Phase I first-in-human study of CUDC-101, a multi-targeted inhibitor of HDACs, EGFR and HER2 in patients with advanced solid tumors

Toshio Shimizu,1 Patricia LoRusso,2 Kyri Papadopoulos,1 Amita Patnaik,1 Muralidhar Beeram,3 Lon S. Smith,3 Drew W. Rasco,1 Theresa A. Mays,1 Glenda Chambers,1 Anna Ma,4 Jing Wang,4 Robert Laliberte,4 Maurizio Voi,4 Anthony W. Tolcher1

1 START (South Texas Accelerated Research Therapeutics),
San Antonio TX, 78229, U.S.A

2 Karmanos Cancer Institute, Wayne State University,
Detroit MI, 48201, U.S.A

3 STOH (South Texas Oncology Hematology),
San Antonio TX, 78229, U.S.A

4 Curis, Inc.,
Lexington, MA, 02421, U.S.A

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Address for correspondence:
Anthony W. Tolcher, M.D., FRCPC.
START (South Texas Accelerated Research Therapeutics),
San Antonio TX, 78229, U.S.A
Phone: (210)-593-5255
Fax: (210)-615-1121
Email: atolcher@start.stoh.com

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Translational Relevance

CU Denver-101 represents a first in class small-molecule multi-targeted inhibitor of both receptor tyrosine kinases (RTKs) and class I/II Histone Deacetylase enzymes (HDAC enzymes). The impetus for the clinical development of CU Denver-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, CU Denver-101 can be safely administered to patients with advanced solid tumors at doses up to 275 mg/m² IV 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 CU Denver-101 effectively inhibited HDAC activity at the 275 mg/m² dose level. Continued clinical development of CU Denver-101 is supported by early evidence of anti-tumor and PD activity observed in this early phase clinical study.
Abstract

**Purpose:** This first-in-human phase I study evaluated dose-limiting toxicities (DLTs) and defined a phase II recommended dose (RD) for CUDC-101, a multi-targeted inhibitor of HDACs, EGFR and HER2 as a 1-hour intravenous (i.v.) infusion for 5 consecutive days every 2 weeks (q2wk).

**Experimental Design:** Twenty-five patients with advanced solid tumors received escalating doses of CUDC-101 (range: 75 to 300 mg/m²/day) following a standard 3+3 dose escalation design.

**Results:** The MTD was determined to be 275 mg/m². Common grade 1/2 adverse events included nausea, fatigue, vomiting, dyspnea, pyrexia and dry skin. DLTs occurred in 1 patient in the 275 mg/m² dose cohort (grade 2 serum creatinine elevation, n = 1) and 3 patients in the 300 mg/m² 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 (Cmax), clearance (CL), volume of distribution at steady-state (Vdss), area-under-the-curve (AUC) and terminal elimination half-life (t1/2) at the MTD dose of 9.3 mg/L, 51.2 L/h, 39.6 L, 9.95 hr*ng/mL and 4.4 hours, respectively. Acetylated histone H3 induction was observed in post-treatment skin samples from 3 patients in the 275 mg/m² 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 q2wk was generally well tolerated with preliminary evidence of antitumor activity. A dose of 275 mg/m² is recommended for further clinical testing.
Introduction

Multi-targeted 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, due to 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 non-histone 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 anti-tumor 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 potential benefit of combining HDAC and RTK inhibitors for treatment of cancer patients. Regarding the rationale for increased sensitivity to HER2 inhibition by co-treatment 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 co-treatment with LAQ824 and trastuzumab (12).

CUDC-101 (7-(4-(3-ethynylphenylamino)-7-methoxyquinoxalin-6-yloxy) N-hydroxyheptanamide) 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 4nM. 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: 1) inhibition of HDAC activity (histone acetylation), 2) inhibition of EGFR and HER2 phosphorylation, 3) inhibition of tumor cell proliferation (decrease of Ki67 levels), and 4) induction of apoptosis (Caspase 3 induction) (18). The therapeutic efficacy in xenograft models is likely due to the improved potency of the kinase inhibitory activities and the synergy achieved by the combined RTK and HDAC inhibitory activity within cancer cells. Additionally, CUDC-101 and single-targeted HDAC inhibitors reduce HIF-1α 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, multi-center 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 IV infusion on 5 consecutive days every 14 days; to assess the safety and tolerability, the PK profile, PD measurements, and preliminary evidence of anti-tumor activity in patients with advanced solid malignancies.
Materials and Methods

Patients 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 0-2; previous therapy discontinued ≥4 weeks prior to study treatment; absolute neutrophil count (ANC) ≥1500/L; platelets ≥100,000/L; creatinine ≤1.5x upper limit of normal (ULN) or calculated creatinine clearance ≥60mL/min/1.73m²; total bilirubin ≤1.5x ULN; aspartate aminotransferase (AST) / alanine aminotransferase (ALT) ≤2.5x ULN or ≤5x ULN if documented liver metastases present; prothrombin time ≤1.5x 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 ≤10mg 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 the principles of the Declaration of Helsinki and the 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. Prior to treatment, a solution was prepared by reconstitution with sterile water to a concentration of 30mg/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 IV
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/m²/day, which was 1/6th of the highest non-significantly 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 DLT in the first two 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 two or more patients experience a DLT in the first two cycles (28 days). DLT was defined as any grade 3 or 4 non-hematological 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/uL of any duration or < 50,000/uL 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 (v 3.0).

**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, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, albumin, total protein, total bilirubin, electrocardiogram (ECG), and relevant radiologic studies were performed prior to treatment. During the study, radiologic analysis of disease status was repeated every four 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 pre-dose 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 pre-treatment prior to C1D1 and at C1D5 after the EOI for evaluating the HER2 and EGFR phosphorylation. Vital signs were monitored pre-dose, 30 min, 1 hour and 4 hours post-infusion initiation C1D1 only, and pre-dose and post-dose at all visits. Electrocardiogram assessments were performed at pre-dose, 1 hour and 4 hours post-infusion initiation on C1D1 and C1D5 only, and only pre-dose at all other visits. A pre-study 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 1-5 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 pre-
infusion and at 30 minutes after the beginning of infusion, just prior to the end of infusion (EOI), and then 30 minutes, 1, 2, 3, 5, 7, 9, 23 hours after the EOI on Day 1 and Day 5, and pre-infusion on days 2-4, for the measurement of plasma concentrations of CUDC-101 and its metabolite, CUDC-101 Met-M1. Each blood sample was collected in a pre-labeled lithium heparinized tube that was immediately placed on ice until centrifugation. Tubes were centrifuged for 10 minutes at approximately 1000 x g at 0 to 5°C within 1 hour of collection. Each plasma sample was transferred into duplicate pre-labeled 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 (0-24 hr 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 non-compartmental methods was utilized to determine the PK parameters of CUDC-101 and CUDC-101 Met-M1. The plasma concentration data was processed using WinNonlin software.

Pharmacodynamic Analyses

Paired skin biopsies were obtained from patients at baseline and 30 minutes post the 5th 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 employed 2 fields of view and evaluated 200
cells per sample. The Pannoramic NuclearQuant IHC quantification software was also used for digital pathological quantitation, evaluating over 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 x percentage of strongly staining nuclei + 2 x 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 (UK) 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 (UK) 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). 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 IV 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/m², including 1 patient who discontinued early due to progressive disease and was replaced. No DLTs were observed in the starting 75 mg/m² or the 150 mg/m² dose cohorts. Two patients enrolled in the 300 mg/m² dose cohort experienced DLTs (Grade 3 pericarditis, Grade 2 serum creatinine elevation for each
The next lower dose cohort (150 mg/m²) was expanded and intermediate dose levels of 225 and 275 mg/m² were selected for further evaluation. Four additional patients were enrolled at 150 mg/m² without DLT, including 1 patient who withdrew and was replaced. Intermediate dose levels at 225 and 275 mg/m² were explored without DLTs (4 and 3 patients/cohort, respectively). Following this, (after IRB approval) the 300 mg/m² 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/m² dose level was then expanded and one of six patients experienced dose limiting increased serum creatinine. The 275 mg/m² dose level was thus determined to be the MTD for CUDC-101 when administered on the indicated dosing schedule. Two patients discontinued due to AEs that were considered definitely related to study treatment: 1 patient in the 225 mg/m² cohort (AE of Grade 2 vomiting) and 1 patient in the 300 mg/m² cohort (AE of Grade 2 increased serum creatinine). One patient in the 300 mg/m² cohort discontinued treatment due to a serious AE (also a DLT) of Grade 4 pericarditis, which was considered possibly related to study treatment.

Non-Hematologic 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 two highest dose levels (275 mg/m² and 300 mg/m²). The onset of the drug-related elevations in serum creatinine occurred within 2-3 days of CUDC-101 infusions and recovered by day 8 with appropriate hydration in all patients. Electrocardiogram (ECG) recordings were obtained prior to 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 pre-dose 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 msec, 3 patients (12%) had a QTc increase >60 msec, and 2 patients (8%) had QTc >500 msec. Increases >60 msec from baseline in mean QTc were seen in the 275 and 300 mg/m² cohorts. These increases were most prominent at the 1-hour and 4-hour post-infusion 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 AEs 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 AEs 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 AUC and $C_{max}$ values for each patient are shown in Table 4 and Figure 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/m²). CUDC-101 systemic clearance was low with a high volume of distribution, which may be interpreted as a high distribution of drug into tissues from the central compartment. Consistent with its relatively short half-life (Day 1: 2.9 – 6.3 hours; Day 5: 5.4 – 8.1 hours), there appeared 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 $V_{dss}$ 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 $t_{1/2,β}$ 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/m² 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 marker of drug activity. Accumulation of histone H3 acetylation was evidenced in the post-treatment skin samples from all 3 patients in the 275 mg/m² cohort (Figure 2A). H-score analysis was used to assess CUDC-101 induced acetylated histone H3 accumulation in the epithelial compartment by pathologist reading (Figure 2B) and the Pannoramic NuclearQuant IHC quantification software (Figure 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 (Figure 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 due to 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/m² 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. Anti-tumor activity was also observed in two patients with head and neck cancer with radiologic regression >20% in the target lesion (primary lesion of jaw) at the 275 mg/m² dose level and mixed response with a reduction in the size of one target lesion (mediastinal lymph node metastasis) at the 150 mg/m² dose level. Six patients had a best overall response of stable disease with a mean duration of 48.7 days (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/m² dose experienced radiographic stable disease of > 12 weeks. Eight patients experienced progressive disease.
Discussion

CUDC-101 represents a first in class small-molecule multi-targeted 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 anti-proliferative and pro-apoptotic 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 cancer patients 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 IV. Dose levels up to 275 mg/m² 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, one at 275 mg/ m² and three at 300 mg/ m² dose levels. These DLTs included Grade 2 serum creatinine elevation (n=3), and pericarditis and pericardial effusion in one patient. These events occurred within 24
hours following the first dose of CUDC-101, were transient, did not appear 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 (Pharmaceuticals 2010; 3:2751-67). 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/m2 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 post-treatment skin samples from all 3 patients in the 275 mg/m2 dose cohort. No obvious dose-related changes were observed in EGFR, HER2, or phosphorylated-HER2 status, while as the phosphorylated-EGFR in skin biopsy samples were 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 proven to be a suitable surrogate tissue for evaluating HDAC inhibition activities, identification of acetylated histone H3 signal in PBMC samples was failed due to 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/m² IV 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/m² dose level. Therefore, continued clinical development of CUDC-101 is supported by early evidence of anti-tumor and PD activity observed in this trial. A phase Ib expansion study investigating the safety, efficacy, and pharmacokinetics of IV CUDC-101 in patients with advanced head and neck, gastric, breast, liver and non-small cell lung cancer tumors (NCT01171924) and a phase I study of orally administered CUDC-101 to evaluate its bioavailability have been conducted (NCT01702285).
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Disclosure of Potential Conflicts of Interests

None of the authors have any conflict of interest relevant to the subject of this manuscript.

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Authors’ Contributions

Conception and design: Toshio Shimizu, Patricia LoRusso and Anthony W. Tolcher

Provision of study materials or patients: Patricia LoRusso, Amita Patnaik, Muralidhar Beeram, Kyriakos P. Papadopoulos, Lon S. Smith and Anthony W. Tolcher

Collection and assembly of data: Toshio Shimizu, Patricia LoRusso, Anna Ma, Jing Wang, Robert Laliberte, Maurizio Voi and Anthony W. Tolcher

Data analysis and interpretation: Toshio Shimizu, Patricia LoRusso, Anna Ma, Jing Wang, Robert Laliberte, Maurizio Voi and Anthony W. Tolcher

Manuscript writing: All authors

Final approval of manuscript: All authors
**Table 1. Patient characteristics**

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</tr>
<tr>
<td>Gastric, Small Cell Lung, Renal,</td>
<td></td>
</tr>
<tr>
<td>Mesothelioma, Mullerian tumor</td>
<td>1 each</td>
</tr>
<tr>
<td>Dose (mg/m²)</td>
<td>Number of Patients</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------</td>
</tr>
<tr>
<td></td>
<td>Enrolled</td>
</tr>
<tr>
<td>75</td>
<td>4</td>
</tr>
<tr>
<td>150</td>
<td>7</td>
</tr>
<tr>
<td>225</td>
<td>4</td>
</tr>
<tr>
<td>275</td>
<td>6</td>
</tr>
<tr>
<td>300</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>25</td>
</tr>
</tbody>
</table>

DLT; dose-limiting toxicity
* Patient whose dose was reduced to the next lowest dose for toxicity.
** 1 patient with Grade 2 blood creatinine increased.
*** 2 patients with Grade 2 blood creatinine increased; 1 patient with Grade 4 pericarditis.

The MTD was determined to be 275 mg/m². As noted in Table 6.2-1, 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 appear to worsen with continued study treatment, and were managed by dose delay and reduction for 2 patients. One patient was discontinued study after dose limiting creatinine increase.
Table 3. Most Common Treatment-Related Adverse Events (≥2 Patients)

<table>
<thead>
<tr>
<th>Adverse Event / CTCAE Grade</th>
<th>CUDC-101 Related Adverse Events (n, %)</th>
<th>75 mg/m² (N=4)</th>
<th>150 mg/m² (N=7)</th>
<th>225 mg/m² (N=4)</th>
<th>275 mg/m² (N=6)</th>
<th>300 mg/m² (N=4)</th>
<th>Overall (N=25)</th>
<th>Any Relation (N=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>Any</td>
<td>0 (0)</td>
<td>2 (29)</td>
<td>1 (25)</td>
<td>0 (0)</td>
<td>1 (25)</td>
<td>4 (16)</td>
<td>6 (24)</td>
</tr>
<tr>
<td></td>
<td>Grade 3-4</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Any</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (17)</td>
<td>2 (50)</td>
<td>3 (12)</td>
<td>3 (12)</td>
</tr>
<tr>
<td></td>
<td>Grade 3-4</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Dry Skin</td>
<td>Any</td>
<td>3 (75)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (12)</td>
<td>4 (16)</td>
</tr>
<tr>
<td></td>
<td>Grade 3-4</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>AST Incr.</td>
<td>Any</td>
<td>1 (25)</td>
<td>1 (14)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (8)</td>
<td>3 (12)</td>
</tr>
<tr>
<td></td>
<td>Grade 3-4</td>
<td>1 (25)</td>
<td>1 (14)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (8)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Nausea</td>
<td>Any</td>
<td>1 (25)</td>
<td>0 (0)</td>
<td>1 (25)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (8)</td>
<td>6 (24)</td>
</tr>
<tr>
<td></td>
<td>Grade 3-4</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>
Table 4. Mean [SD] and Median Non-Compartmental Pharmacokinetic Parameters of CUDC-101 Cycle 1

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>Variable*</th>
<th>Cmax (mg/L)</th>
<th>Tmax (h)</th>
<th>AUC₀-t** (mg*h/L)</th>
<th>t₁/₂ (h)</th>
<th>AUC₀-∞ (mg*h/L)</th>
<th>Cl (L/h)</th>
<th>Vss (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>N</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Mean/Median</td>
<td>2.39</td>
<td>0.75</td>
<td>2.37</td>
<td>2.9</td>
<td>2.57</td>
<td>50.4</td>
<td>27.9</td>
<td></td>
</tr>
<tr>
<td>%CV/Range</td>
<td>26</td>
<td>0.5-1</td>
<td>37</td>
<td>23</td>
<td>40.3</td>
<td>47.1</td>
<td>12.2</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>N</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Mean/Median</td>
<td>5.04</td>
<td>0.60</td>
<td>5.42</td>
<td>5.6</td>
<td>5.46</td>
<td>49.9</td>
<td>44.5</td>
<td></td>
</tr>
<tr>
<td>%CV/Range</td>
<td>31</td>
<td>0.5-1</td>
<td>36</td>
<td>52</td>
<td>36</td>
<td>49</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>225</td>
<td>N</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Mean/Median</td>
<td>9.21</td>
<td>0.50</td>
<td>9.26</td>
<td>5.2</td>
<td>9.27</td>
<td>44.9</td>
<td>27.3</td>
<td></td>
</tr>
<tr>
<td>%CV/Range</td>
<td>28</td>
<td>0.5-1.1</td>
<td>35</td>
<td>23</td>
<td>34.8</td>
<td>39.3</td>
<td>34.3</td>
<td></td>
</tr>
<tr>
<td>275</td>
<td>N</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Mean/Median</td>
<td>9.23</td>
<td>0.50</td>
<td>9.95</td>
<td>4.4</td>
<td>9.99</td>
<td>51.2</td>
<td>39.6</td>
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<tr>
<td>%CV/Range</td>
<td>18</td>
<td>0.5-1</td>
<td>27</td>
<td>53</td>
<td>27.17</td>
<td>29.51</td>
<td>42.00</td>
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</tr>
<tr>
<td>300</td>
<td>N</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Mean/Median</td>
<td>8.83</td>
<td>1.00</td>
<td>10.5</td>
<td>6.3</td>
<td>10.6</td>
<td>55.4</td>
<td>51.2</td>
<td></td>
</tr>
<tr>
<td>%CV/Range</td>
<td>11</td>
<td>0.5-1</td>
<td>16</td>
<td>24</td>
<td>16</td>
<td>12</td>
<td>9.2</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Cmax: maximum plasma concentration; Tmax: time to maximum concentration; AUC: area under the concentration time curve; t₁/₂: half-life; Cl: clearance; Vss: volume of distribution at steady state

* Geometric mean and coefficient of variation (%CV) are presented for all PK parameters with the exception of Tmax for which the median and range are reported.

** AUC₀-t was calculated as area under the curve to last time point with a measurable concentration.
FIGURE LEGENDS

Figure 1.

Individual $\text{AUC}_{(0-24\text{hr})}$ vs Dose (left) and Individual $C_{\text{max}}$ vs. Dose (right)

Data presented includes both Day 1 and Day 5 AUC and $C_{\text{max}}$ values for each individual subject.

No significant difference was observed between day 1 and day 5 values.

Figure 2.

CU DC-101 induces the accumulation of acetylated histone H3 in skin biopsies in the 275 mg/m² cohort

(A) Acetylated histone H3 immunohistochemistry staining of skin biopsy at baseline and half an hour post the 5th doses of CU DC-101 treatment.

(B and C) H-score quantification of Acetylated histone H3 immunohistochemistry staining by pathologist (B, $p = 0.02$) and by the Pannoramic NuclearQuant IHC quantification software (C, $p = 0.03$).
Figure 1.

Individual $\text{AUC}_{(0-24\text{hr})}$ vs Dose (left) and Individual $C_{\text{max}}$ vs. Dose (right)

Data presented includes both Day 1 and Day 5 AUC and $C_{\text{max}}$ values for each individual subject. No significant difference was observed between day 1 and day 5 values.
Figure 2.

A

Pt #114

Pt #115

Pt #116

Pre-treatment

Post-treatment

B

H-Score

Pre-Treatment

Post-Treatment

C

H-Score

Pre-Treatment

Post-Treatment
Phase I first-in-human study of CUDC-101, a multi-targeted inhibitor of HDACs, EGFR and HER2 in patients with advanced solid tumors

Toshio Shimizu, Patricia M. LoRusso, Kyriakos Papadopoulos, et al.

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