Phase 2 trial of romidepsin in patients with peripheral T-cell lymphoma

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Romidepsin (depsipeptide or FK228) is a histone deacetylase inhibitor, one of a new class of agents active in T-cell lymphoma. A phase 2 trial was conducted in cutaneous (CTCL) and peripheral (PTCL) T-cell lymphoma. Major and durable responses in CTCL supported the approval of romidepsin for CTCL. Forty-seven patients with PTCL of various subtypes including PTCL NOS, angioimmunoblastic, ALK-negative anaplastic large cell lymphoma, and enteropathy-associated T-cell lymphoma were enrolled. All patients had received prior therapy with a median of 3 previous treatments (range 1-11); 18 (38%) had undergone stem-cell transplant. All patients were evaluated for toxicity; 2 patients discovered to be ineligible were excluded from response assessment. Common toxicities were nausea, fatigue, and transient thrombocytopenia and granulocytopenia. Complete responses were observed in 8 and partial responses in 9 of 45 patients, for an overall response rate of 38% (95% confidence interval 24%-53%). The median duration of overall response was 8.9 months (range 2-74). Responses were observed in various subtypes, with 6 responses among the 18 patients with prior stem-cell transplant. The histone deacetylase inhibitor romidepsin has single agent clinical activity associated with durable responses in patients with relapsed PTCL. This study has been registered at clinicaltrials.gov as NCT00007345. (*Blood*. 2011; 117(22):5827-5834)

Introduction

Histone deacetylase (HDAC) inhibitors are epigenetic therapies that induce acetylation of histones. As they also lead to increased acetylation of other proteins such as nuclear transcription factors, they may be more accurately referred to as protein deacetylase inhibitors. Exposure of cancer cells to HDAC inhibitors results in growth arrest, cellular differentiation, and apoptosis.[1-4] Their antitumor effects have been hypothesized to occur through modulation of gene expression; however, their other cellular effects may be more important.[5-6] Romidepsin (FK228, previously FR901228 or depsipeptide) is a potent HDAC inhibitor and was isolated from *Chromobacterium violaceum*.[7-8] In phase 1 trials of romidepsin, responses were observed in patients with T-cell lymphoma.[9-11] Consequently, a phase 2 trial in patients with T-cell lymphoma was initiated with the primary goal of determining response rate and toxicity profile. In patients with cutaneous T-cell lymphoma (CTCL) treated with romidepsin, including 71 enrolled on this trial and 96 treated on a separate registration trial, response rates of 35% and 34% were noted with median durations of 11.1 and 15.4 months, respectively.[12-13] These results supported the approval of romidepsin for CTCL in 2009. The activity of romidepsin in patients with CTCL appears to be a class effect with other HDAC inhibitors also demonstrating activity in this disease, including vorinostat, which also received Food and Drug Administration (FDA) approval.[12,14-15]

Non-Hodgkin lymphomas arise from B cells in 85% of patients, from T cells in 10% of patients, or from other cells such as NK or precursor cells. Lymphomas derived from a mature, postthymic, T-cell clone in which a T-cell receptor gene rearrangement can be detected are referred to as peripheral T-cell lymphomas and are distinguished from immature T lymphoblastic lymphoma. Several subtypes are defined based on clinical features, nodal versus extranodal sites of involvement, and immunophenotypic markers.[16] Lymphomas that do not fit into a defined category are referred to as PTCL, not otherwise specified (PTCL NOS) and comprise the largest sub-classification with 34% of all patients with T-cell or NK/T-cell lymphoma.[17] PTCL is associated with a poor prognosis; patients with the main subtypes such as PTCL NOS, angioimmunoblastic, and enteropathy-associated T-cell lymphoma have a 5-year overall survival of less than 32%. Anaplastic large cell lymphoma (ALCL) associated with a t(2;5) or variant chromosomal translocation, referred to as ALK-positive ALCL, is a subtype associated with a better prognosis with a 5-year survival of 70%. These patients have a better response rate to front line therapy and are known to respond to stem cell transplantation in the relapsed setting.[17] Conversely, the majority of patients with PTCL experience recurrence of their disease and have a poor response to further treatment, highlighting the need for better treatment options. Pralatrexate

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recently received accelerated approval from the FDA for the treatment of patients with relapsed PTCL.[18]

A patient with PTCL NOS enrolled on the phase 1 trial of romidepsin was observed to have a durable complete response, leading to the enrollment of additional patients with T-cell lymphoma, confirming its activity in these patients.[11] The multiinstitutional phase 2 trial that is the subject of this report was then initiated, with separate cohorts for patients with CTCL and PTCL, to evaluate the efficacy of romidepsin in patients with T-cell lymphoma. Secondary goals of this study included evaluation of long-term safety of romidepsin. Results in patients with CTCL were previously reported[12] and this analysis is limited to the patients enrolled with PTCL.

Methods

Patient eligibility

Initially, the study was restricted to patients with relapsed or refractory PTCL NOS or primary cutaneous anaplastic large cell lymphoma who had not received more than 2 systemic cytotoxic chemotherapy regimens. Therapies such as PUVA or topical chemotherapy; systemic therapies including steroids, retinoids, interferon, or denileukin diftitox; and alemtuzumab and other nonradiolabeled antibodies were not considered cytotoxic chemotherapy – prior therapy with any number of these therapies was allowed. The observed activity led us to amend and adapt the trial to include additional centers and to enroll patients with other mature T-cell lymphoma subtypes and patients who had previously received more than 2 cytotoxic therapies. Pathology underwent central review. The protocol, informed consent, and subsequent amendments were approved by the Institutional Review Boards of all participating institutions. All patients signed informed consent in accordance with the Declaration of Helsinki. (ClinicalTrials.gov Identifier: NCT00020436, Depsipeptide to Treat Patients with Cutaneous T-Cell Lymphoma and Peripheral T-Cell Lymphoma).

Inclusion criteria required measurable disease, age ≥ 18 years, ECOG performance status 0-2, and life expectancy of > 12 weeks. Required laboratory values included absolute neutrophil count (ANC) $\geq 1 \times 10^9$ cells/L, platelets $\geq 100 \times 10^9$/L, bilirubin $\leq 1.5\times$ the institutional upper limit of normal (ULN), AST $\leq 3\times$ ULN, and creatinine $\leq 1.5\times$ ULN. Exclusion criteria included CNS involvement, HIV infection, or prior therapy with an HDAC inhibitor. Patients who were pregnant were excluded and use of effective birth control was required. Four weeks were required between prior chemotherapy and protocol enrollment. A stable dose of steroids at protocol entry was allowed in patients with nonresponding lesions with tapering after initiation of therapy. Patients with unstable angina, myocardial infarction within the previous 12 months, left ventricular ejection fraction below normal ($< 45\%$ if performed by MUGA or $< 50\%$ if performed by echocardiogram or cardiac MRI), or corrected QT interval of > 480 ms were excluded. A bone marrow biopsy was required, except for patients who tested positive subsequent to their last treatment regimen or patients who had a negative marrow within 3 months of study entry.

Trial design and treatment plan

Romidepsin, NSC 630176, was provided by the Cancer Therapy Evaluation Program (CTEP), National Cancer Institute (NCI). Romidepsin was administered as a 4-hour infusion on days 1, 8, and 15 of a 28-day cycle with a starting dose of 14 mg/m^2.[10] Treatment for the first 2 patients enrolled was initiated at 18 mg/m^2 on days 1 and 5 of a 21-day cycle, the schedule originally studied at the NCI.[9] The schedule was changed for improved tolerability. Doses were held for grade 3 or worse nonhematologic toxicity, ANC under 0.5×10^9 cells/L, or platelet count under 50×10^9/L. Doses were reduced from 14 mg/m^2 to 10.5, dose level-1 (DL-1), or from 10.5 to 8 (DL-2) for ANC between 0.5 and 1×10^9 cells/L or platelet count between 50 and 75×10^9/L on days 8 or 15. A later amendment allowed escalation to 17.5 mg/m^2 (DL+1) in the absence of toxicity. The NCI Common Toxicity Criteria Version 2.0 were used.

Supportive care

As hypomagnesemia and hypokalemia are associated with T-wave and ST segment abnormalities and QT interval prolongation, electrocardiographic findings also associated with HDAC inhibitor therapy, the protocol was amended to mandate supplementation of electrolytes to achieve serum magnesium and potassium levels over 0.85mM and 4.0mM, respectively, before administration of romidepsin.[19] The protocol was also amended to exclude medications known to either prolong the QTc or interfere with CYP3A4 metabolism. The latter exclusion was added after it was found that romidepsin may be metabolized in part by CYP3A4.[20] Antiemetics were administered to prevent nausea.

Response evaluation

Responses in patients with nodal disease were assessed using the International Working Group (IWG) guidelines.[21] Responses in patients with skin or visceral lesions were assessed using Response Evaluation Criteria in Solid Tumors (RECIST).[22] Response assessments were performed every 2 cycles; every 3 cycles for patients considered to be in CR. Complete response required clearing of known sites of disease. Partial response required documented response in skin or lymph nodes. The data cutoff for this report was February 22, 2010.

Pharmacokinetic analysis

Blood samples were collected with the first dose before drug administration, at the end of infusion (4 hours), and at 6, 11, 13, 15, 18, and 22 hours after start of infusion. Samples were centrifuged for 5 minutes at 1200*g* and collected plasma stored at -80°C. Samples were analyzed using a sensitive analytical liquid chromatography-mass spectrometry assay validated for the range of 2-1000 ng/mL.[23] Noncompartmental pharmacokinetic data analysis was performed using WinNonLin v.5. The AUC from time zero to time of final quantifiable sample (AUC_{last}) was calculated using the linear trapezoidal method, while AUC_{inf} was calculated by extrapolation to infinity. Volume of distribution during the terminal phase (V_z) was estimated during the terminal phase and systemic clearance (Cl_{obs}), was calculated as dose divided by AUC_{inf}.

Statistical methods

The trial began as a single institution analysis of romidepsin in patients with CTCL or PTCL after no more than 2 prior cytotoxic therapies evaluated in separate cohorts. The Simon 2-stage design[24] for the first cohort required a response in 2 of 16 patients to accrue the full cohort of 25 to target a response rate of 30% and rule out a 10% response rate, with 10% probabilities of accepting a poor agent and of rejecting a good agent. Duration of response was determined by the Kaplan-Meier method. Patients in long-term remission who discontinued therapy for reasons other than disease progression or adverse events were allowed to restart therapy if disease recurred. Response durations were censored at the time of first disease recurrence.

Results

Patient characteristics

Forty-seven patients with PTCL were enrolled. The protocol was initially restricted to patients who had not received more than 2 systemic cytotoxic chemotherapeutic regimens. The protocol exceeded the targeted first stage criteria, with responses being observed in 6 of the first sixteen patients enrolled. Subsequently, by amendment, the protocol was expanded to

Table 1. Patient characteristics

Characteristic	No. of patients
Sex	N = 47
Male	25
Female	22
Age, y	
Median	59
Range	27-84
> 60	23
ECOG Performance Status	
0	20
1	23
2	4
Extent of disease at enrollment	
Stage II	2
Stage III	11
Stage IV	34
Bone marrow involvement	14
Elevated LDH	26
Primary Diagnosis	N = 47
PTCL, unspecified or NOS	27 (57%)
Angioimmunoblastic*	7 (15%)
Anaplastic large T-cell, ALK pos	2 (4%)
Anaplastic large T-cell, ALK neg	2 (4%)
Primary cutaneous anaplastic large T-cell	2 (4%)
Cutaneous γδ T-cell	2 (4%)
Hepatosplenic PTCL	1 (2%)
Enteropathy associated T-cell lymphoma	1 (2%)
PTCL, unspecified of the skin	1 (2%)
CD30 lymphoproliferative disorder	1 (2%)
Diffuse large B-cell lymphoma*	1 (2%)

*Two patients were evaluable for toxicity assessment and excluded from response assessment: 1 with angioimmunoblastic T-cell lymphoma found to be ineligible for enrollment after the first dose and 1 whose T-cell lymphoma was reclassified as DLBCL.

allow enrollment of patients with PTCL of any subtype who had received any number of prior treatment regimens. Patient characteristics and PTCL classification based on central review for the patients enrolled can be found in Table 1. PTCL NOS was the most common subtype. Patients had received a median of 3 (1-11) prior regimens, at least 1 consisting of cytotoxic chemotherapy, with 18 (38%) patients having undergone stem-cell transplantation (Table 2). Other prior therapies included radiation therapy (40%), interferon (6%), bexarotene (6%), or other investigational agents (6%). Patients had extensive stage disease with 34 patients having stage IV disease, including bone marrow involvement in 14.

Two patients were discovered to be ineligible for study. One patient diagnosed with PTCL NOS experienced a partial response (PR) but a biopsy of new nodules appearing in cycle 5 led to a revision of the diagnosis as diffuse large B-cell lymphoma. Another patient was found to have a baseline prolonged corrected QT interval (QTc) of over 480 milliseconds rendering the patient ineligible for study. This patient received only a single dose. These 2 patients were included in the toxicity analysis but were excluded from the response analysis.

Patients received a median of 3 (1-57) cycles and 9 (1-169) doses (Table 3). Throughout 373 cycles, 1062 doses were administered; 510 (48%) were full doses, 135 (13%) were escalated doses, and 417 (39%) were reduced. 4 doses in 3 patients were held because of thrombocytopenia ($< 50 \times 10^9$/L) and 1 dose because of neutropenia ($< 0.5 \times 10^9$/L). Seven additional doses in 5 patients were held because of ongoing adverse events. These included infection, fever with the suspicion of infection, or

Table 2. Prior therapy

Prior therapy, no. of pts (%)	N = 47
Cytotoxic chemotherapy	47 (100)
Interferon	3 (6)
Bexarotene	3 (6)
Experimental and other*	3 (6)
Stem-cell transplant†	18 (38)
Radiation Therapy	19 (40)
Median number of prior therapies (range)	3 (1-11)
Median number of prior cytotoxic therapies (range)	2 (1-6)
Prior cytotoxic regimens in 47 patients (no.)	n = 118
CHOP	32
ICE	11
Methotrexate (single agent)	6
CVAD	5
EPOCH	5
Various other regimens	59
Other combination regimens	45
Other single agent regimens	14

*Includes denileukin diftitox, UCN01, pralatrexate, and 506U78.
†Includes both patients with ALK-positive anaplastic large T-cell lymphoma.

elevated LFTs. Protocol-mandated dose reductions were required in 20 patients: 93 doses in 16 patients because of thrombocytopenia (> 50, $< 75 \times 10^9$/L), 24 doses in 6 patients because of neutropenia (> 0.5, $< 1 \times 10^9$ cells/L), and 5 additional doses in 3 patients because of both. An additional 15 dose reductions occurred because of ongoing adverse events in 6 patients, including fatigue (9 doses), elevated LFTs (2 doses), anorexia, diarrhea, infection, or fever, 1 dose each. The remainder of the doses below 14 mg/m^2 (280) were administered to patients who had a dose held or had 1 or more protocol mandated dose reductions.

Table 3. Administered therapy

Treatment	
Total no. of cycles	373
Cycles per patient, no. of cycles	
Median	3
Range	1-57
Cycles per patient, no. of patients	
≤ 2	22
3-5	9
≥ 6	16
No. of doses per patient	
Median	9
Range	1-169
No. of doses	
Total	1062
Full	510
Dose escalated	135
Reduced, total*	417
Reduced due to toxicity	137
Held†	5
Dose administered	
Cumulative dose, mg/m^2	
Median	112
Range	14-1176
Cumulative dose, mg	
Median	196
Range	25-2391

*Total number of dosese reduced includes those on a stable dose reduction, required to prevent recurrence of toxicity.
†According to protocol criteria.

Table 4. Pharmacokinetics

Parameter	n	Geometric mean	95% CI
Half-life (h)	19	3.04*	2.30-3.78
C_{max} (ng/mL)†	36	427.0	342.4-511.5
AUC_{last} (hr·ng/mL)	36	1498	937.2-2058
AUC_{inf} (hr·ng/mL)	19	1899	1293-2505
$V_{z\ obs}$ (L/m^2)	19	19.01	13.55-24.47
Cl_{obs} (L/hr/m^2)‡	19	7.37	4.96-9.77

C_{max} indicates maximum plasma concentration; AUC_{last}, area under the curve from time zero to time of final quantifiable sample; AUC_{inf}, area under the curve extrapolated to infinity; V_z, volume of distribution during the terminal phase; and Cl, systemic clearance.

Pharmacokinetic analysis was not possible in ten patients, and a full analysis was not possible in 17 patients. One patient was excluded from this table due to receiving a dose of 18 mg/m^2.

*The median half-life was 3.14 h (range: 1.04-6.77).

†Cmax is reported as observed value.

‡Clearance is expressed as L/hr/m^2 due to the BSA dosing employed.

Pharmacokinetics

First dose pharmacokinetics were evaluable in a total of 37 patients; 36 patients received 14 mg/m^2 romidepsin and 1 patient received 18 mg/m^2. Full pharmacokinetic parameters are provided in Table 4. Only 19 of the 36 PTCL patients who received romidepsin at 14 mg/m^2 had sufficient data to estimate the slope of the terminal phase during noncompartmental analysis. From this calculation, half-life, AUC_{inf}, volume of distribution in the terminal phase (V_z), and total body clearance (Cl) can be calculated. All parameters listed in Table 4 have values that are not significantly different from the results obtained with the same romidepsin dose (14 mg/m^2) in patients with CTCL.[12,28]

Toxicities

Toxicities commonly observed were similar to those observed in the phase 1 trials of romidepsin and reported for other HDAC inhibitors.[25-26] First cycle toxicities are presented in Table 5. Side effects (any grade) included fatigue (40%), nausea (51%), vomiting (19%), and anorexia (21%). Hematologic abnormalities included leucopenia (47%), granulocytopenia (45%), lymphopenia (17%), thrombocytopenia (47%), and anemia (40%). Transient elevations of liver function tests, AST or ALT, were observed in 10 patients with 4 additional patients experiencing isolated hyperbilirubinemia. Hyperuricemia was noted in 10 patients, 7 grade 1 and 3 grade 3 events. Hypophosphatemia was noted in 2 patients.

Overall, infections occurred in 17 (36%) patients over 28 cycles (8%), including bacterial infections of the skin; bacteremia; sepsis; and upper respiratory, pulmonary, and urinary tract infections. Also included were oral candidiasis, viral URI, and herpes zoster in 3 patients. Reactivation of hepatitis B and the emergence of EBV-associated lymphoproliferative disorder resulted in discontinuation in 2 patients.[27] One patient, reported previously,[19] was noted to have an asymptomatic, nonrecurrent, 12-beat run of ventricular tachycardia while on telemetry placed for a QTc over 500 milliseconds observed on the routine ECG obtained after the second dose of cycle 5. At the time of this event, this patient had abnormal magnesium and potassium levels that may have been related to her lymphoma, prior chemotherapy, and underlying celiac disease.

Two deaths occurred among patients with PTCL while on study and 5 within 30 days of removal from study. Deaths on study included 1 patient with rapidly progressing disease, complicated by a pericardial effusion, who was enrolled but deteriorated and died 5 days after his first dose. The second patient had a past cardiac history significant for extensive atherosclerotic disease including hypercholesterolemia, hypertension, diabetes, myocardial infarction, carotid endarterectomy, and renal stents. This patient experienced a CR on romidepsin but expired in his sleep 3 days after the second dose of the fifth cycle. As a result of this death on study, protocol enrollment criteria were changed to exclude patients with significant cardiovascular disease as well as other patients at risk for sudden death. Of the deaths in patients within 30 days of study, 4 patients died because of disease progression. One patient, who had achieved a CR, developed high-grade fevers, thrombocytopenia, and elevated LFTs after the first dose of his 15th cycle. An elevated EBV viral load was detected. Biopsies of lymph node and liver detected an aggressive EBV-positive NK-T lymphoma, and the patient died of hemophagocytosis syndrome.[27]

Table 5. Cycle 1 toxicities*

	Grade 1	Grade 2	Grade 3	Grade 4
Hematologic				
Leukopenia	6%	11%	26%	4%
Granulocytopenia	6%	13%	17%	9%
Lymphopenia			17%	
Thrombocytopenia	28%	4%	15%	
Anemia	13%	21%	6%	
Nonhematologic				
Fatigue	28%	4%	9%	
Headache	15%	2%		
Fever	11%	9%		
Infection w/o neutropenia		4%		2%
Nausea	43%	6%	2%	
Vomiting	11%	6%	2%	
Anorexia	17%	4%		
Dysgeusia	4%	2%		
Constipation	6%	2%		
Diarrhea	9%	2%		
ECG T-wave changes	53%	11%		
Laboratory				
Hypoalbuminemia	15%	4%	2%	
Hyperbilirubinemia	6%	6%	2%	
AST	11%	4%	4%	
ALT	9%	4%		
Hyperglycemia	9%	4%		
Hyperuricemia	15%			6%
Hypocalcemia	9%	26%	2%	
Hypokalemia	9%			
Hypomagnesemia	9%			
Hyponatremia	9%			

*Toxicities occurring in cycle 1 in $>$ 5% of patients, with an attribution of possibly, probably or definitely related to drug.

Responses

Among the 45 patients with PTCL included in the response analysis, 8 patients experienced complete responses (CR) and an additional 9 patients experienced partial responses (PR), for an overall response rate of 38% (95% CI, 24% to 53%). Details of these responses are presented in Tables 6 and 7. The overall median duration of response was 8.9 months (range 2-74). The median time to response was 1.8 months, with all patients achieving a response within 2 months except 3 patients at 4, 8, and 11 months. Among the 18 patients who had undergone prior SCT, 6 experienced a response to therapy, including 3 CR and 3 PR. Only 1 patient had disease confined to the skin; the patient enrolled with stage IV disease and experienced a complete response. Seven patients were

Table 6. Response and duration

	Cohort 1, N = 45*	
Response category	No. (%)	Duration (mo)
CR	8 (18%)	3, 3, 6, 12, 13+, 17, 49+, 74
PR	9 (20%)	2, 3, 3, 5, 6, 8, 9, 12, 23+
SD	5 (11%)	3, 3, 6, 6, 12
PD	18 (40%)	
NE	5 (11%)	

*Two patients were excluded from response assessment: one patient discovered to be ineligible for enrollment after receiving the first dose and one patient whose T-cell lymphoma was reclassified as DLBCL.

enrolled who were receiving oral prednisone at study entry. 4 had disease progression within 2 cycles; 1 patient was later found to be ineligible, after his disease was reclassified as diffuse large B-cell lymphoma; and 2 patients received on-going steroids that could not be discontinued for previously diagnosed pneumonitis induced by prior chemotherapy (1 with stable disease on study 12 months and 1 with PR on study 7 months).

Complete responses were noted in 8 patients (18%) with a median duration of response of 29.7 months (range 3-74). Three patients remain on protocol with 2 continuing in CR. The third patient experienced re-emergence of disease 74 months after achieving a CR (53 months after discontinuing therapy) and has restarted therapy as allowed by protocol. Responses in 2 of these patients are shown in Figure 1A and B. A fourth patient, 84 years old, died because of worsening of his pre-existing aortic stenosis 14 months after discontinuing romidepsin. One patient discontinued study because of progression of disease, and 1 patient died unexpectedly, as noted above. A seventh patient, discussed in "Toxicities,", developed a new EBV-positive NK-T lymphoma. The eighth patient was noted to have elevated LFTs on routine laboratory evaluations after 11 cycles of therapy. Evaluation demonstrated a reactivation of hepatitis B. Details of these latter 2 patients have been reported.[27]

Partial responses were also observed in 9 patients (20%) with a median duration of response of 5.2 months (range 2-23+). Eight of 9 patients discontinued treatment because of progression of disease, with a median time to progression of 7.4 months; 1 patient remains on study.

Stable disease was noted in an additional 5 patients with a median duration of 6 months (range 3-12). Eighteen patients without response to treatment were categorized as having progressive disease (PD). Five patients were considered unevaluable, as response could not be assessed. One patient was enrolled with rapidly progressing disease complicated by a pericardial effusion and died on study, as noted in "Toxicities," after receiving only 1 dose. Four patients were removed from study after 1 dose in cycle 2 because of adverse events or concurrent illness including thrombocytopenia, thrombocytopenia accompanying probable disease progression, initiation of conflicting concomitant medication, and hemochromatosis with the development of elevated LFTs. A Kaplan-Meier plot of time to progression by category of response is presented in Figure 2.

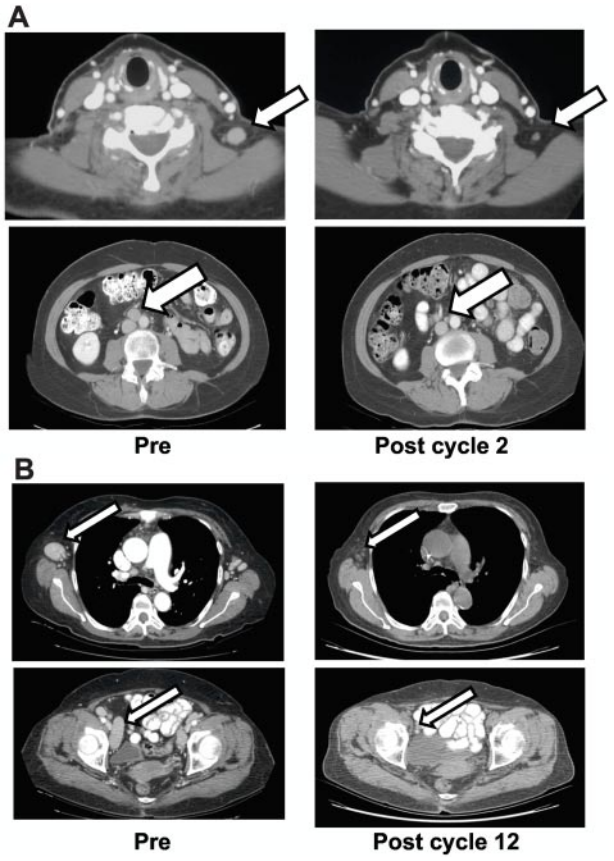

Figure 1. Patient responses. (A) Patient had recurrent PTCL, subtype not otherwise specified (NOS) after cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP); etoposide, methylprednisolone, cytarabine, and cisplatin (ESHAP); and autologous stem cell transplant. PET scan at 8 months showed no evidence of disease. The response was scored as CR and therapy was discontinued after 2 years with the patient remaining free of disease for another 53 months. (B) Patient with PTCL, subtype not otherwise specified (NOS), had prior CHOP and pralatrexate. Patient, who was declared a PR after 2 cycles of romidepsin and a CR afer 12 cycles of romidepsin, remained on study at 24 months as of data cutoff.

Discussion

This phase 2 trial was initiated after the observation of responses in patients with CTCL and PTCL treated on a phase 1 trial of romidepsin.[11] The index patient, with PTCL NOS, was observed to have a durable complete response to romidepsin and remains alive at 10 years. Durable major responses were observed in the CTCL cohort enrolled on the phase 2 trial.[12] Pharmacokinetic analysis of romidepsin revealed a 2-compartment model with moderate interindividual variability.[12,28] Although the half-life is relatively short at 3 hours, exploratory pharmacodynamic studies demonstrated increased histone acetylation in PBMCs extending from 4 to 48 hours.[29] These studies, which included both patients from the CTCL cohort and 18 of the 47 patients with PTCL reported here,

Table 7. Response and duration by classification

	No. enrolled	No. of responses	Duration of CR (mo)	Duration of PR (mo)
PTCL, unspecified or NOS	27	11	3, 3, 12, 13+, 74	2, 3, 5, 6, 9, 12
Angioimmunoblastic	6	1		23+
Anaplastic large T-cell, ALK neg	2	2	6	3
Enteropathy associated T-cell lymphoma	1	1		8
PTCL, u of the skin	1	1	17	
CD30 lymphoproliferative disorder	1	1	49+	

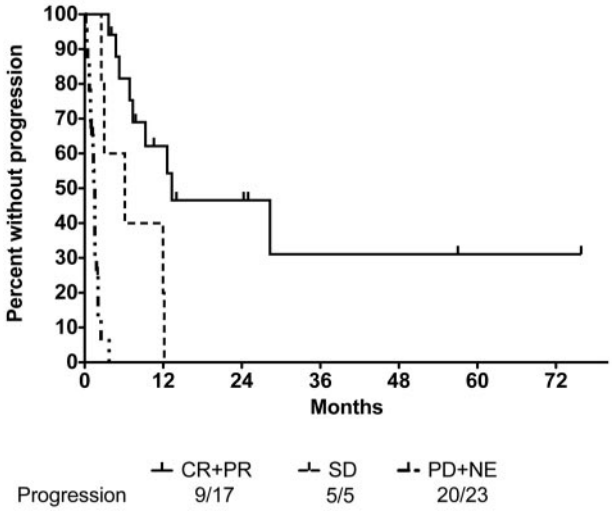

Figure 2. Time to progression. (A) Kaplan-Meier plot of time to progression is shown for descriptive purposes, separated by response category. The median time to progression was 13.0 months for CR+PR, 4.6 for SD and 1.4 for PD+NE. Note that TTP is biased in this analysis because PD or NE could occur at any time, while a categorical response necessitated a 2-cycle interval before disease reassessment occurred.

also demonstrated increases in *ABCB1* as a marker of gene expression in PBMCs and in biopsy samples; and increased hemoglobin F in blood.[29] Increased gene expression subsequent to increased histone aceylation is an expected effect of a HDAC inhibitor. Interestingly, persistence of histone acetylation at 24 hours was associated with both drug clearance and disease response, suggesting that drug exposure may be important for efficacy. Numbers were too few to generate a separate correlation in the PTCL population alone.

Forty-seven patients with PTCL who had received prior systemic cytotoxic chemotherapy were enrolled. The overall response rate was 38% with a median duration of response of 8.9 months. Romidepsin was well tolerated. The toxicities observed were similar to those previously observed[9-10,12] including fatigue, nausea, vomiting, and transient neutropenia and thrombocytopenia. These toxicities appear to be a class effect among the HDAC inhibitors.[25-26] Because of ECG changes observed in the phase 1 trial, characterized by T-wave flattening and ST segment depression, rigorous assessment of the potential cardiac effects of romidepsin were incorporated in the phase 2 trial. We demonstrated that these changes were not associated with myocardial dysfunction based on echocardiography, radionuclide imaging, and troponin assays performed during therapy as well as after long-term follow up.[19] Furthermore, all 17 patients who had received 18 months or more of therapy had no evidence of cumulative cardiac toxicity. Our review of QT interval changes, based primarily on the ECG machine Bazett correction, suggested a median increase of 14 milliseconds; however, recent central review of the ECGs suggests a median increase closer to 5 milliseconds, implying that QT interval prolongation is not of major concern.[30] A review of unexplained deaths in trials with romidepsin, including the death reported here, revealed that each of the patients had risk factors for sudden death.[19] Furthermore, patients with observed ectopy, such as the patient with a 12-beat run of ventricular tachycardia, were found to have electrolyte abnormalities. These findings led to changes in protocol including exclusion of patients with risk factors for sudden death, avoidance of concomitant medications which may interfere with metabolism or potentiate QTc prolongation and the supplementation of magnesium and potassium.[31] To maintain a minimum potassium level of 4.0mM and magnesium level of 0.85mM, preliminary analysis of 429 pretreatment assessments performed in the PTCL patients revealed that supplementation would be required 50% of the time: 13% required potassium; 20%, magnesium; and 17%, both (S.E.B., R.L.P., R.F., S.M.S., manuscript in preparation). Fourteen patients with PTCL were enrolled after exclusion criteria were amended; among these 1 patient developed grade 3 atrial flutter occurring 1 week after his first dose of romidepsin. A wandering atrial pacemaker with premature supraventricular complexes was noted on the pre-enrollment ECG.

Peripheral T-cell lymphomas represent approximately 10% of the non-Hodgkin lymphomas. The largest analysis of these patients was presented by the International T-cell Lymphoma Project (ITLP).[17] Among the notable findings from that study included the observation that 5-year overall survival was less than 50% for all subtypes except for a few notable ones such as ALK-positive ALCL. Patients with the more common subtypes had a 5-year survival of 32% or less. Furthermore, unlike patients with B-cell lymphomas, the inclusion of an anthracycline did not appear to have therapeutic benefit. Lastly, the difference in outcome between T-cell NHL and B-cell NHL has been magnified after the introduction of the monoclonal antibody rituximab, which targets CD20 on B cells. These findings highlight the great need for better treatments for patients with PTCL, both in the front line and salvage settings. The ITLP report concluded that "the clinical outcome for patients with most of these lymphoma subtypes is poor with standard therapies and novel agents and new modalities are needed to improve survival."[17p4124] Pralatrexate, a folate analog, received accelerated approval by the FDA in 2009 for relapsed PTCL, representing the only FDA approved therapy for this disease.[32] In 109 patients enrolled on a phase 2 study, the overall response rate for patients with PTCL was 27%, with 7 patients in CR. The median duration of response was 9.4 months. Although a number of different chemotherapeutics and biologic agents have shown activity in T-cell lymphomas, and a large development pipeline exists, it has been difficult to fully develop therapies for this disease because of its rarity, its aggressiveness, and its heterogeneity.

The HDAC inhibitors represent a new class of antineoplastic agents. While limited activity has been seen in solid tumors, the most striking activity has been observed in patients with T-cell lymphoma.[33] After demonstrating responses in patients with PTCL and CTCL,[11] we initiated the phase 2 trial that is the subject of this report. Based on data from the cohort of patients with CTCL, together with data from a separate study, romidepsin (Istodax, Celgene Corporation) received approval from the FDA in 2009 with relapsed or refractory CTCL as the indication. Other HDAC inhibitors are also in testing in patients with T-cell lymphoma. Vorinostat was approved by the FDA in 2006 for cutaneous manifestations of CTCL. The related hydroxamic acid, belinostat, has also been tested in PTCL, with results reported in abstract form, including 2 CR and 3 PR in 20 patients with PTCL.[34] A separate industry-sponsored study of romidepsin for patients with PTCL has recently completed accrual (NCT00426764). Among the patients treated on this trial, many of the responses demonstrated remarkable durability. Eight patients had complete responses; 3 of these had prior stem cell transplants, 5 demonstrated responses of a year or more, and

7 never had disease progression while on treatment. Romidepsin offers an important new therapeutic option for patients in urgent need of better treatments. While over 10 mechanisms of antitumor activity have been proposed,[35] the mechanism of antitumor activity in patients with T-cell lymphoma remains to be elucidated. Understanding the mechanism of action may enable the identification of patients most likely to derive benefit from romidepsin. An important direction for future research will be to develop combination or sequential therapies that exploit the exquisite activity of romidepsin in T-cell lymphoma and enhance the efficacy of the HDAC inhibitor therapy. Furthermore, it is important to develop clinical trials that have the potential to move promising combinations to the upfront setting where increased rates of remission and depth of responses are so sorely needed.

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Authorship

Contribution: R.L.P. designed and conducted clinical trial, analyzed data, wrote manuscript; R.F. contributed to patient care, collection of clinical trial data, and database quality; H.M.P. and S.L.A. contributed to patient care, collection of clinical trial data, and manuscript revision; M.H.K., J.Z., M.T., S.S., D.J., I.L., L.H., and M.C. contributed to patient care, collection of clinical trial data, and manuscript review; E.S.J. performed central review of pathology and manuscript review; A.L. performed central review of radiology and manuscript review; C.J.P. and W.D.F. performed assay and analysis of pharmacokinetics and manuscript review; S.M.S. performed statistical analysis and manuscript review; A.T.F. designed the clinical trial, reviewed clinical trial data, and reviewed the manuscript; J.J.W. designed and monitored the clinical trial and reviewed the manuscript; and S.E.B. designed and conducted the clinical trial, analyzed data, and wrote the manuscript.

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