A Phase I Pharmacologic and Pharmacogenetic Trial of Sequential 24-Hour Infusion of Irinotecan Followed by Leucovorin and a 48-Hour Infusion of Fluorouracil in Adult Patients with Solid Tumors

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Abstract

Purpose: In preclinical studies, sequential exposure to irinotecan (CPT-11) then fluorouracil (5-FU) is superior to concurrent exposure or the reverse sequence; a 24-hour infusion of CPT-11 may be better tolerated than shorter infusions.

Experimental Design: CPT-11 was first given at four levels (70-140 mg/m²/24 hours), followed by leucovorin 500 mg/m²/0.5 hours and 5-FU 2,000 mg/m²/48 hours on days 1 and 15 of a 4-week cycle. 5-FU was then increased in three cohorts up to 3,900 mg/m²/48 hours.

Results: Two patients had dose-limiting toxicity during cycle 1 at 140/3,900 of CPT-11/5-FU (2-week delay for neutrophil recovery; grade 3 nausea despite antiemetics); one of six patients at 140/3,120 had dose-limiting toxicity (grade 3 diarrhea, grade 4 neutropenia). Four of 22 patients with colorectal cancer had partial responses, two of which had prior bolus CPT-11/5-FU. The mean 5-FU plasma concentration was 5.1 µmol/L at 3,900 mg/m²/48 hours. The end of infusion CPT-11 plasma concentration averaged 519 nmol/L at 140 mg/m²/24 hours. Patients with UDP-glucuronosyltransferase (UGT1A1; TA) 6/6 promoter genotype had a lower ratio of free to glucuronide form of SN-38 than in patients with TA 7 allele. Thymidylate synthase genotypes for the 28-base promoter repeat were 2/2 (13%), 2/3 (74%), 3/3 (13%); all four responders had a 2/3 genotype.

Conclusions: Doses (mg/m²) of CPT-11 140/24 hours, leucovorin 500/0.5 hours and 5-FU 3,120/48 hours were well tolerated.

The rationale for evaluating sequential i.v. infusions of irinotecan (CPT-11) over 24 hours followed by leucovorin-modulated fluorouracil (5-FU) over 48 hours is based on the following considerations. The cytotoxicity of camptothecin analogues is highly dependent on ongoing DNA replication and the fraction of cells in S phase (1, 2). Prolonged exposure to CPT-11 is active in both preclinical and clinical settings, may be better tolerated than 30- to 90-minute infusions, and acute cholinergic symptoms may be diminished (3–7). From a pharmacologic perspective, a slower infusion rate may avoid saturation of glucuronidation of the active metabolite, SN-38, which in turn may be associated with less severe delayed diarrhea, and conversion of CPT-11 to SN-38 by hepatic carboxylesterase may be more efficient (8, 9). In preclinical models, CPT-11 given 6 to 24 hours prior to inhibitors of thymidylate synthase is the most effective sequence (10–17). By depleting TTP pools, thymidylate synthase inhibition in turn inhibits DNA synthesis. Because active DNA synthesis is required to convert the formation of covalent SN-38-topoisomerase I–cleavable complexes to a cytotoxic lesion, inhibition of thymidylate synthase during or prior to formation of the SN-38 cleavable complex is counterproductive. An every-other-week schedule of leucovorin-modulated bolus/infusional 5-FU (LV5FU2) is superior to i.v. daily for 5 days bolus 5-FU/leucovorin every 4 weeks in terms of response rate, time to progression, and toxicity profile (18). A modified schedule using leucovorin before 5-FU on the first day only followed by a continuous infusion of 5-FU over 46 to 48 hours is more convenient than requiring the patient to return on day 2 for additional leucovorin and bolus 5-FU (19, 20).
In addition to identifying the recommended doses of CPT-11 and 5-FU given on this sequential infusion schedule, we planned to evaluate the pharmacokinetics of 5-FU and CPT-11, and its metabolites, SN-38, in the free and glucuronides, and 7-ethyl-10-[4-N-([5-amino-2-pentanoic acid]-1-piperidinyl)-carbonyloxycamptothecin (APC), and polymorphisms in the promoter region of the genes encoding for thymidylate synthase and UDP glucuronosyltransferase 1A1 (UGT1A1).

The human thymidylate synthase promoter has three tandem repeats of a 28-nucleotide G/C-rich sequence in the first 100 nucleotides upstream of the AUG codon (the translation start site), as well as one inverted copy of the repeat starting at −139 (21–24). At least one tandem repeat is needed for efficient translation of thymidylate synthase mRNA; the number of repeats influences the efficiency of translation, and is polymorphic (25–27). Patients may be homozygous for a double or triple tandem repeat (2/2 or 3/3), or be heterozygous (2/3; ref. 28). Several investigators have reported an association between the genotype of the thymidylate synthase enhancer region and response to 5-FU-based therapy in patients with colorectal cancer (29–32).

Glucuronidation of the SN-38 metabolite of irinotecan represents a detoxification mechanism; patients with a reduced capacity to conjugate SN-38 have a higher risk of experiencing severe or life-threatening toxicity with irinotecan therapy (8, 33–35). A polymorphism in the promoter region of the human UGT1A1 gene has been described with variation of a 2-bp doublet, TA; the (TA)6TAA element is the normal pattern (36–38). Patients with Gilbert’s syndrome, a benign form of unconjugated hyperbilirubinemia, are homozygous for the 7/7 allele (38, 39). Recent information suggests that patients who are homozygous (6/7) or homozygous for the TA-7 repeat (7/7) have a decreased capacity to conjugate SN-38, and are more likely to have severe diarrhea and myelosuppression with irinotecan therapy (38–41).

Patients and Methods

Patient selection. This study was activated on April 2001; accrual was completed on May 2003. Patients with locally advanced, but nonresectable primary or recurrent solid tumors, or metastatic disease who have failed standard therapy for their disease or for whom no such therapy is available, and patients with metastatic adenocarcinoma for whom this regimen represents a reasonable initial therapy were eligible. Other inclusion criteria included a performance status of 2 or better; absolute neutrophil count ≥2,000/mcL; platelets ≥100,000/mcL; bilirubin and creatinine ≤1.6 mg/dL, and aspartate aminotransferase ≤4 x normal. Exclusion criteria included known central nervous system primary tumor or metastases, active ischemic heart disease, congestive heart failure class III or IV, symptomatic arrhythmia, active infections, or other serious concurrent medical illness that would jeopardize the ability of the patient to receive this treatment safely. This study had the approval of the Institutional Review Board of the National Cancer Institute (protocol 01-C-0082) and the National Naval Medical Center (project B00-038). All patients gave written informed consent.

Treatment plan. Study drugs were obtained from commercial sources. Chemotherapy was given using an implanted central venous catheter with an ambulatory infusion pump. Warfarin (1 mg daily) was given for prophylaxis against catheter-related thrombosis. The starting doses were CPT-11, 70 mg/m2/24 hours given on days 1 and 15; leucovorin, 500 mg/m2/30 minutes given on days 2 and 16; and 5-FU, 2,000 mg/m2/48 hours given on days 2 and 16. Initially, the doses of leucovorin and 5-FU were held constant, and the dose of CPT-11 was escalated in 25% increments of 90, 110, and 140 mg/m2/24 hours. This initial target for the CPT-11 dose was based on the results of two trials involving a 24-hour CPT-11 infusion (42, 43). In one trial, the recommended dose of CPT-11 was 140 mg/m2/24 hours on day one in combination with uracil/fluorouracil, 400 mg twice daily, on days 1 to 7 every two weeks (42). In the other trial, the recommended dose of CPT-11 given as a weekly 24-hour infusion was 70 mg/m2 (43). 5-FU dose escalation then proceeded in 25% increments. Dose escalation continued until dose-limiting toxicity during the initial cycle was seen in two of three patients at a level defined as an absolute neutrophil count nadir <500/mcL, platelet nadir <25,000/mcL, >grade 3 non-hematologic toxicity, or treatment delay of 2 or more weeks for resolution of toxicity. If dose-limiting toxicity was seen in one patient, one to five additional patients were entered at the same dose level. If dose-limiting toxicity was seen in two patients, no further patients were to be entered at this dose level, and additional patients would be entered one dose level below. Patients received 10 mg dexamethasone and 24 mg ondansetron i.v. 30 minutes prior to the start of irinotecan, and took ondansetron 8 mg orally twice daily for eight doses starting on the evening of irinotecan administration.

Dose modifications on days 15 and 16 within a treatment cycle were as follows: if the absolute neutrophil count was ≥1,000/mcL, the platelets were 50 to 74.9 × 1,000/mcL, or diarrhea was grade 1 in severity, the dose of CPT-11 was decreased by 25%, whereas full doses of 5-FU were given. If the absolute neutrophil count was 500 to 999/mcL, platelets 25 to 49.9 × 1,000/mcL, or diarrhea was grade 2 in severity, the dose of CPT-11 was reduced by 50%, and the dose of 5-FU was decreased by 25%. Both CPT-11 and 5-FU would be held for an absolute neutrophil count <500/mcL, platelets <25,000/mcL, or grade 3 diarrhea.

The next cycle could proceed on day 29 if the absolute neutrophil count was >1,500/mcL, the platelets were >75,000/mcL, and non-hematologic toxicities (excluding alopecia) had resolved. Dose modifications at the start of the next cycle were based on the following criteria. If the absolute neutrophil count nadir and the platelet nadir were ≥1,000/mcL and >50,000/mcL, and nonhematologic toxicity was grade 1 or less, the dose of either CPT-11 or 5-FU could be increased one dose level provided that full doses were given midcycle of the prior cycle, and there was no treatment delay. The doses of either CPT-11 or 5-FU were preferentially reduced depending on which drug was being escalated at the time. If the absolute neutrophil count nadir was between 501 and 999/mcL, the platelet nadir was 25,000 to 49,900/mcL, and nonhematologic toxicity was grade 2 or less in severity, the doses remained the same provided that full doses were given; otherwise, the dose of CPT-11 or 5-FU was decreased by one level. If the absolute neutrophil count was <500/mcL, the platelets were <25,000/mcL, or grade 3 nonhematologic toxicity occurred, the dose of CPT-11 or 5-FU was decreased by one level if full doses were given midcycle, and by two dose levels if full doses could not be given on days 15 and 16 of the prior cycle. If the absolute neutrophil count was <1,500/mcL, platelets were <75,000/mcL, or nonhematologic toxicity was grade 1 or worse after a 1-week delay (day 36), therapy was held for another week. If the absolute neutrophil count was 1,000 to 1,499/mcL, the platelets were 25 to 49.9 × 1,000/mcL, or diarrhea was grade 2 in severity, the dose of CPT-11 was reduced by 25%, whereas full doses of 5-FU were given. If the absolute neutrophil count was 500 to 999/mcL, platelets 25 to 49.9 × 1,000/mcL, or diarrhea was grade 2 in severity, the dose of CPT-11 was reduced by 50%, and the dose of 5-FU was decreased by 25%. Both CPT-11 and 5-FU would be held for an absolute neutrophil count <500/mcL, platelets <25,000/mcL, or grade 3 diarrhea.

Toxicity was assessed by the National Cancer Institute Common Toxicity Criteria, version 2.0. A complete blood count with differential was obtained weekly. A complete metabolic panel, prothrombin time, urinalysis, and pertinent tumor markers were obtained at the start of each 4-week cycle. Restaging studies were repeated every other 4-week cycle; the studies were repeated 4 weeks later to confirm an objective response, and assessed using the RECIST criteria (44).
Pharmacokinetic methodology. Venous blood was collected in 10 mL green-top Vacutainer tubes (BD, Franklin Lakes, NJ) containing heparin. The samples were placed immediately on ice, and then centrifuged at 1,000 × g for 10 minutes at 4°C; the plasma was frozen at −70°C until analyzed. Camptothecin, 5-chlorouracil, and β-glucuronidase were obtained from Sigma Chemical Company (St. Louis, MO). CPT-11, SN-38, and APC was generously provided by Pharmacia (Kalamazoo, MI) through a material transfer agreement with the National Cancer Institute. High-performance liquid chromatography solvents were obtained from Fisher (Fair Lawn, NJ). Chromatographic separation was achieved with a Beckman (Beckman degasser, a 717 plus autosampler, and a 2,475 fluorescence detector. A 3% Tris-borate EDTA-low melting point agarose Genomic DNA Kit (Clontech, Mountain View, CA). The PCR products were separated on a 3% Tris-borate EDTA-low melting point agarose mini-gel electrophoresed at 60 V for 3 hours at 4°C. After ethidium bromide staining of the gel, the number of tandem repeats was determined by visualization of the size of the amplified DNA fragments.

Results. Thirty-two patients were enrolled in the trial; all but two had measurable disease. The performance status was 0, 1, and 2 in 9, 17, and 6 patients, respectively. Male patients predominated three to one. Twenty-two patients had colorectal cancer, three patients each had pancreas, appendix or small bowel, or esophagogastric primaries, whereas one had an unknown primary. Nine patients had no prior chemotherapy, whereas the number with one, two, or three or more prior regimens was seven, eight, and eight, respectively. Nine patients had prior irinotecan, 21 had prior 5-FU or capecitabine, and 10 had prior radiation.

Toxicity. The median number of cycles received was 4.5 (range 1-29). Dose-limiting toxicity during the initial cycle of therapy was not observed at the first two dose levels of CPT-11 (70-90 mg/m2/24 hours) with fixed doses of leucovorin and 5-FU 2,000 mg/m2/48 hours. One of the first three patients enrolled at CPT-11 110 mg/m2/24 hours experienced grade 4 hematologic toxicity, which was the limiting dose of CPT-11. However, the median number of cycles received was 4.5 (range 1-29). Dose-limiting toxicity during the initial cycle of therapy was not observed at the first two dose levels of CPT-11 (70-90 mg/m2/24 hours) with fixed doses of leucovorin and 5-FU 2,000 mg/m2/48 hours. One of the first three patients enrolled at CPT-11 110 mg/m2/24 hours experienced grade 4 neutropenia. An additional three patients were added to this cohort, but none experienced dose-limiting toxicity. The dose of CPT-11 was therefore increased to 140 mg/m2/24 hours.
One of three initial patients experienced grade 3 diarrhea. The cohort was expanded to six patients; no other dose-limiting toxicities were seen. As planned, the dose of CPT-11 was then fixed at 140 mg/m²/24 hours and the dose of 5-FU was subsequently escalated in 25% increments. As only one of six patients who received 5-FU 3,120 mg/m²/48 hours experienced grade 4 neutropenia, the 5-FU dose was escalated to 3,900 mg/m²/48 hours. At this dose, one patient with pancreatic cancer had an unWitnessed death in her sleep at home on day 7 of cycle one. Although the cause of death was unknown, she had reported no side effects to her family, and the relationship to chemotherapy was unlikely. Two other patients experienced dose-limiting toxicity: 2-week treatment delay to permit recovery of the absolute neutrophil count to ≥1,500/mCL, and grade 3 nausea/vomiting despite antiemetic therapy.

A total of 11 patients received one or more cycles of therapy at the recommended doses of CPT-11 140 mg/m²/24 hours and 5-FU 3,120 mg/m²/48 hours (including three patients escalated from lower doses and two patients de-escalated from higher doses; Table 1). Full doses on day 15 were received in 8 of the 11 patients and 28 of 31 cycles. Three patients experienced grade 3 diarrhea, one of which also had grade 4 neutropenia. One patient with known abdominal carcinomatosis experienced grade 3 constipation/ileus. These symptoms recurred in subsequent cycles despite dose reductions in both CPT-11 and 5-FU, suggesting that carcinomatosis was a possible contributing factor. Another patient had 1 day of grade 3 vomiting requiring i.v. hydration on day 4. He had not experienced nausea or vomiting the prior cycle at the same dose. Although it was not clear if this was treatment-related, the 5-FU dose was reduced to 2,500 mg/m²/48 hours with CPT-11 maintained at 140 mg/m²/24 hours. He only experienced one episode of grade 1 nausea/vomiting during the next seven cycles. Among four patients who received three or more cycles at the recommended dose of 140/3,120 mg/m², all received full doses on day 15, suggesting that cumulative toxicity was not a problem.

When the worst nonhematologic toxicity experienced by each patient across all 231 cycles of therapy was considered, four patients had grade 3 diarrhea (12.5%), and grade 2 mucositis occurred in 9.4% of patients. Three patients had grade 3 vomiting (9.4%); in one patient, this occurred in the setting of documented acute pancreatitis. One patient each had grade 3 or 4 ileus/constipation; both had documented abdominal carcinomatosis. No patient experienced acute CPT-11-associated cholinergic symptoms. Hematologic toxicity was not a major problem; one patient had grade 4 neutropenia (3%); the worst platelet toxicity was grade 2 in one patient. One patient had grade 3 anemia.

**Clinical benefit.** Four of 30 patients with measurable disease experienced a confirmed partial response (13%). All responses were seen among 22 patients with colorectal cancer. Two of these had not had prior 5-FU or CPT-11; the other two received prior short infusion of irinotecan with bolus 5-FU/leucovorin and experienced disease progression. Two of the responders were entered at CPT-11/5-FU 110/2,000 mg/m², and two were at 140/2,000 mg/m². The median time to treatment failure for all 32 patients was 178 days (range 6-826 days); 25% had a median time to treatment failure of >1 year.

**Pharmacokinetic analyses.** The mean steady state plasma concentration values and clearances of 5-FU are shown in Table 2. At the recommended dose of 3,120 mg/m²/48 hours, the average steady state plasma concentration was 4.4 nmol/mL. The plasma concentration versus time of CPT-11 and 5-FU was not clear if this was treatment-related, the 5-FU dose was reduced to 2,500 mg/m²/48 hours with CPT-11 maintained at 140 mg/m²/24 hours. He only experienced one episode of grade 1 nausea/vomiting during the next seven cycles. Among four patients who received three or more cycles at the recommended dose of 140/3,120 mg/m², all received full doses on day 15, suggesting that cumulative toxicity was not a problem.

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### Table 1. Worst toxicity per patient treated with CPT-11 140 mg/m² and 5-FU 3,120 mg/m² (11 patients and 33 cycles)

<table>
<thead>
<tr>
<th>Grade</th>
<th>WBC 1</th>
<th>Absolute neutrophil count 4</th>
<th>Hemoglobin 8</th>
<th>Platelets 0</th>
<th>Diarrhea 3</th>
<th>Constipation 2</th>
<th>Mucositis 5</th>
<th>Fatigue 8</th>
<th>Nausea 4</th>
<th>Vomiting 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Grade</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 2. Mean steady state plasma concentrations of 5-FU

<table>
<thead>
<tr>
<th>5-FU dose (mg/m²/48 hours)</th>
<th>No. patients</th>
<th>Plasma concentration (nmol/mL)</th>
<th>Clearance (mL/minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,500</td>
<td>2</td>
<td>2.83 ± 0.63</td>
<td>2.98 ± 0.72</td>
</tr>
<tr>
<td>2,000</td>
<td>19</td>
<td>3.15 ± 1.42</td>
<td>3.81 ± 1.87</td>
</tr>
<tr>
<td>2,500</td>
<td>8</td>
<td>3.15 ± 0.3</td>
<td>4.270 ± 923</td>
</tr>
<tr>
<td>3,120</td>
<td>9</td>
<td>4.44 ± 1.28</td>
<td>4.018 ± 1.588</td>
</tr>
<tr>
<td>3,900</td>
<td>3</td>
<td>5.08 ± 0.61</td>
<td>3.718 ± 806</td>
</tr>
</tbody>
</table>

*NOTE:* The average of the plasma concentration at 22, 23, 45, and 46 hours for each patient were calculated. The data are shown as the mean ± SD; for 1,500 mg, the mean ± 1/2 range is shown.

**Fig. 1.** The plasma concentrations of CPT-11 and its metabolites versus time are shown for 10 patients receiving 140 mg/m². Points, mean; bars, SD.
metabolites at 140 mg/m² are shown in Fig. 1, and the average end of infusion values for all doses are shown in Table 3. At the recommended dose of 140 mg/m²/24 hours, the mean plasma concentration values for CPT-11 was 519 nmol/L. APC and SN-38-total values were about 26% and 4% of that for CPT-11, respectively. The ratio of SN-38-free to SN-38-glucuronide was 33%, and did not appreciably vary across the 2.7-fold range in CPT-11 doses used in this study.

Pharmacodynamic analyses indicated that the percentage of change in neutrophil count did not fit a sigmoidal maximum effect model. Therefore, the data for 5-FU steady state plasma concentration and the biliary index were analyzed according to whether the associated percentage of change in absolute neutrophil count was above or below the median. The distribution of 5-FU plasma concentration values was higher in those patients who had a greater percentage of change in

<table>
<thead>
<tr>
<th>CPT-11 (mg/m²/24 hours)</th>
<th>52.5</th>
<th>70</th>
<th>90</th>
<th>110</th>
<th>140</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Patients</td>
<td>1</td>
<td>7</td>
<td>6</td>
<td>9</td>
<td>23</td>
</tr>
<tr>
<td>SN-38-free (nmol/L)</td>
<td>3.8</td>
<td>3.2 ± 1.7</td>
<td>3.7 ± 2.1</td>
<td>3.6 ± 1.3</td>
<td>4.2 ± 1.6</td>
</tr>
<tr>
<td>SN-38-glucuronide (nmol/L)</td>
<td>9.1</td>
<td>11.6 ± 3.8</td>
<td>13.8 ± 6.4</td>
<td>15.2 ± 12.8</td>
<td>15.7 ± 7.7</td>
</tr>
<tr>
<td>SN-38-total (nmol/L)</td>
<td>13.0</td>
<td>14.8 ± 5.0</td>
<td>17.5 ± 7.1</td>
<td>18.8 ± 12.9</td>
<td>19.9 ± 8.2</td>
</tr>
<tr>
<td>Ratio SN-38-free to SN-38-glucuronide</td>
<td>0.42</td>
<td>0.28 ± 0.10</td>
<td>0.30 ± 0.13</td>
<td>0.30 ± 0.13</td>
<td>0.33 ± 0.23</td>
</tr>
<tr>
<td>APC (nmol/L)</td>
<td>42.3</td>
<td>54.8 ± 21.2</td>
<td>86.7 ± 40.7</td>
<td>117.2 ± 86.4</td>
<td>150.7 ± 180.7</td>
</tr>
<tr>
<td>Ratio APC to CPT-11</td>
<td>0.20</td>
<td>0.24 ± 0.12</td>
<td>0.26 ± 0.14</td>
<td>0.29 ± 0.12</td>
<td>0.26 ± 0.23</td>
</tr>
<tr>
<td>Ratio SN-38-total to CPT-11</td>
<td>0.06</td>
<td>0.07 ± 0.03</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.12</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Biliary index</td>
<td>1,024</td>
<td>824 ± 320</td>
<td>1,246 ± 632</td>
<td>1,305 ± 606</td>
<td>1,875 ± 1,133</td>
</tr>
</tbody>
</table>

NOTE: The plasma concentration for each patient at 21, 22, and 23 hours were averaged. The data are presented as the mean ± SD. Biliary index, AUC CPT-11 × (AUC SN-38-free / AUC SN-38-glucuronide).
absolute neutrophil count (Fig. 2, top). In contrast, there was no significant association between the biliary index and whether the change in neutrophils was greater than or lower than the median. There was a trend for a higher biliary index in those patients who experienced grade 2 to 3 diarrhea (Fig. 2, bottom). Two patients with the highest biliary indices among those who had grade 0 to 1 diarrhea did not receive all the planned doses of 5-FU with CPT-11 (5-FU was held on day 2 in one patient due to tumor-related rectal bleeding; the other patient did not receive either dose of 5-FU due to pump malfunction). There was no relationship between 5-FU steady state plasma concentration and grade of diarrhea.

Pharmacogenetic analyses. Twenty-eight patients had analysis of the number of 28-bp repeats in the thymidylate synthase promoter region. The allelic genotype was 2/2 in 4 patients (14.3%), 2/3 in 20 patients (71.4%), and 3/3 in 4 patients (14.3%). All four of the patients achieving partial response were heterozygotes.

The number of TA repeats in the UGT1A1 promoter region was analyzed in 30 patients. Nine patients were homozygous for 6/6 TA repeats (30%), 18 were heterozygous for the 6/7 genotype (60%), and 3 were heterozygous for a 7/8 genotype (10%); the latter three were all African-American. Patients who were homozygous for two (TA)6 alleles had a significantly lower ratio of SN-38-free to SN-38-glucuronide compared with those who had one or more (TA)7 allele (Fig. 3), indicating that former patients were able to detoxify SN-38 better through glucuronidation.

Discussion

The recommended doses for this regimen are CPT-11 140 mg/m²/24 hours followed by leucovorin 500 mg/m²/0.5 hours, followed by 5-FU 3,120 mg/m²/48 hours. Dose-limiting toxicities that were clearly chemotherapy-related included diarrhea, vomiting, and neutropenia. Overall, the regimen was relatively well tolerated, and grade 3 or worse hematologic toxicity was unusual. Two patients who experienced grade 3 to 4 ileus had known peritoneal carcinomatosis; one of these patients and another with grade 3 fatigue did not have amelioration of their symptoms despite successive dose reductions of CPT-11 and 5-FU. Therefore, these symptoms were not clearly treatment-related.

In this phase I study, the subject population was heterogeneous in terms of histology and prior therapy, and efficacy was not the primary end point. However, four partial responses were seen among 22 patients with colorectal cancer, two of which experienced disease progression on prior bolus CPT-11/5-FU/leucovorin. The median time to treatment failure was nearly 6 months, and 25% of all subjects had a time to treatment failure in excess of 12 months.

Higher 5-FU steady state plasma concentration correlated with a greater percentage of decrease in neutrophils, but not with diarrhea. Although the median biliary index was higher in subjects who had a greater percentage of decrease in neutrophils, it did not reach significance. There was a trend for a higher median biliary index in those individuals who experienced grades 2 to 3 diarrhea.

The literature concerning thymidylate synthase genotype and response to 5-FU-based therapy has yielded inconsistent reports. Some investigators have reported that a genotype of 2/2 for the 28-bp repeats is associated with greater sensitivity to 5-FU-based therapy, whereas in other trials, a 2/2 or 2/3 genotype predicts for response to 5-FU-based therapy (29–32). In this trial, 75% of subjects were heterozygous for double/triple 28-bp repeat alleles in the thymidylate synthase enhancer region; all four responders were heterozygotes, compared with 0/4 in subjects with either the 2/2 or 3/3 genotype. Due to the small numbers, no firm conclusions could be drawn. Because other active agents such as CPT-11, oxaliplatin, bevacizumab, and cetuximab are now routinely being combined with 5-FU or capecitabine, the possible prognostic and/or predictive importance of thymidylate synthase promoter genotype may change. The problem may be exaggerated with heterogeneous patient populations.

Subjects with a (TA)6/6 genotype in UGT1A1 had a significantly lower ratio of free SN-38/SN-38G compared with those with at least one allele containing a (TA)7 repeat, indicating a greater ability to conjugate and thus detoxify SN-38. The three subjects with a (TA)7/8 genotype were African-American.

Acute irinotecan-associated cholinergic symptoms (early diarrhea, rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, and intestinal hyperperistalsis that can cause abdominal cramping) are thought to be due to inhibition of human acetylcholinesterase by the lactone form of irinotecan (47–49). The cholinergic syndrome is more likely to occur at higher irinotecan dose levels and is associated with the onset of peak irinotecan plasma levels. The observation that no patient experienced acute cholinergic symptoms in this trial is likely due to the lower $C_{\text{max}}$ levels compared with administration of 125 mg/m²/90 minutes (8, 50). The infusion rate at the recommended dose of 140 mg/m² of CPT-11 was 5.8 mg/m²/hours, compared with 83 and 125 mg/m²/1.5 hours or 700 and 350 mg/m²/0.5 hours. Nevertheless, the UGT1A1 genotype seemed to retain importance in the detoxification of CPT-11, because subjects who were homozygous for (TA)6 alleles had greater formation of SN-38G. In summary, administration of CPT-11 as a 24-hour infusion in a modified “FOLFIri” regimen was well tolerated and had clinical activity.


A Phase I Pharmacologic and Pharmacogenetic Trial of Sequential 24-Hour Infusion of Irinotecan Followed by Leucovorin and a 48-Hour Infusion of Fluorouracil in Adult Patients with Solid Tumors

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