A Phase 1 Pharmacokinetic and Pharmacodynamic Study of the Histone Deacetylase Inhibitor Belinostat in Patients with Advanced Solid Tumors

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Abstract

Purpose: To determine the safety, dose-limiting toxicity, maximum tolerated dose, and pharmacokinetic and pharmacodynamic profiles of the novel hydroxamate histone deacetylase inhibitor belinostat (previously named PXD101) in patients with advanced refractory solid tumors.

Experimental Design: Sequential dose-escalating cohorts of three to six patients received belinostat administered as a 30-min i.v. infusion on days 1 to 5 of a 21-day cycle. Pharmacokinetic variables were evaluated at all dose levels. Pharmacodynamic measurements included acetylation of histones extracted from peripheral blood mononuclear cells, caspase-dependent cleavage of cytokeratin-18, and interleukin-6 levels.

Results: Forty-six patients received belinostat at one of six dose levels (150-1,200 mg/m^2/d). Dose-limiting toxicities were grade 3 fatigue (one patient at 600 mg/m^2; one patient at 1,200 mg/m^2), grade 3 diarrhea combined with fatigue (one patient at 1,200 mg/m^2), grade 3 atrial fibrillation (one patient at 1,200 mg/m^2; one patient at 1,000 mg/m^2), and grade 2 nausea/vomiting leading to inability to complete a full 5-day cycle (two patients at 1,000 mg/m^2). The maximum tolerated dose was 1,000 mg/m^2/d. I.v. belinostat displayed linear pharmacokinetics with respect to C_{max} and AUC. The intermediate elimination half-life was 0.3 to 1.3 h and was independent of dose. Histone H4 hyperacetylation was observed after each infusion and was sustained for 4 to 24 h in a dose-dependent manner. Increases in interleukin-6 levels were detected following belinostat treatment. Stable disease was observed in a total of 18 (39%) patients, including 15 treated for $\geq$4 cycles, and this was associated with caspase-dependent cleavage of cytokeratin-18. Of the 24 patients treated at the maximum tolerated dose (1,000 mg/m^2/d), 50% achieved stable disease.

Conclusions: I.v. belinostat is well tolerated, exhibits dose-dependent pharmacodynamic effects, and has promising antitumor activity.

Aberrant patterns of histone modification are a feature of cancer cells. Histone deacetylase (HDAC) enzymes catalyze the removal of an acetyl group from the terminal lysine residues of histone proteins, leading to more compact chromatin and repression of associated genes. Inhibitors of HDAC enzymes alter patterns of gene expression, induce cellular differentiation, and promote cell cycle arrest and apoptosis (1). Many inhibitors of apoptosis are repressed by HDAC inhibitors and proapoptotic genes are activated (2). Pharmacologic inhibition of histone deacetylation may therefore regulate gene expression patterns and, subsequently, cellular characteristics, making them attractive anticancer therapies. HDAC inhibitors affect the transcription of only a small number of genes (3), which may be a factor in their selectivity for cancer cells. In addition to this, acetylation of nonhistone proteins [e.g., p53 (4) and Rb (5)] may play a role in their antitumor activity.

Belinostat (previously PXD101) is a novel hydroxamic acid HDAC inhibitor with potent antiproliferative and HDAC inhibitory activities *in vitro* (6). Belinostat has growth inhibitory and proapoptotic activities in a variety of human tumor cell lines at nanomolar concentrations. *In vivo*, belinostat inhibits growth in human tumor xenografts without apparent toxicity to the host mice (6). Growth inhibition *in vitro* and *in vivo* is associated with a marked increase in the level of acetylation of histone proteins (6).

The primary objective of this phase 1 trial was to determine the safety, toxicity, dose-limiting toxicity, and maximum

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Note: N.L. Steele and J.A. Plumb contributed equally to this work.

Preliminary results from this study were presented at the Annual Meeting of the American Society for Clinical Oncology, 2005, and at the National Cancer Institute-AACR-European Organization for Research and Treatment of Cancer Molecular Targets and Cancer Therapeutics Meeting, 2005.

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tolerated dose of i.v. belinostat. In initial human tumor xenograft studies, mice were treated daily with i.p. belinostat for a total of 7 days (6). This led to significant, dose-dependent growth delay without apparent toxicity to the host mice. Toxicology studies using the belinostat clinical formulation in dogs found the maximum tolerated dose of belinostat given as a 30-min infusion daily for 5 days to be 50 mg/kg (human equivalent of 1,000 mg/m^2). Based on these studies, belinostat was administered as a 30-min infusion on days 1 to 5 of a 21-day cycle with a cautious starting dose of 150 mg/m^2/d. Whereas continuous dosing might be desirable to enable prolonged target inhibition, this schedule was chosen as the most practical for protracted i.v. administration based on the available preclinical toxicology. Secondary objectives included pharmacokinetic and pharmacodynamic profiles and antitumor activity.

Patients and Methods

Patient eligibility

The study was approved by the Research Ethics Committees at both participating institutions. Eligible patients were those with histologically or cytologically confirmed advanced malignancy refractory to standard therapy or for whom no standard therapy existed. Other inclusion criteria included age ≥18 years, Eastern Cooperative Oncology Group performance status ≤2, estimated life expectancy of ≥3 months, and written informed consent. Adequate bone marrow, hepatic, and renal function for trial entry was defined as hemoglobin ≥9.0 g/dL, absolute neutrophil count ≥1.5 × 10^9/L, platelets ≥100 × 10^9/L, total bilirubin ≤1.5 × upper limit of normal, aspartate aminotransferase and alanine aminotransferase ≤2.5 × upper limit of normal (or ≤5 × upper limit of normal if liver metastases), and serum creatinine ≤1.5 × upper limit of normal. Female patients of reproductive potential were required to have a negative pregnancy test.

Patients were excluded from the trial if they had received any anticancer therapy within the preceding 4 weeks. Continuation of bisphosphonates, luteinizing hormone–releasing hormone agonists, or corticosteroids was permitted provided dosing was stable before and during the trial. Other exclusions were coexisting illness likely to interfere with trial procedures; uncontrolled brain metastases; persistent neuropathy of any cause, grade ≥2 as measured by National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0; lactating women; and known HIV infection.

Study design

Belinostat was administered i.v. as a 30-min infusion daily on days 1 to 5 of a 21-day cycle. The starting dose was 150 mg/m^2. Sequential cohorts of three to six patients were entered into one of the escalating dose steps. Dose escalation was intended in 100% increments until grade 2 toxicity was observed, at which point dose escalation was to proceed in 50% increments until grade 3 toxicity was seen, following which further dose increments would be of 33%.

Toxicities were graded and reported according to NCI-CTCAE version 3.0 per investigators assessments. Dose-limiting toxicity was based on the toxicities observed in the first 21-day cycle. Dose-limiting toxicity was defined as grade 4 neutropenia for ≥7 days, or with sepsis; grade 4 thrombocytopenia, any drug-related grade 3 or 4 nonhematologic toxicity (except nausea and vomiting, rash, arthralgias, or myalgias without appropriate treatment or prophylaxis); grade 4 diarrhea despite maximal supportive therapy, inability to tolerate a full 5-day dosing course due to toxicity; or any drug-related adverse event resulting in more than 14 days of treatment delay. The maximum tolerated dose was defined as the dose below that in which ≥2 of 3 or ≥2 of 6 patients experienced dose-limiting toxicity.

Patient assessments and response criteria

Pretreatment evaluation included history, physical examination, assessment of performance status, chest X-ray, electrocardiogram, urinalysis, and tumor evaluation. Laboratory studies included full blood count, clotting screen, measurement of CD3, CD4, and CD8, urea, creatinine, electrolytes, phosphate, liver function tests, uric acid, glucose, and cholesterol.

Laboratory studies were repeated on the first day of treatment and weekly thereafter. Although preclinical data did not suggest any adverse effects of belinostat on cardiac function, the electrocardiogram schedule was intensified to include electrocardiograms immediately and 2 h following each infusion of belinostat in response to emerging data about the possible cardiac toxicity of other HDAC inhibitors. Clinical tumor assessment was carried out at each cycle with radiologic evaluation every two cycles. Efficacy was assessed in patients with measurable disease using Response Evaluation in Solid Tumors criteria (7).

Drug preparation

Belinostat was supplied by Topotarget as 10-mL vials, each containing 50 mg/mL belinostat and the solubilizer L-arginine (100 mg/mL). Belinostat was stored below 5°C. The assigned dose of belinostat was added to a 250-mL bag of sterile sodium chloride 0.9% solution or 5% dextrose solution and immediately used.

Pharmacokinetic studies

Pharmacokinetic samples were taken from ≥3 patients at each dose level. Heparinized blood samples (5 mL) were collected pretreatment and at 0, 5, 15, 30, 60, 90 min and 2, 4, 6, 8, and 10 h postinfusion on day 1 of the first treatment cycle. Samples were also collected before treatment on days 2, 3, and 4. On day 5, the pharmacokinetic sampling schedule was the same as on day 1. Samples were also collected on day 6 and on days 8, 15, and 22. Once preliminary results were available, the sampling schedule was simplified, omitting day 3, 4, 6, 8, 15, and 22 samples and restricting day 5 sampling to a pretreatment sample. Samples were placed on ice, then centrifuged at 4°C. Two-milliliter aliquots of plasma were stored at -20°C until analysis.

Belinostat was extracted from human plasma by solid-phase extraction and quantified by a reversed phase high-performance liquid chromatographic method with tandem mass spectrometry detection in electrospray ionization negative mode (317 > 143 m/z). The limit of quantification was 5 ng/mL. Intra-assay precision values, based on coefficients of variation of quality control samples, were ≤19.7%.

The concentration of belinostat was determined in the urine of patients collected over a 12-h period preinfusion and 0 to 8, 8 to 24, and 24 to 48 h postinfusion. Volume of each collection was recorded and 10-mL samples were stored at -20°C until analysis. High-performance liquid chromatography-tandem mass spectrometry was used to quantitate belinostat with a limit of quantification of 5 ng/mL.

Pharmacokinetic calculations were done using a noncompartmental method (WinNonLin version 5.1.0). The area under the plasma concentration time curve (AUC) was calculated from end of infusion using the linear trapezoid method and the elimination half-life by the log-linear method.

Pharmacodynamic studies

Histone acetylation. Histone acetylation was evaluated by Western blotting for histone H4 on histones isolated from peripheral blood mononuclear cells. Samples for histone acetylation were taken on day 1 pretreatment and at 0, 5, 15, 30, 60, 90 min and 2, 4, 6, and 24 h postinfusion. Blood samples (6 mL) were collected in lithium heparin vacutainer tubes, placed on ice and processed immediately. Red cells were lysed in Cell Lysis Buffer (Promega) for 10 min. Samples were centrifuged at 2,000 × g for 10 min and the cell pellet was used for histone isolation. Histone extraction was carried out with a modification of the method of Yoshida et al. (8) and acetylated histones detected by Western blotting as previously described (6). The primary antibody

Table 1. Patient characteristics

Characteristic	No. patients
Total	46
Age (y)	
Median	58.5
Range	25-74
Sex	
Male	26
Female	20
ECOG performance status	
0	11
1	32
2	3
Tumor Type	
Colorectal	12
Melanoma	6
Renal	6
Esophago-gastric	4
Sarcoma	4
Prostate	3
Ovary	2
Thymoma	1
Pseudomyxoma peritonei	1
Breast	1
Carcinoma of unknown primary	1
Germ cell	1
Cervix	1
Mesothelioma	1
Lung (non–small cell)	1
Diffuse large B-cell lymphoma	1

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

was anti–acetyl histone H4 Lys^5, Lys^8, Lys^{12}, and Lys^{16} (Upstate Biotechnologies). Autoradiographs were scanned and bands quantified by densitometry (PDI 420 OE Gel Scanner with PDI MS DOS software). Densitometry results were expressed relative to a control sample (histones extracted from A2780 ovarian cancer cell line treated for 1 h with belinostat 0.2 μmol/L). The same cell line standard was used in all blots. AUC for histone acetylation was calculated by noncompartmental analysis using WinNonLin version 4.0 software (Pharsight) and the key time points for the calculation were identified.

Measurement of caspase-dependent cleaved cytokeratin-18 in plasma. Caspase-dependent cleavage of cytokeratin-18 is a potential serum measure of the level of apoptosis in epithelial tumors (9) and was examined pretreatment on days 1 and 2 and on day 8. The blood was centrifuged at 1,500 × *g* for 10 min and the plasma collected and stored at -70°C. The apoptotic cytokeratin-18 neoepitope level in plasma samples was determined with the M30 Apoptosense ELISA (Peviva AB) according to the manufacturers' instructions.

Cytokine detection. Cytokine expression was determined in plasma samples by an antibody array technique that detects 40 inflammatory factors (RayBio Human Cytokine Array I and RayBio Human Inflammation Array III). Plasma samples were applied to the membranes and incubated overnight at 4°C. They were then processed exactly according to the manufacturers' instructions. Plasma levels of interleukin 6 (IL-6) were quantified by ELISA (RayBio Human IL-6 ELISA Kit) according to the manufacturers' instructions.

Results

Patient characteristics

Forty-eight patients were enrolled in the study between October 2003 and February 2006. Two patients never received treatment due to lack of venous access and detection of brain metastases, respectively. Forty-six patients received a total of 158 cycles (median treatment duration, 2 cycles; range, 1-18) of belinostat. Baseline patient characteristics are summarized in Table 1.

Dose-limiting toxicity and maximum tolerated dose

Patients were treated at a total of six dose levels (Table 2). During the dose escalation phase, dose-limiting toxicities were seen in one patient treated at 600 mg/m^2 (grade 3 fatigue) and in three patients at 1,200 mg/m^2/d. Among the patients in the 1,200 mg/m^2 cohort, one developed palpitations and supraventricular tachycardia shortly after the end of the second infusion. The patient subsequently developed atrial fibrillation with no hemodynamic compromise before reverting spontaneously to sinus rhythm. The second patient had grade 3 diarrhea with fatigue leading to inability to complete the 5-day study drug administration. The third patient had grade 3 fatigue starting on day 21 of cycle 1, resolving on day 25 to grade 1 allowing cycle 2 to commence.

After six patients had been treated at the intermediate dose level of 1,000 mg/m^2/d in the dose escalation phase without any dose-limiting toxicities experienced, this dose level was expanded to include an additional 18 patients. This number of patients was chosen to allow evaluation of possible drug activity in a broad range of tumor types. In addition, expansion to include this number of patients in the cohort allowed us to carry out preliminary studies comparing the pharmacokinetics and pharmacodynamics of i.v. administration of belinostat with a number of schedules of oral administration in the second or subsequent treatment cycles. Among these 18 patients, three dose-limiting toxicities occurred: atrial fibrillation (no sequelae, no specific treatment required), nausea, and vomiting (each leading to inability to complete the 5-day cycle). Based on these data, the maximum tolerated dose (recommended phase II dose) for i.v. belinostat is 1,000 g/m^2/d administered on days 1 to 5 of a 21-day cycle.

Safety and tolerability

The most common treatment-related adverse events (observed in ≥15% of patients) are summarized in Table 3. Other

Table 2. Dose escalation and dose-limiting toxicity

Dose level (mg/m^2/d; days 1-5)	No. patients	Dose-limiting toxicity
Dose escalation phase		
150	4	None
300	4	None
600	6	Fatigue (*n* = 1)
900	3	None
1,200	5	Atrial fibrillation (*n* = 1) Diarrhea + Fatigue (*n* = 1) Fatigue (*n* = 1)
1,000	6	None
Maximum tolerated dose expanded cohort		
1,000	18	Atrial fibrillation (*n* = 1) Vomiting (*n* = 1) Nausea (*n* = 1)

Table 3. Adverse events with a frequency of ≥15% (most severe per patient during entire study treatment time) definitely, probably, or possibly related to belinostat at all dose levels in all cycles, graded according to NCI-CTCAE version 3.0

Dose level:	150 mg/m²			300 mg/m²			600 mg/m²			900 mg/m²			1,000 mg/m²			1,200 mg/m²			Total
No. courses:	9			9			16			22			80			22			158
No. patients:	4			4			6			3			24			5			46
NCI-CTCAE grade:	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	Total AEs (%)
Nausea	1	0	0	2	0	0	5	1	0	1	2	0	10	6	0	4	1	0	33 (72)
Vomiting	1	1	0	1	0	0	4	1	0	1	2	0	13	5	0	3	1	0	33 (72)
Lethargy	2	0	0	2	0	0	2	2	0	1	0	0	4	1	0	0	0	1	15 (33)
Fatigue	1	1	0	1	0	0	0	0	1	1	1	0	3	1	0	2	0	1	13 (28)
Constipation	1	1	0	1	0	0	3	0	0	1	1	0	3	2	0	0	0	0	13 (28)
Flushing	0	0	0	0	0	0	3	0	0	0	0	0	6	0	0	2	0	0	11 (24)
Diarrhea	2	0	0	1	0	0	0	0	0	0	0	0	1	2	0	0	0	1	7 (15)

NOTE: No related grade 4 adverse events were experienced by any patient at any dose level.
Abbreviation: AE, adverse event.

common adverse events (frequency ≥5%) were pyrexia, anorexia, headache, phlebitis, dysgeusia, tachycardia, abdominal pain, chills, hyperhidrosis, rash, hypotension, infusion site phlebitis, and injection site thrombosis.

There were no related grade 4 adverse events observed, and only 6 (13%) patients experienced a related grade 3 event (Table 3), namely, fatigue (two patients), lethargy with diarrhea (one patient), atrial fibrillation (one patient), infection (one patient), and prolonged corrected QT interval (one patient in one single electrocardiogram 23 h after dosing in cycle 5, not observed in subsequent cycles or in 68 other posttreatment electrocardiograms in this patient). No significant drug-related hematologic toxicity was observed at any dose level. Diarrhea was managed using standard antidiarrheal medication. No patient required hospital admission due to diarrhea. Nausea and vomiting occurred most commonly toward the end of the infusion of belinostat and rarely persisted beyond day 5 of each cycle. Symptoms were managed with standard antiemetic therapy according to local unit policy.

The most frequent grade 3/4 laboratory abnormalities (shift from baseline) were hyperglycemia [experienced by 4 (9%) patients; grade 4 in one patient] and hyponatremia [experienced by 3 (7%) patients; no grade 4]. Grade 3 lymphopenia and increased alkaline phosphatase were each experienced by one patient each, and grade 4 hypokalaemia, hypophosphatemia, and increased prothrombin time also by one patient each. No significant drug-related hematologic toxicity was observed at any dose level. No significant consistent changes in CD3, CD4, or CD8 levels were observed in response to belinostat treatment.

Pharmacokinetics

Pharmacokinetic properties of belinostat are summarized in Table 4. Belinostat displays three-compartment pharmacokinetics. The intermediate elimination half-life varied between 0.3 and 1.3 h and was independent of dose. Data did not allow reliable determination of the distribution half-life and the elimination half-life due to the limited number data points available during the distribution and elimination phases. Belinostat displays dose-proportional pharmacokinetics with respect to AUC and C_{max}. Drug accumulation was assessed in a limited number of patients at various dose levels. The average ratio (±SD) between AUC_{INF} day 5 and AUC_{INF} day 1 was found to be 107% (±18.5%). Belinostat appeared in urine during the first 8-h postinfusion collection period. The excreted parent drug ranged from ~0.2% to 2.0% of total belinostat administered. No trend toward altered urinary excretion following repeat dosing was observed.

Table 4. Pharmacokinetic variables for belinostat (i.v. administration; mean ± SD for a single i.v. dose on day 1)

No. patients	Dose (mg/m²)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2(\beta)}$ (h)	V_z (L/m²)	CL_s (L/h/m²)	AUC_{0-t} (ng h/mL)	AUC_{INF} (ng h/mL)
4	150	6,010 (±6,235)	0.02 (±0.02)	0.45 (±0.39)	90.0 (±82.2)	132.1 (±135.3)	1,212 (±1,177)	1,220 (±1,184)
4	300	14,777 (±4,704)	0.00 (±0.00)	0.54 (±0.09)	80.7 (±47.0)	99.4 (±43.2)	3,414 (±1,275)	3,422 (±1,276)
3	600	28,611 (±6,425)	0.00 (±0.00)	0.52 (±0.16)	43.4 (±17.1)	58.9 (±18.4)	11,002 (±3,434)	11,008 (±3,435)
3	900	46,278 (±17,893)	0.00 (±0.0)	0.90 (±0.32)	76.9 (±16.5)	62.3 (±17.7)	15,164 (±3,753)	15,181 (±3,748)
22	1,000	32,124 (±9,128)	0.00 (±0.00)	0.69 (±0.22)	113.9 (±60.9)	110.5 (±34.4)	9,993 (±3,335)	9,990 (±3,420)
5	1,200	53,793 (±11,474)	0.02 (±0.04)	0.79 (±0.25)	89.2 (±58.2)	73.4 (±30.0)	19,210 (±8,894)	19,240 (±8,895)

NOTE: T = 0 h corresponds to the time for end of infusion. $T_{1/2(\beta)}$ is the intermediate elimination half life.

Pharmacodynamic studies

Histone H4 hyperacetylation in peripheral blood mononuclear cells. Low levels of acetylated histone H4 were detected in all pretreatment samples. A marked increase in histone H4 acetylation was observed at the end of infusion at all doses studied. At the lowest dose (150 mg/m^2), the increased acetylation was transient and had returned to pretreatment levels by 2 h after end of infusion (Fig. 1A and B). At higher doses, this increase in H4 acetylation was sustained for longer such that it remained above pretreatment levels up to 24 h after end of the infusion (Fig. 1A). There was no dose-related increase in the level of end-of-infusion H4 acetylation (Fig. 1B). However, the period of time for which the increased acetylation is sustained increased with increasing dose of belinostat (Fig. 1B). There was a dose-related increase in histone H4 acetylation AUC up to ~900 mg/m^2 (Fig. 1C).

Caspase-dependent cleavage of cytokeratin-18. Levels of caspase-dependent cleaved cytokeratin-18 in plasma samples from patients with epithelial tumors taken pretreatment on days 1, 2 and 8 of the first cycle of treatment at all doses are shown in Fig. 1D. Results are separated into two groups depending on response to treatment. M30 epitope levels were significantly increased on days 2 and 8 ($P < 0.02$ and $P < 0.01$, paired t test; $n = 14$) in patients with stable disease but showed no significant difference in patients with progressive disease ($n = 14$). Consistent with this epitope being derived from tumor cells and not normal cells undergoing therapy-induced apoptosis, patients who did not have epithelial cancer showed no change in level of the M30 epitope during treatment.

Analysis of cytokine expression. Initially, two patients were selected who had drug-related fatigue. Both were treated at 1,000 mg/m^2. Samples were taken pretreatment on days 1 and 2 and analyzed using antibody arrays. In both patients, IL-6 was clearly increased in the posttreatment plasma sample (Fig. 1E). Subsequently, IL-6 was measured in plasma samples from 10 available patients treated at 1,000 mg/m^2. IL-6 levels in the pretreatment samples were variable but there was a significant increase (2.7-fold) in IL-6 levels following treatment with belinostat (Fig. 1F; $P < 0.01$, Dunnett's multiple comparisons test).

Clinical outcomes

Eighteen (39%) patients achieved disease stabilisation on belinostat, including 15 who were treated for ≥4 cycles. Five of these patients (all treated at ≥900 mg/m^2/d) had sustained stable disease for longer than would have been expected, raising the possibility that belinostat might have changed tumor behavior in these patients, although this remains speculative in the absence of objective responses. Two patients with soft tissue sarcoma had stable disease for 14 and 7 months, respectively; one patient each with epithelial thymoma (Fig. 2), renal carcinoma, and melanoma had stable disease for 17, 4, and 3 months, respectively.

Discussion

These data describe the first-in-man clinical trial of the potent HDAC inhibitor belinostat (previously PXD101) and show that belinostat can be administered safely to patients with advanced solid tumors as a 30-min infusion daily for 5 days every 3 weeks. Dose-limiting toxicities were fatigue, diarrhea, nausea/vomiting, and atrial fibrillation. The dose

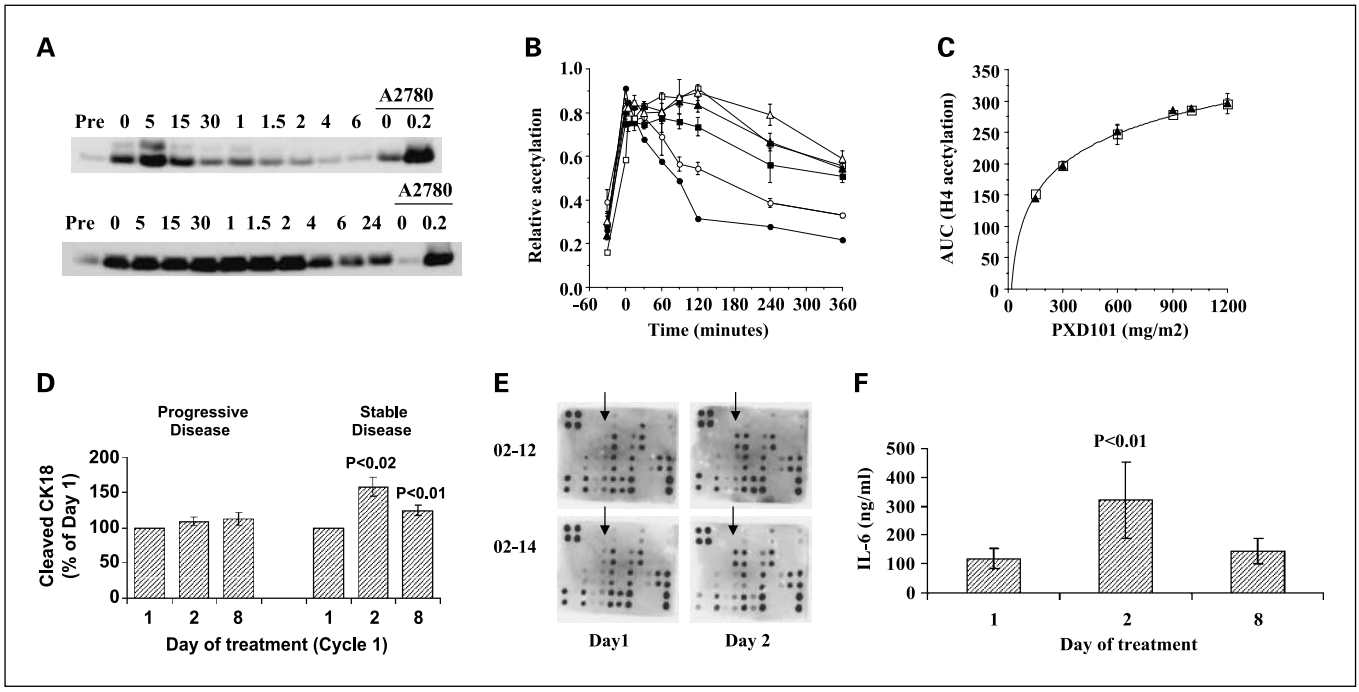

Fig. 1. *A* and *B,* histone H4 acetylation for two patients treated with belinostat (150 and 1,000 mg/m^2, respectively) and mean ± SE for each dose [●, 150 mg/m^2 ($n = 2$) or 300 mg/m^2 ($n = 4$); ■, 600 mg/m^2 ($n = 4$); □, 900 mg/m^2 ($n = 2$); ▲, 1,000 mg/m^2 ($n = 11$); △, 1,200 mg/m^2 ($n = 4$)]. *C,* AUC for histone acetylation calculated at all time points (□) or pretreatment, 5 min, 2 h, and 6 h (▲). *D*, caspase-dependent cleaved cytokeratin-18 (*CK18*) in plasma taken in cycle 1 of treatment from patients with progressive disease ($n = 14$) or with stable disease ($n = 14$) following treatment. *E,* detection of IL-6 (*arrowed*) in plasma taken pretreatment on days 1 and 2 from patients treated at 1,000 mg/m^2. *F,* quantitation of IL-6 in plasma taken pretreatment on days 1, 2, and 8 for 10 patients treated at 1,000 mg/m^2.

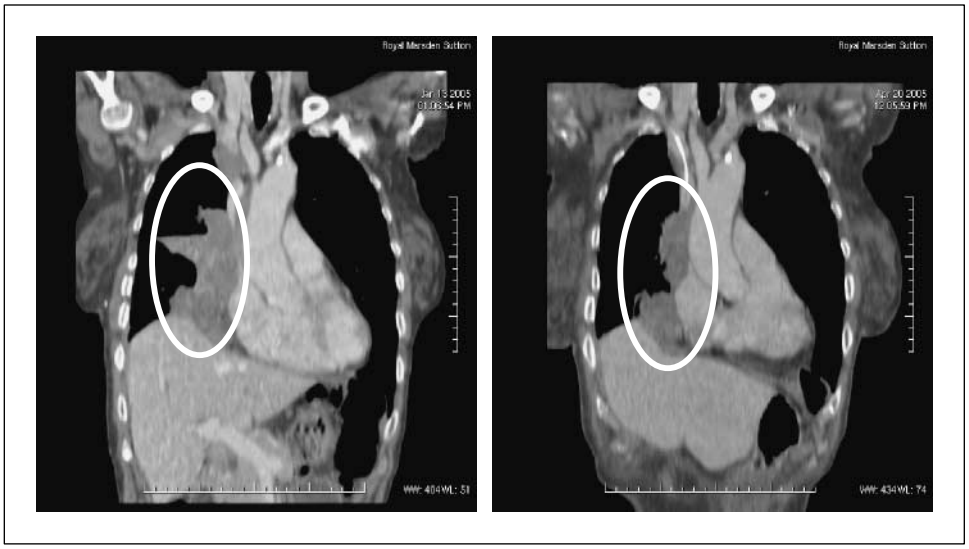

Fig. 2. Shrinkage of tumor mass in response to belinostat in a patient with epithelial thymoma.

recommended for phase II evaluation using this schedule is 1,000 mg/m^2/d.

Belinostat was rapidly cleared from plasma and displayed dose-proportional pharmacokinetics within the dose range studied. A reliable elimination half-life could not be determined with the sampling in this study. Further sampling would not, however, lead to a more reliable elimination half-life because plasma concentration at later time points was expected to be below or close to the limit of quantitation. The intermediate half-life was therefore reported in place of the elimination half-life and was 0.5 to 1.3 h. This was independent of dose, indicating that elimination of the drug was not saturable within the clinically relevant dose range. There was some variability in the pharmacokinetic variables between patients (Table 4). It was attempted to explain the variability by demographic variables or by differences in liver or kidney function. However, AUC/dose and clearance were not found to have significant correlation with height, weight, age, gender, and renal or hepatic function and the reason for the variability is therefore unknown.

Treatment with belinostat was generally well tolerated, the most common treatment-related adverse events being nausea, vomiting, and lethargy/fatigue. There were no related grade 4 adverse events and only 13% of patients experienced a related grade 3 event. No significant myelosuppression was reported, supporting the study of belinostat combined with cytotoxic chemotherapy. Phase I trials evaluating the administration of belinostat with carboplatin and paclitaxel, as well as with 5-fluorouracil, are ongoing and indicate that this HDAC inhibitor is well tolerated in these combinations (10, 11).

Fatigue was a common toxicity, as has been observed with several other HDAC inhibitors (12–15). Symptoms resolved rapidly on withdrawal of the drug. We hypothesized that fatigue, flushing, fever, and chills could be due to cytokine release. Samples taken pretreatment on days 1 and 2 from two patients (treated at 1,000 mg/m^2) who showed clear drug-related fatigue were analyzed by an antibody array that detects 40 inflammatory factors. In both patients, IL-6 was clearly increased in the posttreatment plasma sample (Fig. 1E). Quantitative analysis of these samples showed that IL-6 levels were increased by 2.7- and 5.8-fold posttreatment, which is equal to or greater than the mean increase in IL-6 of 10 patients treated at 1,000 mg/m^2 (2.7-fold; $P < 0.01$; Fig. 1E and F). The number of patients included in the trial was too small to allow correlation of IL-6 levels with fatigue. Nonetheless, because clinical trials of therapeutic recombinant IL-6 resulted in both fatigue (in 56% of patients) and atrial fibrillation (16), further evaluation of IL-6 as a potential mediator of HDAC inhibitor toxicity is now ongoing.

Hyperacetylation of histone H4 was shown in peripheral blood mononuclear cells in blood samples taken from patients treated with belinostat (Fig. 1A). Acetylation was markedly increased at the end of infusion and was sustained in a dose-dependent manner (Fig. 1B). The effects of belinostat on histone acetylation in patient samples were reversible: at the lowest dose, the level of acetylation had returned to pretreatment levels by ~2 h after the end of the infusion. The main effect of increasing belinostat dose was an increased duration of acetylation, seen as an increase in the AUC of histone acetylation (Fig. 1C). Whereas the plasma pharmacokinetics of belinostat showed a linear relationship between dose and AUC, the AUC for histone acetylation approaches a plateau such that by the maximum tolerated dose, there is no further increase in the level or duration of pharmacodynamic effect. It is unclear whether histone acetylation in peripheral blood mononuclear cells from patients treated with belinostat correlates with its biological activity. In a preclinical study of belinostat, we were able to show in mice that histone acetylation measured in blood showed a similar dose response to that seen in the tumor, and both correlated with a dose-dependent increase in tumor growth delay (6). Therefore, it is likely that the maximum biologically effective dose has been achieved at 1,000 mg/m^2.

Encouragingly, stable disease was observed in 39% of patients with a wide range of tumor types, the majority of whom had been heavily pretreated. Patients with stable disease showed a significant increase in caspase-dependent cleavage of cytokeratin-18 in plasma during treatment ($P < 0.02$, Fig. 1D)

whereas those with progressive disease did not. The appearance of the M30 epitope in plasma of patients with refractory disease who develop disease stabilisation supports the notion that belinostat can induce apoptosis in epithelial tumors. These data support the further evaluation of this biomarker in larger randomized trials as a predictor of antitumor activity.

In summary, this first clinical study of the HDAC inhibitor belinostat has shown that belinostat can be administered safely to patients and is well tolerated at biologically active doses for prolonged periods. These data and preliminary indications of antitumor activity support the further clinical development of this agent in single-agent and combination studies.

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