Phase I Trial of the Proteasome Inhibitor PS-341 in Patients With Refractory Hematologic Malignancies

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Purpose: To determine the maximum-tolerated dose (MTD), dose-limiting toxicity (DLT), and pharmacodynamics (PD) of the proteasome inhibitor bortezomib (previously known as PS-341) in patients with refractory hematologic malignancies.

Patients and Methods: Patients received PS-341 twice weekly for 4 weeks at either 0.40, 1.04, 1.20, or 1.38 mg/m^2, followed by a 2-week rest. The PD of PS-341 was evaluated by measurement of whole blood 20S proteasome activity.

Results: Twenty-seven patients received 293 doses of PS-341, including 24 complete cycles. DLTs at doses above the 1.04-mg/m^2 MTD attributed to PS-341 included thrombocytopenia, hyponatremia, hypokalemia, fatigue, and malaise. In three of 10 patients receiving additional therapy, serious reversible adverse events appeared during cycle 2, including one episode of postural hypotension, one systemic hypersensitivity reaction, and grade 4 transaminitis in a patient with hepatitis C and a substantial acetaminophen ingestion. PD studies revealed PS-341 induced 20S proteasome inhibition in a time-dependent manner, and this inhibition was also related to both the dose in milligrams per meter squared, and the absolute dose of PS-341. Among nine fully assessable patients with heavily pretreated plasma cell dyscrasias completing one cycle of therapy, there was one complete response and a reduction in paraprotein levels and/or marrow plasmacytosis in eight others. In addition, one patient with mantle cell lymphoma and another with follicular lymphoma had shrinkage of nodal disease.

Conclusion: PS-341 was well tolerated at 1.04 mg/m^2 on this dose-intensive schedule, although patients need to be monitored for electrolyte abnormalities and late toxicities. Additional studies are indicated to determine whether incorporation of dose/body surface area yields a superior PD model to dosing without normalization. PS-341 showed activity against refractory multiple myeloma and possibly non-Hodgkin's lymphoma in this study, and merits further investigation in these populations.

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INTRACELLULAR PROTEIN degradation in eukaryotes occurs predominantly through the ubiquitin conjugating system and the 26S proteasome.[1-3] The former labels proteins with ubiquitin chains, a prerequisite for recognition and degradation by the 26S proteasome, and the latter contains the 20S multicatalytic proteinase complex,[1-3] where the proteolytic enzymes are found.[4] Ubiquitin-proteasome pathway function is essential to a variety of processes, including timely degradation of cyclins, cyclin-dependent kinases, and cyclin-dependent kinase inhibitors[5]; processing and degradation of transcription factors and short-lived regulatory proteins[1-3]; antigen processing[6]; and angiogenesis.[7] Small molecule, cell-permeable inhibitors have uncovered a role for the proteasome in programmed cell death.[8,9] Exposure of a variety of tumor-derived cell lines to proteasome inhibitors triggers apoptosis, likely as a result of effects on several pathways, including cell cycle regulatory proteins, p53, and nuclear factor kappa B (NF-κB).[8-10]

Many of the initial studies documenting proteasome inhibitor–mediated apoptosis used cells of hematopoietic origin, including monoblasts,[11] T-cell and lymphocytic leukemia cells,[12] lymphoma cells,[13] and promyelocytic leukemia cells.[14] The first demonstration of in vivo antitumor activity of a proteasome inhibitor used a human lymphoma xenograft model.[15] Furthermore, proteasome inhibitors were reported to induce preferential apoptosis of patient-derived lymphoma[15] and leukemia cells,[16] and to preferentially inhibit proliferation of multiple myeloma cells,[17] with relative sparing of control, nontransformed cells. These findings suggested that proteasome inhibitors might be applicable as therapeutic agents in patients with refractory hematologic malignancies.

Bortezomib (Velcade [Millennium Pharmaceuticals, Inc, Cambridge, MA]; previously known as PS-341), a dipeptide boronic acid derivative (Fig 1), was synthesized as a highly selective, potent, reversible proteasome inhibitor with a K_i of 0.6

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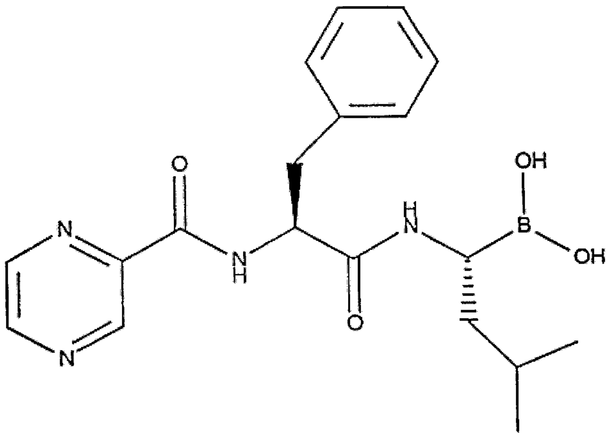

Fig 1. The structure of PS-341.

nmol/L.[18] Using the National Cancer Institute's in vitro screen, PS-341 showed cytotoxicity against a range of tumor lines[18] and had antitumor activity in human prostate[18] and lung cancer[19] xenograft models. In preclinical studies, PS-341 was rapidly cleared from the vascular compartment,[18] suggesting that traditional pharmacokinetic parameters of plasma concentration would not be appropriate for measuring its activity. A pharmacodynamic (PD) assay was therefore developed to measure and monitor activity at the proteasome.[20] To begin to determine the therapeutic potential of proteasome inhibitors in hematologic malignancies, a trial was designed to determine the maximum-tolerated dose (MTD) and dose-limiting toxicity (DLT), and to evaluate the PD of PS-341 in this patient population. The current report details these results and provides preliminary evidence of antitumor activity.

PATIENTS AND METHODS

Patient Selection

Patients with a histologically confirmed hematologic malignancy refractory to standard therapy, or for whom no standard therapy was available, were eligible for enrollment. Inclusion criteria included the following: (1) Eastern Cooperative Oncology Group performance status of 0 to 2; (2) age ≥ 18 years; (3) expected survival ≥ 6 weeks; (4) no radiation/chemotherapy/immunotherapy in the previous 4 weeks, and no major surgery in the previous 2 weeks; (5) absence of active infection; (6) adequate cardiovascular function without ischemia, conduction abnormalities, or myocardial infarction in the previous 6 months; and (7) adequate hepatic function, with bilirubin less than 1.5 times the upper limit of normal, and AST and ALT of less than 2.5 times the upper limit of normal. A total WBC count ≥ 2,000/mm^3, absolute neutrophil count ≥ 1,000/mm^3, hemoglobin ≥ 8.0 g/dL, and a platelet count ≥ 50,000/mm^3 were required. Patients receiving supportive care could continue to do so, but had to be transfusion-independent. An estimated creatinine clearance of ≥ 50 mL/min was required, and was calculated using the formula of Cockroft and Gault.[21] When the activity of PS-341 against multiple myeloma was identified, because renal function had been noted to be a common reason for such patients to be excluded, this was subsequently amended to allow a serum creatinine of ≤ 2.5 mg/dL. Exclusion criteria included (1) pregnancy or breast-feeding; (2) New York Heart Association class III to IV congestive heart failure; and (3) serious medical/psychiatric conditions that could interfere with treatment. All patients had to practice effective birth control and gave written informed consent in accordance with federal and institutional guidelines before treatment.

Study Design

This was a phase I trial designed to determine the MTD and DLT of PS-341 in patients with hematologic malignancies and to study the PD of PS-341. Secondary objectives were to study the response of hematologic malignancies, to correlate toxicity with proteasome inhibition, and to evaluate serum levels of interleukin-6 (IL-6) in patients with multiple myeloma receiving PS-341. DLT was defined using the National Cancer Institute Common Toxicity Criteria Version 2.0[22] as grade 4 hematologic toxicity or hyperbilirubinemia, or a grade 3 toxicity in any other system except for alopecia. MTD was defined as the highest dose studied for which the incidence of DLT was ≤ 33%.

The dosing of PS-341 was in milligrams per meter squared, which was based both on preclinical studies[23] and on preliminary clinical experience from phase I trials conducted in solid tumor patients.[24,25] As an initial dose level, 0.13 mg/m^2 had been chosen because this was one-sixth of the MTD identified in nonhuman primate studies.[23] At the time that this trial was to begin accrual, however, two other phase I studies had already identified this level as being well-tolerated.[24,25] Therefore, after approval by the institutional review board, accrual was begun at 0.40 mg/m^2, the highest level that had at that time been shown to be well tolerated, and further escalation occurred according to a modified Fibonacci schema. Dosing for individuals more than 30% above their ideal body weight was adjusted to reflect 50% of the difference between their actual and ideal weights. For dose escalation, three assessable patients had to complete their first cycle without a DLT. When one DLT was seen, an additional three assessable patients had to be accrued, and further escalation could occur if no additional DLTs were seen. In certain instances, information about the safety profile of PS-341 from phase I trials at other institutions, obtained through Millennium Pharmaceuticals, was used to skip dose levels that were considered biologically inactive, or that had been adequately proven well tolerated, subject to the approval of the institutional review boards at each clinical center. Additional patients were accrued once it was felt that the MTD had been identified to further confirm the safety of this dose.

Drug Administration

Millennium Pharmaceuticals provided PS-341 as a sterile, lyophilized powder containing PS-341 and mannitol. Each vial was reconstituted with normal saline to a final PS-341 concentration of 1 mg/mL, which was administered as an intravenous push over 3 to 5 seconds. Patients were treated twice weekly for 4 weeks on days 1, 4, 8, 11, 15, 18, 22, and 25, followed by a 2-week rest period, with this 6-week period constituting one cycle. Treatment days could be changed by up to 24 hours, provided there was a 72-hour span between doses.

Response Criteria

A complete response (CR) was defined as total resolution of measurable disease parameters, whereas a partial response (PR) was defined as a ≥ 50% resolution without the appearance of new disease. Stable disease was defined as between a less than 50% resolution and a ≤ 25% increase in measurable parameters, again without new disease. Progressive disease was defined as an increase of more than 25% in measurable disease parameters.

PD

In preclinical studies, PS-341 rapidly exited the intravascular compartment and was widely distributed, and serum concentrations approached the limits of detection of the assay within 1 hour. Because pharmacokinetic parameters were not likely to adequately guide dosing and characterize this agent, a PD assay measuring the percentage of inhibition of proteasome function in whole blood[20] was used to provide a more biologically relevant characterization. Blood samples were collected before therapy, and then 1, 6, and 24 hours after each PS-341 dose for measurement of 20S proteasome activity. PD data were then analyzed and modeled using WinNonlin Professional software, Version 3.2 (Pharsight Corp, Mountain View, CA). In addition, serum samples were collected and evaluated for IL-6 with an enzyme-linked immunosorbent assay, which was used according to the manufacturer's specifications (R&D Systems, Minneapolis, MN).

Table 1. Patient Characteristics

Characteristic	No.	%
No. of patients	27	
MSKCC	7	
UNC	20	
Sex		
Male	17	63
Female	10	37
Age, years		
Mean	56	
Range	22-77	
Race		
American Indian	1	4
African American	7	26
White	19	70
Diagnoses		
Hodgkin's disease	4	
Non-Hodgkin's lymphoma	10	
SLL/CLL	2	
DLCL	2	
Mantle cell	3	
Plasma cell dyscrasias	12	
Waldenström's	1	
Multiple myeloma	11	
MDS with excess blasts and POEMS	1	
ECOG performance status		
0	6	22
1	16	59
2	5	19
Prior therapy		
Chemotherapy	27	
Median no. of regimens	3	
Range	1-12	
Radiation therapy	13	
Marrow or stem-cell transplantation	10	

Abbreviations: CLL, chronic lymphocytic leukemia; DLCL, diffuse large-cell lymphoma; MDS, myelodysplasia; MSKCC, Memorial Sloan-Kettering Cancer Center; POEMS syndrome, polyneuropathy/organomegaly/endocrinopathy/monoclonal protein/skin changes; SLL, small lymphocytic lymphoma; UNC, University of North Carolina.

Table 2. Adverse Events Occurring in More Than 10% of All Patients at All Dose Levels Whether Considered Related to PS-341 or Not (N = 27)

Adverse Event	Frequency	
	No.	%
Thrombocytopenia	20	74
Fatigue	16	59
Nausea	14	52
Anemia	13	48
Leukopenia	13	48
Headache	12	44
Diarrhea	10	37
Neutropenia	10	37
Constipation	8	30
Vomiting	8	30
Arthralgia	7	26
Dyspnea	7	26
Hyponatremia	7	26
Fever	7	26
Back pain	6	22
Hypocalcemia	6	22
Rigors	6	22
Abdominal distention	5	19
Decreased appetite	5	19
Dizziness	5	19
Peripheral neuropathy	5	19
Somnolence	5	19
Anorexia	4	15
AST elevation	4	15
Crepitations	4	15
Insomnia	4	15
Malaise	4	15
Myalgia	4	15
Lower extremity edema	4	15
aPTT prolongation	3	11
Alkaline phosphatase increase	3	11
Amylase increase	3	11
Cough	3	11
Depression	3	11
Epistaxis	3	11
Hypokalemia	3	11
Hypotension	3	11
Extremity pain	3	11
Pleural effusion	3	11
PT prolongation	3	11
Rash	3	11

Abbreviations: aPTT, activated partial thromboplastin time; PT, prothrombin time.

RESULTS

Patients

A total of 27 patients at two clinical centers were enrolled between November 1999 and May 2001, with the last patient dosed in June 2001. Two of these patients were treated as protocol exceptions because they had received chemotherapy more recently than 4 weeks before PS-341. One had been treated with cyclophosphamide and prednisone 19 days before PS-341, whereas the second had discontinued thalidomide only 1 week before dosing. The patient demographics are listed in Table 1.

Dose Escalation

The first cohort was enrolled at 0.40 mg/m^2/dose, the second at 1.04 mg/m^2/dose, the third at 1.20 mg/m^2/dose, and the fourth at 1.38 mg/m^2/dose. No DLTs were observed in the initial three cohorts of three patients each at doses ranging from 0.40 to 1.20 mg/m^2/dose. At the 1.38-mg/m^2 dose level, two of five patients experienced DLTs, consisting of two episodes of grade 3 hyponatremia and grade 3 fatigue. In addition, grade 3 thrombocytopenia was seen and PS-341 was withheld because of the anticipation that grade 4 thrombocytopenia was inevitable. Because of these DLTs, the 1.20-mg/m^2 cohort was expanded, but two of four additional patients experienced DLTs, including grade 3 malaise and fatigue in one and grade 3 hypokalemia in another. Therefore, enrollment continued in the cohort at 1.04 mg/m^2 for an additional nine patients to further refine the side-effect profile. Of the 27 patients dosed with PS-341, three received 0.40 mg/m^2/dose, 12 received 1.04 mg/m^2/dose, seven received 1.20 mg/m^2/dose, and five received 1.38 mg/m^2/dose.

Adverse Events

Adverse events were documented in all patients at all dose levels. Among those seen in ≥ 10%, listed in Table-2, cytopenias were prominent, with thrombocytopenia most common and seen in 74%, anemia and leukopenia in 48%, and neutropenia in 37%. Fatigue was seen in 59%, and constitutional symptoms included fevers, rigors, malaise, and anorexia. Gastrointestinal events included nausea in 52%, vomiting in 30%, constipation and diarrhea, and elevations of the AST and amylase levels in 11%. Neurologic symptoms included headache in 44%, back pain in 22%, dizziness or peripheral neuropathy in five patients,

Table 3. PS-341–Related Adverse Events During Cycle 1 of Intensity ≥ Grade 3

	PS-341 Dose Level (mg/m^2)									
	0.40 (n = 3)		1.04 (n = 12)		1.20 (n = 7)		1.38 (n = 5)		Total (N = 27)	
Adverse Event	No.	%	No.	%	No.	%	No.	%	No.	%
At least one adverse event	0		7	58	6	86	4	80	17	63
Blood and lymphatic system	0		5	42	6	86	4	80	15	56
Thrombocytopenia	0		4	33	4	57	2	40	10	37
Anemia	0		1	8	3	43	1	20	5	19
Neutropenia	0		2	17	1	14	1	20	4	15
Leukopenia	0		2	17	1	14	0		3	11
Metabolism and nutrition	0		4	33	1	14	2	40	7	26
Hyponatremia	0		2	17	0		2	40	4	15
Hypokalemia	0		1	8	1	14	0		2	7
Other	0		0		1	14	0		1	4
Malaise	0		0		1	14	0		1	4

and somnolence in some but insomnia in others. Musculoskeletal events including arthralgias, and pulmonary events such as dyspnea were seen in 26%. Finally, electrolyte disturbances were notable, including hyponatremia and hypokalemia.

Thrombocytopenia was the most commonly reported grade 3 event attributed to PS-341, occurring in 10 patients during cycle 1 (37%) (Table 3), and in 11 overall (41%) at some point during therapy, with one patient requiring a platelet transfusion. This study enrolled patients with ≥ 50,000 platelets/mm^3, and those with less than normal platelet counts were at greatest risk for thrombocytopenia, with none completing a full cycle. Patients who entered with normal platelet counts experienced up to grade 3 thrombocytopenia, but only one of nine had a PS-341 dose withheld on this basis. At the MTD of 1.04 mg/m^2, six of 12 patients reached their platelet nadir in week 3 of therapy, whereas the other six did so in week 4, with three patients each doing so on days 15, 18, 22, and 25 of cycle 1. These nadirs tended to persist until the onset of the 2-week rest period, at which point all patients experienced a platelet recovery by day 38 to levels that were generally comparable to, or in two cases even substantially higher than, those before initiation of PS-341 dosing. Across the three higher dose groups, a slight dose-dependent increase in the incidence of grade 3 thrombocytopenia was seen, with 33%, 57%, and 60% of patients at the 1.04-mg/m^2, 1.20-mg/m^2, and 1.38-mg/m^2 cohorts, respectively, experiencing this toxicity. The incidence of nausea also suggested a dose dependence, with 0%, 50%, 57%, and 80% of patients, respectively, developing this adverse event at the four doses. Finally, the incidence of patients suffering any grade 3 or 4 adverse event also was dose dependent, with 33%, 75%, 86%, and 100% of patients reporting such toxicity at the dose levels evaluated, respectively.

Several grade 4 serious adverse events (SAEs) occurred during cycle 1, none of which were felt to be linked to PS-341. The first of these occurred in one patient at 1.20 mg/m^2 with a prior history of coronary artery disease, myocardial infarctions, a decreased ejection fraction, and congestive heart failure, including one episode during previous vincristine, doxorubicin, and dexamethasone (VAD) chemotherapy. During a transfusion initiated for fatigue and anemia, this patient developed a fever and bacteremia with what was later identified as *Stomatococcus mucilaginosus* and coagulase-negative staphylococci, became tachycardic, suffered recurrence of heart failure, and later suffered a myocardial infarction. This patient also had a history of prior episodes of mood alteration and suffered a recurrent grade 4 mood alteration with suicidal ideation that resolved without intervention. One additional grade 4 SAE noted during cycle 1 occurred at 1.20 mg/m^2 in another patient who developed anuria and renal failure. Radiographic evaluation revealed massive progression of perinephric adenopathy with new hydronephrosis, and this event was felt to be because of disease progression. Other SAEs not attributed to PS-341 included two episodes of grade 3 dyspnea, one in a patient with baseline obstructive/restrictive pulmonary disease receiving home oxygen therapy who had a transient worsening in pulmonary status. A second occurred in a patient with a prior pulmonary embolus and recurrent malignant pleural effusion that had been drained by thoracentesis, but which recurred again during therapy.

Overall, five (19%) of the patients developed treatment-emergent peripheral neuropathy felt in three cases to be related to PS-341. In four patients, the neuropathy was assessed as grade 2 in severity and was not dose-limiting, whereas in the sole patient with grade 3 neuropathy this was attributed to progressive disease with spinal cord and nerve root compression. All patients had received prior therapy with other potentially neurotoxic agents, including vinca alkaloids and thalidomide.

Late Toxicity

Ten patients who completed cycle 1 went on to receive one to three additional cycles, with 11 complete and five incomplete courses, and some experienced SAEs. One patient receiving 1.04 mg/m^2 developed a grade 2 maculopapular rash during cycle 1 that, on biopsy, showed a superficial perivascular lymphocytic/eosinophilic infiltrate with prominent dermal mucin deposition felt to represent an unusual hypersensitivity reaction. After resolving spontaneously, this rash reappeared during cycle 2, was more confluent, and was accompanied by dyspnea and arthritis consistent with a systemic hypersensitivity reaction. These manifestations resolved with a brief course of steroids and discontinuation of PS-341. A second event occurred in a patient with Waldenström's and known hepatitis C with normal liver function tests before treatment. During cycle 2 at 1.20 mg/m^2, this patient self-treated with up to 4.0-g acetaminophen daily for abdominal discomfort, and developed grade 2 hyperbilirubinemia and grade 4 ALT and AST elevations. With supportive care, the patient's hepatic enzymes returned to normal. In a third patient, postural hypotension and orthostatic symptoms developed that resolved after discontinuation of PS-341 and initiation

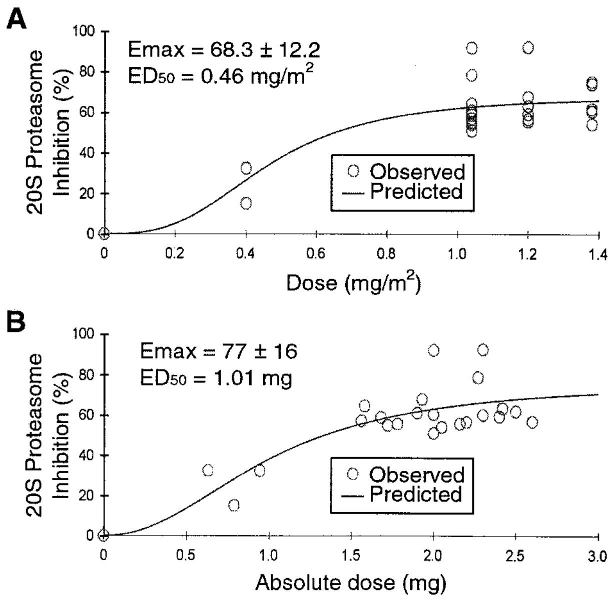

Fig 2. PS-341 pharmacodynamics. (A) 20S proteasome inhibition induced 1 hour after dose 1 of cycle 1 for each patient as a function of dose in milligrams per meter squared. (B) 20S proteasome inhibition as a function of the absolute dose of PS-341 in milligrams.

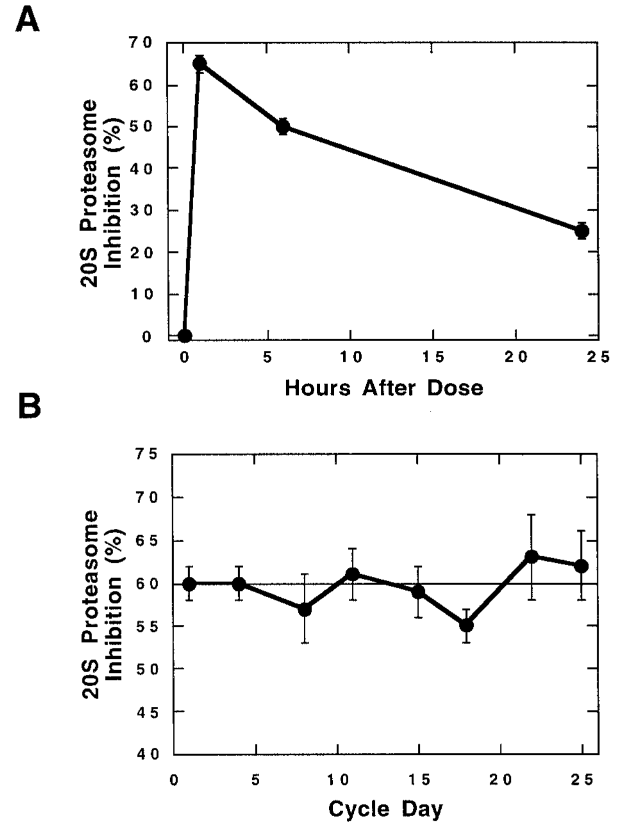

Fig 3. PS-341 pharmacodynamics as a function of time. (A) 20S proteasome inhibition 1, 6, and 24 hours after PS-341 for every patient at 1.04 mg/m² (n = 12) (mean ± SEM). (B) 20S proteasome inhibition 1 hour after each dose of PS-341 during cycle 1 for patients at 1.04 mg/m² (mean ± SEM).

of steroids. None of these patients were rechallenged with PS-341. Finally, one patient treated as an exception at 1.04 mg/m^2 developed a nosocomial pneumonia with hypoxia, and urinary retention because of lumbosacral varicella-zoster reactivation. After recovery, this patient received a third cycle of PS-341 without a recurrence of any of these toxicities.

No patients on this study died during therapy, but one did so within 25 days of their last PS-341 dose. This patient with myeloma and cutaneous plasmacytomas was treated as a protocol exception at 1.04 mg/m^2 and developed progressive abdominal pain with grade 2 transaminase elevations and grade 3 lipase elevations after five PS-341 doses. An evaluation revealed negative hepatitis serologies, but computed tomography showed a mass in the pancreatic head with multiple hepatic and pulmonary nodules suggestive of metastatic pancreatic cancer or visceral plasmacytomas. This patient died within 4 weeks, and no pathologic diagnosis of the underlying disease process was obtained. All other deaths that occurred beyond 30 days from the last PS-341 dose were judged to be because of disease progression or an associated complication, and none were felt to be drug-related.

PD

PS-341 induced a dose-dependent inhibition of 20S proteasome function compared with pretreatment controls, with 0.40-, 1.04-, 1.20-, and 1.38-mg/m^2 doses yielding an inhibition of 36% ± 9%, 60% ± 1%, 65% ± 2%, and 74% ± 2%, respectively. PD data relative to 1 hour after dosing on day 1 for each patient were also suitable for inclusion in a sigmoid maximum effect model, which showed a dose-response relationship between proteasome inhibition and both the dose of PS-341 in milligrams per meter squared (Fig 2A) and the total dose of PS-341 (Fig 2B). Significant inhibition of 20S proteasome activity was seen within 1 hour of dosing, after which the inhibition slowly decayed (Fig 3A), returning toward baseline by 72 hours (not shown). To evaluate the possibility that toxicity was caused by an accumulation of proteasome inhibition, this was followed throughout a treatment cycle (Fig 3B). No significant increase in proteasome inhibition was demonstrable throughout therapy, suggesting that a 72-hour period between dosing was adequate to allow for recovery of normal function.

The ability of proteasome inhibitors to block activation of NF-κB,[8,9] and the important role of this transcription factor in induction of IL-6,[26] led to evaluations of the impact of PS-341 on this cytokine's levels as another PD measure. Interestingly, systemic IL-6 levels increased an average of 24%, 10%, 19%, and 20%, respectively, at the four dose levels evaluated in this study.

Antitumor Activity

Twelve patients with plasma cell dyscrasias were treated on this study, nine of whom completed at least one full cycle and were assessable for response. Patient 4 had an immunoglobulin (Ig) G-kappa multiple myeloma and was treated initially with radiation to a lytic hip lesion. Although the disease first responded to three cycles of VAD chemotherapy, neither additional VAD nor topotecan/dexamethasone resulted in further decreases of the serum monoclonal protein or the bone marrow plasmacytosis. Before starting PS-341, a biopsy showed plasma cells constituting 41% of the total marrow cellularity (Fig 4A), immunoglobulins included an IgA of 25 mg/dL (normally 40 to 390), IgM of 15 (normally 25 to 210), and IgG of 3,011 (normally 525 to 1,650), whereas immunofixation showed large monoclonal IgG heavy-chain and kappa light-chain bands (Fig

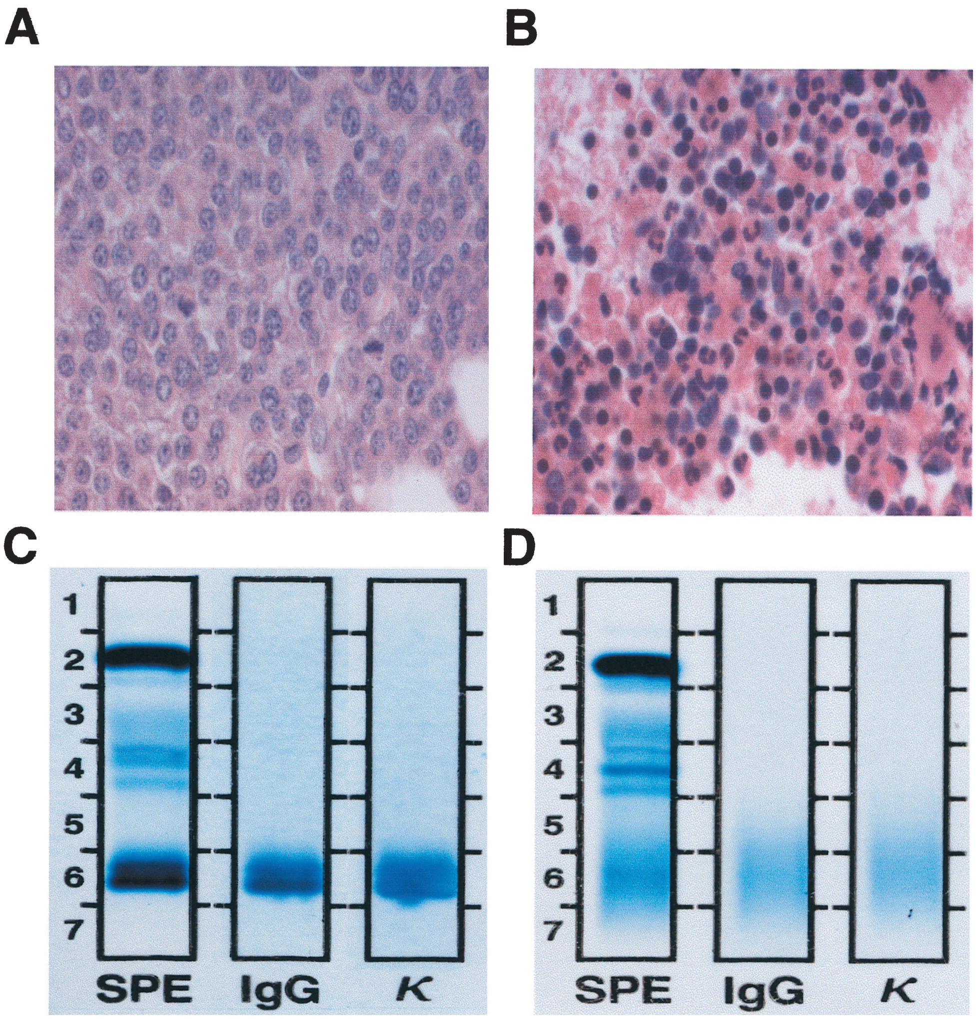

Fig 4. Response of one multiple myeloma patient to PS-341. Patient 4's bone marrow biopsy before therapy (A), and after cycle 1 of PS-341 (B). Also presented are this patient's serum protein electrophoresis (SPE) and immunofixation for IgG and kappa light chains before therapy (C), and after cycle 3 of PS-341 (D).

4C). After cycle 1 of eight doses at 1.04 mg/m^2/dose, the bone marrow plasmacytosis declined to 1% (Fig 4B); IgA and IgM rose to 69 and 44, respectively; and IgG declined to 1,215. This patient went on to receive a total of four cycles, and by completion of cycle 3 the immunofixation took on a polyclonal pattern (Fig 4D), indicative of a CR. In follow-up, a faint monoclonal band reappeared 6 months after discontinuation of therapy, but no additional myeloma-directed chemotherapy has been necessary for 1 year.

Among the eight other patients with myeloma who also received one full cycle at 1.04 mg/m^2/dose, there was additional evidence of antitumor activity. Two patients had a decrease in their serum monoclonal protein of 44% and 27% (Fig 5A), accompanied by a decrease in marrow plasmacytosis of 47% and 91% (Fig 5B), respectively. Three patients had stable disease judged by paraprotein levels, with changes ranging from a 1.5% decrease to a 13% increase, although all had a decline in marrow plasmacytosis ranging from 43% to 82%. One myeloma patient had a mixed response, with a paraprotein decrease of 3.6% but a 138% increase in marrow plasmacytosis. A single patient with light-chain disease had a 31% decrease in their urinary light-chain excretion and a 25% decrease in marrow plasmacytosis. Finally, one patient with Waldenström's was treated whose IgM increased by 34%, but the initial value was felt to be falsely low because it was obtained immediately after plasmapheresis, and on bone marrow evaluation there was a 79% decrease in plasmacytoid lymphocytes. Therapy with PS-341 was continued for all of these patients, but three showed signs of disease progression after cycle 2, and two did so after cycle 3. Two patients discontinued because of SAEs in the form of one hypersensitivity reaction and one episode of transaminitis, as described above, and a third was removed from the study because of a decline in their performance status.

PS-341 also showed activity against non-Hodgkin's lymphomas in a patient with mantle-cell lymphoma who had previously received cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy; autologous stem-cell transplantation; and radiation therapy. Treatment with almost two cycles of PS-341 at 1.38 mg/m^2 induced a PR, and the patient discontinued therapy to receive a nonablative allogeneic transplantation. A second patient at this level with refractory follicular lymphoma who had received cyclophosphamide, vincristine, and prednisone followed by rituximab also responded with a PR on the basis of a greater than 50% shrinkage of mediastinal and intra-abdominal adenopathy. This patient self-discontinued therapy to return to work after four cycles, missing two of 32 doses because of hyponatremia, and has remained in a stable PR for more than 12 months off therapy.

DISCUSSION

Laboratory and preclinical studies have established the proteasome as a potential target for cancer therapy, and it seemed

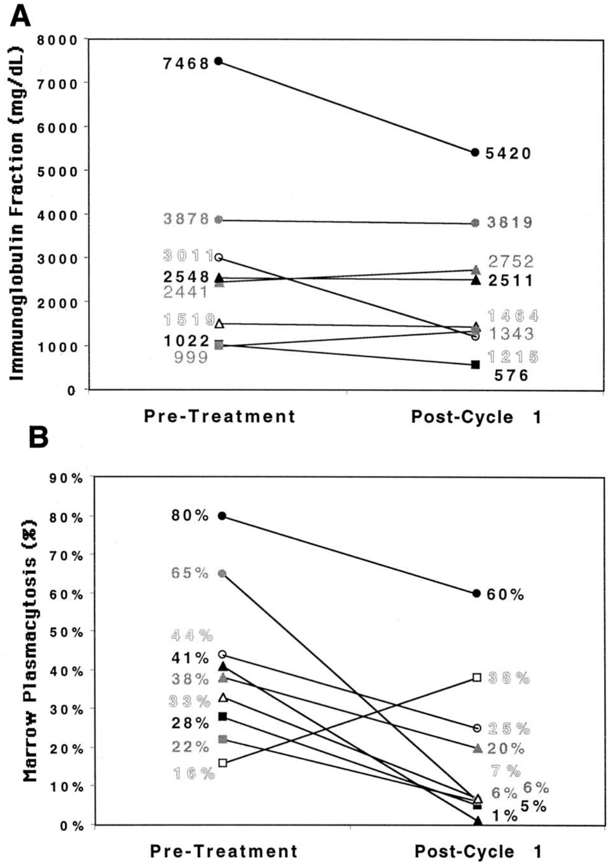

Fig 5. Plasma cell dyscrasia patients receiving one full PS-341 cycle. (A) Changes in the quantitative immunoglobulins of eight patients after cycle 1. One patient with light-chain disease is excluded. (B) Changes in bone marrow plasmacytosis in all nine patients with plasma cell dyscrasias after therapy with cycle 1.

possible that such inhibitors would be effective in patients with refractory hematologic malignancies. This was based on findings that showed that proteasome inhibitors were active agents in models of human non-Hodgkin's lymphoma,[15] chronic lymphocytic leukemia,[16] multiple myeloma,[17] and adult T-cell leukemia.[27] We sought to determine the MTD of the proteasome inhibitor PS-341 in this population and found that a dose of 1.04 mg/m^2 was well tolerated using a dose-intensive, twice-weekly administration for 4 weeks, followed by a 2-week rest. Acute infusion-related side effects were minimal, and routine premedications were not generally required. DLTs at least possibly related to PS-341 included thrombocytopenia, malaise and fatigue, and electrolyte disturbances in the form of hyponatremia and hypokalemia. Thrombocytopenia was not dose-limiting by the protocol definition, but the occurrence of grade 3 thrombocytopenia was relatively common and did influence dosing behavior. Leukopenia and neutropenia were noted, but neither was dose-limiting, and no episodes of febrile neutropenia were seen. Five patients developed a treatment-emergent peripheral neuropathy; however, in the cases in which this was judged to be drug-related, the severity did not surpass grade 2.

Patients who continued on therapy at times developed delayed toxicities, which in some cases seemed likely to be drug-related. One patient with prior hepatitis C developed significant transaminitis and hyperbilirubinemia in the setting of a self-administered acetaminophen ingestion double the 2.0-g limit recommended for patients with viral hepatitides,[28] suggesting the need for close monitoring of patients with hepatitis receiving PS-341. There was a trend for the frequency and severity of the more common toxicities to be dose-related, and because PS-341 induced a dose-dependent inhibition of the 20S proteasome, it is likely that these toxicities were also related to the extent of proteasome inhibition.

This protocol did not include provisions for initiation of colony-stimulating growth factors or for continued therapy with PS-341 in the face of a need to start transfusion support. It is therefore possible that, with management such as platelet support, a higher dose could be safely administered. In addition, the schedule of administration may relate to the induction of toxicity. Among the patients who had to have their cycle 1 therapy interrupted for toxicity, this most commonly occurred during the third week of treatment. Other studies of PS-341 have used twice-weekly therapy for 2 weeks with the third week off, and this schedule, which delivers the same total dosage over 6 weeks, may prove better tolerated. Indeed, this schedule has been chosen for subsequent phase II studies, and appears to allow PS-341 to be well tolerated by myeloma patients at 1.30 mg/m^2.[29]

PD modeling of the 20S proteasome activity data showed dose-dependent proteasome inhibition that was related both to the PS-341 dose in milligrams per meter squared, and to the total dose of PS-341. To determine whether incorporation of dose/body surface area yields a superior PD model to dosing without normalization, analysis of the residuals was conducted using the r^2 method as part of evaluating the robustness of the models. Mean residual squares were 116 for the milligrams per meter squared model and 114 for the total dose model, suggesting that the latter might better predict the extent of proteasome inhibition. Very similar and large SDs of 217 and 208, respectively, were obtained, however, indicating that this feature of the models will require further testing. This in part is a result of the slope of the curves being highly influenced by the small number of data points at the lowest dose of 0.40 mg/m^2, the close proximity of the remaining three doses in the plateau, and the limited number of observations overall. In an attempt to further clarify the possibility that one of these two models could be superior, the occurrence of any DLT, any grade 3 or 4 adverse event, any SAE, or termination because of an adverse event was analyzed in relationship to both the absolute dose and the dose adjusted for body surface area. Logistic regression analysis showed no statistically significant findings for preferring either model for predicting an association between dosing and toxicity. The small data set makes it unfeasible to perform other exploratory analyses such as inclusion of multivariate analysis into the model. In conjunction with other ongoing clinical trials with PS-341, PD data are continually being gathered, and this additional information may allow a future analysis of these two models, and others as appropriate, to arrive at a more definitive conclusion.

Pharmacokinetic studies of PS-341 have been performed in conjunction with other clinical trials of this agent, and have shown that it has a rapid $t_{1/2\alpha}$ of 0.22 to 0.46 hour and a large ($>$ 500 L) volume of distribution.[30] Additional pharmacokinetic studies are being performed in conjunction with trials studying combination regimens with standard chemotherapeutic drugs and PS-341. One area of interest is the dosing of PS-341 in obese patients, which in the current study was adjusted empirically in those who were more than 30% above their ideal body weight.

Subsequent preclinical work in obese mouse models and clinical experience[23] has indicated that this adjustment is not necessary. A second area of particular interest is the dosing of PS-341 in patients with renal impairment, which was allowed in the current study for patients with multiple myeloma and a creatinine of up to 2.5 mg/dL. Patients with even more compromised renal function have been treated on a phase II trial of PS-341,[29] and preliminary results have shown that there was no correlation between 20S proteasome inhibition and reduced creatinine clearance. Also, those patients with impaired renal function did not appear to have increased toxicity, suggesting the possibility that renal function may not need to be considered in the dosing of PS-341. Detailed pharmacokinetic and PD studies are currently underway to address this important question further.

PS-341 showed evidence of antitumor activity in patients with refractory multiple myeloma, with one patient achieving a CR. It is interesting that this CR was associated with a rapid, sustained recovery of the other immunoglobulins to normal levels during therapy, suggesting that proteasome inhibitors may be selectively toxic to transformed cells in vivo, as they have proven in laboratory studies.[15-17] Because adherence of myeloma cells to bone marrow stroma triggers synthesis of the myeloma survival and growth factor IL-6[31] through an NF-κB–dependent mechanism,[32] the ability of proteasome inhibitors to block NF-κB signaling[8,9] may in part explain the effects of PS-341 in this disease. We evaluated the impact of PS-341 on patients' serum IL-6, and found that systemic levels actually increased after therapy, but this does not rule out a possible effect at the level of the marrow microenvironment. More recently, PS-341 has been shown to potently inhibit proliferation and growth pathway signaling, and to directly induce apoptosis in both laboratory- and patient-derived multiple myeloma cells.[17] Furthermore, the ability of PS-341 to enhance the antimyeloma efficacy of dexamethasone in vitro[17] suggests therapy using combinations such as dexamethasone and PS-341 might be particularly effective. This provides a strong argument for further study of such regimens in patients with multiple myeloma, and a phase II trial using this rationale is currently underway.

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