Phase I and Pharmacological Study of CI-980, A Novel Synthetic Antimicrotubule Agent


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ABSTRACT

CI-980 (NSC 613862) is one of a novel class of 1,2-dihydropyrido[3,4-b]pyrazines that inhibits tubulin polymerization, presumably by binding to the colchicine binding site of tubulin. In a Phase I and pharmacological study, 16 patients with advanced solid neoplasms were treated with CI-980 on a continuous 72-h infusion schedule at doses ranging from 3.0–5.4 mg/m²/day every 3 weeks. High rates of central nervous system (CNS) toxicity and neutropenia occurred in both minimally and heavily pretreated patients who were treated with CI-980 doses above 3.75 mg/m²/day, which is the maximum tolerated dose and the recommended dose for additional evaluations. CNS effects, characterized by neurocortical, mood, and cerebellar manifestations, were generally observed toward the end of the infusion and immediately posttreatment and usually resolved within 48 h after the completion of treatment. Toxicity was mild to modest at the 3.75 mg/m²/day dose level. Neither clinical nor pharmacological risk factors that may predispose patients to the development of CNS effects were evident. Although no objective antineoplastic activity was observed in this Phase I study, CI-980 steady-state plasma concentrations achieved at the recommended dose of 3.75 mg/m²/day (mean ± SE, 5.74 ± 0.54 nM) approached and exceeded concentrations that have been associated with significant activity in preclinical studies, indicating that additional disease-directed evaluations of CI-980 may be warranted.

INTRODUCTION

CI-980 [(ethyl (S)-(5-amino-1,2-dihydro-2-methyl-3-phe- nylpyrido[3,4-b]pyrazin-7-yl) carbamate 2-hydroxyethane sulfonate; NSC 613862; Fig. 1A] is the prototype of a family of compounds (1,2-dihydropyrido[3,4-b]pyrazines) initially synthesized and developed as folate antagonists (1). Although profound antineoplastic activity was observed, they did not act as antifols (2). Instead, prominent antimitotic effects were observed in preliminary studies, and this class of compounds was subsequently demonstrated to inhibit tubulin polymerization and compete with colchicine for binding to tubulin (2–4).

CI-980, a chiral S isomer that is approximately 60-fold more potent than its R isomer (5, 6), was selected for further development because it was responsible for all of the antimitotic and cytotoxic effects of the racemic mixture and demonstrated greater cytotoxic potency and aqueous solubility than the other agents in its class (7, 8). CI-980 demonstrated profound cytotoxic activity against a broad spectrum of both human and murine tumor cell lines and in vivo tumor models, including multidrug-resistant tumors (5–8), and the compound was twice as potent as vincristine against P388 leukemia (8). Similar to other tubulin-depolymerizing agents, the cytotoxicity of CI-980 was highly schedule-dependent, with ID₅₀ values against L1210 leukemia that were approximately 50,000-fold higher for 1-h treatment durations compared to 24-h treatment durations (8). In vivo studies using P388 leukemia also demonstrated that regimens utilizing highly fractionated treatment and rest periods generally produce superior activity (8). In toxicological studies in rats and dogs, the most susceptible tissues were hematopoietic and gastrointestinal (8).

Two prior Phase I trials of CI-980 have been performed in patients with advanced solid malignancies, and the preliminary results of these studies have been published (9, 10). In the first trial, CI-980 was evaluated as a 24-h infusion administered every 3 weeks, which resulted in mild to moderate myelosuppression and antitumor activity in previously treated patients with colorectal and ovarian cancers (9). However, severe CNS toxicity consisting of dysequilibrium, loss of consciousness, confusion, tremors, and coma occurred at CI-980 doses from 10.8–19.2 mg/m²/day. This prompted additional evaluations of more prolonged infusion schedules based on the notion that CNS toxicity might be related to the magnitude of peak plasma concentrations. The dose recommended for Phase II trials of CI-980 was 10.8 mg/m² administered over 24 h. The second Phase I trial initially evaluated a 1-h infusion given daily for 3 consecutive days every 3 weeks (10). Again, CNS toxicity, characterized by mood effects, confusion, and cerebellar manifestations, as well as transient hypertension led to progressive prolongation of the duration of the daily infusion to 3 h, then to 6 h, and finally to 24 h. Although adverse CNS effects were

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3 The abbreviations used are: CNS, central nervous system; MTD, maximum tolerated dose; C₅₀, steady-state plasma concentration; DLT, dose-limiting toxicity; ANC, absolute neutrophil count; Cl, clearance rate; HPLC, high-performance liquid chromatography.
Phase I and Pharmacological Study of CI-980

Fig. 1 Structures of CI-980 (A) and the internal standard 6-[(trifluoromethyl)phenyl]thio]pyrido[3,2-d]pyrimidine-2,4-diamine (PD 080658; B).

occasionally noted on the longest infusion schedule (continuous 72-h infusion every 3 weeks), neurotoxicity was mild to moderate and brief in duration. Hematological toxicity, particularly leukopenia, was the principal DLT. The MTD and recommended doses for Phase II evaluations of CI-980 given as a continuous 72-h infusion were 5.4 mg/m²/day for untreated or minimally pretreated patients and 4.35 mg/m²/day for heavily pretreated patients.

This Phase I and pharmacological study was performed to further evaluate the feasibility of administering CI-980 as a continuous 72-h infusion every 3 weeks in patients with advanced solid malignancies. The principal objectives of this trial were: (a) to determine the MTD of CI-980 given as a continuous 72-h infusion every 3 weeks and to recommend a dose for Phase II trials; (b) to characterize the toxicities associated with this schedule of administration; (c) to seek preliminary evidence for antitumor activity; and (d) to evaluate the pharmacological behavior of CI-980 administered on this schedule.

PATIENTS AND METHODS

Eligibility. Patients with histologically documented solid tumors refractory to conventional therapy or for whom no effective therapy existed were candidates for this study. Eligibility criteria included: (a) age ≥ 18 years; (b) an Eastern Cooperative Oncology performance status ≤ 2 (ambulatory and capable of self-care); (c) a life expectancy that enabled the completion of at least 2 courses of therapy; (d) no major surgery within 14 days or wide-field radiotherapy and/or chemotherapy within 28 days of entering onto protocol (6 weeks in those treated with a nitrosourea or mitomycin C); (e) adequate hematopoietic (WBC ≥ 4000/μl and platelets ≥ 100,000/μl), hepatic (total bilirubin ≤ 1.5 mg/dl), and renal (creatinine ≤ 1.5 mg/dl) functions; and (f) no other coexisting medical problems of sufficient severity to limit full compliance with the study. Patients were considered ineligible for this study if they had experienced active psychosis or other CNS disorder precluding the observation, identification, and reporting of adverse CNS effects or had poorly controlled moderate to severe hypertension (consistent systolic blood pressure > 170 mm Hg or diastolic blood pressure > 100 mm Hg). All patients gave informed written consent according to federal and institutional guidelines before treatment.

Dosage and Drug Administration. The starting dose of CI-980 was 3.0 mg/m²/day, which was lower than doses associated with the severe toxicities noted in the previous Phase I studies (9, 10). Doses at successive levels were approximately 20–25% higher than at preceding levels (0.75–0.9 mg/m²/day increments): 3.75, 4.5, and 5.4 mg/m²/day. At least three new patients were entered at each escalated dose level. Retreatment was permitted at a minimum interval of 3 weeks. Dose escalations were permitted in the same subject if the individual received at least two courses at the lower dose level without DLT, and if one new patient had completed treatment at the next higher dose without DLT. Dose reductions by one level were permitted for DLT. If one of three new patients at any dose level experienced DLT, then a maximum of six patients were treated. The MTD or recommended Phase II dose was defined as one dose level below the dose that induced DLT in greater than one-third of new patients (at least two out of a maximum of six new patients). DLT was defined as either: (a) an ANC < 500/μl for greater than 5 days; (b) a platelet count < 50,000/μl; (c) nonhematological toxicity of grade 3–4; and (d) any unresolved toxicity resulting in treatment delays of at least 2 weeks. Toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria (11).

CI-980 was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD) in vials that contained 10 mg of lyophilized drug. CI-980 was reconstituted with 5 ml of sterile water to a concentration of 2 mg/ml CI-980 base equivalent. After reconstitution, the solution was diluted with 100 ml of 5% dextrose solution, which was infused over 24 h.

Pretreatment and Follow-Up Studies. Histories, physical examinations, and routine laboratory studies were performed pretreatment and weekly after treatment. Laboratory studies included a complete blood count, differential WBC count, electrolytes, blood urea nitrogen, creatinine, glucose, total protein, albumin, calcium, phosphate, uric acid, alkaline phosphatase, total and direct bilirubin, aspartate amino transferase, alanine amino transferase, amylase, prothrombin time, and urinalysis. Complete blood counts were also obtained on day 10 and every 2 days if ANCs decreased to ≤ 1000/μl. Mini-mental status examinations were performed before treatment, twice daily during the 72-h drug infusion, and immediately posttreatment. This exam, as described by Folstein et al. (12), consists of 11 questions answered over 5–10 min and scored from 0–30 and has been validated as a screen for dementia and delirium in hospitalized patients. Formal tumor measurements and evaluations were performed pretreatment and after every two courses of therapy. Patients were able to continue treatment if they did not develop progressive disease. A complete response was scored if there was disappearance of all active disease on two measurements separated by a minimum period of 3 weeks, and a partial response required at least a 50% in the sum of the product of the bidimensional measurements of all measurable.
lesions documented by two measurements separated by at least 3 weeks.

For the analysis of hematological toxicity, patients were considered heavily pretreated with therapies that may affect long-term hematopoietic function if they had received large-field radiation to bone marrow-containing areas such as the pelvis or spine or ≥6 courses of chemotherapy containing an alkylating agent or ≥2 courses of a nitrosourea or mitomycin C.

Pharmacological Studies. Extensive plasma sampling was performed only in selected patients during the first course, but measurements of C_{in} were attempted during all courses. In patients who had extensive plasma sampling performed, blood samples were collected in heparinized tubes before the infusion; 5, 10, 15, 20, and 30 min and 1, 1.5, 2, 3.5, 6, 9, 15, 24, and 48 h during the infusion; immediately before the end of the 72-h infusion; and 5, 10, 20, 30, 60, and 120 min after the end of the infusion. Blood sampling for C_{in} was performed at 24 and 48 h during the infusion and immediately before the end of the infusion. The blood samples were centrifuged at 1000 × g for 10 min immediately after collection, and plasma was stored at −20°C until quantitative analysis.

Plasma and urine samples were thawed and centrifuged at 14,000 × g for 4 min before analysis. CI-980 was separated from the biological matrix by solid-phase extraction and then assayed using HPLC, using a modified version of a previously described procedure (13). Solid-phase extraction was accomplished using Bond-Elute C-18 cartridges (Varian, Harbor City, CA), which were conditioned twice with 2 ml of methanol. Five μl of a 1 mm solution of the internal standard 6-[[3-(trifluoromethyl)phenyl][thio]pyrido[3,2-d]pyrimidine-2,4-diamine (PD 080658; Fig. 1B), which was obtained from Parke-Davis Pharmaceutical Research (Ann Arbor, MI), were added to each 2-ml sample. The resultant test sample/internal standard mixture was applied to the conditioned cartridge along with a 1-ml wash of the sample tube and then drawn through the cartridge with 4 mm Hg vacuum pressure (Vac-Elute; Alttech Associates, Chicago, IL). The cartridge was then rinsed with a 30/70 (v/v) acetonitrile/0.2% ammonium acetate solution followed by a second rinse with 0.2% ammonium acetate solution using 5 mm Hg vacuum pressure and then dried for 30 s at full vacuum pressure. Next, the manifold cover of the Vac-Elute was removed, the stems were blotted on clean paper, and excess moisture was wiped from the stems. Microcentrifuge tubes were placed in the apparatus, and elution of the compound was performed with 0.5 ml of a 60/40 (v/v) acetonitrile/0.2% ammonium acetate solution using 5 mm Hg vacuum pressure. The cartridge was then dried for 20 s at full vacuum pressure, and the elution procedure was repeated. The eluate was next evaporated to dryness in a Savant CC120 speed vacuum (Farmingdale, NY) at a medium rate (43°C). The samples were reconstituted in 100 μl of mobile-phase solution consisting of a 62/38 (v/v) solution of 10 mm ammonium dihydrogen phosphate/acetonitrile, vortexed for 30 s, and centrifuged at 14,000 × g for 10 min. The supernatant was then transferred to autoinjector vials for HPLC analysis.

Separation of the sample was accomplished by reverse-phase HPLC. Fifty-ul samples were injected using a Perkin-Elmer ISS 100 autosampler (Norwalk, CT) onto a Spectra Physics HPLC system (San Jose, CA) equipped with a Spectra System P100 pump, two 4.6 mm × 150 mm × 5-μm particle-size C_{18} Zorbax (Chadds Ford, PA) columns maintained at 40°C, and a Guard-Pak precolumn (Waters, Milford, MA) dry-packed with Bondapak Corasil C_{18} (Waters). The mobile phase consisted of a 62/38 (v/v) mixture of ammonium dihydrogen phosphate (10 mm; pH 7.5) and acetonitrile maintained at an isocratic flow rate of 0.75 ml/min. The column effluent was monitored fluorometrically using a LS40 Perkin-Elmer fluorescence detector, with excitation and emission wavelengths set at 388 and 473 nm, respectively. Peak areas were quantitated using a Nelson 2600 integration system (Perkin-Elmer Nelson System, Cupertino, CA), and drug concentrations were determined from linear regression equations derived from calibration curves prepared with samples between 2.5 and 25 nm. Under these conditions, the retention times for CI-980 and the internal standard were 22 and 28 min, respectively. Assay precision for CI-980 based on quality control samples was within ±5.6% with an accuracy of ±4.7%. The minimum quantitation limit for the assay was 0.44 nm.

The Cl of CI-980 was calculated using the formula, Cl = \frac{F \times DV}{C_{in}} with C_{in} equivalent to the CI-980 plasma concentration at the end of the infusion. In four courses, CI-980 concentrations at the end of the infusion were not available, and C_{in} was derived from the 48-h concentration. The drug disposition curves of the selected patients who had complete plasma sampling performed were fit using a pharmacokinetic model characterized by a two-compartment distribution of drug and first-order elimination of drug from the central compartment. Concentration data were weighted by 1/concentration. The values of the following parameters were estimated for each clearance curve: the α and β rate constants and associated half-lives (t_{1/2}), the volume of distribution for the central compartment, the volume of distribution at steady state, and Cl. Pharmacokinetic modeling and parameter estimation was performed using the nonlinear regression program PCNONLIN (SCI Software, Lexington, KY).

RESULTS

Sixteen patients, whose characteristics are displayed in Table 1, were treated with 43 total courses of CI-980 through 4 dose levels (Table 2). All patients had received prior chemotherapy, and five subjects had also previously received radiotherapy. Treatment was discontinued in mid-course in one patient treated with CI-980 at the 4.5 mg/m²/day dose level due to the development of fever and hypotension that were not felt to be drug-related; therefore, this course was not considered to be fully evaluable. The same patient developed severe CNS toxicity during the second course of CI-980, which resulted in the discontinuation of treatment on day 2. The median number of courses administered per patient was 2 (range, 1–10). Two patients were treated at two dose levels; one subject was treated at a reduced dose due to prolonged neutropenia at the higher dose, and another subject was dose-escalated after four courses with minimal toxicity at a lower dose level. Antitumor activity was not observed in this study.

General Toxicity. Both hematological and CNS effects were the principal DLTs of CI-980 as a continuous 72-h infusion every 3 weeks. High rates of intolerable toxicities occurred...
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Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
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</tr>
</thead>
<tbody>
<tr>
<td>No. of patients (evaluable)</td>
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</tr>
<tr>
<td>No. of courses/patient</td>
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</tr>
<tr>
<td>Median</td>
<td>1-10</td>
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<tr>
<td>Sex (male:female)</td>
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</tr>
<tr>
<td>Median age (range)</td>
<td>63 (44-74)</td>
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<tr>
<td>ECOG* performance status</td>
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</table>

Table 2 Dose escalation scheme

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<tr>
<th>CI-980 dose level (mg/m²/day)</th>
<th>No. of patients (evaluable)</th>
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<td>3.00</td>
<td>3</td>
</tr>
<tr>
<td>3.75</td>
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</tr>
<tr>
<td>5.40</td>
<td>1</td>
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<tr>
<td>Total</td>
<td>43 (42)</td>
</tr>
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</table>

“ECOG, Eastern Cooperative Oncology Group.”

at CI-980 doses above 3.75 mg/m²/day, the MTD and recommended dose for additional evaluations.

Hematological Toxicity. Myelosuppression, particularly neutropenia, was the most common toxic effect of CI-980 observed in this study. The onset of neutropenia was generally early, and ANC nadirs were typically observed on days 8-11, whereas effects on platelets usually occurred later, with platelet count nadirs noted on days 10-16. Recovery to baseline was typically complete by day 22, and no patient required a treatment delay for more than 1 week due to unresolved myelosuppression. There was also no evidence of cumulative hematopoietic effects in the 12 patients who received more than 1 course of CI-980.

Table 3 displays the median ANC nadirs and ranges, as well as other pertinent hematological parameters, as a function of dose level. There were no episodes of dose-limiting myelosuppressive effects at the 3.0 and 3.75 mg/m²/day dose levels, although 4 and 2 of 17 courses at the 3.75 mg/m²/day dose level were associated with grade 3 and 4 neutropenia, respectively. Both episodes of grade 4 neutropenia involving 2 patients were brief at the 3.75 mg/m²/day dose level, and ANC nadirs were never below 500 cells/µL for longer than 5 days. In contrast, all 6 new patients treated at the 4.5 mg/m²/day dose level experienced grade 4 neutropenia at some time during treatment, including 4 patients who had ANC nadirs below 500 cells/µL for more than 5 days. One of the four episodes was complicated by the development of fever requiring hospitalization for parenteral antibiotics. Of these four patients, one subject was retreated at the 3.75 mg/m²/day dose level and again developed grade 4 neutropenia lasting less than 5 days, two other patients elected to discontinue treatment due to toxicity, and the fourth subject developed progressive disease. At the 4.5 mg/m²/day dose level, four of the six patients who experienced grade 4 neutropenia and three of the four subjects with grade 4 neutropenia lasting longer than 5 days were considered minimally pretreated. Dose-limiting thrombocytopenia was experienced by two patients during two courses, with grades 3 and 4 thrombocytopenia occurring in one course each.

Neurotoxicity. CNS toxicity, characterized by neurocortical, mood, and cerebellar manifestations, occurred in 13 of 42 courses involving 8 patients. CNS effects were observed during 3 of 7 courses (3 patients) at 3.0 mg/m²/day, 1 of 17 courses (1 patient) at 3.75 mg/m²/day, 6 of 16 courses (4 patients) at 4.5 mg/m²/day, and 0 of 2 courses at 5.4 mg/m²/day. Most episodes (10 of 13) were mild (grade 1) and resolved within several hours after the end of the infusion. Five episodes involving five patients were characterized predominately by mood changes with feelings of listlessness, depressive feelings, and/or irritability. These effects were usually noted at the end of infusion and resolved soon after drug administration. Four patients (six courses) also complained of dysequilibrium, dizziness, and lightheadedness, which were temporally similar to the neurocortical complaints. More severe CNS manifestations were experienced by two patients on two occasions in the pretreatment period of CI-980 at the 4.5 mg/m²/day dose level. Both patients developed severe (grade 3) neurocortical toxicity characterized by profound confusion and delirium. In addition, both patients experienced disorientation, hallucinations, slurred speech, expressive difficulties, and urinary incontinence. In one subject, toxicity was noted approximately 2 h after the second dose of CI-980. The effects completely resolved within 24 h, and the patient was not retreated. For the second patient, CNS effects initially occurred at the end of treatment, were maximal in intensity over 6-12 h posttreatment, and completely resolved within 2 days. Neurological examinations did not reveal focal abnormalities, and computerized tomographic brain scanning was unremarkable. Mini-mental status examinations, which reflect cognitive function, were not helpful in predicting the development of severe CNS toxicity in the two patients who developed severe CNS toxicity, and examination scores were similar to baseline examinations in those patients who were actively experiencing mild to moderate mood and cerebellar disturbances.

Miscellaneous Toxicity. Mild to moderate (grades 1 or 2) nausea and/or vomiting occurred during 11 courses in 9 patients (1 course at 3.0 mg/m²/day, 6 courses at 3.75 mg/m²/ day, and 4 courses at 4.5 mg/m²/day). Complaints were maximal during the last 2 days of the infusion, usually resolved within 24 h posttreatment, and were managed successfully with pro-
steady state averaged 20 ± 3 and 239 + 49 liters/m², respectively. Plasma CI-980 Cₘ values were measured in 13 patients during the first course of therapy and in 8 patients during the second course. A scatterplot of these data, which demonstrate substantial interindividual variability, is displayed in Fig. 3. Respective mean Cₘ and CI values were 5.20 ± 0.81 nM and 966 ± 175 ml/min/m² at 3.0 mg/m²/day, 5.74 ± 0.54 nM and 1117 ± 125 ml/min/m² at 3.75 mg/m²/day, 9.66 ± 1.59 nM and 854 ± 157 ml/min/m² at 4.5 mg/m²/day, and 3.23 ± 0.31 nM and 2624 ml/min/m² at 5.4 mg/m²/day. The aggregate mean (+ SE) CI during the first and second courses was 1220 ± 195 and 1073 ± 181 ml/min/m², respectively. Although mean CI-980 CI values seem to decrease disproportionately as CI-980 doses are increased from 3.75 to 4.5 mg/m²/day, examination of the overall aggregate data does not absolutely support nonlinear pharmacokinetic behavior. The significant interindividual variability, the overall paucity of data, the fact that a probable outlier at the 4.5 mg/m²/day dose level artifically weights mean values at this dose level, and the absence of a trend at the 5.4 mg/m²/day dose level argue against nonlinearity. Although the difference in the aggregate mean values was not statistically significant (P = 0.86, two-tailed Mann-Whitney U test), paired analysis of CI values in seven patients in whom Cₘ was measured during both courses revealed a marginally significant difference (P = 0.07, two-tailed paired t test).

Relationships between CI-980 Cₘ and principal clinical effects were also evaluated. The propensity to develop neurotoxicity of any grade was not related to the magnitude of Cₘ; aggregate mean Cₘ was 7.71 ± 1.7 nM for patients who experienced CNS toxicity and 5.99 ± 0.06 nM for patients without CNS complaints (P = 0.62, Mann-Whitney two-sample test). However, one of the two patients who developed severe (grade 3) CNS toxicity had the highest Cₘ (16.5 nM) in the study, whereas the other patient had the lowest Cₘ (4.27 nM) at the 4.5 mg/m²/day dose level. The relationship between the CI-980 Cₘ and the mean percentage decrease in ANC from baseline was evaluated by examination of the data scatterplot (Fig. 4). Over the CI-980 concentration range of 2.5–8 nM (17 cases), there was marked variability in the percentage decrements in ANC, with values spanning the entire range from 0–100%. For the few subjects with CI-980 Cₘ above 8 nM (four cases), the percentage decreases in ANC are all greater than 75%. Mathematical modeling of the relationship was not possible because of the absence of data in the crucial low CI-980 concentration range as well as the variability in the relationship for the majority of the subjects. The consistent large percentage decreases in ANC in the sub-

**Table 3** Hematological toxicity

<table>
<thead>
<tr>
<th>Dose level (mg/m²/day)</th>
<th>No. of evaluable courses</th>
<th>Nadir ANC (per μL) Median (range)</th>
<th>Neutrophils</th>
<th>Platelets</th>
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<tr>
<td></td>
<td></td>
<td>1–2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>3.00</td>
<td>7</td>
<td>2860 (2520–5776)</td>
<td>0</td>
<td>0</td>
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<tr>
<td>3.75</td>
<td>17</td>
<td>2856 (234–13552)</td>
<td>2</td>
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<tr>
<td>4.50</td>
<td>16</td>
<td>966 (18–2193)</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>5.40</td>
<td>2</td>
<td>1303 (1056–1551)</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

⁺ One course was associated with the development of fever requiring hospitalization for parenteral antibiotics.

**Fig. 2** Plasma drug disposition curve of a patient treated at a CI-980 dose level of 3.75 mg/m²/day. Inset, early sampling times.
jects with CI-980 concentrations above 8 nM suggest that appreciable neutropenia may be expected with C_{ss} at 8 nM or above.

**DISCUSSION**

CI-980, a synthetic antimicrotubule agent that was originally synthesized to be an inhibitor of folate synthesis, is a unique candidate drug for development for several reasons: (a) CI-980 seems to bind to the colchicine binding domain of tubulin, which is not a subcellular target of the antineoplastic agents in broad clinical use (2, 4, 6, 14); (b) the spectrum and degree of cytotoxic activity noted with CI-980 in preclinical studies has been wide, particularly in comparison to other agents that induce microtubule disassembly, such as the Vinca alkaloids (7, 8); (c) in contrast to the Vinca alkaloids, colchicine, and the taxanes, multidrug resistance due to P-glycoprotein overexpression does not seem to confer cross-resistance to CI-980 (7, 8, 15); and (d) preclinical evaluations in mice indicate that CI-980 possesses comparable antitumor activity whether given i.v., i.m., or p.o., which has many potential practical implications in clinical practice (7, 8).

The results of the present study demonstrate that both myelosuppression and CNS effects are the principal toxicities of CI-980 administered as a continuous 72-h infusion. Although toxicities were formidable in previous studies using shorter infusions (9, 10), both hematological and CNS effects were predictable and tolerable at CI-980 doses below 4.5 mg/m^2/day in this study, even in heavily pretreated subjects. In contrast, the MTD and dose recommended for additional Phase II evaluations, 3.75 mg/m^2/day, produced consistent and tolerable toxicity; principally brief, isolated neutropenia. Although other toxic effects were occasionally noted at doses below 4.5 mg/m^2/day, they were mild to moderate (≤grade 2) in severity and were not associated with clinically significant sequelae. These results contrast somewhat with those of a previous Phase I study, which evaluated the effects of progressively longer infusion durations (10). In this study, CNS toxicity and hypertension occurred frequently when CI-980 was administered over 1, 3, and 6 h daily for 3 consecutive days. However, myelosuppression alone precluded further dose escalation of CI-980 above slightly higher doses compared to the present study, 4.35 and 5.4 mg/m^2/day in poor- and good-risk patients, respectively, when the infusion duration was prolonged to 24 h daily (72-h continuous infusion). The preliminary results of two Phase II studies of CI-980 as a 72-h infusion at 4.5 mg/m^2/day in patients with refractory ovarian and colorectal carcinoma have been reported (16, 17). A total of 43–50% of patients experienced grade 4 neutropenia of unspecified duration. Although the majority of patients were also reported to experience mild, reversible CNS toxicities, hallucinations and unresponsiveness were also described (16, 17). However, neither detailed final toxicological results nor pharmacological results of these studies have been published to date; therefore, it is difficult to formally compare these results to the present study.

In contrast, similar toxicities and toxicological susceptibility have been reported in a concurrent Phase I trial of CI-980 on an identical 72-h infusion schedule (18). In this study, prolonged grade 4 neutropenia and grade 4 thrombocytopenia were noted in several patients treated at the 4.2 mg/m^2/day dose level, whereas the 3.5 mg/m^2/day dose level was tolerable. CNS toxicity, generally manifested as transient cognitive dysfunction at the end of treatment, also occurred in two patients with primary CNS malignancies. In the present study, no specific clinical factors such as concomitant medications that may predispose patients to developing CNS toxicity were evident. In fact, both patients who developed severe CNS effects had no objective evidence of brain metastases. Although the development of severe CNS toxicity seemed to be dose-related, no relationship between CI-980 C_{ss} and CNS toxicity was identified. However, one of the two subjects who developed severe CNS toxicity had the highest CI-980 C_{ss} in the study, but the other involved patient’s C_{ss} was among the lowest. These data suggest that there are other as yet unidentified factors that predispose patients to CNS toxicity.

Besides demonstrating a tolerable toxicity profile at the 3.75 mg/m^2/day dose level, CI-980 C_{ss} (mean, 5.74 ± 0.54 nM; range, 2.80–6.57 nM) approached and in most cases exceeded
IC\textsubscript{50} values for most murine and human leukemias and solid tumors in vitro, which are in the nanomolar and subnanomolar range (7, 8). Preclinical studies have demonstrated that the cytotoxicity of CI-980 is highly dependent on the duration of exposure both in vitro and in vivo (7, 8). To illustrate this, the CI-980 ID\textsubscript{50} against L1210 leukemia in vitro CI-980 was approximately 50,000-fold higher for a 1-h treatment period compared to a 24-h treatment period, whereas the cytotoxicity of vincristine varied only 7-fold (7, 8). Therefore, the 72-h schedule may be an optimal schedule for development, not only from a toxicological standpoint, but because of the optimal antitumor activity noted with schedules resulting in more prolonged exposure. However, a concern that was not sufficiently addressed in the present study due to the high proportion of patients who had indwelling venous catheters for drug administration is the potential for CI-980 to induce venous toxicity, which was experienced by several patients. CI-980 has been shown to be a vesicant in a murine model in which i.d. administration of clinically relevant local doses induced ulcerative lesions at the injection site (19). Compared to vincristine, however, local tissue damage was significantly less severe and resolved sooner.

The present study demonstrates that the administration of CI-980 as a continuous 72-h infusion is feasible, confirming the conclusions of other investigations (10, 18). Although both myelosuppression and CNS toxicity are the principal DLTs of CI-980 on this schedule, these effects are typically mild to modest, brief, and rarely associated with complications at the recommended Phase II dose, 3.75 mg/m\textsuperscript{2}/day, even in heavily pretreated patients. If this initial dose is well tolerated, the results of the present study suggest that higher doses could also be administered safely in some subjects; therefore, intraindividual dose escalation may be feasible in some cases. However, formal recommendations for intraindividual dose escalation were not evaluated. Thus, the acceptable toxicity profile of CI-980, the broad antitumor spectrum demonstrated for CI-980 in preclinical studies, and the achievement of biologically relevant plasma concentrations at tolerable doses indicate that the agent may warrant further disease-oriented development in Phase II trials. However, it may also be worthwhile to evaluate alternate administration schedules, such as intermittent dosing or even longer infusions, that may offer advantages from both toxicological and therapeutic standpoints.

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