Combined topoisomerase I inhibition for the treatment of metastatic colon cancer

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The objective of this study was to define the maximally tolerated dose (MTD) and response rate of a combination of two topoisomerase I inhibitors, topotecan and irinotecan, in patients with metastatic colon cancer. Eleven patients, the majority with previously progressive disease on 5-fluorouracil-based regimens, were enrolled onto a phase I/II dose escalation trial utilizing continuous infusion topotecan for 2 weeks and weekly irinotecan × 3 with cycles repeated every 28 days. Dosages of topotecan utilized included 0.2 and 0.25 mg/m^2/day. Irinotecan was administered at a dose of 62 mg/m^2 by i.v. bolus. Patients were followed for toxicity and response. The MTD of the combination of agents was found to be 0.25 mg/m^2/day for topotecan and 62 mg/m^2 for irinotecan. The most common serious toxicities were diarrhea and nausea/vomiting. Only one patient experienced grade III neutropenia. There were no complete or partial responses. However, four patients had prolonged disease stabilization (SD) of up to 324 days and this group remained on protocol therapy for an average of 227 days ($p = 0.0005$ versus patients not achieving SD). We concluded that the MTD for this combination of topoisomerase I inhibitors, given on this particular schedule, has been defined. This combination cannot be recommended as a first- or second-line therapy for patients with metastatic colon cancer based on the responses observed. However, approximately one-third of patients achieved prolonged disease stabilization. Topotecan with irinotecan may be useful as a palliative regimen for a subgroup of colon cancer patients. *Anti-Cancer Drugs* 15:569–574 © 2004 Lippincott Williams & Wilkins.

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Introduction

Irinotecan is a topoisomerase I inhibitor which is a clinically effective agent for patients with colon cancer. Response rates in patients with metastatic disease who have progressed on 5-fluorouracil (5-FU)-based regimens of up 18–23% have been noted with similar effectiveness in chemotherapy-naive patients [1,2]. The combination of irinotecan with 5-FU/leucovorin (IFL) as up-front therapy for patients with stage IV disease is associated with improved survival compared to 5-FU with leucovorin [3]. Another topoisomerase I inhibitor, topotecan, is active in many different types of cancer, including lung [4,5], breast [6], and myelodysplastic syndromes and chronic leukemias [7]. Topotecan has also been utilized as a single agent in patients with metastatic colorectal cancer with disappointing response rates of 10% [8–11]. The best responses were seen when topotecan was administered as a continuous infusion over 3 weeks [8].

Topotecan and irinotecan have similar mechanisms of action (inhibiting topoisomerase I) [12,13], but have distinct activity *in vitro* and *in vivo*. For example, topotecan is active against kidney human tumor colony forming units (HTCFUs), while irinotecan is not, and irinotecan is active against mesothelioma HTCFUs, while topotecan is not [14]. Clinically, irinotecan appears most effective in colon, cervical, esophageal, renal cell, head and neck, and non-small cell lung cancers, while topotecan appears most effective in ovarian, breast and small cell lung cancers, lymphoma, and chronic leukemias.

The dose-limiting toxicity for topotecan is myelosuppression, including granulocytopenia and thrombocytopenia, with minimal non-hematologic toxicities aside from nausea and vomiting [4,5,13]. The dose-limiting toxicity for irinotecan is diarrhea, which often necessitates early intervention with high-dose loperamide [1,13]. Grade 4 neutropenia is seen in approximately 18% of patients receiving weekly irinotecan. Thus, these two agents, despite a similar mechanism of action, display different toxicity profiles. This permitted consideration of concurrent combination therapy.

This trial was designed to make use of the relatively non-overlapping toxicities of topotecan and irinotecan in order

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to combine them and doubly target the topoisomerase I enzyme, define the maximally tolerated dosages of these agents when given in combination, and determine the response rate to this combination in patients with stage IV colon cancer. The overall hypothesis was that more substantial inhibition of tumor topoisomerase I could be achieved through the use of the combination of these two inhibitors than with either agent individually and that this would correlate with clinical responsiveness. The phase I dose-escalation design was developed to test this hypothesis and define the maximally tolerated dose (MTD) while maintaining patient safety. The protocol was reviewed and approved by the on-site National Cancer Institute-sanctioned protocol review committee at the Chao Family Comprehensive Cancer Center. This is the first study reporting dual topoisomerase I inhibition for cancer therapy.

Patients and methods

Patients

Patients with stage IV colon cancer who were newly diagnosed or had progressed on 5-FU-based chemotherapy were eligible for this study. The protocol was initiated in September 1999 and closed to accrual in August 2001, with a total of 11 evaluable and eligible patients enrolled. Patients were eligible if they had histologically defined metastatic colon cancer, bidimensionally measurable disease, normal white blood cell and platelet counts, normal creatinine, and SWOG/ECOG performance status 2 or less. Pregnant patients and patients who had received prior therapy with, or had known hypersensitivity to, a topoisomerase I inhibitor were ineligible. Patients were assigned to a particular dose level according to their order of entry into the study and remained at that dose level throughout treatment on the protocol. This study was approved by the Cancer Center Clinical Trials Protocol Review and Monitoring Committee and the University of California, Irvine Institutional Review Board.

Phase I/II dose-escalation design

This study utilized a phase I dose-escalation design for combination chemotherapy as proposed by Korn and Simon [15]. Because topotecan and irinotecan have distinct toxicity profiles, it was expected that the dose-limiting toxicity would be at organ sites with overlap; in this case, hematopoietic myelosuppression. The targeted, or anticipated, MTD was for a combination which was half of the single-agent MTDs for the expected dose-limiting toxicity. The study utilized a hybrid design of dose escalation of topotecan with irinotecan dose fixed, followed by dual escalation at subsequent dose levels. The MTD for irinotecan is 125 mg/m^2 when given weekly [1,2]. The MTD for topotecan given by continuous infusion for 21 days, defined by Creemers *et al.* [8] in colon cancer patients, is 0.5–0.6 mg/m^2/day. Because of concerns of cumulative myelosuppression on the two-drug regimen, the duration of continuous topotecan infusional therapy was limited to 14 days. The targeted dosages of 62 mg/m^2 for irinotecan and 0.3 mg/m^2/day for topotecan were to be achieved at the third dose level [15] (Table 1).

Table 1 Phase I dose-escalation schedule

Dose level	Topotecan (mg/m^2/day)	Irinotecan (mg/m^2)
I	0.2	62
II	0.25	62
III	0.3	62
IV	0.35	75
V	0.4	100
VI	0.45	125

Toxicity criteria, dose levels and definition of response

Toxicity was defined according to Southwest Oncology Group (SWOG) criteria. The principal toxicities expected were hematologic and gastrointestinal. Dose modifications in individual patients were not allowed under the protocol. Once an individual patient experienced grade III or grade IV toxicity as a consequence of the treatment, the patient was withdrawn from the study and treated according to their physician's discretion. No dose escalation was permitted if the patient did not experience toxicity. Three patients were to be enrolled at each dose level with progression to the next dose level if no grade III or grade IV toxicity was encountered. If one of three patients experienced grade III/IV toxicity, an additional three patients were enrolled at that dose level. If one of six (or zero of three) patients experienced grade III/IV toxicity, the study would progress to the next dose level. Once two or more patients (two out of three or two out of six) at any given dose level experienced grade III or grade IV toxicity, that level was designated as MTD and no further dose escalation was performed. The study was initially designed for treatment of 18 patients with a minimum of 14 at the MTD or MTD – 1 level to assess response under a phase II design.

Response was defined based on the sum of longest diameters (LD) according to RECIST criteria. The sum of the products of bidimensional diameters of all measurable lesions was also recorded as was standard practice at the time of study initiation. Complete response (CR) was defined as the disappearance of all measurable lesions, partial response (PR) as at least a 30% decrease in the sum of the LD, progressive disease (PD) as at least a 20% increase in the sum of the LD and stable disease as neither sufficient shrinkage to qualify as PR nor sufficient increase to qualify as PD.

Statistical considerations

The phase I portion of the study was the primary component of the trial design which drove patient

recruitment goals. Up to 18 patients were likely to be enrolled into the phase I portion. Since the targeted dose level for combination MTD was to be achieved by the third dose level, it was anticipated that 12–18 patients would be treated at the MTD or MTD – 1 dose level in order to provide information for response analysis as part of the phase II component. Means and 95% confidence intervals (CI) were calculated for all groups. Comparisons between groups were made utilizing a two-tailed, unpaired *t*-test with $p < 0.05$ considered statistically significant.

Results

Enrollment and patient characteristics

Between September 1999 and June 2001, 11 patients were enrolled onto this study following informed, written consent. All patients had pathologically confirmed metastatic colon cancer and all met eligibility criteria, and were evaluable for both toxicity and response. Eight patients had received prior 5-FU-based therapy for metastatic disease and one had received 5-FU with leucovorin in the adjuvant setting. Two patients had received no chemotherapy prior to enrollment on this study. Enrollment was terminated at 11 patients because the MTD had been defined and the response rate in 14 patients would have been less than published response rates for other irinotecan-containing regimens [1,2] even if three additional patients each achieved a PR.

Toxicities, MTD determination and duration of therapy

The most frequent toxicity was diarrhea, occurring in 10 of 11 patients and at grade III in one patient. Nausea/vomiting was also common (nine of 11 patients), again with one patient experiencing grade III toxicity. Grade III neutropenia was also encountered in one patient, although this was not a common side-effect and occurred in no other individuals. The only other toxicities encountered at a frequency above 50% were anorexia and fatigue. There were no grade IV or fatal toxicities in any of the patients on protocol. The frequencies of all toxicities recorded are listed in Table 2.

One patient of the first three enrolled at dose level I experienced grade III toxicity (diarrhea). According to the protocol, this led to three additional patients receiving treatment at dose level I. No other grade III toxicities were encountered and subsequent patients were enrolled at dose level II. In the first cohort at this dose level, one patient experienced grade III nausea and vomiting. An additional cohort was then enrolled at dose level II, the first patient of which also experienced grade III toxicity (neutropenia). According to the protocol guidelines, since two patients experienced grade III or higher toxicity at a specific dose level, dose level II was designated as the MTD for this combination of medications.

According to the protocol, treatment was continued for all patients until progressive disease was documented, grade III toxicity was encountered or patients elected to terminate the study early. Evaluation for response or progression was performed after cycle 2 and subsequently after every other cycle. Patients received treatment on protocol for an average of 109 (95% CI 36–181) days. However, patients fell into two distinct subgroups—those receiving therapy for 2 cycles or less and those receiving therapy for more than 2 cycles (Fig. 1). Patients in the former group ($N = 7$) remained on protocol an average of 41 (95% CI 12–69) days and three of these patients were on protocol for 11 (95% CI 0–15) days or less. Each of these three was withdrawn from protocol after experiencing grade III toxicity. The four patients who received more than 2 cycles of treatment remained on protocol for an average of 227 (range 150–324; 95% CI 90–364) days. The difference in protocol treatment duration between those receiving more than two cycles and those receiving 2 cycles or less was highly statistically significant ($p = 0.0005$). The duration of treatment on the protocol did not correlate with whether the patient had received prior chemotherapy for metastatic colon cancer.

Response to treatment

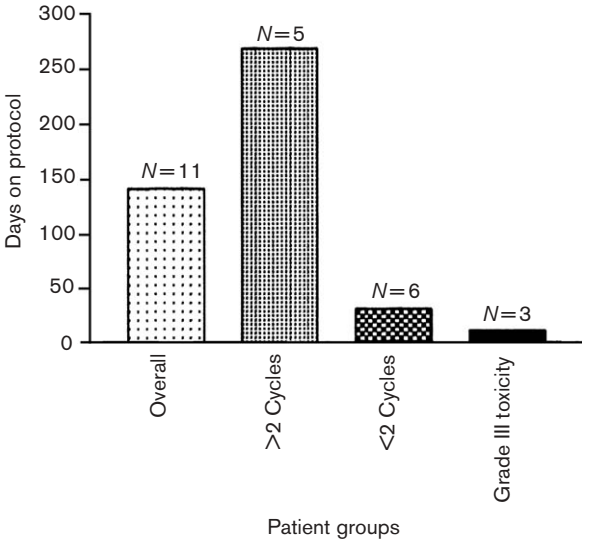
No CRs or PRs were observed at either dose level I or dose level II. Four patients (45%), three of whom

Table 2 Toxicities (%) at dose level I and dose level II

Toxicity	Dose level I (grade)					Dose level II (grade)				
	I	II	III	IV	Total	I	II	III	IV	Total
Diarrhea	33 (2/6)	50 (3/6)	17 (1/6)		100 (6/6)	40 (2/5)	40 (2/5)			80 (4/5)
Neutropenia					0			20 (1/5)		20 (1/5)
Nausea/vomiting	33 (2/6)	67 (4/6)			100 (6/6)	40 (2/5)		20 (1/5)		60 (3/5)
Abdominal cramping	17 (1/6)	33 (2/6)			50 (3/6)					0
Anorexia	50 (3/6)	33 (2/6)			83 (5/6)	20 (1/5)				20 (1/5)
Fatigue	17 (1/6)	67 (4/6)			83 (5/6)	40 (2/5)				40 (2/5)
Insomnia	17 (1/6)	17 (1/6)			33 (2/6)					0
Constipation	33 (2/6)				33 (2/6)					0
Dizziness/vertigo	17 (1/6)	17 (1/6)			33 (2/6)	20 (1/5)	20 (1/5)			40 (2/5)
Gastric ulcer	17 (1/6)				17 (1/6)					0

had previously progressive disease on a 5-FU-based treatment regimen, achieved stabilization of disease for extended periods of time up to 324 days. The maximum number of cycles received by any individual patient while on protocol was 8. Of those with SD, three were treated at dose level I and one at dose level II. This latter patient had received no prior chemotherapy and exhibited stable disease for nearly a year (324 days). Two patients who achieved prolonged stabilization of disease exhibited a reduction in serum CEA which persisted throughout the treatment course (Fig. 2).

Fig. 1

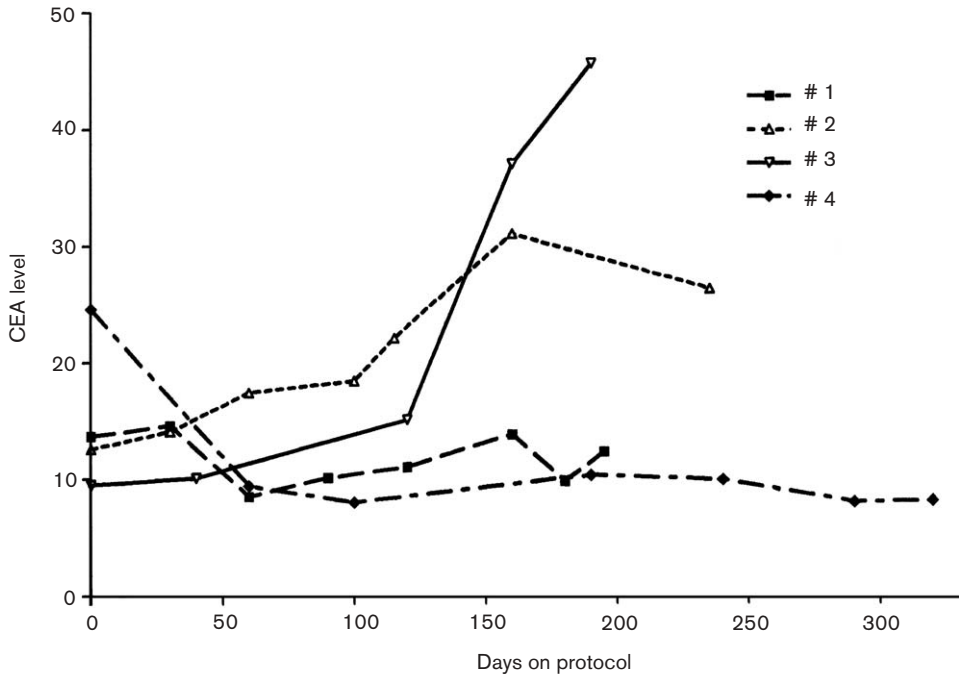


Average duration of protocol treatment for specific subgroups of patients. One subgroup of patients exhibited a significantly longer period of protocol therapy and disease stabilization than the remainder of patients ($p=0.0005$, column 2 versus 3).

Discussion

This protocol was designed to evaluate the toxicities and efficacy of a combination of two drugs which both target topoisomerase I. This enzyme is important in regulating movement of the replication fork during DNA replication in order to relax transcription-associated supercoils [16]. *In vitro*, resistance to topoisomerase I inhibitors occurs because of impaired transport into cells related to overexpression of the BCRP gene, mutations in the topoisomerase I gene which confer resistance to camptothecin activity and alteration in the cellular response to topoisomerase I–DNA ternary complexes [17]. Some of these resistance mechanisms are not cross-reactive among different camptothecin compounds [18,19]. The mechanisms of resistance to topotecan and irinotecan in the clinical setting, however, are not well understood. This is the first report of the combination of topotecan and irinotecan for the treatment of cancer patients.

Fig. 2



Serum CEA levels for patients with prolonged duration of protocol therapy. Day 0 indicates the day of protocol initiation. Patients 1 and 4 (closed square/diamond) exhibited a reduction in serum CEA which persisted throughout the treatment course.

This study has defined the MTD of topotecan by continuous infusion for 14 days with bolus, weekly irinotecan as 0.25 mg/m^2/day and 62 mg/m^2, respectively. The most common, and dose-limiting, toxicities were diarrhea and nausea/vomiting. Myelosuppression, anticipated to be the principal dose-limiting toxicity, was infrequent though neutropenia did result in protocol withdrawal for one patient.

Overall, the combination of topotecan and irinotecan was not particularly active for this patient population, most of which had previously exhibited progressive disease on a 5-FU-based regimen. Of the 11 patients treated, no partial or complete responses were seen. However, a subgroup of patients, about one-third in this study, achieved prolonged disease stabilization which was clinically significant. The basis for this is unclear. It is possible that colon cancers from different patients may express topoisomerase I in higher or lower concentrations and that this may affect the sensitivity to the combined topoisomerase inhibition. Topoisomerase I has been shown to be overexpressed in some colon cancer tissues [20] and sensitivity to camptothecins appears to correlate with topoisomerase I concentrations [21]. However, many additional studies have failed to demonstrate a direct correlation between tumor topoisomerase I levels and sensitivity to topoisomerase I inhibitors [22]. The pharmacokinetics of topotecan and irinotecan may be different in a subgroup of patients, resulting in differences in bioavailability. Topotecan is a directly active drug, whereas irinotecan must be metabolized to liberate the active SN38 camptothecin. In addition, clearance of camptothecins is hepatic and dependent upon uridine diphosphate glycosyltransferase I family polypeptide A1 (UGT1A1). Patients with reduced activity of this enzyme are known to have greatly amplified toxicity to irinotecan [23] and this may influence efficacy as well. The challenge will be to identify, prospectively, the characteristics which define the subgroup of patients that will derive clinical benefit from this treatment approach.

Combined topoisomerase inhibition with topotecan and irinotecan cannot be recommended as first- or second-line therapy for patients with metastatic colon cancer based on the findings in this study. However, some patients do appear to derive significant clinical benefit as represented by lack of progression for a prolonged time period. Use of these agents in combination may provide an additional alternative as palliative treatment for patients who have progressed on other regimens, although whether there is an advantage to combination therapy over monotherapy with irinotecan alone is not addressed here. It is also unclear if there is any increased benefit of treatment at dose level II, compared to treatment at dose level I, although significant toxicity was more common at the higher dose level. Therefore, if combined topoisomerase I inhibition is utilized as a palliative regimen for patients with metastatic colon cancer, we would recommend monthly cycles at dosages of 0.2 mg/m^2/day by continuous infusion for 14 days for topotecan and 62 mg/m^2 by i.v. bolus, weekly $\times$ 3, for irinotecan as an initial starting dose. Dose escalation of topotecan to 0.25 mg/m^2/day can be considered if the combination is well tolerated in individual patients. The combination of topotecan and irinotecan could also be explored in other stage IV malignancies.

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