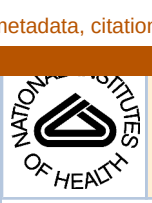

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A Phase I Study of Bortezomib in Combination With Standard 5-Fluorouracil and External-Beam Radiation Therapy for the Treatment of Locally Advanced or Metastatic Rectal Cancer

Bert H. O'Neil[1], **Laura Raftery**[1], **Benjamin F. Calvo**[1], **A. Bapsi Chakravarthy**[3], **Anastasia Ivanova**[2], **Michael O. Myers**[1], **Hong Jin Kim**[1], **Emily Chan**[3], **Paul E. Wise**[3], **Laura S. Caskey**[1], **Stephen A. Bernard**[1], **Hanna K. Sanoff**[1], **Richard M. Goldberg**[1], and **Joel E. Tepper**[1]

[1]Lineberger Comprehensive Cancer Center

[2]Department of Biostatistics, University of North Carolina, Chapel Hill

[3]Vanderbilt University Medical Center, Nashville, TN

Abstract

Background—Standard therapy for stage II/III rectal cancer consists of a fluoropyrimidine and radiation therapy followed by surgery. Preclinical data demonstrated that bortezomib functions as a radiosensitizer in colorectal cancer models. The purpose of this study was to determine the maximum tolerated dose (MTD) of bortezomib in combination with chemotherapy and radiation.

Patients and Methods—Patients with locally advanced rectal adenocarcinomas, as staged by endoscopic ultrasound, were eligible. Bortezomib was administered on days 1, 4, 8, and 11 every 21 days for 2 cycles with 5-fluorouracil at 225 mg/m^2/day continuously and 50.4 Gy of radiation. Dose escalation of bortezomib was conducted via a standard 3 + 3 dose escalation design. A subset of patients underwent serial tumor biopsies for correlative studies.

Results—Nine patients in 2 dose cohorts were enrolled. Diarrhea was the principal dose-limiting toxicity and occurred at the 1.0-mg/m^2 dose level. There was no clear evidence of suppression of nuclear factor-κB target gene expression in biopsy samples.

Conclusion—The MTD of bortezomib in combination with chemotherapy and radiation may be below a clinically relevant dose, limiting the clinical applicability of this combination. Performing biopsies before and during irradiation for determining gene expression in response to radiation therapy is feasible.

Keywords

Maximum tolerated dose; NF-κB; PS-341; Proteasome inhibitors

Address for correspondence: Bert H. O'Neil, MD, UNC Lineberger Comprehensive Cancer Center, 170 Manning Dr., Third Floor, CB#7305, Chapel Hill, NC 27599-7305 Fax: 919-966-6735; bert_oneil@med.unc.edu.

Disclosures

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The remaining authors report no relevant potential conflicts of interest.

Introduction

For locally advanced rectal adenocarcinoma, neoadjuvant chemoradiation with 5-fluorouracil (5-FU) and radiation are widely applied to decrease local recurrence after surgical resection.[1,2] Despite the use of chemoradiation therapy (CRT) and optimal surgery, local recurrence rates vary from as low as 5% to as high as 25%, depending upon clinical features such as T (tumor) and N (node) stage as well as distance from the anal canal. Pathologic response to neoadjuvant chemoradiation therapy assessed at the time of surgical resection has become an accepted surrogate marker for outcome, with current regimens producing a complete pathologic response approximately 10%-20% of the time.3 Based on our preclinical data, we developed a strategy to improve local control and pathologic response rates using continuous infusion 5-FU CRT plus bortezomib, an agent with interesting biologic properties that might increase apoptosis as well as provide radiation sensitization.

Bortezomib is a modified dipeptidyl boronic acid derived from leucine and phenylalanine that acts as an inhibitor of the 26S proteasome, an adenosine triphosphate–dependent multicatalytic protease. The ubiquitin-proteasome pathway is important for intracellular protein degradation in eukaryotic cells, and the 26S proteasome plays a vital role in degrading regulatory proteins that govern the cell cycle, transcription factor activation, apoptosis, and cell trafficking.[4]

An indirect but important target of the proteasome is transcription factor nuclear factor-κB (NF-κB), which associates with an inhibitor protein, IκB protein (IκB), in the cell's cytoplasm. When the proteasome disposes of ubiquitinated IκB, NF-κB is free to enter the cell's nucleus, where it influences the transcription of genes whose proteins encourage survival and proliferation. Restricting NF-κB's activity by preventing degradation of IκB sensitizes cells to chemotherapy and radiation. NF-κB has been shown in colorectal cancer models to be activated in response to chemotherapy and radiation.[5] Specific inhibition of NF-κB activation, either by proteasome inhibition or by insertion of a nondegradable IκB, leads to increased sensitivity of tumor cells to radiation therapy.

On this basis, we designed a phase I study to evaluate the safety and maximum tolerated dose (MTD) of bortezomib when combined with continuous-infusion 5-FU and radiation therapy in the treatment of patients with locally advanced, recurrent, or metastatic rectal cancer. As a single agent at its MTD of 1.3 mg/m^2 on days 1, 4, 8, and 11 of 21, bortezomib causes diarrhea in approximately 50% of patients (grade 3 diarrhea, 7%), peripheral neuropathy in about 40% of patients with myeloma (grade 3 neuropathy, 14%), and thrombocytopenia in about 40% (grade 3 thrombocytopenia, 27%).[6] A dose-escalation study of bortezomib was performed to determine the MTD of bortezomib when it is administered concurrently with standard-dose continuous-infusion 5-FU and external-beam radiation. Response was measured by pathologic evaluation of the surgical specimen. In a subset of patients, we were also able to obtain serial tumor biopsies to assess the transcriptional levels of mRNAs that are regulated by NF-κB to determine whether bortezomib at that dose level could functionally repress NF-κB activation.

Patients and Methods

The Institutional Review Boards of the University of North Carolina and Vanderbilt University approved this clinical trial. Each patient gave written informed consent before enrollment.

Eligibility criteria included patients who had histopathologically confirmed rectal carcinoma, clinically staged II or III (T3, T4, and/or node-positive disease). Patients with known distant metastasis who, according to the treating physician, required local therapy for palliative reasons or in whom potentially curative resection of metastatic disease would be attempted were also eligible. Patients who developed local recurrence of rectal cancer following surgery alone and who were felt to require radiation therapy, regardless of clinical stage, were eligible for this

study. An Eastern Cooperative Oncology Group performance status of 0-2, a life expectancy > 3 months, and the following laboratory requirements at entry were also required: white blood cell count ≥ 4000/μL; absolute neutrophil count > 2×10^9/L; platelet count ≥ 100,000/μL; serum creatinine ≤ 1.5 mg/dL; and total bilirubin ≤ 1.5 mg/dL within 14 days of the start of therapy.

Pregnant women were excluded, as were patients who had previous pelvic radiation therapy. Patients with the following conditions were also ineligible: a serious medical or psychiatric illness that would prevent informed consent or limit survival to < 2 years, a history of refractory congestive heart failure or cardiomyopathy, active coronary artery disease or a history of myocardial infarction within 3 months of treatment, or a history of stroke within 3 months of treatment.

Radiation Therapy

Volumes were planned using computed tomography (CT) conformal planning techniques. The gross tumor volume (GTV) was defined as the gross tumor as identified by all available clinical data including CT/magnetic resonance imaging, endoscopic evaluation, and physical examination. Lymph nodes < 1.5 cm were considered part of the GTV. The clinical target volume (CTV) was defined as the GTV with a 2-cm margin. In addition, the CTV included the presacral space with the superior boundary at the sacral promontory and the inferior border approximately 3-5 cm below the lesion and including the perirectal soft tissue in this range. The CTV included the entire mesorectal soft tissue, although irradiation to the anal canal was minimized as much as possible. A margin for set-up error and/or patient motion was added to the CTV of at least 1 cm. The exact margins were left to the discretion of the treating radiation oncologist and did not have to be uniform in all dimensions.

The tumor dose was calculated at the isocenter of the multiple fields. The dose was delivered using megavoltage accelerators with a minimum energy of 10 MV. Patients were treated using 180 cGy per fraction, 1 fraction per day, 5 days per week (excluding holidays), to give a total of 4500 cGy in 25 fractions to the large pelvic field. Attempts were made to minimize the amount of small bowel in the radiation field and to minimize irradiation of anus, perianal skin, and bladder if this could be done without compromising treatment of the tumor volume. Treatments were given with either a 3-field (posterior-anterior [PA] and laterals) or a 4-field (anterior-posterior [AP]/PA and laterals) technique. An additional 540 cGy, 180 cGy per day, 5 days per week was delivered for an additional 3 fractions to the gross tumor volume while minimizing dose to small bowel in this field. This was generally delivered with paired lateral fields or 3-field techniques (PA and laterals).

Drug Therapy

Doses of 5-FU and bortezomib for planned cohorts are shown in Table 1 and the treatment schema in Figure 1. On day 1, radiation was administered within 3 hours of the bortezomib administration. Bortezomib was given biweekly for 5.5 weeks on weeks 1, 2, 4, and 5 on Monday and Thursday or on Tuesday and Friday. Continuous infusion of 5-FU via ambulatory pump at 225 mg/m^2 was begun on day 2 (to allow for serial tumor biopsy collection for NF-κB target gene analysis in patients who consented to serial biopsies) and continued until radiation was completed.

Patients received weekly physical examinations, blood counts, chemistries, tests of liver function, and urinalysis. Toxicity was assessed according to National Cancer Institute Common Toxicity Criteria, version 2.0. No dose modifications were made for radiation therapy toxicity; if radiation therapy was held, then 5-FU and bortezomib would likewise be held until the toxicity subsided. 5-Fluorouracil was interrupted for 1 week and dose reduced 20% for grade

3 diarrhea or grade 2 diarrhea not controlled by medication, palmar-plantar erthrodysesthesia, stomatitis, or hematologic toxicity. A second dose reduction of 20% followed the first dose reduction if toxicity recurred. If bortezomib was interrupted because of a grade 4 hematologic or grade ≥ 3 nonhematologic toxicity, the same dose was continued if the toxicity resolved after only 1 dose was missed. If 2 doses were missed (either consecutive or out of cycle) then bortezomib dosing was reduced as follows: 1.3- or 1.5-mg/m^2 cohorts had bortezomib reduced to 1.0 mg/m^2; and the 1.0-mg/m^2 cohort was reduced to 0.70 mg/m^2. If the dose was 0.70 mg/m^2, then bortezomib was discontinued.

Dose-limiting toxicity (DLT) was defined as any grade ≥ 3 nonhematologic toxicity or grade 4 hematologic toxicity that did not resolve to grade ≤ 2 within 1 week and thereby led to more than a week's delay in treatment. Grade 3 nausea or vomiting was not considered a DLT unless patients did not respond to appropriate changes in the antiemetic regimen in a subsequent cycle. The MTD was defined as the maximum dose of bortezomib associated with DLT occurring in less than one third of the patients treated in a particular dose cohort.

Surgical Resection

Timing of surgical therapy was not mandated by the protocol, but generally occurred 4-8 weeks after radiation therapy was completed. Evaluation of pathologic response at the time of surgical resection was classified according to the tumor-node-metastasis (TNM) staging system of the American Joint Committee on Cancer/International Union Against Cancer using the prescript "y" (ie, yp). Histologically viable tumor was used to assess the pathologic stage. Pathologic complete response (pCR) was defined as the complete absence of viable tumor cells in the resected specimen. A major pathologic response was defined as the presence only of microscopic residual tumor in the resection specimen (measuring < 1 mm). Any specimen with gross disease was classified as a pathologic poor responder.

Tumor Biopsies and Correlative Studies

Biopsies were performed in the subset of patients who consented at 3 time-points: before the initiation of radiation therapy, 2-6 hours after first radiation therapy fraction and 24 hours after the first radiation therapy fraction. Four to six biopsies were taken by rigid sigmoidoscopy at each interval. Half of the biopsies were frozen for RNA extraction, and half were fixed in formalin.

Quantitative Fluorescence Reverse Transcriptase Polymerase Chain Reaction

Total RNA was isolated from frozen biopsy samples using an RNeasy kit (Qiagen; Valencia, CA). DNA contamination was resolved by treating all RNA isolates with DNase I RNase free (Ambion; Foster City, CA). Samples were diluted to 2 ng/μL, and 5 μL/reaction was used. Samples were run in triplicate.

Applied Biosystem's Primer Express software was used to design primers for the following genes: *BCL2*, *Bcl-x_L*, *cIAP1*, *cIAP2*, *TRAF1*, *TRAF2*, *IκBα*, *IKKε*, *IL-8*, and cyclin D1. Probes were labeled with 5′FAM-3′BHQ-1. One-step reverse transcriptase polymerase chain reaction was performed (40 cycles). Values for relative mRNA expression were derived by calculating the mean cycle number, normalizing by subtracting 40 from the mean (40 = the maximum number of cycles) then calculating e^n where n is the normalized cycle number value.

Statistical Methods

Overall survival was defined as the time from the date of first treatment to the date of death or last contact. The Kaplan-Meier method was used to estimate median overall survival. Statistical analyses were performed using SAS statistical software, version 9.2 (SAS Institute Inc.; Cary

NC). This study was approved by the University of North Carolina at Chapel Hill Committee on the Protection of the Rights of Human Subjects.

Results

Ten patients were enrolled onto the study between November 2003 and August 2008; demographic and disease-related information are shown in Table 2.

Toxicity

Patients were initially treated with bortezomib at 0.7 mg/m^2, with escalations and reductions as show in Table 3. One patient at dose level 1 (DL1) discontinued treatment after 4 weeks because of atypical chest pain and shortness of breath, which was diagnosed as gastroesophageal reflux disease and resolved after a day. The patient withdrew after this of her own accord and was replaced as the toxicity did not meet criteria for DLT. Nine patients completed therapy, with grade 3 and 4 adverse events as shown in Table 4. Doses of bortezomib were not increased above 1.0 mg/m^2 because of grade 3 diarrhea, the most frequently occurring toxicity. In DL2 grade 3 diarrhea was observed in 2 out of 3 patients and resolved by 6 days in both patients. One of these patients also had grade 3 hypoalbuminemia and hypokalemia and was subsequently hospitalized for an ileus, which resolved in 2 days. His therapy was held and bortezomib and 5-FU doses were reduced when therapy recommenced. The second of the patients at DL2 with diarrhea also had dose reductions in bortezomib and 5-FU. Because of this toxicity, the DL1 cohort was expanded by 3 patients to a total of 6; one of the new patients at DL1 had diarrhea that lasted 3 days, prompting dose modification of 5-FU. There was no grade 3 or 4 neuropathy or hematologic toxicity observed in the trial. The MTD of bortezomib in combination with 5-FU and radiation therapy was determined to be 0.7 mg/m^2 day.

Pathologic Response to Therapy and Survival

Of the 9 patients who completed the trial, all 9 underwent resection. Microscopic evaluation of the specimens showed that 1 patient had a pCR, 1 had microscopic residual disease, and 7 had gross residual disease. Clinical staging before surgery was compared with pathologic staging (Table 5). At the time of analysis, 5 patients had no evidence of disease, 1 was alive with disease, and 3 were deceased, having died of the disease at 3 months, 6 months, and 26 months. Three patients were alive more than 2 years out (at 25 months, 25 months, and 29 months). The median follow-up for survivors was approximately 13 months (range, 1-29 months). The median survival time for this cohort of patients was almost 26 months.

Nuclear Factor-κB Target Gene Expression

Baseline and 24-hour (after bortezomib) biopsy pairs from 5 patients were evaluable for NF-κB target gene expression by reverse transcriptase polymerase chain reaction. Two patients (patients 2 and 5) also had biopsies at an early timepoint (2-4 hours post radiation therapy). Results are shown in Figure 2. Interestingly, the only patient who underwent biopsy and who also achieved a complete pathologic remission at the time of surgery had a marked increase in NF-κB target gene mRNA copy number at 24 hours compared with baseline, suggesting that NF-κB was not being inhibited by bortezomib (at 1.0 mg/m^2). This patient experienced a DLT of diarrhea and then had recurrence of grade 3 diarrhea during postoperative 5-FU/leucovorin administration, suggesting sensitivity to 5-FU; he remains disease free at 4 years. For the poor responders (gross residual disease present), the general trend was for a decrease in NF-κB target gene expression, with the exception of one patient who had a substantial increase in *TRAF1* expression at 24 hours. These results would seem to indicate that some degree of repression of NF-κB–related gene expression did occur at the 2 dose levels tested but that this repression did not necessarily result in better clinical response. Statistical analysis was not performed

because of the small numbers of patients assayed, and as such, our conclusions are limited in this regard.

Discussion

Preclinical data suggested a strong biologic rationale to study the use of bortezomib in combination with radiation therapy in the treatment of rectal cancer. NF-κB activation by radiation contributes to radioresistance in vitro and in vivo, and inhibition of NF-κB can sensitize colorectal tumors in model systems.[5] Similarly, in a radioresistant breast cancer model, gene array experiments performed to identify expression changes associated with the development of radioresistance demonstrated increased expression of NF-κB–regulated genes in radioresistant cells compared with parental radiosensitive cells.[7] Furthermore, baseline NF-κB activation as measured by immunohistochemistry has been correlated with response to chemoradiation in esophageal cancer[8] and with response to chemotherapy plus cetuximab in patients with colorectal cancer.[9] This study is the first to attempt to demonstrate the feasibility of combining bortezomib with 5-FU and radiation in patients with advanced rectal cancer. The major toxicity was diarrhea, which limited the MTD to 0.7 mg/m^2, the first dose level evaluated in this study. By comparison, in a phase I trial combining bortezomib and 5-FU, the recommended dose of bortezomib was 0.7 mg/m^2 twice weekly for 4 weeks administered with bolus 5-FU for 4 out of 6 weeks. Neuropathy was not encountered at this dose, but diarrhea was prominent.[10] Similarly, a phase I trial conducted in patients with head and neck cancer reached a dose of only 0.6 mg/m^2 twice weekly with DLTs including dehydration and hyponatremia.[11] In terms of outcome, 1 pCR out of 10 evaluable patients is in line with the 8% pCR rate with 5-FU–based CRT in the study by Sauer et al[12] but not indicative of additivity or synergy between bortezomib at the doses given and the other components of therapy.

In primates the target level of proteasome inhibition should not exceed 80%, which would occur at a dose of 1.96 mg/m^2. A dose of 1.3 mg/m^2 is sufficient to inhibit 60% of proteasome activity.[13] It has been shown that proteasome inhibition is dose dependent.[14,15] Incomplete inhibition of the proteasome by bortezomib or use of alternative pathways which allow survival might in part explain the apparent relative resistance of epithelial malignancies to bortezomib.[16] We reached a dose of only 0.7 mg/m^2 twice weekly in our study and did not proceed to phase II evaluation because of concerns that the dose would not be biologically meaningful. Additionally, only 1 of 9 patients experienced a pCR at the MTD, suggesting that this is not an active regimen.

Conclusion

Although our study was unable to reach a biologically meaningful dose, it demonstrated the feasibility of evaluating the response of a target gene to therapy through serial tumor biopsy. Although biopsies were mandatory in the initial study design, we subsequently made them optional to help increase study participation. In the samples obtained, we were able to purify high-quality RNA for polymerase chain reaction–based studies to evaluate the NF-κB response to bortezomib. Given our small numbers of biopsy samples, we were not able to draw strong conclusions about the effect of bortezomib on NF-κB–dependent gene expression. Parenthetically, the patient with the best response (and significant gastrointestinal toxicity) had tremendous increases in several NF-κB–dependent genes on tumor biopsy at 24 hours after bortezomib administration and radiation; this observation runs counter to our initial hypothesis that increases in NF-κB suppress apoptosis and contribute to radioresistance. It is possible that upreglation of NF-kB–dependent genes is an attempt by the cell to overcome the proapoptotic stimulus of radiation therapy; or that the NF-κB response is proapoptotic rather than antiapoptotic as is generally believed. We have also examined the relationship between NF-κB expression and response to radiation therapy in patients treated with standard 5-FU–based

chemoradiation and have found that, in general, NF-κB induced genes are upregulated by radiation but that baseline NF-κB status does not correlate with pathologic response to radiation therapy (O'Neil, B; manuscript in preparation), further tempering our enthusiasm for pursuing this particular drug-radiation combination.

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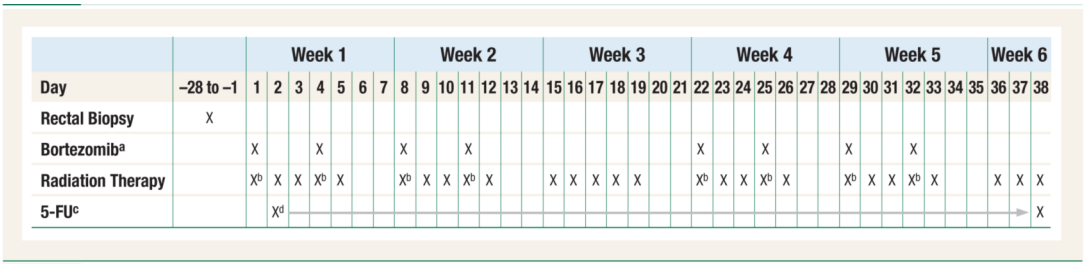
References

1. Gerard JP, Conroy T, Bonnetain F, et al. Preoperative radiotherapy with or without concurrent fluorouracil and leucovorin in T3-4 rectal cancers: results of FFCD 9203. J Clin Oncol 2006;24:4620–5. [PubMed: 17008704]
2. Bosset JF, Calais G, Mineur L, et al. Enhanced tumorocidal effect of chemotherapy with preoperative radiotherapy for rectal cancer: preliminary results–EORTC 22921. J Clin Oncol 2005;23:5620–7. [PubMed: 16009958]
3. Ngan SY, Burmeister BH, Fisher R, et al. Early toxicity from preoperative radiotherapy with continuous infusion 5-fluorouracil for resectable adenocarcinoma of the rectum: a Phase II trial for the Trans-Tasman Radiation Oncology Group. Int J Radiat Oncol Biol Phys 2001;50:883–7. [PubMed: 11429215]
4. Voorhees PM, Dees EC, O'Neil B, et al. The proteasome as a target for cancer therapy. Clin Cancer Res 2003;9:6316–25. [PubMed: 14695130]
5. Russo SM, Tepper JE, Baldwin AS Jr, et al. Enhancement of radiosensitivity by proteasome inhibition: implications for a role of NF-kappaB. Int J Radiat Oncol Biol Phys 2001;50:183–93. [PubMed: 11316563]
6. Millennium Pharmaceuticals. VELCADE (bortezomib) for Injection: Prescribing Information. Millennium Pharmaceuticals, Inc; Cambridge, MA: 2003.
7. Li Z, Xia L, Lee LM, et al. Effector genes altered in MCF-7 human breast cancer cells after exposure to fractionated ionizing radiation. Radiat Res 2001;155:543–53. [PubMed: 11260656]
8. Izzo JG, Wu X, Wu TT, et al. Therapy-induced expression of NF-kappaB portends poor prognosis in patients with localized esophageal cancer undergoing preoperative chemoradiation. Dis Esophagus 2009;22:127–32. [PubMed: 19021681]
9. Scartozzi M, Bearzi I, Pierantoni C, et al. Nuclear factor-kB tumor expression predicts response and survival in irinotecan-refractory metastatic colorectal cancer treated with cetuximab-irinotecan therapy. J Clin Oncol 2007;25:3930–5. [PubMed: 17761976]
10. Iqbal S, Cole S, Yang D, et al. Phase I study of PS-341 (bortezomib) with 5-fluo-rouracil/leucovorin (5-FU/LV) in advanced solid tumors: A California Cancer Consortium study. J Clin Oncol 2004;22 (14 suppl) abstract 2057.
11. Van Waes C, Chang AA, Lebowitz PF, et al. Inhibition of nuclear factor-kappaB and target genes during combined therapy with proteasome inhibitor bortezomib and reirradiation in patients with recurrent head-and-neck squamous cell carcinoma. Int J Radiat Oncol Biol Phys 2005;63:1400–12. [PubMed: 16005577]
12. Sauer R, Becker H, Hohenberger W, et al. Preoperative versus postoperative chemoradiotherapy for rectal cancer. N Engl J Med 2004;351:1731–40. [PubMed: 15496622]
13. Adams J, Palombella VJ, Sausville EA, et al. Proteasome inhibitors: a novel class of potent and effective antitumor agents. Cancer Res 1999;59:2615–22. [PubMed: 10363983]
14. Rajkumar SV, Richardson PG, Hideshima T, et al. Proteasome inhibition as a novel therapeutic target in human cancer. J Clin Oncol 2005;23:630–9. [PubMed: 15659509]
15. Jagannath S, Barlogie B, Berenson JR, et al. Updated survival analyses after prolonged follow-up of the phase 2, multicenter CREST study of bortezomib in relapsed or refractory multiple myeloma. Br J Haematol 2008;143:537–40. [PubMed: 18783399]

16. Allen C, Saigal K, Nottingham L, et al. Bortezomib-induced apoptosis with limited clinical response is accompanied by inhibition of canonical but not alternative nuclear factor-{kappa}B subunits in head and neck cancer. Clin Cancer Res 2008;14:4175–85. [PubMed: 18593997]



		Week 1							Week 2							Week 3							Week 4							Week 5							Week 6		
Day	−28 to −1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
Rectal Biopsy	X																																						
Bortezomib[a]		X			X				X			X											X			X				X			X						
Radiation Therapy		X[b]	X	X	X[b]	X			X[b]	X	X	X[b]	X			X	X	X	X	X			X[b]	X	X	X[b]	X			X[b]	X	X	X[b]	X			X	X	X
5-FU[c]			X[d]	→																																		X

Figure 1. Treatment Schema

[a]Bortezomib is given during weeks 1, 2, 4, and 5 on a Monday/Thursday or Tuesday/Friday schedule.

[b]Radiation should be administered within 3 hours following bortezomib administration.

[c]5-FU continuous infusion by ambulatory pump of choice throughout radiation treatment.

[d]During the first week only 5-FU will be started on day 2 of radiation therapy to allow for biopsies for biologic endpoints in the presence of radiation therapy and bortezomib alone.

Abbreviation: 5-FU = 5-fluorouracil

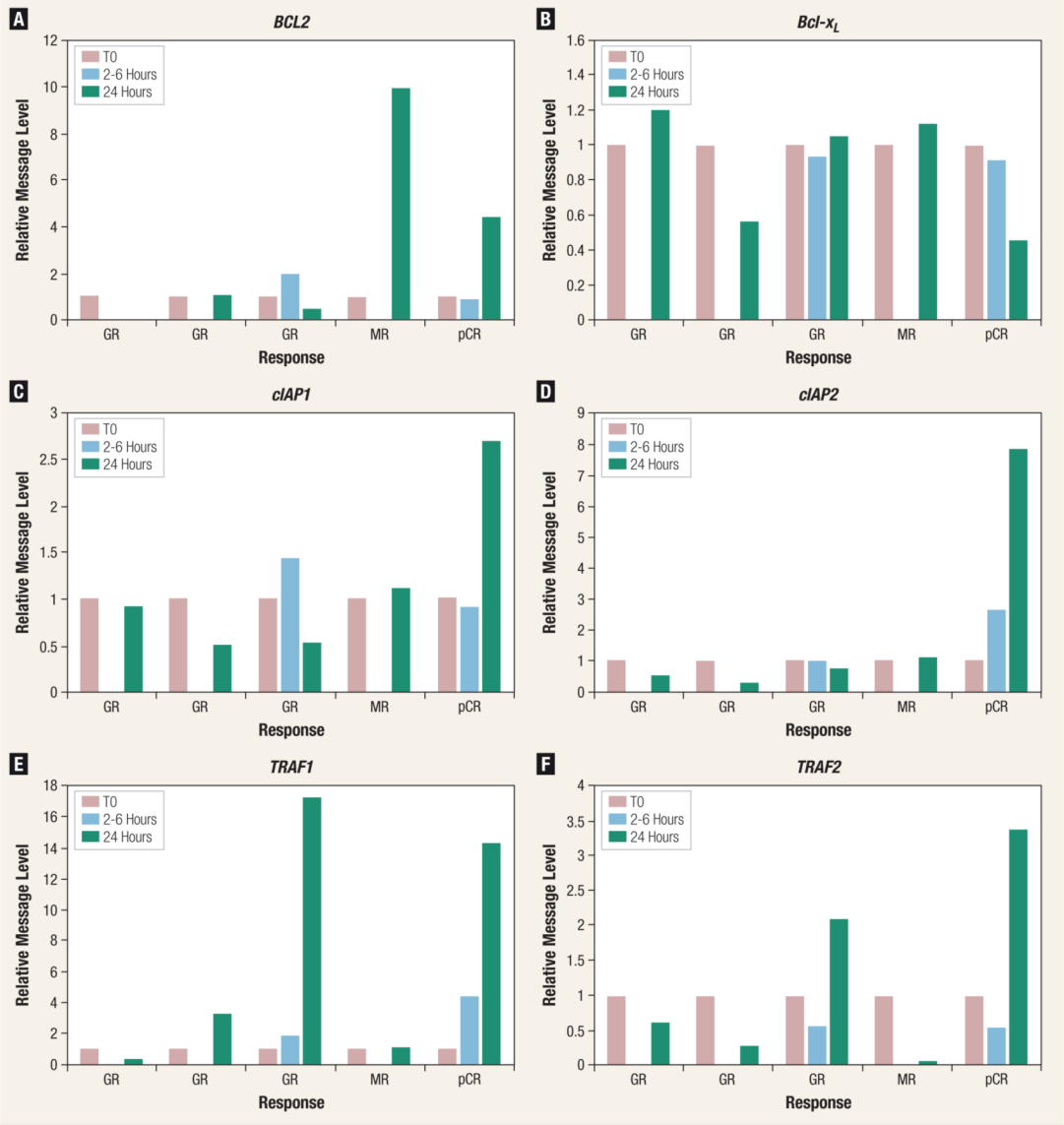


Figure 2. Quantitative Fluorescence Reverse Transcriptase Polymerase Chain Reaction Was Performed Across 5 Patients for 6 Representative Genes That Are Known Target Genes of Activated NF-κB

Results for 6 representative genes are shown here. T0 refers to pretreatment baseline and is compared with the early timepoint (T2-6) and 24-hour timepoint (T24) for each patient. Patients were ordered by pathologic response from gross residual disease (GR) to microscopic residual (MR) to complete response (CR). Patients 2 and 5 in the figure had early timepoints analyzed as well (2-4 hours after radiation therapy). The results indicate lack of suppression of NF-κB target gene transcription.

Abbreviations: NF-κB = nuclear factor-κB; pCR = pathologic complete response

Table 1

Treatment Dose Levels

Cohort/ Dose Level	Bortezomib Dose	5-FU Daily Dose	Radiation Dose/Fraction
Cohort Minus 1	0.70 mg/m^2	185 mg/m^2	180 cGy
Cohort 1	0.70 mg/m^2	225 mg/m^2	180 cGy
Cohort 2	1.0 mg/m^2	225 mg/m^2	180 cGy
Cohort 3	1.3 mg/m^2	225 mg/m^2	180 cGy
Cohort 4	1.5 mg/m^2	225 mg/m^2	180 cGy

Abbreviation: 5-FU = 5-fluorouracil

Table 2

Patient Characteristics (N = 10)

Characteristic	Value
Sex, n	
Female	4
Male	6
Mean Age, Years	59
Range	42-69
Race, n	
Black	1
White	9
Baseline ECOG Scores, n	
0	6
1	2
2	2

Abbreviation: ECOG = Eastern Cooperative Oncology Group

Table 3
Dose Escalation and Dose-Limiting Toxicities

Bortezomib Dose Level, mg/m^2	Number of Evaluable Patients	Number of DLTs	Dose Reduction
0.7	3	0	–
1.0	3	2	20% 5-FU and bortezomib
0.7	3	1	20% 5-FU

Abbreviations: 5-FU = 5-fluorouracil; DLT = dose-limiting toxicity

Table 4
Grade 3/4 Adverse Events by Dose Level

Adverse Event	Bortezomib Dose	
	0.7 mg/m^2 (n = 6)	1.0 mg/m^2 (n = 3)
Chest Pain	1	–
Diarrhea	1	2
Hyperglycemia	1	–
Hypoalbuminemia	–	1
Hypokalemia	–	1
Ileus	–	1
Lymphopenia	1	–

n = number of patients evaluable for toxicity.

Table 5
Postoperative Stage and Pathologic Response for Treated Patients

Patient ID	Preoperative Stage	yp Stage	Pathologic Response
1	T3 Nx M1	ND	Gross residual
2	T3 N1 M0-R	T2 N0 MX	Gross residual (0.3 cm)
3	T3 N1 M0	T2 N0 MX	Gross residual
4	T3 N0 M0	T3 N0 MX	Gross residual
5	T3 N1 M0	T0 N0	CR
7	ND-R	T4 N0 M0	Gross residual
8	T3 N1 M0	T3 N1 M0	Microscopic residual
9	T3 N1 M0	T2 N1 MX	Gross residual
10	T3 N1 M0	T3 N1 MX	Gross residual
11	T3 Nx M0	T2 N0	Microscopic residual

Patient 6 was consented but not treated.

Abbreviations: CR = complete response; ND = not determined (patient initially had T2 N0 disease but at recurrence had bulky disease by computed tomography, so endoscopic ultrasound not felt necessary to recommend chemoradiation and exception was allowed); R = recurrence