Published in final edited form as:
Invest New Drugs. 2009 February ; 27(1): 53–62. doi:10.1007/s10637-008-9154-z.

A phase I study of bortezomib, etoposide and carboplatin in patients with advanced solid tumors refractory to standard therapy

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Summary

Purpose—To evaluate the toxicity, pharmacological, and biological properties of the combination of bortezomib, etoposide, and carboplatin in adults with advanced solid malignancies.

Patients and methods—Patients received escalating doses of bortezomib, etoposide, and carboplatin every 21 days. Surrogate markers of angiogenesis were evaluated.

Results—Twenty-four patients received 64 courses of therapy. The most common treatment-related adverse events were myelosuppression. Dose-limiting grade 3 and 4 neutropenia and thrombocytopenia were observed when bortezomib was given on days 1, 4, 8, 11. With revised dosing, the maximum tolerated dose (MTD) of bortezomib 0.75 mg/m^2 (days 1, 8), etoposide 75 mg/m^2 (days 1–3), and carboplatin AUC 5 (day 1) was well tolerated, and are the recommended doses for further studies with this combination. No objective responses were observed, however stable disease was noted for greater or equal to four cycles in nine highly refractory patients.

Keywords

Bortezomib; Combination chemotherapy; Phase I clinical trial; Proteasome inhibitor

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Introduction

The ubiquitin–proteasome pathway is important for cancer growth and metastasis, as it controls the intracellular degradation of many of the key regulatory proteins that govern cell division, growth, activation, apoptosis, signaling and transcription [1,2]. The 26S proteasome is an ATP-dependent, multicatalytic, multiprotein complex expressed in all eukaryotic cells [3]. It plays a critical role in degrading cyclin D, E, and A-dependent kinase inhibitors including p21 and p27, tumor suppressor proteins such as p53, and IKK, an inhibitor of nuclear factor κ-B (NFκB) activation [4,5] controlling gene expression of endothelial cell surface adhesion molecules involved in tumor metastasis and angiogenesis [5]. The proteasome also regulates angiogenesis *in vivo* [6]. It is therefore an attractive therapeutic target that may potentially arrest the cell cycle, disrupt growth regulatory pathways, induce apoptosis, and inhibit angiogenesis.

Bortezomib (PS-341, Velcade™) is a highly specific, potent boronic acid dipeptide derivative selected for its broad *in vitro* activity when tested for cytotoxicity and proteasome inhibition [7,8]. *In vivo* activity of bortezomib was evident in a number of tumor xenograft models including breast, glioblastoma, prostate, colon, and pancreatic xenografts and in the Lewis lung carcinoma model [9]. Additive to synergistic tumor growth delay was found in combination regimens with 5-fluorouracil, cisplatin, doxorubicin, gemcitabine, taxanes and irinotecan [10–15]. Substantial increases in apoptosis were observed when bortezomib was added to chemotherapy [12,16]. Bortezomib was the first proteasome inhibitor to progress to clinical trials [7].

A variety of phase I single agent clinical trials with bortezomib have been completed, enrolling several hundred patients [17,20]. The maximal tolerated dose (MTD) is treatment schedule-dependent [17,19–21], with dose-limiting toxicities of diarrhea and sensory neuropathy [18]. Other toxicities include fatigue, fever, anorexia, nausea, vomiting, rash, pruritus and headache. Sensory neuropathy was dose-related and dose limiting in these trials, but was more prevalent in patients previously treated with neurotoxic agents [22]. Notable was the lack of any significant myelosuppresion. The maximally tolerated dose and schedule that also demonstrated biological activity in solid tumors was 1.5 mg/m^2 given twice weekly, every 3 weeks [22]. Suggestion of anti-tumor activity was seen in single agent trials [17–20], and due to the highly significant median time to progression favoring bortezomib over daily dexamethasone [23], it was approved for refractory multiple myeloma (13.5 versus 6.2 months, one year survival of 80% versus 60% $p<0.001$) [24]. Subsequent phase II studies did not find objective responses in renal cell, colon, neuroendocrine tumors, or melanoma despite clear documented proteasome inhibition in tumor biopsies and peripheral blood [25–28]. A significant increase in intratumoral HIF-1α was observed without modifying p53, NFκB or IκB expression in the phase II colon cancer study, suggesting that the proteasome may alter the response to tumor hypoxia [28]. In a phase II study in patients with sarcomas, one patient with leiomyosarcoma had a partial response [29]. Although single agent activity in solid tumors is low, bortezomib may restore sensitivity and enhance chemotherapy efficacy. As a result, it has been explored in phase I/II clinical trials in combination therapy, and preliminary results demonstrate tolerability [30,31].

Etoposide is a topoisomerase II inhibitor [32] with broad clinical application. Carboplatin [33] binds DNA covalently to form DNA–DNA inter- and intrastrand cross-links, and DNA protein cross-links to inhibit DNA synthesis, function, and transcription. Although each agent alone has broad anti-tumor activity, and high doses of each are used in stem cell transplantation, combination therapy with etoposide and carboplatin has been used in lung cancer, neuroendocrine cancer, and germ cell tumors [34–37]. The side effects of etoposide and carboplatin commonly include fatigue, myelosuppression (neutropenia, thrombocytopenia,

and anemia), nausea, and alopecia [32,33]. Less commonly observed are transaminitis, sensorineural hearing loss, sensory neuropathy, renal dysfunction, and secondary leukemias [24].

The combination of bortezomib with etoposide and carboplatin was proposed for this trial, as it was felt to confer non-overlapping mechanisms of activity and toxicity, with the potential of bortezomib to enhance chemosensitivity and apoptosis induced by carboplatin and etoposide [9,12,38]. Furthermore, bortezomib preclinical studies demonstrate that bortezomib can restore sensitivity to cytotoxic agents [9,39,40].

The objectives of this phase I study were to determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of bortezomib in combination with etoposide and carboplatin, given intravenously; to investigate pharmacokinetic and biologic interactions between these agents; to seek preliminary evidence of anti-tumor activity in patients with advanced cancer; and to explore the use of surrogate markers of biologic activity.

Materials and methods

Patient selection

Patients with pathologically confirmed advanced solid malignancies with measurable or non-measurable disease without curative options were enrolled in this study. Patients could not have received chemotherapy, radiation, or any other investigational agent for at least 4 weeks prior to study entry (6 weeks for nitrosoureas or mitomycin C) and must have recovered from any toxic effects of previous therapy. Palliative radiation therapy was allowed two or more weeks prior to enrollment. Patients may have received carboplatin and/or etoposide chemotherapy in the past. Patients with a known history of intracranial metastases could be included, provided that they were clinically stable, had no active seizures, and were on stable anti-epileptic or steroid medications for at least 7 days prior to study enrollment. Patients must not have had wide-field radiation therapy to more than 35% of their bone marrow reserve, any pelvic radiation, greater than two courses of mitomycin C, or have undergone stem cell transplant. Additional eligibility criteria included: age≥16 years; Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, and life expectancy of greater than 12 weeks. Study candidates were required to have adequate hematopoietic (ANC≥1,500/μl, hemoglobin≥9 g/dl, platelets≥100,000/μl), hepatic (total serum bilirubin≤1.5 times upper limits of normal; SGOT and SGPT≤2.5 times upper normal limits or lesser or equal to five times upper normal limits for patients with hepatic metastases), and renal (serum creatinine≤ 1.5 mg/dl or creatinine clearance of at least 60 ml/min) function. Women and men consented to the use of an approved method of contraception during study participation. Pregnant or nursing women were excluded. Informed consent was obtained according to federal and institutional guidelines.

Drug administration

Bortezomib was supplied by the National Cancer Institute and was administered as an IV bolus over 3 to 5 seconds initially on days 1, 4, 8, and 11, one hour prior to carboplatin on day 1. Due to unacceptable dose limiting toxicity (primarily neutropenia) noted in early cohorts, this was amended to days 1 and 8 IV bolus dosing. Commercial carboplatin and etoposide were used. Carboplatin was given IV over 30 min on day 1 of each cycle, with dosing calculated using the Calvert formula [41]. Etoposide was given IV over 60 min immediately after carboplatin on day 1 and given alone on days 2 and 3 of each 21 day cycle.

Antiemetic therapy with a 5-HT3 antagonist and dexamethasone (10–20 mg IV) was administered prior to chemotherapy on days 1–3. Additional doses of dexamethasone and a 5-

HT3 antagonist were given on days 4–7 for delayed nausea and/or vomiting as needed. Concomitant hormonal, radiation, experimental, or biologic therapies were not permitted. Prophylactic use of colony-stimulating factors was not allowed initially; however, if neutropenia occurred, G-CSF was allowed according to ASCO guidelines [42]. All concomitant medications were recorded.

The dose escalation scheme for bortezomib, carboplatin and etoposide is shown in Table 1. Three patients were enrolled per dose level and if no patients experienced DLT, dose escalation occurred. If dose-limiting toxicity (DLT) occurred, up to six patients were enrolled at that dose level. If two or more patients at a dose level experienced DLT, then dose escalation was halted. The maximum tolerated dose (MTD) was defined as the highest dose at which zero or one of six patients experienced DLT in the first course. At least three patients enrolled at the MTD underwent a superficial tumor biopsy of easily accessible tumor to obtain additional information for biologic correlative studies. Toxicities were graded according to the NCI's Common Toxicity Criteria (CTC), version 2.0. DLT was defined as (a) any non-hematologic toxicity>grade 3, excluding diarrhea, nausea, or vomiting in the absence of adequate supportive care; (b) grade 4 thrombocytopenia (platelets<25,000/mm^3) or grade 3 thrombocytopenia requiring platelet transfusion to maintain the clinical safety of the patient; (c) grade 4 neutropenia lasting more than 5 days or complicated by fever; or (d) any treatment delay due to toxicity lasting more than 2 weeks.

Interval toxicities were required to resolve to grade 1 or baseline before proceeding with further courses of treatment. All patients must have recovered an ANC≥1,500/mm^3 and platelets≥100,000/mm^3 to begin a new cycle of therapy. Patients with an ANC of <1,500/mm^3 and or platelets<100,000/mm^3 had therapy held for 1 week. If ANC and/or platelets were still low the following week, treatment was delayed another week. Patients were allowed to receive successive courses of treatment until they withdrew consent, exhibited progressive disease, developed an unacceptable adverse reaction, failed to resolve drug-related toxicity within 14 days of the start of the next course, were non-compliant, or if discontinuation of treatment was determined to be in their best interest. Patients with greater than a 2 week delay in treatment were removed from study unless the investigator determined that the delay was not drug related and the patient did not have clinical progression of disease.

Dose modifications

Study therapy was interrupted for clinically significant grade 3 or 4 non-hematologic toxicity until it resolved to <grade 1. For patients with a nadir ANC≤1,500/mm^3 and/or platelet count of ≤100,000/mm^3, the carboplatin dose was lowered one dose level while the bortezomib and etoposide level were maintained at 100%. For patients with a nadir ANC of ≤500/mm^3 and/or platelets≤25,000/mm^3, doses of etoposide and bortezomib were reduced by 25% for the next treatment cycle, and the dose of carboplatin was reduced by one dose level (i.e., to an AUC of 4, if starting at AUC 5). The doses of carboplatin and etoposide were not re-escalated in subsequent cycles, but the dose of bortezomib could be re-escalated back to the original dose level and then to the next higher dose level in subsequent cycles, as long as all toxicities were ≤grade 1. Patients were required to have an ANC≥1,500/mm^3, hemoglobin≥9 g/dl, and platelets≥50,000/mm^3 prior to receiving bortezomib on day 8. If the patient failed to meet count criteria, the scheduled day 8 dose of bortezomib was given on day 15, provided that laboratory abnormalities resolved. Laboratory tests were performed on the next scheduled treatment day or sooner, as clinically indicated. On day 8, if the ANC was ≥1,000/mm^3 and/or the platelets were ≥75,000/mm^3, the starting dose of bortezomib was maintained at 100%. If the ANC was 500–999/mm^3 and/or the platelet count was 50,000–75,000/mm^3 on day 8, bortezomib was reduced to 75% of the starting dose. Finally if the ANC was <500/mm^3 and/or the platelet count was <50,000/mm^3, treatment was held. If count recovery was sufficient by day 15,

bortezomib was given, otherwise it was eliminated for the remainder of that cycle. Due to myelosuppression noted in early cohorts, the dose escalation was modified from 1 to −1 to 1a. Once 1a was determined to be too toxic, adjustment in the bortezomib dose was made (to dose 1b) after discussions with an NCI advisory panel. Subsequent escalation to dose level 2 followed.

Pre-treatment and follow-up clinical assessments

Within 7 days of cycle 1, a complete history and physical, ECOG performance status, electrocardiogram, laboratory tests (including serum or urine β-hCG testing for women of child-bearing potential), serum tumor markers, and metastatic disease biopsies (if relevant) were performed. Within 28 days of cycle 1, relevant baseline radiologic studies and primary tumor biopsies in paraffin blocks (if available) for documentation of tumor status were obtained. On cycle 1 day 1, baseline adverse events, body surface area calculation, performance status, history and physical examination were performed.

While on study, weekly evaluations of symptoms, laboratory results and toxicities were assessed according to the CTC. Before each successive course of treatment, an interval history, physical exam, performance status, electrocardiogram, complete blood count with differential, and serum chemistries were performed. Tumor status was assessed after every other cycle by the same modality used at baseline.

Biological and correlative studies

Patients who were treated at the MTD underwent biopsy of easily accessible tumor tissue within 14 days of the start of therapy and within 7 days of cycle 2, day 1 for the relevant proteasome targets and for apoptosis by Tunel assay. Tissue was obtained by 8 mm punch technique and immediately placed in preservative for processing into paraffin blocks. Follow-up biopsies were obtained and assessed after bortezomib treatment only if diagnostic material was acceptable in the initial biopsy. Blood samples for the 26S proteasome inhibition assay were drawn in cycles 1 and 2 on days 1, 8, 15, and at the end of study.

Immunohistochemistry (p21, p27, CDK2 and IκB)

Paraffin sections from initial diagnostic specimens and punch biopsies were deparaffinized in xylene and subjected to decreasing concentrations of ethanol. Sections were then incubated in 3% hydrogen peroxide to quench endogenous perioxidase activity for 10 min followed by exposure to 20% serum for 30 min to reduce nonspecific background staining. Sections were incubated with the appropriate primary antibody followed by a biotinylated secondary antibody and avidin–biotin peroxidase.

Serum and urine surrogate markers of growth and angiogenesis

Whole blood specimens were collected on days 1 and 15 of cycles 1 and 2 for measurement of surrogate markers of angiogenesis, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), e-selectin, p-selectin. Urine was collected for bFGF at the same time points. Measurements for each variable at each time point were calculated using descriptive statistics as shown in Table 7, and a t-test was used to calculate differences between the means for each time interval pair (Table 8). Two-sided significance was calculated for each paired comparison.

Results

General

The characteristics of 24 patients are depicted in Table 2. One patient discontinued therapy after 3 weeks due to progressive disease and was not evaluable for response; all 24 patients were evaluable for toxicity. The total number of patients and cycles listed by dose level, as well as the overall dose escalation scheme, are presented in Table 3. Nine of 24 patients (38%) received 3 or more cycles of therapy. Eleven of 24 (46%) patients required delay in retreatment due to toxicity in the previous cycle. Nine patients (38%) withdrew consent or discontinued therapy due to treatment-related adverse events and 15 patients (62%) discontinued study due to disease progression.

Adverse events and dose-limiting toxicities

The most common hematologic treatment-related adverse events were neutropenia (77%), thrombocytopenia (59%), and anemia (56%). The most common non-hematologic treatment-related adverse events were fatigue (50%), nausea/vomiting (36%), alopecia (20%), and anorexia (20%). Twenty of the 24 patients (83%) developed grade 3 or 4 treatment-related toxicities that included neutropenia and unexpected toxicity encountered in the initial cohorts required changing the dosing of bortezomib from twice weekly to once weekly. Dose limiting toxicities (DLT) included neutropenia, neutropenia with fever, transaminitis and thrombocytopenia, with neutropenia predominating.

Hematologic toxicity

Neutropenia, febrile neutropenia, thrombocytopenia, and anemia were the predominant hematologic toxicities of this combination. Hematologic toxicities for each dose level in this trial are depicted in Table 4 and 5. Overall, for all patients in all courses, the nadir of the absolute neutrophil count (ANC) typically occurred around day 8 or 15, while the platelet nadir generally occurred on day 15. Overall, neutropenia occurred in 49 (77%) of 64 courses, with grade 3 or 4 neutropenia occurring in 32 (50%) of 64 courses. Neutropenia was dose limiting in one patient at dose level −1, two patients at dose level 1b, one patient at dose level 2, and 2 patients at dose level 1. Myelosuppression was treated with supportive care, including prophylactic broad-spectrum antibiotics for fever, transfusions of packed red blood cells and platelets as clinically indicated, and close clinical monitoring. Neutropenia frequently caused treatment delays and required dose reductions of etoposide and carboplatin. Febrile neutropenia occurred in 3 patients (5%) and grade 3 or 4 febrile neutropenia occurred in 2 patients in the 1b cohort.

Thrombocytopenia was noted in 38 (59%) of 64 courses, but grade 3 or 4 thrombocytopenia, in only 9 (14%) courses. Thrombocytopenia was dose limiting in 2 patients at dose level 1a and in 1 patient at dose level 2. Thrombocytopenia caused 30% of the doses of etoposide and carboplatin to be held or delayed, and dose reduction in one cycle.

Gastrointestinal toxicity

Gastrointestinal (GI) toxicities noted in all courses are depicted in Table 6. The most common toxicities were moderate to severe nausea and/or vomiting (23%) and anorexia (20%). Elevations of alkaline phosphatase (16%) and AST/ALT (9%) also occurred, with 1 patient in cohort 1a experiencing grade 3 AST/ALT elevations. Less frequently observed GI effects (<10%) were constipation, diarrhea, hyperbilirubinemia, hypoalbuminemia, and abdominal pain. Grade ≥3 nausea and/or vomiting, and diarrhea occurred in 1 (1.5%) of 64 courses, respectively.

Non-hematologic, non-gastrointestinal toxicity

There were no dose-limiting non-hematologic, non-gastrointestinal toxicities related to treatment. Fatigue was noted in 32 (50%) of courses as depicted in Table 6, however, grade 3 or 4 fatigue was observed in only three (5%) of all courses. These toxicities did not require any dose omissions or delays. All other toxicities were mild, including alopecia, hypokalemia, edema, myalgias, hyponatremia, and occurred in less than 10% of all courses.

Dose intensity/dose modifications

Nine of 24 patients (37.5%) required delays in re-treatment due to toxicity in the previous cycle. Approximately 10 (16%) of the 64 cycles of etoposide and carboplatin required dose reduction due to hematologic toxicity. The median dose intensity of etoposide and of carboplatin was approximately 94% of planned for each drug. The median dose intensity of bortezomib was 91% of planned. The most common reason for dose reduction of all three drugs was myelosuppression. Modifications in the dose escalation were made to attempt to isolate the individual components' role in the hematologic toxicity noted.

Anti-tumor activity

Response and efficacy were determined in 23 evaluable patients. Nine patients, all with refractory disease, had stable disease lasting more than or equal to four cycles of therapy: one patient each with head and neck (four cycles, cohort 1), NSCLC (four cycles, cohort −1), esophageal adenocarcinoma (four cycles, cohort 1a), breast cancer (six cycles, cohort 1a), endometrial carcinoma (four cycles, cohort 1a), melanoma (five cycles, cohort 1b), and germinoma (five cycles, cohort 1a). No complete or partial responses were observed. Of the nine patients with stable disease lasting more than or equal to four cycles, all patients had prior therapy with a median of three prior regimens (range 1–9) of chemotherapy with or without radiation therapy, and four of the nine had received prior carboplatin and etoposide.

Biological studies

Measurements of plasma VEGF suggested a trend toward decrease during the first 2 weeks of treatment, which reverted to baseline or higher by the start of cycle 2 day 1, however the sample size was too small for statistical significance (n=3). Serum bFGF levels increased slightly between baseline and cycle 2 day 1 (p=0.057), but more substantially between cycle 1 day 15 and cycle 2 day 1 (p=0.002). There was a statistically significant decrease in plasma e-selectin levels between baseline and cycle 1 day 15 (n=12, p<0.001) and between baseline and cycle 2 day 15 (p=0.029). There was a suggestion of a decrease in plasma p-selectin between baseline and cycle 1 day 15 (p=0.056), and an increase between cycle 1 day 15 and cycle 2 day 1 (p=0.015). There were no significant differences in levels of urine bFGF measured over three time points during the study.

Exploratory immunohistochemistry for p21, p27, CDK2, and IκB, and Tunel assays for apoptosis were performed in biopsy samples on three patients treated at the MTD, at baseline and post-treatment (cycle 2 day 1±3 days); the small sample number rendered both immunohistochemistry and apoptosis results inconclusive (Tables 7 and 8).

Discussion

Bortezomib is a potent, reversible, specific proteasome inhibitor with broad activity in a variety of tumor xenograft models. A series of phase I studies have defined the toxicities, the maximal tolerated dose, schedule, and anti-tumor activity of single agent bortezomib. The rationale for combining bortezomib with etoposide and carboplatin in this study included the established role of etoposide and carboplatin in variety of malignancies, and its potential to enhance the

apoptosis generated by etoposide and carboplatin as has been demonstrated in animal models [9,12,38]. This phase I study was designed to determine the maximal tolerated dose and dose-limiting toxicity of bortezomib in combination with etoposide and carboplatin, investigate pharmacokinetic and biologic interactions, and to seek preliminary evidence of anti-tumor activity in patients with advanced cancer.

The principle dose-limiting toxicity in this study was myelosuppression, primarily neutropenia with or without thrombocytopenia. Grade 3 or 4 neutropenia was noted in approximately half of all courses administered. Thrombocytopenia was also prevalent, with three patients experiencing dose-limiting thrombocytopenia. Though at least one other phase I trial of bortezomib had noted thrombocytopenia as a dose limiting adverse event, neutropenia and thrombocytopenia are known complications of etoposide and carboplatin [19]. The addition of bortezomib appears to greatly increase the hematologic toxicity associated with etoposide and carboplatin when compared to other studies evaluating the efficacy of combination chemotherapy with etoposide and carboplatin alone [32,33], although the mechanism of this potential enhanced effect is not known. Though studies of bortezomib have revealed few clinically relevant hematologic toxicities, there was clearly a profound effect of bortezomib when given in combination with etoposide and carboplatin. The degree of myelosuppression was not necessarily limited to the degree of pre-treatment, as patients with little or no treatment prior to study enrollment showed similar toxicities. At higher doses of bortezomib, carboplatin, and etoposide, the incidence of myelosuppression was unacceptably high, with all patients in dose levels 1 and 2 experiencing hematologic DLT in the first cycle. In contrast, the lower dose levels (cohort −1, 1a, 1b) were better tolerated.

In prior phase I studies with bortezomib, the major dose-limiting toxicities were primarily peripheral neuropathy and diarrhea [18], which were quite rare in this study, likely due to the relatively low doses of bortezomib that could be administered in this study. Other adverse events noted in previous studies included fatigue, fever, anorexia, nausea, vomiting, rash, and headache. In this study, the major non-hematologic adverse events were fatigue, nausea/vomiting, and anorexia. In contrast to prior studies, peripheral neuropathy was only associated with 2 of 64 total treatment cycles in this study. It is likely that the lower dose of bortezomib in this trial prevented patients from experiencing its non-hematologic side effects. Overall the nonhematologic toxicities in this study were relatively mild, infrequent, reversible, and well tolerated.

The best observed response to treatment was seen in one patient with breast cancer that exhibited stable disease for 6 cycles of therapy. This patient underwent initial radical mastectomy, and received adjuvant fluorouracil, cyclophosphamide and adriamycin. With subsequent recurrences, she received tamoxifen, taxotere, liposomal doxorubicin, and gemcitabine, interspersed with radiation therapy. Another patient with melanoma who had prior high-dose interferon/DTIC, temozolamide, radiation to sites of bony metastases, and four other experimental regimens exhibited stable disease for 5 cycles of therapy.

The biologic correlates which were able to be analyzed suggest that markers important in angiogenesis may be relevant surrogates for the effects of this combination, although the number of specimens was too small to draw any significant conclusions in most cases. Unfortunately, the samples collected for proteasome inhibition assays could not be completed by the designated lab, and samples were not usable for further testing. Clearly measurement of proteasome inhibition would have been important in helping to determine whether or not target modulation has been effective at the treatment doses, and will be critical to assess in future studies.

This study demonstrated that bortezomib 0.75 mg/m^2, etoposide 75 mg/m^2 (days 1–3), and carboplatin AUC 5 (day 1) every 21 days could be safely administered. Further studies with this combination in patients with good performance status may better reveal anti-tumor effects not identified in this study. Based on the outcomes of this study, the potential clinical relevance of this combination is questionable, due to the relatively low doses of etoposide and carboplatin that were clinically deliverable, however, bortezomib may restore sensitivity and enhance chemotherapy, and thus remains of interest in combination therapy.

Acknowledgments

This project has been funded in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract N01-C0-12400 (22XS047A-P3358). The content of this publication does not necessarily reflect the views of policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US government.

Research support: LG was supported by CA086913 during the conduct of this study.

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Table 1

Doses of bortezomib, carboplatin and etoposide administered every 21 days

Dose level	Bortezomib (mg m^{-2} $dose^{-1}$)[a]	Carboplatin AUC (day1)	Etoposide (mg m^{-2} $dose^{-1}$; day 1, 2, 3)
Days 1, 4, 8, and 11			
−1	0.75	AUC 4	75
1	1	AUC 5	100
1a	0.75	AUC 5	75
Days 1 and 8			
1b	1	AUC 5	75
2	1	AUC 5	100

[a] Dosing of bortezomib reduced from twice weekly to once weekly in cohorts 1b and 2 due to excessive toxicity noted in cohorts 1, −1 and 1a

Table 2

Patient demographics and characteristics

Characteristic	*n* (%)
Number of patients	24
Total number of courses	64
Number courses delivered per patient	
Median	2
Range	1–6
Patients receiving more than or equal to three cycles of therapy	9
Sex	
Male	13 (54)
Female	11 (46)
Age	
Median (in years)	54
Range (in years)	20–75
ECOG performance status[a]	
0	5
1	17
2	2
Previous treatment	
Patients with prior chemotherapy treatments	21 (88)
Median (range) of chemotherapy treatments	2 (0–4)
No prior therapy (including targeted therapy, chemotherapy and radiotherapy)	2
Prior radiotherapy	15 (63)
Tumor type	
Melanoma	4
Upper GI tract[b]	3
Sarcoma[c]	3
Colon	2
Head and neck	2
Pancreatic cancer	2
Other[d]	7

[a] *ECOG* Eastern Cooperative Oncology Group

[b] Includes one each esophageal, gastroesophageal and gastric carcinomas

[c] Includes one each of angiosarcoma, Ewing's sarcoma and leiomyosarcoma

[d] Other tumor types include one each of: adenocarcinoma of the breast, astrocytoma, germinoma, endometrial, ovarian, non-small cell lung, and renal cell carcinoma

Table 3

Summary of patients treated with bortezomib, carboplatin and etoposide every 21 days

Dose level	Number of patients treated	Number of courses	Bortezomib[a] (mg m^{-2} dose^{-1})	Carboplatin AUC (day 1)	Etoposide (mg m^{-2} dose^{-1}; days 1, 2, 3)	Dose-limiting toxicities (number of patients)	Other grade 3 or 4 toxicities (*n*)
1	3	8	1	AUC 5	100	Grade 4 neutropenia (2)	Grade 4 thrombocytopenia (1), grade 3 thrombocytopenia (1)
−1	6	14	0.75	AUC 4	75	Grade 2 neutropenia (2)	Grade 3 neutropenia (1), grade 3 fatigue (1)
1a	7	22	0.75	AUC 5	75	Grade 4 neutropenia > 5 days (2), grade 3 transaminitis (1), grade 3 thrombocytopenia (2)	Grade 3 anemia (4)
1b	5	15	1	AUC 5	75	Grade 4 neutropenia > 5 days (2), grade 3 neutropenic fever (2)	Grade 4 anemia (1), grade 3 thrombocytopenia (2), grade 3 fatigue (2)
2	3	5	1	AUC 5	100	Grade 4 neutropenia > 5 days and grade 3 thrombocytopenia (1)	Grade 3 thrombocytopenia (2), grade 3 anemia (2)
Total	24	64					

[a]Bortezomib dosed on days 1, 4, 8, and 11 in dose level 1, −1, 1a, and on days 1 and 8 in dose level 1b and 2

Table 4

Hematological toxicities attributed to treatment with bortezomib, carboplatin and etoposide administered every 21 days

Cohort	Bortezomib[a] (mg m^{-2} $dose^{-1}$)	Carboplatin AUC (day 1)	Etoposide (mg m^{-2} $dose^{-1}$; days 1, 2, 3)	No. of patients (courses)	Neutropenia (# of courses)			Thrombocytopenia (# of courses)			Anemia (# of courses)		
					Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
−1	0.75	AUC 4	75	6 (14)	5	2	-	3	-	-	4	-	-
1	1	AUC 5	100	3 (8)	2	4	3	7	1	1	4	-	-
1a	0.75	AUC 5	75	7 (22)	4	10	2	14	3	-	12	4	-
1b	1	AUC 5	75	5 (15)	4	2	5	6	2	-	7	1	1
2	1	AUC 5	100	3 (5)	-	-	4	1	2	-	1	2	-
Total				24 (64)	(15)	(18)	(14)	(31)	(8)	(1)	(28)	(7)	(1)

Toxicity assessed according to the National Cancer Institute Common Toxicity Criteria, version 2.0

[a]Bortezomib dosed on days 1, 4, 8, and 11 in dose level 1, −1, and 1a, and on days 1 and 8 in dose level 1b and 2

Table 5

Summary of Nadir hematologic parameters by dose level

Cohort	Bortezomib[a] (mg/m^2)	Carboplatin AUC (day 1)	Etoposide (mg/m^2; day 1,2,3)	No. of patients (courses)	Median nadir ANC (range; /μl)	Median nadir platelets (range; /μl)	Median nadir hemoglobin (range; /μ1)	No. of patients with heme DLT/ total patients
−1	0.75	AUC 4	75	14	1,300 (174–3375)	54 (38–163)	10.1 (8.8–12.9)	1/6
1	1	AUC 5	100	8	267 (131–1,100)	36 (17–51)	10.1 (9.5–14.1)	3/3
1a	0.75	AUC 5	75	22	620 (200–1,520)	75 (13–136)	10.5 (6.9–12.4)	3/7
1b	1	AUC 5	75	15	434 (171–1,242)	112 (35–184)	12.7 (8.8–12.8)	3/5
2	1	AUC 5	100	5	523 (365–6,745)	21 (10–139)	8.7 (8.0–12.2)	3/3

[a]Bortezomib dosed on days 1, 4, 8, and 11 in dose levels −1, 1, and 1a, and on days 1 and 8 in cohorts 1b and 2

Table 6

Non-hematologic toxicities attributed to bortezomib, carboplatin and etoposide administered every 21 days

Dose Level	Bortezomib[a] (mg m^{-2} $dose^{-1}$)	Carboplatin AUC (day 1)	Etoposide (mg m^{-2} $dose^{-1}$; day 1, 2, 3)	Number of patients (courses)	Fatigue (# of courses)		Nausea/vomiting (# of courses)		Anorexia (# of courses)		Alopecia (# of courses)		Alk Phos (# of courses)	
					Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4
−1	0.75	AUC 4	75	6 (14)	7	1	3	-	-	-	1	-	2	-
1	0.75	AUC 5	100	3 (8)	1	-	4	-	3	-	2	-	-	-
1a	0.75	AUC 5	75	7 (22)	12	-	8	-	6	-	9	-	5	-
1b	1	AUC 5	75	5 (15)	9	2	5	1	2	-	1	-	3	-
2	1	AUC 5	100	3 (5)	-	-	2	-	2	-	-	-	-	-
Total				24 (64)	29 (45%)	3 (5%)	22 (34%)	1 (2%)	13 (20%)	0	13 (20%)	0	10 (16%)	0

Grade 1–4 represent National Cancer Institute CTC Version 2.0 grades 1–4

[a]Bortezomib dosing on days 1, 4, 8, and 11 in dose levels 1, −1, and 1a, and on days 1 and 8 in dose levels 1b and 2

Table 7

Biomarker correlates of plasma and urine markers in patients treated with bortezomib, carboplatin and etoposide

Specimen time point	Number of specimens	Mean±SD	Standard error (min-max)
VEGF (pg/ml)			
Cycle 1 day 1	7	85.3957±98.97	37.41 (9.30–236.0)
Cycle 1 day 15	3	30.1933±20.10	11.60 (14.94–52.98)
Cycle 2 day 1	8	142.7712±147.30	52.08 (12.30–420.10)
Cycle 2 day 15	5	12.10±10.78	4.82 (3.04–29.37)
Plasma bFGF (pg/ml)			
Cycle 1 day 1	15	28.1047±30.93	7.99 (0.92–97.83)
Cycle 1 day 15	12	20.5392±15.09	4.36 (1.79–51.59)
Cycle 2 day 1	11	37.5909±26.34	7.94 (1.52–83.13)
Cycle 2 day 15	6	37.0867±41.26	16.84 (7.54–119.04)
Plasma e-selectin (ng/ml)			
Cycle 1 day 1	15	50.9520±26.72	6.90 (24.86–116.36)
Cycle 1 day 15	12	40.2058±24.22	6.99 (15.00–95.97)
Cycle 2 day 1	11	45.6273±25.22	7.60 (21.55–99.23)
Cycle 2 day 15	6	37.5533±26.75	10.92 (14.16–88.01)
Plasma p-selectin (ng/ml)			
Cycle 1 day 1	14	54.4321±52.34	13.99 (13.35–185.06)
Cycle 1 day 15	6	28.8767±16.45	6.71 (14.12–55.87)
Cycle 2 day 1	9	94.9711±72.36	24.12 (16.77–256.22)
Cycle 2 day 15	3	35.0967±7.44	4.29 (29.04–13.40)
Urine bFGF (pg/ml)			
Cycle 1 day 1	8	2.9675±3.76	1.32 (0.11–11.53)
Cycle 1 day 15	9	2.9589±3.19	1.06 (0.32–10.37)
Cycle 2 day 1	8	4.7200±9.32	3.29 (0.20–27.39)
Cycle 2 day 15	6	7.1800±7.20	2.94 (1.12–19.79)

Table 8

Statistical significance of differences between means of biomarker correlatives in patients treated with bortezomib, carboplatin and etoposide

Paired Samples	Paired mean differences	SD	Standard error of the mean	Significance (two-tailed)
Plasma e-selectin (ng/ml)				
Cycle 1 day 1-Cycle 1 day 15	14.8191	12.39612	3.73757	0.003
Cycle 1 day 1-Cycle 2 day 1	7.8850	8.83334	3.60619	0.080
Cycle 1day 1-Cycle 2 day 15	12.2517	9.93155	4.05454	0.029
Cycle 1 day 15-Cycle 2 day 1	−6.4200	7.56046	3.08654	0.092
Cycle 1 day 15-Cycle 2 day 15	−2.0533	8.17020	3.33547	0.565
Cycle 2 day 1-Cycle 2 day 15	4.3667	6.67321	2.72432	0.170
Plasma p-selectin (ng/ml)				
Cycle 1 day 1-Cycle 1 day 15	56.6167	55.99680	22.86060	0.056
Cycle 1 day 1-Cycle 2 day 1	−34.9900	64.31250	26.25547	0.240
Cycle 1 day 15-Cycle 2 day 1	−91.6067	61.93910	25.28653	0.015
Plasma bFGF (pg/ml)				
Cycle 1 day 1-Cycle 1 day 15	3.3100	19.84186	5.98255	0.592
Cycle 1 day 1-Cycle 2 day 1	−12.6636	19.48835	5.87596	0.057
Cycle 1 day 15-Cycle 2 day 1	−15.9736	12.72533	3.83683	0.002