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Phase I First-in-Human Study of CUDC-101, a Multitargeted Inhibitor of HDACs, EGFR, and HER2 in Patients with Advanced Solid Tumors

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Abstract

Purpose: This first-in-human phase I study evaluated dose-limiting toxicities (DLT) and defined a phase II recommended dose (RD) for CUDC-101, a multitargeted inhibitor of HDACs, EGFR, and HER2 as a 1-hour intravenous (i.v.) infusion for 5 consecutive days every 2 weeks.

Experimental Design: Twenty-five patients with advanced solid tumors received escalating doses of CUDC-101 (range, 75–300 mg/m^2/day) following a standard 3 + 3 dose escalation design.

Results: The MTD was determined to be 275 mg/m^2. Common grade 1/2 adverse events included nausea, fatigue, vomiting, dyspnea, pyrexia, and dry skin. DLTs occurred in 1 patient in the 275-mg/m^2 dose cohort (grade 2 serum creatinine elevation, $n = 1$) and 3 patients in the 300-mg/m^2 dose cohort (grade 2 serum creatinine elevation, $n = 2$; pericarditis, $n = 1$), all of which were transient and reversible. CUDC-101 exposure increased linearly with the mean maximum concentration (C_{max}), clearance (CL), volume of distribution at steady-state (Vd_{ss}), area under curve (AUC), and terminal elimination half-life ($t_{1/2}$) at the MTD dose of 9.3 mg/L, 51.2 L/h, 39.6 L, 9.95 h·ng/mL and 4.4 hours, respectively. Acetylated histone H3 induction was observed in posttreatment skin samples from 3 patients in the 275-mg/m^2 dose cohort, suggesting adequate systemic exposure and target inhibition. One patient with gastric cancer had a partial response and 6 patients had stable disease.

Conclusion: CUDC-101 administered by 1-hour i.v. infusion for 5 consecutive days every 2 weeks was generally well tolerated with preliminary evidence of antitumor activity. A dose of 275 mg/m^2 is recommended for further clinical testing. *Clin Cancer Res; 20(19); 5032–40. ©2014 AACR.*

Introduction

Multitargeted drugs and drug candidates currently in clinical development generally affect related members of the same gene family. Inhibitors of HER family RTKs, including erlotinib, gefitinib, and lapatinib, have become important drugs for treating human solid tumors (1, 2). However, because molecular heterogeneity among and within tumors, their efficacy is restricted to a small subset of patients (2). The efficacy of RTK inhibitors is also limited by drug resistance that frequently emerges following treatment (3, 4). Several strategies have been proposed to overcome the limited activity of, and acquired resistance to RTK inhibitors. One particularly promising approach involves modulation of RTK pathway signaling by inhibition of HDACs. By modulating the acetylation of both histone and nonhistone substrates (5–8), HDAC inhibitors can regulate a variety of cell functions through indirect effects on downstream targets. Importantly, many of these targets are key regulators of RTK signaling pathways (6, 7, 9). Several studies also suggest a synergy between RTK or conventional chemotherapeutics and HDAC inhibition in cancer cells (10–14). The combination of the HDAC inhibitor romidepsin (gloucester/fujisawa) with erlotinib demonstrated increased erlotinib sensitivity with synergistic apoptotic effects *in vitro* and in xenograft non–small cell lung cancer (NSCLC) tumor models (15). Synergistic preclinical antitumor activity was also observed for the combination of the HDAC inhibitor, PXD101 (Curagen), and erlotinib. In addition, PXD101 treatment resulted in downregulation of human epidermal growth factor receptor 3 (HER3) protein levels. HER3 can enable cancer cells to escape the effects of conventional EGFR/HER2 inhibitors (16, 17). These preclinical study results further suggest the

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Translational Relevance

CUDC-101 represents a first-in-class small-molecule multitargeted inhibitor of both receptor tyrosine kinases (RTK) and class I/II histone deacetylase enzymes (HDAC enzymes). The impetus for the clinical development of CUDC-101 is based on the critical role that HDAC and RTK inhibitors play as cancer treatments, as well as the synergistic anticancer activity these inhibitors display when combined in preclinical setting. In our first-in-human phase I study, CUDC-101 can be safely administered to patients with advanced solid tumors at doses up to 275 mg/m^2 i.v. daily for 5 consecutive days repeated every 2 weeks and has shown promising single-agent activity. Moreover, using skin as a surrogate tissue, pharmacodynamics (PD) analysis has further confirmed that CUDC-101 effectively inhibited HDAC activity at the 275-mg/m^2 dose level. Continued clinical development of CUDC-101 is supported by early evidence of antitumor and PD activity observed in this early-phase clinical study.

potential benefit of combining HDAC and RTK inhibitors for treatment of patients with cancer. As to the rationale for increased sensitivity to HER2 inhibition by cotreatment with an HDAC inhibitor, LAQ824 (a cinnamic acid hydroxamate) has been shown to modulate the transcription of p21 and HER2 genes. In addition, post-translational effects on HER2, AKT, and c-Raf-1 mediated through LAQ824-induced acetylation of heat shock protein 90 (Hsp90) have been demonstrated. Given that treatment with LAQ824 attenuates the levels and activity of AKT and c-Raf-1, it is therefore possible that resistance to trastuzumab based on HER2-independent increased activity of AKT may be overcome by cotreatment with LAQ824 and trastuzumab (12).

CUDC-101 [7-(4-(3-ethynylphenylamino)-7-methoxyquinazolin-6-yloxy) Nhydroxyheptanamide] is a synthetic small-molecule member of the quinazoline class of compounds with a molecular weight of 434.5 Da. CUDC-101 is a potent inhibitor of EGFR, HER2, class I and class II HDACs, and can disrupt signaling downstream of EGFR, HER2, HER3, c-MET, AXL, and AKT (18–20).

In preclinical *in vitro* experiments, CUDC-101 inhibited HDAC activity and EGFR auto-phosphorylation both with an IC_{50} of 4 nmol/L. In *in vitro* mechanistic studies, CUDC-101 reduced MET expression, MET phosphorylation, and inhibited the AKT signaling pathway in MET-amplified NSCLC H1993 cells (18). *In vivo*, CUDC-101 displayed broad antitumor activity in xenograft tumor models across a wide range of cancer types. Pharmacodynamic analysis of several human xenograft tumors after CUDC-101 treatment demonstrated: (i) inhibition of HDAC activity (histone acetylation), (ii) inhibition of EGFR and HER2 phosphorylation, (iii) inhibition of tumor cell proliferation (decrease of Ki67 levels), and (iv) induction of apoptosis (caspase-3 induction; ref. 18). The therapeutic efficacy in xenograft models is likely because of the improved potency of the kinase inhibitory activities and the synergy achieved by the combined RTK and HDAC inhibitory activity within cancer cells. In addition, CUDC-101 and single-targeted HDAC inhibitors reduce HIF1α protein levels, thus suggesting that antitumor activity could also be accomplished by a combination of antiproliferative and antiangiogenic effects (9, 19).

This first-in-human phase I, open-label, multicenter study was conducted at both START (South Texas Accelerated Research Therapeutics, San Antonio, TX) and Karmanos Cancer Institute, Wayne State University (Detroit, MI). The objectives of this study were to determine the MTD of CUDC-101 administered as a 1 hour intravenous (i.v.) infusion on 5 consecutive days every 14 days, and to assess the safety and tolerability, the PK profile, PD measurements, and preliminary evidence of antitumor activity in patients with advanced solid malignancies.

Materials and Methods

Patient eligibility

Eligible patients had pathologically confirmed solid tumors refractory to standard therapy or for which no standard therapy existed; age ≥18 years; life expectancy ≥12 weeks; an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2; previous therapy discontinued ≥4 weeks before study treatment; absolute neutrophil count (ANC) ≥1,500/L; platelets ≥100,000/L; creatinine ≤1.5× upper limit of normal (ULN) or calculated creatinine clearance (CL) ≥60 mL/min/1.73 m^2; total bilirubin ≤1.5× ULN; aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ≤2.5× ULN or ≤5× ULN if documented liver metastases present; prothrombin time ≤1.5× ULN unless receiving therapeutic anticoagulation; serum magnesium and potassium levels within normal limits; negative pregnancy test, known infection with human immunodeficiency virus, hepatitis B or C, and no coexisting severe medical conditions. Patients with brain metastases were eligible if controlled on a stable dose ≤10 mg prednisone equivalent units/day. Patients gave written informed consent according to federal and institutional guidelines before treatment, and the study was conducted in accordance with accordance with the principles of the Declaration of Helsinki and the accordance with International Conference on Harmonization Guideline for Good Clinical Practice.

Dosage and drug administration

CUDC-101 was supplied in individually sealed vials containing lyophilized CUDC-101, tartaric acid and Captisol (a sulfobutyl ether β-cyclodextrin), and was stored at −20°C. Before treatment, a solution was prepared by reconstitution with sterile water to a concentration of 30 mg/mL of CUDC-101. A final solution for administration was prepared by further dilution with 5% dextrose in sterile water to a total volume of approximately 100 mL. CUDC-101 was administered via peripheral venous line or an

indwelling i.v. catheter with an inline sterile filter over 1-hour on days 1 to 5 of a 14-day cycle. Patients could continue to receive additional cycles until PD, unacceptable toxicity and/or withdrawal of subject consent.

Dose escalation design

This study utilized a standard 3 + 3 dose escalation design. From the starting dose of 75 mg/m^2/day, which was one sixth of the highest nonsignificantly toxic dose (HNSTD) in rats (the most sensitive species), dose levels were increased by doubling at each cohort until new onset (not present at baseline) of grade 2 toxicity was observed. Subsequent cohorts used a modified Fibonacci dose escalation scheme. Three patients were treated at each dose level until the first instance of dose-limiting toxicity (DLT) in the first 2 cycles was observed, after which up to 6 evaluable subjects were treated at that dose level. If a second DLT was observed in up to 6 patients, the DLT dose level would have been reached and additional patients would be added to the previous lower dose level up to a total of 6 patients. The MTD was defined as the dose level immediately below the dose at which 2 or more patients experience a DLT in the first 2 cycles (28 days). DLT was defined as any grade 3 or 4 nonhematologic toxicity (grade 3 nausea or vomiting treated with less than optimal antiemetic therapy; grade 3 diarrhea treated with less than optimal antidiarrheal therapy; grade 3 alopecia was not considered a DLT); thrombocytopenia <25,000/μL of any duration or <50,000/μL with bleeding; grade 4 neutropenia lasting longer than 5 days, or grade 3 or worse neutropenia with fever greater than 101.3°F (38.5°C) or grade 3 or worse neutropenia with infection; and any treatment delay or dose hold for drug-induced toxicity occurring in the first 2 cycles. Toxicity was graded according to the NCI CTCAE (v3.0).

Pretreatment and follow-up studies

A complete medical history, physical examination, and routine laboratory studies including a complete blood count (CBC), blood chemistry including electrolytes, calcium, glucose, blood urea nitrogen (BUN), creatinine, AST, ALT, alkaline phosphatase, albumin, total protein, total bilirubin, electrocardiogram (ECG), and relevant radiologic studies were performed before treatment. During the study, radiologic analysis of disease status was repeated every 4 cycles and assessed by RECIST (v1.0). Confirmatory radiographic analysis for measurement of response was done 4 weeks after the initial documentation of a complete or partial response. Stable disease assessment was confirmed by follow-up measurements performed at a minimum interval of 6 weeks from the baseline assessment. Peripheral blood mononuclear cell (PBMC) samples were collected from patients predose and 4 hours after the end of infusion (EOI) on cycle 1 day 1 (C1D1) and cycle 1 day 5 (C1D5) only, and at any time on cycle 1 day 8 for determination of histone acetylation. Skin biopsies were obtained pretreatment before C1D1 and at C1D5 after the EOI for evaluating the HER2 and EGFR phosphorylation. Vital signs were monitored predose, 30 minutes, 1 hour and 4 hours postinfusion initiation C1D1 only, and predose and postdose at all visits. ECG assessments were performed at predose, 1 hour and 4 hours postinfusion initiation on C1D1 and C1D5 only, and only predose at all other visits. A prestudy MUGA scan was required for all patients with any history of coronary artery disease, cardiomyopathy, congestive heart failure, or clinically significant arrhythmia. Additional scans were required at cycle 2 day 1 and at the end of treatment for these patients. Laboratory tests (CBC and chemistry) were performed on days 1 to 5 and 8 (cycles 1–3 only) and on day 15 (all cycles).

Plasma and urine pharmacokinetic sampling and assay

In cycle 1, blood samples (3 mL) were collected in heparinized tubes immediately preinfusion and at 30 minutes after the beginning of infusion, just before the EOI, and then 30 minutes, 1, 2, 3, 5, 7, 9, and 23 hours after the EOI on day 1 and day 5, and preinfusion on days 2 to 4, for the measurement of plasma concentrations of CUDC-101 and its metabolite, CUDC-101 Met-M1. Each blood sample was collected in a prelabeled lithium heparinized tube that was immediately placed on ice until centrifugation. Tubes were centrifuged for 10 minutes at approximately 1,000 × g at 0°C to 5°C within 1 hour of collection. Each plasma sample was transferred into duplicate prelabeled screw-capped polypropylene tubes and kept frozen at −70°C until analysis. Urine was collected from the time of initial dosing on day 1 until just before dosing on day 2 (0- to 24-hour urine sample). The total urine volume was recorded and then a 10 mL aliquot was stored at −70°C for later analysis. At the completion of the PK sampling, the plasma and urine samples were analyzed by LC/MS-MS. Standard noncompartmental methods was utilized to determine the PK parameters of CUDC-101 and CUDC-101 Met-M1. The plasma concentration data were processed using WinNonlin software.

Pharmacodynamic analyses

Paired skin biopsies were obtained from patients at baseline and 30 minutes after the fifth dose for the measurement of acetylated histone H3 levels, a biomarker of HDAC inhibition. The skin biopsies were placed in 10% neutral buffered formalin within 30 minutes of collection, and incubated for 24 hours. Immunohistochemistry (IHC) staining was carried out on 5-μm sections of paraffin-embedded tissue. Antigen retrieval was performed by incubating slides in Target Retrieval Solution (Dako S1699) at 125°C for 30 seconds followed by 90°C incubation for 10 seconds. The antibodies used were anti-acetyl-histone H3 (CST 9671), and Dako EnVision+ System-HRP Labeled Polymer anti-Rabbit (Dako K4002). IHC quantitation used 2 fields of view and evaluated 200 cells per sample. The Pannoramic NuclearQuant IHC quantification software was also used for digital pathological quantitation, evaluating more than 500 cells from 2 selected areas on each slide. Histology score (H-score) was obtained from both quantification methods. H-score is a method of assessing

the extent of nuclear immunoreactivity, applicable to steroid receptors. The score was obtained by the formula: 3 × percentage of strongly staining nuclei + 2 × percentage of moderately staining nuclei + percentage of weakly staining nuclei, giving a range of 0 to 300. IHC staining with EGFR, phosphorylated EGFR, HER2, and phosphorylated HER2 were conducted by Source BioScience Limited. The antibodies used were anti-EGFR (NovoCastra, NCL-EGFR), anti-pEGFR (CST 2234 for Tyr1068 and Millipore 05-483 for Tyr1173), anti-HER2 (Dako A0485), and anti-pHER2 (CST 2243). PBMC frozen pellets were extracted and analyzed by Source BioScience Limited.

Results

Study population

Twenty-five patients were treated in 1 of 5 dose cohorts between August 2008 and April 2010. Demographic characteristics, prior treatment history, and cancer types for the study participants are provided in Table 1. The study population included 11 males and 14 females with a median age of 60 years (range, 37–79). Performance status ranged from ECOG 0 (4 patients, 16%) to ECOG 1 (21 patients, 84%). Eligible patients had stage IV solid tumors for which they had received at least 1 prior treatment. The most common tumor types were breast ($n = 6$; 24%), head and neck ($n = 4$; 16%), and lung ($n = 4$; 16%). The total number of patients and median number of doses administered at each dose level, as well as the overall dose escalation scheme, are depicted in Table 2. The median number of doses administered per patient was 14 (range, 1–34).

Table 1. Patient characteristics

Characteristic	Number of patients
Number of patients	25
Median age (range)	60 (37–79)
Performance status	
0	4
1	21
Males/females	11/14
Prior chemotherapy	25
Prior radiation therapy	16
Median number of prior therapies (range)	3 (1–11)
Tumor types	
Breast	6
Head and neck	4
Lung (NSCLC)	4
Esophageal	2
Colorectal	2
Pancreatic	2
Gastric, small cell lung, renal, mesothelioma, Mullerian tumor	1 each

Patients discontinued treatment for the following reasons: disease progression ($n = 16$), adverse event unrelated to study treatment ($n = 2$), physician/sponsor decision ($n = 3$), treatment-related adverse event [$n = 3$; pericarditis and pericardial effusion ($n = 1$), increased serum creatinine ($n = 2$)], and other ($n = 1$).

Safety

All 25 patients were included in the safety evaluation. Table 3 lists the most common treatment-related adverse events (≥2 patients) by cohort and severity. The patients were administered the study drug by i.v. infusion over 1 hour on Days 1 to 5 of each treatment cycle. Total treatment cycle duration was 14 days. Four patients were enrolled at the starting dose level of 75 mg/m^2, including 1 patient who discontinued early because of progressive disease and was replaced. No DLTs were observed in the starting 75-mg/m^2 or the 150-mg/m^2 dose cohorts. Two patients enrolled in the 300-mg/m^2 dose cohort experienced DLTs (grade 3 pericarditis, grade 2 serum creatinine elevation for each patient). The next lower dose cohort (150 mg/m^2) was expanded and intermediate dose levels of 225 and 275 mg/m^2 were selected for further evaluation. Four additional patients were enrolled at 150 mg/m^2 without DLT, including 1 patient who withdrew and was replaced. Intermediate dose levels at 225 and 275 mg/m^2 were explored without DLTs (4 and 3 patients/cohort, respectively). Following this, (after IRB approval) the 300-mg/m^2 dose level was again re-explored. Two additional patients were enrolled and treated at this dose level; however, 1 experienced dose-limiting increased serum creatinine, thus confirming that the MTD had been exceeded. The 275-mg/m^2 dose level was then expanded and 1 of 6 patients experienced dose limiting increased serum creatinine. The 275-mg/m^2 dose level was thus determined to be the MTD for CUDC-101 when administered on the indicated dosing schedule. Two patients discontinued because of adverse events that were considered definitely related to study treatment: 1 patient in the 225-mg/m^2 cohort (adverse event of grade 2 vomiting) and 1 patient in the 300-mg/m^2 cohort (adverse event of grade 2 increased serum creatinine). One patient in the 300-mg/m^2 cohort discontinued treatment because of a serious adverse event (also a DLT) of grade 4 pericarditis, which was considered possibly related to study treatment.

Nonhematologic toxicities

The principal toxicities experienced on CUDC-101 treatment were transient reversible nausea (24%), fatigue (24%), dry skin (16%), serum creatinine elevation (12%), and serum AST elevation (12%). The majority of these events were grade 1 or 2 in severity. Reversible elevation in serum creatinine levels was considered to be a DLT and occurred only at the 2 highest dose levels (275 and 300 mg/m^2). The onset of the drug-related elevations in serum creatinine occurred within 2 to 3 days of CUDC-101 infusions and recovered by day 8 with appropriate

Table 2. Dose escalation scheme

Dose (mg/m^2)	Number of patients: Enrolled	Number of patients: Dose reduced[a]	Median number doses (range)	Patients with DLT (first 2 cycles)
75	4	0	17 (9–20)	0/4
150	7	0	13 (1–28)	0/7
225	4	0	13.5 (13–20)	0/4
275	6	1	19 (7–34)	1/6[b]
300	4	1	3.5 (1–8)	3/4[c]
Total	25	2	14 (1–34)	4

NOTE: The MTD was determined to be 275 mg/m^2. A total of 4 patients experienced a DLT, including grade 2 blood creatinine increased in 3 patients and grade 4 pericarditis in 1 patient. Dose-limiting creatinine increases occurred within 24 hours following the first dose of CUDC-101, were transient and did not seem to worsen with continued study treatment, and were managed by dose delay and reduction for 2 patients. One patient was discontinued from the study after dose-limiting creatinine increase.

[a]Patient whose dose was reduced to the next lowest dose for toxicity.

[b]One patient with grade 2 blood creatinine increased.

[c]Two patients with grade 2 blood creatinine increased; 1 patient with grade 4 pericarditis.

hydration in all patients. ECG recordings were obtained before each CUDC-101 infusion and again on day 8 of each cycle. More intensive ECG monitoring was performed on day 1 and day 5 of cycle 1, which included assessments predose and 1 and 4 hours after starting the infusion. The ECGs were evaluated by each investigator and the corrected QT interval (QTc) was reported. Overall, 7 patients (28%) experienced a QTc increase >30 ms, 3 patients (12%) had a QTc increase >60 ms, and 2 patients (8%) had QTc >500 ms. Increases >60 ms from baseline in mean QTc were seen in the 275- and 300-mg/m^2 cohorts. These increases were most prominent at the 1- and 4-hour postinfusion time points. Although a dose-dependent trend was observed in the number of patients with increased QTc interval, no PK correlation was noted and no clinically significant ECG findings or ECG related adverse events were reported.

Hematologic toxicity

Patients treated with CUDC-101 did not experience clinically significant changes in ANC or platelet counts and there were no hematologic adverse events of greater than grade 2 severity.

Pharmacokinetics

Mean plasma pharmacokinetic (PK) parameters for CUDC-101 from each dose cohort as well as day 1 and day 5 area under curve (AUC) and maximum concentration (C_{max}) values for each patient are shown in Table 4 and Fig. 1, respectively. CUDC-101 exhibited a linear PK and there was a dose-proportional increase in CUDC-101 exposure across the dose range examined (75–300 mg/m^2). CUDC-101 systemic CL was low with a high volume of distribution, which may be interpreted as a high distribution of drug into tissues from the central compartment.

Table 3. Most common treatment-related adverse events (≥2 patients)

Adverse event/ CTCAE grade		CUDC-101–related adverse events (*n*, %): 75 mg/m^2 (*N* = 4)	150 mg/m^2 (*N* = 7)	225 mg/m^2 (*N* = 4)	275 mg/m^2 (*N* = 6)	300 mg/m^2 (*N* = 4)	Overall (*N* = 25)	Any relation (*N* = 25)
Fatigue	Any	0 (0)	2 (29)	1 (25)	0 (0)	1 (25)	4 (16)	6 (24)
	Grade 3–4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Creatinine	Any	0 (0)	0 (0)	0 (0)	1 (17)	2 (50)	3 (12)	3 (12)
	Grade 3–4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Dry skin	Any	3 (75)	0 (0)	0 (0)	0 (0)	0 (0)	3 (12)	4 (16)
	Grade 3–4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
AST incr.	Any	1 (25)	1 (14)	0 (0)	0 (0)	0 (0)	2 (8)	3 (12)
	Grade 3–4	1 (25)	1 (14)	0 (0)	0 (0)	0 (0)	2 (8)	3 (12)
Nausea	Any	1 (25)	0 (0)	1 (25)	0 (0)	0 (0)	2 (8)	6 (24)
	Grade 3–4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)

Table 4. Mean (SD) and median noncompartmental PK parameters of CUDC-101 cycle 1

Dose (mg/m^2)	Variable[a]	C_{max} (mg/L)	T_{max} (h)	AUC_{0-t}[b] (mg·h/L)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (mg·h/L)	CL (L/h)	Vd_{ss} (L)
75	*N*	4	4	4	3	3	3	3
	Mean/median	2.39	0.75	2.37	2.9	2.57	50.4	27.9
	%CV/range	26	0.5–1	37	23	40.3	47.1	12.2
150	*N*	7	7	7	7	7	7	7
	Mean/median	5.04	0.60	5.42	5.6	5.46	49.9	44.5
	%CV/range	31	0.5–1	36	52	36	49	25
225	*N*	4	4	4	4	4	4	4
	Mean/median	9.21	0.50	9.26	5.2	9.27	44.9	27.3
	%CV/range	28	0.5–1.1	35	23	34.8	39.3	34.3
275	*N*	6	6	6	6	6	6	6
	Mean/median	9.23	0.50	9.95	4.4	9.99	51.2	39.6
	%CV/range	18	0.5–1	27	53	27.17	29.51	42.00
300	*N*	4	4	4	4	4	4	4
	Mean/median	8.83	1.00	10.5	6.3	10.6	55.4	51.2
	%CV/range	11	0.5–1	16	24	16	12	9.2

Abbreviation: T_{max}, time to maximum concentration.

[a]Geometric mean and coefficient of variation (% CV) are presented for all PK parameters with the exception of T_{max} for which the median and range are reported.

[b]AUC_{0-t} was calculated as area under the curve to last time point with a measurable concentration.

Consistent with its relatively short half-life (day 1: 2.9–6.3 hours; day 5: 5.4–8.1 hours), there seemed to be no accumulation of CUDC-101, as shown by the mean day 5/day 1 C_{max} (0.75–0.99) and AUC ratio (0.83–1.02). The volume of distribution at steady-state (Vd_{ss}) was very large with an average value of 39.6 L at the MTD reflecting significant tissue distribution. At the MTD, the CL averaged 51.2 L/h and the mean $t_{1/2,\beta}$ was 4.4 hours. Plasma exposure for CUDC-101 Met-M1 was slightly more than dose proportional, with minor accumulation observed following 5 days of dosing. CUDC-101 Met-M1 C_{max} and AUC ranged from 4 to 7 mg/mL and 4 to 7 mg·h/mL, respectively. The mean $t_{1/2}$ ranged from 2.9 to 6.3 hours, with a mean value of 4.4 hours for patients receiving the 275 mg/m^2 MTD dose. Urinary excretion of CUDC-101 and CUDC-101Met-M1 metabolite was assessed in a 24-hour urine collection following the first CUDC-101 administration (C1D1). The total amount of CUDC-101 recovered increased linearly with dose, ranging from 0.65 to 3.93 mg and comprising <1% of the total administered dose. Total urine excretion of the CUDC-101 Met-M1 metabolite was also minimal, ranging from a mean of 0.09 to 0.9 mg across the dose cohorts.

Pharmacodynamics

Skin biopsies were collected from patients for analysis of acetylated histone H3 levels as a pharmacodynamics

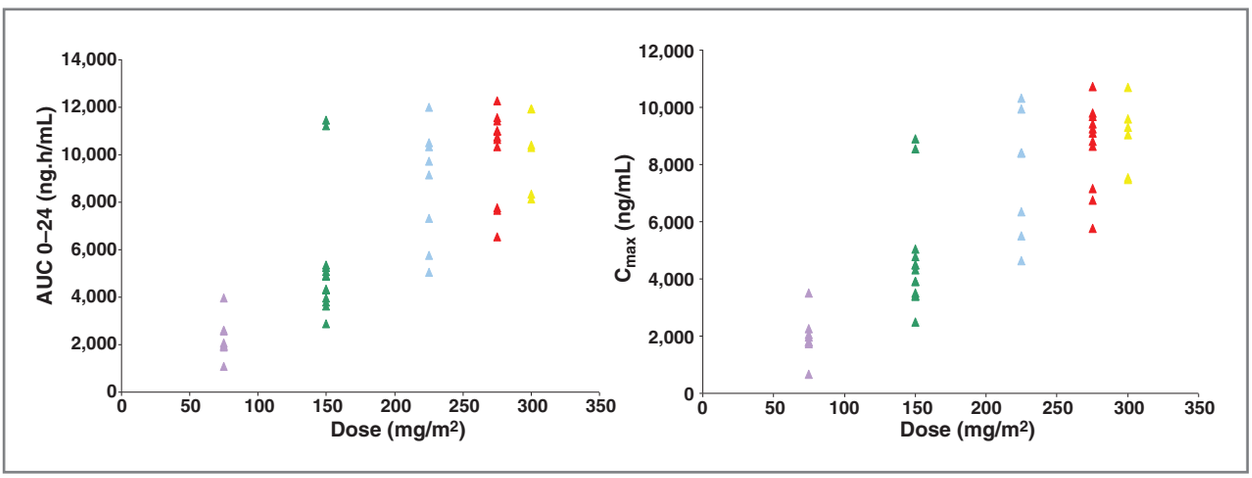

Figure 1. Individual $AUC_{(0-24h)}$ versus dose (left) and individual C_{max} vs. dose (right). Data presented include both day 1 and day 5 AUC and C_{max} values for each individual subject. No significant difference was observed between day 1 and day 5 values.

marker of drug activity. Accumulation of histone H3 acetylation was evidenced in the posttreatment skin samples from all 3 patients in the 275-mg/m^2 cohort (Fig. 2A). H-score analysis was used to assess CUDC-101 induced acetylated histone H3 accumulation in the epithelial compartment by pathologist reading (Fig. 2B) and the Pannoramic NuclearQuant IHC quantification software (Fig. 2C). Significant accumulation of acetylated histone H3 was demonstrated by pathologist quantification ($P = 0.02$) and digital pathology analysis ($P = 0.03$). Elevated nuclear acetylated histone H3 staining was also observed in other skin cells such as endothelial cells and fibroblasts (Fig. 2A). These results indicate that CUDC-101 treatment inhibited HDAC activity in the skin. No obvious dose-related changes were observed in epidermal HER2, phosphorylated HER2, and EGFR protein levels. Analysis of phosphorylated EGFR in skin biopsy samples, as well as all planned analyses of PBMC samples, could not be completed because of technical difficulties.

Efficacy

A total of 15 patients were evaluable for efficacy. One patient with recurrent gastric cancer and measurable abdominal wall metastasis at baseline treated in the 275-mg/m^2 cohort had a confirmed partial response by RECIST lasting 57 days. The target lesion decreased by 56% following 4 cycles of treatment and was sustained at cycle 6. Antitumor activity was also observed in 2 patients with head and neck cancer with radiologic regression >20% in the target lesion (primary lesion of jaw) at the 275-mg/m^2 dose level and mixed response with a reduction in the size of one target lesion (mediastinal lymph node metastasis) at the 150-mg/m^2 dose level. Six patients had a best overall response of stable disease with a mean duration of 48.7 days

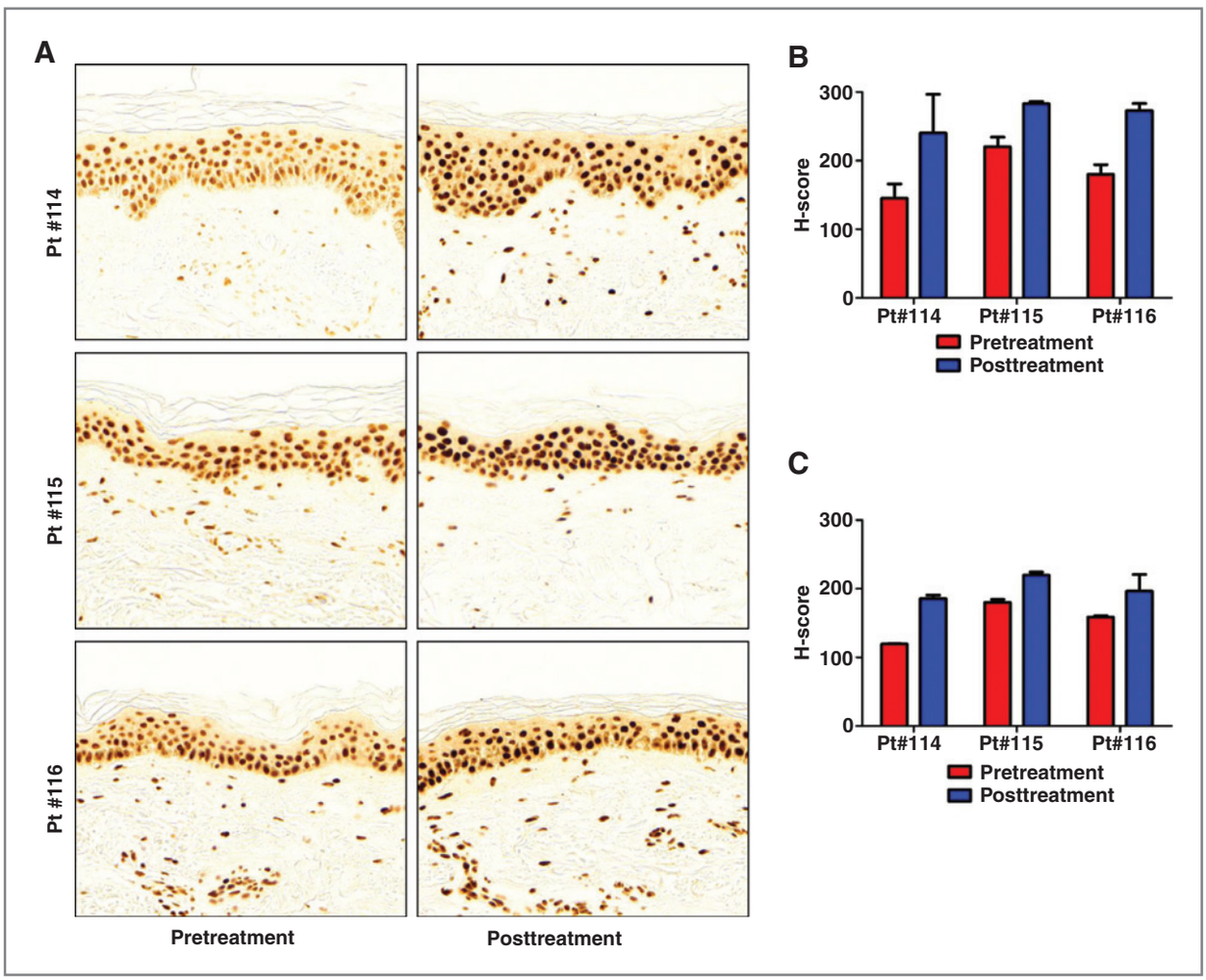

Figure 2. CUDC-101 induces the accumulation of acetylated histone H3 in skin biopsies in the 275-mg/m^2 cohort. A, acetylated histone H3 IHC staining of skin biopsy at baseline and half an hour after the fifth doses of CUDC-101 treatment. B and C, H-score quantification of acetylated histone H3 IHC staining by pathologist (B, $P = 0.02$) and by the Pannoramic NuclearQuant IHC quantification software (C, $P = 0.03$).

(median 44.0 days; range, 28.0–81.0 days). Of these, 1 patient with refractory HER2 overexpressing breast cancer that progressed on prior trastuzumab therapy treated at 150 mg/m^2 dose experienced radiographic stable disease of >12 weeks. Eight patients experienced progressive disease.

Discussion

CUDC-101 represents a first-in-class small-molecule multitargeted inhibitor of both RTKs and HDAC enzymes. The impetus for the clinical development of CUDC-101 is based on the critical role that HDAC and RTK inhibitors play as cancer treatments, as well as the synergistic anticancer activity these inhibitors display when combined in preclinical setting. CUDC-101 displays potent antiproliferative and proapoptotic activity in *in vitro* and *in vivo* drug-resistant tumor models, including erlotinib-sensitive and resistant NSCLC cell lines as well as lapatinib-sensitive (HER2 positive) and resistant (HER2 negative) breast cancer models (18–20). Mechanistic studies have shown that CUDC-101 not only directly inhibits both EGFR and HER2 signaling but also indirectly attenuates signaling mediated by the HER3, MET, AXL, and AKT (18–20). CUDC-101 is an example of the strategy for simultaneous inhibition of multiple, biochemically distinct molecular targets that may address resistance mechanisms encountered by single-targeted therapeutics. Preclinical studies have shown that HDAC inhibition can induce sensitivity to EGFR inhibition (14–16). These studies also indicate that the combination of EGFR inhibition with HDAC inhibitors may benefit patients with cancer not expected to respond to EGFR-directed therapy.

This phase I study was performed to evaluate the safety and tolerability along with the PK profile of CUDC-101 when administered intravenously. Dose levels up to 275 mg/m^2 were well tolerated with the most frequent adverse events being dry skin/rash, nausea, fatigue, constipation, dyspnea, and pyrexia. The type and frequency of adverse events were comparable with those previously reported with administration of erlotinib or vorinostat. Four DLTs were reported in the study, 1 at 275 mg/m^2 and 3 at 300 mg/m^2 dose levels. These DLTs included grade 2 serum creatinine elevation ($n = 3$), and pericarditis and pericardial effusion in 1 patient. These events occurred within 24 hours following the first dose of CUDC-101, were transient, did not seem to worsen with continued study treatment, and were managed in 2 patients by dose delay and reduction. There was no indication of acute or cumulative creatinine increases in other study patients. The etiology and clinical significance of these increased serum creatinine levels has not been determined. Dose limiting increase in creatinine levels has been a common laboratory adverse event reported with single-agent HDAC inhibitors (21). No significant changes in serum chemistry parameters, including serum creatinine, were noted in preclinical toxicology studies. In dog toxicology studies, the kidney was identified as a potential target organ (vacuolation of the proximal tubule epithelium). These findings occurred at 40- and 80-mg/kg/day dose levels and were dose related in incidence and severity. Other than pericarditis observed in a single subject at the 300-mg/m^2 dose level, no clinically significant, treatment-related cardiac events, ECG changes, or MUGA abnormalities were observed on this study. Analysis of pre– and post–CUDC-101 treatment skin biopsy samples were available from 16 patients and were analyzed by IHC for biomarkers of target modulation. Acetylated histone H3 induction was observed in posttreatment skin samples from all 3 patients in the 275-mg/m^2 dose cohort. No obvious dose-related changes were observed in EGFR, HER2, or phosphorylated HER2 status, and the phosphorylated EGFR in skin biopsy samples was not detachable due to technical difficulty. Phosphorylated HER2 positivity in the skin samples was noticeably cytoplasmic in the epidermis without obvious membrane staining, indicating potential issues in sample preservation or IHC staining specificity. Therefore, the EGFR and HER2 inhibition is skin remain inconclusive. Even through PBMC is proved to be a suitable surrogate tissue for evaluating HDAC inhibition activities, identification of acetylated histone H3 signal in PBMC samples was failed because of technical issues. However, the acetylated histone H3 induction in skin samples demonstrated HDAC inhibition and suggests adequate systemic exposure and target inhibition. Promising antitumor activity was seen in various cancer types, including one confirmed partial response in a patient with gastric cancer, stable disease with radiologic regression of >20% in the target lesions in a patient with head and neck cancer and stable disease lasting for more than 3 months in a patient with refractory HER2 overexpressing breast cancer that progressed on prior trastuzumab therapy.

In conclusion, CUDC-101 can be safely administered to patients with advanced solid tumors at doses up to 275 mg/m^2 i.v. daily for 5 consecutive days repeated every 2 weeks and has shown promising single-agent activity. Moreover, using skin as a surrogate tissue, PD analysis has further confirmed that CUDC-101 effectively inhibited HDAC activity at the 275-mg/m^2 dose level. Therefore, continued clinical development of CUDC-101 is supported by early evidence of antitumor and PD activity observed in this trial. A phase Ib expansion study investigating the safety, efficacy, and PKs of intravenous CUDC-101 in patients with advanced head and neck, gastric, breast, liver and NSCLC tumors (NCT01171924) and a phase I study of orally administered CUDC-101 to evaluate its bioavailability have been conducted (NCT01702285).

Disclosure of Potential Conflicts of Interest

T.A. Mays reports receiving speakers bureau honoraria from Genentech. J. Wang and R. Laliberte are employees of Curis Inc. M. Voi is an employee of Novartis. A.W. Tolcher is consultant/advisory board member for AbbVie, Adnexus, Ambit, AP Pharma, Aragon, Ariad, ArQule, Astellas, Astellas Japan, Astex, Bayer, Bind, BioMed Valley Discoveries, Blend Therapeutics, Bristol-Myers-Squibb Japan, Celator, Clovis, Curis, Daiichi Sankyo, Dicerna, Eisai, Emergent Product Development, Five Prime, Galapagos, Janssen R&D, Eli Lilly, MedImmune, Merck Sharp & Dohme, Merus, Micromet, Nanobiotix, Nektar, Neumedicines, Nexus, Novartis, OncoGenex, Onyx, Otsuka, Pfizer, Pharmacyclics, Pierre Fabre, ProNai, Proximagen, Sanofi-Aventis, Santaris,

Symphogen, Vaccinex, and Ztyngenia, and reports receiving fees for the above-mentioned associations through his company, South Texas Accelerated Research Therapeutics, for which he is a co-owner. No other potential conflicts of interest were disclosed.

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