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Phase I Study of Vorinostat in Combination with Bortezomib for Relapsed and Refractory Multiple Myeloma

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Abstract

Purpose: Vorinostat, a histone deacetylase inhibitor, enhances cell death by the proteasome inhibitor bortezomib *in vitro*. We sought to test the combination clinically.

Experimental Design: A phase I trial evaluated sequential dose escalation of bortezomib at 1 to 1.3 mg/m^2 i.v. on days 1, 4, 8, and 11 and vorinostat at 100 to 500 mg orally daily for 8 days of each 21-day cycle in relapsed/refractory multiple myeloma patients. Vorinostat pharmacokinetics and dynamics were assessed.

Results: Twenty-three patients were treated. Patients had received a median of 7 prior regimens (range, 3-13), including autologous transplantation in 20, thalidomide in all 23, lenalidomide in 17, and bortezomib in 19, 9 of whom were bortezomib-refractory. Two patients receiving 500 mg vorinostat had prolonged QT interval and fatigue as dose-limiting toxicities. The most common grade >3 toxicities were myelo-suppression ($n = 13$), fatigue ($n = 11$), and diarrhea ($n = 5$). There were no drug-related deaths. Overall response rate was 42%, including three partial responses among nine bortezomib refractory patients. Vorinostat pharmacokinetics were nonlinear. Serum C_{max} reached a plateau above 400 mg. Pharmacodynamic changes in CD-138+ bone marrow cells before and on day 11 showed no correlation between protein levels of NF-κB, IκB, acetylated tubulin, and p21CIP1 and clinical response.

Conclusions: The maximum tolerated dose of vorinostat in our study was 400 mg daily for 8 days every 21 days, with bortezomib administered at a dose of 1.3 mg/m^2 on days 1, 4, 8, and 11. The promising antimyeloma activity of the regimen in refractory patients merits further evaluation. (Clin Cancer Res 2009;15(16):5250–7)

Multiple myeloma is an incurable plasma cell malignancy. In the United States, there are 19,920 estimated new cases of multiple myeloma and 11,190 deaths annually (1). Autologous transplantation and the introduction of thalidomide, bortezomib, and lenalidomide have improved treatment outcomes. However, despite initial response to various therapies, almost all patients relapse and become refractory to subsequent treatment. Responses are uncommon in refractory myeloma patients, and median survival is <12 months after failure of two or more salvage therapies (2).

The clinical activity of bortezomib in multiple myeloma is well-established. Possible mechanisms of bortezomib activity include diminished nuclear localization of NF-κB due to inhibition of proteasomal degradation of ubiquitinated inhibitor of κB (IκB; refs. 3–5). NF-κB mediates key cellular functions, including growth, survival, and apoptosis of multiple myeloma cells and may play a role in chemoresistance (6–9). Cells exposed to bortezomib form aggregates of ubiquitin-conjugated proteins, or "aggresomes," *in vitro* and *in vivo* (10). Bortezomib-induced aggresome formation may serve a cytoprotective role by allowing cells to dispose of accumulated unfolded proteins that result from proteasome dysfunction.

Histone deacetylase (HDAC) inhibitors regulate gene expression through epigenetic modulation of chromatin structure (11). HDACs also affect other cellular functions through upregulation of death receptors, induction of oxidative injury, and disruption of chaperone protein function (12). Notably, HDACIs, inhibit HDAC6 function and disrupt aggresome formation (13, 14). These events result in endoplasmic reticulum stress and induction of apoptosis *in vitro* (15).

In multiple myeloma cells, bortezomib and tubacin, an experimental HDACI that specifically inhibits HDAC6 and

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Translational Relevance

This is a report of a phase I clinical trial of a novel combination of bortezomib and vorinostat in relapsed/refractory myeloma patients. The trial was based on extensive preclinical work demonstrating *in vitro* synergistic activity for the combination. We defined the maximum tolerated dose and the pharmacokinetics of vorinostat when administered in combination with bortezomib. Pharmacodynamic changes in protein levels of NF-κB, IκB, acetylated tubulin, and p21ClP1 in plasma cells isolated from the bone marrow on days 1 (pretreatment) and 11 of vorinostat and bortezomib in the study did not correlate with the patients' clinical responses. The regimen was well-tolerated and had promising antimyeloma activity. This work is the basis of upcoming phase II and III clinical trials to confirm the overall response in relapsed/refractory multiple myeloma patients.

triggers the acetylation of α-tubulin, exhibited highly synergistic interactions (16). The combination induced significant cytotoxicity in multiple myeloma cells isolated from patients' bone marrow and in adherent multiple myeloma cells, which are resistant to conventional treatments. The relative contributions of NF-κB inhibition versus disruption of aggresome function in synergistic interactions between proteasome and HDAC inhibitors in multiple myeloma are unknown. The combination of bortezomib and vorinostat also diminished NF-κB activation and induction of $p21^{CIP1}$, as was first described by Mitsiades et al. (6) Later studies have shown that HDACIs can increase expression of NF-κB and that acetylation of p65/RelA promotes DNA binding and activation. Conversely, inhibition of NF-κB by IKK inhibitors diminishes nuclear translocation and activation, resulting in down-regulation of NF-κB–dependent survival genes such as XIAP and Bcl-xL (17). Therefore, bortezomib may increase HDACI lethality by opposing NF-κB activation induced by p65/RelA acetylation. A recent study showed synergistic interactions between bortezomib and HDACIs in chronic lymphocytic leukemia cells in association with NF-κB inactivation and XIAP/Bcl-xL down-regulation (18).

Although a phase I trial of vorinostat failed to establish significant single-agent activity in relapsed and refractory myeloma patients, it did show favorable tolerability and evidence of disease stabilization in this heavily pretreated population (19). Based on informative preclinical data and the encouraging safety profile defined in the phase I monotherapy setting, we hypothesized that vorinostat may be clinically synergistic with bortezomib, especially in bortezomib-refractory patients, and that this combination therefore warranted study. A multicenter phase I trial was initiated to determine the maximum tolerated dose (MTD) of vorinostat in combination with bortezomib in patients with relapsed/refractory multiple myeloma. Secondary objectives were to define the pharmacokinetics of vorinostat when administered in combination with bortezomib, to assess the biological effects of vorinostat on bone marrow plasma cells, and to evaluate antitumor activity. Biological effects of vorinostat studied included measurement of protein levels of NF-κB, IκB, acetylated tubulin, and p21CIP1. Additionally, expression of phosphorylated eIF2α and levels of NF-κB–dependent antiapoptotic proteins, including Bcl-xl and XIAP, were evaluated for their potential utility as surrogate markers for response in future trials (20, 21).

Patients and Methods

Eligibility

Patients with relapsed and/or refractory multiple myeloma were eligible. Patients had to have received at least three lines of prior therapies that could have included conventional and high-dose chemotherapy, as well as novel investigational agents. Patients were required to have measurable disease at study entry. Patients could not have received therapy for at least 2 wk before study entry. Patients had to have an Eastern Cooperative Oncology Group (ECOG) Performance Status score of ≤2 and a life expectancy >6 mo. Patients were excluded if they had abnormal liver function (bilirubin, aspartate aminotransferase, or alanine aminotransferase of greater than twice the upper limit of normal) or inadequate marrow function as defined by an absolute neutrophil count of <1,000/mL or a platelet count of <50,000/mL, except if myelosuppression was attributed to marrow plasmacytosis (defined by >80% plasmacytosis). Patients were allowed to receive growth factors. Patients had to have a serum creatinine of <2 mg/dL or a creatinine clearance of >40 mL/min by 24-h urine collection. Patients with grade II or higher peripheral neuropathy were excluded. Women of childbearing potential and all men agreed to use adequate contraception during study participation. The Institutional Review Boards of the participating sites approved the study. Written informed consent was obtained from all patients.

Study design

Therapy was administered on an outpatient basis. Bortezomib was delivered at 1 to 1.3 mg/m^2 i.v. over 3 to 5 s on days 1, 4, 8, and 11. Vorinostat was administered orally 1 h after bortezomib on days 4 to 11. The vorinostat dose regimens evaluated were 100 mg twice daily, 200 mg twice daily, 400 mg once daily, and 500 mg once daily. Cycles were repeated every 21 d and continued for a total of eight cycles or until disease progression, occurrence of unacceptable adverse events or if the patient withdrew consent. Patients removed from study for unacceptable adverse events were followed until resolution or stabilization of the adverse event. After two cycles, dexamethasone was allowed at 20 mg orally daily for 5 d (days 4-8) in patients with less than a partial remission, for a total dose of 100 mg/cycle. All patients were to receive antiviral prophylaxis. Antidiarrheal therapy was prescribed if diarrhea occurred.

Three patients were enrolled in each cohort; dose escalation proceeded if there were no dose-limiting toxicities (DLT) in the first cycle. If one of three patients experienced DLT in cycle 1, dose escalation was stopped, and three additional patients were enrolled into the cohort. The dose was not escalated if two of three to six patients in a cohort experienced DLT. DLT was defined as any grade 3, or higher, nonhematologic toxicity excluding grade 3 fatigue unless persisting for 7 d after stopping vorinostat. Any grade 4 hematologic toxicity that persisted for >7 d was considered a DLT except for thrombocytopenia that responded to transfusion support. The MTD was the highest dose that produced ≤1 DLT in a cohort of six patients.

If grade 2 or higher toxicity was suspected to be due to bortezomib, the drug was held until the toxicity resolved and then restarted at 1 mg/m^2. If toxicity recurred, the subsequent dose of bortezomib was decreased to 0.7 mg/m^2. If toxicity did not resolve to less than grade 2 within 2 wk of onset or recurred on 0.7 mg/m^2 of bortezomib, the patient was removed from the study. The vorinostat dose was decreased by 50% for each toxicity grade, and was stopped if the lowest vorinostat dose of 100 mg daily was not tolerated. For overlapping toxicities such as hematologic and gastrointestinal, bortezomib was decreased first.

Pretreatment evaluation included a complete history and physical examination, ECOG performance status, complete blood counts,

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electrolytes, hepatic, renal, and thyroid function tests, urinalysis, pregnancy test (if appropriate), chest X-ray, electrocardiogram, bone marrow examination, and computerized tomography scan for patients with extramedullary disease. Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria (CTCAE version 3.0).[6] Any grade 3 or 4 nonhematologic toxicity was reported to the Institutional Review Board and the National Cancer Institute. Multiple myeloma response and progression were assessed using International uniform response criteria for multiple myeloma (22).

Vorinostat and bortezomib were provided by Merck & Co., Inc., and Millennium: The Takeda Oncology Company, Inc., respectively, through the National Cancer Institute, Cancer Therapy Evaluation Program, which supported the trial. Patients were treated at two centers, University of Maryland (n = 21) and Cornell (n = 2).

Pharmacokinetic studies

Twenty-one patients had blood samples drawn on day 4 of cycle 1 to characterize the pharmacokinetics of orally administered vorinostat. Five milliliters of blood were collected, without anticoagulant, before and at 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h after oral administration of vorinostat. Blood samples were allowed to clot at 4°C for 20 to 30 min and then centrifuged at 2,000× g for 15 min at 4°C. The serum was stored at -70°C until assayed for drug content. Vorinostat concentrations in serum were quantified by a validated liquid chromatography-electrospray ionization tandem mass spectrometric method (23). The maximum serum vorinostat concentration (C_{max}) and time to reach the maximum concentration (t_{max}) were determined by visual inspection of serum vorinostat concentration versus time curves. The Lagrange function (24), as implemented by the LAGRAN computer program (25), was used for noncompartmental estimation of the area under the vorinostat serum concentration versus time curve (AUC), with extrapolation to infinity, and for estimation of vorinostat half-life ($t_{1/2}$). The AUC was used to calculate clearance (CL/F), which was defined as dose/AUC. Statistical analyses for pharmacokinetics were done using SPSS 15.0 for Windows (SPSS, Inc.). The relationship between vorinostat dose and Cmax was evaluated with the Student's t test. The relationship between vorinostat dose and CL/F was evaluated with linear regression. P values of <0.05 were considered significant.

Pharmacodynamic studies

Isolation of myeloma cells from bone marrow. Bone marrow aspirates (5-10 mL) were obtained from patients at baseline (day 1) and on day 11 of the first cycle of therapy, 2 to 3 h after both drugs were given. Multiple myeloma cells were isolated by using a magnetic cell sorter and anti-CD138 antibody-coated magnetic microbeads (Miltenyi Biotec), as described previously (26, 27). Bound (CD138+) and unbound cell fractions were collected, washed in PBS, and pelleted. Approximately 50,000 cells were diluted into 300 μL PBS and used to prepare cytospins with a Shandon Cytospin I centrifuge (Thermo Scientific). The remaining cells were pelleted and frozen at -80°C for subsequent Western blot analysis.

Protein extraction and Western blot analysis. Frozen CD138+ cell pellets were resuspended in ice cold cell lysis buffer containing a final concentration of 4 mmol/L Tris-Hcl (pH 7.5), 30 mmol/L NaCl, 0.2 mmol/L EDTA, 0.2 mmol/L EGTA. 0.2% Triton, 0.2 mmol/L sodium pyrophosphate, and 0.2 mmol/L β-glycerophosphate with protease and phosphatase inhibitors (F. Hoffmann-La Roche Ltd.) and sonicated using a Misonix sonicator 3000. Total cellular protein was quantified using a Bio-Rad protein assay. Protein (30-50 mg) was loaded onto a 4% to 12% NuPAGE gels and separated using a NuPAGE Novex bis-tris gel electrophoresis system (Invitrogen). Each blot was divided into two membranes depending on the molecular weight of the proteins to be probed, using anti–glyceraldehyde-3-phosphate dehydrogenase polyclonal antibody (Sigma-Aldrich) as the loading controls for the analysis. Phospho-eIF2α (Ser51) rabbit monoclonal antibody, phospho-IκBα (Ser32/36) mouse monoclonal antibody, and BclxL polyclonal rabbit antibody from Cell Signaling Technology, poly ADP ribose polymerase (PARP) mouse monoclonal antibody from BIOMOL International, Inc., Bcl2 mouse monoclonal antibody from DAKO North America, Inc., $p21^{CIP1}$ and XIAP monoclonal antibodies from BD Transduction Laboratories, and NFκ B/p65 subunit polyclonal antibodies from Millipore were used. Secondary antibodies were peroxidase-labeled affinity-purified antibodies to rabbit and mouse IgG from KPL. Signals were detected by enhanced chemiluminescence (SuperSignal WestPico chemiluminescent substrate, Pierce Biotechnology, Inc.), and quantitative analysis was done using the FluoChem 8800 Imaging System (Alpha Innotech). Two-dimensional spot densitometric images were obtained and analyzed with Alpha Ease FC software (Alpha Innotech). Each protein band on the Western blots was assigned an average pixel value from a scale of 1 to 200 adjusted to arbitrary unit 1 in presamples. Changes in protein expression were normalized to the loading control protein (glyceraldehyde-3-phosphate dehydrogenase) to determine the percent increase or decrease.

Results

Patient characteristics. Twenty-three patients were enrolled between June 2006 and October 2007; the final analysis was carried out in July 2008. Demographics, disease characteristics, and baseline laboratory values are shown in Table 1. All patients had an ECOG performance status of 0 or 1. All patients were heavily pretreated for multiple relapses. The median number of prior regimens was 7 (range, 3-13). Twenty patients had had prior autologous stem cell transplantation, including six who received tandem transplants, and one who also had an allogenic transplant. All 23 patients had received thalidomide, 11 as maintenance after transplantation, and 12 for relapsed disease; of the latter group, 5 were refractory. Nineteen patients

Table 1. Patient characteristics

	No.	Median (range)
Age (y)		54 (39-78)
Sex: male/female	15/8	
Race: Caucasian/Blacks	18/5	
Isotype: Ig G/A/LC	11/4/8	
No. of prior regimens		7 (3-13)
SCT-Autologous/tandem/allo	20/6/1	
Prior thalidomide/lenalidomide	23/17	
Bortezomib	19	
Duration (mo)		6 (2-40)
No. of regimens 1/2/3	8/5/6	
Primary refractory	9	
Responsive then progressive	10	
ECOG		1 (0-2)
Time from diagnosis to study (y)		5.7 (1.8-9)
Time from last therapy (d)		20 (15-39)
Creatinine (mg/dl)		1.3 (0.7-2.1)
Hgb (g/dl)		11 (8.5-14)
Platelets (x10^3/μL)		148 (62-253)
Albumin (g/dl)		3.8 (2.5-4.7)
LDH (Units/L)		209 (104-619)
β-2-Microglobulin (mg/L)		3 (1.3-6)
Baseline peripheral neuropathy	13	
Grade 1/2	11/2	
Abnormal karyotype	14	
11;14	6	
Hypodeploidy: del(13), del(17)	7/3	
Dup, der, del(1)	9	

[6] http://ctep.info.nih.gov

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Table 2. Bortezomib and vorinostat CTC-version 3 toxicities for cycles 1 and cumulative for cycles 2 to 8

Toxicity	Cycle 1					Cycle 2-8				
	No	Grade				No	Grade			
		1	2	3	4		1	2	3	4
Anemia	7	2	4	1	—	16	4	7	3	2
Neutropenia	4	2	—	2	—	7	1	3	3	—
Platelets	12	3	3	3	3	17	3	1	7	6
Diarrhea	12	9	1	2	—	11	8	1	2	—
Nausea	11	9	1	1	—	12	6	6	—	—
Vomiting	5	2	2	1	—	7	4	2	1	—
Constipation	1	1	—	—	—	3	3	—	—	—
Fatigue	8	4	3	1	—	11	2	4	5	—
Fever	5	2	2	1	—	4	3	1	—	—
Peripheral neuropathy	13	11	2	—	—	14	5	9	1	—
Dyspnea	0	—	—	—	—	5	1	1	3	—
Hypotension	0	—	—	—	—	5	3	1	1	—
Atrial fib.	0	—	—	—	—	1	—	1	—	—
Prolonged QTC	10	7	2	1	—	0	—	—	—	—
Pneumonia	0	—	—	—	—	3	—	—	3	—
Shingles	0	—	—	—	—	2	—	2	—	—
Creatinine	7	2	5	—	—	8	4	3	1	—
Hyponatremia	6	5	1	—	—	10	6	—	4	—
Hypokalemia	2	—	—	2	—	5	3	—	2	—
Hypocalcemia	1	1	—	—	—	5	2	2	1	—
LDH	5	3	2	—	—	4	4	—	—	—
AST	4	3	1	—	—	5	4	—	1	—
ALT	3	3	—	—	—	2	—	2	—	—

Abbreviations: LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

had received bortezomib; the median number of bortezomib-containing regimens was 2 (range, 1-3). Nine patients were bortezomib-refractory and 10 had progressive disease (PD) after an initial response lasting a median of 6 months (range, 3-35) and 4 were bortezomib-naïve. Seventeen patients had received lenalidomide, among whom 9 were refractory and 8 progressed after an initial response lasting a median of 4 months (3–18). Overall, 16 patients were refractory to their last therapy.

Patients were treated at five dose levels. Three patients were treated with bortezomib 1.0 mg/m^2 on days 1, 4, 8, and 11, with 8 days of vorinostat 100 mg twice daily. The bortezomib dose was then escalated to 1.3, with 8 days of vorinostat at doses of 100 mg (n = 3) and 200 mg (n = 3) twice daily, and 400 mg (n = 3) and 500 mg (n = 3) once daily. Eight patients were then treated at the MTD to better define toxicity and efficacy. A total of 112 cycles of therapy were administered, with a median of 3 cycles per patient (range, 1-8). Three patients completed the maximum allowed eight cycles of therapy.

Safety and tolerability. DLTs occurred in two patients treated at the vorinostat 500 mg dose. One DLT was grade 3 fatigue and the other was prolongation of the corrected QT interval. The MTD for the combination was 400 mg of vorinostat administered oral daily for 8 days with bortezomib at 1.3 mg/m^2 given on days 1, 4, 8, and 11, with cycles repeated every 21 days.

The most common drug-related adverse events and laboratory abnormalities observed in cycle 1 were diarrhea (52%), nausea (48%), fatigue (35%), peripheral neuropathy (57%), and increased creatinine (30%). These events were mostly grades 1 and 2. Hematologic toxicities including thrombocytopenia (52%), anemia (30%), and neutropenia (17%) were reversible, with no bleeding or life-threatening infections. Once CTEP reported on prolonged QT interval in patients receiving vorinostat on other trials, we monitored patients entering the trial with electrocardiograms 3 hours after vorinostat administration. Ten patients had electrocardiograms at baseline and after vorinostat. Prolongation of QTc was noted in nice patients, all on 400 mg daily, and was grade 1 in seven and grade 2 in two, One patient had grade 3 prolongation of cQT; baseline QTc was 394 ms, and cQT was 633 ms after vorinostat infusion and 389 ms at 24 hours. None of the patients, including the one with the grade 3 event, had a prior history of arrhythmias, cardiac disease, and none were taking concomitant medications that might prolong the QT.

Several other grade 3 serious adverse events occurred after cycle 1, and thus did not contribute to defining DLTs. These included pneumonia (n = 3), varicella zoster (n = 2), dyspnea (n = 3), hyponatremia (n = 4), fatigue (n = 5), and diarrhea (n = 2; Table 2). The two patients who developed zoster were not compliant with their prescribed acyclovir prophylaxis. Several metablic abnormalities were noted, including hypokalemia, hyponatremia, and hypocalcemia. Hypotension was noted in five patients, only one of whom required 24-hour hospital admission for hydration. Atrial fibrillation, occurred in the setting of pneumonia in one patient, with conversion to normal sinus rhythm with β-blockers.

Eleven patients (48%) had adverse events that required dose modification. These included eight patients in whom bortezomib was decreased from 1.3 mg/m^2 to 1 mg/m^2 for grade 3 peripheral neuropathy (n = 4) or grade 3 thrombocytopenia (n = 4), and an additional three patients who required a decrease in vorinostat dose for grade 3 diarrhea. After dose modification, discontinuation, or interruption, all patients recovered from these events within 2 weeks. One of the eight patients treated at the MTD stopped vorinostat after two doses in cycle 1 due to mild nausea and during cycle 2 due to diarrhea, but then continued therapy for three subsequent cycles at a vorinostat dose of 200 mg/day.

Responses. Table 3 summarizes responses. Overall, 9 of 21 (42%) evaluable patients achieved objective responses, including 2 very good PR and 7 PR. Three of the nine responses occurred in bortezomib-refractory patients. Although the numbers were small, more responses occurred at the MTD (vorinostat 400 mg daily), with 6 of 11 patients achieving a PR or better (55%) compared with lower vorinostat doses, at which 3 of 12 patients achieved responses (25%). Dexamethasone was

Table 3. Overall response to vorinostat and bortezomib

Prior Bortezomib	No of Pts (n = 23)	Response				
		VGPR	PR	SD	PD	NE
Naïve	4	1	2	1		
Pretreatment	10	1	2	5	1	1
Refractory	9		3	4	1	1
Pts treated at MTD, n = 11)						
Naïve	2		2			
Pretreatment	3		2	1		
Refractory	6		2	4		

Abbreviations: Pts, patients; VGPR, very good PR; NE, not evaluable.

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Table 4. Vorinostat pharmacokinetics

Dose	C_{max} (mmol/L)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{INF} (mmol/L*h)	CL/F (l/h)	CL/F (l/min)
100 mg BID (n = 6)						
Mean	0.31	1.3	1.3	0.76	605	10.1
SD	0.14	0.4	0.5	0.43	246	4.1
200 mg BID (n = 3)						
Mean	0.65	1.3	2.4	2.03	383	6.4
SD	0.35	0.6	2.3	0.40	82	1.4
400 mg daily (n = 10)						
Mean	1.42	2.8	1.6	5.13	313	5.2
SD	0.32	1.6	0.5	1.22	87	1.4
500 mg daily (n = 3)						
Mean	1.33	3.8	4.4	8.10	246	4.1
SD	0.15	1.9	3.0	2.17	73	1.2

added for patients with less than a partial response (PR) after cycle 2 (n = 6) or cycle 4 (n = 5) and in cycle 6 for two patients with PD. There was no improvement in response with the addition of dexamethasone to the regimen in any patient. This lack of response to dexamethasone in these heavily pretreated patients, despite prior response, does not mean that the addition of dexamethasone to the combination of bortezomib and vorinostat would not be beneficial in less heavily pretreated patients.

Three patients completed the eight cycles on the study and remained in remission for 9, 6, and 6 months. Six patients discontinued therapy due to toxicity, including two patients during cycle 1 due to DLT, two patients, both in PR, after cycle 4 due to worsening peripheral neuropathy and two patients, both with stable disease (SD), in cycles 3 and 5 due to fatigue. Fourteen patients went of study due to PD. At last follow-up, 15 patients had PD. The median time to progression from study entry was 4 months; the corresponding 95% confidence interval lower limit was 3.12 months, and the upper limit has not been reached yet. Twelve patients had died, among them three from aggressive transformation to plasma cell leukemia.

Pharmacokinetics. Pharmacokinetic data from patients treated with 100, 200, 400, and 500 mg of vorinostat are presented in Table 4. Vorinostat C_{max} increased with doses between 100 and 400 mg, but was not statistically different between 400 and 500 mg (P = 0.35). Vorinostat t_{max} varied between 0.5 and 6 hours, and vorinostat $t_{1/2}$ varied between 0.8 and 7.6 hours. Although vorinostat AUC increased with dose, the increase was greater than proportional, and vorinostat CL/F decreased with increasing dose [the slope of Cl/F (L/min) versus dose (mg) was -0.014; P = 0.001].

Pharmacodynamic studies. Baseline marrow samples were collected from 12 patients, median total cell count was 19.9×10^6 (range, 3.6-157 $\times 10^6$) with a median CD138+ cell of 3.05×10^6 (range, 0.36-97 $\times 10^6$). Three patients refused day 11 bone marrow aspiration; for the rest, total cell count was 15.1 $\times 10^6$ (range, 4.1-31 $\times 10^6$) with a median CD138+ fraction of 4.2×10^6 (range, 1.7-7.5 $\times 10^6$). Protein extraction at baseline and day 11 samples yielded adequate amounts for analysis in eight patients.

Overall expression for various proteins did not correlate with response. For example, cells from the patient GCC-016, PD, displayed increased expression of $p21^{CIP1}$, acetylated tubulin and total p65/RelA, and a slight increase in Bcl-xL posttreatment (Fig. 1A and B). Disparate responses were observed in cells obtained from two responding patients. Patient GCC-19 displayed a marked reduction in the expression of PARP, phospho-eIF2α, acetylated tubulin, $p21^{CIP1}$, Bcl-2, XIAP, Bcl-xL, p65/RelA, and phospho-IκBα. Whereas patient GCC-20 displayed an increase in PARP and $p21^{CIP1}$ and a modest decrease in phospho-eIF2α. Cells from patients with SD displayed heterogeneous responses, with increases in certain proteins (e.g., $p21^{CIP1}$ and p65/RelA). The only discernible trend was a posttreatment decrease in Bcl-xL in patients with SD and PR.

Discussion

This is the first clinical trial describing the safety and response profile of a regimen combining an HDAC inhibitor, vorinostat, with a proteasome inhibitor, bortezomib, in heavily pretreated relapsed and refractory multiple myeloma patients. The MTD of oral vorinostat was determined to be 400 mg once daily for 8 days (days 4-11) with bortezomib 1.3 mg/m^2 administered on days 1, 4, 8, and 11 of a 21-day cycle. A twice-daily dosing regimen was used initially to prolong the duration of HDAC inhibitor activity. Subsequently, a daily dosing schedule was used to achieve higher peak HDAC inhibitor concentrations following bortezomib administration to enhance potential synergism between the drugs. There were no apparent differences in responses or toxicities between the once daily and twice daily regimens.

The toxicity profile of vorinostat and bortezomib supports the concept that both proteasome inhibitors and HDAC inhibitors selectively target malignant cells, rather than normal cells (28, 29). The most common toxicities were hematologic, gastrointestinal, and fatigue. The events were mild-to-moderate in severity and were similar to those observed in previous trials with vorinostat (19, 30–32). Patients rapidly recovered from the majority of these events, within 2 to 3 weeks of dose modification, interruption or discontinuation of the drug.

Prolonged QTc was noted in a number of our patients, but was not clinically significant. In the single patient with grade 3 QTc prolongation, the event was asymptomatic, with reversal seen in follow-up electrocardiograms. QT interval prolongation is a recently recognized side effect of molecularly targeted drugs (33). At least four HDAC inhibitors examined to date-including vorinostat (34), panobinostat (35), depsipeptide (36, 37), and LAQ82411-have shown evidence of QTc prolongation in phase I and/or II studies. Although QTc prolongation is associated with a risk of severe cardiac arrhythmias such as torsade de pointes, it is important to note that the QT abnormalities observed in HDAC inhibitor clinical trials have not been associated with any symptomatic or clinically significant events. The actual incidence and, more importantly, the significance of QTc prolongation has not been clearly defined for any HDAC inhibitor including vorinostat (38). Until a definitive study and more data are available, it seems reasonable to obtain baseline and periodic electrocardiograms as a precaution during treatment with vorinostat. Moreover, this combination should be used with particular care in patients with congenital long QT syndrome or those taking medications that may lead to QT prolongation. Additionally, potassium and magnesium levels should be monitored, and hypokalemia and hypomagnesemia should be corrected before drug administration in all patients.

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Vorinostat pharmacokinetics were not altered by coadministration of bortezomib. The values for vorinostat C_{max}, AUC, $t_{1/2}$, and CL/F are consistent with those previously published for single-agent vorinostat, suggesting no interactions with bortezomib (39). This was not surprising, as the two drugs have different metabolic pathways, and is consistent with the observation that bortezomib is an excellent partner in many combination therapies.

We have documented encouraging activity for the combination of vorinostat and bortezomib in relapsed, as well as refractory, multiple myeloma patients. Although limited in number, the responses occurring in bortezomib-refractory patients are clearly of interest. The precise mechanism underlying the favorable interaction between vorinostat and bortezomib is unknown. Notably, HDAC inhibitors have been shown to inhibit NF-κB, among their numerous effects, in multiple myeloma cells.(6) As NF-κB activation represents a critical factor in the pathogenesis of multiple myeloma (40), it is conceivable that in bortezomib-resistant cells, combined treatment may reduce NF-κB levels below those necessary for survival. Resolution of this issue will require a better understanding of the mechanisms by which multiple myeloma cells develop resistance to bortezomib, and determination of whether such factors are circumvented by HDAC inhibitors. Of potential relevance to this question is the recent observation that constitutively activated NF-κB in multiple myeloma cells may be intrinsically insensitive to the effects of proteasome inhibitors such as bortezomib, at least when they are administered *ex vivo* (41). Whether a similar phenomenon occurs in cells exposed to bortezomib *in vivo* remains to be established.

We attempted to determine whether pharmacodynamic changes induced by HDAC and/or proteasome inhibitors in preclinical studies could be documented in posttreatment specimens. These included assessment of modulation of proteins

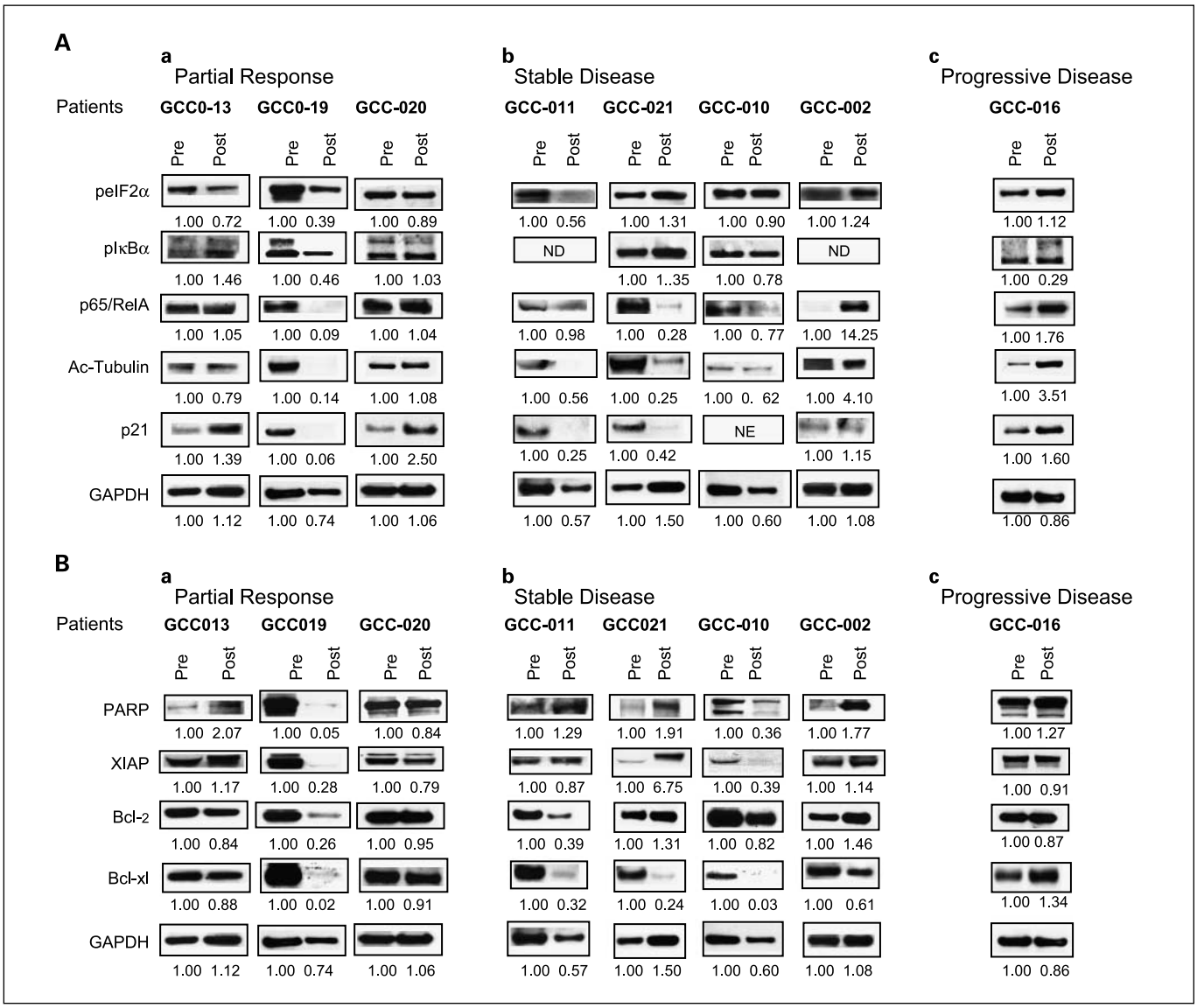

Fig. 1. Western blot analysis of biomarkers corresponding to HDAC inhibitor–related proteins (*A*) and apoptosis-related proteins (*B*) from CD138+ myeloma cells. *A, B,* and *C,* results for baseline and day 11 bone marrow samples following vorinostat-bortezomib therapy in relationship to clinical responses. Numbers reflect densitometric scans of each protein normalized to pretreatment values, arbitrarily designated as 1.00.

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involved in regulation of cell cycle or apoptosis (e.g., PARP cleavage, alterations in Bcl-2 or $p21^{CIP1}$ expression; refs. 16, 42), changes in expression of p65/RelA or IκBα (43, 44), down-regulation of the NF-κB–dependent proteins Bcl-xL and XIAP (18), induction of ER stress (eIF2α phosphorylation; ref. 45), and acetylation of tubulin (10), by Western blotting. Preclinical evidence suggests that the basis for interactions between bortezomib and vorinostat may be multifactorial and involve, among other factors, enhanced inactivation of NF-κB, down-regulation of antiapoptotic NF-κB–dependent proteins, disruption of aggresome function, and induction of ER stress (15, 18, 46). In our pharmacodynamic studies, which are in the context of this phase I trial, necessarily preliminary and should be considered exploratory, there was no clear signal or association with response for the various proteins evaluated. The results of the Western blotting analyses suggest that some of the anticipated changes in multiple myeloma cell protein expression following *in vivo* administration of vorinostat and bortezomib occurred in a subset of patients, but with a high degree of variability. In view of multiple factors, including the small sample size, the limited number of specimens for analysis and differences in drug doses, conclusions cannot be drawn regarding pharmacodynamic findings and their lack of correlation with response. It should also be noted that acetylation of histones H3 and H4 was not monitored in this trial, in part because the results of some preclinical studies suggest that this process does not correlate closely with HDAC inhibitor–mediated lethality (47). It is possible that NF-κB activation status as determined by EMSA analysis, rather than expression of p65/RelA or IκBα, may correlate more closely with responses to a regimen combining bortezomib and a HDAC inhibitor. However, limited sample availability precluded such assays. Furthermore, dynamic considerations could influence the abundance of proteins such as IκBα. For example, although proteasome inhibition would be expected to promote IκBα accumulation, inhibition of NF-κB may limit its synthesis (48). Of the multiple laboratory correlates examined, down-regulation of Bcl-xL seemed to correlate best with lack of disease progression. Finally, a recent study has shown that Myc regulates the sensitivity of multiple myeloma cells to bortezomib and vorinostat *in vitro* and that its expression directly correlates with the percentage of aggresome-positive cells and cell death (49). Whether the expression of Myc or other proteins will correlate with responsiveness to vorinostat/bortezomib therapy will best be addressed in successor phase II trials that use uniform drug dosing and involve a significantly larger number of patients.

In summary, results of this phase I trial indicate that vorinostat can be administered at a dose of 400 mg daily for 8 days in conjunction with bortezomib at 1.3 mg/m^2 to patients with relapsed multiple myeloma, with manageable toxicity. Encouraging activity was observed in this trial, with responses in patients who were previously proven bortezomib-unresponsive. Based on these results, further evaluation of vorinostat and bortezomib in patients with relapsed and relapsed/refractory myeloma is warranted. Multicenter phase II and III trials have been initiated.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Authors' Contributions: A. Badros designed the study, did the research, analyzed the data, and wrote the manuscript. A.M. Burger processed BM. S. Philip and C. Harris followed the study patients. R. Niesvizky contributed patients to the study. J.L. Holleran and M.J. Egorin did the pharmacokinetic analyses and wrote the PK section. O. Goloubeva and M.R. Baer analyzed the data and helped write the article. J. Zweibel, J.J. Wright, and I.E. Delgado provided guidance into the study design and reviewed the data. S.S. Kolla and S. Grant did the pharmacodynamic analyses and wrote the PD section.

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