Phase I Clinical Trial of Histone Deacetylase Inhibitor: Suberoylanilide Hydroxamic Acid Administered Intravenously[1]

Wm. Kevin Kelly,[2] Victoria M. Richon, Owen O'Connor, Tracy Curley, Barbara MacGregor-Curtelli, William Tong, Mark Klang, Lawrence Schwartz, Stacie Richardson, Eddie Rosa, Marija Drobnjak, Carlos Cordon-Cordo, Judy H. Chiao, Richard Rifkind, Paul A. Marks, and Howard Scher

Genitourinary Oncology Service [W. K. K., H. S.], Lymphoma Service [O. O.], Departments of Medicine [W. K. K., O. O., H. S.], Nursing [T. C., B. M-C.], Pharmacy [M. K.], Radiology [L. S.], Epidemiology and Biostatistics [S. R.], and Pathology [M. D., C. C-C.], Laboratory of Analytical Pharmacology [W. T.], and Cell Biology Program of Sloan-Kettering Institute [R. R., P. A. M.], Memorial Sloan-Kettering Cancer Center and Joan and Sanford Weill Medical College of Cornell University, New York, New York 10021, and Aton Pharma, Inc., Tarrytown, New York [V. M. R., E. R., J. H. C.]

ABSTRACT

Purpose: **To evaluate the safety, pharmacokinetics, and biological activity of suberoylanilide hydroxamic acid (SAHA) administered by 2-h i.v. infusion in patients with advanced cancer.**

Experimental Design: **SAHA was administered for 3 days every 21 days in part A and 5 days for 1–3 weeks in part B. Dose escalation proceeded independently in patients with solid tumor and hematological malignancies (part B only). Pharmacokinetic studies were performed along with assessment of acetylated histones in peripheral blood mononuclear cells and tumor tissues.**

Results: **No dose-limiting toxicities were observed in 8 patients enrolled in part A (75, 150, 300, 600, and 900 mg/m^2/day). Among 12 hematological and 17 solid tumor patients enrolled in part B (300, 600, and 900 mg/m^2/day), therapy was delayed ≥1 week for grade 3/4 leukopenia and/or thrombocytopenia in 2 of 5 hematological patients at 600 mg/m^2/day × 5 days for 3 weeks. The maximal-tolerated dose was 300 mg/m^2/day × 5 days for 3 weeks for hematological patients. One solid patient on 900 mg/m^2/day × 5 days for 3 weeks developed acute respiratory distress and grade 3 hypotension. The cohort was expanded to 6 patients, and no additional dose-limiting toxicities were observed. Mean terminal half-life ranged from 21 to 58 min, and there was dose-proportional increase in area under the curve. An accumulation of acetylated histones in peripheral blood mononuclear cells up to 4 h postinfusion was observed at higher dose levels. Posttherapy tumor biopsies showed an accumulation of acetylated histones by immunohistochemistry. Four (2 lymphoma and 2 bladder) patients had objective tumor regression with clinical improvement in tumor related symptoms.**

Conclusions: **Daily i.v. SAHA is well tolerated, inhibits the biological target *in vivo*, and has antitumor activity in solid and hematological tumors.**

INTRODUCTION

Histone acetylation is a posttranslational modification of the core nucleosomal histones that affects chromatin structure and gene expression. The acetylation status of histones is determined by the opposing activities of HATs[3] and HDACs (1). Defects in both HAT activities and HDACs have been described in a variety of cancers. Genes that encode HAT enzymes are translocated, amplified, overexpressed, and/or mutated in cancers, including both hematological and epithelial malignancies. HDACs have been shown to be involved in oncogenic transformation by mediating the function of transcription factors. For example, in acute promyelocytic leukemia, the oncogenic translocation product PML-RAR α

Received 1/31/03; revised 4/7/03; accepted 4/8/03.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: Memorial Sloan-Kettering Cancer Center and Columbia University jointly hold patents on the hydroxamic acid-based hybrid polar compounds, including SAHA and related compounds, which are exclusively licensed to Aton Pharma, Inc. (Tarrytown, NY), of which P. A. M., V. M.R., and R. A. R. are founders. P. A. M. and R. A. R. are members of the Board of Directors of Aton Parma, Inc. Both institutions and the founders have an equity position in Aton Pharma, Inc. V. M. R., E. R., and J. H. C. are employed by Aton Pharma, Inc. The remainder of the authors do not have any disclosures or financial interests in Aton Pharma, Inc.

[1] Supported, in part, by CaPCURE, the Japanese Foundation for the Prevention of Cancer Research, the DeWitt Wallace Fund for Memorial Sloan Kettering Cancer Center, the Kleberg Foundation, David Koch Foundation, and the National Cancer Institute.

[2] To whom requests for reprints should be addressed, at Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021. Phone: (646) 422-4473; Fax: (212) 988-0701; E-mail: kellyw@mskcc.org.

[3] The abbreviations used are: HAT, histone acetyltransferase; HDAC, histone deacetylase; RAR, retinoic acid receptor; SAHA, suberoylanilide hydroxamic acid; MM, multiple myeloma; DLT, dose-limiting toxicity; KPS, Karnofsky performance status; AST, aspartate aminotransferase; EKG, electrocardiogram; CT, computed tomography; HPLC, high-performance liquid chromatography; AUC, area under the curve; anti-Ac, antiacetylated histone; PBMN, peripheral blood mononuclear.

represses transcription by associating with a corepressor complex that contains HDAC activity (2).

Inhibitors of HDAC activity induce differentiation, growth arrest, and/or apoptosis of transformed cells in culture and inhibit tumor growth in animals (1). Linear hydroxamic acids such as SAHA are inhibitors of HDAC activity (3). Mammalian HDACs have been grouped into three classes (1, 4). Class I human HDACs are homologous to the yeast HDAC Rpd3 and include HDAC1, HDAC2, HDAC3, HDAC8, and HDAC11. Class II HDACs include HDAC4, HDAC5, HDAC6, HDAC7, HDAC9, and HDAC10 and are homologous to the yeast HDAC Hda1. The third class of human HDACs consists of homologues of yeast Sir2 and requires NAD^+ for activity. SAHA has been shown to inhibit the activity of HDACs in both class I and class II but does not inhibit the activity of hSirT1 (class III HDAC; Ref. 1).

SAHA induces differentiation, growth arrest, or apoptosis of transformed human cells in culture at micromolar concentrations. SAHA was originally identified based on its ability to induce differentiation of murine erythroleukemia cells (5). Subsequently, it was found to induce differentiation of human breast adenocarcinoma cells and growth arrest in human prostate carcinoma and bladder transitional cell carcinoma cells (3, 6). SAHA induces apoptosis in transformed hematopoietic cells, including Jurkat, CEM cells, and ARP-1 cells (1).

The antitumor activity of SAHA was demonstrated in several *in vivo* models of cancer, including a xenograft model of human prostate cancer (CWR22; Ref. 6), a transgenic mouse model of leukemia (promyelocytic leukemia zinc-finger-retinoic acid receptor α), and a carcinogen-induced tumor model in rodents (*N*-methylnitrosourea-induced mammary tumors; Ref. 7). SAHA showed activity when administered either by daily i.p. injections (CWR22 and PLZF-RAR α) or oral (p.o.) administration by incorporation into the diet (the carcinogen-induced tumor model; Ref. 1).

Initial toxicology studies in mice and rabbits showed that a 2-h infusion of SAHA at the maximal administered dose of 3000 mg/m^2/day for 3 days produced no drug related deaths or organ toxicities. On the basis of this study and studies regarding the efficacy of SAHA in a mouse tumor xenograft model (efficacy at 150 mg/m^2/day), we calculated a safe and biologically active starting dose in humans to be 75 mg/m^2/day for 3 days. This study reports the experience of i.v. administered SAHA in patients with advanced solid tumors and hematological malignancies.

PATIENTS AND METHODS

Patient Eligibility and Pretreatment Evaluation

Patients with histologically documented, progressive advanced stage, primary, or metastatic solid tumors that were refractory to standard therapy or for which no curative standard therapy existed were considered eligible for the study. After we established some of the preliminary dosing and toxicity parameters, the study was amended to include patients with hematological malignancies (refractory or relapsing leukemia, MM, indolent or aggressive non-Hodgkin's lymphoma, mantle cell lymphoma, or Hodgkin's disease). The diagnosis for hematological malignancies were confirmed at Memorial Sloan-Kettering Cancer Center and, where indicated, patients must have failed, relapsed, or not be eligible for a peripheral blood stem cell transplant. To help distinguish the two groups, group A refers to the patients with solid tumors, whereas group B includes those patients with hematological malignancies.

All patients were required to have a KPS $\geq$ 70%, total bilirubin and AST $\leq 1.5 \times$ upper limit of normal, creatinine $\leq$ 2.0, and prothrombin time $\leq$ 14 s. A platelet count $\geq$ 100,000 cells/mm^3 and WBC $\geq$ 3,500 cells/mm^3 were required for all patients with solid tumors, whereas patients with hematological malignancies were required to have a platelet count $\geq$ 25,000 cells/mm^3.

All patients were required to have recovered from the acute toxicities of any prior therapy and could not receive chemotherapy, radiation therapy, cytokine therapy, or other investigational anticancer therapeutic drugs for at least 4 weeks before entry into the trial. Patients with leukemia and lymphoma or MM may have received hydroxyurea or steroids, respectively, up to 2 weeks before starting therapy.

Patients were required to be at least 18 years of age and were informed of the potential risk of procreation while participating in this trial and required to use effective contraception during the entire study period. All patients signed an informed consent. Patients with clinically significant cardiac disease (New York Heart Association Class III or IV) or severe debilitating pulmonary disease, active central nervous system or epidural tumor, active infection requiring i.v. antibiotic treatment, or severe medical problems that would increase the patient's risk for toxicity were not eligible to participate in the study. Pregnant women and lactating females were excluded because of the unknown teratogenic effects of the compound and because the excretion of the compound into breast milk has not been characterized.

The pretreatment evaluation included a complete history and physical examination with a baseline KPS. Laboratory studies included an automated blood and platelet count (CBC), serum electrolytes, and comprehensive screening profile (alkaline phosphatase, lactate dehydrogenase, aspartate transglutaminase, blood urea nitrogen, creatinine, calcium, phosphorus, uric acid, total protein, albumin, total bilirubin, and electrolytes). Prostate cancer patients had serum acid phosphatase and prostate-specific antigen levels. Imaging studies included a chest, abdominal, and pelvic CT or magnetic resonance imaging scan, bone scan, and chest radiograph as indicated. All patients had a baseline EKG and additional cardiac work-up if indicated. Patients with lymphoma, leukemia, and MM had a baseline bone marrow aspiration and biopsy as clinically indicated to monitor their disease status.

Trial Design and Treatment

Patients received SAHA by a 2-h i.v. infusion through a central venous catheter. The study was divided into parts A and B as outlined in Table 1. Part A investigated 3 consecutive days of SAHA administration starting at a dose of 75 mg/m^2/day and escalated to 900 mg/m^2/day repeated every 21 days. Starting at 300 mg/m^2, part B increased the number of days the infusion was given to 5 days and the number of consecutive weeks of treatment to 3 weeks then subsequently increased the dose of SAHA to 600 and 900 mg/m^2 as outlined in Table 1. An

Table 1 Dose escalation schedule

Part A Cohort	Dose (mg/m^2)	No. of days/week	No. of consecutive weeks	Observation period (wk)	No. of patients
Solid tumor patients					
1a	75	3	1	2	1
2a	150	3	1	2	1
3a	300	3	1	2	1
4a	600	3	1	2	1
5a	900	3	1	2	4
Part B					
Cohort	Dose (mg/m^2)	No. of days/week	No. of consecutive weeks	Observation period (wk)	No. of patients
Solid tumor patients					
1b	300	5	1	2	1
2b	300	5	2	2	3
3b	300	5	3	1	3
4b	600	5	3	1	3
5b	900	5	3	1	7
Cohort	Dose (mg/m^2)	No. of days/week	No. of consecutive weeks	Observation period (wk)	No. of patients
Hematological patients					
3b	300	5	3	1	7
4b	600	5	3	1	5

accelerated titration design was used in part A of the study. A single patient was entered and observed at each specified dose level for at least one treatment cycle until a grade 2 or greater toxicity (other than hemoglobin-anemia) was encountered in the first cycle or when two different patients experienced a grade 2 toxicity (other than hemoglobin-anemia) during any course of treatment. This marked the end of the accelerated phase and standard dose escalation was initiated. In the standard dose escalation phase, 1 patient was entered in cohort 1b, and 3 patients were entered in subsequent cohorts. In the event of a DLT, the cohort was expanded to 6 patients.

Starting at cohort 3b, the trial was amended to allow the participation of patients with hematological malignancies. Dose escalation proceeded independently in patients with solid tumors (group A) and patients with hematological malignancies (group B).

A cycle of therapy was defined as the treatment period (1–3 weeks) plus the observation period (1–2 weeks). For solid tumor patients, the DLT was defined as grade 4 neutropenia or thrombocytopenia, grade 3 neutropenia with fever or any grade 3 or 4 nonhematological toxicity using the National Cancer Institute Common Toxicity Criteria during the first cycle of therapy. All patients with solid tumors must have recovered from the toxicity (grade 0 or 1) before receiving the next cycle of therapy. For patients with hematological malignancies, the DLT was defined as a grade 3 or 4 nonhematological toxicity using the National Cancer Institute Common Toxicity Criteria during the first cycle of therapy. Patients with hematological malignancies could be re-treated if nonhematological toxicity and WBC recovered from toxicity (grade 0 or 1) and platelet count $\geq$ 25,000 cells/mm^3. If the treatment was delayed secondarily from toxicity $>$1 week, this was also considered a DLT. Maximum-tolerated dose was defined as the highest dose with an observed incidence of DLT in no more than 1 of 6 patients treated at a particular dose level.

Drug Preparation

SAHA was manufactured at Southern Research Institute (Birmingham, AL) and ChemSyn Laboratories (Lenexa, KS). The i.v. solution was prepared in the investigational pharmacy at Memorial Sloan-Kettering Cancer Center. The SAHA solution was prepared in a sterile isotonic solution of water for injection and sodium chloride (~300 mOsm) at pH 11.2 with a buffer capacity of 0.006 mol/l/pH unit. The protocol for preparation of 100 ml of a 5 mg/ml SAHA formulation for i.v. infusion was as follows: add 25 ml of NaOH (0.25 N) to 0.5 g of SAHA and stir until dissolved without heating. Add 25 ml of water for injection and 0.55 g of NaCl and stir until dissolved. Add 0.1 N HCl slowly until the pH of the solution is 11.2. The volume was adjusted to 100 ml. The pH was checked and maintained between 11.0 and 11.2. Solution was subsequently sterilized by filtration through a cellulose acetate (0.22 μm) filter before administration.

Posttreatment Evaluation

Patients were evaluated weekly with a medical history, physical exam, toxicity assessment, CBC, comprehensive screening profile, coagulation profile (prothrombin time, partial thromboplastin time, and international normalized ratio) and urinalysis. Tumor markers, including acid phosphatase and prostate-specific antigen, were repeated every 2 weeks while on the study. Imaging studies were repeated every 8 weeks in patients as clinically indicated. An EKG was repeated at baseline and before every cycle of therapy. Bone marrow aspiration and biopsy were performed at baseline and repeated as indicated.

All patients registered were eligible for assessment of toxicity and response if they received any treatment. Toxicities were graded on the Common Toxicity Criteria from the Cancer Therapy Evaluation Program (version 2) of the National Cancer Institute (Bethesda, MD). Outcomes were assessed independ-

ently using CT or magnetic resonance imaging scans for measurable lesions, radionuclide bone scans, and posttherapy changes in serum tumor markers (8). In patients with measurable disease, standard Phase II response criteria (9) were used, and radiographs were reviewed independently, with the reviewer (L. S.) blinded to the clinical outcome.

The time to progression was calculated from the start of the therapy to the off study date defined by the criteria for progression based on measurable or osseous disease or removal from the study because of toxicity or death.

Pharmacokinetics

Pharmacokinetic studies were performed on the first and last day of i.v. therapy in the first cycle of therapy. Ten ml of heparinized blood from a peripheral vein were collected at baseline, 30 min, and 1 h during the infusion and at end of the 2-h infusion. Postinfusion blood samples were drawn at 15 min and 0.5, 1, 1.5, 2, 3, and 4 h. SAHA samples were placed on ice and refrigerated until they were centrifuged to separate the plasma. The plasma (3–5 ml) was transferred to labeled conical 15-ml polypropylene screw top tube and stored at −20 C° until they were analyzed by HPLC in the laboratory of Analytical Pharmacology. The HPLC method involved the addition of 10 μl of 1 N hydrochloric acid to 200 μl of plasma. After mixing, 200 μl of methanol were then added to precipitate the protein that was isolated by centrifugation. The supernatant was analyzed with an XDB-C18 Eclipse column (3 × 250 mm; HP) with the mobile phase A consisting of 14% acetonitrile in 50 mM potassium phosphate buffer with 0.025% triethylamine and mobile phase B consisting of 30% acetonitrile in 50 mM potassium buffer with 0.025% triethylamine, delivered at a flow rate of 0.5 ml/min. The eluate was monitored at 240 nm, and the lower limit of detection was 31.25 ng/ml.

Twenty-four-h urine collection was performed in each patient starting at the initiation of the i.v. therapy on day 1. Aliquots of urine were frozen at −20 C° for evaluation by HPLC. Pharmacokinetic parameters were calculated according to a noncompartmental analysis using WinNonLin version 3.1 (Pharsight Corp., Mountain View, CA). *AUC* was estimated using the linear trapezoid method. The first order rate constant (λz) associated with the terminal phase was estimated by linear regression of at least three terminal plasma concentration-time data points. Clearance (*Cl*) was calculated by the standard formula (*i.e.,* total dose divided by $AUC_{0-\infty}$). Volume of distribution based on the terminal phase was calculated using *Cl* and *z* (*i.e., Cl*/lambda z).

Correlative Studies

Effect on Histone Acetylation in Mononuclear Cells. Ten to thirty ml of peripheral blood were obtained in heparinized tubes. PBMN cells were isolated from the whole blood by centrifugation using Ficoll-Paque (Pharmacia, Peapack, NJ) at a bedside laboratory according to the manufactures guidelines. Nuclei from mononuclear cells were then isolated by lysis in buffer containing 10 mM Tris-HCl (pH 6.5), 50 mM sodium bisulfite, 1% Triton X-100, 10 mM $MgCl_2$, 8.6% sucrose, and Dounce homogenization. Histones were isolated from the mononuclear cells as described by Yoshida *et al.* (10). Equal amounts of partially purified proteins (1–5 μg) were electrophoresed on 15% SDS-PAGE minigels (Bio-Rad, Hercules, CA) and transferred to Hybond-P filters (Amersham). Filters were blocked with 3% milk. Purified rabbit polyclonal (anti-Ac-H4) antibody or anti-Ac-H3 antibody (Upstate Biotechnology, Inc., Lake Placid, NY) were used to detect acetylated histone H4 and H3, respectively. Levels of acetylated histone were visualized using a horseradish peroxidase-conjugated goat antirabbit antibody (1:5000) and the Super Signal chemiluminescent substrate (Pierce, Rockford, IL). As a control for the amount of protein loading, parallel gels were stained with Coomassie blue stain.

During cycle 1, mononuclear cells for histone acetylation were evaluated pretreatment, end of the infusion, and then between 15 min and 4 h after infusion. These studies were repeated on the last day of therapy in the first cycle.

Effect on Histone Acetylation on Tumor Tissues. Tumor biopsies were performed pretreatment and during the last week of therapy of cycle 1 in patients willing to undergo a tumor biopsy. The posttherapy biopsy was performed within 6 h of the completion of the SAHA infusion. Informed consent was obtained for these biopsies. Biopsies were formalin fixed and paraffin embedded. Slides (5 μm) were sectioned from the paraffin block immediately before immunostaining. The slides were stained using a rabbit polyclonal anti-Ac-H3 antibody (Upstate Biotechnology, Inc.) and titrated with serial dilutions of 1:1000, 1:2500, and 1:5000. Standard streptavidin techniques were used. 3,3′-Diaminobenzidine was used as the counterstain. The samples were read using the pretherapy sample as the control for histone H3 accumulation and graded the posttherapy samples as a grade +1, +2, or +3 increase in histone H3 acetylation.

RESULTS

Patient Characteristics

Thirty-nine patients were registered to the study; 2 patients with Hodgkin's disease had rapid progression and were withdrawn from the study before receiving SAHA. Thirty-seven patients (25 solid tumor and 12 hematological malignancies) received a total of 1010 (range, 1–134) doses of i.v. SAHA from February 2000 to August 2002, and the median number of doses was 15. Baseline characteristics for solid tumor and hematological patients are listed in Table 2. KPS was good for both groups of patients, but the median age was substantially less for the hematological patients (39 years; range, 19–77 years) than patients for the solid tumors (63 years; range, 42–81 years). The majority of patients were heavily pretreated, and in the case of the hematological patients, 67% of the patients had prior peripheral blood stem cell transplants.

SAHA Dose Escalation

In part A of the study, 1 patient was entered at the 75, 150, 300, and 600 mg/m^2 dose level. No grade 2 or greater toxicity was seen, but it was elected to expand the 900 mg/m^2 dose level to evaluate this dose in a larger number of patients. Four patients were entered, and 1 patient was withdrawn from the study after the first dose of SAHA for noncompliance. No DLTs were observed.

In part B, there were no DLTs in cohorts 1b through 4b in

Table 2 Patient characteristics

	n (%)
Solid tumor patients	25
Male	17 (68%)
Female	8 (32%)
White, non-Hispanic	21 (84%)
Black, non-Hispanic	2 (8%)
Asian	2 (8%)
Tumor type	
Prostate	8 (32%)
Bladder	6 (24%)
Breast	4 (16%)
Colon	4 (16%)
Ovarian	1 (4%)
Renal	2 (8%)
Median age, yr (range)	63 (42–81)
Median KPS	80 (70–90)
Median no. of prior chemotherapies and biological therapies (range)	5 (2–15)
Hematological tumor patients	12
Male	7 (58%)
Female	5 (42%)
White, Non-Hispanic	11 (92%)
Tumor type	
Hodgkin's lymphoma	5 (42%)
Non-Hodgkin's lymphoma	6 (50%)
Multiple myeloma	1 (8%)
Median age (range)	39 (19–77)
Median KPS	80 (70–90)
Median no. of prior chemotherapies and biological therapies (range)	7 (4–15)
Stem cell transplants	8 (67%)
1	7 (58%)
2	1 (8%)

patients with solid tumors. In the initial 3 patients in cohort 5b (900 mg/m^2 $\times$ 5 days $\times$ 3 weeks), 1 patient with metastatic breast cancer with lymphangitic lung metastasis developed an acute adult respiratory distress syndrome and grade 3 hypotension possibly related to SAHA prompting an expansion of the cohort. This patient recovered and was taken off study for progression of disease. One additional patient in cohort 5b had an acute cardiac event during his observation week and died. This patient had a long history of cardiopulmonary disease. He suddenly ceased smoking and self-medicated with nicotine patches on his week off therapy. Before his death, EKGs showed no acute changes from his baseline EKG, and electrolytes were within normal. There was no grade 3 or 4 toxicities, and the only grade 2 toxicities observed in this patient were anemia and thrombocytopenia. A postmortem exam was not performed. The death was considered unlikely related to the study drug. An additional 4 patients (the later patient was replaced. and 3 were enrolled as per protocol) were treated in cohort 5b. To further explore the possible cardiac effects from the drug, EKGs were performed in these patients before therapy while receiving the i.v. infusion and posttreatment. There were nonspecific ST changes noted on EKG, but no acute cardiac events or DLTs were seen in the additional 4 patients enrolled in cohort 5b. Additional dose escalation of IV SAHA was suspended at this juncture because of the availability of an oral formulation of SAHA.

In patients with hematological tumors, 12 patients were treated in two cohorts (3b and 4b). Two of 5 patients in cohort 4b developed DLT. One patient with Hodgkin's disease and prior history of two peripheral stem cell transplants developed grade 4 nonfebrile neutropenia for 6 days. Her treatment was delayed for >1 week. The patient subsequently had her dose reduced to 300 mg/m^2 $\times$ 5 days $\times$ 3 weeks, and she tolerated therapy without any additional neutropenia. A second patient with diffuse large-cell lymphoma and history of one prior peripheral stem cell transplant developed a fever. On admission, the patient had grade 3 leukopenia (grade 1 neutropenia) and thrombocytopenia that lasted 3 days. No bleeding was noted in this patient. Despite a full course of antibiotics, the fever persisted, and it was felt this represented tumor fever. The patient was rechallenged at the same dose of i.v. SAHA but again developed leukopenia. Subsequently, this patient had increasing fatigue and dyspnea related to his progressive metastatic disease and was removed from the study. Because the patient had treatment delay >1 week, which was considered a DLT, no additional patients were enrolled at this dose. Three additional patients were treated in the proceeding dose level (cohort 3b: 300 mg/m^2 $\times$ 5 days $\times$ 3 weeks). One patient with MM after 1 week of therapy fell and fractured his shoulder. This patient was removed from the study, and 1 additional lymphoma patient was treated. No additional DLTs were seen in these additional 3 patients treated at 300 mg/m^2 $\times$ 5 days $\times$ 3 weeks, and this dose level was considered to be the maximum-tolerated dose for the hematological patients.

Adverse Events

The most common adverse events for solid tumor and hematological patients are listed in Tables 3, *a* and *b*. There was an increased incidence of leukopenia and thrombocytopenia associated with the treatment of patients with lymphoma compared with the solid tumor patients. The nonhematological adverse events were similar between the groups.

Hematological. Treatment delay longer than 1 week because of leukopenia or thrombocytopenia was the DLT in patients with hematological malignancies. Considering all cycles of therapy given to the hematological patients, 1 patient developed a grade 4 leukopenia and a grade 3 thrombocytopenia, 1 had a grade 4 leukopenia/neutropenia, and 1 was observed to have grade 3 thrombocytopenia. No significant first-cycle leukopenia or thrombocytopenia was seen in the solid tumor patients. There was evidence of cumulative marrow toxicity seen in solid tumor patients with 44, 12, and 4% developing grade 1, 2, or 3 thrombocytopenia, respectively. One solid tumor patient developed a grade 3 thrombocytopenia after four cycles of therapy. The platelet count would typically rebound within 7 days after the discontinuation of SAHA.

Cardiovascular/Pulmonary. One patient suffered an acute myocardial infarction as described above, but no other acute cardiac events were noted in other patients. EKGs were performed weekly before therapy, during therapy, and after treatment, subsequently. Nonspecific asymptomatic ST changes were observed in patients but considered not clinically significant. No patients had any cardiac dysrhythmias while on study. One patient with metastatic breast cancer with diffuse lymphangitic disease to the lung developed hypotension and acute respiratory distress syndrome. After discontinuation of SAHA

Table 3 Common adverse events

a. Solid tumors

Adverse event	Grade 1+	2+	3+	4+	5+
Cardiac/thromboembolic					
ARDS[a]	0 (0%)	0 (0%)	0 (0%)	1 (4%)	0 (0%)
Cardiac ischemia	0 (0%)	1 (4%)	0 (0%)	0 (0%)	1 (4%)
Hypotension	0 (0%)	1 (4%)	1 (4%)	0 (0%)	0 (0%)
Thrombosis	0 (0%)	0 (0%)	0 (0%)	1 (4%)	0 (0%)
Hematological					
Hemoglobin	7 (28%)	14 (56%)	2 (8%)	0 (0%)	0 (0%)
Leukocytes	8 (32%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Platelets	11 (44%)	3 (12%)	1 (4%)	0 (0%)	0 (0%)
Metabolic					
Creatinine	4 (16%)	4 (16%)	0 (0%)	0 (0%)	0 (0%)
Hyperglycemia	13 (52%)	6 (24%)	4 (16%)	0 (0%)	0 (0%)
AST	8 (32%)	1 (4%)	0 (0%)	0 (0%)	0 (0%)
ALT	5 (24%)	2 (8%)	0 (0%)	0 (0%)	0 (0%)
Gastrointestinal					
Anorexia	13 (52%)	2 (8%)	0 (0%)	0 (0%)	0 (0%)
Constipation	10 (40%)	4 (16%)	0 (0%)	1 (4%)	0 (0%)
Diarrhea	15 (60%)	1 (4%)	1 (4%)	0 (0%)	0 (0%)
Vomiting	6 (24%)	4 (16%)	0 (0%)	0 (0%)	0 (0%)
General					
Abdominal pain/cramping	3 (12%)	1 (4%)	1 (4%)	1 (4%)	0 (0%)
Dyspnea	5 (20%)	6 (24%)	1 (4%)	0 (0%)	0 (0%)
Fatigue	13 (52%)	1 (4%)	1 (4%)	0 (0%)	0 (0%)
Tumor pain	0 (0%)	0 (0%)	2 (8%)	0 (0%)	0 (0%)

b. Hematological malignancies

Adverse event	Grade 1+	2+	3+	4+
Cardiac/thromboembolic				
Hypotension	1 (8%)	2 (17%)	0 (0%)	0 (0%)
Pulmonary, other	0 (0%)	0 (0%)	1 (8%)	0 (0%)
Thrombosis	0 (0%)	1 (8%)	2 (17%)	0 (0%)
Hematological				
Hemoglobin	2 (8%)	5 (42%)	5 (42%)	0 (0%)
Leukocytes	1 (8%)	1 (8%)	0 (0%)	2 (17%)
Platelets	4 (33%)	2 (17%)	2 (17%)	0 (0%)
Metabolic				
Creatinine	2 (17%)	1 (8%)	0 (0%)	0 (0%)
Hyperglycemia	8 (67%)	2 (17%)	0 (0%)	0 (0%)
AST alanine	3 (25%)	0 (0%)	0 (0%)	0 (0%)
Aminotransferase	3 (25%)	0 (0%)	0 (0%)	0 (0%)
Gastrointestinal				
Anorexia	6 (50%)	1 (8%)	0 (0%)	0 (0%)
Constipation	4 (33%)	0 (0%)	1 (8%)	0 (0%)
Diarrhea	4 (33%)	0 (0%)	1 (8%)	0 (0%)
Vomiting	1 (8%)	2 (17%)	0 (0%)	0 (0%)
General				
Abdominal pain/cramping	1 (8%)	1 (8%)	0 (0%)	0 (0%)
Dyspnea	3 (25%)	2 (17%)	3 (25%)	1 (8%)
Fatigue	3 (25%)	4 (33%)	3 (25%)	0 (0%)
Tumor pain	1 (8%)	0 (0%)	0 (0%)	0 (0%)

[a] Acute respiratory distress syndrome.

and steroid treatment, symptoms resolved to her baseline respiratory status. This event was considered possibly related to the SAHA administration.

Gastrointestinal. Transient mild nausea, constipation and diarrhea were seen in patients postinfusion that was self limiting and did not require medical intervention.

Metabolic. Significant hepatotoxity was not encountered. Five patients (4 solid tumor and 1 hematological patient) developed grade 2 renal insufficiency after median of 4 months of therapy. On discontinuation of the drug, the renal insufficiency returned to the baseline. Hyperglycemia (>160 mg/dl, grade $\geq$ 2) was seen in 17–40% of the patients, but medical

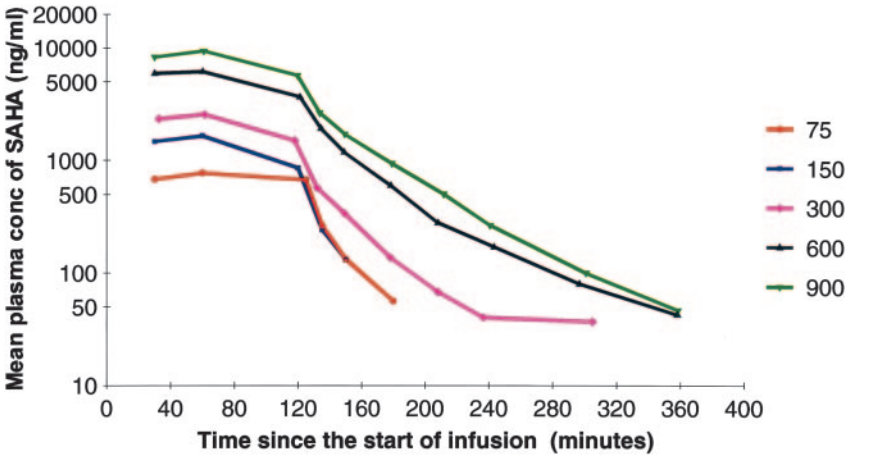

Fig. 1 Cycle 1, day 1 mean plasma concentrations over time for escalating doses of i.v. SAHA administered over 2 h.

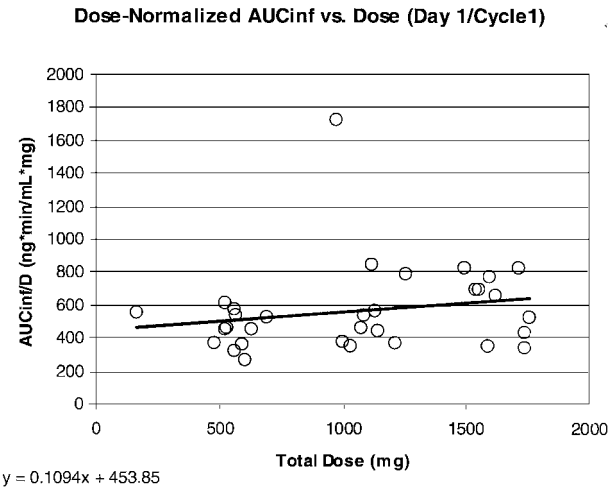

Fig. 2 AUC_{inf} on cycle 1, day 1 of therapy normalized to dose.

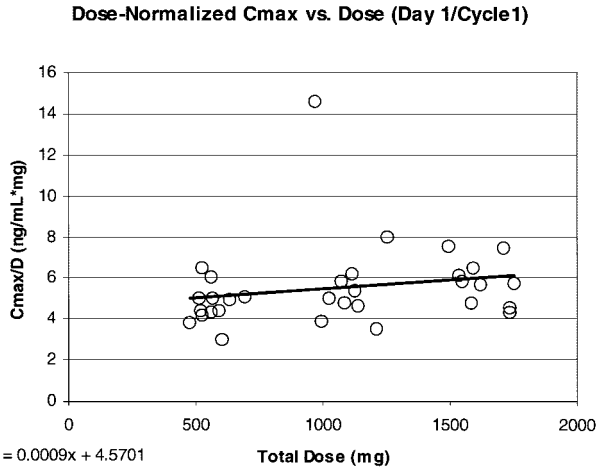

Fig. 3 Maximum plasma concentration (C_{max}) on cycle 1, day 1 of therapy normalized for dose.

interventions were not required for any patient, except for diet control and close monitoring.

General. The most common adverse event was fatigue (grade 1 or 2). In general, the fatigue increased as the number of consecutive weeks of therapy and dose was increased. The fatigue resolved during the observation period or discontinuation of the therapy. In 3 patients, there was an increase in tumor-related pain with the administration of i.v. SAHA. Tumor pain flares were seen in 3 cases (2 solid tumor and 1 hematological patient) and required narcotics to complete the infusion in the 2 solid tumor patients that had large tumor burdens. The pain flares were most severe on the first day of the infusion and decreased on subsequent days. The rate of the infusion did not affect the intensity of the pain. One patient with metastatic colon cancer with peritoneal disease had an increase in abdominal pain and cramping associated with the infusion requiring additional narcotics.

Pharmacokinetics

Samples for pharmacokinetic analysis were obtained in all patients. Representative semilogarithmic plots of mean plasma SAHA concentration-time profiles are shown in Fig. 1. Higher maximum plasma concentration (C_{max}) and AUC_{inf} were achieved with higher doses and there was linearity between total dose and exposures (Figs. 2 and 3).

The C_{max}, terminal half-life ($T_{1/2}$), AUC_{inf}, volume of distribution (V_z) and Cl per dose level are summarized in Table 4. The SAHA concentration peaked at the median of 60 min (range 25–120 min) from the beginning of the infusion. The explanation for the decline of SAHA concentration during the constant infusion is not clear. To further evaluate whether this was related to rapid fall immediately after the discontinuation of therapy at 120 min, end of infusion levels were drawn at 115 min. This trend for lower SAHA concentrations at the end of infusion persisted. Mean estimates for the $T_{1/2}$ ranged from 21 to 58 min on the first day of dosing. Within a dose group, estimates of terminal C_{max}, terminal $T_{1/2}$, and AUC_{inf} were consistent on different dosing days. The few patients on each dose level precludes a reliable assessment of the relationship between pharmacokinetic parameters and adverse events.

Table 4 Pharmacokinetic parameters

a. i.v. SAHA

Dose level	*n*	C_{max} (ng/ml) Mean ± SD	$T_{1/2}$ (min) Mean ± SD	AUC_{inf} (h × ng/ml) Mean ± SD	V_z liters/m^2 Mean ± SD	*Cl* liters/h/m^2 Mean ± SD
75 mg/m^2						
Day 1	1	770	21	1501	24	49
Day 3	1	683	A[b]	A	A	A
150 mg/m^2						
Day 1	1	1649	A	A	A	A
Day 3	1	1631	21	2862	28	56
300 mg/m^2						
Day 1	11	2646 ± 588	33 ± 27	4285 ± 1150	62 ± 56	75 ± 21
Day 3	1	2339	18	4027	33	75
Day 5	1	2408	38	4634	59	65
Day 12	3[a]	2721 ± 944	21	4592	32	65
Day 19	6	2963 ± 559	37 ± 12	5053 ± 1159	57 ± 27	62 ± 13
600 mg/m^2						
Day 1	9	6334 ± 3086	58 ± 38	11233 ± 6928	103 ± 101	66 ± 25
Day 3	1	4167	35	7584	67	79
Day 19	5	8628 ± 5475	72 ± 30	16697 ± 11523	85 ± 49	45 ± 16
900 mg/m^2						
Day 1	11	9525 ± 1611	46 ± 28	16611 ± 4533	66 ± 50	60 ± 19
Day 19	6	14169 ± 2010	50 ± 27	29198 ± 8723	42 ± 22	36 ± 16

b. i.v. SAHA for solid tumor and hematological patients

Dose level	*n*	C_{max} (ng/ml) Mean ± SD	$T_{1/2}$ (h) Mean ± SD	AUC_{inf} (h × ng/ml) Mean ± SD	V_z liters/m^2 Mean ± SD	*Cl* liters/h/m^2 Mean ± SD
Solid (300 mg/m^2)						
Day 1	8	2650 ± 656	22 ± 4	4434 ± 1278	40 ± 21	74 ± 24
Day 19	3	3298 ± 438	28 ± 10	5725 ± 1298	35 ± 10	54 ± 12
Hematologic (300 mg/m^2)						
Day 1	4	2638 ± 515	53 ± 39	4026 ± 1002	101 ± 81	78 ± 17
Day 19	3	2627 ± 501	47 ± 5	4382 ± 564	78 ± 17	69 ± 9
Solid (600 mg/m^2)						
Day 1	4	5674 ± 545	38 ± 12	9341 ± 1107	59 ± 17	65 ± 8
Day 19	2	5347 ± 760	80 ± 29	10832 ± 500	107 ± 44	55 ± 3
Hematologic (600 mg/m^2)						
Day 1	5	6862 ± 4246	74 ± 45	12748 ± 9414	137 ± 129	66 ± 34
Day 19	3	10815 ± 6459	66 ± 36	20606 ± 14426	70 ± 55	38 ± 18

[a] Includes number of patients having pharmacokinetics studies during cycles 1 and 2.
[b] A, missing data points to adequately calculate parameters.

Correlative Studies

Effect on Histone Acetylation in Mononuclear Cells. In 37 patients, 162 peripheral blood samples were analyzed for histone acetylation in PBMN cells. Ninety-eight percent (158 of 162) of these samples had sufficient material for histone extraction for Western analysis. An increase in the accumulation of acetylated histones was observed at the completion of the 2-h infusion at day 1 and last day of therapy (days 3, 5, 12, and 19) of the first cycle in all patients. The plasma concentration of SAHA during the infusion exceeded 2.5 μM at all dose levels. This correlates with the concentration of SAHA that inhibits cell proliferation *in vitro* and causes accumulation of acetylated histones. To determine the duration of the accumulation of acetylated histones, blood for analysis was drawn 2 and/or 4 h after the completion of the therapy. An increased accumulation of acetylated histones was not consistently detected 2 h after infusion at the lower dose levels (75–300 mg/m^2). At the higher dose levels (600 and 900 mg/m^2), accumulation of acetylated histones was seen consistently at 2 and 4 h after infusion, respectively (Fig. 4). These effects on acetylation of histones correlate with the observed short $T_{1/2}$ of SAHA and the reversible nature of the SAHA inhibition of HDAC activity.

Effect on Histone Acetylation on Tumor Tissues. All patients were approached to obtain pre- and posttherapy tumor biopsies, although this was not a requirement to participate in the study. Eleven of 37 patients (30%) consented to the pre- and posttherapy tumor biopsies. Five of the 11 had sufficient amount of tumor in the pre- and posttherapy specimens to make a comparison. The remaining 6 patients had inadequate material in the pre- or postbiopsy specimen or refused follow-up biopsy. Using serial dilutions (1:1000, 1:2500, and 1:5000) of the anti-acetylated H3 antibody, 3 of 5 patients showed an increased accumulation of acetylated histones in the posttreatment biopsy sample. Fig. 5 shows an increase in the proportion and intensity of immunohistochemical staining using an anti-Ac-H3 antibody in the posttherapy tumor biopsy of a metastatic prostate skin nodule. This suggests SAHA inhibited HDAC activity in the tumor.

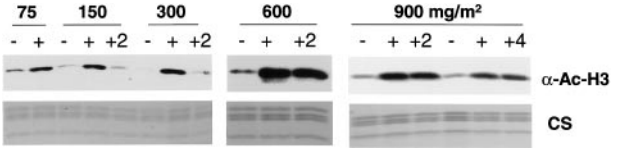

Fig. 4 Peripheral whole blood was collected from patients before (−), at the end of the 2-h infusion of SAHA (+), and at either 2-h after infusion (+2) or 4 h after infusion (+4). Western blot analysis was performed as described using a rabbit purified polyclonal antiacetylated histone H3 antibody (α-Ac-H3). As a loading control for the histone proteins, parallel gels were run and stained with Coomassie. An accumulation of acetylated histones was observed at each dose level (75, 150, 300, 600, and 900 mg/m^2) after infusion. At 600 and 900 mg/m^2, an accumulation of acetylated histones was seen 2 h after the completion of the infusion, and an accumulation of acetylated histones was seen at 4 h after infusion at 900 mg/m^2 dose level. Similar results were obtained in the second cycle of therapy. Evaluation of differences in the level of acetylation of histones in peripheral mononuclear cells was performed by densitometric scanning of the films. The increase in density of the signal intensity after SAHA infusion was 2.5–5.6-fold greater than preinfusion samples.

Clinical Outcomes. Median duration of therapy was 6.4 (range, 1.6–40) weeks for all 37 patients, and the median duration of therapy for solid tumor and hematological malignancy patients was 7 (range, 2.1–35.6) weeks and 6.7 (range, 1.6–40) weeks, respectively. Two patients with refractory metastatic bladder cancer treated at the 600 mg/m^2 × 5 days × 3 weeks dose level had a minor objective response on CT scans (17 and 32% reduction of pelvic tumor mass and lymphadenopathy) with clinical improvement of tumor-related pain. These 2 patients were maintained on therapy for 6 and 7 months, respectively. Both patients showed disease progression and return of pain within 2 months after they were taken off therapy because of line or urinary sepsis. A refractory breast cancer patient did show transient inhibition of the CA-125 breast cancer marker while on SAHA therapy. Antitumor activity was also observed in 2 patients with refractory Hodgkin's disease that previously failed autologous transplants. One patient had a 30% objective tumor regression in her multiple lung lesions and marked improvement in her pulmonary and overall performance status on the 600 mg/m^2 dose level. This was maintained for 3 months, then the disease progressed while she was off therapy secondarily to systemic infection. An additional patient on the 300 mg/m^2 dose level with refractory Hodgkin's disease was maintained on therapy for 8 months, returned to work full time, had stable disease on CT scan, and her positron emission tomography scan normalized. This patient was subsequently converted to the oral SAHA formulation for ease of administration.

DISCUSSION

This is the first report of a clinical trial of the novel HDAC inhibitor SAHA. This study showed that SAHA could be safely administered using a daily 2-h i.v. infusion for 5 consecutive days a week for 3 consecutive weeks followed by 1 week of rest. An accumulation of acetylated histones in PBMN cells was observed in all patients at each dose level immediately after infusion. Posttreatment tumor tissue obtained within 4 h after SAHA infusion showed an accumulation of acetylated histones when compared with a pretherapy tumor biopsy. Although no partial or complete tumor responses were seen, tumor regression on radiographs with clinical improvement in cancer-related symptoms were observed, suggesting that SAHA has antitumor activity when administered as a daily 2-h i.v. infusion.

Unique to this Phase I trial was the inclusion of refractory lymphoma patients in the later stages of the study and the independent dose escalation in patients with solid tumor and hematological malignancies. A 3-fold higher dose of SAHA was safely administered to patients with solid tumors compared with the hematological patients, although the pharmacokinetic parameters were similar between groups (Table 4). The treatment delay due to leukopenia/neutropenia and thrombocytopenia observed in the lymphoma patients is most likely related to the limited marrow reserve in these heavily pretreated patients because 67% of the patients had prior peripheral stem cell transplants for their disease.

The daily infusions of SAHA were well tolerated, but in 2 cases, an exacerbation of tumor-related pain was observed, which was similar to what has been previously described with *Vinca* alkaloids (11). They occurred in patients with large tumor burdens; it was not related to the rate of infusion, and pain went back to pretreatment baseline once therapy was discontinued. The explanation for these tumor pain flares is not clear but has been previously been reported with other HDAC inhibitors (12).

As the duration of treatment of SAHA was escalated from 1 week to 3 weeks, mild to moderate fatigue increased along with cumulative thrombocytopenia and renal insufficiency, which were observed in several patients that were on therapy for >4 months. Both the fatigue and renal insufficiency were reversible within 1–2 weeks after discontinuing the drug. There were two serious adverse events noted in the study that included 1 cardiac death that occurred on study after the completion of 3 weeks of therapy, but this was not considered related to the administration of SAHA. Additional cardiac monitoring was implemented but revealed no additional significant cardiac events. One patient developed adult respiratory distress syndrome that was considered likely to be related to the administration of SAHA, however, the etiology of this pulmonary complication is not known.

The pharmacokinetic analysis demonstrated that SAHA is rapidly eliminated and has linear pharmacokinetics with dose-proportional increases in C_{max} and *AUC* in the dose range of 75–900 mg/m^2. The C_{max} at the lowest dose level (75 mg/m^2) exceeded the optimal concentration for differentiation in the murine erythroleukemia cell lines (2.5 μM), indicating that the effective *in vitro* concentration of SAHA could be achieved *in vivo*. Clearance ranged from 49 to 75 liters/h/m^2 across the dose range of 75 to 900 mg/m^2 on the first day of dosing. The interpatient variability of the clearance was 28–38% (coefficient of variation), suggesting that elimination of the drug was not saturable in the dose range studied. The time to reach the maximal plasma concentration was less than the 120-min infusion and could be because of sampling time errors, drug instability, or *in vivo* effects of drug metabolism and *Cl*. To eliminate the possible sampling time errors, patients had pharmacokinetics samples drawn 5 min before the completion of the 2-h infusion. The lower levels of SAHA persisted at the end of the infusion, suggesting the possibility of an *in vivo* effect.

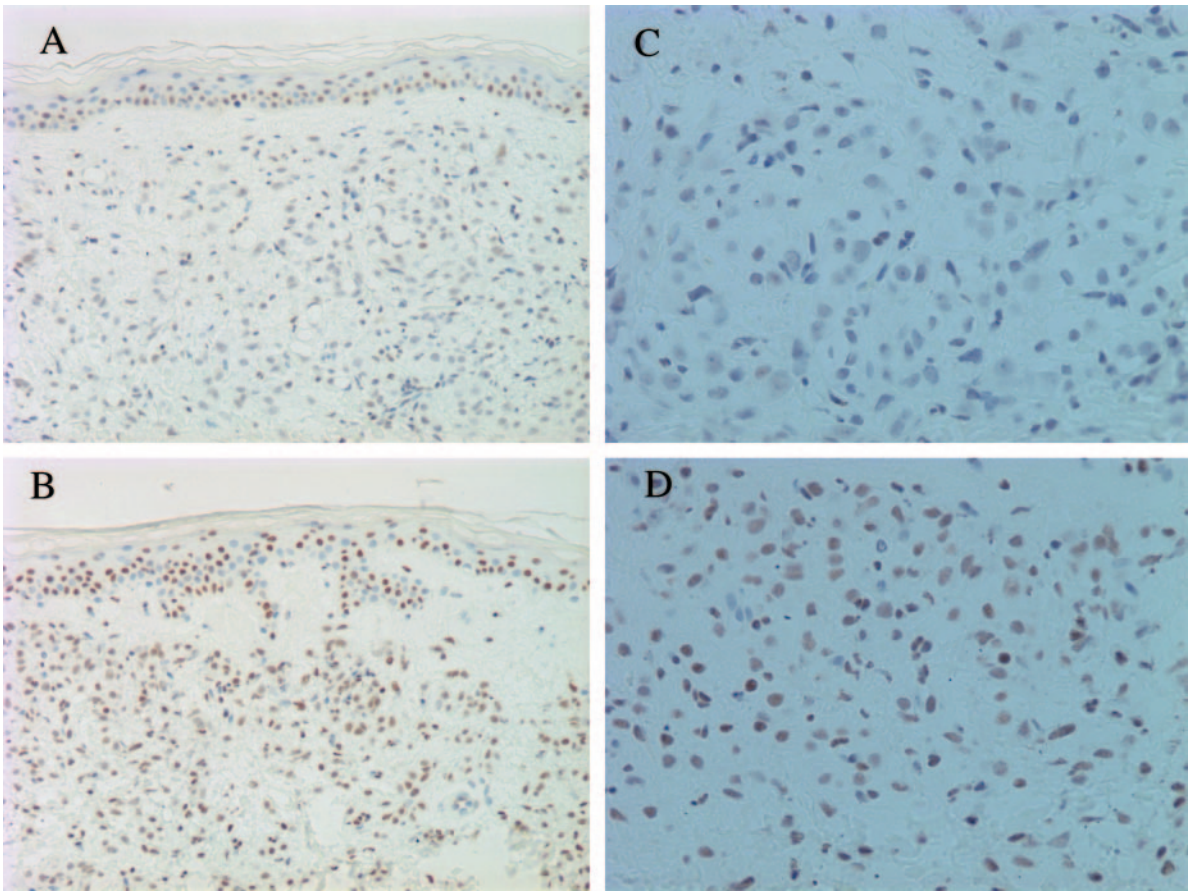

Fig. 5 Pre- and posttherapy biopsy of metastatic skin lesion in a patient with metastatic castrate prostate cancer. The posttherapy sample was obtained within 4 h after infusion of 300 mg/m^2 of i.v. SAHA. A rabbit polyclonal anti-Ac-H3 antibody (1:2500) was used to access the changes in accumulation of acetylated histones. At low magnification, there is an accumulation of acetylated histones observed in the posttherapy sample (*B*) compared with the pretherapy biopsy (*A*). These results are further identified at higher magnification showing increased accumulation posttherapy (*D*) compared with the pretherapy (*C*) biopsy.

Cell lines cultured with SAHA results in the accumulation of acetylated histones as early as 1 h after the addition of the HDAC inhibitor (3). It is believed, based on these studies, that histone acetylation is a useful biological marker of SAHA activity at the cellular level. To assess the biological activity of SAHA in normal tissue and tumors, we evaluated PBMN cells and posttreatment tumor samples for the accumulation of acetylated histones. Western blot analysis demonstrated an increase in accumulation of acetylated histones in PBMN cells immediately after therapy in all patients treated with SAHA, and at higher dose levels (600 and 900 mg/m^2), an accumulation of acetylated histones was detected at 4 h after infusion. We also observed the accumulation of acetylated histones in PBMN cells drawn immediately after infusion after 3 weeks of treatment, suggesting that inhibition of HDAC by SAHA did not diminish after repeated dosing. To establish that we did achieve a biological level of SAHA in the tumor, pre- and posttherapy tumor biopsies were obtained in 11 patients. Five of these patients had sufficient pathological material to perform immunohistochemical analysis. Three patients showed an increase in accumulation of acetylated histones in the posttherapy samples. Two of the 5 patients showed no difference in the immunohistochemical staining pattern comparing pre- and postbiopsy samples. This may be the result of inadequate levels of SAHA in tumor or be related to the handling of the pathological material. Although studies with other HDAC inhibitors showed an increased histone acetylation in peripheral mononuclear cells (13), this is the first trial to demonstrate an increase in histone acetylation in human tumor tissues. Unlike many other biological assays, assessing the histone acetylation status in normal and malignant tissue has been a valuable aide in confirming that SAHA can inhibit target enzymes in normal and malignant tissues *in vivo*. The study was limited in determining the changes associated with acetylated histone H3 or H4. In the future, the use of this biological marker will be further explored by examining the patterns of expression of acetylation of other histones in tumor tissue before the therapy, correlating the clinical outcome to changes in acetylation, developing specific antibodies to all of the HDACs, and elucidating the downstream effects from these agents on the expression of genes.

Other HDAC inhibitors such as phenylbutyrate, depsipeptide, and CI-994 have been evaluated in patients (12, 13–16).

Phenylbutyrate has been extensively evaluated, using a prolonged i.v. infusion. Somnolence and confusion were DLTs in these studies (15). Central nervous system adverse effects have not been observed with i.v. SAHA. Whether SAHA crosses the blood-brain barrier in humans has not been established, but studies in rodents reveal that SAHA can cause the accumulation of acetylated histones in the brain (17), suggesting diffusion across the blood-brain barrier. Thrombocytopenia has been a dose-limiting factor with depsipeptide and CI-994 (13, 16). In our study, thrombocytopenia was mild in most patients and only required stopping drug administration in 1 solid tumor patient. The thrombocytopenia was rapidly reversible, suggesting an inhibitor effect on the bone marrow precursors rather than a cidal effect. In our study, we observed cumulative thrombocytopenia and renal insufficiency that was not reported with the other HDAC inhibitors. This may be related to patient selection or the duration that some patients were treated. Although fatigue was common among patients treated with SAHA, this was not dose limiting as seen in 3 of 8 patients treated at the highest dose level of depsipeptide (13). This may be related to the differences in the potency between drugs or this may be related to the duration of the HDAC inhibition *in vivo* because depsipeptide has a longer terminal $T_{1/2}$ ranging from 4.3 to 49.5 h *versus* SAHA (0.35–1.3 h). Dose-limiting fatigue has also been seen with another hydroxamic acid HDAC inhibitor, pyroxamide, when administered as a 7-day continuous infusion (L. Saltz *et al.,* personal communication). These data suggest that prolonged HDAC inhibition may contribute to the fatigue and intermittent dosing schedules might be better tolerated. Cardiac toxicity was suggested in the preclinical data with depsipeptide, however, after extensive cardiac evaluation and monitoring in the Phase I study of depsipeptide, dysrhythmia was seen in 1 patient, and nonsymptomatic EKG changes occurred in the majority of the cases (13). Although this SAHA study had less intensive cardiac monitoring, nonspecific EKG changes after therapy were also observed but were not clinically significant. These data suggested that short-term administration of HDAC inhibitors appears to have minimal cardiac toxicity, however, long-term effects of these drugs on the myocardium are not known.

This study has established that SAHA can be administered safely to patients at doses that inhibit HDAC activity *in vivo* with evidence of antitumor activity. To improve the convenience of the administration of SAHA, an oral formulation has been developed and is currently being evaluated in clinical trials.

REFERENCES

1. Marks, P., Rifkind, R., Richon, V., Breslow, R., Miller, T., and Kelly, W. K. Histone deacetylase and cancer: causes and therapies. Nat. Rev., *1:* 194–202, 2001.

2. Warrell, R. P., Jr., He, L. Z., Richon, V., Calleja, E., and Pandolfi, P. P. Therapeutic targeting of transcription in acute promyelocytic leukemia by use of an inhibitor of histone deacetylase. J. Natl. Cancer Inst., (Bethesda) *90:* 1621–1625, 1998.

3. Richon, V. M., Emiliani, S., Verdin, E., Webb, Y., Breslow, R., Rifkind, R. A., and Marks, P. A. A class of hybrid polar inducers of transformed cell differentiation inhibits histone deacetylases. Proc. Natl. Acad. Sci. USA, *95:* 3003–3007, 1998.

4. Kelly, W. K., O'Connor, O., and Marks, P. Histone deacetylase inhibitors: from target to clinical trials. EOID, *11:* 1695–1713, 2002.

5. Richon, V. M., Webb, Y., Merger, R., Sheppard, T., Jursic, B., Ngo, L., Civoli, F., Breslow, R., Rifkind, R. A., and Marks, P. A. Second generation hybrid polar compounds are potent inducers of transformed cell differentiation. Proc. Natl. Acad. Sci. USA, *93:* 5705–5708, 1996.

6. Butler, L. M., Agus, D. B., Scher, H. I., Higgins, B., Rose, A., Cordon-Cardo, C., Thaler, H. T., Rifkind, R. A., Marks, P. A., and Richon, V. M. Suberoylanilide hydroxamic acid, an inhibitor of histone deacetylase, suppresses the growth of prostate cancer *in vitro* and *in vivo*. Cancer Res., *60:* 5165–5170, 2000.

7. Cohen, L. A., Amin, S., Marks, P. A., Rifkind, R. A., Desai, D., and Richon, V. M. Chemoprevention of carcinogen-induced mammary tumorigenesis by the hybrid polar cytodifferentiation agent, suberanilohydroxamic acid (SAHA). Anticancer Res., *19:* 4999–5006, 1999.

8. Scher, H. I., Mazumdar, M., and Kelly, W. K. Clinical trials in relapsed prostate cancer: defining the target. J. Natl. Cancer Inst. (Bethesda), *88:* 1623–1634, 1996.

9. Miller, A. B., Hoogstraten, B., Staquet, M., and Winkler, A. Reporting results of cancer treatment. Cancer (Phila.), *47:* 207–214, 1981.

10. Yoshida, M., Kijima, M., Akita, M., and Beppu, T. Potent and specific inhibition of mammalian histone deacetylase both *in vivo* and *in vitro* by trichostatin A. J. Biol. Chem., *265:* 17174–9, 1990.

11. Rowinsky, E., and Donehower, R. Vinca alkaloids and epipodophyllotoxins. *In:* M. Perry (ed.), The Chemotherapy Source Book, Vol. 1, pp. 359–383. Baltimore: Williams & Wilkins, 1992.

12. Carducci, M., Gilbert, J., Bowling, M., Noe, D., Eisenberger, M., Sinibaldi, V., Zabelina, Y., Chen, T., Grochow, L., and Donehower, R. A Phase I clinical and pharmacological evaluation of sodium phenylbutyrate on an 120-h infusion schedule. Clin. Cancer Res., *7:* 3047–3055, 2001.

13. Sandor, V., Bakke, S., Robey, R., Kang, M. H., Blagaskonny, M., Brooks, R., Piekarz, R., Tucker, E., Figg, W. D., Chan, K. K., Goldspiel, B., Sausville, E., Balcerzak, S. P., and Bates, S. E. Phase I trial of a novel antitumor depsipeptide (FR 901228) in patients with refractory neoplasms. Clin. Cancer Res., *8:* 718–728, 2002.

14. Gilbert, J., Baker, S. D., Bowling, M. K., Grochow, L., Figg, W. D., Zabelina, Y., Donehower, R. C., and Carducci, M. A. A Phase I dose escalation and bioavailability study of oral sodium phenylbutyrate in patients with refractory solid malignancies. Clin. Cancer Res., *7:* 2292–2300, 2001.

15. Carducci, M., Bowling, M. K., Eisenberger, M., Sinibaldi, V., Chen, T., Noe, D., Grochow, L., and Donehower, R. Phenylbutyrate (PB) for refractory solid tumors: Phase I clinical and pharmacologic evaluation of intravenous and oral PB. Anticancer Res., *17:* 3972–3973, 1997.

16. Prakash, S., Foster, B., Meyer, M., Wozniak, A., Heilbrun, L., Flaherty, L., Zalupski, M., Radulovic, L., Valdivieso, M., and LoRusso, P. Chronic administration of CI-994: a Phase I study. Investig. New Drugs, *19:* 1–11, 2001.

17. Hockly, E., Richon, V., Woodman, B., Smith, D., Zhou, X., Rosa, E., Sathasivam, K., Ghazi-Noori, S., Mahal, A., Lowden, P., Steffan, J., Marsh, J., Thompson, L., Lewis, C., Marks, P., and Bates, G. Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, ameliorates motor deficits in a mouse model of Huntington's Disease. Proc. Natl. Acad. Sci., USA, *100:* 2041–2046, 2003.

Clinical Cancer Research

Phase I Clinical Trial of Histone Deacetylase Inhibitor: Suberoylanilide Hydroxamic Acid Administered Intravenously

Wm. Kevin Kelly, Victoria M. Richon, Owen O'Connor, et al.

Clin Cancer Res 2003;9:3578-3588.