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A phase I trial and pharmacokinetic evaluation of CI-980 in patients with advanced solid tumors

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Abstract

CI-980 is a synthetic mitotic inhibitor that binds to the colchicine binding site of tubulin. It demonstrates broad activity against human and murine tumor models and shows no cross resistance with tumor models whose mechanism of resistance is mediated by P-glycoprotein (MDR-1). A phase I study was completed in 25 patients with solid tumors using a 24-hour infusion schedule, with courses repeated every 3 weeks. Eight dose levels were tested between 1.2 and 15.6 mg/m^2. The maximum tolerated dose was 14.4 mg/m^2. Neutropenia was dose-related but not dose-limiting; thrombocytopenia was infrequent. CNS toxicities were dose-limiting and consisted of dizziness, headache, loss of coordination, loss of consciousness, nervousness, and other symptoms. These events occurred near the end of the infusion and were reversible, usually within 24 hours. One patient who was to be treated at dose level 8 (intended dose was 19.2 mg/m^2; actual dose was 15.6 mg/m^2) became encephalopathic prior to completion of the infusion. Other adverse events included gastrointestinal toxicities (nausea, vomiting, anorexia, constipation, stomatitis, dyspepsia, bleeding, cheilitis), IV site erythema, fever, and fatigue. A partial response was observed in one patient with colon cancer and reductions in CA-125 levels were observed in 2 patients with ovarian cancer. Pharmacokinetics were linear and dose-proportional. Results indicate high systemic clearance and wide tissue distribution. Mean pharmacokinetic parameter values: $T_{1/2}$ = 5.52 hours, plasma clearance 1163 mL/min/m^2, and Vd_{ss} 376 L/m^2.

Introduction

CI-980, ethyl (S)-(5-amino-1, 2-dihydro-2-methyl-3-phenylpyridol[3,4-b]pyrazin-7-yl)carbamate 2-hydroxyethanesulfonate (1:1) (Figure 1), is a synthetic antineoplastic agent that acts as a mitotic inhibitor by blocking tubulin polymerization [1]. It binds to the colchicine binding site on tubulin, distinct from that of the vinca alkaloids and taxanes. CI-980 was more potent and more active than vincristine in *in vitro* testing against murine and human tumor cell lines. *In vivo* testing demonstrated similar activity when compared to vincristine in many tumors, including P388 and L1210 leukemias, B16 melanoma, and several colon cancer lines [2].

In preclinical studies, CI-980 retained activity regardless of the route of drug administration and the site of tumor implantation, a characteristic not found in currently approved mitotic inhibitors. Unlike the vinca alkaloids, no cross-resistance to CI-980 was found in a large number of drug-resistance tumor models,

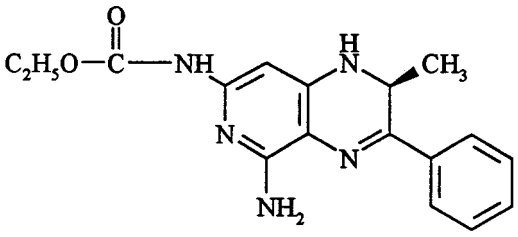

Figure 1. CI-980 structure.

whether tested *in vitro* or *in vivo*. This implies that CI-980 may not be susceptible to pleiotrophic drug resistance and therefore could potentially be a useful agent in patients previously treated with anthracyclines, vinca alkaloids, etc. CI-980 exhibited marked schedule dependency *in vitro* and *in vivo*. Using a clonogenic assay, long exposure times (i.e., 24 hours and continuous) were approximately 10^5 times more effective than shorter exposure times (i.e., 1 and 4 hours) in inhibiting colony formation of L1210 cells. In addition, various CI-980 treatment schedules were studied in mice implanted with P388 leukemia or mammary 16/C tumor. The optimal schedules appeared to be those that provided a prolonged exposure to CI-980 [3].

In animals, the toxicologic profile of CI-980 was consistent with the action of mitotic inhibitors, affecting primarily rapidly dividing cell populations. Target organ effects included bone marrow suppression, lymphoid depletion, enteropathy, epithelial necrosis, testicular tabular degeneration, and degenerative urinary bladder lesions [4]. In addition, endosteal new bone formation and stromal proliferation were observed in rats [5]. Peripheral neuropathy could not be evaluated in the animal studies but was an anticipated toxicity in the clinical trials, because of the similarity in the mechanism of action with vinca alkaloids.

Based on these findings, four CI-980 Phase I studies have been conducted, each designed to provide a prolonged period of exposure. One study treated patients over a 3-day period using intravenous infusions of various durations [6, 7]. Two studies treated patients using a continuous intravenous infusion for 72 hours [8, 9]. The study reported here utilized a 24-hour infusion of CI-980, with courses repeated every 3 weeks. The objectives of this study were to determine the tolerance and pharmacokinetic parameters of CI-980 when administered using this schedule in patients with solid tumors. Preliminary results have been presented [10].

Patients and methods

Patient selection

Patients were eligible if they had a histologically confirmed advanced solid tumor which was refractory to standard chemotherapy. Measurable or evaluable disease was required, as was a performance status of 0–2 (ECOG) and an expected survival of at least 8 weeks. Laboratory values were obtained within one week prior to the start of CI-980 therapy and were required to be as follows: BUN $\leq$ 30 mg/dL, serum creatinine $\leq$ 1.5 mg/dL, bilirubin and SGOT < 1.5 times the upper limit of normal, normal serum calcium, sodium $\geq$ 130 mEq/L, white blood count $> 3.0 \times 10^3/\mu$l, absolute granulocyte count $\geq 1.5 \times 10^3/\mu$l, platelet count $\geq 100 \times 10^3/\mu$l. All patients were informed of the investigational nature of the study and provided written informed consent. The study was approved by the institutional review board of Montefiore Medical Center.

Due to the similarity in the mechanism of action between CI-980 and the vinca alkaloids and taxanes, it was anticipated that CI-980 might cause peripheral neuropathy. Therefore, to allow an assessment of CI-980's toxicities, limitations were placed on the amount of prior chemotherapy allowed with these agents. Patients were excluded if they had received prior treatment with more than one vinca alkaloid, a vinca alkaloid and paclitaxel, or had a clinically significant peripheral neuropathy. Also excluded were patients with brain metastases unless they were clinically stable for 4 weeks. Because acute hypertension was reported in another Phase I study [7], patients were excluded if they had hypertension that required therapy with agents other than thiazides. Patients were also excluded if they had received chemotherapy within 4 weeks prior to the start of the study, or mitomycin or nitrosourea therapy within 6 weeks. Small port radiotherapy within 4 weeks was allowed for symptomatic relief.

Patient evaluation

Preliminary patient evaluation included a history and physical examination including a detailed neurologic review. Baseline studies included a complete blood count, electrolytes, renal and liver function tests, calcium magnesium, chest x-ray, ECG, and appropriate scans to identify disease sites. Levels of tumor markers were analyzed where applicable. Interim blood counts were obtained three times between treatment and day 15 of each course, and chemistry and electrolyte values were checked weekly. A chest x-ray was obtained on day 1 of each course in most patients. Relevant x-rays and scans were repeated after every 2–3 cycles to follow the course of the disease. During the first course, patients underwent blood pressure monitoring using an ambulatory blood pressure monitor.

A complete response was defined as complete disappearance of all known disease for at least 28 days, a partial response as $\geq$ 50% decrease in the sum of

the products of the greatest perpendicular diameters of bidimensionally measurable lesions, and progressive disease as a $\geq$ 25% increase in any tumor lesion. Toxicities were graded according to NCI Common-Toxicity Criteria. The MTD was defined as that dose which produced dose-limiting toxicity in at least one-half of the patients initially treated at that dose level. Nonhematologic dose-limiting toxicity was defined as grade 3 or 4 toxicity, or grade 1 or 2 toxicity lasting $\geq$ 6 weeks. Hematologic dose-limiting toxicity was defined as a granulocyte count nadir $\leq 0.5 \times 10^3/\mu$l, a platelet count nadir $\leq 50 \times 10^3/\mu$l, or failure to recover to $\geq 1.5 \times 10^3/\mu$l granulocytes or to $\geq 100 \times 10^3/\mu$l platelets within 6 weeks after treatment. Depending on the severity of toxicities encountered, the recommended Phase II dose would be either 75% or 100% of the MTD.

Drug formulation and administration

CI-980 was supplied by Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company (Ann Arbor, MI), as a sterile preservative-free lyophilized powder containing 10 mg of CI-980 base equivalent. The drug was reconstituted with Water for Injection to a concentration of 2 mg/mL and then further diluted with 5% Dextrose in Water to a concentration of 50 to 500 μg/mL, depending on the dose level. Doses were prepared in a 60cc Becton-Dickinson polyethylene syringe, primed through a Baxter microvolume tubing extension set, and administered via a Medfusion 2001 syringe pump over 24 hours. Because CI-980 adheres to certain plastics, infusions were administered through a peripheral vein using Teflon angiocaths.

The starting dose for this Phase I study, 1.2 mg/m^2, was determined from a series of toxicology studies. This dose was equivalent to 1/10 the LD_{10} following administration of a single intravenous bolus dose in mice, and was also the no-effect dose following administration of either a single intravenous bolus dose or a 24-hour continuous intravenous infusion in dogs (0.06 mg/kg).

Three new patients were treated at each dose level. Doses were escalated based on the modified Fibonacci escalation scheme. Courses were repeated every 3 weeks provided the patient recovered from drug-attributable adverse events. In the absence of progressive disease or toxicity, patients received the highest currently open dose level in subsequent courses.

Sample collection

CI-980 pharmacokinetics were studied during the first treatment course. Heparinized blood samples were collected at 0, 4, 8, 16, 23.5, 24 (end of infusion), 24.5, 25, 26, 28, 32, 36 and 48 hours following the start of the infusion. Plasma were harvested after centrifugation and the resulting samples were frozen at -70°C until analyzed. Urine samples were collected in Teflon or stainless steel containers at intervals of 0–6, 6–12, 12–24, 24–36, and 36–48 hours following the start infusion. In a few patients, urine was collected in two intervals: 0–24 and 24–48 hours. Total volume of urine collected over each interval was recorded and a 5 mL aliquot was removed, stored in Teflon vials, and frozen at -70°C until analyzed.

Analytical methods

Plasma CI-980 concentrations were determined with a validated HPLC assay with fluorescence detection [11]. Following the addition of PD 080658 (internal standard-IS) samples were vortexed and applied to Bond-Elut^{TM} C-18 cartridges preconditioned with methanol and water. Cartridges loaded with samples were washed with acetonitrile mixed with 0.2% ammonium acetate buffer (30:70). CI-980 and IS were eluted using a 60:40 mixture of acetonitrile and 0.2% ammonium acetate buffer. The eluate was evaporated to dryness and reconstituted in mobile phase. CI-980 was resolved from the IS using two Zorbax Rx C-18 columns connected in series and maintained at 30°C and a mobile phase of acetonitrile and 10 mM ammonium dihydrogen phosphate buffer (38:62). Detection was achieved using a fluorescence detector with an excitation wavelength of 388 nm and an emission wavelength of 473 nm. The range of the calibration curve was 0.2–25 ng/mL. Accuracy and precision of quality controls during validation of the assay were within 4.7% and 5.6%, respectively.

Urine CI-980 concentrations were determined in a manner similar to those of plasma concentrations. The main difference was that 1 mL of serum albumin (BSA) solution was added to every 5 mL of urine before the addition of PD 080658. Following this the IS was added and the samples were vortexed for 30 seconds. Urine samples were processed in the same fashion as plasma. The eluate from solid phase extraction was evaporated to dryness and reconstituted in mobile phase which is 40:60 of acetonitrile mixed with 10 mM ammonium dihydrogen phosphate buffer. A Zor-

bax Rx C-18 column maintained at 30–35°C was used to chromatograph the compound and internal standard. Detection was achieved using a UV detector at a wavelength of 380 nm. The range of calibration curve was 1–100 ng/mL. Accuracy and precision of quality controls during validation of assay were within 1.5% and 4.3%, respectively.

Pharmacokinetic methods

CI-980 pharmacokinetic parameters were determined from CI-980 plasma and urinary concentration-time data using noncompartmental methods. Maximum plasma CI-980 concentration (C_{max}) was recorded as observed. AUC(0–tldc) and AUMC(0–tldc), the AUC and AUMC from time zero to the time of the last detectable plasma concentration (ldc), respectively, were determined by Lagrange polynomial interpolation using LAGRAN [12]. The apparent elimination rate constant (λz) was estimated as the absolute value of the slope of the least squares linear regression line of the natural logarithm (ln) of plasma concentrations as a function of time during the terminal phase of drug disposition. Apparent elimination half-life ($T_{1/2}$) values were calculated as $0.693/\lambda z$. AUC(0–∞) values were determined by summing AUC(0–tldc) and $ldc/\lambda z$ values. AUMC(0–∞) values were determined from the sum of AUMC(0–tldc) and ($ldc/\lambda z^2 + ldc - tldc/\lambda z$). Total plasma clearance (CL_{TOT}) was calculated as dose/AUC(0-∞). Mean residence time (MRT) was calculated as ((AUMC(0–∞)/AUC(0–∞))-(T/2)) where T is the length of the constant intravenous infusion. Volume of distribution at steady state was calculated as CL_{TOT}-MRT. Renal clearance (CL_R) was determined by dividing the amount of CI-980 excreted in urine from 0 to 24 hours (Ae(0–24)) by the AUC from 0 to 24 hours (AUC(0–24)). CI-980 blood clearance was calculated as the product of the calculated plasma clearance and the ratio of plasma to blood concentrations.

Pharmacodynamic analysis

The pharmacodynamics of CI-980 were evaluated to correlate CNS toxicity and myelosuppression with some measure of CI-980 plasma exposure. CNS adverse event data were analyzed as a function of C_{max}, dose, and the time the plasma CI-980 concentration was above 7 ng/mL. The relationships between pharmacokinetic parameters (dose, AUC($0 - \infty$), C_{max}, CL_{TOT}) and the grade of CNS toxicity or precent decrease in neutrophils from baseline values were evaluated by linear regression analysis.

Table 1. Patient characteristics

Male/female	15/10
Median age (range)	62 (30–78)
Performance status	
0	2
1	19
2	4
Primary tumor type	
Adenocarcinoma of colon	10
Renal cell	3
Sarcoma	2
Ovarian	2
Small cell lung	1
Nonsmall cell lung	1
Squamous cell esophagus	1
Squamous cell head/neck	1
Squamous cell vulva	1
Melanoma	1
Mesothelioma	1
Breast	1
Prior treatment	
None	1
Chemotherapy	12
Chemotherapy + radiotherapy	11
Radiotherapy	1

Results

Treatment

Twenty-five patients with 11 different solid tumor diagnoses participated in this trial. Patient characteristics are shown in Table 1. This was a heavily pretreated patient population, having received a median of three chemotherapy regimens prior to entering the study. All patients met the eligibility requirements described in "Patients and methods".

One hundred-twelve courses of CI-980 treatment were administered over the dose range 1.2 to 15.6 mg/m^2 (range 1 to 24 courses per patient, median 3). Using the modified Fibonacci dose escalation scheme, 8 dose levels were required to reach the MTD (Table 2). Three new patients were treated at each dose level until toxicities at dose level 8 became apparent. Additional patients were then treated at dose level 6 (1 patient)

Table 2. CI-980 treatment dose and schedule

Dose level	Dose (mg/m^2)	Percent increase[#]	New patients	Total patients	Number of courses
1	1.2	– –	3	3	5
2	2.4	100	3	4	8
3	4.0	67	3	4	6
4	6.0	50	3	6	10
5	8.4	40	3	9	17
6	10.8	29	4	13	26
7	14.4	33	5	9	39
8	19.2[*]	33	1	1	1
Total			25		112

[*] Actual total dose administered = 15.6 mg/m^2.
[#] Percent increase over prior dose level.

and dose level 7 (2 patients) to confirm the toxicity findings.

Twelve patients received escalated doses and two patients received deescalated doses in subsequent courses. Six patients remained on study for an extended treatment of $\geq$ 7 courses, including one patient who received 24 courses of CI-980 (total dose of 568 mg).

Adverse events

Neurotoxicity. Acute reversible neurotoxicity principally affecting the central nervous system (CNS) was the dose-limiting toxicity. The most common neurologic adverse events were those suggestive of cerebellar dysfunction, including dizziness, loss of coordination, ataxia, and tremors. Eleven of 25 patients (44%) reported neurologic adverse events. Many of these events were first reported after the decision was made to deescalate the dose following the adverse events described below at the 19.2 mg/m^2 dose level.

Table 3 shows the dose-relationship of neurologic adverse events that were considered to be related to study treatment. The lowest dose that was associated with any neurologic adverse event was 6.0 mg/m^2, where three separate grade 1 events were reported. At 10.8 mg/m^2, several grade 1 and 2 neurologic adverse events were reported, including dizziness, headache, ataxia, loss of coordination, and one grade 3 event (loss of consciousness). The largest dosing experience was obtained at the 14.4 mg/m^2 dose level where a total of 39 courses of treatment were given. Seven of 9 patients treated at this dose level reported at least one neurologic adverse event, including three grade 3 events of dizziness, loss of consciousness, and hand tremors. One patient was treated at the 19.2 mg/m^2 dose level and experienced multiple neurologic adverse events, described below.

CNS symptoms usually began during or within one day following the CI-980 infusion. All CNS adverse events were reversible, usually within a few days after completion of the infusion. Neurotoxicity commonly occurred after the first course of treatment and did not appear to be cumulative in those patients who were treated with multiple courses.

Two patients experienced a total of 3 episodes of loss of consciousness. One patient's initial episode occurred on day 2 following treatment at 14.4 mg/m^2, and a second episode occurred on day 2 following a subsequent course of treatment at 10.8 mg/m^2. The patient quickly recovered consciousness following both episodes. There was no seizure activity or incontinence associated with these events. Another patient began CI-980 treatment at the 19.2 mg/m^2 dose level and experienced significant CNS toxicities. Within several hours of starting the infusion, the patient described a band-like pain across the chest and abdomen, followed by severe peripheral vasoconstriction, diaphoresis, agitation, confusion, and hypertension. Because of worsening symptoms, the CI-980 infusion was terminated after 19 hours, delivering an estimated total dose of 15.6 mg/m^2. Within one-half hour of discontinuing the infusion, the diaphoresis and skin changes resolved. However, during the next day the patient's neurologic condition deteriorated, exhibiting hyperreflexia, flacidity, a right gaze preference, and Babinski signs. The patient's level of consciousness fluctuated over the ensuing four days before recovering completely. Because there was a reduction in this ovarian cancer patient's CA-125 levels following this first CI-980 treatment, the patient insisted on being retreated. She experienced no recurrence of CNS adverse events following additional treatment courses at 10.8 mg/m^2.

Myelosuppression. Meylosuppression was dose-related but not dose-limiting (Table 4). Neutropenia did not occur following doses of 1.2–8.4 mg/m^2. At 14.4 mg/m^2, grade 3 or 4 neutropenia occurred in 4 of 39 courses. Nadirs occurred early, usually between day 6 and 12 of each course; the median time to recovery was day 14. Thrombocytopenia was infrequent and generally was not clinically significant. One patient treated at 14.4 mg/m^2 and one patient treated at 15.6 mg/m^2 developed grade 2 thrombocytopenia.

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