

A Phase I Trial of the Novel Proteasome Inhibitor PS341 in Advanced Solid Tumor Malignancies¹

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ABSTRACT

Purpose: The purpose of this study was to evaluate the toxicity and pharmacodynamic behavior of the novel proteasome inhibitor PS341 administered as a twice weekly i.v. bolus for 2 weeks, followed by a 1-week recovery period in patients with advanced solid tumor malignancies.

Experimental Design: In this Phase I trial, 43 patients were treated with PS341 in doses ranging from 0.13 to 1.56 mg/m²/dose. A standard Phase I design was used. Pharmacodynamic studies were performed to access 20S proteasome activity.

Results: Forty-three patients were treated with 89 cycles of PS341. Patients were heavily pretreated. Dose-limiting toxicities on this schedule were diarrhea and sensory neurotoxicity. Other side effects seen were fatigue, fever, anorexia, nausea, vomiting, rash, pruritus, and headache. There was no dose-limiting hematological toxicity. A dose-related inhibition of 20S proteasome activity with increasing dose of PS341 was seen. There was one major response in a patient with refractory non-small cell lung carcinoma.

Conclusions: Given the results of this trial, it is safe and reasonable to recommend treatment with PS341 on the schedule used in this trial at 1.56 mg/m²/dose in Phase II trials. Particular care should be taken with patients with preexisting neuropathy. Further testing in Phase II trials is warranted.

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INTRODUCTION

The ubiquitin proteasome pathway is a highly conserved intracellular pathway for the degradation of proteins. Many of the short-lived regulatory proteins that govern cell division, growth, activation, signaling, and transcription are substrates that are temporally degraded by the proteasome. The proteasome therefore represents a novel target for chemotherapy. PS341 is a dipeptidyl boronic acid inhibitor with high specificity for the proteasome. It is the first member of this new class of antitumor agents to come to human trials.

The ubiquitin-proteasome pathway plays a significant role in neoplastic growth and metastasis. The ordered and temporal degradation of numerous key proteins such as cyclins, cyclin-dependent kinase inhibitors, and tumor suppressors is required for cell cycle progression and mitosis (1). The proteasome is also required for activation of NF- κ B³ by degradation of its inhibitory protein, I κ B (2). NF- κ B is required, in part, to maintain cell viability through the transcription of inhibitors of apoptosis, in response to environmental stress or cytotoxic agents (3–6). Stabilization of the I κ B protein and blockade of NF- κ B activity has been demonstrated to make cells more susceptible to apoptosis (3–5). Furthermore, NF- κ B has been implicated in controlling the cell surface expression of adhesion molecules such as E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 (7, 8). Proteasome inhibitors have also been shown to overcome Bcl-2-mediated protection from apoptosis (9–11).

Using the National Cancer Institute's preclinical *in vitro* cytotoxicity screen of 60 cell lines derived from multiple tumors (12), PS341 was found to have potent and wide-ranging antitumor activity (13). There was excellent correlation between intrinsic potency against the proteasome and cell cytotoxicity among tested proteasome inhibitors. Moreover, in this assay PS-341 was also found to have a unique pattern of growth inhibition and cytotoxicity, suggesting that PS-341 represents a novel class of cytotoxic compounds (13). Human xenograft studies demonstrated antitumor activity in nude mice injected s.c. with HT-29 (colon), PC-3 (prostate), LoVo (colon), and PANC-1 (pancreatic), and Lewis lung carcinoma cells (13, 14).

In animal models, PS341 is rapidly removed from the vascular compartment and distributed widely, quickly approaching the limits of detection. It is not yet possible to correlate plasma concentration of the drug with the degree of proteasome inhibition in blood samples. A pharmacodynamic assay has therefore been developed that provides a reliable measure of 20S proteasome activity in peripheral leukocytes, whole blood, and tissue biopsy that is sensitive, accurate, and reproducible

³ The abbreviations used are: NF- κ B, nuclear factor- κ B; MTD, maximum tolerated dose; ECG, electrocardiogram; KPS, Karnofsky performance status.

(15). This assay allows for measurement of PS341 pharmacodynamics and reflects its target activity.

In preclinical toxicology studies in rodents and primates, the main side effects seen were gastrointestinal (16). In primates, treated twice weekly for 4 weeks, this was manifested as a decrease in food intake (anorexia), emesis, and diarrhea, all of which occurred in a dose-related manner. Three doses were tested, 0.54, 0.8, and 1.2 mg/m². The 0.8-mg/m² dose was determined to be the MTD. No other significant toxicity was seen. Modest effects of PS341 were seen in the spleen and the thymus, where lymphocytic depletion was observed. PS341 did not induce bone marrow toxicity. The 20S proteasome activity in WBCs was significantly decreased in all treated groups 1 h postdose as compared with vehicle-treated animals. Recovery to vehicle control levels occurred within 72 h. The percentage of proteasome inhibition at the MTD dose of 0.8 mg/m² was 76%. Beyond this level, the animals developed hypotension and death.

The principal objectives of this Phase I and pharmacodynamic study were to: (a) characterize the toxicities of PS341 administered twice weekly for 2 weeks, followed by a 1-week recovery period in patients with advanced solid tumor malignancies; (b) determine the MTD and recommended dose for subsequent Phase II trials; (c) characterize the pharmacodynamics of 20S proteasome activity in patients treated with PS341; and (d) seek preliminary evidence of antitumor activity in patients with advanced cancer.

PATIENTS AND METHODS

Patient Selection. Patients with histologically documented solid tumor malignancies refractory to standard therapy or for whom no effective therapy existed were eligible for this trial. Other eligibility criteria were: (a) age ≥ 18 years; (b) Karnofsky performance status ≥ 70 ; (c) adequate bone marrow function (WBC $\geq 3,000/\text{mm}^3$, absolute neutrophil count $\geq 1,500/\text{mm}^3$, platelets $\geq 100,000/\text{mm}^3$, and hemoglobin ≥ 8 g/dl), hepatic function (aspartate aminotransferase and alanine aminotransferase levels ≤ 2.5 times upper limit of institutional normal, total bilirubin ≤ 1.8 mg/dl), and renal function (serum creatinine ≤ 1.5 mg/dl or calculated or measured creatinine clearance ≥ 50 ml/min); (d) normal ejection fraction ($>50\%$) by multiple gated acquisition scan or echocardiography; (e) no major surgery within 2 weeks of study entry; (f) no chemotherapy, radiation therapy, or immunotherapy within 4 weeks of study entry; (g) no significant atherosclerotic disease (defined as peripheral vascular disease requiring surgical management, history of myocardial infarction or congestive heart failure, history of cerebrovascular event, and/or history of transient ischemic attack); (h) no ECG evidence of acute ischemia or significant conduction abnormality (bifascicular block, defined as left anterior hemiblock in the presence of right bundle branch block; 2nd or 3rd degree AV blocks); (i) no orthostatic hypotension; (j) no current or concurrent use of calcium channel blockers, beta blockers, or alpha blockers; (k) no inflammatory bowel disease; (l) no diabetes mellitus requiring insulin or oral hypoglycemics for >2 years; (m) no uncontrolled central nervous system metastases (controlled defined as radiographically stable lesions, asymptomatic, and does not require steroids); and (n) no serious medical or psychiatric illness that would limit full compliance

with the study. Informed consent was obtained according to federal and institutional guidelines.

Drug Administration. PS-341 was administered twice weekly for 2 consecutive weeks with a 1-week recovery period. Treatments in a particular week were either given on Monday and Thursday, Monday and Friday, or Tuesday and Friday. PS-341 was administered as a rapid i.v. bolus into the side arm of a running i.v. infusion of normal saline at 100 ml/h. In primate studies, using a schedule of twice weekly for 4 weeks, the highest PS-341 dose not associated with severe irreversible toxicity was 0.8 mg/m²/dose. Because the pharmacodynamics of PS341 was not known in patients, PS-341 was administered in this trial twice weekly for 2 weeks, followed by a 1-week recovery period. The starting dose of 0.13 mg/m²/dose represents one-sixth the MTD of 0.8 mg/m²/dose in primates. Toxicities were graded according to the National Cancer Institute's Common Toxicity Criteria version 2.0. Dose-limiting toxicity was defined as grade 4 hematological toxicity and any grade ≥ 3 nonhematological toxicity, except alopecia, occurring during the first cycle of therapy. Three to six patients were entered at each dose level. The highest dose level of 1.56 mg/m² was expanded to 12 patients to gather further toxicity information. No inpatient dose escalation was allowed. All patients at a particular dose level had to complete one cycle of therapy (3 weeks) before escalation to the next dose level could occur. If one patient experienced dose-limiting toxicity, 3 additional patients were added to the dose level. If 2 of 6 patients experienced dose-limiting toxicity, the previous dose level was declared the MTD. If only 1 of 6 patients experienced dose-limiting toxicity, dose escalation was permitted to continue.

PS-341 (*N*-pyrazinecarbonyl-L-phenylalanine-L-leucine boronic acid) was supplied by Millennium Pharmaceuticals, Inc. (Cambridge, MA) in 5-ml glass vials as lyophilized white cake for reconstitution in normal saline. Each sterile single-use vial contained 2.5 mg of PS-341 and 25 mg of mannitol USP. Each vial was reconstituted with 2.5 ml of normal (0.9%) saline, Sodium Chloride Injection USP, so that the reconstituted solution contained PS-341 at a concentration of 1 mg/ml. The reconstituted solution is clear and colorless, with a final pH of 5–6. The reconstituted solution was administered promptly and in no case >8 h after reconstitution.

Pretreatment and Follow-Up Studies. Pretreatment evaluation consisted of history and physical exam, assessment of KPS, ECG, measurement of left ventricular ejection fraction (by multiple gated acquisition scan or echocardiography), chest radiograph, urinalysis, CBC, serum chemistries (electrolytes, glucose, blood urea nitrogen, creatinine, magnesium, calcium, phosphate, albumin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and total bilirubin), and documentation of measurable disease. During cycle one of therapy, patients were seen before the administration of drug each day for physical exam, determination of KPS, and toxicity assessment. A physical exam and toxicity assessment was also done once during the rest week. A toxicity assessment was done on day 2. Before each day of treatment in cycle one, routine laboratory studies (CBC and serum chemistries) and ECG were performed. During subsequent cycles, patients were seen before the administration of drug each week, and a telephone interview for toxicity was performed during the rest of the week. On day 1 of subsequent cycles, assessment of performance status, phys-

Table 1 Patient characteristics

	No. of patients
Total registered patients	43
Sex	
Male	19
Female	24
Age, yr	
Median	53
Range	34–75
KPS	
Median	80
Range	70–90
Tumor type	
NSCLC ^a	8
Colon	6
Head and Neck	5
Melanoma	4
Ovary	4
Prostate	4
Renal	4
Pancreas	2
Bladder	1
Cervix	1
Endometrial	1
Esophagus	1
Gastric	1
Unknown primary	1
Number of prior chemotherapy regimens	
Median	4
Range	1–16
Prior radiotherapy	
Definitive	12
Palliative	12

^a NSCLC, non-small cell lung cancer.

ical exam, toxicity assessment, routine laboratory studies, ECG, and urinalysis were performed.

Evaluation of response was performed after every two treatment cycles. A complete response was defined as resolution of all measurable disease, signs, symptoms, and biochemical changes related to the malignancy, without the appearance of new lesions for at least 4 weeks. A partial response was defined as the reduction by at least 50% in the sum of the products of the perpendicular diameters of all measurable lesions without the appearance of new lesions for at least 4 weeks. Patients were allowed to continue treatment in the absence of disease progression or intolerable toxicity.

Pharmacodynamics. In animal model studies, PS-341 was rapidly removed from the vascular compartment and distributed widely, quickly approaching the limits of detection. Therefore, a pharmacodynamic assay that results in a calculation of percentage of inhibition of the proteasome was used as an alternative to pharmacokinetic measurements. The method for determination of 20S proteasome activity was developed by Millennium Pharmaceuticals, Inc. (15). Blood samples were collected in sodium heparin-containing tubes and inverted several times before freezing at -80°C . Samples were sent frozen to Millennium Pharmaceuticals, Inc. for 20S proteasome inhibition determination. The blood cells were lysed with 5 mM EDTA (pH 8.0) for 1 h and then centrifuged at $6600 \times g$ for 10 min at 4°C . The resultant whole blood lysate samples were then used in the 20S proteasome assay as described previously (15). Briefly, samples (10 ml) were added to 2 ml of

Table 2 Dose levels, number of patients, and number of cycles

Dose level (mg/m ² /dose) ^a	Total no. of patients	Total no. of cycles	Range (no. of cycles)
0.13	3	6	2
0.25	4	9	1–4
0.40	5	8	1–2
0.60	4	8	1–4
0.75	3	9	2–4
0.90	6	17	2–6
1.08	3	6	2
1.30	3	7	1–4
1.56	12	19	1–3

^a PS341 was administered as an i.v. bolus twice weekly for 2 consecutive weeks with a 1-week rest period.

substrate buffer (20 mM HEPES, 0.5 mM EDTA, 0.05% SDS, and 60 mM Ys substrate-Suc-Leu-Leu-Val-Tyr-AMC; Bachem, King of Prussia, PA). The reaction was carried out at 37°C for 5 min, and the rate of substrate cleavage per 20S proteasome activity was determined. The protein content of the samples was determined using a Coomassie protein assay (Pierce Corp., Rockford, IL). Data are represented as $\pm\text{SE}$ with statistical significance of $P < 0.05$. Two samples were obtained prior to study to establish a baseline for each patient. During the first cycle, samples were drawn on the following schedule: (a) day 1: 0, 1, and 24 h; (b) day 4: 0 and 1 h; (c) day 8: 0, 1, and 24 h; and (d) day 11: 0 and 1 h.

RESULTS

Patient Characteristics. Forty-three patients were enrolled into the study. All patients were assessed for toxicity. Three patients failed to complete a full cycle of therapy (1 for possible drug toxicity and 2 for rapid progression of disease). Patient characteristics are listed in Table 1. The median KPS was 80% (range, 70–90). Patients were heavily pretreated. The median number of prior chemotherapy regimens was 4, with a range of 1–16. Twelve of the patients had definitive radiotherapy as part of primary therapy for their malignancy. An additional 12 patients had palliative radiotherapy given some time during the course of their disease before starting this trial.

Drug Delivery. Forty-three patients received a total of 89 cycles of PS-341 at doses ranging from 0.13 to 1.56 mg/m²/dose. The dose escalation schema, as well as the number of patients and courses administered as a function of dose level, are reported in Table 2. The median number of courses administered per patient was two (range, one to six).

Toxicity. Both hematological and nonhematological toxicity were minimal at the first five dose levels (0.13–0.75 mg/m²/dose). Toxic effects that are reported were scored as being possibly, probably, or definitely drug related. These are reported as the worst toxicity per category exhibited by a patient during their treatment course with PS-341. Toxicities that were scored as not being drug related have been reviewed and have not been included if they were clearly not associated with the experimental treatment.

Hematological Toxicity. Hematological toxicity is summarized in Table 3. There was no dose-limiting hematological toxicity seen in the trial. There was an association between increased dose and the development of thrombocytopenia and neu-

Table 3 Hematological toxicities

Dose levels (mg/m ²), no. of patients	Total no. of cycles	Hemoglobin				Platelets				Neutrophils			
		1	2	3	4	1	2	3	4	1	2	3	4
0.13, n = 3	6	0	1	0	0	0	0	0	0	0	0	0	0
0.25, n = 4	9	0	1	0	0	0	0	0	0	0	0	0	0
0.40, n = 5	8	0	4	0	0	0	0	0	0	0	0	0	0
0.60, n = 4	8	0	0	0	0	0	0	0	0	0	0	0	0
0.75, n = 3	9	1	1	0	0	0	0	0	0	0	0	0	0
0.90, n = 6	17	1	0	0	0	1	1	0	0	0	0	1	0
1.08, n = 3	6	0	2	0	0	1	0	0	0	0	1 ^a	0	0
1.30, n = 3	7	2	0	1	0	1	1	0	0	0	0	0	0
1.56, n = 12	19	5	3	1	0	3	2	2	0	0	0	1	0

^a Episode associated with febrile neutropenia.

Table 4 Nonhematological toxicities

Dose Levels (mg/m ²), no. of patients	Total no. of cycles	Nausea				Vomiting				Diarrhea				Fatigue				Fever				Rash				Prurits				Neuropathy-Sensory				Headache														
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4															
0.13, n = 3	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
0.25, n = 4	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
0.40, n = 5	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
0.60, n = 4	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0.75, n = 3	9	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
0.90, n = 6	17	1	0	0	0	1	0	0	0	1	0	0	0	1	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.08, n = 3	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
1.30, n = 3	7	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.56, n = 12	19	8	1	0	0	5	1	0	0	1	4	2	0	6	3	0	0	3	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	2	0	6	0	0	0	0	0	0	0	0	

tropenia. Anemia was more difficult to interpret because of the many patients entering the trial with anemia related to multiple factors. These factors include anemia of chronic disease (secondary to advanced malignancy), ongoing blood loss from known tumor sites in the gastrointestinal and genitourinary tracts, and bone marrow involvement with tumor. As most patients in this trial progressed, the above factors progressed as well. It was therefore very difficult to assess the relative contribution of PS-341 to the progressive anemia. Hemoglobin levels reported in Table 3 represent nadir values/patient during the course of the study and are not necessarily a result of drug use.

There was no thrombocytopenia seen during at the first five dose levels (0.13–0.75 mg/m²/dose). There was no clinical sequelae associated with the thrombocytopenia episodes seen at higher levels, such as bleeding or need for transfusion. There was no neutropenia seen at the first five dose levels (0.13–0.75 mg/m²/dose). At the 0.9-mg/m²/dose level, there was one grade 3 neutropenia seen (nadir value, 0.7). This was in a patient with known bone marrow involvement with metastatic prostate cancer. At the 1.08-mg/m²/dose level, one patient experienced a grade 2 neutropenia (nadir value, 1.2) that resulted in an admission for febrile neutropenia. The neutropenia resolved within 24 h. The patient had infiltrates on chest radiograph that were treated with antibiotics with resolution.

Nonhematological Toxicity. Nonhematologic toxicity is summarized in Table 4. The two dose-limiting toxicities seen were diarrhea and a painful sensory neuropathy. Two of the 12 patients treated at the highest dose level of 1.56 mg/m²/dose of PS341 developed grade 3 diarrhea. The first patient developed

grade 3 diarrhea that lasted for 36 h after his third dose of PS341 (day 8) of cycle one. He eventually took two loperamide tablets with resolution. He took loperamide prophylactically with his fourth dose of drug (day 11) and had no further diarrhea. He was taken off study after one cycle secondary to the development of a small bowel obstruction related to disease progression and adhesions (from prior radiotherapy and surgery). The second patient had grade 3 diarrhea on day 15 of cycle one. She had continuous diarrhea for an 18-h period. It eventually resolved with the use of loperamide and Pepto-Bismol. She was dose reduced to 1.3 mg/m²/dose with her second cycle and experienced grade 2 diarrhea. Four additional patients at this dose level had grade 2 diarrhea, and one patient grade 1 diarrhea. All patients described the diarrhea as watery, profuse, associated with abdominal bloating and cramping, and self-limiting within 24–36 h. It was not hemorrhagic. Three patients at lower dose levels reported grade 1 diarrhea (one each at the 0.75, 0.90, and 1.30 mg/m²/dose levels). These results suggest a relationship between increasing dose of PS341 and development of diarrhea.

Two of 12 patients at the 1.56 mg/m²/dose level experienced grade 3 sensory neuropathy. Both patients had preexisting neuropathy from heavy prior treatment that worsened while on treatment with PS341. The first patient entered the trial with grade 1 sensory neuropathy manifested by tingling in his feet. His prior treatment for bronchioloalveolar carcinoma included known neurotoxic agents such as paclitaxel and carboplatin. After two cycles of PS341, he reported increased tingling in his feet (still grade 1). After three cycles of PS341, he developed numbness and tingling in his hands and feet as well as an acute onset of severe pain in his

feet, legs, buttocks, and low back. His symptoms prevented him from sleeping and going to work. It made it difficult for him to walk. His neurological exam revealed absent deep tendon reflexes and decreased pinprick sensation in his fingers and toes. He was placed on narcotic analgesics and amitriptyline to help control his symptoms. He was taken off study after the three cycles because of this toxicity (typical stocking glove sensory neuropathy with a severe neuropathic pain component). His symptoms improved 2–3 months after stopping PS341. His symptoms remain grade 2 7 months from the completion of therapy. The second patient had a preexisting grade 2 neuropathy upon entering the trial. She had received 11 prior regimens for refractory granulosa cell tumor of the ovary including cisplatin and paclitaxel. After one cycle of PS341, she noted an increase in neuropathy to the point that it interfered with function (grade 3). PS341 was stopped, and her symptoms returned to baseline within 3 weeks. A third patient on the 1.56-mg/m²/dose level had worsening neuropathy on treatment with PS341. This patient had a preexisting grade 1 sensory neuropathy upon entering the trial. He had received five prior regimens for pancreatic cancer including extensive prior treatment with cisplatin. He had an increase in his neuropathy to grade 2 after one cycle of therapy. He was not rechallenged. His neuropathy improved to baseline in the 3 months after stopping PS341.

One of the three patients at the 1.30-mg/m²/dose level developed a grade 3 sensory neuropathy after four cycles of PS341. His symptoms were identical to the patient with bronchioloalveolar carcinoma described above in that there was a predominant component of neuropathic pain. He was heavily pretreated for nasopharyngeal cancer including treatment with cisplatin and paclitaxel. He had grade 1 neurosensory toxicity upon study entry. After 4 cycles of PS341, he began to develop painful tingling in his arms and legs that rapidly progressed to grade 3. The pain limited his ability to perform his activities of daily living and confined him to his home. He felt weak as a result of the pain and had difficulty walking. He was treated with narcotic analgesics and gabapentin capsules. On neurological exam he had graded loss of pinprick sensation to the level of the wrists and one-third up the legs. He had severely impaired vibration in his feet as well as impaired proprioception the level of the ankle. Vibration was modestly impaired in the fingers. His gait was wide-based and tentative. He was unable to perform toe-walking or tandem gait. Electromyography and nerve conduction studies were performed and consistent with a severe generalized sensory-motor axonal polyneuropathy. The patient did not have a detailed neurological exam by a neurologist or electromyography and nerve conduction studies before study entry that would allow for a direct comparison. His symptoms improved to grade 2 over the 3 months after drug was stopped.

One patient at the 1.08-mg/m²/dose level experienced grade 1 neuropathy after two cycles of therapy. This resolved when PS341 was discontinued. There were no other events of neurological toxicity on the study.

There were no other grade 3 nonhematological toxicities seen. One patient at the 0.90-mg/m²/dose level reported grade 1 nausea and vomiting. At the 1.56-mg/m²/dose level, 8 of 12 patients experienced grade 1 nausea. Five of these 8 patients had grade 1 vomiting as well. One patient developed grade 2 nausea and vomiting. No prophylactic antiemetics were given in the trial. Nine of the 43 patients developed fever (not associated

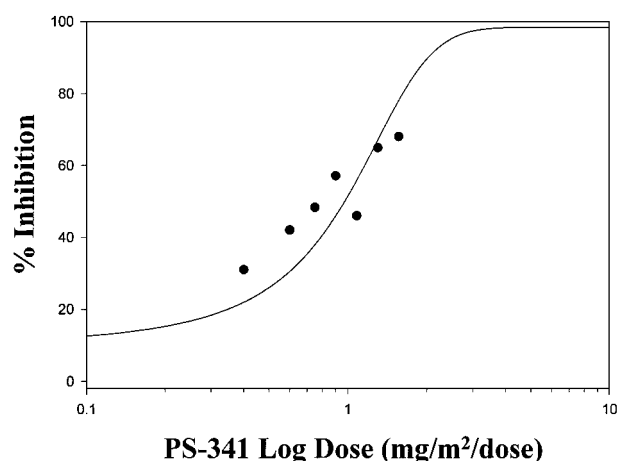


Fig. 1 The percentage of inhibition of the proteasome at 1 h after i.v. dosing.

with infection or neutropenia) either during drug administration or within the 36 h after drug administration. The development of fever was not associated with increasing dose of drug. Three patients developed a diffuse maculopapular rash (one each on the 0.90-, 1.08-, and 1.56-mg/m²/dose levels). Two patients complained of pruritus in the absence of rash (one at the 0.90-mg/m²/dose level and one at the 1.56-mg/m²/dose level). Six of the 12 patients at the 1.56-mg/m²/dose level complained of mild headache, which occurred intermittently during the period of the study, most pronounced in the 24 h after dosing.

One patient treated with two doses (days 1 and 4) of PS341 at the 1.56-mg/m²/dose level experienced asymptomatic ECG changes suggestive of cardiac ischemia (grade 2). She entered the trial with a normal ECG. ECG on day 4 before treatment revealed T-wave inversions in the anterior leads. These were thought to be nonspecific. ECG on day 8 revealed more pronounced T-wave inversions in the anterior leads, and we could not rule out antero-septal ischemia. The patient was taken off study. An echocardiogram revealed no change from pretreatment baseline. It was significant for pulmonary hypertension with right ventricular enlargement. One week later her ECG returned to normal. Although the patient most likely has intermittent T-wave changes based on known pulmonary hypertension with right ventricular strain, this is being reported as possibly related secondary to the temporal relation to drug administration.

Pharmacodynamics. The results of the 20S proteasome inhibition determinations at 1 h after dosing are shown in Fig. 1. Relative proteasome activity (compared with baseline) is shown as a function of PS341 dose (mg/m²/dose). A clear dose-related inhibition of 20S proteasome activity with increasing dose of PS341 was seen. There was no significant difference in the mean percentage of inhibition 1 h after dosing on days 1, 4, 8, and 11. Assays performed on samples drawn immediately before treatment on days 4, 8, and 11 consistently showed complete recovery of proteasome activity to baseline. These findings suggest that as long as baseline 20S proteasome levels are allowed to return to normal between dosing, there is no apparent change in the sensitivity toward PS341-induced proteasome

inhibition. The mean percentage of inhibition of the proteasome at 1 h by dose level were as follows: 0.4 mg/m²/dose, 31%; 0.6 mg/m²/dose, 42%; 0.75 mg/m²/dose, 48%; 0.9 mg/m²/dose, 57%; 1.08 mg/m²/dose, 46%; 1.3 mg/m²/dose, 65%; and 1.56 mg/m²/dose, 68%. Proteasome activity was also evaluated at 24 h after day 1 and day 8 dosing. At 24 h, the mean percentage of inhibition of the proteasome had recovered but not to baseline; the values by dose level are as follows: 0.4 mg/m²/dose, 6.5%; 0.6 mg/m²/dose, 6%; 0.75 mg/m²/dose, 17.5%; 0.9 mg/m²/dose, 29.5%; 1.08 mg/m²/dose, 24.8%; 1.3 mg/m²/dose and 1.56 mg/m²/dose, 32.8%.

Response. There was one partial response seen in a male patient with non-small cell lung cancer (bronchioloalveolar type). The patient had received six cycles of paclitaxel and carboplatin with initial stable disease and then progressed while still on therapy. He subsequently received two cycles of gemcitabine, three cycles of mitomycin and vinblastine, four weekly doses of docetaxel, and then eight weekly doses of methotrexate and progressed on all of the above regimens. His tumor symptoms of cough, bronchorrhea, and intermittent hemoptysis resolved after one cycle of PS341. Computed tomography of the chest after two cycles of therapy revealed a 50% reduction in bilateral pulmonary infiltrative masses. The patient had to stop therapy after three cycles of therapy secondary to the development of neuropathy with neuropathic pain. The duration of the partial response, as measured from the start of therapy until relapse, was 3 months. Three patients (one each with nasopharyngeal carcinoma, malignant melanoma, and renal cell carcinoma) had stable disease as their best response. The median duration of stable disease was 4 months, with a range of 2.5–5 months.

DISCUSSION

The ubiquitin-proteasome pathway plays an essential role in the degradation of most short- and long-lived intracellular proteins in eukaryotic cells. At the heart of this degradative pathway is the 26S proteasome, an ATP-dependent, multicatalytic protease. Proteolytic degradation of damaged, oxidized, or misfolded proteins is part of the “housekeeping” role for the 26S proteasome. In addition, the 26S proteasome also plays a vital role in degrading regulatory proteins that govern the cell cycle, transcription factor activation, apoptosis, and cell trafficking. Inhibitors of the proteasome represent a novel approach to treating human malignancies. PS341 is a reversible, highly selective inhibitor of the proteasome with promising *in vitro* and *in vivo* activity. This Phase I and pharmacodynamic trial was designed to study the feasibility of administering PS341 as a rapid i.v. infusion twice weekly for 2 weeks, followed by a 1-week recovery period, to patients with advanced solid tumor malignancies.

Patients who entered this trial were heavily pretreated and often had a high level of baseline symptomology from their cancers. There was no significant toxicity seen in the first six dose levels tested (0.13–0.90 mg/m²/dose). There was no dose-limiting hematological toxicity seen, although there was an association between increasing dose and the development of nonclinically significant neutropenia and thrombocytopenia. The effect of PS341 on the development of anemia was difficult

to assess in this trial. The nonhematological toxicity seen was largely as predicted by preclinical testing. Patients at the highest dose level tested of 1.56 mg/m²/dose experienced gastrointestinal toxicities similar to those described in preclinical toxicology studies. Patients experienced anorexia, nausea, vomiting, and diarrhea. These symptoms were generally mild. Diarrhea was the most significant of the gastrointestinal toxicities. Two of the 12 patients treated at the highest dose level developed grade 3 diarrhea, with an additional 4 patients developing grade 2 diarrhea and 1 patient developing grade 1 diarrhea. Episodes of diarrhea were self-limiting and did not require hospital admission or parenteral hydration. Because of the few events, it is difficult to comment with certainty, but loperamide seemed to provide symptomatic relief and in some cases prevention upon retreatment. The most serious side effect seen was sensory neuropathy. Two patients experienced this as a painful, grade 3 neurotoxicity that limited their activities and required medication including narcotic analgesia. A third patient had escalation from a significant grade 2 to grade 3 sensory neurotoxicity, but this promptly resolved to baseline with early discontinuation of drug after only one cycle. It should be noted that all patients who experienced neuropathy had prior neuropathy from paclitaxel- and/or platinum-based regimens. Of the 12 patients treated on the highest dose level of 1.56 mg/m²/dose, only 1 patient had no preexisting neuropathy upon study entry, 10 patients had grade 1 neuropathy, and 1 patient had grade 2 neuropathy. Of the 11 patients with preexisting neuropathy, 3 had worsening. It would be prudent to monitor closely for neuropathy when treating patients with PS341, especially in patients with prior exposure to neurotoxic agents and when combining PS341 with other known neurotoxic antineoplastic agents. The neuropathy in both patients described above whose sensory neurotoxicity was troublesome presented with an atypical neuropathic pain syndrome that was first thought to be myalgias or arthralgias and then settled into a more typical stocking glove distribution. Other side effects seen were fatigue, fever, rash, pruritus, and headache. On the basis of these results, a reasonable approach to Phase II dosing would be to start at 1.56-mg/m²/dose level range, with the above-mentioned precautions. Escalation may be possible in less heavily pretreated patients.

The ideal dosing schedule for PS341 is yet to be determined. Several other Phase I trials are in progress to address this question. The following schedules are under investigation in both solid tumor and hematological malignancies: once weekly for four consecutive weeks, followed by a 2-week recovery period; twice weekly for 4 weeks, followed by a 2-week recovery period; and twice weekly on alternating weeks. In addition to defining an ideal dosing schedule, the target population to pursue Phase II testing in, and the appropriate drugs to combine with, PS341 in Phase I combinations are yet to be defined. PS341 holds great promise in the treatment of hematological malignancies. PS341 is a potent inducer of apoptosis in lymphocytes from untreated chronic lymphocytic leukemia patients, as well as cells from chronic lymphocytic leukemia patients resistant to other drugs (such as fludarabine). PS341 seems to induce cytochrome *c* release and caspase activation in these chronic lymphocytic leukemia cells (17, 18). In both multiple myeloma cell lines and multiple myeloma cells from patients, PS341 acts directly to alter cellular interactions and cytokine

secretion in the bone marrow microenvironment to inhibit tumor cell growth, induce apoptosis, and overcome drug resistance (19, 20). In a Phase I trial being performed at the University of North Carolina at Chapel Hill in patients with hematological malignancies, using a twice-weekly schedule for 4 weeks followed by a 2-week recovery period, 2 patients with multiple myeloma entered at early dose levels experienced clinically significant responses (21). Hematological malignancies also allow easy access to malignant cells for correlation of *in vitro* and clinical response as well as investigation of other translational endpoints. This strongly supports further testing of PS341 in multiple myeloma and other hematological malignancies.

Antitumor activity of PS341 in solid tumor malignancies is supported by its activity in National Cancer Institute *in vitro* cancer cell and hollow fiber screens, activity in murine tumor models (B16 melanoma and Lewis lung carcinoma), and activity in human xenograft models (HT-29 colon, NCI-H23 lung, and PC-3 prostate; Refs. 13, 16). Furthermore, the preclinical activity of PS341 makes it a promising agent for combination therapy in overcoming chemotherapy resistance. Preclinical testing has shown synergy between PS341 and the topoisomerase I inhibitor irinotecan (16). Activation of the transcription factor NF- κ B by ionizing radiation (5), irinotecan (22), cisplatin (23), and other chemotherapeutic agents has been found to protect from cell killing. Proteasome inhibitors have been shown to inhibit drug-induced NF- κ B, and in doing so, sensitize the treated cells to drug-induced apoptosis. Proteasome inhibition has also been shown to enhance the radiosensitivity of cell lines *in vitro* (24).

The results of this Phase I trial demonstrate that PS341 on a twice-weekly for 2-week cycle every 21 days results in predictable toxicity and proteasome inhibition associated with preclinical activity. In lieu of a sensitive pharmacokinetic assay, the 20S proteasome pharmacodynamic assay has proved useful and predictive for the dose escalation in this trial. The assay confirms inhibition of the biochemical target, the proteasome, and provides insight into how long the target is affected by PS341. In the future, it is hoped that this assay will be used to determine proteasome inhibition in tumor biopsies, thereby providing data in biologically relevant tissues. Given the antitumor activity noted in a patient with refractory non-small cell lung cancer and preliminary reports of activity in multiple myeloma, further evaluation of PS341 is warranted.

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