NPI-0052 Enhances Tumoricidal Response to Conventional Cancer Therapy in a Colon Cancer Model

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Abstract

Purpose: In the current study, we examine the effects of a novel proteasome inhibitor, NPI-0052 (salinosporamide A), on proteasome function and nuclear factor-κB activation and evaluate its ability to enhance treatment response in colon cancer xenografts when administered orally.

Experimental Design: The effects of treatment on nuclear factor-κB activation, cell cycle regulation, and apoptosis were determined. The pharmacodynamic effect of NPI-0052 on 20S proteasome function was assayed in vivo following oral and i.v. drug administration and compared with treatment with bortezomib. The effect of combined treatment with chemotherapy was determined in a colon cancer xenograft model.

Results: We found that NPI-0052 is a potent, well-tolerated proteasome inhibitor that has pharmacodynamic properties distinct from bortezomib in that it achieves significantly higher and more sustained levels of proteasome inhibition. When combined with chemotherapy, NPI-0052 increases apoptosis and shifts cells toward G2 cell cycle arrest. When added to chemotherapy in vivo [using combinations of 5-fluorouracil (5-FU), CPT-11, Avastin (bevacizumab), leucovorin, and oxaliplatin], NPI-0052 significantly improved the tumoricidal response and resulted in a 1.8-fold increased response to CPT-11, 5-FU, and leucovorin triple-drug combination (P = 0.0002, t test), a 1.5-fold increased response to the oxaliplatin, 5-FU, and leucovorin triple-drug combination (P = 0.013, t test), and a 2.3-fold greater response to the CPT-11, 5-FU, leucovorin, and Avastin regimen (P = 0.00057).

Conclusions: The high level of proteasome inhibition achieved by NPI-0052 is well tolerated and significantly improves the tumoricidal response to multidrug treatment in a colon cancer xenograft model. Further evaluation of this novel proteasome inhibitor in clinical trials is indicated.

The proteasome has been identified as an attractive target for cancer therapy because of the central role it plays in the regulation of proteins that control cell cycle progression and cancer cell survival (1). The direct effects of proteasome inhibition on cancer cells include cell cycle arrest with accumulation of cells typically in the G2 phase as well as the direct induction of apoptosis (2). In addition, proteasome inhibition has been shown to augment the cancer cell response to chemotherapy and radiation by decreasing proteasome-dependent regulatory proteins involved in treatment resistance such as Bcl2, the caspases, and the transcription factor nuclear factor-κB (NF-κB; refs. 1, 3, 4).

Currently, bortezomib/PS-341 (Velcade), a selective, reversible proteasome inhibitor that targets the 20S proteasome, is the only drug that targets proteasome function and is Food and Drug Administration approved for clinical use (2). We have previously shown that bortezomib overcomes treatment resistance induced by the chemotherapeutic agent CPT-11 (3) or γ-irradiation (4) by suppressing stress-induced activation of the transcription factor NF-κB and expression of genes involved in cancer cell survival. The suppression of NF-κB activation by proteasome inhibition occurs primarily through the stabilization of the NF-κB inhibitor IκBα, which is phosphorylated in response to genotoxic stress and then targeted for ubiquitination-dependent degradation by the proteasome (1, 2). In the current study, we examine a novel proteasome inhibitor, NPI-0052 (salinosporamide A), which is a natural product isolated from a marine microorganism that irreversibly inhibits all three active sites within the 20S core proteasome (5–7). Unlike bortezomib, which reversibly inhibits the chymotryptic site within the 20S core particle, NPI-0052 inhibits the chymotryptic, tryptic, and caspase sites of the proteasome (7). Our results show that NPI-0052 is a potent, orally active proteasome inhibitor with unique pharmacodynamic properties that achieve high levels of proteasome inhibition in vivo and is well tolerated. Furthermore, NPI-0052 is an effective adjuvant to anticancer therapies that enhances the apoptotic response to...
drug treatment and augments the tumoricidal response to multidrug treatment regimens in preclinical models of human colon cancer.

Materials and Methods

Cell culture, plasmids, and transfections. The human colorectal cancer cell line LoVo and the breast cancer cell line BT-474 were obtained from American Type Culture Collection (Rockville, MD) and grown according to American Type Culture Collection instructions. Cell cultures were maintained in 5% CO2 at 37°C. HeLa cells were grown in Eagle's MEM with 2 mmol/L L-glutamine and Earle's balanced salt solution adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mmol/L nonessential amino acids, 1.0 mmol/L sodium pyruvate, and 10% heat-inactivated horse serum at 37°C and 5% CO2. The NF-κB/Luc cells were prepared by selecting transfected HeLa cells that carry a luciferase reporter gene under the regulation of 5'-GGGATTCCC-3'. Six milligrams of nuclear extracts were separated on a 5% polyacrylamide gel with preincubation of 1 mg of poly(deoxyinosinic-deoxycytidylic acid) and 20,000 cpm of 32P-labeled DNA probe in a binding buffer at room temperature for 15 minutes. Positive control was BT-474 cells treated with 10 μg/mL doxorubicin (Sigma Co., St. Louis, MO) for 2 hours.

Drug treatment of HEK 293 reporter cells and lystate preparation. HEK 293 NF-κB/Luc cells were treated with 50 or 500 nmol/L of NPI-0052 for 1 hour and then stimulated with 10 ng/mL of recombinant human tumor necrosis factor-α (TNF-α; US Biological, Swampscott, MA) for 30 minutes. DMSO was used as a control. Cells were then washed once with ice-cold 1× Ca++/Mg++-free Dulbecco’s PBS and lysed on ice in radioimmunoprecipitation assay buffer containing 0.9% NaCl, 50 mmol/L Tris-HCl (pH 7.4), 1% Triton X-100, 1 mmol/L EDTA, 0.25% Na-deoxycholic acid, 2 mmol/L Na3VO4 and 1× protease inhibitor cocktail (Calbiochem, San Diego, CA). Cell lysates were cleared by centrifugation at 14,000 rpm for 10 minutes, 4°C. Protein concentration of cell lysates was determined with bichinchoninic acid kit (Pierce Biotechnology, Inc., Rockford, IL) before SDS-PAGE loading.

Western blotting of HEK 293 reporter cells and detection of NF-κB-dependent lucerase activity in HeLa cells. Equal protein concentrations of cell lysates were resolved on 10% NuPage MES precast gels (Invitrogen, Carlsbad, CA) and transferred to nitrocellulose membranes according to the instructions of the manufacturer. Membranes were blocked in 5% nonfat dry milk in 1× Dulbecco’s PBS containing 0.1% Tween 20 at room temperature for 2 hour. Western blot analyses were done with primary antibodies against IκBα, phospho-IκBα (Cell Signaling Technology, Beverly, MA), and tubulin (Lab Vision, Fremont, CA). Antibodies against P21, P27, and P53 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and used at a concentration of 1:500. A horseradish peroxidase– conjugated sheep anti-mouse antibody (Amersham Biosciences, Piscataway, NJ) was used as the secondary antibody and horseradish peroxidase activity was visualized by enhanced chemiluminescence detection system (Pierce Biotechnology). The NF-κB-lucerase reporter construct, pNFκB-Luc, was purchased from BD Biosciences (Palo Alto, CA). HeLa cells were transiently transfected with pNFκB-Luc using FuGENE 6 (Roche, Indianapolis, IN). Twenty-four hours posttransfection, NF-κB-lucerase HeLa cells were pretreated with varying concentrations of NPI-0052 or bortezomib for 1 hour and then treated with 10 ng/mL of TNFα (Calbiochem/EMD Biosciences, San Diego, CA) for 6 hours. Four hours later, lucerase activity was measured using the luciferase reporter assay kit from BD Biosciences as previously described (6).

Cell death ELISA. The Cell Death Detection ELISAlus assay (Roche Applied Sciences) was used to evaluate the effect of proteasome inhibition using NPI-0052 on the apoptotic response to combination therapies. Three different drug combinations were examined including (a) SN-38, the active metabolite of CPT-11 (1 mg/mL; Pfizer, Inc., Groton, CT), 5-fluorouracil (5-FU; 10 mg/mL; America Pharmaceutical Partners, Inc., Schauburg, IL), and leucovorin (1 mmol/L; Bedford Laboratories, Bedford, OH); (b) oxaliplatin (25 mmol/L; Sanofi-Synthelabo, Inc., New York, NY), 5-FU (10 mg/mL), leucovorin (1 mmol/L); and (c) SN-38 (1 mg/mL), 5-FU (10 mg/mL), leucovorin (1 mmol/L), and Avastin (10 μg/mL; Genentech, Inc., South San Francisco, CA). Each drug was sequentially added to the culture medium 3 hours after the prior drug treatment. When used, NPI-0052 (200 nmol/L) was the last drug added. Whole-cell lysates were obtained from harvested cells 48 hours after exposure to the last drug treatment. The assay was done according to the instructions of the manufacturer and the results were measured at 405 nm with a reference wavelength of 490 nm on a spectrophotometer. The experiment was done in triplicate and apoptosis enrichment was determined as fold change compared with the control group.

Cell cycle analysis by flow cytometry. LoVo cells were synchronized by serum starvation for 72 hours, then treated with SN38 (active metabolite of CPT-11) at a concentration of 1 mg/mL without or with pretreatment (or posttreatment) of NPI-0052 at a concentration of 200 nmol/L. Forty-eight hours after treatment with SN38, cells were collected and fixed with 80% ethanol overnight. Cells were then resuspended in 1 mL of 1% bovine serum albumin in PBS and 25 mL of 40% propidium iodide (500 mg/mL; Sigma) and 25 mL of RNase A (10 mg/mL) were added for 30 minutes. Analysis was done using a FACScan flow cytometer (BD Biosciences, San Jose, CA). Area under the curve was calculated using ModFit LT version 3.1 software (Varity Software, Topsham, ME).

In vivo proteasome activity assay. NPI-0052 was dissolved in 100% DMSO and serially diluted with 5% Solotol (Solotol HS 15; polyethylene glycol 660 12-hydroxyestearate; BASF, Shreveport, LA), yielding a final concentration of 2% DMSO. Vehicle control consisted of 2% DMSO and 98% (5% Solotol). Female C57BL/6 mice (n = 5) were treated i.v. and male Swiss-Webster mice (n = 5) were treated orally with various concentrations of NPI-0052 at a volume of 10 mL/kg. Bortezomib (1 mg/kg) was administered i.v. also at a dose volume of 10 mL/kg. Twenty microliters of blood were collected via the tail vein after creating a small puncture using a 25-gauge needle. Blood was immediately lysed with 0.5 mmol/L EDTA for 30 minutes on ice and centrifuged at 6,600 g for 10 minutes at 4°C. The lysates were then removed and analyzed by a fluorescent 20S proteasome assay as previously described (8). Protein content of the samples was determined by a Coomassie protein assay (Pierce). Data are represented as percent inhibition compared with vehicle control treated animals.

In vivo evaluation of tumor growth. Tumor growth was assessed in a LoVo xenograft model. The tumors were established by injecting 5 million cells into the flank of 6-week-old female nu/nu mice. Treatment was initiated once the tumors reached a mean diameter of 8 to 10 mm. CPT-11 (33 mg/kg) was administered i.v. via tail-vein injection twice weekly whereas 5-FU (33 mg/kg), leucovorin (90 mg/kg), and Avastin (2.5 mg/kg) were injected i.p. twice weekly. Oxaliplatin (10 mg/kg i.p.) was injected once during the treatment course and NPI-0052 (0.25 mg/kg) was delivered by oral gavage twice weekly. The maximum tolerated dose of NPI-0052 in mice was 0.50 mg/kg (oral) and 0.25 mg/kg (i.v.). Vehicle was used for the corresponding control group. Tumor size was measured every 4 days and calculated using the formula TV = 4/3πr3, where r = 1/2 (mean tumor diameter measured in two dimensions). All experiments were performed on April 15, 2017. © 2006 American Association for Cancer Research.
**Results**

**Effects of NPI-0052 treatment on TNF-α- and chemotherapy-induced NF-κB activation.** TNF-α and some chemotherapy agents are known to induce the transcription factor NF-κB, resulting in the expression of a variety of known genes to be involved in the inhibition of apoptosis (9). To investigate the effects of NPI-0052 treatment on the activation of NF-κB, we exposed LoVo cells to NPI-0052 either before or after treatment with SN-38 (Fig. 1A). The electrophoretic mobility shift assay showed that at both 2 and 4 hours, chemotherapy-induced activation of NF-κB was almost completely inhibited by NPI-0052. Following exposure to TNF-α and chemotherapy, the inhibitor of NF-κB, called IκBα, is typically phosphorylated, ubiquitinated, and then targeted for degradation by the proteasome. To determine if the inhibition of NF-κB was due to changes in its inhibitor IκBα, NF-κB/luciferase HEK 293 cells were exposed to combinations of TNF-α and NPI-0052 (Fig. 1B). In these experiments, IκBα decreased and phospho-IκBα increased with increasing concentrations of NPI-0052. The addition of NPI-0052 resulted in an accumulation of phospho-IκBα rather than its degradation by the proteasome. Furthermore, at the dosage range tested, NPI-0052 was ~2-fold more potent inhibitor of TNF-α-induced NF-κB activation compared with bortezomib as measured by the NF-κB luciferase reporter assay in HeLa cells (Fig. 1C).

**Cell cycle response to NPI-0052 and effects on chemosensitivity in vitro.** To determine if NPI-0052 treatment affects the apoptotic response to combination chemotherapy regimens in cultured LoVo cells, it was added to three different treatment regimens containing combinations of SN38, oxaliplatin, 5-FU, leucovorin, and Avastin (Fig. 2A). The addition of NPI-0052 resulted in a significant enhancement of apoptosis in both the SN38/5-FU/Leuk and the oxali/5-FU/Leuk treatment groups. In two of the SN38-based regimens, the addition of NPI-0052 enhanced the apoptotic response ~1-fold, whereas in the oxaliplatin-containing regimen, the addition of NPI-0052 increased apoptosis ~3.5-fold. Interestingly, the addition of Avastin to the SN38-based regimen had no effect on apoptosis in vitro as would be expected.

In addition to the promotion of apoptosis, proteasome inhibition may also affect cell cycle regulators. To further evaluate this possibility, flow cytometric analysis of LoVo cells treated with SN-38 and NPI-0052 was done (Fig. 2B). Once again, an increase in apoptosis was observed when NPI-0052 was added to the treatment groups. Even more pronounced, however, was the large shift in the G2-M cell population compared with the untreated controls (8.6% versus 39.2%). One possible explanation for this dramatic shift toward G2 arrest was identified in the Western blot analysis of the cell cycle regulators p21, p27, and p53 (Fig. 2C), which showed an increase in these cell cycle regulators in response to treatment with chemotherapy and proteasome inhibition (using either bortezomib or NPI-0052).

**Effects of NPI-0052 on proteasome function in vivo.** To evaluate the pharmacodynamics of drug treatment and determine if the level of proteasome inhibition was dose dependent, increasing concentrations of NPI-0052 were administered to mice i.v. and 20S proteasome function was assayed at 1.5 and 24 hours (Fig. 3). The degree of proteasome inhibition achieved increased with increasing concentrations of the drug, with a peak level of proteasome inhibition (90%) measured at a drug concentration of 0.2 mg/kg. In comparison, ~55% of proteasome inhibition was achieved after bortezomib was administered at the maximum tolerated dose. Furthermore, bortezomib treatment resulted in a higher level of proteasome inhibition at the
earlier 1.5 hour time point. In contrast, proteasome inhibition following NPI-0052 treatment gradually increased over the sampled time period and was actually higher at the 24 hour time point for each dosage tested.

To further evaluate the time course of proteasome recovery following NPI-0052 treatment, mice were given a single oral administration of NPI-0052 at 0.25 or 0.5 mg/kg. Blood was collected at 90 minutes and 1, 2, 3, and 7 days post dosing.
and proteasome activity was determined (Fig. 4). Bortezomib at 1 mg/kg (i.v.) was included in the same experiment for comparison. Results showed that a single oral administration of NPI-0052 leads to a sustained inhibition of the chymotrypsin-like activity of the 20S proteasome. Significant recovery of the proteasome catalytic activity (albeit not to baseline levels) was observed by day 7. In contrast, a single administration of bortezomib (Fig. 4C) resulted in inhibition of the chymotrypsin-like activity with significant recovery by 24 hours.

The effect of repeated NPI-0052 oral dosing on the 20S proteasome activity in whole blood cells was evaluated using a twice-weekly dosing regimen. In addition, the weights of the mice were monitored to assess tolerability of the treatment schedules. NPI-0052 was administered orally at either 0.25 or 0.5 mg/kg at the days indicated for up to seven treatments. Blood was collected 24 hours after the last treatment and the chymotrypsin-like activity of the 20S proteasome in whole blood cells was determined. The results from this study are shown in Fig. 5A and B and illustrate that repeated dosing of NPI-0052 at 0.25 mg/kg resulted in a sustained inhibition (73–85%) of the whole blood 20S proteasome activity. This dose and treatment schedule was very well tolerated by the animals as reflected in minimal body weight loss and even a weight gain from day 15 on. Repeated oral dosing with NPI-0052 at 0.5 mg/kg resulted in a sustained whole blood 20S proteasome inhibition as high as 90% to 99%, with a maximal weight loss of only 11.5%. Our results indicate that repeated oral dosing of mice with NPI-0052 at 0.25 or 0.5 mg/kg is well tolerated.

Effects of NPI-0052 on the tumoricidal response to combination therapy. Results from in vitro studies showed that treatment of LoVo cells with NPI-0052 leads to an increase in apoptosis and cell cycle arrest in G2 (Fig. 2B). To determine if these effects would result in an enhanced tumoricidal response when NPI-0052 is combined with conventional colon cancer therapy in vivo, a LoVo xenograft model was used (Fig. 6). The results showed that the addition of NPI-0052 (0.25 mg/kg, orally administered twice weekly) significantly enhanced the tumoricidal response to each treatment regimen ($P < 0.05, t$ test). The maximum response leading to an actual decrease in the size of the tumors was achieved in the treatment group receiving NPI-0052 in combination with CPT-11, 5-FU, leucovorin, and Avastin (2.3-fold; $P < 0.001$). However, the addition of NPI-0052 improved the tumoricidal response in each case and resulted in a 1.8-fold improved response to CPT-11, 5-FU, and leucovorin ($P < 0.001$) and a 1.5-fold improved response to oxaliplatin, 5-FU, and leucovorin ($P < 0.05$).

Discussion

The proteasome has recently been identified as an attractive target for anticancer molecular therapies, owing to its involvement in the maintenance of cell cycle regulatory proteins and apoptosis (1). To date, only one inhibitor of the proteasome, bortezomib/PS-341 (Velcade), has received Food and Drug Administration approval and continues to undergo evaluation in clinical trials. In the current study, we present our results obtained when using the novel proteasome inhibitor NPI-0052 that has distinctly different chemical properties and physiologic activity when compared with bortezomib. In contrast to bortezomib, which reversibly targets the chymotryptic site of the proteasome, NPI-0052 targets all three active sites (chymotryptic, tryptic, and caspase) within the 20S core proteasome and does so in an irreversible fashion. The current experiments were done specifically to assess the ability of this novel compound to inhibit proteasome function in vitro as well as in vivo when administered i.v. and orally, and to evaluate the potential role of NPI-0052 as an adjunct to conventional colon cancer therapy.

It has previously been shown that inhibitors of NF-$\kappa$B (including proteasome inhibitors), when combined with conventional therapies, augment chemosensitivity by suppressing the NF-$\kappa$B-mediated antiapoptotic response (1–3, 10, 11). We hypothesize that higher levels of proteasome inhibition will result in a higher degree of NF-$\kappa$B inhibition and therefore a greater treatment response. Interestingly, both preclinical and clinical studies using bortezomib have shown that the level of proteasome inhibition achieved at the maximum tolerated dose ranges between 75% and 80%.4 Following bortezomib administration in vivo in preclinical studies, lethal toxicity ensues when the level of inhibition of proteasome function exceeded ~80% (data not shown). In the current study, in vivo analysis determined that NPI-0052 was significantly more potent than bortezomib in inhibiting 20S proteasome activity. Somewhat surprisingly, we also found that higher levels of proteasome inhibition (approaching 100%) were achieved using NPI-0052, without apparent toxicity.

We also found that NPI-0052 effectively suppressed chemotherapy-induced NF-$\kappa$B activation and augmented apoptosis in vitro, suggesting that the combination of NPI-0052 with conventional chemotherapy may enhance treatment response by muting the NF-$\kappa$B-dependent cancer cell survival signals (3). The cell death ELISA assays and flow cytometric analysis support this hypothesis by showing that the addition of NPI-0052 to chemotherapy in vitro enhanced apoptosis and led to a moderate shift in the cell population toward G2 arrest. Combined, these data show that higher levels of proteasome inhibition may be achieved with NPI-0052 without inducing nonspecific cytotoxicity. These findings further suggest that this

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4 Unpublished data.
A novel compound may be both better tolerated and potentially more effective when used in vivo compared with bortezomib. Our animal studies show that i.v. and orally administered NPI-0052 is a potent inhibitor of proteasome in vivo, which has unique pharmacodynamic properties compared with bortezomib. We observed a dose- and time-dependent increase in 20S chymotrypsin-like proteasome inhibition in NPI-0052-treated mice. A single dose of NPI-0052 resulted in as high as 90% inhibition of the proteasome compared with 70% inhibition achieved after bortezomib administered at the maximum tolerated dose. NPI-0052 treatment resulted in proteasome inhibition that progressively increased over 24 hours whereas bortezomib reached a maximum level of proteasome inhibition at the 1.5 hour time point and then significantly decreased over the subsequent 24 hours. Further examination of the response over time showed in the case of NPI-0052 that proteasome inhibition after a single dose peaked at 24 hours and remained essentially unchanged for 72 hours. In the case of bortezomib, the proteasome recovers to baseline at 72 hours following bortezomib treatment. The prolonged treatment response seen with NPI-0052 seems to be due to the irreversible binding of the drug to the proteasome compared with reversible binding with bortezomib (1, 2, 5, 7, 12). Importantly, repeated oral dosing of NPI-0052 resulted in 99% inhibition of proteasome function. Surprisingly, this extremely high level of proteasome inhibition was well tolerated and resulted only in a modest amount of weight loss in the

![Graph A](image1)

A 0.25 mg/kg NPI-0052, PO

![Graph B](image2)

B 0.5 mg/kg NPI-0052, PO

![Graph C](image3)

C 1 mg/kg Bortezomib, IV

Fig. 4. The effect of proteasome inhibition over time was assessed in mice treated with either NPI-0052 (A and B) or bortezomib (C). The inhibition of the 20S chymotrypsin-like proteasome in mice was determined at 1.5, 24, 48, 72, and 168 hours following oral administration of NPI-0052 or i.v. administration of bortezomib. *, P < 0.05, compared with 72 hours; **, P < 0.05, compared with 90 minutes.

![Graph D](image4)

![Graph E](image5)

Fig. 6. A and B, the effect of orally administered NPI-0052 on the inhibition of the 20S proteasome activity in whole blood cells was determined. Mice (n = 5) were administered NPI-0052 orally at 0.25 mg/kg (A) or 0.5 mg/kg (B) on the days indicated. Twenty-four hours after the last treatment, blood was collected, whole blood cell lysates were prepared, and the chymotrypsin-like activity of the 20S proteasome was determined using the peptide substrate sucb-LVY-AMC. The average percent change in body weights at the time of sacrifice is indicated. Body weights were measured to determine potential toxic effects of treatment on animals.
animals. This runs counter to our experience with bortezomib where proteasome inhibition exceeding 80% was uniformly lethal in animals within 24 hours (data not shown).

The final and perhaps most important finding from these studies is the ability of NPI-0052 to augment the response to multidrug combination chemotherapy regimens without significantly increasing the toxicity of these complex treatment regimens. Whereas the addition of oxaliplatin, CPT-11, and Avastin to 5-FU-based treatment has contributed to improved survival in patients with metastatic colorectal cancer, the vast majority of patients will ultimately manifest treatment resistance (13, 14). The development of new drug combinations that address chemotherapy resistance is essential to further improvement in survival in metastatic colorectal cancer (13–17). Owing to the multiple systems affected by proteasome function, proteasome inhibition is a logical adjuvant to chemotherapy treatment. Proteasome inhibition not only enhances chemosensitivity via the inhibition of chemotherapy-induced NF-kB activation (3, 18) but also decreases vascular endothelial growth factor expression and tumor microvessel density (19) and arrests cancer cell proliferation through effects on cell cycle regulators p21 and p27 (20). Our results show that NPI-0052 effectively augments the therapeutic response to the most commonly used colon cancer multidrug treatment regimens currently used in the clinic. In addition, our study suggests that NPI-0052 may be a superior choice for proteasome inhibition in this treatment paradigm. In summary, our data show that (a) the novel proteasome inhibitor NPI-0052 inhibits proteasome activity in vitro and in vivo; (b) NPI-0052 is orally bioactive; (c) NPI-0052 has a different pharmacodynamic profile compared with bortezomib; (d) NPI-0052 suppresses chemotherapy-induced NF-kB activation and overcomes chemotherapy resistance; and (e) high levels of proteasome inhibition induced by NPI-0052 are achievable in vivo and well tolerated. Together, these findings provide the rationale for clinical trials to evaluate the ability of NPI-0052 to enhance treatment efficacy and overcome drug resistance in combination chemotherapy regimens in patients with metastatic colon cancer.

References

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