A Phase I Study to Assess the Safety, Tolerability, and Pharmacokinetics of CXD101 in Patients With Advanced Cancer

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BACKGROUND: In the current study, the authors sought to determine the maximum tolerated dose (MTD) of the novel class 1 selec¬tive histone deacetylase inhibitor CXD101 in a dose escalation study in patients with advanced solid tumors or recurrent/refractory lymphoma. METHODS: The authors escalated the dose of CXD101 from 1 mg twice daily orally for 5 days in a 21-day cycle (3+3 de¬sign). RESULTS: A total of 39 patients were enrolled, 36 of whom received CXD101. Of the 30 patients in the escalation cohort, 29 were evaluable for determination of the dose-limiting toxicity (DLT). DLTs were noted at doses of 16 mg twice daily (1 of 6 patients), 20 mg twice daily (1 of 6 patients), and 24/25 mg twice daily (2 of 5 patients, both of whom developed neutropenic fever). The MTD was 20 mg twice daily, which achieved maximal plasma concentrations (±standard deviation) of 231 ± 76 nM to 342 ± 126 nM, which was within the biologically active range. Six patients received 20 mg twice daily in an expansion cohort. The most frequent adverse events were fatigue, nausea, and reversible cytopenia. Key grade 3 to 4 adverse events (according to Common Terminology Criteria for Adverse Events criteria [version 4.03]) included thrombocytopenia (11%), neutropenia (17%), and neutropenic fever (2%) across the 133 CXD101 cycles given. The toxicity profile was similar to that of licensing studies with other histone deacetylase inhibitors. In 22 evaluable patients receiving a dose of ≥16 mg twice daily (17 of whom had lymphoma and 5 of whom had solid tumors), 3 partial responses (2 in patients with classic Hodgkin lymphoma after allogenic stem cell transplantation and 1 in a patient with angioimmu-noblastic T-cell lymphoma) and 1 complete response (in a patient with follicular lymphoma) were noted (overall response rate of 18%) in addition to 9 patients who achieved durable stable disease. Responses were noted predominantly among patients with lymphoma (tumor reduction noted in 63% of patients on standard computed tomography). CONCLUSIONS: The MTD in the current study was found to be 20 mg twice daily. Encouraging and durable activity was observed in patients with Hodgkin lymphoma, T-cell lymphoma, and follicular lymphoma. Cancer 2019;125:99-108. © 2018 American Cancer Society.

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Additional supporting information may be found in the online version of this article.

We thank the patients and their families.

INTRODUCTION

Histone deacetylase inhibitors (HDACis) have been studied across numerous cancers in recent years. HDACs are dys-regulated in patients with solid and hematological cancers. High HDAC expression is associated with poorer prognosis in, for example, patients with myeloma.[1] The molecular effects of HDACs are diverse and involve multiple signaling pathways and other biologic phenomena that are important in tumorigenesis.[2] HDACis hyperacetylate nucleosomal histones, which tighten chromatin coiling with resultant silencing of gene expression, and are implicated in cell survival regulation, proliferation, differentiation, and apoptosis.[3] HDACis have pleiotropic cellular effects that include inducing proapoptotic genes/protein expression and causing cellular differentiation and/or cycle arrest, and impact numerous other cellular processes including interference with tyrosine kinases and steroid receptors.[4]

Approved HDACis

Several HDACis have entered trials across the field of oncology, with the most success observed in patients with recurrent or refractory (R/R) peripheral T-cell lymphoma (PTCL) and cutaneous T-cell lymphoma (CTCL). Two licensing phase 2 studies[5,6] demonstrated an overall response rate (ORR) of 25% to 34% after romidepsin monotherapy in patients with R/R PTCL and CTCL with acceptable toxicity.

Belinostat is the latest class 1, 2, and 4 HDACi licensed after a trial of 129 patients with R/R PTCL.[7] The ORR was 25.8% (complete response [CR] rate of 10.8%) and the median duration of response (DOR) was 13.6 months, with some DORs reported to last >3 years. Grade 3 to 4 toxicity was primarily hematological.

The class 1 to 2 HDACi vorinostat has been assessed at various schedules. A total of 8 partial responses (PRs) occurred in 33 patients with CTCL.[8] A dose of 400 mg once daily was found to be best tolerated and subsequently was assessed in 74 patients with R/R CTCL.[9] The ORR was 29.7% and the DOR was ≥185 days. Common drug-related adverse events (AEs) were diarrhea (49%), fatigue (46%), nausea (43%), and anorexia (26%), with mostly grade 2 events reported. A phase 2 trial of the pan-HDACi abexinostat at a dose of 80 mg twice daily[10] noted an ORR of 56% in patients with follicular lymphoma (FL) and an ORR of 40% in patients with PTCL. The DOR in patients with FL was 16.0 months, and was 11.5 months in patients with PTCL.

The HDAC class inhibited by and the dosing schedule, ORR, DOR, and toxicity of licensed HDACis are summarized in Supporting Table S1. Overall, responses occur in 25% to 33% of patients, with noteworthy DORs. Toxicities include cytopenias, nausea, diarrhea, and fatigue. HDACi-related discontinuations occur in 10% to 15% of patients. Despite noteworthy durable remissions, to the best of our knowledge predictive biomarkers correlating responses have not been described to date.

Outcomes in patients with solid tumors who are treated with monotherapy are unimpressive,[11] with no licensed indications noted. Combination studies currently are ongoing in patients with solid and hematological tumors (eg, the combination of romidepsin and carfil-zomib in patients with R/R PTCL [ClinicalTrials.gov identifier NCT03141203] and the combination of moce-tinostat and durvalumab in patients with solid tumors [ClinicalTrials.gov NCT02805660]).

Predictive Biomarkers?

Recent data have demonstrated that certain mutations are associated with epigenetic modifiers in patients with subcutaneous panniculitis-like TCL.[12] Epigenetic modifiers were found to be mutated in 13 of 18 cases and included genes involved in chromatin organization, DNA methylation, and histone modification. To our knowledge, the relevance of epigenetic mutational screening as a potential predictor biomarker for HDACis is unclear.

Qu et al recently described the chromatin accessibility profiles of CTCL and analyzed the dynamic

changes associated with HDACi response and resis-tance.[13] Responses were found to be strongly associated with concurrent gains in chromatin accessibility. Such profiling assays could be analyzed as predictive biomark-ers in future studies.

CXD101

CXD101 is a novel, class 1–selective HDACi (HDAC1 [half maximal inhibitory concentration (IC-50) of 63 nM], HDAC2 [IC-50 of 570 nM], and HDAC3 [550 nM IC-50]) and has no activity against class 2 HDAC (unpublished data).

Although some HDACis are overtly nonselective, many (eg, romidepsin) have a nonexclusive bias toward class 1 HDACs.[14] As such, it is hypothesized that predominant antitumor activity is via class 1–dependent mechanisms. Class 1–specific HDAC1/HDAC2is, such as SK-7041 and SK-7068, demonstrate potent antipro-liferative activity against various cancer cells in vitro,[15] and another class 1–specific inhibitor, 4SC-202, induces dramatic G_2-M phase arrest in colorectal cancer cells while sparing the epithelium.[16] Class 2/4 HDACs have tissue specificity for smooth muscle, heart, brain, liver, and colon.[17]

CXD101 has been tested in vitro in colon, lung, non-Hodgkin lymphoma, and myeloma cell lines. IC-50s ranged from 0.2 to 15 μM. Pharmacodynamic (PD) effects were associated with apoptosis and the inhibition of cellular proliferation. CXD101 substantially reduced tumor size in murine xenograft lung (A549a) and colon (HT29) models at a dose of 50 mg/kg. Tumor reductions were found to be associated with increased histone acetyl-ation and decreased HDAC enzyme activity. CXD101 has a pharmacokinetic (PK) and PD profile in mice and canines with relatively reduced interindividual variations in these parameters (unpublished data). In view of the class 1–specific HDACi, the selective bias toward class 1 HDACis of some licensed HDACis, and the reduced in-terindividual PK/PD profiles noted with CXD101, it was hypothesized that the CXD101 toxicity profile may be improved compared with licensed HDACis by minimizing class 2 target effects, such as cardiac toxicity, while retaining antitumor activity.

HR23B

HR23B protein expression by immunohistochemistry has been suggested as a potential biomarker for HDACi response. A genome-wide loss-of-function screen identified HR23B, a protein that shuttles ubiquitinated cargo proteins to the proteasome for degradation, as a

sensitivity determinant for HDACi-induced cell apop-tosis.[18] Proteasome activity is deregulated by HDACis through a HR23B-dependent mechanism, and HDACis sensitize CTCL cells to the effects of proteasome inhibitors. HR23B expression was found to affect sensitivity to HDACi-induced apoptosis, and specific manipulation of HR23B levels within CTCL cells in vitro altered HDACi sensitivity.[19] High HR23B expression by immunohisto-chemistry in patients with R/R CTCL in situ correlated with responses to HDACi.[6] HR23B expression in 65 biopsies from patients with CTCL had a positive predictive value of 71.7%.[19] These results suggest that proteasome deregulation contributes to the anticancer activity of HDACis and raised the formal hypothesis that HR23B may provide HDACis with a predictive biomarker. Evidence for this association in patients with solid tumors includes an exploratory analysis that demonstrated that patients with hepatocellular carcinoma with stable disease after treatment with belinostat demonstrated high and low HR23B expression in 58% and 14% of patients, respectively (*P*=.036).[20]

The current phase 1 trial was conducted to determine the safety, tolerability, PDs/PKs, and maximum tolerated dose (MTD) of CXD101 in patients with advanced malignancies. The hypothesis that HR23B expression could provide a biomarker for identifying HDACi-sensitive tumors was preliminarily assessed.

MATERIALS AND METHODS

Study Design

This single-arm dose escalation trial determined the safety, tolerability, and dose-limiting toxicities (DLTs) and therefore the MTD of CXD101. The MTD was predefined as the dose below which ≥2 patients within a 3-patient cohort or ≥2 patients in an expanded 6-patient cohort experience an unacceptable, predefined DLT. The incidence and severity of AEs (graded according to Common Terminology Criteria for Adverse Events criteria [version 4.03]), vital signs, electrocardiogram parameters, biochemistry, hematology, and urinalysis were recorded to determine tolerability. The starting dose was 1 mg twice daily for 5 days per 21-day cycle. Patients were evaluated for safety on day (D) 1, D2, D5, D8, and D15 of cycle (C) 1 and C2 and D1 and once between D8 and D15 from C3 onward. Dose escalation proceeded ac-cording to a standard 3+3 design, with dose escalation meetings conducted prior to each escalation. DLTs were defined within C1 as any drug-related, nonhematologi-cal toxicity of ≥grade 3 excluding grade 3 fatigue (unless

there was an increase from baseline of ≥grade 2; grade 3 gamma–glutamyl transferase increase; or grade 3 nausea, vomiting, or diarrhea if inadequately treated); drug-related grade 4 neutropenia for ≥7 days; ≥grade 3 febrile neutropenia; grade 3 infection with ≥grade 3 neutrope-nia; or grade 4 thrombocytopenia or grade 3 thrombocy-topenia with associated bleeding. Patients continued to receive CXD101 at the dose allocated until disease progression, unacceptable toxicity, or withdrawal of consent. If a maximum of grade 1 toxicity attributable to CXD101 was noted, the CXD101 dose was escalated by 100% between cohorts. If a CXD101-related grade 2 toxicity occurred in subsequent dose escalation cohorts, the dose was increased by ≤50%.

Eligibility

The trial was conducted at the Churchill Hospital in Oxford in the United Kingdom. Inclusion criteria were patients aged >18 years with an evaluable malignant tumor who had the potential to benefit from HDACi and who had undergone standard therapy (criteria of Cheson et al[21] and Response Evaluation Criteria In Solid Tumors [RECIST; version 1.1][22]) and had an Eastern Cooperative Oncology Group performance status of 0 to 1, a life expectancy of ≥12 weeks, and minimal residual toxicity from prior treatment (persistent grade 1 toxicity was permitted). Patients provided informed consent for trial entry and HR23B testing.

Eligible patients met the following criteria: aspar-tate aminotransferase or alanine aminotransferase and alkaline phosphatase ≤2.5 times the upper limit of normal (ULN), bilirubin ≤1.5 times the ULN, creatinine ≤1.25 times the ULN, white blood cell count $\geq 2\times10^9$/L, neutrophil count $\geq 1.5\times10^9$/L, hemoglobin ≥10 g/dL (supported by transfusion if required), and platelet count $\geq 100\times10^9$/L (later amended to $\geq 75\times10^9$/L). Patients with hematological parameters below these criteria due to bone marrow infiltration were eligible. Staging was based on examination and computed tomography scan of the neck, chest, abdomen, and pelvis and unilateral bone marrow biopsy as indicated. Patients with solid tumors only in the expansion cohort required high HR23B.

Exclusion criteria included: 1) anticancer therapy within ≤28 days; 2) previous receipt of a HDACi; 3) presence of brain metastases, unless stable (symptomatically and/or radiologically) over ≥2 months; patients could not receive concurrent anticancer therapy while on the study, although low stable-dose steroids were allowed; 4) significant, uncontrolled major medical condition(s); 5) major surgery within <4 weeks; 6) mean corrected QT (QTc)

>450 milliseconds; 7) positive status for human immunodeficiency virus, hepatitis B, or hepatitis C; 8) unwilling to use contraception; 9) pregnant or breastfeeding (all subjects consented to use contraception during treatment and for 16 weeks after discontinuation of treatment); and 10) gastrointestinal disease precluding adequate oral medication.

Dosing Schedule

After oral dosing in murine and canine models, peak plasma concentrations $_{(Cmax)}$ were reached 1 to 2 hours after the dose and terminal half-lives were 6 hours and 8 hours, respectively. After murine oral [^{14}C]-CXD101 at a dose of 1.6 mg/kg (4 μmol/kg), tissue radioactivity peaked 3 to 6 hours after the dose and declined slowly thereafter with CXD101-related material still present in tissue 21 days after the dose (unpublished data). As such, a decision to dose at 21-day cycles was made. The starting dose of 1 mg twice daily was calculated, in part, from the MTD in canine and murine experiments (see Supporting Materials).

CXD101 was given orally twice daily for 5 consecutive days every 21 days. The primary aim was to assess the MTD and subsequent recommended phase 2 dose (RP2D). Intrinsic to this endpoint was an evaluation of the safety and tolerability of CXD101.

Toxicity

CXD101 is a specific class 1 HDACi and is hypothesized to have reduced toxicity versus nonspecific HDACis. Given the theoretical risk of retinal toxicity, patients were screened <4 weeks prior to the administration of CXD101 with a slit lamp and fundoscopy assessment. QTc prolongation is associated with the use of HDACis. Therefore, triplicate electrocardiograms (≥1 minute apart) were performed at screening. Patients developing a QTc >500 milliseconds or with an interval increase from baseline of ≥60 milliseconds permanently discontinued treatment. Dosing was interrupted if the QTc increased to >470 milliseconds and only resumed at a reduced dose (at the investigator's discretion) if the QTc resolved to <450 milliseconds.

AEs were analyzed according to the total number of CXD101 cycles (133 cycles) across all patients received as the denominator. The trial management group (TMG) believed this represented the most accurate method of describing the true burden of AEs experienced given the variable number of cycles each patient was exposed to.

PK Studies

PK analysis was a key secondary endpoint. Peripheral

blood was collected for PK analysis on 5 days over C1 to

C2. A total of 4 mL was drawn into EDTA anticoagulant and spun to produce plasma for the determination of the CXD101 concentration. Samples were drawn at 0, 0.5, 1, 2, 3, 4, 6, and 8 hours after dosing on D1 and D5 of C1 to C2. A trough level predose on D2 of C1 also was taken. Samples were frozen (<-60°C) and analyzed in batches. CXD101 was measured using a validated method by liquid chromatography–tandem mass spectrometry (see Supporting Materials for more details). The Cmax, lowest plasma concentration during the 0.5-hour to 8-hour period $_{(Cmin(0.5\text{-}8h))}$ and time after the dose at which the Cmax was observed $_{(Tmax)}$ were measured. The area under the curve between predose and 8 hours after the dose $_{(AUC(0\text{-}8h))}$ for each cycle and day was calculated using the linear trapezoidal rule using noncompart-mental methods, with no extrapolations made.

Response Evaluation

Secondary endpoints included a preliminary assessment of antitumor activity (ORR; criteria of Cheson et al[21] and Response Evaluation Criteria In Solid Tumors [RECIST; version 1.1][22]). Radiological assessment was made by standard computed tomography. The TMG provided expert consensus that a post hoc preliminary assessment of efficacy should include 22 patients dosed at ≥16 mg twice daily. This represented a reasonable compromise with which to provide adequate numbers of patients for response assessment at a dose that was close enough to the MTD. Patients without an event were censored at the time of their last assessment.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue was stained automatically with a BOND-MAX autostainer (Leica Microsystems Inc, Buffalo Grove, Illinois), using a commercial mouse monoclonal anti-HR23B antibody (BD Transduction Laboratories, Franklin Lakes, New Jersey). Two independent histopathologists evaluated HR23B im-munoreactivity. Each was blinded to patient outcome and scored samples for the combined nuclear and cytoplasmic expression (1 indicates <5%, 2 indicates 25%-50%, 3 indicates 50%-75%, and 4 indicates >75%) and intensity (0 indicates negative, 1 indicates weak, 2 indicates moderate, and 3 indicates strong). For a representative example of intensity scores, see Supporting Figures S1a to S1c. The methodology was similar to a prior HR23B analysis in patients with hepatocellular carcinoma,[20] although the percentage expression was scored in categories rather than as a multiplication factor of intensity. This change aimed to simplify the scoring and enhance reproducibility. Within the expansion cohort, patients with solid tumors were

eligible according to positive expression (6-7 of 7 was considered positive). Archival or recent formalin-fixed, paraffin-embedded tissue was used when available. The study was conducted by the Oxford Early Phase Clinical Trials Unit and was supported by the Experimental Cancer Medicine Centre and Celleron Therapeutics. The study was approved by the Oxford Research Ethics Committee (REC:10/H0604/85) and was conducted in accordance with the Declaration of Helsinki.

Statistical Analysis

Given the intrinsic patient heterogeneity and dose studied, the data are summarized by descriptive methods. Because this was an uncontrolled, nonrandomized study with a relatively small number of patients, hypothesis testing was not preplanned.

RESULTS

Baseline Characteristics

A total of 39 patients were enrolled between March 2014 and March 2016. Three patients did not receive a dose due to acute renal failure (1 patient), immune thrombocy-topenia (1 patient), and an elevated alkaline phosphatase level (1 patient). Thirty patients received CXD101 during the escalation phase and 6 received it during the expansion phase. Data were censored in July 2016. The trial recruited 14 patients with solid tumors and 22 patients with lymphoma (Table 1). The median age for all patients was 58 years (range, 21-79 years). Patients across all tumor types were heavily pretreated (median, 4 prior therapies). Tumor HR23B was assessed in 33 patients and was unavailable in 3. Tumors were positive for HR23B in 24 of the 33 patients.

During the escalation phase, responses were observed in patients with lymphoma. In view of this and the demonstrable HDACi responses in the literature for patients with lymphoma, these patients were included in the expansion phase regardless of HR23B status. Patients with R/R lymphoma (4 patients), esophageal cancer (1 patient), and systemic anaplastic meningioma (1 patient) were recruited in the expansion phase.

Safety

The first 4 dosing cohorts (1 mg twice daily, 2 mg twice daily, 4 mg twice daily, and 8 mg twice daily) were completed with no DLTs. The third patient in cohort 5 (16 mg twice daily) developed a DLT (grade 4 QTc prolongation). Consequently, the cohort was expanded to 6 patients with no additional DLTs noted. No clinically significant QTc prolongation was observed in the

other 35 patients recruited. In view of the DLT, grade 2 toxicities, and the need to expand the cohort at the dose of 16 mg twice daily, the dose was escalated to 20 mg twice daily. The second patient in cohort 6 (20 mg twice daily) developed a DLT (grade 4 neutropenia). No further DLTs were observed in an expanded cohort of 6 patients receiving a dose of 20 mg twice daily. In view of grade 4 neutropenia noted at a dose of 20 mg twice daily in the expanded cohort, the dose was escalated to 24 mg twice daily. An expanded seventh cohort (24 mg twice daily) was considered to be non-tolerated due to 2 episodes of grade 4 neutropenia associated with grade 4 neutropenic infection (occurring in the second and fifth patients in the cohort). Patient 5 received a dose of 25 mg twice daily due to a temporary lack of 1-mg capsules. As such, the MTD was determined to be 20 mg twice daily and this also was the RP2D and expansion phase dose. The expansion phase was opened only after the final cohort in the escalation phase was complete; DLTs were assessed and the RP2D determined.

CXD101 typically was well tolerated (Table 2). No CXD101-related deaths were observed. Across all the cumulative total cycles, AEs that were possibly, probably, or definitely related to CXD101 in >10% of the total of 133 cycles of 21 days each were as follows: thrombocyto-penia (grade 1-2 in 20% of cycles and grade 3-4 in 11% of cycles), neutropenia (grade 1-2 in 19% of cycles and grade 3-4 in 17% of cycles), anemia (grade 1-2 in 11% of cycles), nausea (grade 1-2 in 18% of cycles), diarrhea (grade 1-2 in 11% of cycles), vomiting (grade 1-2 in 11% of cycles), anorexia (grade 1-2 in 12% of cycles), fatigue (grade 1-2 in 27% of cycles), and QTc prolongation (grade 1-2 in 19% of cycles). Other notable grade 3 to 4 AEs included neutropenic fever (2%), bronchial infection (2%), and fatigue (2%). Frequently observed AEs were consistent with the known AE prolife of HDACis, and all were manageable.

Efficacy

In 22 patients with both solid tumors and lymphoma who received a dose of ≥16 mg twice daily, 3 PRs (2 in patients with classical Hodgkin lymphoma [cHL] after allogenic stem cell transplantation [SCT] who were treated at doses of 16 mg twice daily and 20 mg twice daily, respectively, and 1 in a patient with angioimmuno-blastic T-cell lymphoma who was treated at a dose of 16 mg twice daily) and 1 confirmed CR (in a patient with FL who was treated at a dose of 20 mg twice daily) were noted (ORR of 18%), in addition to 9 patients with stable disease (see Supporting Fig. S2).

TABLE 1. Baseline Characteristics of the Patients (N =36)

Characteristic	Solid Tumor N=14	Hematologic Malignancy N=22	Combined N=36
Sex			
Male	5 (36%)	13 (59%)	18 (50%)
Female	9 (64%)	9 (41%)	18 (50%)
Median age (range), y	58.5 (42-70)	53.5 (21-79)	58 (21-79)
Median no. of prior lines of therapy (range)	4 (1-17)	4 (1-10)	4 (1-17)
ECOG performance status			
0	3 (21%)	7 (32%)	10 (28%)
1	11 (79%)	15 (68%)	26 (72%)
Histology (escalation in 30 patients)	NA	Colorectal: 3 (21%) Lung: 2 (14%) Cervical: 2 (14%) Breast: 1 (7%) Pancreatic: 1 (7%) Ovarian: 1 (7%) Head and neck: 1 (7%) Endometrial: 1 (7%) Esophageal: 1 (7%)	Hodgkin lymphoma: 7 (32%) Angioimmunoblastic T-cell lymphoma: 4 (18%) Diffuse large B-cell lymphoma: 3 (14%) Peripheral T-cell lymphoma NOS: 2 (9%) Lymphoplasmacytic lymphoma: 1 (5%) Intermediate-type NHL: 1 (5%)
Histology (expansion in 6 patients)	NA	Follicular lymphoma: 1	Hodgkin lymphoma: 3 (14%)
	Systemic anaplastic (5%) meningioma: 1 (7%)		
Stage of disease[a]			NA
	Stage III: 1 (7%) Stage IV: 13 (93%)	Stage II: 3 (14%) Stage III: 2 (9%) Stage IIIS: 4 (18%) Stage IV: 12 (55%)	
International prognostic index			
0-1		3 (14%)	
2	NA	8 (36%)	NA
3		9 (41%)	
4-5		1 (5%)	
NA		1 (5%)[a]	
HR23B			
Positive (6-7)	10 (71%)	14 (64%)	24 (67%)
Negative (0-5)	2 (14%)	7 (32%)	9 (25%)

Not available	2 (14%)	1 (5%)	3 (8%)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; NA, not applicable; NHL, non-Hodgkin lymphoma; NOS, not otherwise specified. [a]Not applicable (lymphoplasmacytic lymphoma).

In 17 patients with lymphoma who were treated at a dose of ≥16 mg twice daily, the ORR was 23.5%, with 6 additional patients achieving beneficial stable disease. A reduction in tumor volume was observed in 63% of evaluable patients with lymphoma (Fig. 1). In those patients who responded (3 with a PR and 1 with a CR), the DORs was 203 days, 161 days, 173 days, and 441 days, respectively. No PRs were noted among the 5 patients with solid tumors who were treated with a dose of ≥16 mg twice daily and 2 patients with solid tumors experienced stable disease. No clear correlation was noted when reduction in tumor volume or progression-free survival (data not shown) were stratified according to HR23B status (see Supporting Fig. S3).

Pharmacokinetics

Peak plasma concentrations (Tmax) were reached 1 to 4 hours after dosing and the CXD101 half-life was estimated at 5 to 12 hours, with a trend toward a longer half-life at steady state for both cycles measured. The last data point at 8 hours was not long enough to observe the terminal phase of CXD101 elimination before the next daily dose. However, the mean accumulation ratio (ratio between AUC(0 -8h) on D5 and D1) of 2.9 was consistent with a terminal half-life of approximately 19.5 hours.

CXD101 was undetectable by liquid chromatography in predose plasma samples (trough levels) for C2. For all cycles and days of analysis, there was a linear relationship noted between dose and AUC(0-8h), Cmax, and Cmin (0.5-8h) (Figs.

TABLE 2. Treatment-Related (Possibly, Probably, or Definitely) AEs and SAEs Across a Cumulative Total of

133 Cycles

Category	All Grades (Total No. of	%	Grade 1 to 2	%	Grade 3 to 4	%	SAEs
Hematology							
Neutropenia	47	35	25	19	22	17	
Thrombocytopenia	40	30	26	20	14	11	
Anemia	19	14	16	12	3	2	
Leukopenia	13	10	7	5	6	5	
Neutropenic fever	4	3	1	1	3	2	4
Gastrointestinal							
Nausea	24	18	24	18	0	0	
Anorexia	16	12	16	12	0	0	[1]a

Diarrhea	15	11	14	11	1	1	
Vomiting	15	11	15	11	0	0	[1]a
Infections Bronchial	8	6	6	5	2	2	1
General/constitutional							
Fatigue	38	29	36	27	2	2	[2]a
Flu-like symptoms	4	3	4	3	0	0	
Cardiovascular							
QTc prolongation	27	20	25	19	2	2	

Abbreviations: AE, adverse event; QTc, corrected QT interval; SAE, serious adverse event.

[a]Anorexia, fatigue, and vomiting were reported as a single SAE.

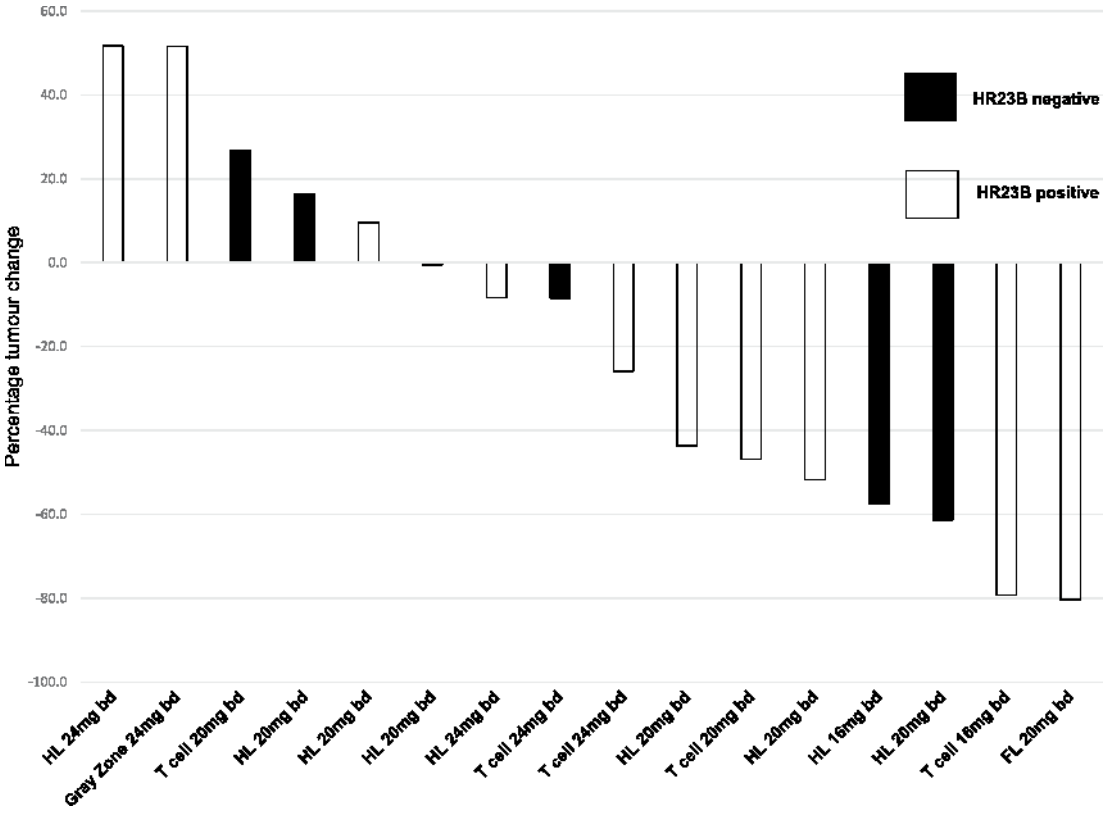

Figure 1. Best tumor volume response (in percentage) for doses of ≥16 mg twice daily (bd) in patients with lymphomas only. Note that a single patient with lymphoma with progressive disease who was treated with a dose of 16 mg bd was not evaluable for changes in tumor volume due to a conflomerate, complex mesenteric mass. FL indicates follicular lymphoma; HL, Hodgkin lymphoma.

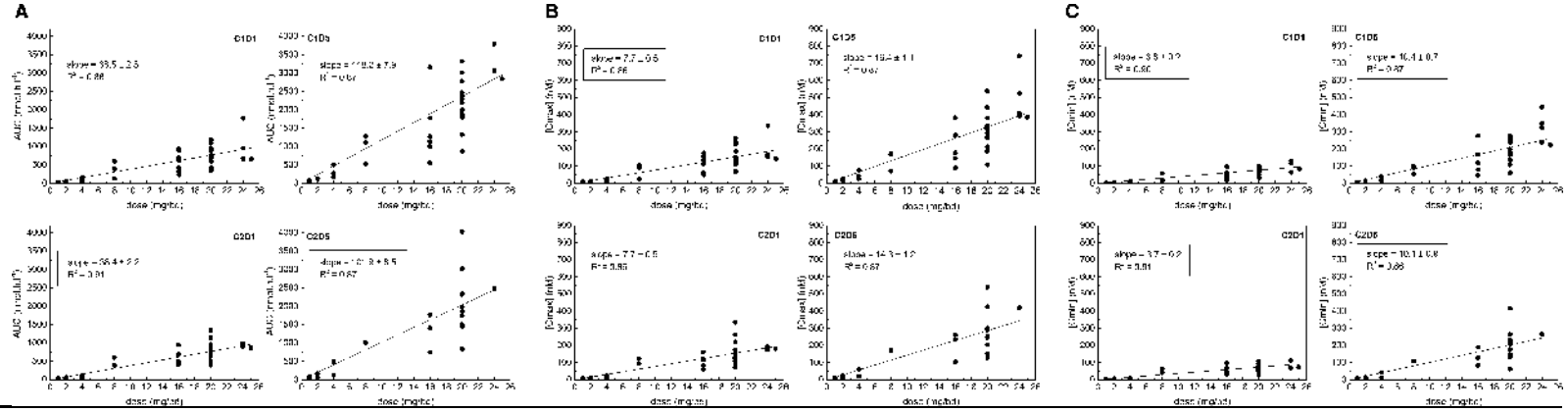

Figure 2. (A) Area under the curve (AUC), (B) peak plasma concentration $_{(Cmax)}$, and (C) lowest plasma concentration $_{(Cmin)}$ at cycle 1, day 1 (C1 D1), C1 D5, C2 D1, and C2 D5 with coefficient of determination (R^2) values.

2A-2C) although, as is normal in a phase 1 trial, there was a degree of interindividual variation in PK parameters (coefficient of determination [R^2], 0.86-0.91). The average (±standard deviation) plasma concentrations for both cycles at steady state (between $C_{min(0.5-8h)}$ and C_{max}) at a dose of 20 mg twice daily ranged from 231±76 nM to 342±126 nM, which were well within the biologically active range in vitro. PK parameters (AUC, C_{max}, and C_{min}) did not appear to differ in the 4 responders compared with nonresponders (data not shown).

HR23B

HR23B assessment was performed successfully in 33 of 36 patients and in all cases for which tissue was available. Agreement between the 2 pathologists was good (identical scores in 23 of 33 patients and a discrepancy of ≤1 point in 32 of 33 patients) (see Supporting Table S1).

DISCUSSION

The current phase 1 trial reached its primary endpoint of determining that the MTD and RP2D of CXD101 of 20 mg twice daily was well tolerated with manageable AEs, which is consistent with previous reports regarding HDACis. However, it should be noted that, when compared with, for example, panobinostat (a nonselective HDACi) in patients with R/R cHL,[23] cytopenias and gastrointestinal toxicity may be lower with the class 1–selective CXD101 (see Supporting Table S1). For example, the rate of any grade of diarrhea was 11% (per total cycle) compared with 66% with panobinostat (per patient). The rate of any grade of vomiting was 11% (per total cycle) compared with 33% (per patient) with panobinostat. There appears to be no appreciable difference in the effect on QTc between CXD101 and other HDACis. In the current study, approximately 19% of patients (per total cycle) had grade 1 to 2 QTc prolongation. With panobinostat, no relevant QTc prolongations were observed whereas when romidepsin was administered to patients with R/R PTCL, approximately 4% experienced grade 1 to 2 QTc prolongation.[5] However, given the heterogenous nature of the patients, the interpatient dose variability, and the relatively small number of patients in the current study, we believe it is too early to specifically address whether the toxicity profile is superior to those of other less selective HDACis. Ultimately, this will require larger phase 2 trials before a definitive judgment can be made. For now, it is reasonable to conclude that there were no new concerning safety signals and that the toxicity profile appears similar to those for other HDACis. It is important to note that no clinically significant events related to prolonged QTc or retinal toxicity were observed.

To our knowledge, the current study is the first trial of a class 1–selective HDACi that has attempted to integrate potentially predictive biomarker assessment into its analysis. HR23B assessment was performed successfully in all cases for which tissue was available, and score concordance was very good. The scoring system was useable, and staining was reliable and reproducible. We found no clear correlation between HR23B status and response. HR23B correlative analysis at this juncture was limited by the range of histologies; the variable dose, which also encompassed subtherapeutic doses; the limited number of patients; and biopsy time lag (median, 1.65 months; range, 0.07-7.6 years). The stability of expression over time currently is unknown, and may have changed prior to C1 D1 in some patients. Gaining an understanding of HR23B stability represents an active area of ongoing investigation. The staining, scoring, and analysis represent preliminary data in a setting that was not designed primarily to definitively evaluate the value of HR23B but rather to provide a platform for testing the discriminative

power of its pre-enrolment expression as a HDACi sensitivity marker in larger, homogenous, phase 2 trials.

To the best of our knowledge, few data assessing HR23B in patients with lymphoma and solid tumors exist. In light of responses noted among patients with lymphoma and the novel nature of the scoring used, the TMG took the view that it was reasonable to enroll patients with lymphoma into the expansion phase regardless of their HR23B status and that a post hoc analysis of patients with a range of HR23B expression may have provided a more valid tool for HR23B assessment. Given the lack of initial responses among patients with solid tumors, the TMG believed it was reasonable to enroll HR23B-positive patients in an attempt to enrich those with potentially HDACi-sensitive disease.

Despite the rationale for CXD101 dosing outlined above, tissue radioactivity was present at 21 days after dosing. Other established HDACis with shorter half-lives, such as romidepsin, are dosed daily and con-tinuously.[5] It is possible that alternative schedules such as continuous dosing could be investigated in separate monotherapy or combination trials; however, safety, MTD, tolerability, and efficacy would require a similar rigorous assessment. Because no additional schedule was assessed in the current study, it is unknown whether a superior continuous schedule with an improved toxicity and efficacy profile exists.

Although the current study was not designed to assess efficacy, there were no noteworthy responses observed in patients with solid tumors. HDACis have been reported to have modest activity in solid tumor–specific trials. For example, no CRs were noted when vorinos-tat was evaluated in a phase 2 trial of patients with various R/R solid tumor histologies.[11] Disappointing results also have been reported with vorinostat in patients with breast cancer.[24] Such modest effects likely are related to tumor-specific upregulation of resistance pathways, although specific mechanisms underpinning HDACi resistance in patients with solid tumors remain speculative. Triple-negative breast cancer cells treated with vorinostat are reported to upregulate antiapoptotic genes, including BCL-2 and MCL1.[25] Epithelial ovarian cells exposed to HDACis upregulate proangiogenic interleukin 8 via nuclear factor κB activation, which in vitro serves as an effective resistance mechanism. Other mechanisms include decreased reactive oxygen species–mediated DNA damage or increased MAPK and PI3K signaling via HDACi-induced p53 acetylation.[26] Prosurvival mutations due to HDACi–selective pressure remain targets for rational combination therapy. Consequently, the evaluation of HDACis in combination with DNA-damaging chemotherapy, radiotherapy, and even checkpoint inhibitors currently is ongoing.[4]

Conversely, the results of the current study demonstrate clear evidence of CXD101 activity in a range of lymphomas. Responses were noted in patients with R/R cHL after allogenic SCT, a setting in which to the best of our knowledge there are no current standard therapeutic options. Panobinostat demonstrated modest activity in patients with R/R cHL after autologous SCT,[23] although only 13 patients had undergone prior alloge-neic SCT. There is a conspicuous lack of data assessing HDACi after allogenic SCT. Overall, the provisional efficacy data for our specific class 1 HDACi appear similar to those for nonselective HDACis (see Supporting Table S1).[5,7,27] A durable PR was observed in patients with an-gioimmunoblastic T-cell lymphoma, for which there are data supporting HDACi,[5,27] and a single CR was noted in a patient with FL. Responses were durable, a feature again described previously. Based on the results of the current study, a phase 2 trial to further assess CXD101 efficacy and HR23B in a broad range of patients with R/R lymphoma is merited.

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CONFLICT OF INTEREST DISCLOSURES

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AUTHOR CONTRIBUTIONS

Toby A. Eyre: Formal analysis, data curation, methodology, project administration, writing–original draft, and writing–review and editing. **Graham P. Collins, Avinash Gupta, Nicholas Coupe,** and **Semira Sheikh**: Formal analysis, data curation, project administration, writing–original draft, and writing–review and editing. **John Whittaker**: Conceptualization, funding acquisition, methodology, project administration, and writing–review and editing. **Lai Mun Wang, Leticia Campo**, and **Elizabeth Soilleux**: Formal analysis, data curation, methodology, project administration, and writing–review and editing. **Finn Tysoe and Richard Cousins**: Data curation, project administration, and writing–review and editing. **Nick La Thangue**: Conceptualization, funding acquisition, methodology, project administration, and writing–review and editing. **Lisa K. Folkes and Michael R.L Stratford**: Formal analysis, data curation, methodology, project administration, and writing–review and editing. **David Kerr**: Conceptualization, funding acquisition, methodology, project administration, and writing–review and editing. **Mark R. Middleton**: Conceptualization, formal analysis, funding acquisition, and writing–review and editing.

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