A Phase I Study of Intravenous LBH589, a Novel Cinnamic Hydroxamic Acid Analogue Histone Deacetylase Inhibitor, in Patients with Refractory Hematologic Malignancies

Francis Giles,[1] Thomas Fischer,[2] Jorge Cortes,[1] Guillermo Garcia-Manero,[1] Joachim Beck,[2] Farhad Ravandi,[1] Eric Masson,[3] Patricia Rae,[3] Glen Laird,[3] Sunil Sharma,[3] Hagop Kantarjian,[1] Margaret Dugan,[3] Maher Albitar,[4] and Kapil Bhalla[5]

Abstract

Purpose: LBH589 is a novel histone deacetylase inhibitor that inhibits proliferation and induces apoptosis in tumor cell lines. In this phase I study, LBH589 was administered i.v. as a 30-minute infusion on days 1 to 7 of a 21-day cycle.

Experimental Design: Fifteen patients (median age, 63 years; range, 42-87 years) with acute myeloid leukemia (13 patients), acute lymphocytic leukemia (1 patient), or myelodysplastic syndrome (1 patient) were treated with LBH589 at the following dose levels (mg/m^2): 4.8 (3 patients), 7.2 (3 patients), 9.0 (1 patient), 11.5 (3 patient), and 14.0 (5 patients). The levels of histone acetylation were measured using quantitative flow cytometry and plasma LBH589 concentrations were assayed.

Results: Four dose-limiting toxicities (grade 3 QTcF prolongation) were observed, four at 14.0 mg/m^2 and one at 11.5 mg/m^2. QTcF prolongation was asymptomatic and reversed on LBH589 discontinuation. Other potentially LBH589-related toxicities included nausea (40%), diarrhea (33%), vomiting (33%), hypokalemia (27%), loss of appetite (13%), and thrombocytopenia (13%). In 8 of 11 patients with peripheral blasts, transient reductions occurred with a rebound following the 7-day treatment period. H3 acetylation increase was significant in B-cells (CD19$^+$; $P = 0.02$) and blasts (CD34$^+$; $P = 0.04$). The increase in H2B acetylation was highest in CD19$^+$ and CD34$^+$ cells [3.8-fold ($P = 0.01$) and 4.4-fold ($P = 0.03$), respectively]. The median acetylation of histones H2B and H3 in CD34$^+$ and CD19$^+$ cells significantly increased on therapy as did apoptosis in CD14$^+$ cells. Area under the curve increased proportionally with dose with a terminal half-life of $\sim$11 hours.

Conclusion: Intravenous administration of LBH589 was well tolerated at doses $<$11.5 mg/m^2 with consistent transient antileukemic and biological effects.

Epigenetic changes are modifications in the pattern of gene expression mediated by mechanisms other than alterations in the primary nucleotide sequence (1). Epigenetic deregulation is integral to the pathophysiology of leukemia causing aberrant transcription of genes involved in cell growth, proliferation, differentiation, and apoptosis (2). There is an increasing focus on the mechanisms underlying epigenetic change in leukemia as potential therapeutic targets because these changes may be reversible (3). These mechanisms alter the accessibility of DNA to regulators of transcription. Nucleosomes are regularly repeating units of chromatin in which 146 bp of DNA are tightly wrapped around the core histone octamer (4). Nucleosomes consist of two units each of core histone H2A, H2B, H3, and H4. Each nucleosome is connected to its neighbor by a short segment of linker DNA. Histone H1 binds and stabilizes the linker DNA. Alterations in chromatin structure are required to allow gene expression and to change DNA from a compact inaccessible chromatin, which does not facilitate binding of RNA polymerase, to a more accessible template DNA.

Covalent modifications of the core histones are an important mechanism rendering chromatin more accessible. Core histone subunits have NH_2-terminal tails, which are lysine rich and positively charged, and protrude from the nucleosome. Posttranslational covalent modifications at specific amino acid residues in the NH_2-terminal histone tails are carried out by a variety of enzyme families and are a major determinant of chromatin structure and gene activity (5). One important set of modifications include the acetylation and deacetylation of

Authors' Affiliations: [1]Department of Leukemia, University of Texas M.D. Anderson Cancer Center, Houston, Texas; [2]Johannes-Gutenberg-Universität, Mainz, Germany; [3]Novartis Pharmaceuticals Corporation, East Hanover, New Jersey; [4]Quest Diagnostics Nichols Institute, San Juan Capistrano, California; and [5]H. Lee Moffitt Cancer Center, Tampa, Florida

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Requests for reprints: Francis J. Giles, Department of Leukemia, The University of Texas M.D. Anderson Cancer Center, 1400 Holcombe Boulevard, Box 428, Houston, TX 77030. Phone: 713-792-7305; Fax: 713-794-4297; E-mail: frankgiles@aol.com.

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lysine residues by histone acetyltransferases and histone deacetylases, respectively (6). Histone acetylation is usually associated with "active" chromatin, facilitating transcriptional activity, whereas deacetylated histones are associated with condensed chromatin and reduced transcription. Three different classes (class 1-3) of histone deacetylases have been identified, which differ in their intracellular localization, pattern of substrates, and binding sites inhibitors (3). As evidence of aberrant histone deacetylase activity in leukemia has accumulated, so has the focus on developing both naturally occurring and synthetic histone deacetylase inhibitors as potential therapeutic agents. These inhibitors are structurally diverse and differ in the classes of histone deacetylase that they inhibit. Histone deacetylase inhibitors generally induce cell cycle growth arrest in G_1 or G_2-M differentiation and or apoptosis of malignant cells with relatively little effect on normal cells. Apoptosis induced by histone deacetylase inhibitors involves both the intrinsic (mitochondrial-initiated) and the extrinsic (death ligand – mediated) pathways. Hydroxamic acids derivatives, among the most potent inhibitors of both class 1 and class 2 histone deacetylase, include suberoylanilide hydroxamic acid, which has single-agent anticancer activity (7).

LBH589 is a novel cinnamic hydroxamic acid analogue histone deacetylase inhibitor that induces apoptosis in a dose-dependent manner on human chronic myelogenous leukemia blast crisis K562 cells and acute leukemia MV4-11 cells with the activating length mutation of FLT-3 (8). Exposure to LBH589 is associated with hyperacetylation of H3, H4, and heat shock protein 90, an increase in p21 levels, and induction of cell cycle G_1 phase accumulation. In MV4-11 cells, this is associated with marked attenuation of the protein levels of p-FLT-3, FLT-3, p-AKT, and phospho-extracellular signal – regulated kinase 1/2. In K562 cells, exposure to LBH589 attenuates Bcr-Abl, p-AKT, and phospho-extracellular signal – regulated kinase 1/2. Treatment with LBH589 also inhibits the DNA binding activity of signal transducers and activators of transcription-5 in both K562 and MV4-11 cells; LBH589 also depletes mutant Bcr-Abl (including the point mutant Bcr-Abl T315I) and induces apoptosis in imatinib-refractory leukemia cells (8). It also inhibits the chaperone association of heat shock protein 90 with Bcr-Abl and FLT-3, promoting their polyubiquitylation and proteasomal degradation (8).

As other histone deacetylase inhibitors have shown clinical activity, including phenylbutyrate in a patient with acute promyelocytic leukemia and depsipeptide in patients with acute myeloid leukemia (AML) or chronic lymphocytic leukemia, a phase I study of LBH589 was conducted in patients with refractory hematologic malignancies (9, 10).

Patients and Methods

The study was approved by the ethics committee or institutional review board of all participating institutions. All patients gave signed informed consent as per institutional guidelines. The study was designed to determine the maximum-tolerated dose (MTD) and dose-limiting toxicity (DLT) of LBH589 as a single agent when administered as a 30-minute i.v. infusion once daily on days 1 to 7 of a 21-day cycle and to characterize the pharmacokinetic profile of LBH589 in plasma. The pharmacodynamic profile of LBH589 was also assessed by measuring changes in histone acetylation in malignant and normal progenitor cells taken from bone marrow and/or blood, as well as bone marrow biopsy sample, and changes in biomarkers of apoptosis (Annexin V) in malignant and normal progenitor cells taken from bone marrow and/or peripheral blood.

Patient eligibility. Patients of ages ≥18 years, with WHO performance status of <2, and with relapsed or refractory leukemias for which no standard therapy was anticipated to result in a durable remission were eligible for study entry. Patients with untreated leukemia electing not to receive standard therapy were also eligible. Patients with active controlled infection including chronic hepatitis were eligible. Other eligibility criteria included serum bilirubin of ≤1.5 mg/dL; aspartate aminotransferase or alanine aminotransferase levels equal or less than three times the upper limit of normal; and serum creatinine of ≤2.0 mg/dL. In the absence of rapidly progressive disease, the interval from prior treatment with myelosuppressive cytotoxic agents could not be less than 2 weeks, and patients requiring hydroxyurea for control of peripheral blood cell counts must have discontinued the hydroxyurea at least 48 hours before treatment on study. Patients were allowed no cytotoxic therapy other than hydroxyurea in the 2 weeks preceding study drug administration. All patients of childbearing potential agreed to use adequate contraception for the duration of the study. Pregnant or nursing patients were excluded and any woman of childbearing potential required a negative pregnancy test within the week before study entry. Additional ineligibility criteria included a history of central nervous system involvement, acute or chronic liver disease, confirmed diagnosis of HIV, or impaired cardiac function (left ventricular ejection fraction <45% as determined by multigated acquisition scan or echocardiogram); complete left bundle branch block; obligate use of a cardiac pacemaker; ST depression of >1 mm in two or more leads and/or T wave inversions in two or more contiguous leads; congenital long QT syndrome; history of or presence of significant ventricular or atrial tachyarrhythmias; clinically significant resting bradycardia (<50 beats per minute); QTcF >480 ms on screening electrocardiogram; or other clinically significant heart disease (e.g., unstable angina pectoris, congestive cardiac failure, myocardial infarction within 6 months).

Treatment and study design. LBH589 was supplied by Novartis Pharmaceuticals Corporation (East Hanover, NJ) as a clear, colorless, slightly viscous, sterile, nonaqueous solution in 5-mL vials containing 25 mg of LBH589. LBH589 vials were stored under refrigeration at 2° to 8°C (36-46°F). LBH589 was diluted in 5% dextrose in water for i.v. administration to patients over 30 minutes once daily on days 1 to 7 of a 21-day cycle.

The starting dose of 4.8 mg/m^2 was determined following a review of data from the ongoing first-time-in-man dose-escalation study in patients with solid tumors. This two-arm, multicenter dose-escalation study of LBH589 (CLBH589A2101) in solid tumors evaluated two schedules of administration. In arm 1 of this study, LBH589 was given on days 1 to 3 and days 8 to 10 of a 21-day cycle. In arm 2, LBH589 was to be given on days 1 to 3 and days 15 to 17 of a 28-day cycle. At the time of dose selection for the current study, arm 2 had not yet started enrolling patients; thus, dose selection was done using arm 1 data. Dose escalation in arm 1 proceeded according to a modified Accelerated Titration Design (11), enrolling one to six patients per dose cohort. At the time of review, a total of nine patients have been treated at 1.2, 2.4, 4.8, or 7.2 mg/m^2 and no DLTs had been reported. The median number of cycles was 2 cycles (range, 1-6 cycles). At doses ≥4.8 mg/m^2, LBH585-related adverse events were grade 1 thrombocytopenia, transient grade 3 lymphopenia, and transient grade 3 neutropenia (that did not meet the definition of DLT). There were no other Common Toxicity Criteria for Adverse Events (CTCAE) grade 3 or 4 adverse events. Preliminary pharmacokinetic analysis of the 1.2 and 2.4 mg/m^2 doses indicated that there was no drug accumulation using this dosing schedule. Analysis of histones (H3) from normal lymphocytes revealed acetylation for up to 6 hours following the start of a 30-minute LBH589 infusion in two of two patients treated at the 2.4 mg/m^2 dose level. No acetylation was seen at 24 hours post dose. Therefore, a starting dose of 4.8 mg/m^2 given on days 1 to 7 every 21 days was chosen for the current study, as it was thought that a longer 7-day dosing period might be required to control disease in acute leukemias, in contrast to solid tumors. In addition, it was

anticipated that the hematologic toxicity seen in the solid tumor trial using the 4.8 mg/m^2 dose would be more pronounced using a 7-day dosing regimen. Using a 4.8 mg/m^2 starting dose in the 7-day regimen was expected to result in histone acetylation without an excessive risk of severe toxicities in a patient population with advanced hematologic malignancies.

Patients were evaluated on the day of therapy and at least thrice weekly and as clinically indicated while on study. At each evaluation, patients were assessed for toxicity, and complete blood count with platelets and differential, serum chemistries, liver function tests, and serial electrocardiograms were obtained.

Toxicity was graded on a scale of 0 to 5 using the National Cancer Institute Common Toxicity Criteria (NCI-CTC version 3.0). All patients who received therapy on study were considered evaluable for toxicity. DLT was defined as any clinically significant grade 3 or 4 non-hematologic toxicity; myelosuppression manifested as grade 4 neutropenia or thrombocytopenia with bone marrow hypoplasia at day 56 with a bone marrow cellularity of ≤5% and no evidence of leukemia, a serum creatinine of ≥2.0 × upper limit of normal, and a total bilirubin >2 × upper limit of normal; grade 3 AST or ALT for >7 days; or grade 4 AST or ALT of any duration, grade 3 or 4 vomiting, or grade 3 nausea despite standard antiemetics; or grade 3 or 4 diarrhea despite optimal antidiarrhea medication. MTD was defined as the dose level at which none or one of six patients experiences DLT with at least two patients experiencing DLT at the next higher dose level.

Measuring histone acetylation. Quantitative flow cytometry was used to measure the levels of H2B and H3 acetylation in cells. The flow cytometry approach allowed gating on specific cell populations and quantification of the levels of acetylated histone in that cell population. Permeabilization and intracellular staining were used to minimize the cell membrane disturbance. Briefly, whole blood was stained with CD19, CD3, and CD34 (BD Biosciences, San Jose, CA) cell-surface markers by incubating for 15 minutes, followed by fixation and permeabilization by adding 100 μL of Intraprep Reagent 2 (Beckman Coulter, Fullerton, CA) and incubation for 5 minutes at room temperature in the dark. Acetylated H2B (Lys$^{5/12/15/20}$) or acetylated H3 (Lys$^{9/14}$; Santa Cruz Biotech, Santa Cruz, CA) antibody was added and the mixture was vortexed and incubated for 15 minutes at room temperature in the dark. After washing once with PBS, 5 μL of goat anti-rabbit phycoerythrin were added (1:1) for labeling (BD Biosciences). After 15-minute incubation at room temperature in the dark, cells were washed with PBS. Cells were analyzed on the FACSCalibur (BD Biosciences). Standardization was done before acquisition of each sample using QuantiBRITE phycoerythrin beads (Becton Dickinson, San Jose, CA). Flow cytometry output was converted in terms of antibodies bound per cell with QuantiCALC software (Becton Dickinson, San Jose, CA). Analysis gates were placed on the target populations to evaluate the percentage of cells positively stained with specific antibodies using CellQuest Pro software. The number of antibodies bound per cell (antibody binding capacity) in the positive cells was multiplied by the percentage. The number of molecules detected in 100 total cells as well as the number of molecules detected in positive cells was studied. Because the number of molecules in 100 cells reflects both percentage and intensity per cell, all data presented here are number of molecules in 100 total cells.

Mitochondrial potential measurement (DePsipher assay). Red cells were lysed and washed twice. DePsipher (0.5 μL; Trevigen, Inc., Gaithersburg, MD) was added and incubated at 37C in 5% CO_2 for 20 to 30 minutes. Cells were then washed with PBS and immediately analyzed on FACSCalibur.

Measurement of Annexin V. Cells were isolated using double-density Histopaque 1119 and 1077 to isolate both mononuclear and polymorphonuclear cells. The two cell populations were mixed, washed, and stained with Annexin V and propidium iodide as recommended by the manufacturer (Becton Dickinson, Mansfield, MA). Costaining with CD14 and CD34 was also done.

Measurement of bromodeoxyuridine incorporation. Briefly, cells were washed twice and 0.5 mL of 1× PBS-sodium azide was added with 4 mL of RPMI medium. Similar number of cells was also prepared in different wells. Bromodeoxyuridine was then added (1 μL/mL) to one well and incubated for 45 minutes. Cells were washed and costaining with CD34 was done following standard procedure.

Results

Patient characteristics. Fifteen patients (median age, 63 years; range, 42-87 years) with either AML (13 patients), acute lymphocytic leukemia (1 patient), or myelodysplastic syndrome (1 patient) received one cycle of LBH589 at the following dose levels (mg/m^2) as detailed in Table 1: 4.8 (3 patients), 7.2 (3 patients), 9.0 (1 patient), 11.5 (3 patients), and 14.0 (5 patients).

Toxicity. Potentially LBH589-related grade 3 and 4 adverse events by dose level and incidence are summarized in Table 1. To be evaluable for DLT, patients must either have received at least six of the scheduled seven doses of LBH589 administered over ≤9 days with no more than 72 hours between doses, have completed all required safety evaluations, or have been observed for at least 21 days or have experienced DLT before day 21. At the first dose level of 4.8 mg/m^2, three patients received all required doses and there were no DLTs. The dose was then escalated to 7.2 mg/m^2. Two patients experienced grade 2 LBH589-related toxicity at the 7.2 mg/m^2 dose level. One of the grade 2 toxicities was a grade 2 QT prolongation corrected using the Bazett's correction factor (QTcB). When the same electrocardiogram was corrected using the Fredericia correction factor (QTcF), the QT interval was no longer prolonged. The QTcF is considered more accurate for patient with a slower heartbeat (11). Following a review by an independent cardiologist, the QTcF value was used for dose escalation purposes and the decision was made to escalate the dose to 9.0 mg/m^2 and to continue to use one-patient cohorts for the evaluation of safety. Therefore, only one patient was enrolled at the subsequent dose level of 9.0 mg/m^2.

The one-patient 9.0 mg/m^2 cohort was completed without DLT, although the patient did experience a grade 2 QTcB prolongation on day 5 of dosing, which was grade 1 by QTcF correction and did not recur despite repeated dosing on days 6 and 7.

The dose was then escalated to 14.0 mg/m^2, representing a 50% dose increase over the previous dose level. Of the five patients treated at this dose, only one patient received all of the scheduled doses, three patients experienced grade 3 QTcF prolongations, and one patient died of pulmonary hemorrhage resulting from sepsis while on study. The three patients with grade 3 QTcF prolongation were all considered to have DLT. Following the first grade 3 QTcF prolongation, two patients were treated simultaneously and both experienced grade 3 QTcF prolongations during the same time period.

Following review of the data from the 14.0 mg/m^2 cohort, the dose was reduced to 11.5 mg/m^2, the mid-point between the 9.0 and the 14.0 mg/m^2 dose levels. One of the three patients treated at this dose level experienced a DLT of a grade 3 QTcF prolongation. The study was halted after the first three patients were treated at 11.5 mg/m^2 due to safety concerns about QTcF prolongation at this dose level.

A total of 368 electrocardiograms were collected on 15 patients. Of these, 17 electrocardiograms in five patients were considered outliers (QTcF that was either >500 or 60 ms greater than the average of six baseline electrocardiograms). All outlier

Table 1. LBH589 dose levels and possibly related grade 3 or 4 adverse events

Dose level (mg/m^2)	Dose level sequence*	No. patients treated	Patients receiving all doses†	No. DLT patients
4.8	1	3	3	0
7.2	2	3	1‡	0
9.0	3	1	1	0
11.5	5	3	2	1
14.0	4	5	1	3

NCI-CTCAE criteria	Grade 3, *n* = 15 (dose, mg/m^2)	Grade 4, *n* = 7 (dose, mg/m^2)
Hepatic		
Elevated ALT	1 (11.5)	
Blood/bone marrow		
Thrombocytopenia		1 (14.0)
Neutropenia		1 (11.5)
Anemia	1 (11.5)	
Cardiac		
QTcF prolongation	4 (11.5, 14.0—3 pts)*	
Pericardial effusion		1 (14.0)
Metabolic/laboratory		
Hypokalemia	1 (14.0)	

*Indicates chronological order dose levels were tested.
†Received at least six of the scheduled seven doses of LBH589 administered over ≤9 days with no more than 72 hours between doses.
‡Two patients received five of the seven scheduled doses before experiencing grade 2 LBH589-related adverse events (atrial fibrillation and QTcB prolongation, respectively) that did not meet the protocol definition of DLT.

electrocardiograms were observed in cycle 1 of dose administration between days 1 and 8 of the cycle. QTcF values range from 348 to 541 ms whereas the difference from baseline ranged from 65 ms below baseline to 126 ms above baseline. Other asymptomatic electrocardiogram abnormalities observed on study were T-wave changes observed in 12 of 15 (80%) patients and at all dose levels; ST segment changes in 3 of 15 (20%) patients; and 1 patient with adenomatous polyposis coli and sinus bradycardia. Patients that were followed after day 11 with undetectable LBH589 levels had resolution of all ST and T-wave changes.

Although QTcF was related to the dose administered, QTcF did not seem to be related to plasma pharmacokinetic variables including C_{max} and area under the curve (AUC).

The most commonly reported potentially related noncardiac adverse events (all grades) occurring in >10% of patients were as follows: nausea in 6 of 15 (40%) patients, diarrhea in 5 of 15 (33%) patients, vomiting in 5 of 15 (33%) patients, hypokalemia in 4 of 15 (27%) patients, anorexia in 2 of 15 (13%) patients, and thrombocytopenia in 2 of 15 (13%) patients. Adverse events were generally mild to moderate with the exception of thrombocytopenia, which was grade 4. Grade 1 to 2 nausea and diarrhea were observed in patients treated at most dose levels ranging from 4.8 to 14.0 mg/m^2. Grade 1 to 2 vomiting, hypokalemia, and anorexia, as well as grade 4 thrombocytopenia, were observed in patients treated at the highest dose levels of 11.5 and 14.0 mg/m^2. One patient treated at 14.0 mg/m^2 died of pulmonary hemorrhage while septic. A white cell differentiation syndrome, which was manifested by pericardial effusion and resolved on corticosteroid therapy, was observed in one patient with refractory AML treated at 14.0 mg/m^2, who had an associated transient decrease in peripheral blast counts.

Serum chemistry variables were reviewed for new or worsening abnormalities. Decreased potassium levels were noted as clinically significant, with 27% of patients showing new or worsening incidences of grade 3 or 4 hypokalemia (Table 2). Except for one patient at the lowest dose level, all patients required potassium supplementation during treatment and within 5 days of treatment completion. Requirements for potassium seemed to increase at doses ≥7.2 mg/m^2. Potassium replacement was frequently required up to 5 days following the last dose of LBH589 treatment (Table 2). Blood urea nitrogen and creatinine also tended to increase during the 7-day treatment period. Serum creatinine values increased during treatment to grade 1 in three patients and to grade 2 in one patient. Blood urea nitrogen values increased to more than the upper limit of normal to 1.5 × upper limit of normal in four patients and >1.5 × upper limit of normal to 2.5 × upper limit of normal in three patients (although two of these patients were more than the upper limit of normal at baseline).

The MTD dose level was exceeded at 14.0 mg/m^2 where DLTs were grade 3 QTcF prolongations in three patients. The study was halted after three patients were treated at 11.5 mg/m^2 due to safety concerns about QTcF prolongation in this study and in a separate study being done with LBH589 administered i.v. in patients with solid tumors.

Response. One patient treated at 11.5 mg/m^2 met the protocol-defined response criteria for hematologic improvement in platelet variables at the end of cycle 1. Additional cycles

of therapy were not given due to safety concerns and the patient's platelets subsequently declined. In 8 of 11 patients with blasts in the peripheral blood, treatment with LBH589 resulted in transient reductions in peripheral blasts during the 7-day treatment period. Six of these patients received all 7 days of treatment with LBH589 and one patient each received 4 and 5 days of treatment with LBH589, respectively. Reductions in peripheral blasts were seen at all dose levels tested. Blast counts were reduced by a maximum of 66% (one patient), 70% (one patient), 89% (one patient), and 100% (five patients). Counts generally rebounded following the 7-day treatment period. In 14 of 15 patients, bone marrow aspirate blasts were elevated at baseline. In contrast to findings in the peripheral blood, only 1 of the 14 patients had a reduction in the bone marrow blasts (from 16% to 5%) during the 7-day treatment period. Following the 7-day treatment period, the patient's blasts returned to their baseline value. As patients with prior overt AML progression, lack of response, or DLT did not have the end of cycle bone marrow assessment, only six patients had a day 21 end of cycle bone marrow assessment for response.

Biomarker changes. To monitor the biological effects of the therapy, apoptosis, proliferation, and levels of histone acetylation in bone marrow and peripheral blood cells were measured. Techniques used included quantitative flow cytometry and antibodies against acetylated H3 (ac-H3) and acetylated H2B (ac-H2B) to measure histone acetylation and Annexin V with mitochondrial potential to measure apoptosis. Proliferation was measured by bromodeoxyuridine incorporation. Costaining with CD34, CD3, and CD19 allowed gating on specific cell population and the monitoring of changes in blasts, T cells, and B cells. Pre- and post-therapy samples were analyzed. The median acetylation of histones H2B and H3 in $CD34^+$ cells in peripheral blood increased on therapy from 353 to 1,547 and from 10,397 to 37,339 molecules in 100 cells, respectively. The levels of ac-H2B and ac-H3 in $CD34^+$ cells in peripheral blood increased significantly ($P = 0.03$ and $P = 0.04$, respectively) by day 7 on therapy (Fig. 1). The median intensity of ac-H2B increased 4-fold. The highest increase in both ac-H2B and ac-H3 was in $CD19^+$ cells. The median percentage of cells staining positively for ac-H2B and ac-H3 increased in $CD19^+$ cells from 20% to 81% ($P = 0.02$) and from 17% to 80% ($P = 0.05$), respectively. The intensity of staining was not evaluable as insufficient normal $CD19^+$ cells were detectable. A dose effect was also observed. More samples were available at day 7 of therapy from patients treated with 7.2 and 11.5 mg/m^2 (three of each). In $CD34^+$ cells, there was greater induction of ac-H2B on day 7 at 11.5 mg/m^2 as compared with 7.2 mg/m^2 ($P = 0.05$; Fig. 2). This dose effect was also seen in H3 acetylation in $CD3^+$ cells ($P = 0.03$) but not in $CD19^+$ cells. When all patients were considered, the increase in histone acetylation at day 7 was associated with a significant increase in apoptosis in $CD14^+$ cells ($P = 04$) but not in $CD34^+$ or $CD19^+$ cells.

Pharmacokinetic findings. Plasma pharmacokinetic profiles were analyzed on day 1 and day 5 or 7. The pharmacokinetic variables of LBH589 derived from the plasma concentration time curves after first doses are summarized in Table 3. Maximum plasma concentration and drug exposure increased proportionally with dose (Fig. 3) with higher values for both variables seen on day 7 versus day 1. LBH589 plasma concentration peaked either at midpoint or at the end of the 0.5-hour infusion, then dropped rapidly within the first 4 hours to reach terminal phase between 4 and 24 hours. The terminal half-lives ranged from 8 to 16 hours. After repeated daily doses, LBH589 accumulated slightly as evidenced by the accumulation ratio ranging from 1.3 to 1.7 on average. This accumulation ratio was similar to the predicted accumulation index based on the dosing interval and terminal half-life.

Discussion

The purpose of this phase I study was to define reasonable i.v. doses of LBH589 that may be examined in the phase II setting in patients with refractory leukemia. The DLT observed with a 30-minute i.v. administration was asymptomatic, reversible QTcF prolongation. The cardiac toxicities observed in this study were somewhat predictable from preclinical data. *In vitro*

Table 2. Hypokalemia grade by dose level and incidence and days of potassium supplementation by dose level

Test/LBH589 cohort (mg/m^2)	Total, *n*	CTCAE grade 3		CTCAE grade 4	
		Total	*n* (%)	Total	*n* (%)
Hypokalemia					
4.8	3	3	1 (33.3)	3	0 (0)
7.2	3	3	0 (0)	3	0 (0)
9.0	1	1	1 (100)	1	0 (0)
11.5	3	3	1 (33.3)	3	0 (0)
14.0	5	5	0 (0)	5	1 (20.0)
Total	15	15	3 (20.0)	15	1 (6.7)

Dose level (mg/m^2)	Average no. days on LBH589 therapy	Average no. days patients required potassium supplementation
4.8	7	1.3
7.2	5.6	4.3
9.0	7	10
11.5	5.3	8
14.0	5	4

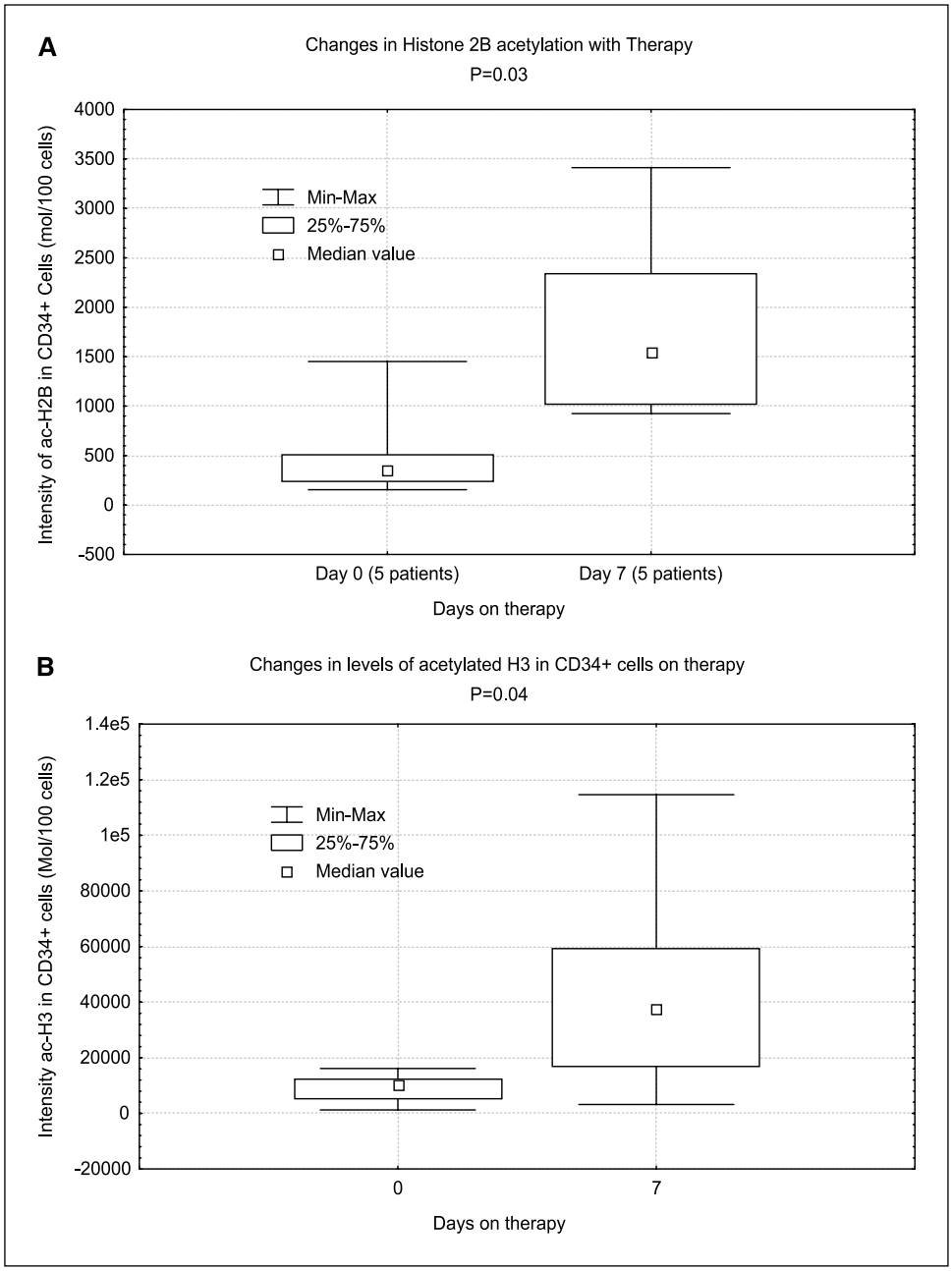

Fig. 1. Changes in histone H2B acetylation (*A*) or histone H3 acetylation in $CD34^+$ cells (*B*) on LBH589 therapy.

electrophysiology data showed that LBH589 had an IC_{50} of 3.9 μmol/L in the hERG channel assay and induced a benign increase of action potential duration in the isolated rabbit heart assay at concentrations in the lower micromolar range, indicating the potential to cause QTcF prolongation. In addition, in animal multiple-dose toxicity studies, the primary target organs for toxicity were identified as erythropoietic and myelopoietic systems and lymphatic organs and tissues. In addition to effects on the hematopoietic system, dogs developed diarrhea at high doses and residual follicular hypertrophy in the thyroid was observed at the end of the 4-week recovery period.

Decreased potassium levels were also associated with i.v. LBH589 administration, with 27% of patients showing new or worsening grade 3 or 4 hypokalemia. All patients, except one, treated at the lowest dose level required potassium supplementation during treatment and within 5 days of treatment completion. Requirements for potassium seemed to increase at doses $\geq$7.2 mg/m^2 compared with the lowest dose level; in some cases, potassium replacement was still required up to 5 days following the last dose, indicating a prolonged effect. Hypokalemia is a known risk factor for QT prolongation but no relationship between LBH589-associated hypokalemia and QTcF prolongation could be established. QT prolongation has been reported with other histone deacetylase inhibitors and thus seems to be a true class effect (3, 11).

Mild or moderate (grade 1 and 2) vomiting and diarrhea were also observed and may have contributed to potassium loss. In addition, changes in renal variables blood urea nitrogen and creatinine, although not sufficient to cause significant potassium loss on their own, may have contributed to overall

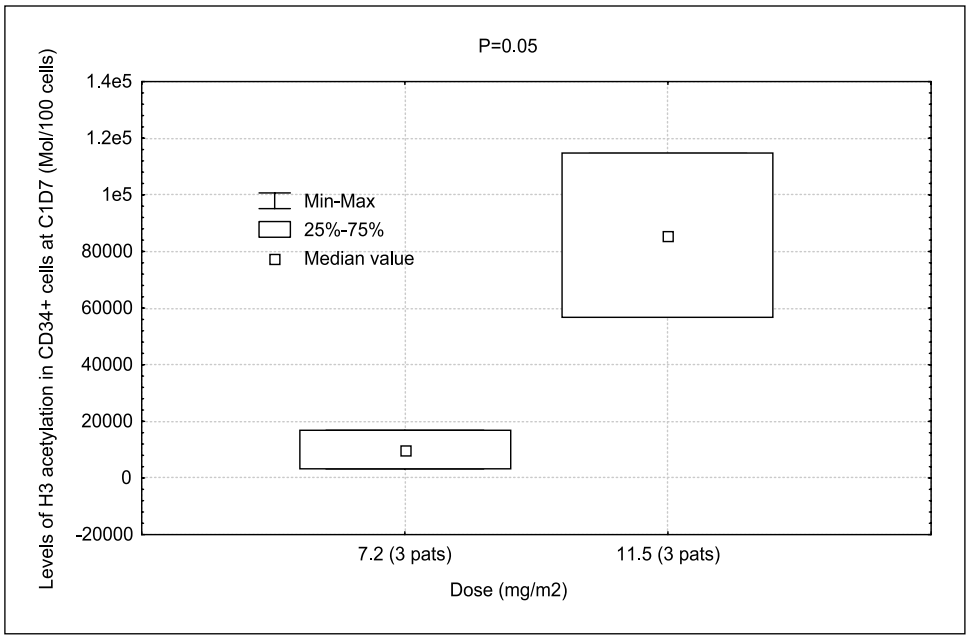

Fig. 2. Induction of H3 acetylation in $CD34^+$ cells by increasing dose of LBH589.

potassium loss when combined with vomiting and diarrhea. However, as no other electrolytes showed the same pattern of loss as potassium, an LBH589-induced mechanism that is specific for potassium consumption and/or wasting cannot be excluded. Other notable toxicities were nausea, loss of appetite, and thrombocytopenia.

MTD was not defined for this study. The MTD dose level was exceeded at 14.0 mg/m^2 wherein dose-limiting adverse events were grade 3 QTcF prolongations in three patients. The MTD dose level was not sufficiently explored at the reduced dose level 11.5 mg/m^2, wherein one of the three patients treated had a grade 3 QTcF prolongation. This dose level was never expanded to six patients to define the DLT dose level. The study was halted after three patients were treated at 11.5 mg/m^2 due to safety concerns about QTcF prolongation in this study and the LBH589 solid tumor study. In the solid tumor trial also using the i.v. formulation, central tendency analysis (mean change from baseline), a dose related increase in QTcF of ≤20 ms was detected after day 3 of dosing. The frequency of QTcF >500 ms (grade 3) increased with dose. At doses ≤ 9 mg/m^2, only one patient (1 of 32) had QTcF >500 ms, whereas at doses >9 mg/m^2, 5 of 13 patients had QTcF >500 ms.

Although there is a clear relationship between dose administered and reversible, asymptomatic grade 3 QTcF prolongation, this relationship with QTcF was not reflected in the plasma pharmacokinetic variables C_{max} and $AUC_{0\text{-}24}$. It may be that LBH589 is initiating a physiologic process that prolongs QTcF but has no temporal dependency on LBH589 plasma concentration or exposure.

LBH589 exhibited transient antileukemic activity at all doses tested. In 7 of 11 patients with blasts in the peripheral blood, counts were reduced during the treatment period but rebounded shortly after treatment completion. One patient treated at 11.5 mg/m^2 met the protocol-defined response criteria for hematologic improvement in platelet variables at the end of cycle 1. She was the last patient assigned to the study and additional cycles of therapy were not given due to concerns over QTcF prolongation in this trial and in a concurrent trial of LBH589 in solid tumors. The patient's platelet count subsequently declined. In addition, a white cell differentiation syndrome (reminiscent of those observed during treatment with retinoic acid), which was manifested by pericardial effusion (and was successfully treated with high-dose steroids), was observed in one patient.

The demonstration of a significant increase in acetylation of the H2B and H3 histones in the leukemic blast ($CD34^+$) cells is consistent with LBH589 reaching its target. The lack of demonstrable significant increase in apoptosis in $CD34^+$ cells indicates resistance of some leukemic cells to this single agent. The demonstration of apoptosis in some subsets of cells in the context of reductions in circulating leukemic cells in some patients reflects activity, which was not of a sufficient degree to cause major tumor load reduction. The ongoing studies with oral LBH589 will address the issue of single agent optimization—

Table 3. Pharmacokinetic variable estimates by a noncompartmental analysis after the first dose (mean ± SD)

Day	Dose (mg/m^2)	T_{max} (h)	C_{max} (ng/mL)	$AUC_{0\text{-}24}$ (h ng/mL)	$AUC_{0\text{-}8}$ (h ng/mL)	$t_{1/2}$ (h)
1	4.8 (n = 3)	0.4 ± 0.1	182.3 ± 62.7	143.1 ± 62.6	158.3 ± 75.8	7.8 ± 5.0
	7.2 (n = 3)	0.4 ± 0.1	120.7 ± 10.1	134.9 ± 7.09	150.7 ± 9.5	8.0 ± 2.1
	9.0 (n = 1)	0.5	201.0	194.5	240.9	13.9
	11.5 (n = 3)	0.4 ± 0.2	249.3 ± 147.4	295.5 ± 97.3	322.8 ± 122.3	9.5 ± 6.3
	14.0 (n = 5)	0.4 ± 0.1	565.6 ± 450.9	372.9 ± 196.9	459.6 ± 242.6	12.0 ± 2.8

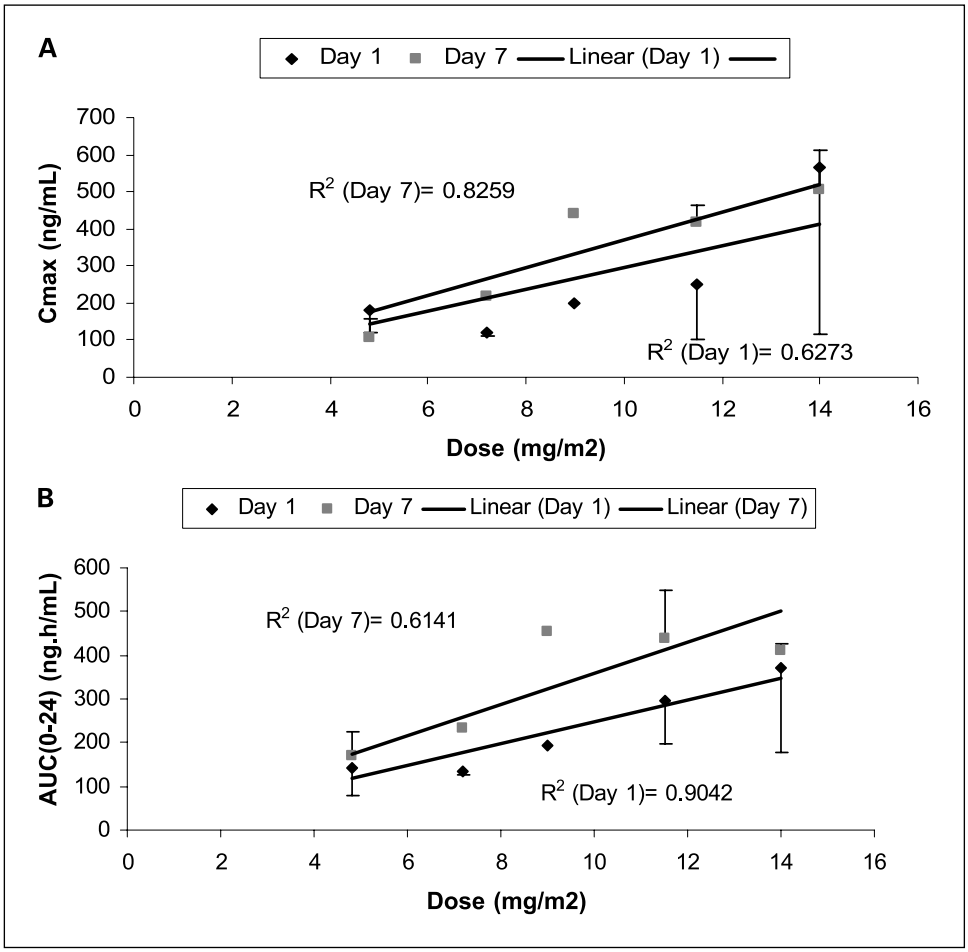

Fig. 3. Dose proportionality of C_{max} (*A*) and $AUC_{0\text{-}24}$ (*B*) on days 1 and 7.

combination studies with other epigenetic modifiers (e.g., hypomethylating agents) have a clear preclinical rationale (3).

Whereas showing increased acetylation in histones is important and may play a role in the cell killing, recent studies suggest that increased acetylation in other cellular proteins, such as nuclear factor κB or other chaperons, may also be critical for inducing cell killing (3). The demonstration of increased apoptosis in $CD14^+$ cells and the transient decreases in peripheral blood blasts support the further exploration of increasing acetylation as therapy in patients with AML. The antileukemic activity observed in this study was transient, suggesting that a more prolonged dosing schedule may be required to see durable clinical responses. Unfortunately, prolonged dosing with the i.v. formulation used in this study was not possible, as QTcF prolongation became more common as additional daily doses were delivered. Preliminary results using the oral formulation of LBH589 in patients with cutaneous T-cell lymphoma show clinical activity on a study in which QTcF interval duration has been measured by central tendency analysis (mean change from baseline): >800 post-dose electrocardiograms have shown a <10-ms effect with variable dose response over the doses tested (15-30 mg/d). In addition, there have been no QTcF prolongation DLTs. The oral LBH589 studies will also allow further exploration of the discrepancies between acetylation and apoptosis in different cellular subfractions observed in this study. The results of the currently reported study in patients with AML and myelodysplastic syndrome show that targeting the acetylation mechanism may be a useful approach, which will be further explored using the oral formulation of LBH589.

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