A phase I study of sequential irinotecan and 5-fluorouracil/leucovorin

R. M. Goldberg¹, S. H. Kaufmann¹, P. Atherton¹, J. A. Sloan¹, A. A. Adjei¹, H. C. Pitot¹, S. R. Alberts¹, J. Rubin¹, L. L. Miller² & C. Erlichman¹*

¹Department of Oncology, Mayo Clinic, Rochester, MN; ²Pharmacia Corporation, Peapack, NJ, USA

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Background: Irinotecan (CPT-11) and 5-fluorouracil (5-FU)/leucovorin are active agents in colorectal cancer. A sequence-dependent synergism of SN-38 followed by 5-FU/leucovorin in vitro led us to conduct a phase I trial of CPT-11 followed by 5-FU/leucovorin to determine the maximum tolerated dose (MTD) and toxicities of this regimen and to obtain preliminary indications of its activity in patients with advanced solid tumors.

Patients and methods: Fifty-six patients were enrolled in sequential cohorts to receive escalating doses of CPT-11 (90 min infusion) on day 1, followed by leucovorin 20 mg/m² (intravenous push) and 5-FU (90 min infusion) on days 2–5 of each 21-day cycle.

Results: A total of 347 treatment cycles (median 4, range 1–25) were administered. Dose-limiting toxicities were diarrhea, neutropenia and fatigue. Nine patients with colorectal cancer and one with gastric cancer had partial or minor responses. Eight of the 10 had prior chemotherapy.

Conclusions: CPT-11 and 5-FU/leucovorin, as constituents of this novel mechanism-based schedule, have promising activity in patients who have received prior chemotherapy. The recommended phase II/III starting doses are CPT-11 275 mg/m² over 90 min on day 1, and 5-FU 400 mg/m² plus leucovorin 20 mg/m² on days 2–5 every 21 days. This combination can be administered safely to this schedule if there is strict adherence to the 90 min infusion time for both CPT-11 and 5-FU.

Key words: 5-fluorouracil, irinotecan, leucovorin, phase I

Introduction

Irinotecan (CPT-11) is a semisynthetic analog of camptothecin, which has greater activity in vivo and milder toxicity [1–6]. CPT-11 is converted in vivo to SN-38, which is 1000 times more potent than the parent drug [7–9]. SN-38 targets topoisomerase I, covalently stabilizing the enzyme–DNA complexes [10], resulting in strand breakage and subsequent cytotoxicity [11–14]. Phase II trials have shown that CPT-11 has activity in a wide spectrum of human neoplasms [15–22], and recent phase III studies indicate that CPT-11 can increase survival in patients with 5-fluorouracil (5-FU)-resistant metastatic colorectal cancer [23, 24].

5-FU cytotoxicity is mediated primarily by the inhibition of thymidylate synthase (TS) [25], which requires the formation of a ternary complex between 5-fluoro-2′-deoxyuridine monophosphate (5-FdUMP), TS and 5,10-methylene tetrahydrofolate (MTHF) [26]. Leucovorin (LV) increases the level of MTHF [26]. This led to the demonstration that 5-FU/LV increases the response rate in patients with advanced colorectal cancer compared with 5-FU alone [27]. Furthermore, adjuvant treatment with 5-FU/LV resulted in increased survival in patients with stage III colon cancer compared with no therapy [28–31].

The activity of CPT-11 and 5-FU in colorectal cancer, and the different mechanisms of action of each drug has sparked considerable interest in combining these drugs. Saltz et al. [32] and Douillard et al. [33] have reported that the combination of CPT-11, 5-FU and LV is more effective than 5-FU/LV alone. Concomitant with this improvement in response and survival, however, significantly increased toxicity of the combination was observed, particularly with the combination reported by Saltz et al. [34, 35].

Preclinical studies raised concern that the sequence of administration might be important for CPT-11/5-FU combinations. 5-FU might inhibit the DNA synthesis required for the cytotoxicity of SN-38; and SN-38 might cause cells to accumulate in G₂, where they would be resistant to 5-FU [36, 37]. Results from our group demonstrated sequence-dependent synergy of these agents in vitro, with the SN-38→5-FU...
sequence being most efficacious [36]. This sequence dependence reflected SN-38-induced S-phase slowing, inhibition of TS and increased deoxythymidine triphosphate (dTTP) pools [36]. Others [38–40] have reported a similar sequence-dependent synergy of this combination.

Based on our preclinical results, we designed a CPT-11/5-FU/LV regimen in which CPT-11 was administered on day 1 and 5-FU/LV was administered on days 2–5 of a 21-day cycle to determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT). Both CPT-11 and 5-FU were administered as 90-min infusions because Parnes et al. [41] had reported severe diarrhea when the drugs were combined, and Machover et al. [42] had demonstrated that prolonging the infusion of 5-FU allowed increased doses to be delivered. This regimen not only maintains the sequence of administration that appeared most promising in our preclinical studies, but also combines a standard schedule of CPT-11 administration [43, 44] with a modified Mayo Clinic schedule of 5-FU and LV [29].

Patients and methods

Patient selection

Patients with histologic evidence of metastatic or locally advanced cancer for which there was no established curative or life-prolonging therapy were eligible for this study. Eligibility criteria included: (i) age ≥18 years; (ii) ECOG performance status ≤2; (iii) measurable or evaluable disease; (iv) life expectancy ≥12 weeks; and (v) informed consent. Exclusion criteria included: (i) history of lymphoma or leukemia; (ii) less than two prior chemotherapy regimens; (iii) prior treatment with CPT-11 or another camptothecin analog; (iv) prior chemotherapy in the last 4 weeks; (v) prior exposure to nitrosourea or mitomycin C; (vi) prior abdominal or pelvic radiation therapy to >25% of bone marrow (except in a separate cohort of patients with prior pelvic irradiation); (vii) other active medical problems such as known central nervous system metastases, interstitial lung disease, pleural effusions or ascites causing more than grade 2 dyspnea; (viii) less than four loose stools per day; and (ix) severe comorbid disease. Prior to study enrollment, patients were required to sign a Mayo Clinic Institutional Review Board-approved informed consent form.

Study design and treatment

Irinotecan and loperamide were supplied by the Pharmacia Corporation (Peapack, NJ). 5-FU and LV were obtained commercially. CPT-11 was administered intravenously over 90 min on day 1. LV (20 mg/m²) was administered by intravenous push each day just before 5-FU, which was given intravenously over 90 min on days 2–5. Both 5-FU and CPT-11 were infused by electronic pump. This 5-day regimen was repeated every 3 weeks. At the first sign of loose stools, anti-diarrhea therapy was implemented for at least 12 h or until diarrhea resolved [44]. Antiemetic prophylaxis consisted of granisetron 1 mg i.v. before treatment, followed by granisetron 1 mg p.o. every 12 h for 24 h as required.

Sequential cohorts of patients were treated at progressively higher starting doses of CPT-11 and 5-FU. At least three patients were entered at each dose level, with an additional three patients added if one of the patients experienced a DLT during the first cycle of treatment. Dose escalation was not allowed in individual patients.

The MTD was defined as one dose level below the dose that induced DLT in more than one-third of patients (at least two of a maximum of six patients). Using the National Cancer Institute common toxicity criteria (NCI-CTC; version 1.0), DLT was defined as the occurrence of any of the following during the first cycle of therapy: (i) grade 4 vomiting or diarrhea despite maximal antiemetic and anti-diarrheal therapy; (ii) other grade 3/4 non-hematologic toxicity; (iii) febrile neutropenia; (iv) absolute neutrophil count (ANC) <500/µl or platelet count <25 000/µl for >5 days; or (v) a treatment delay exceeding 3 weeks. Before each treatment, ANC had to be ≥15 000/µl, platelet count ≥100 000/µl and hemoglobin ≥28.0 g/dl. All other toxicity must have resolved or improved to less than grade 2 before re-treatment. If treatment was withheld due to toxicity for >3 weeks, patients were taken off study.

Six additional patients with prior pelvic irradiation were recruited once the MTD had been determined because of concerns about myelosuppression and increased risk of diarrhea after bowel irradiation. Three patients were treated at one dose level below the MTD and, if this proved tolerable, a further three patients were treated at the MTD.

Patient evaluation

Complete histories, physical examinations, adverse event assessments, complete blood counts (CBCs), and serum chemistries were performed at baseline and prior to each cycle of therapy. CBCs were performed weekly. Disease measurements were made at baseline and after every two cycles of therapy to assess tumor response. A complete tumor response (CR) required the total disappearance of all evidence of tumor. A partial response (PR) was defined as a ≥25% reduction in the sum of the products of the largest perpendicular diameters of single or multiple indicator lesions chosen before therapy. A minor response (MR) was defined as a 25% and 50% reduction in the sum of the products of the largest perpendicular diameters of single or multiple indicator lesions chosen prior to therapy. CR, PR or MR had to be maintained for at least 4 weeks. Tumor progression was defined as the appearance of new lesions, the reappearance of lesions that had become undetectable, a 25% increase in the minimum size of measurable indicator lesions or clear worsening of evaluable lesions. Disease status was classified as stable when the criteria for CR, PR, MR or progression were not met.

Statistical methods

This was a standard cohort-of-three phase I clinical trial in which the primary end point of the trial was determination of the MTD. Analyses of patient characteristics, MTD, incidence of adverse events, treatment administration and tumor responses were descriptive. Correlations were assessed using both parametric (Pearson’s) and non-parametric (Spearman’s) procedures, as appropriate. Duration of response and time to tumor progression were calculated using Kaplan–Meier methods from the day that patients first received protocol chemotherapy until progressive disease was documented.

Results

Between June 1996 and August 1999, 56 patients were recruited to a trial of CPT-11 and 5-FU/LV administered in the sequence suggested to be most efficacious in preclinical studies [36, 43–45]. Patient characteristics are summarized in Table 1. The median age of the study participants was 61.5 years (range 34–81 years). Twenty-nine patients had an
ECOG performance status of 0. Forty patients had received prior chemotherapy. Nine patients had received prior irradiation, including three patients with prior radiation therapy to <25% of bone marrow who were enrolled into the initial dose escalation portion of the study and the six patients with prior pelvic radiotherapy who were enrolled once the MTD had been determined. The most common tumor type was metastatic colorectal cancer in 33 patients.

Thirteen dose levels were studied (Table 2). Three hundred and forty-seven courses of therapy were administered, with a median of 4 and range of 1–25. Patients with no prior pelvic radiation also received a median of 4 courses (range 1–25) and the six patients with prior pelvic radiotherapy received a median of 3.5 courses (range 2–7).

At a dose of CPT-11 150 mg/m² and 5-FU 300 mg/m², one patient experienced grade 4 diarrhea necessitating expansion of the cohort to six. As no further DLT occurred, escalation continued until the 13th dose level (CPT-11 300 mg/m² and 5-FU 400 mg/m²), where four of six patients experienced DLTs. At this dose level, one patient had grade 4 fatigue, one had grade 4 diarrhea, one had grade 4 vomiting, and one patient experienced vomiting, neutropenia, sepsis, mucositis and diarrhea. The latter patient died of *Escherichia coli* sepsis.

Dose level 12 (CPT-11 275 mg/m², 5-FU 400 mg/m²) was considered the MTD. Ten patients treated at that dose received a median of 6.5 courses (range 1–22). Dose reduction of CPT-11 to 250 mg/m² with 5-FU maintained at 400 mg/m² occurred in one of the eight patients treated at the MTD who received a second treatment cycle. Among the seven patients without prior pelvic radiotherapy enrolled at this dose level, one had DLT of grade 4 diarrhea and vomiting. An additional six patients with prior pelvic irradiation were treated, three with CPT-11 250 mg/m² and 5-FU 400 mg/m², and three with CPT-11 275 mg/m² and 5-FU 400 mg/m². One of these patients experienced grade 4 neutropenia at CPT-11 250 mg/m² and 5-FU 400 mg/m².

Non-hematologic toxicity was moderate. Figure 1 summarizes the frequency of major toxicities attributed to treat-
ment grouped by body system. There were 33 grade 3 events and 16 grade 4 events. The most common non-hematologic toxicities were gastrointestinal. Only five patients had grade 3 or 4 nausea or vomiting, and four patients had grade 3 or 4 diarrhea. There were three patients with grade 3 or 4 mucositis and 13 patients with grade 1 mucositis. Four patients had fatigue and four had dehydration. Other grade 3 events occurred in no more than one patient.

Neutropenia was the principal hematologic toxicity of the combination. Hematologic toxicities on cycle 1 are summarized by dose level in Table 3. There were five episodes of grade 3 neutropenia and three of grade 4 neutropenia. Grade 4 thrombocytopenia occurred in one patient only. There was a weak correlation between dose level and neutrophil nadir on all courses of therapy (Spearman’s ρ = −0.29, P = 0.03). In the six patients with a history of prior pelvic irradiation, three patients had 11 grade 3 events and one grade 4 event during all cycles of therapy. These events included toxicities of nausea, vomiting, anorexia, mucositis, fatigue and neutropenia. None of these events was considered dose limiting, and none except neutropenia could be attributed directly to previous pelvic irradiation.

Six patients achieved PRs, four patients had MRs and 34 patients had stable disease. The characteristics of these patients with responding tumors are provided in Table 4. Nine patients had colorectal cancer and one patient had gastric cancer. The median duration of response was 2.09 months (range 1.38–6.08 months). The median time to tumor progression for all patients enrolled in the trial was 85 days (range 22–491 days). Among the 10 patients who were treated at the recommended phase II starting dose level, eight had colorectal cancer. Three of these patients had PRs.

**Discussion**

CPT-11 administered every 3 weeks prolongs survival in patients with 5-FU-refractory colorectal cancer [23, 24]. The administration of 5-FU/LV given over several consecutive days is a common treatment schedule, with efficacy in metastatic

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**Table 3. Hematologic toxicities on course 1**

<table>
<thead>
<tr>
<th>CPT-11/5-FU dose (mg/m²)</th>
<th>ANC nadir (×10⁹/l)</th>
<th>Platelets (×10⁹/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>75/250</td>
<td>3.36</td>
<td>3.04–5.00</td>
</tr>
<tr>
<td>100/250</td>
<td>3.54</td>
<td>3.02–6.16</td>
</tr>
<tr>
<td>125/250</td>
<td>2.11</td>
<td>1.82–2.32</td>
</tr>
<tr>
<td>125/300</td>
<td>3.83</td>
<td>1.94–4.19</td>
</tr>
<tr>
<td>150/300</td>
<td>3.19</td>
<td>1.26–5.50</td>
</tr>
<tr>
<td>175/300</td>
<td>3.43</td>
<td>2.48–4.15</td>
</tr>
<tr>
<td>175/350</td>
<td>2.19</td>
<td>1.14–3.02</td>
</tr>
<tr>
<td>200/350</td>
<td>3.92</td>
<td>2.32–4.91</td>
</tr>
<tr>
<td>225/350</td>
<td>2.59</td>
<td>1.65–3.48</td>
</tr>
<tr>
<td>250/300</td>
<td>1.95</td>
<td>0.63–2.61</td>
</tr>
<tr>
<td>250/400</td>
<td>5.18</td>
<td>0.53–9.27</td>
</tr>
<tr>
<td>275/400</td>
<td>1.72</td>
<td>1.05–3.54</td>
</tr>
<tr>
<td>300/400</td>
<td>3.27</td>
<td>0.10–7.23</td>
</tr>
</tbody>
</table>

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**Figure 1.** Frequency of toxicities seen in patients, categorized according to body system affected. Grading is according to NCI-CTC version 1.0.
Table 4. Characteristics of patients with tumor responses

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female), n</td>
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</tr>
<tr>
<td>Median age, years (range)</td>
<td>60 (35–75)</td>
</tr>
<tr>
<td>ECOG performance score, n</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Previous treatment, n</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>5</td>
</tr>
<tr>
<td>Chemotherapy + radiotherapy</td>
<td>3</td>
</tr>
<tr>
<td>No prior chemotherapy or radiotherapy</td>
<td>2</td>
</tr>
<tr>
<td>Primary tumor type, n</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>9</td>
</tr>
<tr>
<td>Gastric</td>
<td>1</td>
</tr>
<tr>
<td>Starting dose level (CPT-11/5-FU doses in mg/m²)</td>
<td></td>
</tr>
<tr>
<td>(75/250)</td>
<td>1</td>
</tr>
<tr>
<td>(100/250)</td>
<td>1</td>
</tr>
<tr>
<td>(200/350)</td>
<td>1</td>
</tr>
<tr>
<td>(250/400)</td>
<td>2</td>
</tr>
<tr>
<td>(275/400)</td>
<td>3</td>
</tr>
<tr>
<td>(300/400)</td>
<td>2</td>
</tr>
</tbody>
</table>

colorectal cancer and in the adjuvant therapy setting [27–29]. The regimen described in this phase-I dose-escalation study combines these two treatments in a sequence that builds on this past clinical experience with the individual agents [23, 28, 29], and also resulted in drug synergy in vitro [36, 38–40].

Parnes et al. previously evaluated a similar schedule of varying doses of CPT-11, 5-FU and LV [41]. Severe diarrhea occurred with relatively low doses of the drugs. Because of this experience and concern about the potential for overlapping toxicity, the initial doses of CPT-11 (75 mg/m² on day 1) and 5-FU (250 mg/m² on days 2–5) were very conservative and the dose escalation was undertaken cautiously. Furthermore, to minimize the potential for diarrhea, we administered the 5-FU over 90 min instead of by bolus. Machover et al. had demonstrated that, with short infusions, the dose of 5-FU could be escalated to 500 mg/m²/day in the presence of LV [42]. This dependence of clinical toxicity on duration of 5-FU infusion has been recognized clinically for many years [46]. In order to maximize the dose of each cytotoxic agent relative to its single-agent dose, escalations in CPT-11 and 5-FU starting doses were alternated.

The principal toxicities of clinical significance were gastrointestinal (primarily nausea, vomiting, diarrhea and mucositis) and neutropenia. The recommended starting dose of CPT-11 established in this trial, i.e. 275 mg/m², is 79% of the single-agent starting dose of 350 mg/m² when CPT-11 mono-therapy is given every 3 weeks [23, 24]. The recommended 5-FU starting dose derived from the current trial of 400 mg/m² for 4 days (given with 20 mg/m² of LV) is 94% of the conventional daily dose administered when 5-FU is given with LV for 5 consecutive days, i.e. 425 mg/m²/day [29, 47], or 80% of that reported by Machover [42]. As the treatment schedule is q3 weeks, the dose of 5-FU administered in the present CPT-11/5-FU/LV regimen over four cycles (the median in this study) equals the dose of 5-FU administered with LV on the 5-day schedule [29, 47]. The observation that most patients enrolled in this trial had received prior cytotoxic therapy and tolerated this combination schedule over multiple cycles indicates that nearly full doses of each cytotoxicity is feasible if one is attuned to the infusion time of both CPT-11 and 5-FU, and treats toxicity promptly and aggressively.

These results led to the incorporation of this schedule of CPT-11 and 5-FU into the original design of intergroup trial N9741 for patients with advanced colorectal cancer [47]. Sixty-one patients were entered on CPT-11 + 5-FU/LV using this schedule; and six deaths were reported due to toxicity. Although it is not clear why this schedule of CPT-11 and 5-FU/LV was so toxic in N9741 but not in this trial, it is possible that procedures ensuring 5-FU infusion over 90 min, such as use of electronic pumps rather than bolus injection, were not implemented in the cooperative group setting. Careful evaluation of 5-FU infusion duration in those cases in which deaths occurred might elucidate this.

Recent reports have described the efficacy and safety data with other schedules of the combinations of CPT-11/5-FU/LV that differ from the present regimen [33, 48]. The Saltz regimen was empirically selected to combine four weekly infusions of CPT-11 with simultaneously administered 5-FU/LV [49]. It was based on the CPT-11 schedule used in the USA [16, 17] and the Roswell Park schedule of 5-FU/LV [50] given weekly. A major difference from the Roswell Park 5-FU/LV regimen was a reduction in the dose of LV to 20 mg/m² from 500 mg/m², despite demonstration that when 5-FU is given weekly, 25 mg/m² of LV does not improve the response rate of the combination [50]. Furthermore, Saltz gave four weekly treatments followed by 2 weeks of rest, rather than 6 consecutive weeks of therapy followed by a 2-week rest.

In phase III, the Saltz regimen has demonstrated improved tumor control and longer survival [48]. However, weekly CPT-11/5-FU/LV therapy was associated with more grade 3/4 vomiting (10% compared with 4%) and diarrhea (23% compared with 13%) than 5-FU. Grade 3/4 mucositis (2% compared with 17%), grade 4 neutropenia (24% compared with 43%) and neutropenic fever (7% compared with 15%) were lower with CPT-11 and 5-FU/LV than 5-FU/LV, respectively. This toxicity difference may be related to the ability to omit or modify doses of CPT-11 and 5-FU on a weekly schedule, which is not feasible with the intensive course of 5-FU. Saltz et al. reduced doses in >50% of patients during course 1 of therapy; and during course 2 the actual doses of CPT-11 and
5-FU administered were <50% of the day 1/cycle 1 doses, clearly indicating that the full doses of CPT-11 and 5-FU could not be administered on this schedule in most patients [38]. This is further supported by the reports that the weekly schedule was associated with significant toxicity [35] both in N9741 and in the Cancer and Leukemia Group B (CALGB) adjuvant trial C89803. The death rate, regardless of attribution within 60 days of protocol entry, was 4.8% in N9741 and 2.2% in C89803.

Douillard et al. [33] compared CPT-11 combined with the de Gramont schedule [51] or the Arbeitsgemeinschaft Internische Onkologie (AIO) [52] schedule of 5-FU/LV infusion with the 5-FU/LV infusional schedules alone. The CPT-11, 5-FU/LV combinations produced significant improvements in response rate and time to tumor progression, and significantly longer survival. The addition of CPT-11 to infusional 5-FU/LV was associated with more toxicities compared with infusional 5-FU/LV alone. Relative toxicities rates included: grade 3/4 vomiting (6% compared with 3%, respectively), grade 3/4 diarrhea (23% compared with 11%), grade 4 neutropenia (9% compared with 1%) and neutropenic fever (5% compared with 1%).

We have demonstrated the feasibility of administering nearly full dose CPT-11 and 5-FU when each is infused over 90 min. There is no doubt that the combination of CPT-11, 5-FU and LV is an effective therapy, but the therapeutic window is narrow, taking into account the toxicity data of N9741 and the results of Saltz and Douillard. The infusion time of 5-FU is probably pivotal in any schedule of this combination. Combining CPT-11 with infusional 5-FU is one way of alleviating the problem of toxicity.

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References


