The proteasome inhibitor NPI-0052 is a more effective inducer of apoptosis than bortezomib in lymphocytes from patients with chronic lymphocytic leukemia

Stacey Ruiz,1 Yelena Krupnik,2 Michael Keating,2 Joya Chandra,3 Michael Palladino,4 and David McConkey1

Departments of 1Cancer Biology, 2Leukemia, and 3Pediatric Research, The University of Texas M.D. Anderson Cancer Center, Houston, Texas and 4Nereus Pharmaceuticals, Inc., San Diego, California

Abstract
Proteasome inhibitors are potent inducers of apoptosis in isolated lymphocytes from patients with chronic lymphocytic leukemia (CLL). However, the reversible proteasome inhibitor bortezomib (PS-341; Velcade) did not display substantial antitumor activity in CLL patients. Here, we compared the effects of bortezomib and a new irreversible proteasome inhibitor (NPI-0052) on 20S chymotryptic proteasome activity and apoptosis in isolated CLL cells in vitro. Although their steady-state (3 hours) IC50s as proteasome inhibitors were similar, NPI-0052 exerted its effects more rapidly than bortezomib, and drug washout experiments showed that short exposures to NPI-0052 resulted in sustained (≥24 hours) 20S proteasome inhibition, whereas 20S activity recovered in cells exposed to even 10-fold higher concentrations of bortezomib. Thus, brief (15 minutes) pulses of NPI-0052 were sufficient to induce substantial apoptosis in CLL cells, whereas longer exposure times (≥8 hours) were required for commitment to apoptosis in cells exposed to equivalent concentrations of bortezomib. Commitment to apoptosis seemed to be related to caspase-4 activation, in that cells exposed to bortezomib or NPI-0052 could be saved from death by addition of a selective caspase-4 inhibitor up to 8 hours after drug exposure. Our results show that NPI-0052 is a more effective proapoptotic agent than bortezomib in isolated CLL cells and suggest that the chemical properties of NPI-0052 might also make it an effective therapeutic agent in CLL patients. [Mol Cancer Ther 2006;5(7):1836–43]

Received 2/2/06; revised 4/12/06; accepted 5/12/06. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: David McConkey, Department of Cancer Biology, The University of Texas M.D. Anderson Cancer Center, Box 173, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: 713-792-8591; Fax: 713-792-8747. E-mail: dmcconke@mdanderson.org

Copyright © 2006 American Association for Cancer Research. doi:10.1158/1535-7163.MCT-06-0066
apoptosis via mechanisms that are unique from those evoked by bortezomib (19). NPI-0052 has undergone extensive preclinical toxicity studies and entered phase I this year.

The ability of bortezomib to bypass BCL-2-mediated resistance prompted investigators to evaluate its effects on apoptosis in isolated CLL lymphocytes (20–24). Overall, these preclinical studies confirmed that proteasome inhibitors display uniquely high potency against untreated and fludarabine-refractory CLL cells in vitro (24). However, in a recently completed phase II trial of the drug in patients with fludarabine-refractory CLL, the clinical activity of the drug failed to match preclinical expectations (21). Given that bortezomib displays a very short serum half-life, we wondered whether the lack of clinical activity of the drug might be related to the fact that bortezomib is a reversible proteasome inhibitor that is cleared from the serum within minutes. We speculated that the properties of NPI-0052 as an irreversible proteasome inhibitor might make it a more potent inducer of apoptosis in this disease model. The present study was undertaken to address this hypothesis.

Materials and Methods

Reagents

Bortezomib was purchased from The University of Texas/M. D. Anderson Cancer Center pharmacy (Houston, TX). NPI-0052 was provided by Nereus Pharmaceuticals (San Diego, CA). The chymotryptic activity substrate (Suc-LLVY-AMC) was purchased from A.G. Scientific, Inc. (San Diego, CA), the caspase-like substrate (Z-LLE-AMC) was from A.G. Scientific, the tryptic substrate (Boc-LLR-AMC) was from BioMo (Plymouth Meeting, PA), and the anti-procaspase-4 antibody was purchased from Stressgen (Victoria, British Columbia, Canada).

Isolation of CLL Cells

Freshly isolated peripheral blood from CLL patients were fractionated by Ficoll-Paque (Pharmacia Biotech, Piscataway, NJ) sedimentation. The mononuclear cellular layer was then resuspended in RPMI 1640 containing inactivated fetal bovine serum, 10 mmol/L HEPES (pH 7.5), sodium pyruvate, L-glutamine, and antibiotics.

Quantification of Apoptosis

Apoptosis was measured by propidium iodide (PI) staining and fluorescence-activated cell sorting (FACS) analysis as described previously (24). Harvested primary CLL cells were pelleted by centrifugation and resuspended in a PBS containing 50 μg/mL PI, 0.1% Triton X-100, and 0.1% sodium citrate. Samples were stored at 4°C for 16 hours and gently vortexed before FACS analysis (FL-3 channel).

20S Proteasome Activity Assay

CLL cells were pelleted by centrifugation and washed once in PBS. Cells were then resuspended in cold lysis buffer [20 mmol/L Tris (pH 7.5), 0.1 mmol/L EDTA (pH 8.0), 20% glycerol, 0.05% NP40, 1 mmol/L 2mercaptoethanol, 1 mmol/L ATP] and frozen and thawed thrice on dry ice followed by centrifugation at 1,500 rpm for 1 minute. Lysates were then transferred to a 96-well plate, in which substrate buffer [50 mmol/L HEPES (pH 7.5), 5 mmol/L EGTA (pH 7-8) containing 50 μmol/L peptide substrate] was added at a 1:1 dilution and mixed by pipetting up and down. Samples were read 1 hour after addition of substrate buffer. Fluorescence was read on a SpectraMax Gemini EM fluorimeter (Molecular Devices, Sunnyvale, CA) at an excitation wavelength of 380 nm and an emission wavelength of 465 nm.

Table 1. Clinical characteristics of the CLL lymphocytes used in this study

<table>
<thead>
<tr>
<th>Patient</th>
<th>Rai*</th>
<th>WBC</th>
<th>β2M</th>
<th>ZAP-70†</th>
<th>Previous treatment‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>25</td>
<td>5.2</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>254.5</td>
<td>5.3</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>II</td>
<td>190</td>
<td>3.9</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>I</td>
<td>75.9</td>
<td>3.3</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>II</td>
<td>135.3</td>
<td>1.6</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>I</td>
<td>64</td>
<td>5.5</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>II</td>
<td>42.3</td>
<td>4</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>89.9</td>
<td>3.3</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>34.2</td>
<td>3.1</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>III</td>
<td>338</td>
<td>4.3</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>92.4</td>
<td>2.7</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>III</td>
<td>72.2</td>
<td>7.7</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>102.8</td>
<td>5.7</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>37.4</td>
<td>2</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>53.9</td>
<td>3.2</td>
<td>ND</td>
<td>FNDx2</td>
</tr>
<tr>
<td>16</td>
<td>I</td>
<td>88.5</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>0</td>
<td>103.7</td>
<td>2.2</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>0</td>
<td>141</td>
<td>3.4</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>II</td>
<td>98.4</td>
<td>3.3</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>IV</td>
<td>47.1</td>
<td>2.2</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>I</td>
<td>54.9</td>
<td>ND</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>0</td>
<td>141</td>
<td>2.6</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>IV</td>
<td>73</td>
<td>6.2</td>
<td>ND</td>
<td>IDEC</td>
</tr>
<tr>
<td>24</td>
<td>I</td>
<td>27.7</td>
<td>1.9</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>I</td>
<td>57.2</td>
<td>ND</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>IV</td>
<td>35.6</td>
<td>5.6</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>0</td>
<td>90.7</td>
<td>4</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>I</td>
<td>140</td>
<td>3.4</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>I</td>
<td>56.9</td>
<td>3.3</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>II</td>
<td>123.6</td>
<td>1.7</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>I</td>
<td>69</td>
<td>3.3</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>I</td>
<td>84.2</td>
<td>2.2</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>0</td>
<td>26</td>
<td>1.4</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>I</td>
<td>27.5</td>
<td>2.7</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>II</td>
<td>254.5</td>
<td>5.3</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>0</td>
<td>29.1</td>
<td>2.5</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>II</td>
<td>70</td>
<td>3.8</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Lymphocytes were isolated from peripheral blood mononuclear cells as described in Materials and Methods. Note that, although the cells were heterogeneous in terms of Rai stage and ZAP-70 status, almost all of them had not received previous chemotherapy. Levels of apoptosis induced by the proteasome inhibitors did not correlate with any of the variables shown above (data not shown), consistent with our previous work (22).

Abbreviations: β2M, β2 microglobulin levels; ND, not determined; IDEC, IDEC-152 (αCD23, lumilisib); FNDx2, fludaravine + mixoxantrone (novantrone) + dexamethasone.

*Tumor stage.

†Indicates whether a given isolate expressed the T-cell-associated ZAP-70 protein tyrosine kinase.

‡Previous chemotherapy (protocol is indicated, all others were untreated).
an emission wavelength of 460 nm. Protein content was determined for each sample using the Bradford reagent (Bio-Rad, Hercules, CA), and fluorescent units were standardized to the protein concentration in each sample.

Detection of Procaspase-4 by Immunoblotting
Cells (1 × 10^5) were incubated with 10 nmol/L bortezomib or 10 nmol/L NPI-0052 in the presence or absence of the caspase-4-selective inhibitor LEVDfmk or the pan-caspase inhibitor zVADfmk for 16 hours. Cells were harvested by centrifugation and lysed as described previously (25). Approximately 25 μg total cellular protein from each sample were subjected to SDS-PAGE, proteins were transferred to nitrocellulose membranes, and the membranes were blocked with 5% nonfat milk in a TBS solution containing 0.1% Tween 20 for 1 hour. The blots were then probed overnight with anticaspase-4 (StressGen), washed, and probed with a donkey anti-rabbit secondary antibody coupled to horseradish peroxidase. Immunoreactive material was detected by enhanced chemiluminescence (West Pico, Pierce Biotechnology, Inc., Rockville, IL). Blots were then reprobed with a polyclonal antibody specific for actin (Sigma Chemical Corp., St. Louis, MO) to confirm equal protein loading.

Statistical Analyses
All statistics were done using GraphPad Instat, version 3.05 (GraphPad Software, Inc., San Diego, CA). Mean comparisons were evaluated for significance using the paired t test.

Results
Effects of Bortezomib and NPI-0052 on 20S Proteasome Activity
We compared the time- and concentration-dependent effects of bortezomib and NPI-0052 on 20S proteasome chymotryptic activity in isolated CLL cells (Table 1). First, we exposed CLL cells to increasing concentrations of each drug (0.1-25 nmol/L) for 3 hours, prepared cell extracts, and incubated them with fluorogenic peptide substrates that measure the chymotryptic, caspase-like, and tryptic activities of the 20S proteasome. The results showed that a 10 nmol/L concentration of either drug produced maximal inhibition of the chymotryptic activity of the proteasome (Fig. 1A). Because bortezomib is rapidly cleared from the serum, we next compared the effects of increasing concentrations of bortezomib and NPI-0052 on 20S proteasome activity under steady-state conditions (3 h; A). Because bortezomib is rapidly cleared from the serum, we next compared the effects of increasing concentrations of bortezomib and NPI-0052 on 20S proteasome activity.

Figure 1. Concentration-dependent effects of bortezomib and NPI-0052 on 20S proteasome activity. A, steady-state inhibition of 20S activity was determined by incubating CLL cells with the indicated concentrations of bortezomib or NPI-0052 for 3 h, and 20S activity was quantified as described in Materials and Methods. Columns, mean (n = 4; patients 31-34); bars, SE. B, the concentration-dependent acute effects of bortezomib and NPI-0052 were determined by incubating cells with the indicated concentrations of each drug for 5 min and quantifying 20S activity as described in Materials and Methods. Columns, mean (n = 3; patients 15-17); bars, SE. C, cells were exposed to 10 nmol/L bortezomib or NPI-0052 for the times indicated, and 20S activity was measured as described in Materials and Methods. Columns, mean (n = 5; patients 18-22); bars, SE. Note that 10 nmol/L bortezomib or NPI-0052 produced indistinguishable effects on 20S activity under steady-state conditions (3 h; A). D, effects of bortezomib or NPI-0052 on the caspase-like or tryptic activities of the proteasome. Cells were exposed to 10 nmol/L of each inhibitor for 3 h, and 20S proteasome activities were measured as described in Materials and Methods. Columns, mean (n = 4; patients 27-30); bars, SE.
chymotryptic activity measured 5 minutes after cell exposure. NPI-0052 significantly inhibited the proteasome at 10 nmol/L, whereas 10-fold higher concentrations of bortezomib were required to produce the same effect (Fig. 1B). A more complete kinetic analysis of the effects of bortezomib showed that 30 minutes of exposure to the drug (10 nmol/L) produced significant proteasome inhibition but that maximal inhibition was only achieved after 1 hour (Fig. 1C).

Recent studies in multiple myeloma indicate that bortezomib and NPI-0052 exhibit unique effects on the caspase-like and tryptic activities of the 20S proteasome, which may explain their different mechanisms of action in multiple myeloma cells (19). We therefore compared the effects of 10 nmol/L bortezomib and 10 nmol/L NPI-0052 on the caspase-like and tryptic activities of the proteasome in whole CLL cells. Consistent with the results obtained in the earlier report with peripheral blood mononuclear cells exposed to the drugs in vivo, NPI-0052 produced greater inhibition of the trypsin-like activity of the proteasome than bortezomib, whereas the drugs had more similar (partial) effects on caspase-like activity (Fig. 1D). Neither the trypsin-like activity nor the caspase-like activity was inhibited as completely as the chymotryptic activity was, which is also consistent with the previous in vivo results (19).

We then did washout experiments to assess the durability of the proteasome inhibition produced by bortezomib or NPI-0052. Because bortezomib is a reversible inhibitor and NPI-0052 is thought to be irreversible, we expected that the effects of the latter would be more sustained. Consistent with our expectations, short (15 minutes) pulses of NPI-0052 (10 or 100 nmol/L) were sufficient to produce maximal 20S proteasome inhibition, measured 24 hours after drug exposure (Fig. 2A). In contrast, 15 minutes of exposure to bortezomib had no significant sustained effect on 20S proteasome inhibition (Fig. 2A), and proteasome activity also largely recovered in cells exposed to the drug for 2 hours (Fig. 2B).

**Effects of Bortezomib and NPI-0052 on Apoptosis**

We exposed CLL cells continuously to increasing concentrations of bortezomib or NPI-0052 and quantified apoptosis by PI/FACS 24 hours later. Bortezomib induced a modest increase in apoptosis at concentrations as low as 1 nmol/L with more significant cell death observed at concentrations of 10 nmol/L and higher (Fig. 3A). Although 1 nmol/L NPI-0052 did not induce cell death, NPI-0052 displayed activity very similar to bortezomib at higher concentrations (Fig. 3A). Thus, there was a close correlation between the concentration-dependent effects of both drugs on steady-state (3 hours) proteasome activity (Fig. 1A) and cell death measured at 24 hours. However, kinetic studies showed that NPI-0052 stimulated cell death more rapidly than bortezomib ($P < 0.01$; Fig. 3B), consistent with the observation that it also inhibited the proteasome more rapidly than bortezomib (Fig. 1B and C).

We next did washout studies to define the minimal exposure time required for bortezomib- or NPI-0052-induced apoptosis. Again, short (15 minutes) exposures to 10 nmol/L NPI-0052 produced significant increases in apoptosis, whereas longer exposure times (≥8 hours) were required for commitment to bortezomib-induced cell death ($P < 0.01$). Short pulses of 10-fold higher concentrations of bortezomib induced significant apoptosis, but maximal induction of cell death still required longer exposure times compared with NPI-0052 ($P < 0.01$; Fig. 4).

In an attempt to better define the biochemical basis for the 4 to 8 hours of lag period observed between proteasome inhibition and apoptosis (Fig. 3B), we investigated the effects of caspase inhibitors on bortezomib- and NPI-0052-induced DNA fragmentation. Preliminary experiments confirmed that both bortezomib and NPI-0052 stimulated the proteolytic processing of procaspase-4 (Fig. 5A), consistent with our recent observation that bortezomib-induced cell death requires caspase-4 activation (26). A selective peptide inhibitor of caspase-4 (LEHDfmk) and the pan-caspase inhibitor zVADfmk both blocked bortezomib- or NPI-0052-induced procaspase-4 processing (Fig. 5A).
The caspase-4 inhibitor also blocked DNA fragmentation in a concentration-dependent fashion (Fig. 5B), whereas only the pan-caspase inhibitor blocked the DNA fragmentation induced by staurosporine (Fig. 5C), which does not kill cells via a caspase-4-dependent mechanism (26). The caspase inhibitors also completely inhibited proteasome inhibitor–induced DNA fragmentation when they were added to cells up to 8 hours after bortezomib or NPI-0052 (Fig. 5C), whereas, by 12 hours, both inhibitors only partially prevented apoptosis. By comparison, the commitment to death in cells exposed to staurosporine was much more rapid, in which, by 4 hours, the effects of the pan-caspase inhibitor zVADfmk on DNA fragmentation were completely lost (Fig. 5C). Inhibitors of either caspase-8 (IETDfmk) or caspase-9 (LEHDfmk) produced equivalent effects on DNA fragmentation induced by bortezomib or NPI-0052 (Fig. 5D), consistent with previous results showing that both caspases are activated by these proteasome inhibitors (16, 19, 27).

Cell death induced by bortezomib and other agents that induce endoplasmic reticulum stress are mediated via JNK activation (16, 28). We therefore compared the effects of the JNK pathway inhibitor SP60025 on the DNA fragmentation induced by bortezomib and other agents that induce endoplasmic reticulum stress.

**Discussion**

Studies from several different laboratories have concluded that proteasome inhibitors exhibit unique potency against isolated CLL lymphocytes in vitro (27, 29–33). Evidence has been presented that the natural product lactacystin (which is structurally similar to NPI-0052) is more potent than other classes of proteasome inhibitors (31), but in a previous study, we showed that bortezomib induced high levels of apoptosis in almost all of the CLL isolates we examined (n > 300), whether they were from untreated patients or from patients who had developed fludarabine-refractory disease (24). All of these observations prompted the CLL Consortium to do a multicenter phase II clinical trial of bortezomib in patients with refractory CLL. Although parallel laboratory correlative studies suggested that the drug had some modest effects on apoptosis (24), most of the patients progressed on therapy, and the clinical performance of bortezomib did not match preclinical expectations. An explanation for the discrepancy between the in vitro and in vivo effects of bortezomib in CLL cells has not been identified to date.

We speculated that the short serum half-life of bortezomib, coupled with the fact that its effects on the proteasome are reversible, might account for the discrepancy. In this study, we confirm that the effects of bortezomib on 20S proteasome activity in primary CLL lymphocytes are reversible, and our data indicate that exposure times of ≥8 hours are required for commitment to caspase activation and DNA fragmentation in cells exposed to induced by bortezomib or NPI-0052 in CLL cells. The inhibitor blocked the proteasome inhibitor–induced endonuclease activation in cells exposed to either agent (Fig. 6). Together with the results from the caspase-4 analyses, these data strongly suggest that bortezomib and NPI-0052 have common effects on endoplasmic reticulum stress-mediated apoptosis.
concentrations of the drug (10 nmol/L) that produce maximal steady-state proteasome inhibition. Based on our other recent work, the simplest explanation for this lag period is that proteasome inhibitors kill cells by preventing the proteasome-mediated clearance of misfolded or damaged proteins, leading to protein aggregation and endoplasmic reticulum stress (26, 28). The endoplasmic reticulum–resident caspase, caspase-4, plays a central role in proteasome inhibitor–induced cell death, as shown by the observation that small interfering RNA–mediated silencing of caspase-4 prevents downstream caspase-3 activation and DNA fragmentation in other models (26). This pathway of apoptosis is also critically dependent on JNK activation (16, 28); here, we show that, in CLL cells, the selective chemical JNK pathway inhibitor SP600125 attenuated cell death, consistent with the involvement of endoplasmic reticulum stress. Clearly, these proteasome inhibitors probably affect several other cellular pathways that are important for apoptosis (i.e., at the level of the mitochondrion), and ongoing efforts are aimed at dissecting their involvement in more detail. Importantly, our data also show that NPI-0052 inhibits the proteasome more rapidly and in a more sustained fashion than bortezomib does in CLL cells, and relatively short (15 minutes) exposures to the drug induce maximal apoptosis. Coupled with the fact that the drug is orally available and poised to enter phase I clinical trials, these observations strongly suggest that the clinical activity of NPI-0052 in CLL should be examined.

Figure 5. Effects of caspase inhibitors on proteasome inhibitor–induced apoptosis. A, effects of bortezomib or NPI-0052 on processing of procaspase-4. Cells were incubated with the indicated concentrations of bortezomib (PS) or NPI-0052 (NPI) for 16 h in the presence or absence of 100 μmol/L LEVD-CHO (LEVD) or 10 μmol/L zVADfmk (ZVAD), and procaspase-4 expression was measured by immunoblotting. Immunoblotting for actin served as a loading control. Results (from patient 26) are representative of those obtained in three separate experiments. B, concentration-dependent effects of LEVD-CHO on DNA fragmentation. Cells were exposed to bortezomib or NPI-0052 for 24 h in the presence or absence of the indicated concentrations of LEVD-CHO, and DNA fragmentation was measured by PI/FACS. Representative results from patient 26. C, effects of delayed addition of caspase inhibitors on proteasome inhibitor–induced cell death. Cells were exposed to the indicated agents in the presence or absence of LEVD-CHO (100 μmol/L) or zVADfmk (100 μmol/L), added 1 h before addition of the proteasome inhibitors or 4, 8, or 12 h after addition of the proteasome inhibitors, and DNA fragmentation was measured at 24 h by PI/FACS. Columns, mean (n = 3; patients 26, 36, and 37); bars, SE. D, effects of peptide-based inhibitors of caspase-8 or caspase-9 on DNA fragmentation. Cells were preincubated with 100 μmol/L IETDfmk (caspase-8) or LEHDfmk (caspase-9) for 1 h and then exposed to 10 nmol/L bortezomib (PS-341) or NPI-0052 for an additional 24 h, and DNA fragmentation was measured by PI/FACS. Columns, mean (n = 4; patients 27-30); bars, SE.
Although our data provide an attractive explanation for why bortezomib did not display significant clinical activity in CLL, it is likely that limitations associated with working with CLL lymphocytes in vitro also played an important role. For example, in vitro responsiveness to fludarabine-induced apoptosis does not always correlate with fludarabine responsiveness in vivo (34), and recent studies suggest that the host microenvironment provides critical survival support for CLL lymphocytes via adhesion-dependent and adhesion-independent mechanisms (35–38). On the other hand, studies in multiple myeloma showed that bortezomib and NPI-0052 overcame the survival signals provided by stromal cells to induce apoptosis (19). Further comparisons of the effects of bortezomib and NPI-0052 in stromal cell coculture models in vitro and in relevant in vivo models (i.e., the TCL-1 transgenic mouse; ref. 39) should be undertaken to determine whether the tumor stroma limits drug activity in CLL.

Figure 6. Effects of a JNK pathway inhibitor on proteasome inhibitor–induced DNA fragmentation. Cells were preincubated for 2 h with 25 μmol/L SP60025 and then exposed to 10 nmol/L bortezomib or 10 nmol/L NPI-0052 for 24 h, and DNA fragmentation was measured by PI/FACS. Columns, mean (n = 4; patients 27–30); bars, SE.

References


Molecular Cancer Therapeutics

The proteasome inhibitor NPI-0052 is a more effective inducer of apoptosis than bortezomib in lymphocytes from patients with chronic lymphocytic leukemia

Stacey Ruiz, Yelena Krupnik, Michael Keating, et al.

*Mol Cancer Ther* 2006;5:1836-1843.

Updated version  Access the most recent version of this article at: http://mct.aacrjournals.org/content/5/7/1836

Cited articles  This article cites 38 articles, 19 of which you can access for free at: http://mct.aacrjournals.org/content/5/7/1836.full.html#ref-list-1

Citing articles  This article has been cited by 21 HighWire-hosted articles. Access the articles at: /content/5/7/1836.full.html#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.