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PHASE I STUDIES

A phase I study of vorinostat in combination with bortezomib in patients with advanced malignancies

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Summary *Background* A phase I study to assess the maximum-tolerated dose (MTD), dose-limiting toxicity (DLT), pharmacokinetics (PK) and antitumor activity of vorinostat in combination with bortezomib in patients with advanced solid tumors. *Methods* Patients received vorinostat orally once daily on days 1–14 and bortezomib intravenously on days 1, 4, 8 and 11 of a 21-day cycle. Starting dose (level 1) was vorinostat (400 mg) and bortezomib (0.7 mg/m^2). Bortezomib dosing was increased using a standard phase I dose-escalation schema. PKs were evaluated during cycle 1. *Results* Twenty-three patients received 57 cycles of treatment on four dose levels ranging from bortezomib 0.7 mg/m^2 to 1.5 mg/m^2. The MTD was established at vorinostat 400 mg daily and bortezomib 1.3 mg/m^2. DLTs consisted of grade 3 fatigue in three patients (1 mg/m^2,1.3 mg/m^2 and 1.5 mg/m^2) and grade 3 hyponatremia in one patient (1.5 mg/m^2). The most common grade 1/2 toxicities included nausea (60.9 %), fatigue (34.8 %), diaphoresis (34.8 %), anorexia (30.4 %) and constipation (26.1 %). Objective partial responses were observed in one patient with NSCLC and in one patient with treatment-refractory soft tissue sarcoma. Bortezomib did not affect the PKs of vorinostat; however, the Cmax and AUC of the acid metabolite were significantly increased on day 2 compared with day 1. *Conclusions* This combination was generally well-tolerated at doses that achieved clinical benefit. The MTD was established at vorinostat 400 mg daily × 14 days and bortezomib 1.3 mg/m^2 on days 1, 4, 8 and 11 of a 21-day cycle.

Keywords SAHA · Vorinostat · PS-341 · Bortezomib · Phase I

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Introduction

Histone deacetylation plays a key role in the epigenetic regulation of gene expression and has been implicated in the development and progression of cancer. Gene expression is influenced by chromatin structure. DNA that is wrapped around condensed, non-acetylated histones is transcriptionally inactive, whereas acetylation of N-terminal histone lysine residues exposes DNA to important transcription factors that promote transcriptional activity [1, 2]. The dynamic equilibrium between histone acetylation and deacetylation is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs promote transcriptional activity by catalyzing the acetylation of N-terminal histone lysine residues [1, 2], while HDAC activity results in chromatin condensation and silencing of various genes, including those involved in cell survival, proliferation, differentiation, and apoptosis [3]. In tumor cells, HDACs

also target many non-histone proteins such as tumor suppressor genes and proteins that control proliferation, migration, death and angiogenesis [4] and provide a unique mechanistic approach for anti-cancer therapy.

Vorinostat (suberoylanilide hydroxamic acid (SAHA) or MK-0683, Zolinza®, Merck, Whitehouse Station, NJ) is a small molecule inhibitor of class I and II HDAC enzymes that promotes cell cycle arrest and apoptosis in a wide variety of human hematopoietic cells [4–11] and carcinoma cell lines [12–17]. Clinical activity has been observed in a number of hematologic tumors, and vorinostat is currently approved by the Food and Drug Administrations (FDA) for use in patients with refractory cutaneous T-cell lymphoma [18].

Bortezomib (Velcade, PS-341, Millennium, Cambridge, MA) is a modified dipeptidyl boronic acid that reversibly inhibits the 26S proteasome, a large protease complex that degrades ubiquinated proteins. Altered degradation of transcription factors and cell cycle control proteins can result in uncontrolled cell division that promotes cancer growth and spread. Inhibition of targeted proteolysis with bortezomib increases turnover of proteins involved in cell cycle progression and survival, including the p21 cyclin-dependent kinase inhibitor, cyclins and NF-κB, resulting in cell cycle arrest, apoptosis, and inhibition of angiogenesis [19]. In addition, bortezomib causes the sequestration of ubiquitin-conjugated proteins into aggresomes in pancreatic cells [20], which may participate in a cytoprotective response by shuttling ubiquitinated proteins to lysosomes for degradation [21]. In vivo, bortezomib delays tumor growth and enhances the cytotoxic effects of radiation and chemotherapy [22]. Bortezomib is currently FDA approved for use in multiple myeloma and mantle cell lymphoma, and activity has also been seen in solid tumors [23, 24].

Accumulating evidence suggests that HDAC inhibitors and proteasome inhibitors may act synergistically in malignancies. In cultured retinoblastoma cells, treatment with sodium butyrate, an HDAC inhibitor, increased 26S proteasome activity and decreased p53, N-myc and IκBα protein levels [25]. Addition of the proteasome inhibitor, MG132, potentiated the apoptotic effect of sodium butyrate, possibly by blunting the effects on p53, N-myc and IκBα levels and increasing Bax expression [25]. Similar findings were observed when vorinostat or sodium butyrate was combined with bortezomib in leukemia cell lines where a pronounced increase in mitochondrial injury, caspase activation, PARP degradation and reactive oxygen species (ROS) production was observed [26]. More recent studies suggest that HDAC inhibitors may disrupt the aggresome formation induced by proteasome inhibitors, resulting in enhanced endoplasmic reticulum stress and apoptosis [20]. Consistent with these findings, synergistic activity between HDAC and proteasome inhibitors has been observed in vitro in multiple myeloma [27], pancreatic cancer [20], lung cancer [28], hepatocellular carcinoma [29] and colon cancer cell lines [30, 31]. The combination of a histone deacetylase inhibitor with a proteasome inhibitor represents a novel, molecularly targeted combination with non-overlapping toxicities that has strong preclinical support.

Based on preclinical data supporting synergistic activity between HDAC inhibitors and proteasome inhibitors, a phase I study was conducted to determine the safety and tolerability of vorinostat in combination with bortezomib in patients with refractory solid tumors. In addition, pharmacokinetic (PK) analyses were performed.

Materials and methods

Patient selection Eligible patients had a histologically documented, advanced solid malignancy refractory to standard therapy or for which no curative therapy existed. Other inclusion criteria included: age ≥ 18 years; Eastern Cooperative Oncology Group performance status 0 to 2; adequate hematologic, hepatic and renal functions (WBC ≥ 3,000/μl, absolute neutrophil count ≥ 1,500/μl, platelets ≥ 100,000/μl, total bilirubin within institutional normal limit, AST/ALT ≤ 2.5 × the institutional upper limit of normal, creatinine ≤ 1.5 mg/dl or creatinine clearance ≥ 60 ml/min/1.73 m^2 for patients with creatinine levels above institutional normal); and life expectancy greater than 12 weeks.

Exclusion criteria included untreated brain metastasis; chemotherapy or radiation therapy within 4 weeks; history of myocardial infarction; severe pulmonary disease requiring oxygen supplementation; active infection; and any serious concomitant conditions that would place the patient at excessive or unacceptable risk of toxicity. Patients were required to practice effective birth control.

Patients provided written informed consent. The protocol was approved by the Health Sciences Institutional Review Board at the University of Wisconsin-Madison.

Study design and patient evaluation This was a phase I, dose-escalation trial. A fixed dose of vorinostat (400 mg) was administered orally on days 1–14. During cycle 1, increasing doses of bortezomib were administered as an IV bolus on days 2, 5, 9 and 12 to evaluate vorinostat pharmacokinetics alone and in combination with bortezomib. In all subsequent cycles, bortezomib was administered on days 1, 4, 8 and 11. Cycle length was 21 days. Four dose levels of bortezomib were evaluated: 0.7, 1, 1.3 and 1.5 mg/m^2. No intra-patient dose escalation occurred. Dose escalation of bortezomib followed the standard 3+3 rule. The MTD was defined as the highest safely tolerated dose at which no more than one patient out of six experienced dose-limiting toxicity, with the next higher dose having at least two out of six patients experience dose DLT.

Adverse events were evaluated using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), v3.0. DLTs were defined as one of the following adverse events occurring during the first cycle: absolute neutrophil count ≤ 500 for ≥ 7 days; febrile neutropenia or ≥ grade 3 neutropenic infection; platelets ≤ 25,000 or thrombocytopenic bleeding; nonhematologic toxicity ≥ grade 3 except nausea, vomiting, or diarrhea associated with suboptimal premedication and/or management; AST/ALT elevations ≥ grade 3 or higher for > 7 days; toxicity leading to two or more missed doses per cycle; and toxicity resulting in the delay of the subsequent cycle by > 7 days. Response was assessed using the Response and Evaluation Criteria in Solid Tumors (RECIST) 1.0.

Dose modification For dose-escalation to occur, three assessable patients had to complete their first cycle without DLT. With each DLT, three additional patients were accrued, and further escalation could occur if no more DLTs were observed. Patients who experienced DLT were delayed by 1-week intervals until recovery and then allowed to continue on study with dose reduction in either vorinostat or bortezomib. Patients were removed from study following a delay of more than 2 weeks for recovery from toxicity related to treatment. In addition, patients were required to have an absolute neutrophil count ≥ 1,000/mm^3 and a platelet count ≥ 50,000/mm^3 on day 8 of each cycle.

Pretreatment and follow-up studies History, physical examination, weight, estimation of ECOG performance status, and laboratory studies were obtained at baseline and at the beginning of subsequent cycle. Serum pregnancy testing for women of childbearing age and an EKG were obtained at baseline.

Patients who completed at least one cycle followed by 2 weeks of observation were considered evaluable for toxicity. Baseline imaging was performed within 28 days prior to the start of treatment, and all tumor assessments were re-evaluated every 6 weeks thereafter. All patients with responding tumors (CR and PR) were required to have response confirmed 4 weeks after the first documented response.

Duration of treatment Study treatment continued until disease progression, unacceptable adverse event, withdrawn consent, or changes in the patient's condition including intercurrent illness rendering the continuation of study treatment unacceptable.

Pharmacokinetic analysis Blood samples for vorinostat PK analysis were collected on cycle 1, day 1, in the absence of bortezomib, and on and days 2 and 12, with bortezomib. PK sampling was performed before and 0.25, 0.5, 0.75, 1, 2, 4, 6, and 8 h following vorinostat administration. Concentrations of vorinostat and its metabolites (vorinostat glucuronide and 4-anilino-4-oxobutanoic acid) were quantitated with a liquid chromatography-electrospray ionization tandem mass spectrometric method as previously described [32].

Statistical methods The primary outcome measure of this study was assessment of toxicity. The number and severity of toxicity incidents determined the level of tolerance for vorinostat and bortezomib and were categorization via CTC standard toxicity grading. The number of treatment anti-tumor responses served as the secondary outcome measure and were summarized by simple descriptive summary statistics delineating complete and partial responses as well as stable and progressive disease.

Pharmacokinetic analysis for vorinostat and its metabolites was performed by noncompartmental methods using the WinNonlin program, version 5.2 (Pharsight, Cary, NC), and data were summarized using means ± standard deviations. The comparison of PK parameters between time points was performed using a non-parametric Wilcoxon sign rank test. The comparison of PK parameters between patients with a DLT and patients without a DLT was performed using a non-parametric Wilcoxon rank sum test. Statistical data analyses were two-sided and were performed using SAS statistical software (version 9.2, SAS Institute Inc, Cary, NC), and P-values <0.05 were considered significant.

Results

Patient characteristics Twenty-three patients were enrolled and received a total of 57 cycles of therapy (median, 2; range 1 to 6). Demographics and pretreatment characteristics are shown in Table 1. One patient at level 2 was unevaluable, but all patients were included in the safety analysis. The dose escalation schema and the number of PK dosing days are listed in Table 2.

Dose escalation and toxicity Four dose levels ranging from bortezomib 0.7 to 1.5 mg/m^2 with a fixed dose (400 mg) of vorinostat were evaluated (Table 2). The most common toxicities are shown in Table 3. No DLTs were observed at the first dose level. At dose level 2 (bortezomib 1 mg/m^2), one patient was unevaluable due to pneumonia preventing completion of cycle 1, and one patient experienced a DLT (grade 3 fatigue). Three additional patients were enrolled at this dose level without significant toxicity in cycle 1. Dose-limiting grade 3 fatigue occurred during cycle 1 in the first person enrolled at dose level 3 (bortezomib 1.3 mg/m^2). This dose level was expanded to six patients without further DLTs. Two of three patients enrolled at dose level 4 (bortezomib 1.5 mg/m^2) experienced DLTs (grade 3 fatigue and asymptomatic grade 3 hyponatremia). Therefore, the MTD was vorinostat 400 mg and bortezomib 1.3 mg/m^2. Dose level 3 (the MTD)

Table 1 Patient characteristics

Characteristic	No.	%
No. of patients	23	
Median age, year	61	
Range	22–74	
Sex		
Male	14	61
Female	9	39
Performance status		
0	3	13
1	18	78
2	2	9
Primary tumor type		
Colorectal	6	26
Sarcoma	4	17
Pancreas	2	9
Non-small cell lung	2	9
Head and neck	2	9
Other[a]	7	30
Prior systemic[b] therapy		
0	0	0
1	4	17
2	2	9
3	6	26
4	3	13
5	2	9
≥6	6	26

[a] One each of bladder, gastric, GIST, ovarian, germ cell, mesothelioma and lymphoma

[b] Includes conventional chemotherapy, cytokine-based immunotherapy, and experimental cytotoxic chemotherapy

was expanded to 10 total patients in order to further characterize PKs and toxicity.

Safety The most frequent adverse events at least possibly related to study drugs during cycle 1 are described in Table 3. Thrombocytopenia and anemia were the most common hematologic toxicities. Most hematologic events were grade 1 or 2, but grade ≥ 3 thombocytopenia was seen during three cycles of bortezomib at 1.3 mg/m^2. Grade 1 or 2 nausea, vomiting, fatigue, constipation, anorexia, diaphoresis and diarrhea were the most common non-hematologic toxicities encountered. Few adverse events were reported at dose level 1, but toxicities increased in frequency and severity with escalating doses of bortezomib. Three patients (one at dose level 2 and two at dose level 3) reported grade 1/2 sensory neuropathy during the first or second cycles. Another patient at dose level 2 developed grade 2 neuropathic pain during cycle 6 necessitating discontinuation of therapy despite clinical benefit. Cumulative toxicities included low-grade nausea, fatigue and sensory neuropathy, but there did not appear to be an affect on myelosuppression with prolonged treatment.

Efficacy Two of twenty-two evaluable patients had confirmed partial responses (PR), and one had evidence of stable disease (SD). One patient with metastatic high grade malignant fibrous histiocytoma who had multiple resections, prior radiation, and systemic therapy with doxorubicin, ifosfamide and VP-16 had a confirmed PR at level 2 with a 37.2 % decrease in tumor size following 2 cycles and > 50 % decrease after 6 cycles that was durable for > 12 months. Treatment was discontinued following cycle 6 due to grade 2 neuropathic pain that persisted for 18 months. A second patient with previously-treated moderately-differentiated squamous cell carcinoma of the lung with bilateral pulmonary nodules and a right-sided malignant pleural effusion at dose level 3 and had a confirmed PR with resolution of a malignant pleural effusion and > 35 % shrinkage of pulmonary nodules following 2 cycles which lasted 8 months. Treatment was discontinued after 4 cycles due to grade 2 fatigue. A patient with heavily-pretreated metastatic colorectal cancer had SD following 2 cycles but ultimately elected to stop treatment during cycle 4 due to worsening fatigue and sensory neuropathy.

Table 2 Dose escalation schema and frequency of dose limiting toxicities

Dose level	n	Vorinostat (mg)[a]	Bortezomib (mg/m^2)[b]	Courses No.	No. of Patients with DLTs (cycle 1)	Description of DLTs (cycle 1)
1	3	400	0.7	8	0	–
2	7[c]	400	1	17	1	Gr.3 Fatigue
3[d]	10	400	1.3	24	1	Gr.3 Fatigue
4	3	400	1.5	8	2	Gr.3 Fatigue; Gr.3 Hyponatremia

[a] Administered orally once daily on days 1–14

[b] Administered i.v. on days 1, 4, 8 and 11 of a 21 day cycle

[c] One patient was unevaluable

[d] MTD

Table 3 Drug-related adverse events, worst grade per patient during Cycle 1

Bortezomib (mg/m^2)	0.7			1.0			1.3			1.5			Total, % (n=23)
Selected toxicities	G1	G2	G3	G1	G2	G3	G1	G2	G3	G1	G2	G3	
Hematologic													
Thrombocytopenia								2	3[a]		2		7 (30.4)
Anemia		1			1			1			1		3 (13)
Non-Hematologic													
Nausea	2			5			4	1		2		1	15 (65.2)
Fatigue				3		1	2	2	1		1	1	11 (47.8)
Constipation			1	3			2			1			7 (30.4)
Anorexia	1			1	1		2	1			1		7 (30.4)
Diaphoresis/flushing					3		3			2			8 (34.8)
Diarrhea							2	2		1			5 (21.7)
Vomiting				1			2		1			1	5 (21.7)
Hyponatremia												1	1 (4.3)

[a] Two Grade 3 and One Grade 4 Thrombocytopenia

G Grade

Vorinostat pharmacokinetics Pharmacokinetics are presented in Table 4. Evaluation of day 1 plasma concentrations compared with day 2 plasma concentrations to assess the influence of bortezomib on vorinostat PKs showed no difference in vorinostat or its glucuronide metabolite plasma concentrations between the days. However, the AUC and Cmax values for the acid moiety were significantly higher following administration of bortezomib on day 2 of cycle 1 (AUC: $p<0.05$; Cmax: $p<0.05$). Day 1 (vorinostat single dose) plasma concentrations were compared to day 12 (vorinostat steady state) plasma concentrations to assess accumulation with chronic dosing. Both vorinostat and its acid metabolite had significantly higher AUC and Cmax values on day 12 when compared to day 1, cycle 1 when vorinostat was administered alone (AUC: $p<0.05$; Cmax: $p<0.05$).

The relationship of vorinostat plasma concentrations to toxicity was also assessed (Table 5). Both the vorinostat AUC and C_{max}, but not the acid or glucuronide metabolites, were significantly higher in individuals experiencing a DLT (AUC: $p<0.05$; Cmax: $p<0.05$) on all days of treatment when compared to those subjects who did not experience DLTs.

Table 4 Pharmacokinetic parameters in plasma and in patients receiving vorinostat in combination with bortezomib

	Cmax (ng/mL)	Tmax (hr)	AUC (ng/mL x hr)	T1/2 (hr)	Cl/F (L/min)
Vorinostat					
C1D1 (n=19)	299±153	1.4±1.0	1049±444	1.7±0.7	6.4±3.2
C1D2 (n=19)	303±204	1.0±0.9	1115±507	1.3±1.0	11±4.1
C1D12 (n=14)	323±307#	2.0±1.0	1412±924#	1.9±1.0	13±3.3
Vorinostat glucuronide					
C1D1 (n=7)	1153±922	1.9±1.0	4843±3034	1.9±1.4	NA
C1D2 (n=7)	1225±496	3.2±1.8	5283±2915	1.6±0.5	NA
C1D12 (n=4)	849±479	2.0±1.0	4169±1548	2.0±0.8	NA
Vorinostat acid					
C1D1 (n=15)	815±282	2.8±1.4	17495±4153	13.8±33.9	NA
C1D2 (n=22)	1098±439*	3.2±1.5	9298±4841*	5.01±4.41	NA
C1D12 (n=14)	960±272#	3.5±2.1	9714±5069#	5.73±4.17	NA

*$p<0.05$, comparing Day 1 to Day 2 (vorinostat alone to vorinostat + bortezomib)

#$p<0.05$, comparing Day 1 to Day 12 (vorinostat single dose to vorinostat steady state)

Cmax concentration maximum; *Cl* clearance; *AUC* area under the plasma concentration time curve from 0–∞, infinity; *T1/2* half-life; *Tmax* time of maximum concentration

Table 5 Vorinostat pharmacokinetic parameters and dose limiting toxicities

Day	DLT (n)	Mean AUC (ng/mL x hr)	Cmax (ng/mL)
C1D1	No DLT (16)	999±438	322±118
	DLT (3)	1450±277	551±174
C1D2	No DLT (16)	983±418	340±1 17
	DLT (3)	1788±436	675±363
C1D12	No DLT (13)	1414±962	448±314
	DLT (1)	2229	905

C cycle; *D* day; *DLT* dose limiting toxicity; *AUC* area under the curve; *cmax* concentration maximum

Discussion

This phase I study showed that vorinostat with bortezomib is well-tolerated up to standard doses of each agent. The MTD was established as vorinostat 400 mg PO daily on days 1–14 and bortezomib 1.3 mg/m^2 IV on days 1, 4, 8 and 11 of a 21 day cycle. Dose limiting toxicities included fatigue and hyponatremia. The most common grade 1/2 toxicities were nausea, fatigue, diaphoresis, anorexia and constipation, which is consistent with documented side effects of these agents in other single-agent studies [33, 34] and were not more frequent or severe when given in combination. The most common hematologic toxicities included anemia and thrombocytopenia. The grade and frequency of myelosuppression was consistent with observations from single agent bortezomib studies. Vorinostat has not been associated with significant myelosuppression, and our results do not suggest that vorinostat exacerbated the expected myelosuppression of bortezomib. The uncommon occurrence of sensory neuropathy, a DLT of bortezomib, was likely related to the minimum duration of therapy in this phase I study.

While this combination was well tolerated, patients only received a mean number of two cycles of therapy. One patient at dose level 1 received four cycles without difficulty and was discontinued due to PD. Another at dose level 2 received six cycles and ultimately elected to stop treatment due to persistent grade 2 neuropathic pain. Two patients at dose level 3 received four cycles. One patient elected to stop treatment due to persistent grade 2 fatigue, and the other patient tolerated treatment well without dose modifications and came off study due to PD. One patient at dose level 4 tolerated 6 cycles and came off of study with disease growth. Based on these results, the MTD is the recommended phase 2 dose. However, it is possible that more pronounced cumulative toxicities, including myelosuppression, fatigue and sensory neuropathy, will be observed with prolonged dosing in a different patient population.

Two patients in this study had objective responses. One patient with chemotherapy-refractory malignant fibrous histiocytoma enrolled at level 2 had a confirmed PR following 2 cycles, and another patient with previously-treated advanced NSCLC (squamous) enrolled at dose level 3 had a confirmed PR following 2 cycles lasting 8 months. While vorinostat or bortezomib monotherapy is efficacious in hematologic malignancies, limited clinical activity has been observed in solid tumors in single-agent studies. A phase II study of bortezomib in metastatic soft tissue sarcomas was stopped early when only one PR was observed in 21 evaluable patients [35]. Likewise, no objective antitumor activity was detected in a phase II study of vorinostat in refractory NSCLC [36]. Our responses suggest that these agents may have additive or synergistic activity in solid tumors and warrant further evaluation.

Consistent with findings reported by Ramalingam and colleagues [37], plasma levels of vorinostat accumulated with chronic dosing. Interestingly, vorinostat plasma concentrations were statistically associated with toxicity. Both the Cmax and AUC were higher in patients experiencing a DLT across all days of treatment. This demonstrates that a standard dose results in variable plasma concentrations and suggests that individualization of vorinostat dosing may be helpful in decreasing toxicity. In this study, both vorinostat and bortezomib were administered on standard doses and schedules. An alternate dosing schedule of vorinostat was evaluated on a second portion of this study which is reported in an accompanying to determine whether treatment would be better tolerated with varying doses of vorinostat administered around bortezomib administration. Metabolite concentrations did not predict toxicity, although we only characterized the glucuronide in seven subjects and the sample size may not have been sufficient to identify a difference. Additionally, the Cmax values for the acid metabolite were significantly higher following administration of bortezomib on day 2, when compared to day 1 when vorinostat was administered as a single agent (AUC: $p < 0.05$; Cmax: $p < 0.05$, non-parametric Wilcoxon signed rank test, two-tailed). This can be explained by the long half-life of the acid metabolite, with mean baseline plasma concentration on Day 2 of 130±68 ng/mL. The clinical significance of this finding is unclear, as plasma concentrations of the acid metabolite were not associated with toxicity.

Based on the clinical activity observed in this study, two phase II clinical trials are currently being conducted using this combination, one in advanced soft tissue sarcoma and one in advanced NSCLC. In both studies, vorinostat and bortezomib will be administered at the MTD doses established in this trial. We are also expanding this phase I study in advanced solid tumors to evaluate an alternate dosing schedule of vorinostat given twice daily on days 1–4 and 8–11 along with bortezomib, with the aim of further

optimizing the potential synergistic effect of these agents while minimizing toxicity.

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