A Phase I Study of Bortezomib Plus Irinotecan in Patients With Advanced Solid Tumors

David P. Ryan, MD[1]
Bert H. O'Neil, MD[2]
Jeffrey G. Supko, MD[1]
Carlo M. Rocha Lima, MD[3]
E. Claire Dees, MD[2]
Leonard J. Appleman, MD[5]
Jeffrey Clark, MD[1]
Phinos Fidias, MD[1]
Robert Z. Orlowski, MD[2]
Oscar Kashala, MD[6]
Joseph P. Eder, MD[5]
James C. Cusack, Jr., MD[1]

[1] Massachusetts General Hospital Cancer Center, Boston, Massachusetts.

[2] University of North Carolina at Chapel Hill, Chapel Hill, North Carolina.

[3] University of Miami and Sylvester Cancer Center, Miami, Florida.

[5] Dana-Farber Cancer Institute, Boston, Massachusetts.

[6] Millennium Pharmaceuticals, Inc., Cambridge, Massachusetts.

Presented in part at the 38th Annual Meeting of the American Society of Clinical Oncology, May 18–21, 2002, Orlando, Florida, and the 39th Annual Meeting of the American Society of Clinical Oncology, May 31 to June 3, 2003, Chicago, Illinois.

Supported in part by Millennium Pharmaceutical Inc. and Johnson & Johnson Pharmaceutical Research and Development, LLC.

We thank E. Regan (Massachusetts General Hospital, Boston, Massachusetts) for her contribution to this study.

BACKGROUND. The authors conducted a Phase I dose-finding trial to study the use of combined bortezomib plus irinotecan in patients with advanced solid tumors.

METHODS. Patients who had received $\geq$1 prior chemotherapy regimen were eligible. Patients received bortezomib (1.0 mg/m^2, 1.3 mg/m^2, or 1.5 mg/m^2) on Days 1, 4, 8, and 11 and received irinotecan (from 50 mg/m^2 to 125 mg/m^2) on Days 1 and 8 of each 21-day cycle for a maximum of 8 cycles. Bortezomib followed irinotecan on coadministration days in Cycle 1 and Cycles 3 through 8 but preceded irinotecan in Cycle 2 to assess the effect of administration sequence on bortezomib pharmacodynamics.

RESULTS. Fifty-one enrolled patients with malignancies, including colorectal cancer (n = 23 patients), lung cancer (n = 6 patients), gastroesophageal cancer (n = 6 patients), and pancreatic cancer (n = 3 patients), received $\geq$1 dose of study drug. Nausea, vomiting, and diarrhea were the principal dose-limiting toxicities and led to the maximum tolerated doses of 1.3 mg/m^2 bortezomib and 125 mg/m^2 irinotecan. The most common grade $\geq$3 bortezomib-related nonhematologic adverse events were fatigue (n = 5 episodes), diarrhea (n = 4 episodes), and nausea (n = 4 episodes). grade $\geq$3 bortezomib-related hematologic adverse events included neutropenia (n = 6 episodes) and thrombocytopenia (n = 4 episodes) and rarely were dose limiting. Of 34 evaluable patients, no objective responses according to the Response Evaluation Criteria in Solid Tumors were seen; 10 patients achieved stable disease. The degree of proteasome inhibition in whole blood indicated that the biologic activity of bortezomib was unaffected by irinotecan coadministration.

CONCLUSIONS. The results of this Phase I study in patients with solid tumors indicated that bortezomib at a dose of 1.3 mg/m^2 on Days 1, 4, 8, and 11 plus irinotecan at a dose of 125 mg/m^2 on Days 1 and 8 every 21 days were the recommended Phase II doses. ***Cancer* 2006;107:2688–97.** © *2006 American Cancer Society.*

KEYWORDS: bortezomib, colorectal cancer, irinotecan, proteasome inhibitor, solid tumors.

Bortezomib (VELCADE®; Millennium Pharmaceuticals, Inc. and Johnson & Johnson Pharmaceutical Research and Development, LLC), a modified dipeptidyl boronic acid derived from leucine and phenylalanine, is the first proteasome inhibitor to be selected for clinical development as an anticancer agent.[1] The proteasome is a

David P. Ryan is on the Speaker's Bureau at Pfizer Pharmaceuticals. Carlo M. Rocha Lima is on theSpeaker's Bureau at Pfizer Pharmaceuticals and has received honoraria from Pfizer. Leonard J. Appleman has received honoraria from Millenni um Pharmaceuticals. Oscar Kashala is a stockholder in and an employee of Millennium Pharma-ceuticals.

Address for reprints: David P. Ryan, MD, Division of Hematology/Oncology, Massachusetts General Hospital, 100 Blossom Street, COX640, Boston, MA 02114; Fax: (617) 724-3166. E-mail: dpryan@partners.org

Received June 16, 2006; revision received August 29, 2006; accepted August 30, 2006.

DOI 10.1002/cncr.22280
Published online 30 October 2006 in Wiley InterScience (www.interscience.wiley.com).

multicatalytic enzyme that degrades diverse intracellular proteins. Substrates for the proteasome include IκB, an endogenous inhibitor of nuclear factor κB (NF-κB); the cyclin-dependent kinase inhibitors p21 and p27; cyclins; and the tumor suppressor protein p53.[2–4] Proteasome inhibition with bortezomib leads to an accumulation of the growth-inhibitory molecules p21 and p27 and cell cycle arrest.[5,6] Moreover, stabilization of IκB inhibits NF-κB activity, which may lead to enhanced susceptibility of multiple myeloma cells to chemotherapy-mediated cytotoxicity and sensitization of resistant cells to conventional chemotherapy.[3,7] Objective antitumor responses were noted in Phase I and II clinical trials of single-agent bortezomib in patients with hematologic malignancies and solid tumors.[8–13] In a randomized, international, Phase III trial that compared bortezomib with high-dose dexamethasone, bortezomib demonstrated a higher response rate, a longer time to disease progression, and better overall survival in patients with multiple myeloma after they had received from 1 to 3 prior therapies,[14] leading to the recent full approval of bortezomib by the US Food and Drug Administration for the treatment of patients with multiple myeloma who have received at least 1 prior therapy and similar approval by the European Medicines Evaluation Agency; however, patients must be ineligible for or previous recipients of stem cell transplantation.[15] Unlike patients with multiple myeloma, bortezomib has limited or no activity in patients with solid tumors.

Irinotecan, which is a water-soluble prodrug for parenteral administration, undergoes enzymatic conversion by carboxylesterases, predominantly in the liver, to the biologically active metabolite 7-ethyl-10-hydroxy-camptothecin (SN-38).[16] SN-38 is a potent inhibitor of topoisomerase I, a nuclear enzyme that reduces the torsional stress of supercoiled DNA during the replication, recombination, transcription, and repair of DNA. The camptothecins bind to and stabilize a normally transient, covalent complex that is formed between DNA and topoisomerase I. The presence of this ternary, drug-enzyme-DNA complex results in the generation of irreversible, double-strand breaks in DNA during ongoing DNA synthesis, the accumulation of which ultimately leads to cell death. Irinotecan is approved for the treatment of advanced colorectal cancer as follows: as first-line therapy in combination with 5-fluorouracil, as salvage treatment for 5-fluorouracil-refractory disease, and in combination with cetuximab for epidermal growth factor receptor-expressing colorectal cancer that is refractory to irinotecan-based chemotherapy.[17] Promising antitumor activity was observed in Phase II trials that evaluated treatment with irinotecan plus cisplatin in patients with small cell and nonsmall cell lung cancer, with response rates from 30% to 50%. The irinotecan/cisplatin combination is undergoing Phase II testing against other tumor types, including ovarian, cervical, and gastric cancers.[16]

In studies of colon cancer cells, pretreatment with bortezomib inhibited the activation of NF-κB induced by SN-38 and reduced the growth of colon tumor xenografts implanted in nude mice by greater than 90%, which was a greater-than-additive effect.[2] A similarly impressive antitumor effect of the bortezomib and irinotecan combination was observed in mice bearing BXPC3 pancreatic tumor xenografts.[18] Those findings suggested that bortezomib may enhance irinotecan-mediated cytotoxicity by preventing NF-κB activation.

The results of a Phase I clinical trial to evaluate the safety and biologic effects of bortezomib and irinotecan coadministered to patients with advanced solid tumors are described in this report. This study was designed to establish the maximum tolerated dose (MTD) of bortezomib on Days 1, 4, 8, and 11 together with irinotecan on Days 1 and 8 of each 21-day cycle. Pharmacodynamic studies also were undertaken to evaluate the effect of concurrently administered irinotecan on proteasome inhibition in peripheral blood cells.

MATERIALS AND METHODS

Participant Protection

In accordance with the Declarations of Helsinki, the protocol for this clinical trial was reviewed and approved by the appropriate Institutional Review Board at each participating study site. All patients were required to provide written informed consent prior to study participation according to institutional and federal guidelines.

Patient Selection

Patients were required to have histologically confirmed, locally advanced or metastatic, solid malignancies that were either measurable or otherwise evaluable and for which there was no known curative therapy. Eligibility requirements included the following: aged 18 years or older, Karnofsky performance status (KPS) $\geq$60% (Eastern Cooperative Oncology Group score, 0–2), life expectancy $>$3 months, prior treatment with at $\geq$1 chemotherapy regimen, absolute neutrophil count $\geq$1500/mm^3, platelet count $\geq$100,000/mm^3, serum total bilirubin concentration within normal limits, serum alanine aminotransferase and aspartate aminotransferase $\leq$2.5 times the

upper limit of normal ($\leq$5 times the upper limit of normal for patients with liver metastases), and serum creatinine concentration $\leq$1.5 mg/dL.

Patients were excluded from the study if they had received chemotherapy within 4 weeks, chloroethylnitrosourea within 6 weeks, or antibody therapy within 8 weeks of study enrollment. Patients who had received radiation therapy to >35% of the bone marrow within 4 weeks of enrollment and patients who had not recovered from the toxic effects of any previous chemotherapy, radiation therapy, or antibody therapy were ineligible for the study.

Patients who had undergone major surgery within the previous 4 weeks or who had received strontium-89 within 12 weeks of enrollment were not allowed to participate. Other exclusion criteria included electrocardiographic evidence of acute ischemia or new cardiac-conduction abnormalities, a history of myocardial infarction within the previous 6 months, a history of inflammatory bowel disease, a history of grade 4 toxicity while receiving an irinotecan-containing chemotherapy regimen, or the presence of uncontrolled brain metastases or nonneoplastic central nervous system disease. Women who were pregnant or breastfeeding also were excluded, and all men and women of reproductive potential were required to use an acceptable method of birth control for the duration of the study.

Treatment Plan

Patients underwent a complete history and physical examination at baseline and a symptom-directed physical examination prior to bortezomib and irinotecan administration on Days 1 and 8 of each treatment cycle. A complete blood count with differential and a measurement of serum electrolytes were obtained at baseline; prior to bortezomib administration on Days 1, 4, 8, and 11 of each treatment cycle; and at the end-of-study and follow-up visits. A serum chemistry panel was obtained at baseline and prior to the administration of medication on Days 1 and 8 of each treatment cycle.

Irinotecan hydrochloride (CAMPTOSAR®; Pharmacia & Upjohn, Kalamazoo, MI) was administered as a 90-minute intravenous (i.v.) infusion on Days 1 and 8 of each 21-day treatment cycle. Bortezomib was supplied as a sterile, lyophilized powder in glass vials that contained 2.5 mg or 3.5 mg of drug and 25 mg or 35 mg of mannitol, respectively, for reconstitution with 0.9% sodium chloride injection (US Pharmacopeia) to a final drug concentration of 1 mg/mL. Bortezomib was administered as a rapid intravenous bolus injection over 3 to 5 seconds on Days 1, 4, 8, and 11. On days during which both drugs were given, bortezomib was administered 1 hour after patients completed the irinotecan infusion, with the exception of Cycle 2, when bortezomib was given 1 hour before patients started the irinotecan infusion to assess the effect of the administration sequence on bortezomib pharmacodynamics (Fig. 1). A maximum of 8 treatment cycles (32 bortezomib doses and 16 irinotecan doses) were to be administered per patient. All patients were premedicated with antiemetics 30 minutes before they received irinotecan. Loperamide was used for the management of late-onset diarrhea induced by irinotecan. The prophylactic use of loperamide was not recommended, and the routine use of colony-stimulating factors was prohibited.

The bortezomib dose was fixed initially at 1.0 mg/m^2, and the dose of irinotecan was escalated from 50 mg/m^2 by a constant increment of 25 mg/m^2 until the MTD was identified, up to a maximum dose of 125 mg/m^2 (Table 1). Groups of 3 patients were entered for treatment into each dose cohort. In the absence of dose-limiting toxicity (DLT) in the initial 3 patients during a period of 21 days after they began the first cycle of treatment, dose escalation proceeded in the next cohort of patients. However, an additional 3 patients were entered into a dose cohort if a DLT occurred in 1 of the initial 3 patients treated. Dose escalation continued if no DLTs occurred in these additional patients. The MTD was considered to be exceeded if at least 2 patients in a cohort of 3 to 6 experienced a DLT during the first cycle of therapy, in which case, the preceding dose was considered the MTD. Upon establishing the MTD of irinotecan with bortezomib 1.0 mg/m^2, 10 additional patients were to be enrolled at this dose level. Thereafter, in a similar manner, the MTD of irinotecan combined with bortezomib 1.3 mg/m^2 was established, beginning with irinotecan at a dose 1 level below the MTD when given with bortezomib 1.0 mg/m^2. Finally, in a similar manner, the MTD of irinotecan combined with 1.5 mg/m^2 of bortezomib also was determined.

All treatment was to be held upon the appearance of grade $\geq$2 diarrhea that was not controllable with loperamide or any grade $\geq$3 treatment-related toxicity. Upon improvement to grade 1 or resolution of the toxicity, treatment was resumed at a 25% reduced-dose. In the event of grade 2 neutropenia, the irinotecan dose was reduced by 25%. After Cycle 1, bortezomib was to be held on Days 4 and 11 if the platelet count decreased below 20,000/mm^3. Treatment was to be discontinued upon failure to improve of any grade $\geq$3 toxicity that mandated withholding of treatment, upon evidence of disease progression, or after 8 cycles of therapy.

Evaluation of Toxicity

All patients were assessed for toxicity prior to and after each treatment cycle, and events were graded

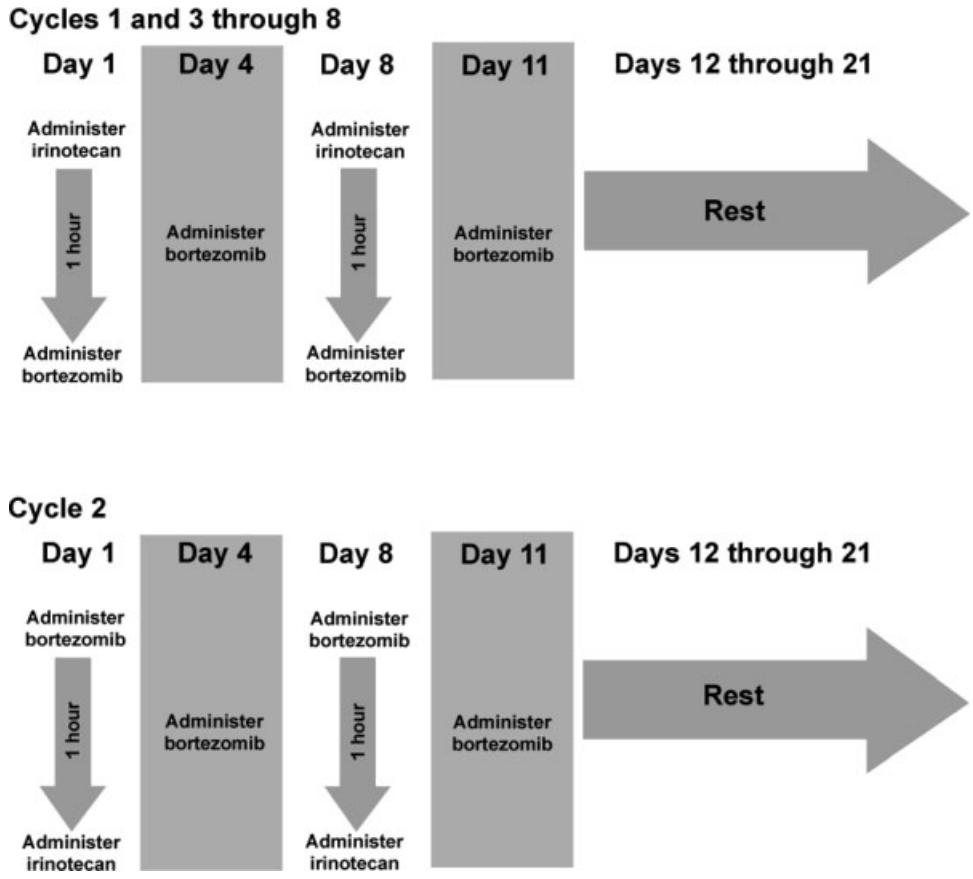

FIGURE 1. This is a schematic diagram of the 21-day bortezomib and irinotecan administration schedule.

according to the National Cancer Institute Common Toxicity Criteria (version 2.0). Patients who received at least 1 dose of study medication were considered evaluable for toxicity. A DLT was defined initially as any adverse event of grade $\geq$3 nonhematologic or hematologic toxicity related to bortezomib. The criteria for DLT were amended twice during the protocol. After the second dose cohort was completed, grade 3 neutropenia was not considered a DLT. For the cohort of patients receiving bortezomib 1.5 mg/m^2 plus irinotecan 125 mg/m^2, thrombocytopenia was considered a DLT only if the platelet count was $<$20,000/mm^3, whereas all other DLT criteria remained the same in each dose cohort. In addition, the use of any concomitant medications, procedures, or supportive therapies was recorded.

TABLE 1
Dose-Escalation Schedule and Number of Patients with Dose-Limiting Toxicities

Dose cohort	Bortezomib dose (mg/m^2)	Irinotecan dose (mg/m^2)	No. of patients	No. with DLT
1	1.0	50	3	0
2	1.0	75	6	1
3	1.0	100	3	0
4	1.0	125	15	3
5	1.3	100	3	0
6	1.3	125	15	2*
7	1.5	125	6	3

DLT indicates dose-limiting toxicity.
* These 2 patients (1 in the original cohort and 1 in the expanded cohort) were classified with DLT after the study was completed.

Evaluation of Response

Computed tomographic scans were obtained at baseline to provide initial documentation of measurable disease. Lesions were categorized as either measurable or evaluable (i.e., nonmeasurable lesions accompanied by detectable tumor markers that would allow response assessment). Tumors also were identified either as target lesions or as nontarget lesions, according to the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines.[19]

Tumor response evaluations were based on computed tomographic scans that were obtained every 6

weeks and at the end of the study. Patients who had an objective response or stable disease but not progressive disease needed a confirmation of response by a second series of scans 6 weeks later.

Pharmacodynamic Studies

The pharmacodynamics of bortezomib were determined by using an ex vivo spectrofluorometric assay of the activity of the 20S proteasome in lysed blood cells, as described previously.[20] Samples for this assay were obtained immediately before and 1 hour after administration of bortezomib on Days 1 and 8 of Cycles 1 and 2.

RESULTS

Patient Characteristics and Disposition

Fifty-two patients were enrolled between May 31, 2001, and January 10, 2003, at 4 study centers (Massachusetts General Hospital, Boston, MA; Dana-Farber Cancer Institute, Boston, MA; H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL; and the University of North Carolina at Chapel Hill, Chapel Hill, NC), and; 51 patients received at least 1 dose of study drug and were evaluable for safety. Thirty of 51 patients (59%) were male, and the median age was 56 years (range, 25–76 years) (Table 2). Most patients had a good performance status at baseline (median KPS, 90%; range, 70–100%). The most frequent primary malignancies were colorectal (n = 23 patients), nonsmall cell lung cancer (n = 6 patients), and adenocarcinoma of the esophagus or gastroesophageal junction (n = 6 patients). Forty-nine of 51 patients (96%) had received prior chemotherapy; and most had been pretreated heavily (median, 3 prior regimens; range, 0–7 prior regimens), including 22 patients (43%) who previously had received irinotecan and 1 patient who previously had received bortezomib. Two patients who had no documentation of receiving prior chemotherapy in their case report forms were considered protocol violations; however, according to the patient narratives within the clinical study report, 1 of these patients previously had received chemotherapy regimens that included irinotecan. Twenty-one of 51 patients (41%) had received prior radiation therapy.

TABLE 2
Demographic and Baseline Clinical Characteristics for All Dose Cohorts (N = 51)

Characteristic	No. of patients (%)
Median age, [range], y	56 [(25–76)]
Sex	
Men	30 (59)
Women	21 (41)
Race	
White	48 (94)
Black/Hispanic	2 (4)
Asian/Pacific Islander	1 (2)
Primary tumor	
Colon or rectum	23 (45)
Lung	6 (12)
Gastroesophageal	6 (12)
Pancreas	3 (6)
Other*	13 (25)
Median KPS (range), %	90 (70–100)
Median no. of prior regimens (range)	3 (0–7)
No. of prior therapies (%)	
Chemoradiotherapy†	49 (96)
Radiation	21 (41)
Surgery	48 (94)

KPS indicates Karnofsky performance score.

* Synovial sarcoma (n = 1 patient), malignant melanoma (n = 1 patient), neuroendocrine carcinoma with unknown primary (n = 1 patient), and tumors of the stomach (n = 1 patient), ovary (n = 3 patients), small bowel (n = 2 patients), cervix (n = 1 patient), anus (n = 1 patient), kidney (n = 1 patient), and urinary bladder (n = 1 patient).

† Two patients who did not receive prior chemotherapy were not assessable for response, but they were assessable for safety.

Determination of the MTD

The irinotecan dose was increased from 50 mg/m^2 to 125 mg/m^2 in 25-mg/m^2 increments through the first 4 planned dose levels with bortezomib 1.0 mg/m^2 (Table 1). One patient who was treated in Dose Cohort 2 (irinotecan 75 mg/m^2 and bortezomib 1.0 mg/m^2) experienced grade 3 neutropenia, which was defined as a DLT, resulting in expansion of the cohort to 6 patients, and no additional DLT was observed in that cohort. Subsequently, the protocol was amended to redefine dose-limiting neutropenia as a grade 4 event. In the absence of DLT among the initial 3 patients in Dose Cohort 4 (irinotecan 125 mg/m^2 and bortezomib 1.0 mg/m^2), that cohort was expanded to a total of 15 patients so that toxicity could be determined at the maximum irinotecan dose prior to escalating the bortezomib dose. Three DLTs were reported in 3 patients in the expanded cohort (grade 3 diarrhea in 2 patients and grade 3 thrombocytopenia in 1 patient). The bortezomib dose was increased to 1.3 mg/m^2 in Cohort 5, and the irinotecan dose was lowered to 100 mg/m^2, and all 3 patients were accrued without DLT. In Cohort 6 (irinotecan 125 mg/m^2 and bortezomib 1.3 mg/m^2), 0 of 3 patients experienced a DLT; however, 1 patient was classified later with a DLT (grade 3 fatigue). Cohort 6 was expanded to include 12 additional patients, including 1 patient who experienced an adverse event (grade 3 increase in blood amylase levels) that was classified as a DLT after the study was completed. The bortezomib dose was escalated further to 1.5 mg/m^2 with irinotecan 125 mg/m^2. Three patients were enrolled, and 1

TABLE 3
Bortezomib-Related Grade 3 or 4 Adverse Events

Variable	No. of adverse events							
	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5	Cohort 6	Cohort 7	All Cohorts
Bortezomib/irinotecan dose (mg/m^2)	1.0/50	1.0/75	1.0/100	1.0/125	1.3/100	1.3/125	1.5/125	—
No. of adverse events*	3	6	3	15	3	15	6	51
No. of patients with at least 1 Grade 3 or 4 adverse event	0	3	1	8	2	9	4	27
Gastrointestinal disorders								
Diarrhea	0	0	1	2	0	0	1	4
Nausea	0	0	0	1	0	0	3	4
Emesis	0	0	0	0	0	0	3	3
Constipation	0	0	1	0	0	0	0	1
Hyperamylasemia	0	0	0	0	0	2	0	2
Hyponatremia	0	0	1	0	0	0	0	1
Infections								
Candida	0	1	0	0	0	0	0	1
Esophageal candidiasis	0	1	0	0	0	0	0	1
Postural hypotension	0	0	0	0	0	0	1	1
Pleural effusions	0	0	1	0	0	0	0	1
Hematologic disorders								
Anemia	0	1	0	0	0	0	0	1
Leukopenia	0	0	0	0	1	1	0	2
Lymphopenia	0	0	0	0	0	1	0	1
Neutropenia	0	1	0	1	0	4	0	6
Thrombocytopenia	0	0	0	1	0	3	0	4
Abnormal WBC count	0	0	0	0	0	1	0	1
Rash or skin eruption	0	0	0	0	1	0	1	2
Neurologic disorders								
Neuralgia	0	1	0	0	0	0	0	1
Hypoesthesia	0	1	0	0	0	0	0	1
Paresthesia	0	1	0	0	0	0	0	1
General disorders								
Fatigue	0	0	1	2	0	2	0	5
Aggravated fatigue	0	0	0	1	0	0	0	1
Weakness	0	0	0	1	0	0	0	1
Musculoskeletal disorders								
Arthralgia	0	0	0	0	0	1	0	1
Pain in limb	0	0	0	0	0	1	0	1

WBC indicates white blood cells.

* More than 1 adverse event may have occurred in a single patient.

patient experienced DLT with grade 3 nausea/vomiting. According to the study schema, 3 additional patients were enrolled to that cohort, and 2 of those patients experienced DLTs (1 with grade 3 nausea/vomiting, 1 with grade 3 rash and diarrhea). Because of DLTs observed in 3 of 6 patients enrolled in Cohort 7 (irinotecan 125 mg/m^2 and bortezomib 1.5 mg/m^2), treatment at that dose level was stopped. Thus, we determined that the MTD was bortezomib 1.3 mg/m^2 and irinotecan 125 mg/m^2, and the study was closed for enrollment.

Toxicity

At least 1 grade 3 or 4 adverse event that possibly or probably was related to treatment was reported in 27 of 51 patients (53%) (Table 3). The most common grade $\geq$3 treatment-related, nonhematologic adverse event was fatigue (5 of 51 patients; 10%), followed by other primarily gastrointestinal events: diarrhea (4 of 51 patients; 8%), nausea (4 of 51 patients; 8%), and vomiting (3 of 51 patients; 6%). The incidence of treatment-related grade 3 or 4 neurotoxicity was low: grade 3 hypoesthesia (numbness in toes) and grade 3 paresthesia (tingling in toes) were reported together in 1 patient with bortezomib 1.0 mg/m^2; neither event was considered a DLT, because neither occurred during the first cycle of treatment.

The most common grade 3 or 4 treatment-related hematologic adverse events were neutropenia (6 of 51 patients; 12%) and thrombocytopenia (4 of 51 patients; 8%). Other treatment-related grade 3 or

TABLE 4
Treatment Administration, Patient Disposition, and Response by Dose Group

Variable	No. of patients (%)						
	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5	Cohort 6	Cohort 7
Bortezomib/irinotecan dose (mg/m^2)	1.0/50	1.0/75	1.0/100	1.0/125	1.3100/	1.3/125	1.5/125
Total no. of patients	3	6	3	15	3	15	6
No. of cycles completed							
0	0	0	0	0	0	0	2
1	0	2	0	5	0	1	2
2	3	0	2	4	2	7	0
≤3	0	4	1	6	1	7	2
Early discontinuations							
Adverse events	0	1	0	1	1	0	3
Progressive disease	3	3	3	9	0	11	2
Poor health status	0	0	0	2	0	0	1
Patient request	0	1	0	1	0	3	0
Other*	0	0	0	0	1	1	0
No. of evaluable patients	2	4	2	12	1	11	2
No. with stable disease†	0	2	1	3	1	3	0
Primary tumor site	—	Colon, ovary	GE junction	Rectum, pancreas, lung	Lung	Colon	—

GE indicates gastroesophageal.

* One patient withdrew because of a bortezomib-related grade 3 rash, and the other patient withdrew to participate in a different investigational study of bortezomib.

† Seventeen patients were not evaluable for efficacy because of insufficient data.

4 hematologic adverse events that occurred in <5% of patients included leukopenia (n = 2 patients), lymphopenia (n = 1 patient), and anemia (n = 1 patient). These events generally were short-lived and did not require hospitalization. Adverse events of any grade, regardless of their relation to treatment, that were reported in >20% of patients were fatigue (42 of 51 patients; 82%), diarrhea (39 of 51 patients; 76%), nausea (38 of 51 patients; 75%), vomiting (29 of 51 patients; 57%), constipation (24 of 51 patients; 47%), abdominal pain (21 of 51 patients; 41%), anorexia (18 of 51 patients; 35%), anemia (15 of 51 patients; 29%), decreased appetite (11 of 51 patients; 22%), dizziness (11 of 51 patients; 22%), dyspnea (11 of 51 patients; 22%), pyrexia (11 of 51 patients; 22%), and thrombocytopenia (11 of 51 patients; 22%).

Response

There were no objective responses. Of the 51 patients enrolled, 34 patients were evaluable for response (i.e., patients who had received at least 1 dose of bortezomib or irinotecan, had a baseline imaging scan, and at least 1 postbaseline assessment); 17 patients were not evaluable because of insufficient data. The best response to treatment according to RECIST criteria occurred in 10 of 34 patients (29%) who achieved stable disease (Table 4), and 4 of those patients previously had received irinotecan.

The numbers of cycles administered to each cohort appear in Table 4. Most patients (49 of 51 patients; 96%) completed at least 1 cycle of treatment with bortezomib and irinotecan, and 21 patients (41%) completed 3 or more treatment cycles. The average number of cycles of bortezomib completed at the 1.0 mg/m^2 or 1.3 mg/m^2 dose was approximately 3. Two patients were enrolled in an extension trial under a separate protocol, including 1 patient with nonsmall cell lung cancer from Cohort 4 who received 10 additional cycles of treatment (irinotecan 165 mg once weekly for the first 2 weeks of the 3-week cycle and bortezomib 1.0 mg/m^2 for 2 cycles, which was increased to 1.3 mg/m^2 for the last 8 cycles). Disease progression was the primary reason for study discontinuation, as shown in Table 4.

Pharmacodynamics

The maximum inhibition of 20S proteasome activity measured at 1 hour postdose on Day 1 was similar to that observed on Day 8 of the same cycle (Fig. 2). The mean percentages of 20S proteasome inhibition were similar among the 3 bortezomib dose groups (1.0 mg/m^2, 1.3 mg/m^2, and 1.5 mg/m^2). No apparent differences were observed among the various irinotecan dose groups. With regard to recovery of proteasome function, in most cohorts, the mean percentage of proteasome inhibition predose on Day 8 was reduced by approximately 50% compared with postdose values on Day 1.

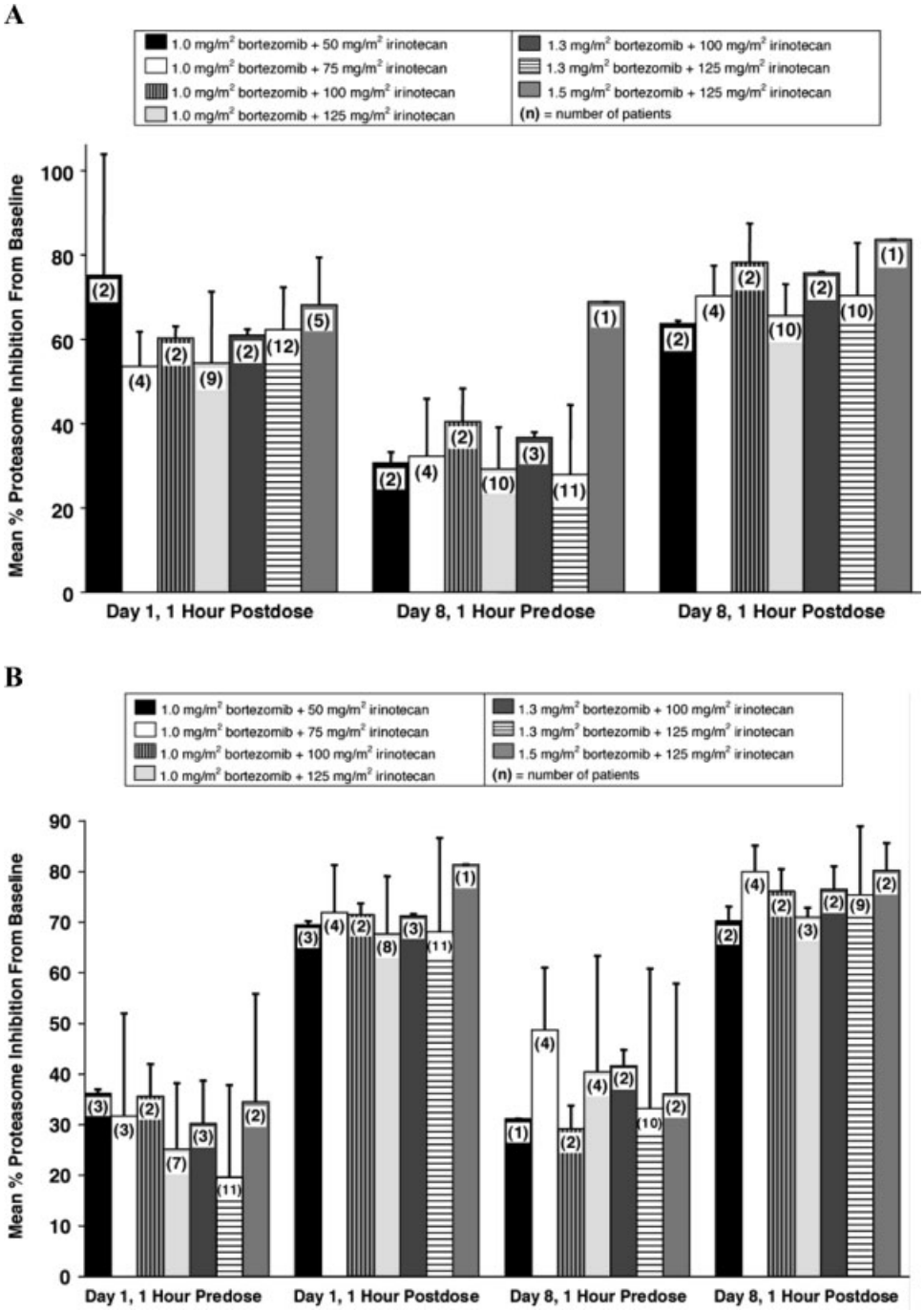

FIGURE 2. The mean percentage proteasome inhibition (compared with baseline levels) was measured on Days 1 and 8 in each dose cohort. (A) Cycle 1. (B) Cycle 2. Bars indicate standard deviation. Numbers in bars indicate the number of patients.

Therefore, approximately 50% of proteasome function had recovered 4 days after the previous administration of bortezomib on Day 4. Although the administration of bortezomib followed that of irinotecan during Cycle 1, and vice versa during Cycle 2, the pharmacodynamics of bortezomib did not appear to be altered by drug sequencing.

DISCUSSION

An understanding of mechanisms underlying irinotecan resistance is emerging rapidly.[21] Up-regulation of NF-κB-directed transcription of antiapoptotic factors, such as Bcl-2, is associated with irinotecan resistance. In a murine model of colorectal cancer, bortezomib combined with irinotecan resulted in enhanced

apoptosis in LoVo tumor xenografts.[2] The NF-κB effect that was observed in mice also may occur in human colorectal tumors, potentially circumventing tumoral resistance to irinotecan.

In the current study, the combination of bortezomib and irinotecan generally was tolerated well with manageable toxicities. The MTD for the combination regimen was bortezomib 1.3 mg/m^2 and irinotecan 125 mg/m^2. Overall, the most common grade 3 or 4 nonhematologic adverse events were fatigue, diarrhea, nausea, and vomiting, whereas neutropenia was the most common hematologic event.

The majority of DLTs were gastrointestinal in nature (specifically, diarrhea, nausea, and vomiting). Given the overlapping toxicities of irinotecan and bortezomib, this finding was not unexpected. grade $\geq$3 diarrhea is a dose-related and dose-limiting toxicity of irinotecan and has been reported in 24% to 37% of patients in other studies treated at irinotecan doses similar to those used in the current trial, albeit with a more intense administration schedule (4 of every 6 weeks).[22,23] grade $\geq$3 diarrhea was reported in 8% of patients who were treated with bortezomib at the doses used in this study[12] and was dose-limiting at higher doses.[8] In contrast, hematologic events (i.e., neutropenia and thrombocytopenia) rarely were dose limiting in our study.

Grade 3 peripheral neuropathy was reported in only 1 of 51 patients (2%), a frequency lower than that observed in earlier Phase I trials using single-agent bortezomib treatment in patients with solid tumors or hematologic neoplasms.[8,9] However, fewer patients received bortezomib 1.3 mg/m^2, and the frequency of neuropathy was lower at the 1.0-mg/m^2 dose than at the 1.3-mg/m^2 dose in a previous Phase II trial[11]; the 1.0-mg/m^2 dose was received by the majority of patients in the current study.

With respect to the pharmacodynamics of the bortezomib/irinotecan combination, the mean percentages of proteasome inhibition at the time points evaluated were similar among all bortezomib dose groups (1.0 mg/m^2, 1.3 mg/m^2, and 1.5 mg/m^2). This is consistent with the described sigmoid maximum-effect model for bortezomib, in which proteasome inhibition has a steep dose response that reaches a plateau and does not increase significantly beyond 60% to 70% with doses increasing from 1.0 mg./m^2 to 2.0 mg/m^2.[9,24] The range of proteasome inhibition that we observed at doses of 1.0 mg/m^2, 1.3 mg/m^2, and 1.5 mg/m^2 was similar to that reported in other studies of bortezomib at similar doses in patients with solid tumors or hematologic neoplasms.[8,9] No apparent differences were observed when bortezomib was combined with 4 dose groups of irinotecan, suggesting that irinotecan has a negligible effect on the ability of bortezomib to inhibit proteasome activity. However, contrary to findings from previous studies that evaluated the pharmacodynamics of single-agent bortezomib in advanced malignancies,[8,9] we observed that proteasome activity did not recover fully to pretreatment baseline levels at the predose assessments (Day 8 for Cycle 1 and Days 1 and 8 for Cycle 2). The mechanism and implication of this prolonged inhibitory effect on proteasome activity are unknown and warrant further investigation in both preclinical and clinical settings.

In the current study, 22 patients had received prior treatment with irinotecan, and 4 of those patients achieved stable disease after treatment with irinotecan and bortezomib. Three of those patients had achieved stable disease as their best response to prior irinotecan treatment; for the remaining patient, response information to prior treatment regimens was not documented on the case report form. Although stable disease was noted, no objective responses were observed in this study after treatment with irinotecan and bortezomib. Because bortezomib has limited or no activity in solid tumors, this finding raises concerns that bortezomib may not enhance irinotecan activity, like what was observed in preclinical studies. It is unclear whether this is because of alternative mechanisms of irinotecan resistance, such as the up-regulation of breast cancer resistance protein 1 (BRCP-1),[25] excision repair genes like ERCC1,[26] and multidrug resistance-associated protein 4,[27] or the unimportance of NF-κB in irinotecan resistance. However, the observation of prolonged stable disease in patients who received previously treatment with irinotecan may indicate that some modulation of irinotecan resistance was occurring.

Proteasomal degradation of topoisomerase I in response to irinotecan inhibition and the consequential transcriptional arrest because of stalled topoisomerase I is postulated as a primary irinotecan-resistance mechanism (for a review, see Xu and Villalona-Calero).[28] Preclinical studies using tumor cell lines have demonstrated synergy between irinotecan and proteasome inhibitors, leading to strong antitumor activity against various malignancies.[29,30] The lack of objective tumor responses at the MTD in the current trial suggests that additional therapeutic strategies, such as optimization of the schedule and/or sequence of administration, may be required to circumvent drug resistance to irinotecan.

The combination of bortezomib 1.3 mg/m^2 and irinotecan 125 mg/m^2 was tolerated with and had a manageable toxicity profile. The use of bortezomib in combination with irinotecan did not appear to

result in additive gastrointestinal or hematologic toxicities. Administration of irinotecan did not interfere with the pharmacodynamic activity (proteasome inhibition) of bortezomib. These results warrant further investigation of combination treatment with bortezomib and irinotecan, especially in cancers that are known to be responsive to irinotecan therapy.

REFERENCES

1. Adams J. Development of the proteasome inhibitor PS-341. *Oncologist*. 2002;7:9–16.
2. Cusack JC Jr., Liu R, Houston M, et al. Enhanced chemosensitivity to CPT-11 with proteasome inhibitor PS-341: implications for systemic nuclear factor-kappaB inhibition. *Cancer Res*. 2001;61:3535–3540.
3. Hideshima T, Richardson P, Chauhan D, et al. The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. *Cancer Res*. 2001;61:3071–3076.
4. Ling YH, Liebes L, Jiang JD, et al. Mechanisms of proteasome inhibitor PS-341-induced G(2)-M-phase arrest and apoptosis in human non-small cell lung cancer cell lines. *Clin Cancer Res*. 2003;9:1145–1154.
5. An B, Goldfarb RH, Siman R, Dou QP. Novel dipeptidyl proteasome inhibitors overcome Bcl-2 protective function and selectively accumulate the cyclin-dependent kinase inhibitor p27 and induce apoptosis in transformed, but not normal, human fibroblasts. *Cell Death Diff*. 1998;5:1062–1075.
6. Blagosklonny MV, Wu GS, Omura S, el Deiry WS. Proteasome-dependent regulation of p21WAF1/CIP1 expression. *Biochem Biophys Res Commun*. 1996;227:564–569.
7. Mitsiades N, Mitsiades CS, Richardson PG, et al. The proteasome inhibitor PS-341 potentiates sensitivity of multiple myeloma cells to conventional chemotherapeutic agents: therapeutic applications. *Blood*. 2003;101:2377–2380.
8. Aghajanian C, Soignet S, Dizon DS, et al. A phase I trial of the novel proteasome inhibitor PS341 in advanced solid tumor malignancies. *Clin Cancer Res*. 2002;8:2505–2511.
9. Orlowski RZ, Stinchcombe TE, Mitchell BS, et al. Phase I trial of the proteasome inhibitor PS-341 in patients with refractory hematologic malignancies. *J Clin Oncol*. 2002;20: 4420–4427.
10. Stevenson J, Nho CW, Schick J, et al. Phase II clinical/pharmacodynamic trial of the proteasome inhibitor PS-341 in advanced non-small cell lung cancer [abstract 810]. *Proc Am Soc Clin Oncol*. 2003;22:202.
11. Jagannath S, Barlogie B, Berenson J, et al. A phase 2 study of two doses of bortezomib in relapsed or refractory myeloma. *Br J Haematol*. 2004;127:165–172.
12. Richardson PG, Barlogie B, Berenson J, et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. *N Engl J Med*. 2003;348:2609–2617.
13. Kondagunta GV, Drucker B, Schwartz L, et al. Phase II trial of bortezomib for patients with advanced renal cell carcinoma. *J Clin Oncol*. 2004;22:3720–3725.
14. Richardson PG, Sonneveld P, Schuster MW, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. *N Engl J Med*. 2005;352:2487–2498.
15. Twombly R. First proteasome inhibitor approved for multiple myeloma [abstract]. *J Natl Cancer Inst*. 2003;95:845.
16. Garcia-Carbonero R, Supko JG. Current perspectives on the clinical experience, pharmacology, and continued development of the camptothecins. *Clin Cancer Res*. 2002;8:641–661.
17. Pfizer Inc.Camptosar (brand of irinotecan hydrochloride injection) prescribing information. Available at: http://www.pfizer.com/download/uspi_camptosar.pdf. 2004.
18. Shah SA, Potter MW, McDade TP, et al. 26S proteasome inhibition induces apoptosis and limits growth of human pancreatic cancer. *J Cell Biochem*. 2001;82:110–122.
19. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst*. 2000; 92:205–216.
20. Lightcap ES, McCormack TA, Pien CS, Chau V, Adams J, Elliott PJ. Proteasome inhibition measurements: clinical application. *Clin Chem*. 2000;46:673–683.
21. Rasheed Z, Rubin EH. Mechanisms of resistance to topoisomerase I-targeting drugs. *Oncogene*. 2003;22:7296–7304.
22. Rothenberg ML, Eckardt JR, Kuhn JG, et al. Phase II trial of irinotecan in patients with progressive or rapidly recurrent colorectal cancer. *J Clin Oncol*. 1996;14:1128–1135.
23. Verschraegen CF, Levy T, Kudelka AP, et al. Phase II study of irinotecan in prior chemotherapy-treated squamous cell carcinoma of the cervix. *J Clin Oncol*. 1997;15:625–631.
24. Papandreou CN, Daliani DD, Nix D, et al. Phase I trial of the proteasome inhibitor bortezomib in patients with advanced solid tumors with observations in androgen-independent prostate cancer. *J Clin Oncol*. 2004;22:2108–2121.
25. Kawabata S, Oka M, Shiozawa K, et al. Breast cancer resistance protein directly confers SN-38 resistance of lung cancer cells. *Biochem Biophys Res Commun*. 2001;280:1216–1223.
26. Di Nicolantonio F, Mercer SJ, Knight LA, et al. Cancer cell adaptation to chemotherapy. *BMC Cancer*. 2005;5:78.
27. Norris MD, Smith J, Tanabe K, et al. Expression of multidrug transporter MRP4/ABCC4 is a marker of poor prognosis in neuroblastoma and confers resistance to irinotecan in vitro. *Mol Cancer Ther*. 2005;4:547–553.
28. Xu Y, Villalona-Calero MA. Irinotecan: mechanisms of tumor resistance and novel strategies for modulating its activity. *Ann Oncol*. 2002;13:1841–1851.
29. Desai SD, Li TK, Rodriguez-Bauman A, Rubin EH, Liu LF. Ubiquitin/26S proteasome-mediated degradation of topoisomerase I as a resistance mechanism to camptothecin in tumor cells. *Cancer Res*. 2001;61:5926–5932.
30. Catley L, Tai YT, Shringarpure R, et al. Proteasomal degradation of topoisomerase I is preceded by c-Jun NH2-terminal kinase activation, Fas up-regulation, and poly(ADP-ribose) polymerase cleavage in SN38-mediated cytotoxicity against multiple myeloma. *Cancer Res*. 2004;64:8746–8753.