Development of the Proteasome Inhibitor PS-341

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ABSTRACT

Over the last decade, the critical role of the proteasome in cell-cycle regulation has become increasingly apparent. The proteasome, a multicatalytic protease present in all eukaryotic cells, is the primary component of the protein degradation pathway of the cell. By degrading regulatory proteins (or their inhibitors), the proteasome serves as a central conduit for many cellular regulatory signals and, thus, is a novel target for therapeutic drugs. PS-341 is a small molecule that is a potent and selective inhibitor of the proteasome. In vitro and mouse xenograft studies of PS-341 have shown antitumor activity in a variety of tumor types, including myeloma, chronic lymphocytic leukemia, prostate cancer, pancreatic cancer, and colon cancer, among others. Although PS-341 rapidly leaves the vascular compartment, a novel pharmacodynamic assay has shown that inhibition of proteasome—the biologic target—is dose dependent and reversible. These studies provided the rationale for a twice-weekly dosing schedule employed in ongoing clinical studies. Phase I trials in a variety of tumor types have shown PS-341 to be well tolerated, and phase II trials in several hematologic malignancies and solid tumor types are now in progress. Efficacy and safety data from the most advanced of these, a phase II multicenter trial in myeloma, will be available in early 2002. *The Oncologist 2002;7:9-16*

INTRODUCTION

In addition to the recycling of damaged or obsolete proteins, protein degradation is a mechanism for controlling the availability of regulatory proteins in the cell. The 26S proteasome is a primary component of the protein degradation pathway of the cell (over 80% of all cellular proteins are processed by this enzyme), and the proteasome's rapid and irreversible elimination of targeted proteins is key to the activation or repression of many cellular processes, including cell-cycle progression and apoptosis. This fundamental role for the proteasome singles it out as a unique target for anticancer therapy, and PS-341 is the first proteasome inhibitor to enter human trials. In preclinical studies, this potent and specific inhibitor has shown significant antitumor activity as a single agent and in combination with other cytotoxic drugs [1-6]. PS-341 treatment has also proven to be well tolerated in phase I trials at doses that result in significant proteasome inhibition and show preliminary evidence of biologic activity, justifying a large-scale phase II program in a variety of tumor types, both solid and hematologic [7, 8]. This review provides a summary of the clinical experience with PS-341, as well as the preclinical rationale that supports the potential of PS-341 as a novel treatment for hematologic and solid tumor malignancies.

THE PROTEASOME AND PROTEASOME INHIBITION

The proteasome is a large, multiprotein particle—present in both the cytoplasm and the nucleus of all eukaryotic cells—composed of two functional components: a 20S core catalytic complex and a 19S regulatory subunit. Proteins that are to be degraded are marked with ubiquitin chains, which bind to a receptor on the 19S complex (Fig. 1). Once recognized by the regulatory complex, the ubiquitin chain is removed and the protein denatured in preparation for degradation. The protease activity resides in a channel at the center of the 20S complex, which is formed from four stacked, multiprotein rings. The outer α subunit rings form a narrow channel that allows only denatured proteins to enter the catalytic chamber formed by the central β subunit rings [9-11]. Inside the catalytic chamber, proteins are surrounded by six protease-active sites (three on each β subunit ring). The proteasome protease functions similarly to serine proteases but is unique since it relies on a threonine residue in the active site. Proteins processed by

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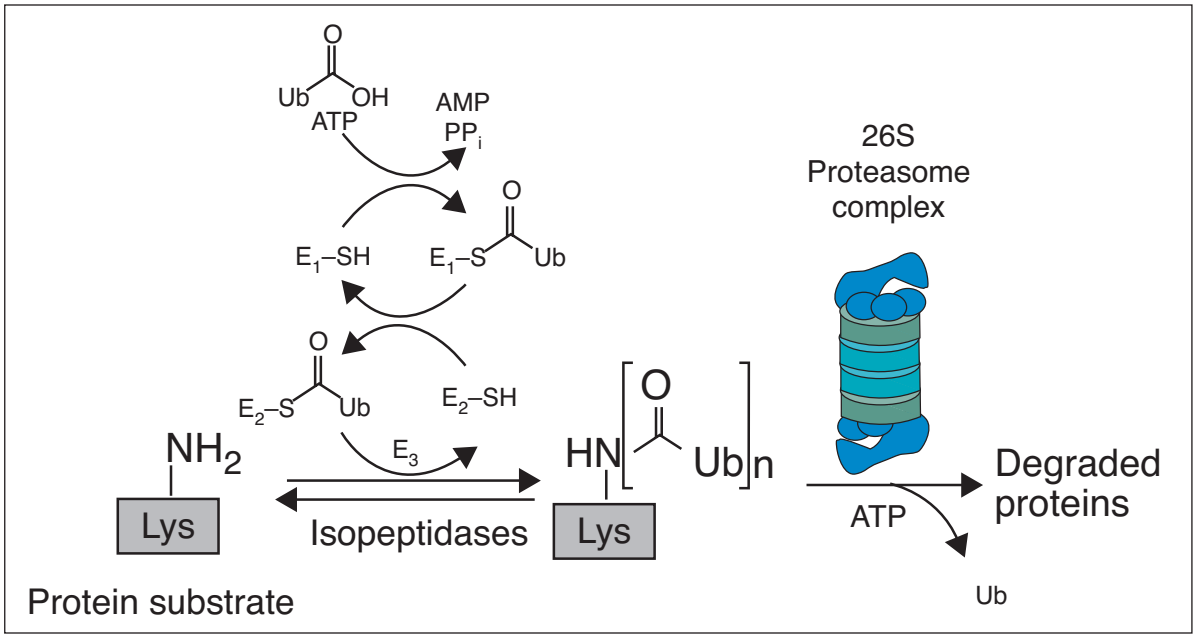

Figure 1. Ubiquitin-proteasome protein degradation. *Prior to degradation, doomed proteins are first marked on specific lysine residues with a polyubiquitin chain. Marking the protein is a three-step process in which ubiquitin is activated by a ubiquitin-activating enzyme (E_1), then transferred to a ubiquitin-conjugating enzyme (E_2). E_3s (the ubiquitin-protein ligases) recognize degradation motifs on specific substrates, and catalyze the transfer of ubiquitin from the E_2 to the target. These polyubiquitinated substrates are then recognized and degraded by the 26S proteasome.*

the proteasome are reduced to small polypeptides 3 to 22 residues in length [12].

Proteolysis by the 26S proteasome is a fundamental metabolic process, and complete blockade of the proteasome activity with an inhibitor results in death for cells and organisms. Many laboratories have demonstrated the effects of proteasome inhibition on the stability of various cell-cycle regulatory proteins, especially those that are short lived [1, 5, 13-15]. Cyclins, cyclin-dependent kinase inhibitors, and tumor suppressors (e.g., cyclin B1, $p21^{Waf1/Cip1}$, p27, p53) are all substrates for the ubiquitin-proteasome pathway (Table 1), and inhibiting their requisite degradation has been clearly implicated in sensitizing cells to apoptosis [16-18]. Camptothecin-bound topoisomerase I cleavable complexes are also substrates for the proteasome [19]; inhibition of the proteasome thus stabilizes these

Table 1. Cell cycle–related proteins degraded by the proteasome and their functions[a]

Class of proteins	Protein	Protein function
Cyclins and related proteins	Cyclins A, B, D, E Cyclin-dependent kinase (CDK) inhibitors	Cell-cycle progression Regulation of cyclin activity
Tumor suppressor	p53	Transcription factor
Oncogenes	c-fos/c-jun c-myc N-myc	Transcription factor Transcription factor Transcription factor
Inhibitory proteins	IκB p130	Inhibitor of NF-κB Inhibitor of E2F-1
Enzymes	cdc25 phosphatase Tyrosine amino transferase (TAT)	CDK1/cyclin B phosphatase Tyrosine metabolism
Others[b]	Ki-67	PCN degradation

[a] *Adams et al.* [1]

[b] *Wu et al.* [15]

complexes and improves the effectiveness of camptothecin treatment [3].

However, not all cells respond in the same way to proteasome inhibition, and those studying proteasome inhibitors soon discovered that transformed cells are much more sensitive to blockade of the proteasome than are normal cells. Although actively dividing cells are more sensitive to proteasome-induced apoptosis than are nonproliferating cells [20], this effect is not simply a consequence of the high replication rate of malignant cells. When confronted with the disruption in cell cycle–regulator turnover that accompanies proteasome inhibition, checkpoint mechanisms arrest cell division in normal cells and allow division to resume only after proteasome activity has been restored. However, in malignant cells, the genetic changes that accompany transformation disable these protective checkpoint mechanisms. Thus, the pro-apoptotic effect of lactacystin (a proteasome-specific protease inhibitor) was more pronounced in malignant lymphocytes harvested from chronic lymphocytic leukemia (CLL) patients than in their normal counterparts from healthy individuals [21]. While the exact mechanism for this differential susceptibility is not fully understood, proteasome inhibition may reverse some of the changes that permit proliferation and suppress apoptosis in malignant cells. For example, the cyclin-dependent kinase inhibitor p27 is degraded by the proteasome [18], and the decreased protein levels observed in some malignant cells are achieved by upregulation of proteasome-mediated p27 degradation [22]. When simian-virus-40-transformed fibroblasts are treated with a proteasome inhibitor, p27 levels increase substantially and apoptosis ensues [23].

The NF-κB signaling pathway may also be a critical target for proteasome inhibitors. Transcription by NF-κB is prevented in quiescent cells through binding of a specific inhibitor protein, IκB, which sequesters the NF-κB p50/p65 heterodimer in the cytoplasm [24]. This repression is released in response to cellular stresses (including chemotherapy and radiation; Fig. 2), which cause targeted degradation of IκB: phosphorylation of specific serine residues in the N-terminus of IκB prompt ubiquitination of internal lysine residues [25-27]; ubiquitinated IκB is then recognized and degraded by the proteasome, and NF-κB is released [28-32]. Once the transcription factor is released, it is rapidly translocated to the nucleus where it binds cognate sites in the promoter regions of genes encoding cytokines (e.g., tumor necrosis factor, interleukin-1, interleukin-6), stress response enzymes (COX2, NO, 5-LO), and cell adhesion molecules (intracellular adhesion molecule, vascular cell adhesion molecule, E-selectin) and can increase the expression of anti-apoptotic proteins (inhibitor of apoptosis and the Bcl-2 family) [33-36]. Importantly, NF-κB also drives its own transcription and the transcription of IκB, effectively acting as a molecular amplifier that maintains its own transcriptional activity [37]. Dysregulation of NF-κB signaling is an important feature of some malignancies [38, 39], and activation of this pathway can stimulate proliferation and/or reduce the effectiveness of chemotherapy or radiation [40]. These observations of the

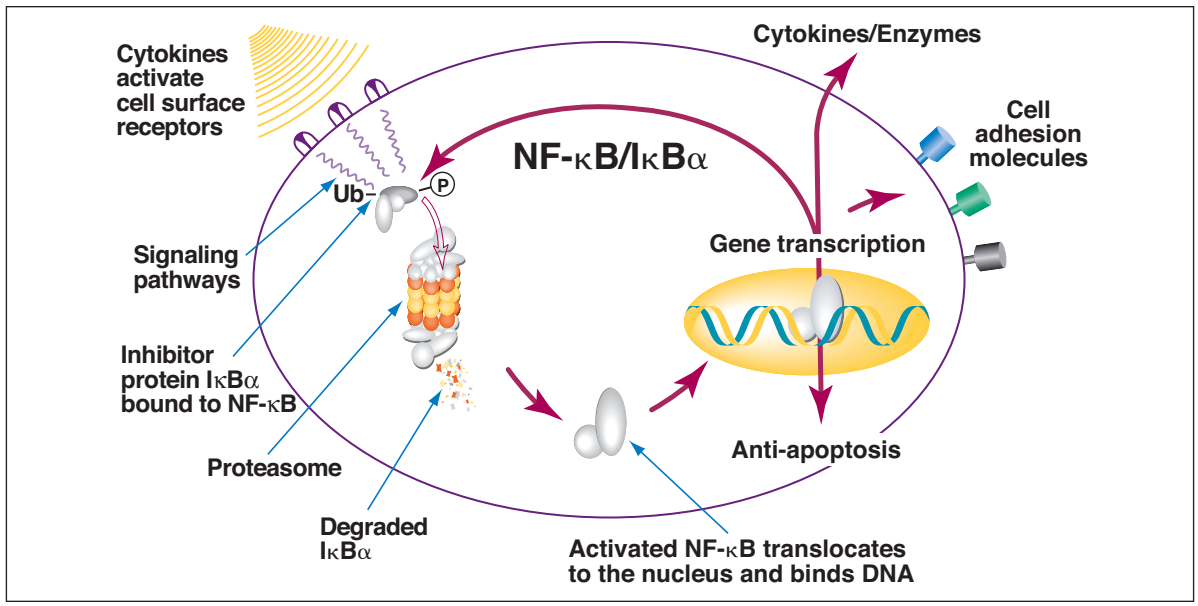

Figure 2. NF-κB activation pathway. *Viruses, growth factors, antigens, radiation, or chemotherapeutic drugs activate signaling pathways that lead to the degradation of IκB by the proteasome. Once released from IκB inhibition, NF-κB translocates to the nucleus to activate transcription of genes that protect the cell from apoptosis and promote cell growth and differentiation. The synthesis of growth factors, cell-adhesion molecules, angiogenesis factors, and antiapoptotic factors is increased.*

consequences of NF-κB activation in tumor cells led several investigators to test the effect of preventing NF-κB activation in transformed cells, usually through inhibition of IκB degradation. *Cusack et al*. [41] used an engineered form of IκB that is not ubiquitinated (i.e., the serines that are recognized by ubiquitinating enzymes were mutated to alanines) and, therefore, cannot be degraded by the proteasome; this superrepressor maintains inhibition of NF-κB under circumstances that would otherwise lead to its activation. In colorectal cancer cells transfected with the IκB superrepressor, apoptosis is induced by SN38 (the active metabolite of CPT-11) to a greater extent than in the untransfected parental cell line. *Masdehors et al*. demonstrated the potential clinical benefits of NF-κB repression through treatment with a proteasome inhibitor using lymphocytes isolated from CLL patients at diagnosis [21]. These samples included both radiation-resistant and radiation-sensitive isolates, but when treated with lactacystin, all isolates were equally sensitive.

Preclinical Studies with PS-341

Initial screening of the National Cancer Institute's (NCI) tumor cell lines revealed that PS-341 is active against a broad range of tumor types [1], prompting further exploration of the activity of PS-341 in cell culture and in murine and human xenograft models. In these models, PS-341 exhibited many of the properties seen in preclinical studies of other proteasome inhibitors such as lactacystin: activity as a single agent, enhancement of apoptosis induced by chemotherapy or radiation, and specificity for transformed cells. In cell culture, PC-3 prostate cancer cells undergo growth arrest and apoptosis when treated with PS-341, and PS-341 reduces the growth of PC-3 tumors on nude mice [1]. Additionally, combination therapy with PS-341 increases the effectiveness of CPT-11 (Fig. 3) or radiation in colon tumor xenografts (Fig. 4) [42]; and in pancreatic tumor xenografts, significant tumor responses are observed when PS-341 is used in combination with gemcitabine [2] or CPT-11 [5]. In each of these studies, the authors were able to identify changes in the expression of proteasome targets, including $p21^{Waf1/Cip1}$ [1, 3], IκB [5], and NF-κB [42]. Finally, the 50% inhibitory concentration (IC_{50}) of myeloma cells isolated from patients was reached at PS-341 concentrations that had no effect on the peripheral blood mononuclear cells from healthy volunteers. Cultured bone marrow stromal cells (BMSCs) were equally unresponsive to PS-341: the IC_{50} of BMSCs was at least 170 times that of the myeloma cells used in this experiment [4].

Another observation that arose from preclinical studies is the infrequency of resistance to PS-341 and the drug's effectiveness even in the presence of known drug resistance factors [4, and *D. McConkey*, personal communication]. A systematic check of multidrug resistance transporters revealed that PS-341 is a poor substrate for this class of proteins, and *Hideshima et al*. recently showed that PS-341 can arrest the growth of multiple myeloma cells that are resistant to conventional therapies [4]. Other mechanisms that result in drug resistance are also insufficient to forestall PS-341-induced apoptosis. PC-3 cells are sensitive to PS-341 even though they do not express p53 [1], and overexpression of Bcl-2 also appears to be incapable of preventing PS-341-induced apoptosis (Fig. 5) [*D. McConkey*, personal communication].

Pharmacology of PS-341

In initial pharmacokinetic studies, PS-341 was found to rapidly exit the plasma compartment (>90% is cleared within 15 minutes of i.v. administration) [43, and Millennium data on file]. To allow for accurate dosing in phase I

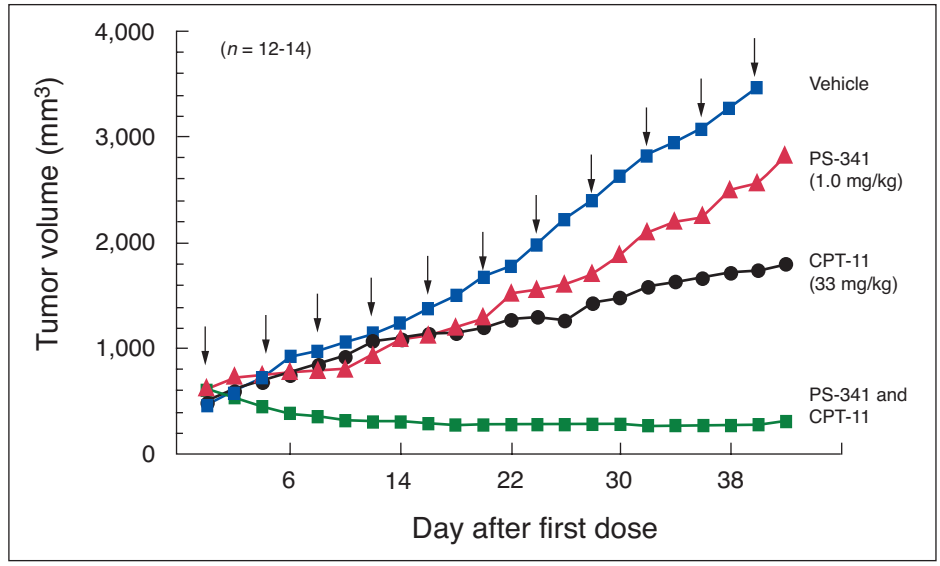

Figure 3. PS-341 activity in combination CPT-11. *CPT-11 treatment triggers IκB degradation and activation of NF-κB transcription, resulting in decreased effectiveness of this compound. Blockade of NF-κB activation by PS-341 should increase the cytotoxicity of CPT-11. To test this hypothesis, LOVO colon cancer xenografts were grown in the flanks of nude mice; after these tumors were established, the mice were treated twice weekly (indicated by arrows) with PS-341 or saline before administration of CPT-11. Either treatment, when given as a single agent, results in a slight reduction in tumor growth. In contrast, the combination of PS-341 and CPT-11 actually reduces the size of the tumor [3].*

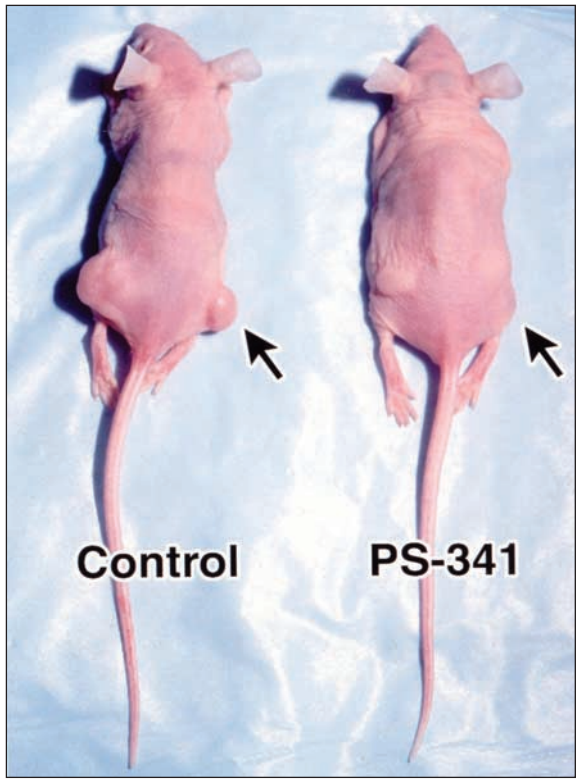

Figure 4. PS-341 activity in combination with radiation. *PS-341 treatment, in combination with radiation, significantly reduces the growth of LOVO colorectal cancer xenografts in nude mice. The mouse on the left received a single saline injection, while the mouse on the right received a single injection of PS-341 (1 mg/kg). Tumors on the right flank (marked with arrows) of each mouse were then irradiated (6 Gy). The combination of PS-341 and radiation resulted in greater antitumor activity than PS-341 or radiation treatment alone [42].*

trials, a rapid and reliable bioassay has been developed to measure residual proteasome activity following PS-341 treatment [44]; for convenience, proteasome activity can be determined from whole blood or white blood cells, but biopsied tissues can also be used to determine the effect of the drug at the tumor site. Using this assay on samples from healthy volunteers, proteasome activity was found to be similar among study participants, and intrasubject variation was minimal [Millennium data on file]. Furthermore, this assay and whole-body autoradiography of [^{14}C]PS-341–treated rats revealed that most organs received roughly the same amount of drug; the central nervous system, testes, and eyes, however, are protected from PS-341 [1].

The proteasome bioassay facilitated pharmacologic and toxicity studies in animals. In all species studied, baseline proteasome activity was restored within 48 to 72 hours of PS-341 treatment. Toxicology studies conducted in rodents and primates indicated that gastrointestinal side effects (anorexia, emesis, and diarrhea) were the primary adverse events associated with PS-341 treatment. Notably, bone marrow toxicity was not observed in primates [1]. The gastrointestinal toxicity is dose related, and overall, treatment is well tolerated until 80% proteasome inhibition is exceeded. In primates, gastrointestinal toxicity becomes significantly more pronounced, and changes in blood pressure and heart rate occur at proteasome inhibition greater than 80%.

In human studies, the ex vivo proteasome bioassay is an integral part of the phase I and phase II protocols. In Figure 6, proteasome activity following PS-341 treatment is plotted. The dose-related inhibitory effect of PS-341 is shown, and the dose that would result in 80% proteasome inhibition (the maximum safe level of inhibition identified in preclinical experiments) can be estimated to be 1.96 mg/m^2. Further characterization of the response to PS-341 treatment using the bioassay reveals that proteasome inhibition is dose dependent and varies very little, even among the diverse phase I patient population. Given the consistency of this response, the bioassay will probably be unnecessary outside of the clinical trial setting.

Clinical Studies with PS-341

Six phase I clinical trials for PS-341 in hematologic cancers or solid tumors have been completed or are in progress,

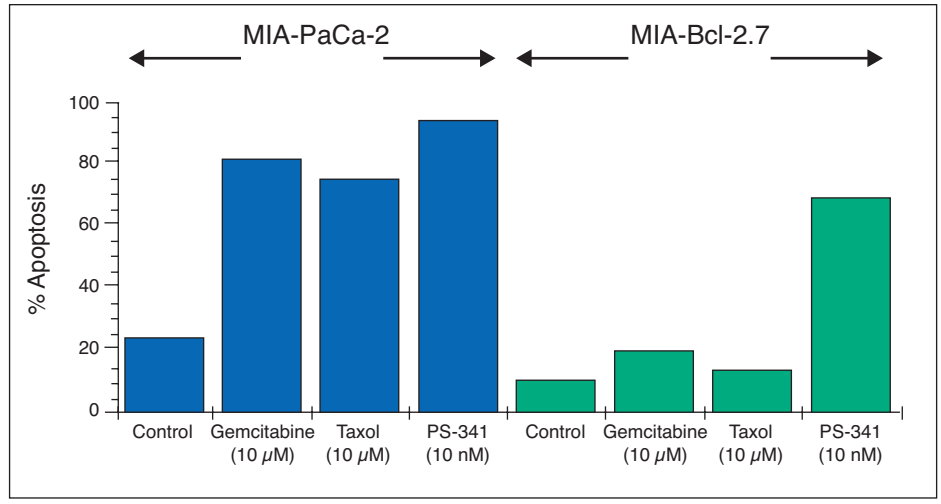

Figure 5. Apoptosis in cells that overexpress Bcl-2. *Expression of Bcl-2 reduces the fraction of cells that undergo apoptosis following gemcitabine or paclitaxel treatment (compare MIA-PaCa-2 with MIA-Bcl-2.7). In contrast, PS-341 retains most of its activity even in cells expressing Bcl-2 [*D. McConkey, *personal communication].*

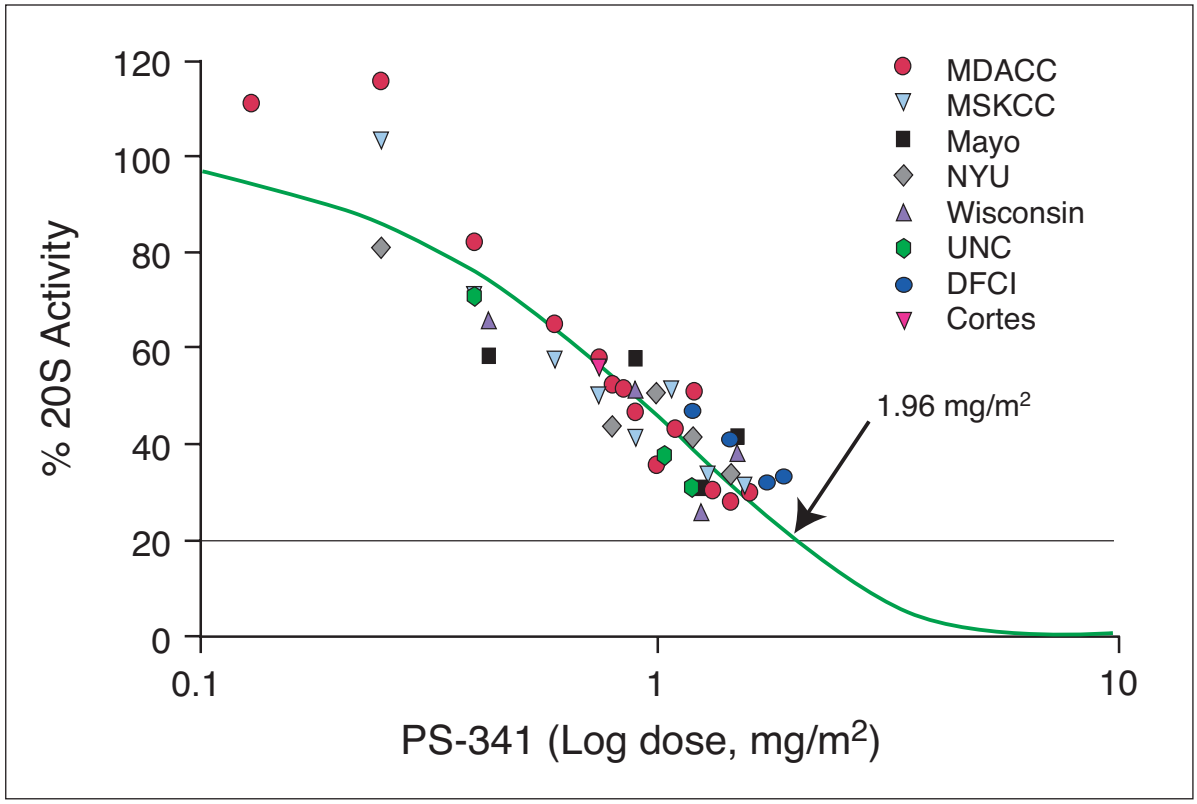

Figure 6. Combined 20S proteasome activity from phase I study centers. *Proteasome activity was determined in blood samples taken from study participants 1 hour after PS-341 treatment. A semi-log plot of the data fits to a theoretical binding isotherm, and there is a clear dose-dependent inhibitory effect. Using these data, proteasome inhibition would exceed 80% at doses above 1.96 mg/m^2. Abbreviations: MDACC = M. D. Anderson Cancer Center; MSKCC = Memorial Sloan-Kettering Cancer Center; Mayo = Mayo Clinic; NYU = New York University; Wisconsin = University of Wisconsin; UNC = University of North Carolina; DFCI = Dana-Farber Cancer Institute; Cortes (M. D. Anderson Cancer Center).*

and the safety and efficacy of PS-341 treatment for refractory multiple myeloma and refractory CLL is being tested in two ongoing phase II trials. Additional phase II trials are planned for solid tumor indications. The NCI is also sponsoring 19 additional trials through the Cancer Therapy Evaluation Program. The first phase I trial, conducted at M.D. Anderson Cancer Center, used a conservative dosing regimen of once-weekly PS-341 for 4 weeks, followed by a 2-week rest. This study continues to accrue patients, and the maximum tolerated dose has not yet been reached [45]. Additional phase I trials were designed to evaluate twice-weekly PS-341 treatments.

Approximately 200 patients have now been treated in the phase I setting, and PS-341 has been well tolerated. Patients experience low-grade fever and/or fatigue after several cycles of PS-341 at doses above 1.0 mg/m^2. Thrombocytopenia is also observed, but this toxicity has not been dose limiting or required supportive transfusions. As predicted from animal studies, some patients develop low-grade diarrhea that can be averted with prophylactic loperamide treatment. In addition, several patients have experienced peripheral neuropathy (PN); however, most of these patients had been treated previously with platinum or taxane regimens. Participants in ongoing trials are being closely monitored to determine whether prior neurotoxic chemotherapy can sensitize patients to PN during PS-341 treatment. Skin rash has also been reported infrequently.

Because phase I trials are not designed to test efficacy, tumor responses in these trials can only be considered anecdotal. Nevertheless, the preclinical data, combined with the tolerability of PS-341 in phase I trials, provide a compelling rationale for further clinical development of PS-341. On the once-weekly treatment regimen, several patients with androgen-independent prostate cancer experienced a decrease in prostate-specific antigen levels [8]. A patient with non-small-cell lung cancer achieved a partial response (>50% reduction in tumor mass) on twice-weekly PS-341 at a dose of 1.56 mg/m^2 [7]. At a dose that results in 68% proteasome inhibition, lung metastases in two melanoma patients also responded to a lower dose of PS-341 given on the twice-weekly treatment regimen [46]. Significant activity has also been evident in multiple myeloma, with patients showing significant reductions in myeloma-related immunoglobulins and marrow plasmacytosis

[47]; a 7-month remission was achieved in one of these patients after four cycles of therapy.

Conclusion

PS-341 represents the first of a potentially promising class of agents. In addition to antitumor effects, proteasome inhibitors may also have applications for diseases caused or exacerbated by inflammation, as the mediators of inflammation are often affected by proteasome-mediated protein degradation. The proteasome, which was largely an unknown enzyme until very recently, is thus proving to be an attractive target for drug development. In addition, the regulatory events upstream of the proteasome—those that control the phosphorylation and ubiquitination of proteasome substrates—are actively being explored as potential drug targets. The utility of drugs whose development is based on the elucidation of these pathways is currently unknown but is potentially far reaching.

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