The Proteasome Inhibitor PS-341 in Cancer Therapy

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ABSTRACT

The anticancer activity of the boronic acid dipeptide proteasome inhibitor PS-341 was examined in vitro and in vivo. PS-341 was a potent cytotoxic agent toward MCF-7 human breast carcinoma cells in culture, producing an IC$_{50}$ of 0.05 µM on 24 h of exposure to the drug. In the EMT-6 tumor cell survival assay, PS-341 was equally cytotoxic administered p.o. or by i.p. injection up to a dose of 2 mg/kg. PS-341 was also toxic to the bone marrow colony-forming unit-granulocyte macrophage. PS-341 increased the tumor cell killing of radiation therapy, cyclophosphamide, and cisplatin in the EMT-6/Parent tumor, but was not able to overcome the in vivo resistance of the EMT-6/CTX and EMT-6/CDDP tumors. In the tumor growth delay assay, PS-341 administered p.o. had antitumor activity against the Lewis lung carcinoma, both primary and metastatic disease. In combination, regimens with 5-fluorouracil, cisplatin, Taxol and adriamycin, PS-341 seemed to produce primarily additive tumor growth delays against the s.c. tumor and was highly effective against disease metastatic to the lungs. The proteasome is an interesting new target for cancer therapy, and the proteasome inhibitor PS-341 warrants continued investigation in cancer therapy.

INTRODUCTION

The regulation of protein activities by the proactive synthesis and degradation of specific protein molecules is vital to cellular metabolic integrity and proliferation. It is becoming well-established that the proteasome, a large, multimeric protease complex, has a central role in cellular protein regulation through catabolism of a wide variety of proteins, resulting in the activation of certain pathways and the blocking of others (1–8). The proteasome degrades proteins that have been conjugated to multiple units of the polypeptide ubiquitin; thus, this process is often called the ubiquitin-proteasome pathway (1–8).

The ubiquitin-proteasome pathway may be critical to regulation of the amount of activated signal transduction proteins and protein activators of transcription (STAT proteins) in cells (9). Rock et al. (10) showed that the ubiquitin-proteasome pathway has a role in the processing and presentation of MHC class I-restricted antigens. Palombella et al. (6) and several subsequent studies (4, 11) established a role for the ubiquitin-proteasome pathway in the processing of the p105 nuclear factor-kB precursor into the active p50 subunit of the transcriptional activator. There is evidence that the ubiquitin-proteasome pathway may be critical in cell cycle regulation by degrading cyclins that act in different phases of the cell cycle (1, 12–15) and in maintaining p53 levels (2). The ubiquitin-proteasome pathway has been implicated in the degradation of abnormal proteins such as the progressive multifocal leukoencephalopathy-retinoic acid receptor, an oncoprotein in acute promyelocytic leukemia (3), as well as in the degradation of the platelet-derived growth factor β-receptor complex (5).

Both naturally occurring and synthetic inhibitors of the ubiquitin-proteasome pathway have been identified (8, 16–22). The most widely studied inhibitors are: (a) lactacystin, a streptomycetes metabolite, which is metabolized to lactacystin β-lactone, the active proteasome inhibitor (8, 16–22); (b) peptide aldehydes, such as carbobenzoxyl-leucinyl-leucinyl-leucinal-H$_2$(MG-132) and others (23, 24); and (c) boronic acid peptides (8, 23). Dipeptide boronate derivatives, which are potent proteasome inhibitors and are suitable for administration in vivo being p.o. bioavailable and relatively stable under physiological conditions, have been prepared (8, 25).

PS-341 is a boronic acid dipeptide derivative (Fig. 1). Boronic acid peptides have been shown to inhibit serine proteases (e.g., thrombin, elastase, dipeptidyl protease IV; Refs. 26–31). Dipeptide boronate derivatives are potent proteasome inhibitors presumably through the stability of the boron-Thr’Og dative bond that forms at the active site of the proteasome. The $K_s$ for PS-341 is 0.6 nm (8). The dipeptide boronates have a high degree of selectivity for the proteasome and are not inhibitors of many common proteases.

The current study was undertaken to initiate investigation of the boronate dipeptide ubiquitin-proteasome pathway inhibitor PS-341 in cancer therapy.

MATERIALS AND METHODS

Drugs. PS-341 was prepared by ProScript, Inc. (Cambridge, MA). Cyclophosphamide, CDDP, 5-fluorouracil, and Taxol were purchased from Sigma Chemical Co. (St. Louis, MO). Adriamycin was purchased from the Dana-Farber Cancer Institute pharmacy.

PS-341 was prepared as a stock solution in DMSO. For administration to the animals, solutions were prepared fresh daily by diluting the stock with 0.9% saline so that the final vehicle contained <0.5% DMSO.

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2 The abbreviation used is: CDDP, cisplatin; FBS, fetal bovine serum; CFU-GM, colony-forming unit-granulocyte macrophage.
Cytotoxicity Experiment. MCF-7 is a human adenocarcinoma of the breast, developed by Dr. M. Rich of the Michigan Cancer Foundation (Detroit, MI). This line is estrogen receptor positive and retains certain characteristics of breast adenocarcinoma. MCF-7 has been used as a model for in vitro studies of breast carcinoma (32). MCF-7 human breast carcinoma cells grow as monolayers in DMEM supplemented with antibiotics, l-glutamine, and 10% FBS. This cell line has a plating efficacy of 25–40%.

Cells in exponential growth were concentrated to a density of 10^6 cells/ml. The samples were centrifuged for 10 min at 1000 × g, and the supernatant was removed. The cells were then resuspended in Waymouth's medium supplemented with 10% FBS and 100 units/ml of penicillin and streptomycin for 24 or 48 h. After exposure to the agent, the cells were washed three times with 0.9% PBS, then plated in duplicate at three dilutions in monolayer for colony formation, as described above. Results were expressed as the surviving fraction of treated cells, as compared with vehicle-treated control cells (32–34).

Tumor System. The EMT-6/Parent mouse mammary carcinoma grown as a solid tumor in the flanks of female Balb/C mice (Taconic Farms, Germantown, NY) has been used widely in radiobiology and chemotherapy studies. The alkylating agent-resistant EMT-6 tumor lines were established by repeated treatment of tumor-bearing animals with CDDP (20 mg/kg) or CTX (300 mg/kg) injected i.p. 24 h before passage of each tumor line into fresh host animals 10 times (35). The parent tumor line was passaged in the same manner in the absence of drug treatment. The alkylating agent sublines designated EMT-6/CDDP (resistant to CDDP) and EMT-6/CTX (resistant to CTX) were maintained as frozen tumor brei in liquid nitrogen and used for experiments during the second and third tumor passages (35–37).

Tumor Cell Survival Assay. The EMT-6 murine mammary carcinoma is an in vivo-in vitro tumor system (35). The EMT-6/Parent and alkylating agent-resistant tumors were grown in female Balb/C mice. For the experiments, 2 × 10^6 tumor cells prepared from a brei of several stock tumors were implanted s.c. into the hind legs of Balb/c mice, 8–10 weeks of age. Tumor cell survival was performed when the tumors had reached a volume of ∼150 mm^3 (day 9 after tumor implantation). Animals bearing the EMT-6/Parent tumor were untreated, treated with PS-341 (0.3, 0.6, 1, 2, or 5 mg/kg) p.o. or by i.p. injection on day 8, or were treated with PS-341 (1 mg/kg) by i.p. injection, followed by radiation therapy (5, 10, 15, or 20 Gy), on day 8. Other animals bearing the EMT-6/Parent, EMT-6/CTX or EMT-6/CDDP tumors were untreated, treated with cyclophosphamide (100, 300, or 500 mg/kg) or with CDDP (10, 20, or 30 mg/kg) by i.p. injection on day 8, or treated with PS-341 (0.1 mg/kg) p.o. twice/day on days 0–8, along with cyclophosphamide (100, 300, or 500 mg/kg) or with CDDP (10, 20, or 30 mg/kg) by i.p. injection on day 8.

A 24-h interval was incorporated before the mice were killed to allow for the full expression of drug cytotoxicity and repair of potentially lethal damage. Mice were immersed briefly in 95% ethanol, and the tumors were excised under sterile conditions in a laminar flow hood and minced to a fine brei with two scalpels. Four tumors were pooled to make each treatment group. Approximately 400 mg of tumor brei were used to make each single cell suspension. All reagents were sterilized with 0.22-μm Millipore filters and were added aseptically to the tumor cells.

Each sample was washed in 20 ml of Waymouth’s medium (Mediatech, Pittsburgh, PA), after which the liquid was gently decanted and discarded. The samples were resuspended in 450 units of collagenase/ml (Sigma Chemical Co.) and 0.1 DNase/ml (Sigma Chemical Co.) and incubated for 10 min at 37°C in a shaking water bath. The samples were resuspended, as described above, and incubated for another 15 min at 37°C. Next, 1 ml of 1 mg/ml DNase was added, and incubation was continued for 5 min at 37°C. The samples were then filtered through a 70-μm cell strainer (Fisher, Pittsburgh, PA). The

![Fig. 1 Structure of PS-341.](image)

Table 1  Tumor growth delay and number and size of lung metastases in animals bearing Lewis lung carcinoma after treatment with p.o. administered PS-341

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Tumor growth delay (days)</th>
<th>Lung metastases on day 20, n (% large)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td>27 (48)</td>
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<tr>
<td>PS-341 (p.o.)</td>
<td></td>
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</tr>
<tr>
<td>1 mg/kg, d 0–18</td>
<td>4.4 ± 0.3</td>
<td>10 (38)</td>
</tr>
<tr>
<td>1 mg/kg, d 4–18</td>
<td>3.8 ± 0.3</td>
<td>13 (30)</td>
</tr>
<tr>
<td>1 mg/kg, d 7–18</td>
<td>1.7 ± 0.3</td>
<td>18 (38)</td>
</tr>
<tr>
<td>0.3 mg/kg, 2 × d 0–18</td>
<td>3.9 ± 0.3</td>
<td>9 (48)</td>
</tr>
<tr>
<td>0.3 mg/kg, 2 × d 4–18</td>
<td>3.4 ± 0.3</td>
<td>9 (30)</td>
</tr>
<tr>
<td>0.3 mg/kg, 2 × d 7–18</td>
<td>2.7 ± 0.3</td>
<td>9 (33)</td>
</tr>
<tr>
<td>0.3 mg/kg, d 0–18</td>
<td>4.7 ± 0.3</td>
<td>12 (20)</td>
</tr>
<tr>
<td>0.3 mg/kg, d 4–18</td>
<td>2.8 ± 0.3</td>
<td>14 (42)</td>
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<tr>
<td>0.3 mg/kg, d 7–18</td>
<td>2.2 ± 0.3</td>
<td>15 (41)</td>
</tr>
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<td>0.1 mg/kg, d 0–18</td>
<td>4.9 ± 0.4</td>
<td>5.5 (20)</td>
</tr>
<tr>
<td>0.1 mg/kg, d 4–18</td>
<td>4.4 ± 0.3</td>
<td>8 (20)</td>
</tr>
<tr>
<td>0.1 mg/kg, d 7–18</td>
<td>4.0 ± 0.3</td>
<td>12 (30)</td>
</tr>
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<td>13 (33)</td>
</tr>
<tr>
<td>1 mg/kg, alt d 7–18</td>
<td>2.6 ± 0.3</td>
<td>15 (42)</td>
</tr>
</tbody>
</table>
samples were washed twice, then resuspended in Waymouth’s medium supplemented with 15% newborn calf serum. These single-cell suspensions were counted and plated at six different cell concentrations for the colony-forming assay. No significant difference was observed in the total cell yield from the pooled tumors in any treatment group. After 1 week, the plates were stained with crystal violet and colonies of >50 cells were counted. The untreated tumor cell suspensions had a plating efficiency of $8\text{--}14\%$. The results were expressed as the surviving fraction ($\pm$ SE) of cells from the untreated groups as compared with untreated controls.

**Bone Marrow Toxicity.** Bone marrow was taken from the same animals used for the tumor excision assay. A pool of marrow from the femurs of two animals was obtained by gently flushing the marrow through a 23-gauge needle, and the CFU-GM assay was carried out as described previously (38). Bone marrow cells were suspended in supplemented McCoy’s 5A medium containing 15% FBS, 0.3% agar (Difco, Detroit, MI), and 10% conditioned medium as a source of colony-stimulating activity. The colony-stimulating activity supplement was prepared by incubating L-929 mouse fibroblasts (2500 cells/ml; Microbiological Associates, Bethesda, MD) with 30% FBS in McCoy’s 5A medium for 7 days at 37°C in a humidified atmosphere containing 5% CO$_2$. The supernatant containing the colony-stimulating activity was obtained by centrifugation of the medium at 10,000 x g for 10 min at 4°C, followed by filtration under sterile conditions. The bone marrow cell cultures were incubated for 7 days at 37°C and were fixed with 10% glutaraldehyde. Colonies of at least 50 cells were scored. The results of three experiments, in which each group was measured at six-cell concentrations, were averaged. The results were expressed as the surviving fraction ($\pm$ SE) of treated groups as compared with untreated controls.

**Tumor Growth Delay.** The Lewis lung tumor was carried in male C57BL mice (Taconic Farms, Germantown, NY). For the experiments, $2 \times 10^5$ tumor cells prepared from a brei of several stock tumors were implanted s.c. into the hind legs of male mice, 8–10 weeks of age. By day 4 after tumor-cell implantation, Lewis lung tumors had begun neovascularization. Animals bearing Lewis lung tumors were treated with PS-341 (0.1, 0.3, or 1 mg/kg) p.o. on days 0–18, 4–18, or 7–18 after tumor-cell implantation or with PS-341 (0.3 mg/kg) p.o. twice daily on days 0–18, 4–18, or 7–18 or with PS-341 (1 mg/kg) p.o. alternate days on days 0–18, 4–18, or 7–18. Other groups of animals bearing Lewis lung tumors were treated with PS-341 (0.1 or 0.03 mg/kg) p.o. on days 4–18 alone or along with cytotoxic therapy. When the tumors were ~100 mm$^3$ in volume, day 7 after tumor cell implantation, cytotoxic therapy was initiated. CDDP (10 mg/kg) was administered i.p. on day 7 after tumor implantation. 5-Fluorouracil (30 mg/kg) was administered i.p. on days 7–11 after tumor implantation. Taxol (24 mg/kg) was administered i.v. on days 7–11 after tumor implantation. Adriamycin (1.75 mg/kg) was administered i.p. on days 7–11 after tumor implantation. Radiation was delivered locally to the tumor-bearing limb as 3-Gy fractions daily on days 7–11 using a Gamma Cell 40 (Atomic Energy of Canada, Ltd.)

Other groups of Lewis lung carcinoma-bearing animals were treated with 5-fluorouracil (20, 25, or 30 mg/kg) by i.p. injection on days 7–11 after tumor cell implantation alone or along with PS-341 (0.01, 0.03, or 0.1 mg/kg) administered by i.p. injection on days 4–18 after tumor cell implantation.

The progress of each tumor was measured thrice weekly until it reached a volume of 500 mm$^3$. Tumor growth delay was calculated as the days taken by each individual tumor to reach 500 mm$^3$ compared with untreated controls. Each treatment group had five animals. Days of tumor growth delay are the mean $\pm$ SE for the treatment group compared with the control (39, 40).

**Lung Metastases.** External lung metastases from animals treated as described above on day 20 after tumor implantation were counted manually and scored as $\geq$3 mm in diameter. The numbers in parentheses in Tables 1 and 2 indicate the percentages of the lung metastases that were large ($>3$ mm in diameter) enough to be angiogenic (39, 40).

**Statistical Analysis.** Data were analyzed using a two-tailed t test after applying ANOVA to the data.

**RESULTS**

PS-341 was a potent cytotoxic agent toward human MCF-7 breast carcinoma cells in monolayer culture. On 24 h or 48 h of exposure to PS-341, MCF-7 cells were killed in a manner that increased logarithmically with logarithmic increase in drug concentration (Fig. 2). Ninety percent of MCF-7 cells were killed...
by 0.05 μM PS-341 in 24 h, whereas in 48 h 0.05 μM PS-341 killed 99% of the MCF-7 cells.

The cytotoxicity of PS-341 in vivo was assessed in animals bearing the EMT-6/Parent murine mammary carcinoma. PS-341 is p.o. bioavailable (8); therefore, the toxicity of PS-341 toward EMT-6/Parent tumor and toward bone marrow CFU-GM was compared in animals receiving the drug p.o. and animals who received injections of the drug i.p. (Fig. 3). Doses of PS-341 were selected that produced no toxicity, as determined by weight loss or lethality. Up to a single dose of 2 mg/kg, PS-341 was equally cytotoxic toward EMT-6/Parent tumor cells whether administered p.o. or by i.p. injection. By extrapolation, 90% of the tumor cells were killed by 3 mg/kg PS-341 administered by i.p. injection, whereas >5 mg/kg would be required to kill 90% of the tumor cells with PS-341 administered p.o. Administering PS-341 p.o., there was no significant difference in cytotoxicity toward EMT-6/Parent tumor cells and bone marrow CFU-GM. However, when PS-341 was administered by i.p. injection, there was a 1 log greater kill of EMT-6/Parent tumor cells than of bone marrow CFU-GM at an PS-341 dose of 5 mg/kg.

As an initial combination study, a single dose of PS-341 (1 mg/kg) was administered by i.p. injection before a single dose of radiation therapy (Fig. 4). Administration of PS-341 increased the tumor cell killing by the radiation therapy, resulting in a dose-modifying factor of 1.2. In another study, PS-341 (0.1 mg/kg) was administered p.o. twice/day to animals bearing the EMT-6/CTX tumor or the EMT-6/CDDP tumor on days 0–8 after tumor cell implantation alone or along with cyclophosphamide on day 8 (Fig. 5). The EMT-6/Parent tumor was more sensitive to cyclophosphamide than was the EMT-6/CTX tumor, which was made resistant to cyclophosphamide in vivo, and the EMT-6/CDDP tumor, which was made resistant to CDDP in vivo. Administration of PS-341 to animals bearing the EMT-6/Parent tumor increased the tumor cell killing by the lower doses of cyclophosphamide by about 3-fold, did not alter the cyclophosphamide killing of the EMT-6/CTX tumor cells, and increased the killing of EMT-6/CDDP tumor cells from 6-fold to 40-fold over the dosage range of cyclophosphamide studied. PS-341 administration increased the toxicity of cyclophosphamide toward bone marrow CFU-GM in animals bearing each of the three tumors in a manner that was dose modifying (i.e., the magnitude of the increased toxicity was greater at higher doses of cyclophosphamide). When PS-341 was administered along
with cyclophosphamide, toxicity toward bone marrow CFU-GM increased from 3.5- to 10-fold in animals bearing the EMT-6/Parent tumor, from 8- to 10-fold in animals bearing the EMT-6/CTX tumor, and from 3- to 10-fold in animals bearing the EMT-6/CDDP tumor.

The EMT-6/Parent tumor was more sensitive to CDDP than was the EMT-6/CTX tumor or the EMT-6/CDDP tumor (Fig. 6). Treating animals bearing each of these three tumors with PS-341 (0.1 mg/kg) p.o. twice/day on days 0–8, along with CDDP administered on day 8, did not significantly alter the toxicity of CDDP toward the tumors. CDDP is not very toxic toward bone marrow CFU-GM; however, administering PS-341 (0.1 mg/kg) p.o. to the animals twice daily on days 0–8 increased the toxicity of CDDP to the animals bearing each of the tumors, but most markedly increased the toxicity of CDDP toward the bone marrow CFU-GM from animals bearing the EMT-6/CTX tumor.

The Lewis lung carcinoma was selected for the initial in vivo tumor growth delay studies with PS-341 because this tumor metastasizes avidly to the lung, thus allowing response of the primary tumor and response of systemic disease to be assessed. PS-341 was administered p.o. beginning on the same day (day 0) of tumor implantation, beginning on day 4 after tumor implantation when tumor angiogenesis had begun, or beginning on day 7 after tumor implantation when the tumor was well-established (Table 1). The dose of PS-341 varied from 1 mg/kg/day to 0.1 mg/kg/day; however, there was no significant effect of PS-341 dose on the growth delay of the Lewis lung carcinoma (P > 0.4). There was a significant effect of tumor burden so that starting PS-341 administration on day 0 when the tumor burden was minimal was more effective than starting PS-341 administration on day 7 when the tumor was well-established (P < 0.05). Overall, the tumor growth delay produced by PS-341 treatment was 4.3
days, 3.6 days, and 2.6 days for drug administration initiated on day 0, day 4, or day 7 after tumor implantation, respectively. PS-341 administration was highly effective in decreasing the number of lung metastases present in the animals on day 20 after tumor implantation (Table 1). Beginning PS-341 treatment on day 0 decreased the number of lung metastases to 22–35% of the number found in the control animals. Greater numbers of lung metastases were present on day 20 in animals in whom PS-341 was started later; however, the number of lung metastases found in animals treated with PS-341 beginning on day 7 were still significantly less than in the control animals, ranging from 33–67% of the control number ($P < 0.05$). There was a trend toward a lower percentage of large (vascularized) lung metastases in the PS-341-treated animals, indicating that PS-341 slowed the growth of the lung metastases that formed.

For initial combination therapy regimens, PS-341 (0.1 or 0.03 mg/kg) was administered p.o. to animals bearing the Lewis lung carcinoma on days 4–18 after tumor implantation (Table 2). The cytotoxic therapies 5-fluorouracil, cisplatin, Taxol, Adriamycin, and fractionated radiation therapy, were administered beginning on day 7 after tumor implantation. Each of the cytotoxic therapies and PS-341 were effective in producing a measurable growth delay in the Lewis lung carcinoma. The tumor growth delay produced by combination regimens indicated that PS-341 produced primarily additive antitumor activity with each of the cytotoxic therapies. PS-341 was effective against the metastatic disease in these animals, as were the cytotoxic therapies to varying degrees. Administering PS-341 along with the cytotoxic therapies reduced the number of lung metastases on day 20 further such that, in animals treated with 5-fluorouracil and PS-341, there were 1.5–2.5 (0% large) lung metastases on day 20 compared with 33 (45% large) lung metastases in the control animals.

To assess direct systemic administration of PS-341 and chemotherapy, lower doses of PS-341 were administered by i.p. injection, along with a range of doses of 5-fluorouracil to animals bearing Lewis lung carcinoma (Table 3). PS-341 (0.1 mg/kg) administered by i.p. injection on days 4–18 after tumor cell implantation was somewhat less effective against both the primary and metastatic disease than the same dose and schedule of PS-341 administered p.o. The combinations of PS-341 and 5-fluorouracil resulted primarily in additive tumor growth delay in the s.c. tumor and were highly effective against the metastatic disease.

**DISCUSSION**

Inhibition of the proteasome represents a new and unique target for cancer therapy. The consequences of proteasome inhibition in a malignant cell can be manifold, including inhibition of proliferation and inability to actively mount a response to stress. Cell culture studies suggest that the proteasome has a significant role in maintaining cell viability and controlling cell proliferation (20, 41). PS-341 is a potent proteasome inhibitor in cell culture and is p.o. bioavailable (8). PS-341 was a potent cytotoxic agent toward human MCF-7 breast carcinoma cells in culture and demonstrated cytotoxic activity toward EMT-6 mammary carcinoma tumor cells in vivo when administered p.o. or i.p. and produced some toxicity toward bone marrow CFU-GM. Although administration of PS-341 increased the cytotoxicity of cyclophosphamide, radiation therapy and CDDP in sensitive EMT-6 tumors, administration of this proteasome inhibitor was not able to overcome *in vivo* resistance in the resistant EMT-6 tumors. The tumor cell survival assay coupled with the bone marrow CFU-GM assay provide an indication of therapeutic index, assuming that the bone marrow CFU-GM is a representative sensitive normal tissue. Whereas combined treatment with PS-341 and cyclophosphamide did not significantly increase the toxicity of cyclophosphamide to the bone marrow CFU-GM, when administration of PS-341 was added to treatment with CDDP there was a marked increase in the killing of bone marrow CFU-GM. CDDP does not have dose-limiting toxicity related to bone marrow when administered as a single agent; however, combination with an agent such as PS-341 may alter the normal tissue toxicity patterns seen with standard chemotherapeutic agents.

Administered p.o., PS-341 had antitumor activity against the Lewis lung carcinoma and was highly effective against lung metastatic disease, when administered i.p. PS-341 seemed to be somewhat less effective than when administered p.o., although only one dose level was administered by both routes. In tumor growth delay studies, PS-341 did not seem to add to the toxicity of the chemotherapeutic agents administered in combination with it and seemed to produce additive tumor growth delay with the other drugs in combination. There was no evidence of dose modification when PS-341 was administered with 5-fluorouracil over a dosage range reinforcing the notion that these two drugs...

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**Table 3** Growth delay and number of lung metastases in animals bearing Lewis lung carcinoma after treatment of the animals with i.p. administered PS-341 and 5-fluorouracil

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Tumor growth delay (days)</th>
<th>Mean number of lung mets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td>31</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 mg/kg, i.p., d 7–11</td>
<td>4.0 ± 0.4</td>
<td>4</td>
</tr>
<tr>
<td>25 mg/kg, i.p., d 7–11</td>
<td>2.5 ± 0.3</td>
<td>4</td>
</tr>
<tr>
<td>20 mg/kg, i.p., d 7–11</td>
<td>1.8 ± 0.3</td>
<td>4</td>
</tr>
<tr>
<td>PS-341</td>
<td></td>
<td></td>
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<tr>
<td>0.1 mg/kg, i.p., d 4–18</td>
<td>1.8 ± 0.3</td>
<td>12</td>
</tr>
<tr>
<td>0.03 mg/kg, i.p., d 4–18</td>
<td>1.6 ± 0.3</td>
<td>16</td>
</tr>
<tr>
<td>0.01 mg/kg, i.p., d 4–18</td>
<td>0.9 ± 0.3</td>
<td>17</td>
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<td>PS-341 (0.1 mg/kg) + 5-fluorouracil</td>
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<td></td>
</tr>
<tr>
<td>30 mg/kg, i.p., d 7–11</td>
<td>5.0 ± 0.4</td>
<td>2</td>
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<tr>
<td>25 mg/kg, i.p., d 7–11</td>
<td>4.2 ± 0.3</td>
<td>2</td>
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<td>3.7 ± 0.3</td>
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<td>25 mg/kg, i.p., d 7–11</td>
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<td>25 mg/kg, i.p., d 7–11</td>
<td>2.8 ± 0.3</td>
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<tr>
<td>20 mg/kg, i.p., d 7–11</td>
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operate by completely independent noninteractive mechanisms. PS-341 alone and in combination was highly effective against disease metastatic to the lungs. This effect may be a result of tumor burden or may result from a specific tissue effect in the lungs.

The proteasome represents an interesting new target for cancer chemotherapy. PS-341 was chosen as the first representative of this new class of agents to enter the clinic and is currently under Phase I clinical evaluation (24). Further exploration of the proteasome inhibitor PS-341 in this setting is clearly warranted.

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The Proteasome Inhibitor PS-341 in Cancer Therapy
Beverly A. Teicher, Gulshan Ara, Roy Herbst, et al.


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