A Phase I Pharmacokinetic and Pharmacodynamic Study of CHR-3996, an Oral Class I Selective Histone Deacetylase Inhibitor in Refractory Solid Tumors

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Abstract

Purpose: This clinical trial investigated the safety, tolerability, pharmacokinetic (PK), and pharmacodynamic (PD) profile of CHR-3996, a selective class I histone deacetylase inhibitor.

Patients and Methods: CHR-3996 was administered orally once a day. This phase I trial used a 3 + 3 dose-escalation design. PK profiles were analyzed by liquid chromatography–tandem mass spectroscopic methods and PD studies were conducted using ELISA studying histone H3 acetylation in peripheral blood mononuclear cells.

Results: Thirty-nine patients were treated at dose levels of 5 mg (n = 3), 10 mg (n = 4), 20 mg (n = 3), 40 mg (n = 10), 80 mg (n = 10), 120 mg (n = 4), and 160 mg (n = 5) administered orally once daily. The dose-limiting toxicities seen were thrombocytopenia (160 mg), fatigue (80 and 120 mg), plasma creatinine elevation (80 and 120 mg), and atrial fibrillation (40 mg). The area under the curve was proportional to the administered dose and a maximal plasma concentration of 259 ng/mL at a dose of 40 mg exceeded the concentrations required for antitumor efficacy in preclinical models. Target inhibition measured by quantification of histone acetylation was shown at doses of 10 mg/d and was maximal at 40 mg. A partial response was seen in one patient with metastatic acinar pancreatic carcinoma.

Conclusions: Taking the toxicity and PK/PD profile into consideration, the recommended phase II dose (RP2D) is 40 mg/d. At this dose, CHR-3996 has a favorable toxicologic, PK, and PD profile. CHR-3996 has shown preliminary clinical activity and should be evaluated in further clinical trials. Clin Cancer Res; 18(9); 2687–94. ©2012 AACR.

Introduction

Epigenetic regulation of gene function controls differentiation, angiogenesis, and apoptosis in cancer cells (1). Amino acid residues on histone tails are acetylated by histone acetyl transferase and deacetylated by histone deacetylase (HDAC), leading to differential access of transcription factors to gene promoter regions (2). HDACs are classified into 4 main categories including class I HDACs (HDAC1, HDAC2, HDAC3, and HDAC8), class II HDACs (HDAC4, HDAC5, HDAC6, HDAC7, HDAC9, and HDAC10), class III HDACs (Sirtuins 1–7), and class IV (HDAC11; refs. 1, 3). Vorinostat is a pan-HDAC inhibitor in clinical practice, and there are a number of pan-HDAC inhibitors in various stages of clinical evaluation (1). To improve the therapeutic window, there are now attempts to develop isoform-specific HDAC inhibitors (4) and romidepsin, a cyclic peptide, is a class I–specific HDAC inhibitor that is now licensed for use in cutaneous T-cell lymphoma (5). However, romidepsin is administered intravenously weekly (6), and efforts were made to try and develop oral class I–specific HDAC inhibitors. CHR-3996 (see structure: Supplementary Fig. S1) is a potent class I–selective HDAC inhibitor which is 3,000- to 7,000-fold more active against HDAC 1, 2, and 3 compared with HDAC 6 in biochemical assays (7). This was corroborated by the absence of tubulin acetylation when cancer cells were exposed to CHR-3996.
Translational Relevance

There are multiple pan-histone deacetylase (HDAC) inhibitors in clinical development, including Vorinostat, which is licensed for use in cutaneous T-cell lymphoma (CTCL). Efforts were made to develop class I–specific HDAC inhibitors to improve toxicity profiles and romidepsin, an intravenous class I HDAC inhibitor administered weekly, is licensed for use in CTCL. CHR-3996 is the first oral class I–specific HDAC inhibitor to be reported; its toxicity profile was favorable and CHR-3996 achieved plasma concentrations that caused growth inhibition in preclinical models, and at the recommended dose, there was consistent evidence of histone H3 acetylation in peripheral blood mononuclear cells, suggestive of target inhibition. A patient with acinar pancreatic cancer achieved a partial response. The oral route of administration, favorable toxicity, and pharmacokinetic/pharmacodynamic profile warrant further clinical evaluation of CHR-3996.

(7). Most hydroxamate HDAC inhibitors such as vorinostat, panobinostat, belinostat, JNJ-26481585, and ITF-2357 are pan-HDAC inhibitors and seem to lack this selectivity when assessed in biochemical or cellular assays (8). CHR-3996 inhibits growth in a wide range of human cell line models and has synergistic effects on growth inhibition when combined with anticancer agents such as erlotinib, decitabine, and tosedostat (7). In vivo, CHR-3996 accumulates in tissue and is active against a range of xenograft models (7). Its favorable preclinical profile led us to investigate this oral, class I–specific HDAC inhibitor in a phase I trial.

Patients and Methods

Eligibility criteria

Patients were eligible if they had advanced or metastatic solid tumors which were refractory to standard therapy, age 18 years or older, an Eastern Cooperative Oncology Group (ECOG) performance score 2 or less, and adequate bone marrow, hepatic, and renal function. The main exclusion criteria were significant cardiovascular disease, a mean QTc interval greater than 450 msec, and inability to give informed consent. Detailed inclusion and exclusion criteria are documented in the Supplementary Data.

Study design

This study was an open-label, standard 3 + 3, dose-escalating, phase I study of oral once daily administration of CHR-3996. Toxicity was assessed using National Cancer Institute Common Toxicity Criteria (NCI-CTC) Version 3 and the occurrence of a dose-limiting toxicity (DLT) was recorded in all patients. Cohorts of 3 to 6 patients were treated at increasing doses to establish the MTD based on DLTs. The MTD was defined as the dose at which less than 33% of patients developed a DLT. All patients signed informed consent, and the protocol was reviewed by institutional ethics and research committees.

Definition of DLT

DLT was defined as any of the following events that was possibly, probably, or definitely related to CHR-3996 and which occurred during the first 28 days of treatment: absolute neutrophil count (ANC) < 0.5 x 10^9/L for ≥ 7 days; ANC < 0.5 x 10^9/L with sepsis; platelets < 25 x 10^9/L; any drug-related nonhematologic grade 3/4 toxicity, except vomiting, nausea, diarrhea, alopecia, myalgia, or arthralgia, unless appropriate medication was administered. In addition, toxicity of any grade preventing the administration of 75% of the proposed dose in the first 28 days was included in the definition of a DLT.

Patient evaluation

Patients were monitored for adverse events (AE) and underwent safety assessments throughout the study, including 28 days after the last dose of CHR-3996. Safety assessments included full physical examination, hematology, coagulation, blood biochemistry, ECG, MUGA, or ECHO scans and urinalysis. Tumor evaluation was conducted at baseline and at the end of every second cycle, or at the end of treatment/discontinuation visit. Response Evaluation Criteria in Solid Tumors (RECIST; ref. 9) were used to evaluate target lesions. Positron emission tomography studies were not carried out.

Pharmacokinetic methods

Pharmacokinetic (PK) samples were collected on days 1 and 28 of the first treatment cycle at predose, 0, 0.5, 1, 2, 4, 6, 8, and 24 hours postdose. CHR-3996 was quantified by validated liquid chromatography–tandem mass spectrometry assays. PK parameter estimation was carried out using a single compartment model (WinNonLin PK Software v5.1; Pharsight). Details of the PK analysis are documented in the Supplementary Data. The r^2 describing dose linearity was calculated using a Pearson’s test.

Pharmacodynamic methods

Histone acetylation in PBMCs. Blood samples were collected from all patients at predose, then 1, 4, and 24 hours postdose on day 1 and predose, 1 and 4 hours postdose on day 28 of cycle 1. Histone H3 acetylation was measured in peripheral blood mononuclear cells (PBMC) by an ELISA at The Institute of Cancer Research, Sutton, UK.

The ELISA assay used 96-well plates (L1.3XA-6; Meso Scale Discovery) coated with a mouse pan-histone monoclonal antibody (MaB3422; Millipore). After addition of the sample, rabbit acetylated H3 polyclonal antibody (06-599; Millipore Watford) was used to bind to acetylated H3 captured by the pan-histone antibody. Electrochemiluminescent MSD SULFO-TAG–labeled secondary detection antibody (ruthenium-labeled goat anti-rabbit IgG, R32AB-1; MSD) was then added. MSD Read Buffer (R92TC-2; MSD) was added and the plate read on an MSD SECTOR 2400 Imager (MSD). Difference in histone H3...
acetylation pre- and posttreatment were compared using Wilcoxon’s rank test.

**Histone acetylation in hair follicles.** Patients enrolled in the study at one of the study centers (The Royal Marsden Hospital) were asked to provide plucked hairs (eyebrows) for an additional measure of histone acetylation before dosing on day 1 and on day 28 (4 hours after dose) in the first cycle of treatment. Histone H3 acetylation in hair follicles was studied by confocal microscopy, and details of the methods of analysis are documented in the Supplementary Data.

**Results**

**Demographics**

Forty patients were enrolled and 39 patients received treatment. Thirty-eight patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and one patient had an ECOG performance status of 2. The median age was 56 years (range: 24–77). A range of cancers was represented in the trial, with the most common being cancers of the upper GI tract (7), lower GI tract (9) pancreas, and hepatobiliary system (6).

**Dose escalation**

CHR-3996 was administered orally once a day. Three patients were treated at the starting dose of 5 mg and the dose was then doubled to 10 mg as no DLTs were seen. There were no DLTs in the first 3 patients at 10 mg, and a fourth patient was dosed within the 10 mg cohort, as they had signed informed consent and it was deemed unethical to require them to wait until the next cohort. The 40-mg cohort was expanded to 6 patients after one DLT was seen; however, no further DLT was noted at that dose and the dose was doubled to 80 mg. No DLTs were seen in 3 patients at 80 mg, and the dose was doubled to 160 mg. A DLT was noted at 160 mg and a cohort expansion of 6 patients was planned. A further DLT was noted at this dose and the cohort enrolment was stopped at 5 patients. The dose was deescalated to an intermediate dose of 120 mg, in which a DLT was noted in the first 3 patients and a cohort expansion of 6 was planned; however, a further DLT was seen and the cohort expansion was stopped at 4 patients. The 80-mg cohort was expanded to 10 patients and 2 DLTs were seen. A further 4 patients were recruited at 40 mg to further characterize tolerability, PK, and PD profiles. Thus, the number of patients entered into the different cohorts was as follows: 5 mg (n = 3), 10 mg (n = 4), 20 mg (n = 3), 40 mg (n = 10), 80 mg (n = 10), 120 mg (n = 4), and 160 mg (n = 5).

**Toxicity**

The DLTs are summarized in Table 1, with all but one DLT occurring above the recommended phase 2 dose (RP2D) of 40 mg. The grade 4 thrombocytopenia at 160 mg was uncomplicated and spontaneously recovered within 4 to 11 days of stopping medication. Two DLTs of CTC grade 3 fatigue (one at 80 mg and the other at 120 mg) were seen. In addition, 2 DLTs of CTC grade 1 and 2 of inability to complete one cycle of treatment due to an increase in plasma creatinine were seen (one at 80 mg and the other at 120 mg) At 40 mg, one patient developed an atrial fibrillation on day 23 which recovered spontaneously and did not recur on rechallenge at 20 mg. This patient had very low levels of plasma CHR-3996 [area under curve (AUC) 22.8 ng.h/mL], making it less likely that the atrial fibrillation was related to CHR-3996, although a relationship to the study drug could not be excluded.

Toxicities that occurred in more than 10% of patients are summarized in Table 2. Nausea was the most common drug-related AE, mostly grade 1–2, with one episode of grade 3 nausea, which was successfully treated with antinemetics. Fatigue (mostly grade 1–2) was reported in 59% of patients and vomiting was noted in 38% of patients and was well controlled with antinemetics. Grade 3–4 fatigue was considered a DLT only if it occurred within the first cycle (28 days) and was thought not to be due to progression of disease. The most frequent biochemical abnormality was increased plasma creatinine, which occurred in 33% of patients; although no patient developed a grade 3–4 creatinine rise, there were 4 cases of a grade 2 rise in creatinine at dose levels of 80 mg and above. At the recommended dose of 40 mg, 3 of 10 patients experienced a grade 1 elevation of creatinine within the first cycle, but treatment was not interrupted as a result of this. The most frequent hematologic toxicities included thrombocytopenia and anemia. Thrombocytopenia was CTCAE grade 3–4 at 120 mg and 160 mg but grade 1–2 at 40 mg. Anemia was also grade 1–2 in the majority of cases and was not higher than grade 1–2 at the recommended dose.

Eighteen of the 39 patients (46%) had ST segment depression in one or more postdose ECGs. Thirteen patients (33%) had altered T-waves in at least one postdose ECG (12 patients with flat, 4 with biphasic, and 5 with inverted T-waves). No cases of chest pain were documented at the time ECG changes were noted. The QT interval was reported, adjusted for heart rate using Fridericia’s formula.

### Table 1. DLTs seen during the trial

<table>
<thead>
<tr>
<th>Dose level (mg/d)</th>
<th>Number of events</th>
<th>CTC grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>1</td>
<td>3</td>
<td>Atrial fibrillation</td>
</tr>
<tr>
<td>80</td>
<td>1</td>
<td>3</td>
<td>Fatigue</td>
</tr>
<tr>
<td>80</td>
<td>1</td>
<td>1</td>
<td>Inability to take 75% of dose within the first 28 days due to rise in plasma creatinine</td>
</tr>
<tr>
<td>120</td>
<td>1</td>
<td>3</td>
<td>Fatigue</td>
</tr>
<tr>
<td>120</td>
<td>1</td>
<td>2</td>
<td>Inability to take 75% of dose within the first 28 days due to rise in plasma creatinine</td>
</tr>
<tr>
<td>160</td>
<td>2</td>
<td>4</td>
<td>Thrombocytopenia</td>
</tr>
</tbody>
</table>
(QTcF). Two patients had a QTcF more than 450 msec. One patient had an ECG in which QTcF was 451 at end of treatment (mean QTcF at baseline was 431 msec). The second patient had a QTcF varying between 455 to 469 msec and a reading of 469 msec was recorded in a pre-treatment ECG making the variation unlikely to be drug related. The patient with a DLT of atrial fibrillation had a normal QTcF. No other episodes of atrial fibrillation or any ventricular tachycardia were seen on the study.

Echocardiograms were done at baseline and at day 28 and showed a decrease in ejection fraction to abnormal values in one patient dosed at 120 mg (55%–49% with a lower limit of normal defined as 50%).

At the recommended dose of 40 mg, there was one DLT (Table 1). Nine patients completed 2 cycles of treatment, and of these, a further 2 went on to the third cycle of treatment. No patient who started at a dose of 40 mg completed 4 cycles of treatment. The details of toxicity seen at this dose are presented in Table 2. Progressive disease in patients with advanced solid tumors limited the study of long-term toxicity of CHR-3996, however it was noted that a patient with a neuroendocrine tumor who had stable disease at first response started treatment at 120 mg and was dose reduced to 80 mg and then 40 mg, which they tolerated for 17 cycles (patient still on treatment when the manuscript was submitted).

### Pharmacokinetics

Plasma concentration in relation to time after ingestion of drug and PK parameters from day 1 are shown in Fig. 1A and Table 3. There were no significant differences between day 1 and day 28 PK parameters (data not shown). The intersubject variability in systemic exposure was pronounced. The AUC appeared broadly proportional over the dose range $R^2 = 0.8872$ (Fig. 1B). The maximum concentration ($C_{\text{max}}$) ranged from 18.5 ng/mL at 5 mg to 774.3 ng/mL at 160 mg (Table 3). The plasma concentration that achieved growth inhibition in xenografts was 10 ng/mL, and it was possible to achieve this concentration at 40 mg for approximately 8 hours. The median terminal elimination half-life ($t_{1/2}$) for CHR-3996 was 1.8 hour (range 1.1–7.8 hours) and the median $t_{\text{max}}$ was 1 hour (range 0.33–4.12 hours) indicating rapid absorption following oral dosing.

### Pharmacodynamics

Histone acetylation was measured in PBMCs. The maximal changes were seen at 4 hours posttreatment.

### Table 2. Adverse events in 10% or more of patients

<table>
<thead>
<tr>
<th>CHR-3996 dose in mg</th>
<th>n = number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mg</td>
<td>n = 3</td>
</tr>
<tr>
<td>10 mg</td>
<td>n = 4</td>
</tr>
<tr>
<td>20 mg</td>
<td>n = 3</td>
</tr>
<tr>
<td>40 mg</td>
<td>n = 10</td>
</tr>
<tr>
<td>80 mg</td>
<td>n = 10</td>
</tr>
<tr>
<td>120 mg</td>
<td>n = 4</td>
</tr>
<tr>
<td>160 mg</td>
<td>n = 5</td>
</tr>
<tr>
<td>All patients</td>
<td>n = 39</td>
</tr>
</tbody>
</table>

| Preferred term       | G1-2 | G3-4 | G1-2 | G3-4 | G1-2 | G3-4 | G1-2 | G3-4 | G1-2 | G3-4 | G1-2 | G3-4 | G1-2 | G3-4 |
|----------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Fatigue              | 2 (66.7) | 1 (33.3) | 2 (60) | 1 (25) | 2 (66.7) | 0 | 3 (30) | 3 (30) | 7 (70) | 1 (10) | 2 (50) | 1 (25) | 2 (40) | 1 (20) | 20 (71.4) | 8 (28.6) |
| Nausea               | 1 (33.3) | 0 | 3 (75) | 0 | 2 (66.7) | 0 | 6 (60) | 5 (50) | 1 (10) | 4 (100) | 0 | 5 (100) | 0 | 26 (66.3) | 1 (3.7) |
| Vomiting             | 0 | 0 | 2 (50) | 1 (25) | 1 (33.3) | 0 | 4 (40) | 0 | 4 (40) | 0 | 2 (50) | 0 | 3 (60) | 1 (20) | 16 (88.9) | 2 (11.1) |
| Anorexia             | 1 (33.3) | 1 (33.3) | 2 (50) | 0 | 2 (66.7) | 0 | 4 (40) | 0 | 4 (40) | 0 | 2 (50) | 0 | 1 (20) | 0 | 16 (84.1) | 1 (5.9) |
| Blood creatinine     | 0 | 0 | 0 | 0 | 0 | 1 (33.3) | 0 | 2 (20) | 0 | 5 (50) | 0 | 3 (75) | 0 | 4 (80) | 0 | 15 (100) | 0 |
| Constipation         | 1 (33.3) | 0 | 1 (25) | 0 | 1 (33.3) | 0 | 5 (60) | 0 | 2 (20) | 0 | 1 (25) | 0 | 1 (20) | 0 | 12 (100) | 0 |
| Anemia               | 0 | 0 | 0 | 0 | 3 (100) | 0 | 1 (10) | 1 (10) | 2 (20) | 2 (20) | 0 | 0 | 0 | 1 (20) | 6 (60) | 4 (40) |
| Pyrexia              | 0 | 0 | 1 (25) | 0 | 1 (33.3) | 1 (33.3) | 2 (20) | 0 | 2 (20) | 1 (10) | 0 | 0 | 2 (40) | 0 | 8 (80) | 2 (20) |
| Thrombocytopenia     | 0 | 0 | 0 | 0 | 0 | 1 (10) | 1 (10) | 2 (20) | 0 | 1 (25) | 1 (25) | 1 (20) | 2 (40) | 5 (55.6) | 4 (44.4) |
| Dyspnea              | 1 (33.3) | 0 | 0 | 0 | 1 (33.3) | 1 (33.3) | 0 | 0 | 1 (10) | 1 (10) | 1 (25) | 0 | 1 (20) | 0 | 5 (71.4) | 2 (28.6) |
| Abdominal pain       | 0 | 0 | 0 | 0 | 1 (33.3) | 1 (33.3) | 0 | 0 | 1 (10) | 1 (10) | 1 (25) | 0 | 1 (20) | 0 | 4 (66.7) | 2 (33.3) |
| Cough                | 0 | 0 | 0 | 0 | 0 | 1 (33.3) | 0 | 1 (10) | 0 | 2 (20) | 0 | 2 (50) | 0 | 0 | 0 | 6 (100) | 0 |
| Diarrhea             | 0 | 0 | 1 (25) | 0 | 0 | 0 | 1 (10) | 0 | 1 (10) | 0 | 2 (50) | 0 | 1 (20) | 0 | 6 (100) | 0 |
| AST increased        | 1 (33.3) | (33.3) | 0 | 0 | 1 (10) | 0 | 2 (20) | 0 | 0 | 0 | 0 | 0 | 0 | 4 (80) | 1 (20) |
| Chills               | 1 (33.3) | 0 | 0 | 0 | 1 (33.3) | 0 | 0 | 0 | 1 (10) | 0 | 1 (25) | 0 | 1 (20) | 0 | 5 (100) | 0 |
| Abdominal pain, upper, pain, upper | 1 (33.3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (10) | 0 | 2 (50) | 0 | 0 | 0 | 4 (100) | 0 |
| Blood LDH increased  | 2 (66.7) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (10) | 1 (10) | 0 | 0 | 0 | 0 | 3 (75) | 1 (25) |
| Dysgeusia            | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 (50) | 0 | 2 (40) | 0 | 4 (100) | 0 |
| Headache             | 0 | 0 | 1 (25) | 0 | 1 (33.3) | 0 | 0 | 0 | 0 | 0 | 2 (50) | 0 | 0 | 0 | 4 (100) | 0 |
| Rash                 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (10) | 0 | 0 | 1 (25) | 0 | 2 (40) | 0 | 4 (100) | 0 |

**Abbreviations:** AST, aspartate aminotransferase; LDH, lactate dehydrogenase.
Figure 1. PK profile of CHR-3996. A, plasma concentrations of CHR-336 after ingestion of a single dose on cycle 1. The dashed line (—) refers to plasma concentrations that caused inhibition of tumor growth in xenograft models (7). B, the correlation of dose in mg/d and AUC.

Table 3. PK profile of CHR-3996

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dose mg/d</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>80</th>
<th>120</th>
<th>160</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = number of patients</td>
<td>n = 3</td>
<td>n = 4</td>
<td>n = 3</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 4</td>
<td>n = 5</td>
</tr>
<tr>
<td>Mean ( t_{\text{max}} ) h (range)</td>
<td>4.0 (2.0–4.0)</td>
<td>1.2 (0.3–1.1)</td>
<td>4.0 (0.3–4.0)</td>
<td>1.0 (0.33–4.00)</td>
<td>1.5 (0.33–4.00)</td>
<td>1.0 (0.37–4.00)</td>
<td>1.0 (0.37–4.00)</td>
<td></td>
</tr>
<tr>
<td>Mean ( C_{\text{max}} ) ng/mL (CV)</td>
<td>18.5 (121%)</td>
<td>33.9 (91%)</td>
<td>54.3 (68%)</td>
<td>259.0 (100%)</td>
<td>358.9 (167%)</td>
<td>340.4 (114%)</td>
<td>774.3 (74.4%)</td>
<td></td>
</tr>
<tr>
<td>Mean AUC_{0-t} ng.h/mL (CV)</td>
<td>49.0 (104%)</td>
<td>87.9 (69%)</td>
<td>201.9 (104%)</td>
<td>561.0 (83%)</td>
<td>1,084.0 (150%)</td>
<td>849.0 (104%)</td>
<td>1,966.0 (66.8%)</td>
<td></td>
</tr>
<tr>
<td>Mean AUC_{0-\infty} ng.h/mL (CV)</td>
<td>110.7 (56.8%)</td>
<td>93.3 (73.9%)</td>
<td>630.2 (138.1%)</td>
<td>1,214.6 (138.1%)</td>
<td>887.3 (103.2%)</td>
<td>1,985.0 (65.5%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: \( t_{\text{max}} \), time in hours taken to reach maximum concentration; \( C_{\text{max}} \), maximum concentration achieved; AUC; CV, coefficient of variation.

\(^{a}\)No reliable estimate of terminal elimination rate constant for this cohort.

\(^{b}\)No CV% recorded as only one reliable estimate of terminal elimination rate constant.
when compared with baseline across all dose ranges (Fig. 2B). At 40 mg, a significant increase in histone acetylation at 1 and 4 hours compared with baseline was observed (1.95 vs. 1; $P = 0.0078$ and 2.35 vs. 1; $P = 0.0039$, respectively, Fig. 1A). Interestingly, the fold change in histone acetylation at 4 hours was maximal at 40 mg/d and did not increase at further dose levels (median changes 1, 1.64, 1.73, 2.35, 1.75, 1.77, 2.17 at 5, 10, 20, 40, 80, 120, and 160 mg/d, respectively). The interaction between CHR-3996 concentration and histone acetylation in PBMCs was modeled using an $E_{\text{max}}$ sigmoid response model and the EC$_{50}$ concentration of CHR-3996 was 27 ng/mL, which is below the plasma levels achieved at 40 mg/day (259 ng/mL; see Table 1 and Fig. 2C).

### Efficacy

Thirty-nine patients were treated over a total of 105 treatment cycles; mean 2.7 cycles per patient (median 2, range 1–18 with one patient ongoing after 18 cycles). Twelve patients were on study treatment for at least 3 cycles. Stable disease was recorded as the first response in 9 of 39 patients, and the median time that patients stayed on study was 56 days. One patient had a confirmed partial response and continued treatment for 12 months (Fig. 3).

**Declaration of recommended phase II dose (RP2D)**

The dose of 80 mg was the protocol-defined MTD with 2 of 10 patients incurring a DLT. In addition, 4 patients developed a grade 2 rise in plasma creatinine at 80 mg, 2 of these outside the 28-day window. Multiple factors were taken into consideration when recommending the phase II dose: (i) the drug was likely to be used in a chronic daily dosing schedule, thus grade 2 rises in plasma creatinine at 80 mg outside the 28-day window, although not technically DLTs, were likely to result in dose interruptions; (ii) the
AUC of 561 ng.h/mL at 40 mg was above that required for activity in preclinical models; (iii) the pharmacodynamic (PD) changes of histone H3 acetylation at 4 hours peaked at 40 mg/d and did not increase further at the higher doses. Thus, taking into consideration the toxicity profile, PK, and PD results, the recommended phase II dose was 40 mg/d.

**Discussion**

CHR-3996 is a class I selective, orally delivered hydroxamate HDAC inhibitor. Hydroxamate HDAC inhibitors usually target both class I and class II HDACs, but CHR-3996 has little or no activity against HDAC 6 (7). Other class I–specific HDAC inhibitors include the cyclic peptide romidepsin, which is administered weekly intravenously and is licensed for the treatment of cutaneous-T-cell lymphoma (10).

In this phase I study, CHR-3996 was administered to humans orally, once daily, for the first time. The doses ranged from 5 mg to 160 mg/d, while the dose schedule was continuous once daily. The recommended dose for phase II studies was 40 mg/d. The DLTs seen during the trial were similar to those seen for other HDAC inhibitors and included thrombocytopenia (grade 4), fatigue (grade 3), and elevated plasma creatinine (grade 1–2). Thrombocytopenia is a common toxicity seen with HDAC inhibitors such as vorinostat (11), panobinostat (12) but not in others such as belinostat (13). Fatigue was a DLT in a number of phase I trials of HDAC inhibitors such as vorinostat (11), belinostat (13) MGCD0103 (14), and romidepsin (5) with related DLTs such as “asthenia” also being noted with MS-275 (15). Increase in plasma creatinine, although only reaching grade 1–2 in cycle 1, was seen as a DLT in 2 cases, as they required dose interruption to prevent deterioration of renal function, thus meeting the DLT criteria of inability to complete at least 75% of planned dose during the first cycle of treatment. Renal function improved on stopping and/or reducing CHR-3996 in both instances and did not recur on rechallenge at a lower dose. Elevation of serum creatinine has been noted in HDAC inhibitors such as vorinostat (11) and panobinostat (12). Finally, a DLT of atrial fibrillation was seen at 40 mg/d. This patient had very low CHR-3996 plasma levels and the atrial fibrillation did not recur on rechallenge. In addition, the patient did not have a prolonged QTcF. However, it was not possible to exclude a relationship of this event to administration of CHR-3996. Cardiac arrhythmias are well described in phase I trials with other HDAC inhibitors (16).

The toxicity profile of CHR-3996 has some similarities and differences in comparison with other HDAC inhibitors. Similarities include DLTs such as thrombocytopenia, fatigue, and ECG changes. The differences include less GI toxicity, such as a lower incidence of diarrhea, and generally well-controlled nausea and vomiting (11, 17). Also, CHR-3996 showed reversible grade 1–2 elevation in creatinine and this has not been widely reported with other HDAC inhibitors, apart from the licensed pan-HDAC inhibitor vorinostat (11).

The PKs of CHR-3996 is acceptable for a once- or twice-daily dosing regimen. CHR-3996 has a median half-life of 1.8 hours and shows rapid absorption. The mean $C_{\text{max}}$ at 40 mg/d (259 ng/mL) was significantly higher than the level showing activity in mouse xenograft experiments (10 ng/mL; ref. 7). The maximal increase in histone acetylation in PBMCs occurred in the 4-hour samples, consistent with rapid absorption of the drug. The increase in histone acetylation was maximal at 40 mg/d. Modeling of the PK/PD relationship estimated an EC$_{50}$ for histone acetylation of 27 ng/mL, which is approximately one-tenth of the mean $C_{\text{max}}$ at 40 mg (259 ng/mL), thus the recommended dose is capable of achieving pharmacologically relevant plasma drug levels in humans. In preclinical models, there was a 10-fold accumulation of CHR-3996 in tumor compared with plasma (7) and, although it was not possible to measure drug concentration in tumor tissue in this trial, we showed histone acetylation in hair follicles at 40 mg in a limited number of patients, suggesting target inhibition in extravascular tissue. Although proof-of-mechanism
biomarkers (histone H3 acetylation) were carried out and confirmed target engagement, these do not directly predict efficacy. As no pre- and posttumor biopsies were taken during the trial, proof-of-concept biomarkers studying effects on cell cycle or apoptosis such as quantification of p21 induction or caspase-3 cleavage were not done and would be of added benefit in future clinical trials.

A population of patients with advanced solid tumors was enrolled in this study, and the majority of patients progressed at assessment after 2 cycles. Response to HDAC inhibitors in hematologic malignancies occurs at 3 to 6 months, and it is often unrealistic to expect meaningful responses in rapidly growing solid tumors after assessment at 2 cycles. Interestingly, one patient with a neuroendocrine tumor achieved stable disease after 2 cycles of CHR-3996 and is still on treatment at 21 cycles, and a patient with acinar pancreatic cancer who had a partial response at 4 months maintained the partial response for another 6 months. HDAC inhibitors are licensed for use as single agents. There are currently many studies of HDAC inhibitors in combination with both conventional chemotherapeutic agents or targeted agents (18). Combinations of CHR-3996 which have been successfully explored in preclinical models include the epithelial growth factor receptor inhibitor, erlotinib (7), the DNA methyltransferase inhibitor, decitabine (7), and the aminopeptidase inhibitor, tosedostat (7, 19). Given that CHR-3996 is orally admin-
istered and has a favorable toxicity profile, it likely to be tolerable in combination with other agents.

In conclusion, the recommended phase II dose of CHR3996 is 40 mg/d. CHR-3996 can be dosed once a day continuously with a manageable toxicity profile and favorable PK and PD properties. This, in conjunction with early signs of clinical response, warrants further evaluation of CHR-3996 in clinical trials.

**Disclosure of Potential Conflicts of Interest**

CHR-3996 was developed by Chroma Therapeutics in collaboration with The Institute of Cancer Research (ICR). ICR has received financial benefit from Chroma Therapeutics. U. Banerji, M. Tall, A. Stewart, F. Raynaud, M. D. Garrett, and J. S. De Bono are employees of The Institute of Cancer Research. In addition, D. Papadatos-Pastos and R. Kristeleit had honorary contracts with The Institute of Cancer Research. J. S. De Bono has been on a Chroma Therapeutics advisory board meeting. P. Debnam, M. Toal, and L. Hoofman are employees of Chroma Therapeutics. The authors disclosed no potential conflicts of interest.

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