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Phase II Trial of Bortezomib Alone or in Combination with Irinotecan in Patients with Adenocarcinoma of the Gastroesophageal Junction or Stomach

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Abstract

Purpose—To determine the effectiveness of bortezomib plus irinotecan and bortezomib alone in patients with advanced gastroesophageal junction (GEJ) and gastric adenocarcinoma. We also sought to explore the effect of these therapeutics on tumor and normal gene expression in vivo.

Methods—Forty-one patients with advanced GEJ (89%) or gastric (11%) adenocarcinoma received bortezomib (1.3 mg/m² days 1, 4, 8, 11) plus irinotecan (125 mg/m² days 1, 8) every 21 days as first line therapy (N=29), or bortezomib alone as second line therapy (N=12). The trial was designed to detect a 40% response rate for the combination, and 20% response rate for bortezomib alone. Affymetrix HU133A gene chip arrays were used for gene expression studies.

Results—Objective response occurred in 3 of 29 patients (10%, 95% confidence intervals [CI] 2%, 27%) treated with bortezomib plus irinotecan, and in 1 of 12 patients (8%, 95% CI 0%, 39%) with bortezomib alone. Due to the limited number of responders, there were no significant correlations with response found in the gene expression profiles of 12 patients whose tumors were

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Conflicts of Interest:

The authors declare that they have no conflict of interest.

sampled before and 24 hours after therapy with bortezomib alone (N=2) or the combination (N=10).

Conclusions—We conclude that bortezomib is not effective for the treatment of advanced adenocarcinoma of the GEJ or stomach, whether used alone or in combination with irinotecan, in an unselected patient population.

Keywords

Bortezomib; Proteasome inhibitor; Irinotecan; Gastroesophageal Junction; Adenocarcinoma; Combination Therapy; Microarray

INTRODUCTION

Adenocarcinoma of the gastroesophageal junction (GEJ)[1] is increasingly prevalent in industrialized countries, with a 6-fold rise in the United States from 1975–2000 [2]. Most patients with GEJ or gastric adenocarcinoma present with regionally advanced or metastatic disease, for whom median survival remains less than one year. For a minority of patients with HER2 positive cancers, the addition of trastuzumab does improve survival [3,1]. Unfortunately, despite the many cytotoxic therapies available, most treatments are short lived, with early development of resistance and subsequent disease progression [4]. New therapeutic agents exploiting novel targets are desperately needed to improve clinical outcomes in this disease.

The proteasome and topoisomerase I both represent validated therapeutic targets. The ubiquitin proteasome system (UPS) mediates degradation of intracellular proteins involved in multiple cellular processes, including cell-cycle division, DNA repair, growth, differentiation, and regulation of membrane receptors and ion channels [5]. Ubiquitination of intracellular proteins mark them for degradation by the 26S proteasome [6]. Bortezomib is a potent, reversible proteasome inhibitor that is the prototype for this drug class, with activity in multiple myeloma [7]. Bortezomib promotes apoptosis via the stabilization of p53, p21, p27, Bax, and IkappaB_alpha, resulting in nuclear factor kappaB inhibition [8], and has been shown to markedly potentiate the effectiveness of cytotoxic agents in multiple myeloma and solid tumor cell lines, including squamous cell carcinoma [9] and adenocarcinoma [10]. Moreover, bortezomib has been specifically shown to enhance the effectiveness of the topoisomerase I inhibitor, irinotecan, in a BxPC3 pancreatic carcinoma cell line and xenograft model [10], and in a human colorectal carcinoma LOVO cell line and xenograft model [11]. Irinotecan has activity in metastatic colorectal cancer and other cancers, including gastric cancer [12, 13]. A phase II trial of irinotecan (125 mg/m2 intravenously over 90 min weekly for 4 of 6 weeks) which included 43 patients with previously untreated esophagogastric adenocarcinoma revealed objective response in 6 patients (14%; 95% CI, 4–24%), indicating some efficacy in this disease. We performed a phase II trial to examine the efficacy and safety of bortezomib and bortezomib in combination with irinotecan in GEJ and gastric adenocarcinoma. Single agent bortezomib was examined in patients with gastric/GEJ cancers that had progressed on at least one line of therapy for metastatic disease. The combination of irinotecan and bortezomib was examined in patients previously untreated for metastatic disease.

METHODS

This study was performed in accordance with “good clinical practice” and followed applicable patient privacy requirements and the guiding principles of the Declaration of Helsinki. The study protocol was approved by the medical ethical committee at all participating institutions, and patients provided written informed consent prior to participation.

Patient Selection

Eligible criteria included histologically or cytologically confirmed adenocarcinoma of the gastroesophageal junction (GEJ) or stomach with measurable metastatic disease by Response Evaluation Criteria in Solid Tumors (RECIST, Version 1.0). Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status score of 0, 1, or 2, be 18 years of age or older, and provide written informed consent. Other requirements included adequate bone marrow function (leukocyte count $> 3500/\mu\text{L}$, neutrophil count $> 1500/\mu\text{L}$, platelets $> 150,000/\mu\text{L}$), kidney function (normal serum creatinine), and normal hepatic function (normal bilirubin and aspartate aminotransferase and alanine aminotransferase < 2.5 times the upper limit of normal). Patients were excluded if they had brain metastases, chemotherapy within 4 weeks of registration (6 weeks for nitrosoureas), another malignancy (except early stage squamous cell carcinoma of skin or cervix), an uncontrolled intercurrent illness (eg, infection, congestive heart failure, unstable angina, cardiac arrhythmia, congenital or acquired immune deficiency, or psychiatric illness that could potentially impact compliance), were pregnant or breastfeeding.

Treatment Regimen

Patients who had no prior chemotherapy for metastatic disease were assigned to arm A (bortezomib plus irinotecan), whereas those who had prior chemotherapy for metastatic disease were assigned to arm B (bortezomib alone). All patients received bortezomib 1.3 mg/m² intravenous (IV) push on days 1, 4, 8 and 11 every 21 days. Patients in arm A also received irinotecan 125 mg/m² as an IV infusion over 90 minutes on days 1 and 8 every 21 days. Treatment was continued until progression of disease, unacceptable toxicity, intercurrent condition causing decline of performance status preventing further treatment, or patient withdrawal.

For hematologic toxicity, bortezomib was held if the neutrophil count was $< 750/\mu\text{L}$, hemoglobin < 7.5 gm/dL, or platelets $< 50,000/\mu\text{L}$. Irinotecan was held if the neutrophil count $< 1500/\mu\text{L}$ or platelets $< 75,000/\mu\text{L}$. For non-hematologic toxicity, the bortezomib dose was modified (25–50% dose reduction for grade 2–3 toxicity) or discontinued (for grade 3 or 4 sensory neuropathy or neuropathic pain). Irinotecan was held for grade 2–4 diarrhea, grade 3–4 nausea/vomiting, or a decrease in ECOG performance status by more than 2 levels.

Tumor and/or Adjacent Normal Tissue Biopsies for Gene Expression

Fourteen patients consented to optional tumor biopsies obtained by esophagogastroscope before the first bortezomib dose and approximately 24 hours after the first dose. Tumor

and/or normal tissue were collected at the time of biopsy and stored immediately in RNase free polypropylene tubes containing RNAlater (Qiagen, Valencia, CA). Samples were kept at room temperature for 1 hour, refrigerated overnight, and then frozen at -80°C until processed for RNA. Tissue was homogenized using a Roto-Stator and total RNA was extracted using Qiagen RNeasy miniprep kits according to the manufacturer's protocol (Qiagen). RNA was assessed for quality and purity using the Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE) and the Agilent Bioanalyzer 2100 (Agilent, Santa Clara, Ca). Total RNA (100–250 ng) was amplified, labeled with biotin and fragmented using the Affymetrix small sample II protocol. Samples were hybridized to human HG-U133A chips (Affymetrix) according to the manufacturer's instructions and Affymetrix GeneChip 5.0 was used for image acquisition.

Response and Toxicity Evaluation

Computed tomography or magnetic resonance imaging scans of measurable lesions were obtained at baseline and every 8 weeks thereafter. Responses were classified according to RECIST criteria (version 1.0)[14]. National Cancer Institute Common Adverse Events Criteria version 3.0 was used to grade toxicity.

Statistical Considerations for Clinical Trial

For previously untreated patients (arm A), the trial was designed to distinguish between a response rate of 20% vs. 40% using Simon's two-stage minimax design ($\alpha = 0.05$, $\beta = 0.80$). At least 5 responses were required among the first 18 evaluable patients in order to continue accrual to at least 33 evaluable patients; at least 11 responses were required to consider the regimen promising. For previously treated patients (arm B), the trial was designed to distinguish between a response rate of 10% vs. 30% using Simon's two-stage minimax design ($\alpha = 0.05$, $\beta = 0.80$). At least 2 responses were required among the first 15 patients in order to continue accrual to at least 25 evaluable patients; at least 6 responses were required to consider the regimen promising.

Progression-free survival (PFS) and overall survival (OS) were also estimated using the Kaplan-Meier method, and 95% confidence intervals were constructed for median PFS and OS. PFS was defined as the time between registration and either progression of disease or death from any cause. All analyses were performed in SAS version 9.1 (SAS Institute, Inc., Cary, NC) and Stata version 10.0 (Stata Corporation, College Station, TX). All patients had progressive disease or had discontinued therapy at the time of the analysis.

Statistical Methods for Gene Expression Analysis

Quality-control (QC) measures were applied to raw data using Bioconductor packages (affyQCReport) and in-house R code[15]. QC results included signal box plots and histograms before normalization for all hybridizations, the 3':5' ratio for GAPDH/ β -actin genes, percentage of present gene calls and average background levels, box plots of the positive and negative control elements on the outer edges of the arrays, as well as a heatmap for spearman rank correlation between all pairs of hybridizations. Problematic arrays were visually inspected using these plots. Thirty-eight of 44 chips passed QC and were carried over to the next stage analysis. In order to compare expression profiles of interested groups

(tumor tissues pre- vs. post- bortezomib treatment; tumor tissues pre- vs. post-bortezomib-irinotecan treatment, and pre- treatment normal vs. tumor tissue), related chips were picked out to assemble three sub-datasets. The gene expression measures from these three subsets were then normalized by the robust multi-array analysis (RMA) approach[16] in the Expression Console v1.1 (Affymetrix) software package, separately. Ranking of genes by degree of differential expression was performed using Bioconductor package Limma and in-house R code.[17] Data was analyzed using general linear models, adjusting for matched pairs and including unmatched subjects. Selection of significantly different gene expression profiles between the two experimental conditions was based on the empirical Bayes moderated t-statistic and Benjamini-Hochberg method was applied to correct for multiple testing. Significant genes were identified by adjusted-P value ≤ 0.05 . Ingenuity Pathway Analysis (version 8.5, Ingenuity Systems, Redwood City, CA) was also performed evaluating differences in gene expression between tumor and normal tissue (N=8 patients). The details of the microarray data analysis followed the Minimum Information About a Microarray Experiment (MIAME) guidelines issued by the Microarray Gene Expression Data Group [13]. The microarray data have been deposited in the Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo) and the accession number is GSE52138.

Informed Consent and Regulatory Approval

The study was reviewed and approved by the Cancer Evaluation Therapy Program of the National Cancer Institute (P5941), and by the institutional review board at each participating institution in the New York Cancer Consortium (Montefiore Medical Center and New York Presbyterian Hospital), Fox Chase Cancer Center and Princess Margaret Hospital (ClinicalTrials.gov identifier NCT00061932). All patients provided written informed consent.

RESULTS

Patient Characteristics

Forty-one patients were enrolled and treated from April 2003 through September 2006 at 6 participating institutions. The characteristics of the 41 treated patients are shown in Table 1, including 29 patients treated with bortezomib and irinotecan (arm A), and 12 patients with prior chemotherapy for advanced disease treated with bortezomib alone (arm B). Considering the 41 patients in both treatment strata, 36 patients (89%) had GEJ adenocarcinoma, 33 (81%) were white, 31 (76%) had an ECOG performance status of 0 or 1, and 29 (71%) were male.

Response and Progression-Free Survival

Objective response occurred in 3 of 29 patients (10.3%; 95% C.I. 2.2%, 27.4%) with no prior chemotherapy for advanced disease treated with the combination of bortezomib plus irinotecan (arm A), and median PFS was 1.9 months (95% C.I. 1.2 months, 2.6 months). Accrual continued beyond the 18 patients prespecified in the first stage because 12 of 29 patients accrued in this stratum discontinued therapy prior to the first tumor evaluation for reasons other than progressive disease. Objective response occurred in 1 of 12 patients (8.3%; 95% confidence interval [95% C.I.] 0.2%, 38.5%) who had prior chemotherapy for advanced disease treated with bortezomib alone (arm B), and the median progression free

survival (PFS) was 1.5 months (95% C.I. 0.9 months, 1.8 months). The primary efficacy endpoint for each cohort was not met.

Treatment Administered

For the 29 patients in arm A treated with bortezomib plus irinotecan, the median number of treatment cycles given was 3 (range 1–13 cycles), and reasons for discontinuation of therapy included disease progression in 13 patients (45%) after a median of 4 cycles (range 2–10), toxicity or patient withdrawal in 13 patients (45%) after a median of 2 cycles (range 1–5), and other reasons in 3 patients (10%) after a median of 4 cycles (range 2–13).

For patients in arm B treated with bortezomib alone, the median number of treatment cycles given was 2 (range 1–3 cycles), and reasons for discontinuation of therapy included disease progression in 8 patients (67%) after a median of 2 cycles (range 1–2), toxicity or patient withdrawal in 3 patients (25%) after a median of 2 cycles (range 1–2), and other reasons in 1 patient (8%).

Adverse Events

The incidence of grade 3–4 adverse events occurring in at least 5% of patients (or any grade 4 event occurring in at least one patient) in both treatment arms is shown in Table 2. No patient in either arm died of treatment-associated toxicity. In arm A, the most common grade 3 adverse events included neutropenia (28%), diarrhea (24%), vomiting (21%), dehydration (17%), fatigue (17%), anemia (17%), nausea (17%), hypoalbuminemia (14%), febrile neutropenia (14%), bowel obstruction (14%), abdominal pain (10%), thrombocytopenia (10%), hypocalcemia (7%), hypotension (7%), and rash (7%). Grade 4 events included diarrhea in 1 patient (3%), gastric perforation in 1 patient (3%), and cardiac ischemia in 1 patient (3%). For arm B, the most common grade 3 adverse events included dehydration (25%), thrombocytopenia (16%), and multiple other adverse events occurring in 1 patient each. There was one patient with edema, a grade 4 toxicity.

Gene Expression Analysis

Results from Limma adjusted for multiple comparisons indicated that no differentially expressed genes were identified in the pre vs. post-treatment specimens for bortezomib alone or bortezomib plus irinotecan. Similarly, no significant differentially expressed genes were identified in pre-treated normal vs. pre-treated tumor samples.

DISCUSSION

We performed a phase II trial of the proteasome inhibitor bortezomib in patients with advanced GEJ or gastric adenocarcinoma, used either alone in patients who had progressive disease after prior cytotoxic therapy, or in combination with irinotecan in patients who had no prior chemotherapy for advanced disease. Approximately 90% of patients in the study had GEJ adenocarcinoma. Objective response was observed in only 8% of those treated with bortezomib alone in patients with chemotherapy pretreated disease, and in 10% of those treated with bortezomib plus irinotecan as first-line therapy for advanced disease. The study, therefore, failed to achieve the pre-specified 30% response rate target for monotherapy and

the 40% response rate target for combination therapy. It is worthwhile to note that 45% of the patients in arm A stopped treatment because of toxicity. This could have potentially been due to the irinotecan dosing schedule which was 125 mg/m² on days 1 and 8 every 21 days. Using a single dose of irinotecan at 125 mg/m² every 2 weeks may have reduced the observed toxicities and allowed patients to continue therapy. It is also possible that the use of bortezomib with irinotecan (125 mg/m²) may have potentiated the increased toxicity levels seen in arm A of this study. It is possible that lower doses of either drug may be warranted when used in combination therapy.

Other studies have also evaluated the role of bortezomib in adenocarcinoma arising in the GEJ or stomach. Shah et al. reported that none of 15 patients with advanced GEJ carcinoma responded to bortezomib therapy in the first line setting [18]. Jatoi et al. reported a phase II trial of bortezomib (1.2 mg/m² on days 1, 4, and 8) used in combination with carboplatin (AUC6) and paclitaxel (175 mg/m²) every 3 weeks in 35 patients with metastatic esophageal, gastric, and GEJ carcinoma. Objective response occurred in 23%, which was below the targeted response rate and prompted premature closure of the study. In advanced colorectal cancer, Kozuch et al. reported a randomized phase II trial comparing bortezomib alone with bortezomib plus irinotecan in patients with prior chemotherapy for advanced colon cancer (including 84% who had prior irinotecan therapy), with response observed in 0 of 45 treated with bortezomib and 2 of 57 patients (4%) treated with the bortezomib-irinotecan combination [19]. Taken together, these results indicate that bortezomib is not effective when used as a single agent in GEJ cancer, and that there is no clinical evidence that bortezomib enhances the effectiveness of irinotecan in unselected patients with advanced GEJ or colorectal cancer.

We also evaluated the effects of bortezomib alone or in combination with irinotecan on gene expression by performing tumor biopsies before and 24 hours after treatment. We found no significant change in expression of any gene in the pre-treatment vs. post-treatment analysis when adjusted for multiple comparisons.

In conclusion, the proteasome inhibitor bortezomib has limited activity in advanced gastric or GEJ carcinoma and does not enhance the effectiveness of irinotecan in unselected gastric/GEJ adenocarcinoma. Bortezomib based therapy also did not induce significant changes in gene expression when tumors were sampled 24 hours after treatment.

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Table 1

Patient characteristics

Characteristic		Stratum A: Bortezomib plus Irinotecan		Stratum B: Bortezomib Alone	
No.		29		12	
Age (years)	Median	58.5 (33–87)		54.5 (33–77)	
Gender	Male	22	76%	7	58%
	Female	7	24%	5	42%
Race	White	24	83%	9	75%
	Asian	2	7%	2	17%
	Black	3	10%	0	0
	Unknown	0	0	1	
Ethnicity	Non-Hispanic	27	93%	12	100%
	Hispanic	1	3%	0	0
	Unknown	1	3%	0	0
ECOG PS	0	6	21%	2	17%
	1	16	55%	7	58%
	2	2	7%	0	0
	3	0	0	1	8%
	Unknown	5	17%	2	17%
Primary site	GEJ	25	86%	11	92%
	Stomach	4	14%	1	8%
No. Prior Chemo Regimens	0	27	93%	0	0
	1	2 (adjuvant)	7%	9	75%
	2	0	0	1	8%
	3	0	0	2	17%

Table 2

Adverse events

Adverse Event	Stratum A: Bortezomib + Irinotecan (N=29)				Stratum B: Bortezomib alone (N=12)			
	Grade 3		Grade 4		Grade 3		Grade 4	
	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
Hematologic								
Hemoglobin (anemia)	5	17	0	0	1	8	0	0
Leukocytopenia	5	17	3	10	0	0	0	0
Neutropenia	8	28	0	0	0	0	0	0
Thrombocytopenia	3	10	0	0	2	16	0	0
Non-Hematologic								
Albumin, serum-low	4	14	0	0	0	0	0	0
Anorexia	1	3	0	0	1	8	0	0
Cardiac -Other: Heart stoppage	0	0	1	3	0	0	0	0
Cardiac ischemia/infarction	0	0	1	3	0	0	0	0
Dehydration	5	17	0	0	3	25	0	0
Diarrhea	7	24	1	3	0	0	0	0
Distension/bloating, abdominal	0	0	0	0	1	8	0	0
Dyspnea	0	0	0	0	1	8	0	0
Edema: trunk/genital	0	0	0	0	0	0	1	8
Fatigue	5	17	0	0	1	8	0	0
Febrile neutropenia	4	14	0	0	0	0	0	0
Hemorrhage, GU; Urinary NOS	0	0	0	0	1	8	0	0
Hypocalcemia	2	7	0	0	0	0	0	0
Hypotension	2	7	0	0	0	0	0	0
Infection: Lung (pneumonia)	0	0	0	0	1	8	0	0
Malabsorption	0	0	0	0	1	8	0	0
Nausea	5	17	0	0	1	8	0	0

Adverse Event	Stratum A:				Stratum B:			
	Bortezomib + Irinotecan (N=29)				Bortezomib alone (N=12)			
	Grade 3		Grade 4		Grade 3		Grade 4	
	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
Obstruction: Small bowel NOS	4	14	0	0	1	8	0	0
Pain -Other: Right hip	0	0	0	0	1	8	0	0
Pain: Abdomen NOS	3	10	0	0	1	8	0	0
Rash: desquamation	2	7	0	0	0	0	0	0
Vomiting	6	21	0	0	1	8	0	0