytotoxicity or median effect analysis of drug interactions.

In summary, bortezomib is active in AD and AI prostate cancer cell lines, although AD cell lines, which have lower levels of basal NF-κB activity, seem to be more sensitive to proteasome inhibition. In addition, androgen does not protect prostate cancer cells from bortezomib. No consistent interactions were demonstrated between bortezomib and cytotoxic chemotherapeutic agents, including paclitaxel, doxorubicin, and vinblastine. Bortezomib synergizes with TRAIL and TNFα to inhibit growth of prostate cancer cells. Although TNFα causes major toxicity when administered systemically to animals, TRAIL has been given safely to animals (38, 39). Thus, bortezomib and TRAIL represent a drug combination that should be explored in in vivo models.
Bortezomib in Prostate Cancer

ACKNOWLEDGMENTS

We are grateful to Drs. Mark Pegram and Gottfried Konecny for assistance in median effect/combination index analyses.

REFERENCES


Drug Interactions between the Proteasome Inhibitor Bortezomib and Cytotoxic Chemotherapy, Tumor Necrosis Factor (TNF) α, and TNF-Related Apoptosis-Inducing Ligand in Prostate Cancer

Jiabin An, Yi-Ping Sun, Julian Adams, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/9/12/4537

Cited articles
This article cites 37 articles, 20 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/9/12/4537.full#ref-list-1

Citing articles
This article has been cited by 13 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/9/12/4537.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.