A phase I study of the oral combination of CI-994, a putative histone deacetylase inhibitor, and capecitabine

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Background: This study was conducted to determine the toxicity profile, maximum tolerated dose (MTD) and pharmacokinetics of the putative histone deacetylase inhibitor CI-994 in combination with capecitabine.

Patients and methods: Fifty-four patients were treated according to three different dosing schemes in which the capecitabine dose was fixed and the CI-994 dose was escalated. Capecitabine was administered in twice daily divided doses, and CI-994 was given as a single daily dose. In schedule A, 26 patients were treated with capecitabine 1650 mg/m2/day and CI-994 for 2 weeks of a 3-week cycle. In schedule B, six patients received capecitabine 1650 mg/m2/day for two 3-week cycles and CI-994 for 5 of 6 weeks. In schedule C, 22 patients were treated with capecitabine 2000 mg/m2/day and CI-994 for 2 of 3 weeks.

Results: At the MTD, the principal dose-limiting toxicity was thrombocytopenia. The pharmacokinetics of CI-994 were unaltered by capecitabine, and there was no correlation between body surface area and major pharmacokinetic parameters. Platelet count nadir was best predicted by the observed maximal concentration ($C_{\text{max}}$) of CI-994.

Conclusions: The recommended phase II dose is 6 mg/m2 (or 10 mg) of CI-994 in combination with capecitabine 2000 mg/m2/day for 2 weeks of a 3-week cycle.

Key words: capecitabine, CI-994, dose-limiting toxicity, histone deacetylase inhibitor, pharmacokinetics, phase I study

Introduction

CI-994 ([N-acetyl dinaline, 4-[acetylamino-N-2'- (amino-phenyl) benzamide]) is the active metabolite of the des-acetyl parent compound dinaline. In vivo studies of dinaline and CI-994 have shown an unusual profile with activity in tumors normally refractory to conventional anticancer agents [1–8]. Preclinical studies of CI-994 have shown cytostatic in vitro activity against HCT-8 xenografts and murine mammary 25 and colon 26 cell lines [2–6]. Cytostatic in vivo activity has been demonstrated in colon 9 and 51/A, Dunn osteosarcoma, LNCaP human prostate carcinoma, mammary carcinoma 17/ADR and pancreatic ductal adenocarcinoma 02 and 03 tumor models [7]. CI-994 is cytotoxic in the Brown Norway rat leukemia model [8]. While the mechanism of action of CI-994 is unknown, there is evidence suggesting that it may increase histone acetylation by inhibiting histone deacetylase [9].

Prakash et al. [10] performed a phase I study of single-agent CI-994 using a dose-escalation scheme that increased both the daily dose and the duration of treatment. When given for 8 weeks followed by a 2-week rest period, the maximum tolerated dose (MTD) of CI-994 was 8 mg/m2. The dose limiting toxicities (DLTs) observed were thrombocytopenia and neutropenia, though not occurring concurrently. Stable disease was documented in one patient each with colorectal, non-small-cell lung and renal carcinomas. Furthermore, a partial response lasting over 26 months was seen in a heavily pretreated patient with adenocarcinoma of the lung. In a second phase I study combining gemcitabine with CI-994 given daily for 21 days of a 28-day treatment cycle, thrombocytopenia was again the DLT [11]. Pharmacokinetic analyses of CI-994 from both studies suggested linear kinetics and rapid absorption after oral administration. Gemcitabine did not alter the pharmacokinetics of CI-994.

Capecitabine is currently approved in the USA for the treatment of breast and colorectal cancer and has been shown to have activity, both alone and in combination with other chemotherapeutic agents, in a variety of other tumors including gastric, head...
and neck and renal cell carcinoma [12–14]. Toxicities commonly associated with capecitabine monotherapy include anemia, fatigue, gastrointestinal (diarrhea, nausea, vomiting and stomatitis), hand–foot syndrome and lymphopenia [15–18].

Both capecitabine and CI-994 demonstrate activity in solid tumors. They have distinct mechanisms of action with non-overlapping toxicities; in an in vivo murine colon tumor model, they have an additive antitumor effect when administered together. Furthermore, together they provide a convenient oral antitumor regimen. For these reasons, we conducted a phase I study of the combination of CI-994 and capecitabine in patients with advanced solid tumors.

**Patients and methods**

**Patient selection**

Patients with a histological diagnosis of advanced solid malignancy for which no known effective therapy is available, and for which capecitabine is a reasonable treatment option, were eligible for this study. Other eligibility criteria included age ≥18 years, Eastern Cooperative Oncology Group (ECOG) performance status ≤1 and life expectancy ≥8 weeks. Patients may not have received radiation therapy or chemotherapy within 3 weeks (6 weeks for mitomycin C and nitrosourea), or hormone therapy, immunotherapy, or other biological therapy within 2 weeks of first treatment. Patients needed to be capable of swallowing intact capecitabine tablets and CI-994 capsules and to not have previously received treatment with either drug. Required laboratory values included the following: absolute neutrophil count >1500/μl; platelet count ≥100 000/μl; total bilirubin ≤1.5 times upper limit of normal; and serum creatinine ≤1.5 times the upper limit of normal. Patients were excluded for new brain metastases diagnosed within 3 weeks of first study treatment, myocardial infarction within 6 months of study entry, unstable angina, second- or third-degree heart block, and other non-tumor-related life-threatening illnesses. Pregnant or lactating patients were ineligible for this study. There were no limits on the number of previous treatment regimens. Concurrent leucovorin administration was not allowed. The clinical trial was carried out with the approval of the Institutional Review Board, and written informed consent was obtained from all patients prior to study entry.

**Pretreatment and follow-up evaluation**

Complete history, physical examination, assessment of performance status, electrocardiogram, complete blood count (CBC) with differential, serum chemistry profile and serum pregnancy test, if applicable, were conducted prior to the first treatment. Thereafter, physical examination, assessment of adverse effects and performance status, serum chemistry profile and CBC with differential were performed weekly. Additional CBCs were drawn on days 11 and 18 of the first cycle. Electrocardiograms were obtained on the first day of each cycle and on day 22 for patients on the 6-week dosing schedule. Objective tumor assessment by computed tomography or magnetic resonance imaging was performed prior to starting therapy and every 6 weeks thereafter while the patient was in the study.

**Treatment protocol**

Three dosing schemes were explored in this study. In the first scheme (schedule A), a 3-week treatment cycle was used in which both agents were administered for 2 weeks followed by a 1-week rest period. The starting dose of capecitabine was 1650 mg/m²/day in twice-daily divided doses, which is 66% of the recommended dose when given as monotherapy in solid tumors. The starting CI-994 dose was 4 mg/m² as a single daily dose, which is 50% of the phase II recommended single agent dose when administered for 8 weeks of a 10-week treatment cycle.

The single agent phase I study demonstrated that chronic dosing up to eight consecutive weeks is feasible [10]. Because non-clinical studies of CI-994 demonstrated that the degree of tumor growth inhibition is directly related to the duration of drug exposure [19], it was postulated that prolonged, chronic dosing would be more effective than intermittent administration. For this reason a longer 6-week dosing schedule was studied in the second dosing scheme (schedule B). Capecitabine was administered as before (2 weeks of treatment followed by a 1-week rest period, then repeated), but CI-994 was administered for five consecutive weeks followed by a 1-week drug-free period. The capecitabine dose for this second schedule was again 1650 mg/m²/day, and the starting CI-994 dose level was two dose levels below the MTD of schedule A.

After the establishment of the schedule B MTD, a third dosing scheme (schedule C) was added to increase the administered capecitabine to a dose closer to the recommended single-agent dose. Schedule C returned to the original 3-week treatment cycle but with a capecitabine dose of 2000 mg/m²/day, which was a 25% increase over the initial capecitabine dose and 80% of the recommended single-agent dose in solid tumors. The CI-994 starting dose was again 4 mg/m².

In all three dosing schemes, the capecitabine dose remained fixed at the initial dose of either 1650 or 2000 mg/m²/day for all subsequent dose levels, while the CI-994 dose increased by a fixed increment of 2 mg/m² until establishment of the MTD. Initially, three-patient cohorts were used. When a DLT was observed, cohorts were expanded to six patients. The MTD was established when two or more patients at the same dose level experienced similar DLTs. Dose escalation to a new cohort was not permitted until all patients in the previous cohort had completed the initial 3- or 6-week treatment course. Inpatient dose escalations were not permitted. Capecitabine dose was reduced by 25% for the second occurrence of grade 2 or first occurrence of grade 3 toxicity. CI-994 dose was decreased by 2 mg/m² for toxicity grade ≥3.

Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria, version 2.0. DLT was defined as any of the following occurring in the first treatment cycle: platelet nadir <25 000/μl; absolute neutrophil count <500/μl for 5 days or more or associated with infection or fever; grade ≥2 treatment-related central nervous system toxicities lasting for ≥24 h; grade ≥3 non-hematologic toxicity of any type; failure to recover from treatment-related toxicity with 21 days of discontinuation of therapy; or a terminated or non-compliant treatment cycle with <80% of planned capecitabine or CI-994 doses administered due to treatment-related toxicity.

**Drug administration**

CI-994 was supplied by Parke-Davis Pharmaceutical Research Division (Ann Arbor, MI, USA) as 2.5, 5 and 10 mg gelatin capsules. Capecitabine was commercially available as film-coated tablets in strengths of 150 and 500 mg. Doses of both agents were calculated based on body surface area (BSA) and rounded to the closest available tablet or capsule strength. Patients were instructed to take the CI-994 with the morning capecitabine dose and to swallow the capsules and tablets intact.

**Pharmacokinetic studies**

Samples for pharmacokinetic analysis were only obtained from patients who were treated on schedule A. Venous blood samples were collected prior to and at 0.5, 1, 2 and 4 h after CI-994 and capecitabine dosing on days 1 and 8 of the first cycle. Blood was collected in evacuated tubes containing 72 USP units of sodium heparin and placed on ice for a maximum of 20 min. Plasma was isolated by centrifugation at 4°C and stored at −20°C until analyzed.
Concentrations of CI-994 in plasma were determined using a previously validated high performance liquid chromatography (HPLC)/mass spectrometry method [20]. Briefly, heparinized human plasma samples (0.5 ml) were mixed with an aqueous internal standard solution and passed through a C18 solid-phase extraction sorbent. The compound of interest was eluted using a mixture of equal parts methanol and acetonitrile, evaporated to dryness under N2, reconstituted in mobile phase, and separated on a reverse-phase HPLC column with mass spectrometry/mass spectrometry detection.

Concentration–time profiles were analyzed using conventional population pharmacokinetic methods [21]. Both one- and two-compartment models were evaluated and parameterized in terms of oral clearance (CL/F) and volume of distribution (V/F). The absorption rate (Ka) was modeled as a first order process. Interindividual variability in pharmacokinetic parameters was expressed as log normally distributed. Residual (intra-subject) error was modeled as proportional to concentration.

Response evaluation

Only those patients who underwent appropriate radiological tumor evaluation after at least one cycle of treatment were considered assessable for response. Partial response required a ≥50% decrease in the sum of the products of the longest perpendicular diameters of all measured lesions for a minimum of 4 weeks. Progressive disease was defined as an increase of ≥25% in the sum of the products of the longest perpendicular diameters of all measured lesions or the appearance of new lesions. Patients were considered to have stable disease when the criteria for complete response, partial response or progressive disease were not met during the first 6 weeks of treatment.

Results

Patients

From May 2000 to November 2001, a total of 54 patients were enrolled in this study. Patient characteristics are summarized in Table 1. Fifty-two patients were assessable for toxicity, as two patients withdrew consent prior to completing the first treatment cycle. Forty-seven patients were assessable for response. Patients were withdrawn from the study for progressive disease (n = 42, 78%), intercurrent medical illness or clinical deterioration (n = 7, 13%), toxicity (n = 2, 4%), withdrawal of consent (n = 2, 4%) and death (n = 1, 2%).

The single treatment-related death occurred in a patient with mesothelioma who was treated on schedule A with 8 mg/m2 of CI-994. He developed respiratory distress and hypotension on day 19 of the first treatment cycle and died 2 days later. While the clinical scenario suggested septic shock, the patient was not neutropenic and all blood cultures were negative.

Dose escalation and MTDs

Table 2 details the dose escalation schema. A total of 162 cycles were administered to the 52 patients who were assessable for toxicity. Thirty-seven per cent of patients required dose reduction of CI-994 and/or capecitabine for toxicity. The MTD of CI-994 in combination with capecitabine 1650 mg/m2/day (schedule A) was determined to be 10 mg/m2 after four patients in that dose level developed DLT. Enrollment then switched to the 6-week schedule B. As 50% of the patients treated at the initial dose level experienced DLT, further dose escalation on the 6-week dosing schedule was abandoned; dose escalation of CI-994 proceeded in combination with a fixed capecitabine dose of 2000 mg/m2/day on a 3-week schedule (schedule C). The MTD of this third dosing scheme was determined when both patients enrolled at the 8 mg/m2 dose level experienced DLT.
Hematological toxicity

Thrombocytopenia was the principal DLT observed and was dose limiting at the MTD of all three dosing schemes. Table 3 shows the incidence and grade of thrombocytopenia by dose level as well as the median platelet nadirs and time to nadir. Across all dose levels, 46% of patients assessable for toxicity had a nadir platelet count of <100 000/µl and 17% had a nadir of <50 000/µl in cycle one. Overall, the median time to nadir was 17 days from the start of treatment, and recovery to >100 000/µl occurred between 5 and 22 days after withdrawal of therapy.

There was one episode of significant bleeding. In schedule A, a patient with metastatic gastric carcinoma enrolled at the 10 mg/m² dose level developed a bleed from tumor erosion into the lumen of the stomach in the setting of a platelet count of 66 000/µl.

Episodes of severe anemia and neutropenia in cycle 1 were limited. In addition to the above-described patient, two patients treated with 4 mg/m² of CI-994 in schedule C developed grade 3 anemia. A single episode of grade 3 neutropenia in cycle one was observed at the highest dose level of schedule A. However, one patient each treated with 4, 8 and 10 mg/m² of CI-994 in schedule A required dose reduction in cycles three or four due to grade 3 neutropenia.

Non-hematological toxicity

Table 4 summarizes the incidence of non-hematological toxicity by dose level. There were no grade 4 non-hematological toxicities observed in cycle one. The most common toxicities were gastrointestinal and included anorexia, diarrhea, nausea and vomiting. Fatigue was also commonly reported and was a DLT at the CI-994 8 mg/m² dose level of schedule A. The non-hematological DLTs at the MTD of schedules A, B and C were hand–foot syndrome, diarrhea and stomatitis, respectively.

Pharmacokinetics/pharmacodynamics

Pharmacokinetic evaluation was performed in all 26 patients who were treated on schedule A. A one-compartment model provided an excellent fit to the data. Table 5 details the pharmacokinetic parameters by CI-994 dose administered. Oral clearance did not change significantly with dose, suggesting linear kinetics. The mean CL/F and Vd/F of CI-994 were 5.2 l/h and 69 l, respectively. Interindividual variability in CL/F and Vd/F averaged 15% and 23%, respectively. There appeared to be no correlation between BSA and dose normalized maximal concentration (Cmax), CL/F and Vd/F (P > 0.15, data not shown).

The natural log of the platelet count nadir decreased with increasing CI-994 dose (r = 0.49, P = 0.01; data not shown). However, plasma concentrations were a stronger predictor of thrombocytopenia than dose (Figure 1), with Cmax (r = 0.78, P < 0.0001) having a stronger correlation than AUC (r = -0.56, P = 0.003; data not shown).

Tumor response

One partial response was achieved at the 4 mg/m² dose level of schedule A in a patient with colorectal cancer who had previously received 5-fluorouracil adjuvantly and oral topotecan in the metastatic setting. He received a total of eight cycles of therapy on study, and the duration of response was 173 days. Across all dose levels, 19 patients had stable disease at first tumor assessment. Disease stabilization through at least five cycles was seen in adenocarcinoma of unknown primary, appendiceal cancer, breast cancer, colorectal cancer and mesothelioma.

Discussion

This phase I clinical trial explored the oral combination of CI-994 and capecitabine. The dosing schedules explored were designed around the recommended capecitabine treatment schedule of 2 weeks of therapy followed by a 1-week rest period. MTDs were determined for the two 3-week schedules (A and C) in which both drugs were administered for 2 of 3 weeks. The 6-week schedule B, where CI-994 was administered for 5 of 6 weeks, was abandoned when one-third of patients experienced dose-limiting thrombocytopenia in spite of a CI-994 dose that was two dose levels below the MTD of
The recommended BSA-based phase II regimen is CI-994 6 mg/m² once daily and capecitabine 2000 mg/m²/day in twice-daily divided doses for 2 weeks followed by a 1-week rest period (schedule C).

However, CI-994 clearance, volume and dose-normalized C_{max} did not correlate with BSA. These results, together with the fact that C_{max}, the best predictor of toxicity, increased with the actual CI-994 dose administered (i.e. mg/m² × BSA), lends support to BSA-independent dosing of CI-994. The phase II recommended flat dose of CI-994 is 10 mg when combined with capecitabine. It is also possible that fixed dosing of capecitabine (e.g. 3000 mg/day) could be utilized in future trials of this combination [22].

As was the case in the single-agent study of CI-994, thrombocytopenia was the primary DLT. Although there was only one instance of grade 4 thrombocytopenia, the incidence would likely have been higher had treatment not been withheld per protocol specified guidelines when platelet counts approached unacceptably low levels. When it occurred, thrombocytopenia was usually reversible within 1–2 weeks of CI-994 withdrawal, and patients most often could resume therapy with a dose reduction. Other hematological toxicity was

<table>
<thead>
<tr>
<th>Dosing schedule</th>
<th>A (n=3)</th>
<th>B (n=6)</th>
<th>C (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capecitabine (mg/m²/day)</td>
<td>1650</td>
<td>1650</td>
<td>2000</td>
</tr>
<tr>
<td>CI-994 (mg/m²)</td>
<td>4 (n=3)</td>
<td>6 (n=6)</td>
<td>8 (n=12)</td>
</tr>
<tr>
<td>Grade</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Alopecia</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Anorexia</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Constipation</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Hand–foot syndrome</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Hypotension</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Nausea</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Peripheral edema</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Rash</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Stomatitis</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Vomiting</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

*aAbdominal cramps, dizziness, dry eyes, mouth and skin, erectile dysfunction, increased lacrimation, photosensitivity, and skin ulcer occurred with grade 1 intensity only and in <4% of patients.

<table>
<thead>
<tr>
<th>Table 5. Mean (% coefficient of variance) CI-994 pharmacokinetic parameters in schedule A</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI-994 dose (mg/m²)</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>4</td>
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<td></td>
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<td>6</td>
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<td>8</td>
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<tr>
<td>10</td>
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</tr>
</tbody>
</table>

C_{max}, maximal concentration; V/F, volume of distribution; CL/F, oral clearance; \( K_a \), rate of absorption.

Figure 1. Platelet count nadir versus maximal concentration (\( C_{max} \)) of CI-994.
Table 6. Mean (% coefficient of variance) pharmacokinetic parameters of CI-994 administered with and without capecitabine

<table>
<thead>
<tr>
<th>parameter</th>
<th>Without capecitabine(^a) (n = 47)</th>
<th>With capecitabine(^b) (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{C}_{\text{max}}(\text{l/M}^2))</td>
<td>38.9 (NC)</td>
<td>36.2 (31)</td>
</tr>
<tr>
<td>(V_d/F(\text{l/m}^2))</td>
<td>33 (26)</td>
<td>38 (16)</td>
</tr>
<tr>
<td>(CL/F(\text{l/h/m}^2))</td>
<td>2.9 (44)</td>
<td>2.8 (26)</td>
</tr>
<tr>
<td>(K_a(\text{h}^{-1}))</td>
<td>6.4 (55)</td>
<td>22.1 (125)</td>
</tr>
<tr>
<td>(t_{1/2}(\text{h}))</td>
<td>9.6 (54)</td>
<td>9.7 (33)</td>
</tr>
</tbody>
</table>

While the pharmacokinetic parameters in this study were shown to be independent of body surface area, they are normalized here for the purposes of comparison.

\(^{a}\text{Data obtained from single agent phase I study of CI-994 [10].}\)

\(^{b}\text{C}_{\text{max}}\) normalized by dose (mg/m\(^2\)).

\(C_{\text{max}}\), maximal concentration; \(V_d/F\), volume of distribution; \(CL/F\), oral clearance; \(K_a\), rate of absorption; NC, not calculated.

limited to single instances of grade 3 anemia and neutropenia at the highest CI-994 dose of 10 mg/m\(^2\). Non-hematologic toxicities observed were either grade \(\leq 2\) in severity or well-described toxicities of capecitabine.

Concomitant capecitabine administration did not appear to alter the pharmacokinetic disposition of CI-994, as the pharmacokinetic results were comparable to those obtained from the earlier single agent phase I trial (Table 6). While there appears to be a large difference in \(K_a\), this is likely an artifact of the different sampling schemes used. In particular, sampling during the absorption phase was limited in this study as compared with the previous study. Irrespective of the actual \(K_a\), absorption after oral dosing was rapid, with \(C_{\text{max}}\) occurring within 2 h of drug administration in both studies. On the other hand, the effect of CI-994 on the pharmacokinetics of capecitabine and its active metabolite 5-fluorouracil is unknown, as these data were not collected.

Pharmacodynamic analysis demonstrated that the degree of observed thrombocytopenia increased with increasing CI-994 dose. The correlation between CI-994 AUC and observed thrombocytopenia increased with increasing CI-994 unknown, as these data were not collected.

The correlation between CI-994 AUC and observed thrombocytopenia increased with increasing CI-994 unknown, as these data were not collected.

Acknowledgements

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