A phase 2 study of vorinostat in acute myeloid leukemia

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ABSTRACT

Background

This two-stage, multi-institutional, randomized phase 2 trial assessed the toxicity and response rate associated with two treatment schedules of the histone deacetylase inhibitor, vorinostat (suberoylanilide hydroxamic acid; SAHA) in patients with relapsed acute myeloid leukemia and in selected untreated patients with high-risk acute myeloid leukemia.

Design and Methods

Patients with relapsed or untreated acute myeloid leukemia who were not candidates for chemotherapy entered one of the two treatment arms. In both arms a total dose of 8400 mg of vorinostat was delivered in each 21-day cycle of treatment: in arm A the dose regimen was 400 mg daily whereas in arm B the dose regimen was 200 mg three times daily for 14 days followed by 1 week rest.

Results

Data from all 37 patients were used for the analyses. In arm A (n=15), the confirmed complete remission rate was 0% (95% CI, 0% to 23%); this arm was closed at the planned interim analysis. In arm B (n=22), the confirmed complete remission rate was 4.5% (1 response; 95% CI, 0.4% to 24%), with a duration of response exceeding 398 days. The median time to treatment failure in arm A was 42 days (95% CI, 26 to 57); although a minimum of four cycles of treatment were planned, 11 patients (79%) received no more than two cycles. The median time to treatment failure in arm B was 46 days (95% CI, 20 to 71); 13 patients (59%) received no more than two cycles of treatment.

Conclusions

Vorinostat monotherapy demonstrated minimal activity in this group of patients with acute myeloid leukemia. Therapy was discontinued in many patients before the planned four cycles had been administered, either because of failure of vorinostat to control the leukocyte count or patients' and physicians' preference. Future studies of vorinostat in acute myeloid leukemia should focus on combinations with other drugs with which it might interact pharmacodynamically. *ClinicalTrials.gov Identifier: NCT00305773.*

Key words: acute myeloid leukemia, acute myeloid leukemia, HDAC, histone deacetylase inhibitor, phase 2, SAHA, suberoylanilide hydroxamic acid, vorinostat.

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Introduction

Current chemotherapy regimens lead to long-term disease-free survival in approximately 25-30% of adults with acute myeloid leukemia (AML) younger than 60 years;[1-4] in older patients, fewer than 10% of patients survive 2 years. As many as 30% of newly-diagnosed patients with AML prove refractory to induction therapy, especially those whose disease has evolved from a myelodysplastic syndrome or another antecedent hematologic disorder or is related to exposure to environmental/occupational toxins.[5] Current approaches to these high-risk subsets of AML yield complete remissions in 40% of patients or less; the complete remissions are usually brief (<12 months) and few cures are achieved. Thus, the development of novel treatment approaches remains critical for patients with relapsed and poor-risk untreated AML.[6]

Histone deacetylase (HDAC) inhibitors have undergone early stage investigation in AML over the past several years. HDAC and histone acetyltransferases regulate the acetylation of histone proteins, setting the transcriptional activity of chromatin at specific gene loci. These proteins also regulate the acetylation of non-histone proteins, including transcription factors involved in cell cycle progression and apoptosis.[7-9] HDAC inhibition may help to establish more normal transcription patterns through chromatin remodeling since many cancers, including AML, have aberrant acetylation patterns leading to transcriptional silencing of tumor suppressor genes.[10-17] In addition to their impact on gene transcription, HDAC inhibitors have a variety of potentially important effects, which could be beneficial in the treatment of AML. These include the induction of reactive oxygen species, induction of oxidative damage to DNA, inactivation of HSP90 chaperone function, and an effect on NFκB signaling.[18]

Vorinostat (suberoylanilide hydroxamic acid, SAHA), a hydroxamic acid inhibitor of class I and class II HDAC,[16] was approved in the United States for the treatment of recurrent cutaneous T-cell lymphoma. Vorinostat induces cell cycle arrest and apoptosis in cancer cell lines, has demonstrated activity against leukemia and other hematologic malignancies *in vitro*[19-30] and also improved survival and/or produced antitumor effects in rodent models of leukemia.[31] In a phase I study of vorinostat in patients with myeloid leukemias four of six patients receiving vorinostat orally at a dose of 200 mg t.i.d. 14 days on/7 days off developed clinical responses: complete response (n=1); complete response with incomplete platelet recovery (n=1), clearance of marrow blasts (n=2).[32]

The current phase 2 trial was designed to independently assess the toxicity and response rate associated with this schedule of vorinostat administration in patients with AML. In addition, this trial assessed the toxicity and response rate associated with a lower daily dose administered continuously to deliver the same total dose over 21 days, to explore whether continuous exposure to HDAC inhibitors could be superior to intermittent exposure.

Design and Methods

Study design

National Cancer Institute protocol 6882 was an open-label randomized phase 2 study of two schedules of vorinostat administration in patients with relapsed AML and selected untreated patients with high-risk AML. Patients were planned to receive a minimum of four 21 day 21-day cycles of vorinostat before response was evaluated. Concomitant hydroxyurea was allowed during the first two cycles; however, in patients whose white blood cell (WBC) count could not be maintained under 30×10^9/L without hydroxyurea following two cycles of vorinostat, treatment was considered to have failed. Patients could continue to receive vorinostat for 17 cycles in the absence of disease progression. The protocol was approved by the Institutional Review Boards of the Institutions involved, and all patients provided written informed consent in accordance with the Declaration of Helsinki before enrollment following institutional guidelines.

Eligibility criteria and patients

Patients with relapsed AML, including those with core binding factor leukemias in second relapse or in first relapse following a remission of less than 12 months, were eligible for enrollment. Patients with untreated AML were eligible if one of the following conditions were met: age 65 years or older; AML with antecedent myelodysplastic syndrome (AML with trilineage dysplasia); AML with poor-risk cytogenetics (del5q, monosomy 5, monosomy 7, or complex cytogenetics defined as ≥3 cytogenetic abnormalities). Untreated patients were required to be ineligible for potentially curative therapy or have declined such therapy.

Patients in both groups were required to have normal organ function defined as: total bilirubin within normal institutional limits unless attributed to hemolysis or Gilbert's disease; aspartate and alanine transaminases less than or equal to 2.5 times the institutional upper limit of normal (ULN); and creatinine concentration less than or equal to the institutional ULN or creatinine clearance 60 mL/min/1.73 m^2 or more for patients with creatinine levels above the institutional normal. In addition, all patients were required to be at least 18 years old, have a life expectancy of at least 3 months, and an Eastern Cooperative Oncology Group performance status no greater than 2 or a Karnofsky score of at least 60%.

Patients were not allowed to have received other treatment for AML, including hematopoietic growth factors, within 3 weeks prior to study registration with the exception of hydroxyurea, which was allowed to be administered to patients with a WBC count greater than 30×10^9/L according to the treating institutions' standard clinical practice. Radiotherapy (within 4 weeks of registration), valproic acid (within 2 weeks), and concurrent use of any other investigational agents were also not allowed. Other exclusion criteria included: clinical evidence of central nervous system (CNS) or pulmonary leukostasis, disseminated intravascular coagulation, or CNS leukemia; history of allergic reactions attributed to

compounds of similar chemical or biological composition to vorinostat, uncontrolled intercurrent illness; and known positivity for human immunodeficiency virus.

Treatment plan

Vorinostat was supplied by Merck through the National Cancer Institute Cancer Therapy Evaluation Program. Patients were randomized to one of two treatment arms of oral vorinostat. In arm A, vorinostat was delivered at 400 mg daily for the full cycle of 21 days; in arm B, vorinostat was administered at 200 mg t.i.d. on days 1-14 of each 21-day cycle. In patients receiving concomitant hydroxyurea, the hydroxyurea was withdrawn on day 15 of cycles 1 and 2 to assess WBC count under the influence of vorinostat alone. Patients failing to achieve a WBC count below 30×10^9/L after two cycles were removed from the study and the treatment was considered to have failed. Dose adjustments were allowed for both hematologic and non-hematologic toxicities, with 300 mg daily for arm A and 200 mg b.i.d. for arm B being the first levels of dose reduction.

The patients' responses were assessed using the International Working Group Criteria for responses in AML and included morphologic complete response (CR), partial response (PR) and hematologic improvement (HI).[33] Responses were confirmed if they persisted for two consecutive cycles at least 4 weeks apart. Peripheral blood and bone marrow parameters measured after cycles 4 and 6 were used to determine response, and patients responding at cycle 6 continued the treatment with response measured every three cycles.

Safety was assessed through adverse event reporting and the monitoring of vital signs and laboratory parameters. Hospital admissions and deaths were reviewed and classified using information from the hospital records, death certificates, and interviews with primary care physicians and next-of-kin.

Statistical design

This randomized phase 2 trial with an interim analysis was designed to independently assess the confirmed CR rate in each arm. The same design was used in each arm, and required a minimum of 13 and a maximum of 20 patients to test the null hypothesis that the confirmed CR rate was at most 5% versus a specified alternative hypothesis of at least 25%. An interim analysis was planned once an arm had accrued 13 patients, and an arm was only allowed to continue accrual if at least one patient had an unconfirmed CR, PR or HI at the cycle-4 evaluation. Accrual was not suspended while awaiting results of the interim analysis. The final decision criteria required three successes in the first 20 enrolled patients to claim effectiveness of the treatment arm. A success was defined as a patient with a confirmed CR at any point during treatment. The confirmed CR rate for each arm was calculated as the number of successes divided by the number of eligible patients. The overall power for each arm was 90%, given a true confirmed CR rate of at least 25% and a type I error rate of 7.4%. No formal hypothesis was established to test superiority between the individual arms due to the limited sample sizes considered.

The number of successes was assumed to follow a binomial distribution, and 95% confidence intervals (95% CI) for the confirmed CR rate in each arm were calculated using the method of Duffy and Santner.[34] The duration of response was calculated as the time from date of documentation of first CR to the date of progression. Patients without progression at last follow-up were considered censored. The time to treatment failure (TTF) was defined as the time from date of study registration until the date of treatment discontinuation for any reason. Patients receiving treatment at the time of analysis were considered censored. Overall survival was defined as the time from date of registration until the date of death. Patients alive at the last follow-up were considered censored. All time-to-event curves were estimated using the method of Kaplan and Meier.[35]

Results

Patients' characteristics

A total of 37 patients were enrolled into this trial between January 25, 2006 and August 1, 2007. Fifteen patients were enrolled in arm A, and 22 enrolled in arm B. One patient in arm A was ineligible because of inappropriate use of hydroxyurea for a WBC count less than 30×10^9/L. However, because this use of hydroxyurea was not likely to have affected the outcome of treatment, this patient is included in all the analyses.

Five females were accrued in each arm; the median age was 67 years in each arm (arm A range, 41 to 79; arm B range, 28 to 81). Twelve patients (80%) in arm A and 16 patients (73%) in arm B had relapsed disease. The three untreated patients (20%) in arm A were at least 65 years old. Of the six patients (27%) in arm B with untreated disease, three were at least 65 years old, and three had a history of myelodysplastic syndrome. AML with trilineage dysplasia was the diagnosis for 47% of the patients in arm A and 41% in arm B. The randomization procedure produced a general balance of the patients' characteristics between the arms, with the exception of a higher incidence of infection within the previous 3 months among patients in arm B (36% versus 13%). The characteristics of the patients were similar between those with previously untreated and relapsed AML. The patients' characteristics are summarized in Tables 1 and 2.

Toxicity

Toxicities are listed by arm in Tables 3 and 4. In arm A, grade 5 adverse events were reported in three patients (20%). These events were pneumonia during the first cycle of treatment, disease progression during the second cycle, and death, not otherwise specified during the fourth cycle. CNS hemorrhage, dizziness and fatigue were the only grade 4 non-hematologic adverse events reported. The patient reporting grade 4 dizziness subsequently died of pneumonia, and the patient experiencing grade 4 CNS-hemorrhage also died. Grade 3 non-hematologic adverse events were more common, with 26 events reported for ten patients (67%). Seven of the ten patients experienced at least one grade 3 non-hematologic adverse event considered at least possibly related to treatment. The most common grade 4 hematologic adverse events

reported for arm A were neutropenia and thrombocytopenia, both occurring in ten patients (67%), and all patients had at least one thrombocytopenic event. Seven patients (47%) experienced anemia, and six patients (40%) experienced leukopenia. The most common adverse events (>10%) in arm A, regardless of grade and attribution, were thrombocytopenia (100%), neutropenia (93%), fatigue (93%), anemia (47%), dyspnea (47%), nausea (47%), leukopenia (40%), anorexia (27%), rash (20%), diarrhea (20%), dizziness (13%), febrile neutropenia (13%), and vomiting (13%).

In arm B, grade 5 events were experienced by two patients (9%), both occurring during cycle 1. One patient died from pneumonitis and one experienced sudden death. Four patients (18%) experienced at least one grade 4 non-hematologic adverse event, although fatigue was the only event considered possibly or probably related to treatment. The other grade 4 non-hematologic adverse events were pleuritic pain, CNS hemorrhage, hypoxia and acidosis. The latter two events occurred in the same

Table 1. Characteristics of the patients divided by treatment arm.

Characteristic	Arm A (N=15)	Arm B (N=22)
Age, median (range), years	67.0 (41.0-79.0)	67.0 (28.0-81.0)
Gender		
Female	5 (33%)	5 (23%)
Male	10 (67%)	17 (77%)
Performance Score[1]		
0	7 (47%)	8 (36%)
1	6 (40%)	10 (45%)
2	2 (13%)	3 (14%)
Associated diseases, yes	15 (100%)	22 (100%)
Disease status		
Relapsed	12 (80%)	16 (73%)
Untreated	3 (20%)	6 (27%)
AML disease classification		
All other relapsed AML	12 (80%)	16 (73%)
Untreated AML patients ≥65 years old	3 (20%)	3 (14%)
Untreated AML patients with MDS-AML	0 (0%)	3 (14%)
FAB classification[2]		
AML with trilineage dysplasia	7 (47%)	9 (41%)
M0	1 (7%)	0 (0%)
M1	2 (13%)	5 (23%)
M2	2 (13%)	3 (14%)
M	40 (0%)	2 (9.1%)
M5a	1 (7%)	1 (4%)
M5b	1 (7%)	0 (0%)
M7	0 (0%)	1 (4%)
Previous stem cell transplant[3]	4 (27%)	3 (14%)
WBC ($\times 10^9$/L), median (range)	2.5 (0.9-48.5)	3.8 (0.7-74.3)
WBC counts of less than 5×10^9/L	10(71%)	14 (64%)
WBC counts over 30×10^9/L	2(14%)	2 (9%)
Receiving hydroxyurea	4 (27 %)	4 (18)
Hemoglobin (g/dL), median (range)	9.5 (7.9-12.7)	9.7 (7.8-13.7)
Platelets ($\times 10^9$/L), median (range)	29.0 (5.0-176.0)	28.5 (11.0-141.0)
Absolute neutrophil count ($\times 10^9$/L), median (range)	0.6 (0.1-17.4)	0.5 (0.0-4.9)
% Blasts, median (range)	18.0 (0.0-61.0)	2.0 (0.0-94.0)
% Bone marrow blasts, median (range)	48.5 (21.0-100.0)	37.0 (4.0-100.0)
Cytogenetics		
Not done?	1 (7%)	1 (4%)
Normal	3 (20%)	6 (27%)
+8	0 (0%)	3 (14%)
Complex	4 (27%)	5 (23%)
Other	7 (47%)	7 (31.8%)

[1]One patient in arm B was assessed using the Karnofsky score, and had a score of 90. [2]The FAB classification was not assessable for one patient in each arm. [3]One patient on arm B was not assessed for previous stem cell transplant.

Table 2. Characteristics of the patients divided according to whether they had untreated or relapsed acute myeloid leukemia.

Characteristic	Relapsed (N=28)	Untreated (N=9)
Arm		
A	12 (43%)	3 (33%)
B	16 (57%)	6 (67%)
Age, median (range), years	66.5 (28.0-79.0)	77.0 (66.0-81.0)
Gender		
Female	8 (29%)	2 (22%)
Male	20 (71%)	7 (78%)
Performance Score[1]		
0	14 (50%)	1 (12.5%)
1	11 (39%)	5 (62.5%)
2	3 (11%)	2 (25%)
Associated diseases, yes	28 (100%)	9 (100%)
AML disease classification		
All other relapsed AML	28 (100%)	0 (0%)
Untreated AML patients ≥65 years old	0 (0%)	6 (67%)
Untreated AML patients with MDS-AML	0 (0%)	3 (33%)
FAB classification[2]		
AML with trilineage dysplasia	13 (50%)	3 (33.3%)
M0	0 (0%)	1 (11.1%)
M1	6 (23%)	1 (11.1%)
M2	4 (15%)	1 (11.1%)
M4	1 (4%)	1 (11.1%)
M5a	0 (0%)	2 (22.2%)
M5b	1 (4%)	0 (0%)
M7	1 (4%)	0 (0%)
Previous stem cell transplant	7 (25%)	0 (0%)
WBC ($\times 10^9$/L), median (range)	2.4 (0.7-74.3)	7.6 (0.9-62.6)
WBC counts of less than 5×10^9/L	20 (71%)	4 (44%)
WBC counts over 30×10^9/L	3 (11%)	1 (11%)
Receiving hydroxyurea		
Hemoglobin (g/dL), median (range)	9.5 (7.9-13.7)	9.1 (7.8-12.9)
Platelets ($\times 10^9$/L), median (range)	29.5 (10.0-176.0)	27.0 (5.0-90.0)
Absolute neutrophil count ($\times 10^9$/L), median (range)	0.5 (0.0-17.4)	0.5 (0.1-4.9)
% Blasts, median (range)	9.5 (0.0-94.0)	22.0 (0.0-84.0)
% Bone marrow blasts, median (range)	40.0 (4.0-100.0)	49.0 (22.0-83.0)
Cytogenetics		
Not Done	1 (4%)	1 (11%)
Normal	7 (25%)	2 (22%)
+8	2 (7%)	1 (11%)
Complex	9 (32%)	0 (0%)
Other[3]	9 (32%)	5 (56%)

[1]One untreated patient was assessed using the Karnofsky score, and had a score of 90. [2]The FAB classification was not assessable in one untreated patient. [3]Untreated: 12p deletion; trisomy 13, trisomy 21; 13, deletion 7q; trisomy 11; -7 deletion arm1. Relapsed: 8,21 (20); add(12p); inv(3), -7; del(20); 47,XYdel(1)p(13)[1]; add(1) and DER 15t(1;15); del(11)(p12p14); t(2;3)(p21;q27); 1q21 and 1q32.

patient who subsequently died of pneumonitis. Fifty-three grade 3 non-hematologic adverse events were reported for 17 patients (77%), with 36 of these events (68%) being considered at least possibly related to treatment. Grade 4 neutropenia occurred in 17 patients (77%), and a maximum of grade 3 neutropenia occurred in three other patients (14%). Grade 4 thrombocytopenia was experienced by 13 patients (59%) with an additional six patients (27%) having a maximum grade 3 event, and one patient having a maximum grade 2 event. In addition, anemia was experienced by seven patients (32%), and leukopenia by four patients (18%). In arm B, the most common adverse events (>10%), regardless of grade and attribution, were fatigue (95%), neutropenia (91%), thrombocytopenia (91%), nausea (73%), diarrhea (64%), vomiting (45%), anorexia (41%), dyspnea (32%), anemia (32%), febrile neutropenia (27%), leukopenia (18%), pneumonia (14%), and abdominal pain (14%).

The incidence rates of grade 4 non-hematologic events (20% in arm A versus 23% in arm B) and grade 3 non-hematologic events (67% in arm A versus 68% in arm B) were virtually the same. The incidences of the most common hematologic events were also similar, as revealed by the rates of grade 4 neutropenia (67% versus 77%) and grade 4 thrombocytopenia (67% versus 59%). However, without regard to grade or attribution, there were different rates of certain commonly noted adverse events (25% incidence in at least one arm). While the 90% rates of fatigue in each arm were similar, in arm B there were higher rates of nausea (73% versus 47%), diarrhea (64% versus 20%), anorexia (41% versus 27%), and vomiting (45% versus 13%), whereas the rate of dyspnea was higher in arm A (47% versus 32%).

Dose modifications

In arm A, 31 cycles of treatment were administered to 15 patients. Three cycles were delayed in three separate patients, and another patient had a dose reduction in the second cycle. In arm B, 65 cycles of treatment were administered to 22 patients. Seven patients (32%) had at least one delay of treatment and five patients (23%) had one dose reduction. The main reason for these delays and dose reductions was complication due to adverse events.

Clinical responses

Only seven patients completed at least four cycles of vorinostat treatment (two in arm A, five in arm B, see below). In arm A, the confirmed CR rate was 0% (95% CI, 0% to 23%).The arm was closed at the interim analysis since none of the first 13 patients enrolled had an unconfirmed HI, PR or CR at the cycle-4 evaluation. Arm B passed interim analysis since one patient had an unconfirmed HI at the cycle-4 evaluation. Among all 22 enrolled patients in arm B, the confirmed CR rate was 4.5% (one response; 95% CI, 0.4% to 24%). The single responding patient was a 77-year old woman with untreated AML M4 who presented with a WBC count of 3.4×10^9/L, 36% monocytes, and 34% blasts. Cytogenetic analysis was unsuccessful in this patient. The woman had an improvement in hematologic parameters (platelets and neutrophils) following cycle 2 of treatment. She was first classified as having obtained a CR at the cycle-4 evaluation, and the CR was confirmed at the cycle-6 evaluation more than 6 weeks later. The duration of response was at least 398 days, as the patient was still responding at the last recorded follow-up. No partial responses (unconfirmed or otherwise) have been noted in either arm. Overall, the decision criteria to claim the treatment promising (at least three confirmed CR) was not met in either arm.

Survival models

The median TTF in arm A was 42 days (95% CI, 26 to 57). Eleven patients (79%) received no more than two

Table 3. Maximum severity of adverse events for arm A patients.

Adverse event	Gr.1	Gr.2	Gr.3	Gr.4	Gr.5
Thrombocytopenia	1	0	4	10	0
Neutropenia	1	2	1	10	0
Anemia	1	2	3	1	0
Leukopenia	1	1	1	3	0
Fatigue	2	4	7	1	0
Death NOS	0	0	0	0	1
Disease progression	0	0	0	0	1
Rash/desquamation	0	3	0	0	0
Nausea	2	4	1	0	0
Anorexia	2	2	0	0	0
Diarrhea	2	0	1	0	0
Vomiting	0	2	0	0	0
CNS hemorrhage	0	0	0	1	0
Febrile neutropenia	0	0	2	0	0
Pneumonia NOS	0	0	0	0	1
Dizziness	0	0	1	1	0
Dyspnea	3	1	3	0	0

Results are expressed as worst adverse event (regardless of attribution) per patient using the National Cancer Institute Common Criteria version 3.0 toxicity-grading criteria.

Table 4. Maximum severity of adverse events for arm B patients.

Adverse event	Gr.1	Gr.2	Gr.3	Gr.4	Gr.5
Thrombocytopenia	0	1	6	13	0
Neutropenia	0	0	3	17	0
Anemia	0	3	3	1	0
Leukopenia	0	0	3	1	0
Fatigue	4	7	9	1	0
Sudden death	0	0	0	0	1
Nausea	8	5	3	0	0
Anorexia	2	4	3	0	0
Diarrhea	5	3	6	0	0
Vomiting	4	3	3	0	0
CNS hemorrhage	0	0	0	1	0
Febrile neutropenia	0	0	6	0	0
Pneumonia	0	0	3	0	0
Pneumonitis	0	0	0	0	1
Acidosis	0	0	0	1	0
Pain-abdominal	2	1	0	0	0
Pain-pleuritic	0	0	0	1	0
Hypoxia	0	0	1	1	0
Dyspnea	2	4	1	0	0

Results are expressed as worst adverse event (regardless of attribution) per patient using the National Cancer Institute Common Criteria version 3.0 toxicity-grading criteria.

cycles of treatment, and two patients each (14%) received three and four cycles of treatment. No patients in arm A continued treatment after the cycle-4 evaluation. The median TTF in arm B was 46 days (95% CI, 20 to 71). Thirteen patients (59%) received no more than two cycles of treatment. Four patients (18%) received three cycles of treatment, three patients (14%) received four cycles, one patient received six cycles, and one patient received all 17 cycles of treatment as specified by the protocol. The reasons for discontinuation of treatment were death (3 patients in arm A, 2 patients in arm B), adverse events (1 in arm A, 2 in arm B), alternative treatment (4 in each arm), failure to achieve a WBC count less than 30×10^9/L after two cycles of treatment (2 in each arm), patients' refusal (1 in arm A, 4 in arm B), other medical problems (1 in arm A), physicians' decision (1 in each arm), and disease progression (1 in arm A, 3 in arm B).

TTF and survival were similar in untreated and relapsed patients. The median TTF was 41 days (95% CI: 24 to 53) for untreated patients and 42.5 days (95% CI: 41 to 65) for the relapsed group. The median survival was 41 days (95% CI: 27 to 290) in the untreated group and 149 days (95% CI: 81 to 221) in the relapsed group.

To examine whether the high failure rate was due primarily to the inability of vorinostat to control hyperleukocytosis, we examined the number of cycles of therapy administered to patients based on their presenting WBC counts. Ten patients (71%) in arm A and 14 patients (64%) in arm B began treatment with baseline WBC counts of less than 5×10^9/L; none of these patients was receiving hydroxyurea. Conversely, five patients began treatment with baseline WBC counts over 30×10^9/L (3 in arm A and 2 in arm B). Only two of these patients received more than two cycles of vorinostat (1 in each arm). Eleven patients received hydroxyurea at some point during therapy. Five patients (36%) in arm A and eight patients (36%) in arm B had WBC counts over 30×10^9/L during at least one cycle of treatment. These patients with high WBC counts received a median of two cycles of therapy in either arm with the following ranges: arm A, 1 - 3; arm B, 1- 4. Among the seven patients in either arm who received at least four cycles of therapy, the baseline WBC count was 2.5×10^9/L (range, 1.3 – 5.4). After categorizing patients into those with low baseline WBC counts ($\leq5\times10^9$/L) and high baseline WBC counts ($>5\times10^9$/L), the median number of cycles of treatment administered was two for both the groups with low and high WBC counts in each arm. Patients in arm A with both high and low baseline WBC counts received between one and four cycles of treatment. In arm B, the range for the group with a low baseline count was 1 to 17, while the range for the group with a high baseline WBC count was 1 to 4 cycles.

Fourteen of the 15 patients (93%) in arm A have died, and 21 of the 22 patients (95%) in arm B have died. The median overall survival was 105 days (95% CI, 55 to 223) in arm A and 153 days (95% CI, 58 to 229) in arm B.

Discussion

In this multicenter, open-label, randomized phase 2 trial, the primary objective was to determine the response rate associated with two dosing schedules of vorinostat for the treatment of patients with relapsed AML and selected untreated patients with high-risk AML. Only one confirmed CR was observed in the first 37 patients enrolled in the study. In addition, no partial responses were noted in either arm. Overall, the decision criteria to claim the treatment promising (at least three confirmed CR) was not met in either arm. These results are inferior to those obtained with cytotoxic chemotherapy and other recently reported biological therapies and do not confirm the promising activity noted for a similar dosing schedule reported in a phase I study in AML.[32] In that study, seven of 31 AML patients experienced hematologic improvement, including two CR and two CR with incomplete blood count recovery. Four of the patients with responses were treated with a dose schedule corresponding to that used in arm B of the current trial. That cohort accrued a total of 12 patients in the phase I study. The median number of cycles until response or improvement developed was two (range, 1-8); however, the median duration of response was only 6 weeks (range, 0.1-53 weeks). While it is possible that the different case mix in the present study from that in the previously published phase I study accounts for the difference in results, it is more likely that these reflect the disappointing response rate to vorinostat monotherapy in a *real-world* multicenter phase 2 trial.

It is noteworthy that even in a small consortium of academic institutions, a large number of patients exited the trial prior to receiving the planned four cycles. AML therapy has traditionally been developed based on the achievement of complete remission within one to two cycles of therapy. Physicians and patients may become impatient waiting several cycles for response, particularly in the context of declining performance status. This may have accounted for the low median number of cycles (n=3) delivered to patients with myelodysplastic syndromes in the US registration trial of decitabine despite published data suggesting that four to six cycles were required for a response to be demonstrated.[36] Successful development of agents targeting epigenetic therapies which do not act as rapidly as conventional cytotoxic agents requires thoughtful attention to physicians' and patients' expectations in order to enable sufficient treatment to be administered.

Biological features of the leukemia may account for resistance to vorinostat and other HDAC inhibitors. HL60 cells cloned for HDAC inhibitor resistance (HL-60/LR) cells demonstrate resistance to LAQ824, vorinostat, LBH589, and sodium butyrate. These cells show loss of HDAC6, hyperacetylation of HSP90, an aggressive leukemia phenotype, and collateral sensitivity to 17-AAG.[37] An increased expression of the antioxidant signature in lymphoma and AML patients has been correlated as a biomarker for vorinostat resistance, given the fact that vorinostat-induced cytotoxicity has been shown to be blocked by up-regulation of reactive oxygen species in previous clinical and pre-clinical studies.[38-40]

The results of this study do not enable either of the two dosing schedules to be recommended for further study as monotherapy in AML. Both regimens of vorino-

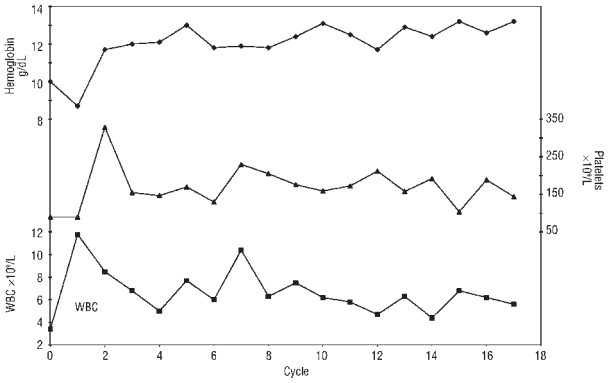

Figure 1. Changes in hematologic parameters over time in the responding patient.

stat were generally well tolerated. Although drug-related adverse experiences were noted in all patients, these events were mostly mild to moderate in severity and rarely resulted in dose reduction or discontinuation in the arm in which 400 mg daily was administered. The group of patients who received 200 mg t.i.d. had a significantly higher incidence of gastrointestinal adverse events that resulted in delays of treatment in almost a quarter of the patients (23%). The single responding patient received intermittent dosing, the schedule which was selected based on responses in the previous phase I trial.[32] The remarkable duration of complete remission for this particular patient confirms the anecdotal observation from the phase I study that isolated patients may in fact respond meaningfully to HDAC inhibitor monotherapy. At present, there is inadequate information about the biology of such patients in order to enable appropriate selection of patients.

Development of epigenetically targeted agents may benefit from pharmacodynamically-driven dose-finding studies rather than classical determination of maximum tolerated dose. However, HDAC inhibitors such as vorinostat have a panoply of molecular effects. Without knowing which molecular end-points drive clinical response (for example, gene re-expression, HSP90 inactivation, generation of reactive oxygen species) selecting a dose and schedule based on a single pharmacodynamic end-point may lead to the problem of underdosing.

The short survival of patients in this trial stands in stark contrast to recent results with 5-azacytidine in patients with high-risk myelodysplastic syndrome. In that study, the 2- year survival was twice that of patients receiving a combined conventional care regimen, despite an extremely low complete remission rate.[45] Whether this reflects a difference between DNA methyltransferase monotherapy and HDAC inhibitor monotherapy, a difference between high-risk myelodysplastic syndrome (including AML with trilineage dysplasia) and, more generally, *AML in the elderly*, which includes patients with more proliferative biology, or differences between a biologically active drug (5-azacytidine) and one which is less active for this disease (vorinostat) remains speculation.

Although vorinostat as monotherapy in these patients did not show sufficient activity to justify further development in AML, a variety of pre-clinical models suggest that HDAC inhibitors may potentiate epigenetic and cytotoxic effects of other agents. These include HSP90 inhibitors,[39] proteosome inhibitors,[41] conventional cytotoxic chemotherapy drugs[42,43] and DNA methyltransferase inhibitors.[44] As of November 2008, there were 102 trials (63 recruiting, 59 completed, terminated or not recruiting) on the use of vorinostat in multiple types of cancer including hematologic malignancies, advanced solid tumors, breast cancer, gliomas, melanoma, kidney cancer, ovarian cancer, prostate cancer, colorectal cancer, and thyroid cancer (*http://clinicaltrials.gov*). Future pre-clinical and clinical trials will need to focus on potential synergistic effects of vorinostat with other agents and to establish low and high vorinostat-resistance profiles, in order to optimize the use of this and other HDAC inhibitors for the treatment of leukemia and other cancers.

Authorship and Disclosures

SDG developed the study concept and design, obtained funding, analyzed and interpreted the data, prepared the manuscript, and supervised the study; AL-B analyzed the data and prepared the manuscript; EWS analyzed the data and prepared the manuscript; MJ, JFD, VR, JS, and IE-D participated in the accrual of patients and supervised the study; WW analyzed the data.

The authors reported no potential conflicts of interest.

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