

Phase I Evaluation of Sequential Topoisomerase Targeting with Irinotecan/Cisplatin Followed by Etoposide in Patients with Advanced Malignancy¹

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ABSTRACT

Purpose: To investigate pharmacologically guided addition of etoposide to a weekly irinotecan/cisplatin chemotherapy.

Patients and Methods: Patients with advanced nonhematologic malignancies were eligible. Treatment consisted of i.v. administration of 50 mg/m² irinotecan and 20 mg/m² cisplatin on days 1, 8, 15, and 22 of a 42-day cycle or on days 1 and 8 of a 21-day cycle. Etoposide was administered in a dose-escalating fashion 2 days after each dose of irinotecan/cisplatin, either i.v. as a single dose or p.o. as two doses administered 12 h apart. Pharmacologic analyses included measurement of plasma concentrations of irinotecan, SN-38, and SN-38 glucuronide, as well as quantitation of topoisomerase protein levels in peripheral blood mononuclear cells (PBMNCs).

Results: A total of 40 patients with a variety of malignancies received 122 cycles of therapy. Dose-limiting toxicities included neutropenia and diarrhea, with the 21-day cycle tolerated better than the 42-day cycle. For the 21-day cycle, the maximum tolerated dose was 75 mg/m² for i.v. etoposide and 85 mg/m² for oral etoposide. Objective responses were observed in four patients with previously treated mesothelioma, gastric, breast, and ovarian cancer, respectively. PBMNC levels of topoisomerase II α were increased at the time of etoposide administration in two

patients, with these patients having the highest SN-38 glucuronide peak-plasma-concentration and area-under-the-curve values among 15 patients with available pharmacokinetic data. One of these patients had a partial response to therapy.

Conclusions: Pharmacologically guided administration of etoposide in combination with irinotecan/cisplatin using a 21-day cycle is associated with acceptable toxicity and significant antitumor activity. The finding that PBMNC topoisomerase II α protein levels increased after irinotecan/cisplatin treatment in two of six patients supports the continued development of sequential topoisomerase targeting in the treatment of malignancy.

INTRODUCTION

Topoisomerase-targeting drugs are important in the treatment of a wide variety of solid and hematologic malignancies. These drugs function by stabilizing a normally transient reaction intermediate that involves covalent linkage of a topoisomerase (Top)³ molecule to DNA (1). The resulting structural DNA abnormalities, which include single-strand breaks for Top1-targeting drugs and double-strand breaks for Top2-targeting drugs, ultimately trigger cell death. Recent data also implicate ubiquitin and ubiquitin-like proteins as determinants in cell death induced by topoisomerase-targeting drugs (2–5). There are five human topoisomerase enzymes: three (Top1, Top2 α , and Top2 β) are known to be effective antitumor drug targets. The anthracyclines and epipodophyllotoxins are among the commonly used Top2-targeting drugs, whereas the camptothecins are currently the only Top1-targeting drugs approved for clinical use.

Several preclinical studies indicate that resistance to drugs that target Top1 confers hypersensitivity to drugs that target Top2 and vice versa (reviewed in Ref. 6). Previous studies exploring this concept in clinical settings involved either concurrent or sequential administration of Top1- and Top2-targeting drugs. Results from some of these studies indicated that Top2 levels are often transiently elevated in peripheral blood mononuclear cells (PBMNCs) obtained from patients treated with camptothecins (7, 8). Xenograft studies indicate that similar transient Top2 protein increases can occur in tumor tissue (9). Because elevations in Top2 protein levels in tumor cells should confer hypersensitivity to Top2-targeting drugs, yet concurrent Top1- and Top2-targeting can be antagonistic (10), we

Received 9/12/02; revised 12/16/02; accepted 12/20/02.

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¹Supported by United States Public Health Service Grant CA71535 (to E. H. R.), the Cancer Institute of New Jersey, and by Pharmacia, Inc.

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³The abbreviations used are: Top, topoisomerase; PBMNC, peripheral blood mononuclear cell; DLT, dose-limiting toxicities; MTD, maximum-tolerated dose; CBC, complete blood count; SN-38G, SN-38 glucuronide; AUC, area(s) under the curve.

favor a sequential rather than a simultaneous topoisomerase-targeting approach.

Studies investigating the concept of sequential topoisomerase-targeting in cell lines indicate that resistance to this strategy can occur, involving both mutation of Top1 and down-regulation of Top2 α (11). However, cells resistant to topoisomerase-targeting drugs were found to be 10-fold hypersensitive to cisplatin (11). This finding provided the rationale for the current trial, involving sequential administration of cisplatin/irinotecan and etoposide using a weekly schedule. A previous study established the feasibility and dosing of weekly cisplatin/irinotecan administration using a 4-weeks-on/2-weeks-off schedule (12). In addition, in our previous study, we analyzed PBMNC topoisomerase protein levels at 15 time points in 11 patients after initiation of daily oral camptothecin (8). Analyses of mean Top2 α protein levels among these 11 patients at a given time point indicated that peak increases occurred 2, 3, and 6 days after initiation of therapy. Therefore, based on these pharmacodynamic data, in the current study, we chose to administer etoposide as a single dose 2 days after each cisplatin/irinotecan treatment (denoted as "PIE" therapy). The major purpose of this study was to determine the maximum-tolerated dose of etoposide using this schedule. In addition, because in a previous study, we found that plasma camptothecin concentrations may be an important determinant in changes in PBMNC Top1 protein levels (8), the study included analysis of topoisomerase protein levels in patient PBMNCs in conjunction with analysis of the pharmacokinetics of irinotecan and its metabolites.

PATIENTS AND METHODS

Patient Selection. Eligibility requirements included the following: (a) histologically documented malignancy, which was either metastatic or inoperable and for which there was no standard effective chemotherapy regimen; (b) age >18 years old; (c) an estimated life expectancy of >2 months; (d) Eastern Cooperative Oncology Group performance status 0–2; (e) no prior surgery for >3 weeks; (f) no prior chemotherapy or radiotherapy for >3 weeks (>6 weeks for prior treatment with a nitrosourea-based agent); (g) no prior high-dose (requiring stem cell support) chemotherapy regimens or radiation therapy involving >30% of the bone marrow reserve; (h) appropriate hematopoietic (neutrophil count >1500/ml, platelets >100,000/ml), hepatic (bilirubin/aspartate aminotransferase/alanine aminotransferase <2-fold the upper limit of normal), and renal function (creatinine <1.6 mg/dl and creatinine clearance >50 ml/min); (i) no evidence of uncontrolled serious medical/psychiatric illness; (j) negative pregnancy test for woman of child bearing potential; and (k) for cohorts receiving oral etoposide, adequate predicted oral absorption (absence of frequent emesis or diarrhea, defined as greater than twice a day). The study was approved by the Robert Wood Johnson Medical School Institutional Review Board. All of the patients were informed of the investigative nature of the study and gave informed consent in accordance with institutional and federal guidelines.

Drug Administration and Dose Escalation. Patients were treated in cohorts of three. On the basis of a previous study (12), the doses of irinotecan and cisplatin were fixed at 50

mg/m² and 20 mg/m², respectively, with these drugs administered i.v. on days 1, 8, 15, and 22 of a 42-day cycle (4 weeks on/2 weeks off). Cisplatin was administered first, followed immediately by irinotecan as a 90-min infusion. All patients received i.v. fluid before the cisplatin infusion, consisting of 500 ml of normal saline containing 2 g of magnesium sulfate and 12.5 mg of mannitol. Etoposide was administered i.v. over 1 h, 2 days after each infusion of irinotecan/cisplatin (days 3, 10, 17, 24), with the etoposide dose escalated in cohorts of three patients. The initial dose of etoposide was 50 mg/m², with dose escalation occurring in 25 mg/m² increments.

In the second and subsequent cohorts, the treatment schedule was modified to a 21-day, 2-weeks-on/1-week-off cycle, with irinotecan/cisplatin given on days 1 and 8 and etoposide on days 3 and 10. Furthermore, the protocol was amended to allow administration of etoposide p.o. as two doses 12 h apart. The initial oral etoposide dose was 50 mg/m² every 12 h, based on a maximum-tolerated dose of 75 mg/m² for i.v. etoposide and an expected bioavailability of about 50% (13). Calculated oral etoposide doses were rounded to the nearest 50 mg. A neutrophil count \geq 1000/ml was required for initiation of each cycle of therapy and for each weekly treatment.

(a) DLT was defined as any of the following toxicities occurring during the first cycle of therapy: absolute neutrophil count <500/ml or platelet count <20,000/ml for >7 days; (b) irreversible grade 2 and any grade 3–5 nonhematologic toxicity (except nausea/vomiting in the absence of aggressive antiemetic therapy); (c) two or more consecutive week dose holds. If one patient in a cohort experienced a DLT, three additional patients would be enrolled at the same dose level. If none of the subsequent three patients experienced a DLT, then the dose of etoposide was escalated. Maximum-tolerated dose (MTD) was defined as the dose level that preceded a dose level at which two or more patients experienced a DLT.

Clinical Evaluation. Pretreatment evaluation included physical examination, complete blood count (CBC), serum chemistries, electrocardiogram, urinalysis, and chest X-ray. All measurable disease was documented by physical examination and/or any appropriate imaging studies before the initiation of protocol therapy. Additionally, any relevant biochemical tumor markers were obtained before therapy. After treatment, a history and physical examination, CBC, serum chemistries, and urinalysis were performed weekly. (CBCs were performed twice weekly for the first two weeks.) Urine dipsticks were obtained before each cycle of therapy and weekly for the first 3 weeks of therapy. A urinalysis was performed if the urine dipstick was abnormal. Disease measurable by physical examination or on plain radiographs was evaluated after each cycle of therapy. Other imaging procedures required for response determination were obtained after every two cycles of treatment. Toxicity was assessed weekly using NCI Common Toxicity Criteria, version 2.0. Responses were evaluated either by physical examination or by appropriate imaging studies according to WHO criteria (14).

Pharmacokinetics. Heparinized blood samples were obtained immediately before starting the irinotecan infusion on day 1 at 30 min after start of the infusion and at 0, 5, 15, and 30 min and 1, 2, 3, 4, 7, and 24 h after the end of the infusion. Total (lactone + carboxylate) concentrations of irinotecan and SN-38 were measured by HPLC with fluorescence detection as de-

scribed previously (12). SN-38 glucuronide (SN-38G) concentrations were determined by measuring the increase in SN-38 concentration after incubation of plasma with β -glucuronidase and heating at 40°C for 15 min.

Irinotecan, SN-38, and SN-38G plasma concentration–time data were analyzed by noncompartmental methods (WinNonlin Standard, Version 1.5). Peak plasma concentrations (C_{max}) were determined by inspection of individual irinotecan, SN-38, and SN-38G concentration–time curves. The apparent elimination half-life ($t_{1/2}$) was determined from linear least-squares regression of plasma-concentration time points in the terminal log-linear region of the plasma concentration–time profiles. The areas under the curve (AUC) from time 0 to 24 h after the end of the infusion (AUC_{0–24}) was calculated using the linear trapezoidal rule (15). Scatter plots and Pearson correlation coefficients were used to compare irinotecan/metabolite pharmacokinetic parameters with neutrophil nadirs.

Analysis of Top1 and Top2 Levels in Peripheral Blood Cells. Mononuclear cells were obtained from ~10 ml of peripheral blood by Ficoll gradient centrifugation immediately before the first dose of irinotecan and at 24 and 48 h after the end of infusion. The 48-h sample was taken immediately before administration of etoposide. The cells were lysed in a buffer containing guanidine isothiocyanate and phenol that allows for the simultaneous isolation of RNA, DNA, and protein (8). Equal amounts of protein from each sample were subjected to SDS-PAGE followed by transfer to nitrocellulose membranes. Top2 α , Top1, and histone immunoblotting was performed sequentially on the blots as described previously (8). Immunoreactive bands were quantified by densitometry using a Bio-Rad Molecular Imager System GS-525. Control studies with purified topoisomerase proteins were performed to ensure that the values obtained from the densitometer were within the linear range of quantitation using this assay.

RESULTS

Patient Characteristics and Cohorts. Twenty-three male and 17 female patients were enrolled in this study (Table 1). All but one patient had received previous chemotherapy, radiotherapy, or both (Table 1). Several patients had non-small lung cancer, with the other patients having a variety of different malignancies (Table 1). These 40 patients received 122 cycles of PIE therapy in seven different cohorts. The initial cohort of patients were treated using a 4-weeks-on/2-weeks-off schedule, involving administration of 20 mg/m² cisplatin and 50 mg/m² irinotecan on days 1, 8, 15, and 22, with 50 mg/m² etoposide given i.v. on days 3, 10, 17, and 24. Because of the occurrence of DLT and frequently missed doses during the first cycle of therapy in this initial cohort, drug administration was modified to a 2-weeks-on/1-week-off schedule in the second cohort. Subsequent cohorts were treated using this schedule, with dose escalation of i.v. etoposide. To enhance patient convenience, when an MTD was defined for i.v. etoposide, additional cohorts of patients were enrolled to define an MTD for oral administration of etoposide.

Toxicity. Among the initial three patients enrolled in the first cohort, none completed a first cycle of therapy. One patient required palliative radiation therapy for pain control and in the

Table 1 Patient characteristics

Characteristic	Number of patients
Male	23
Female	17
Median age in years (range), 58.5 (31–78)	
Previous Therapies	
0	1
1	17
2	15
3	4
3+	2
Previous radiation therapy	21
ECOG ^a Performance Status	
0	16
1	21
2	3
Tumor Histology	
NSCLC	10
Bladder	5
Sarcoma	5
Breast	3
Mesothelioma	2
Ovarian	2
Pancreatic	2
Gastric	2
Renal	2
Other	7

^a ECOG, Eastern Cooperative Oncology Group; NSCLC, non-small cell lung cancer.

two others, doses were held because of neutropenia during week 4. A fourth patient experienced dose-limiting (grade 4) diarrhea at the end of the second week of treatment (day 14). Profound neutropenia also occurred at this time, and the patient died on day 15 despite aggressive use of i.v. antibiotics and antidiarrhea therapy. This patient had bladder cancer and had previously received several cycles of a combination of gemcitabine, carboplatin, and paclitaxel. Among three subsequent patients treated at this dose level, two received all planned doses of therapy during the first cycle, but treatment was held on week two in one patient because of grade 2 diarrhea. An additional three patients were enrolled at this dose level to better characterize the toxicity rate. Two of these last three patients received all planned first cycle doses, with treatment held on week 4 in the third patient because of grade 2 diarrhea. Thus, excluding the patient who did not complete a first cycle because of the need for radiation therapy, only four of nine patients in this cohort were able to complete a full cycle of therapy, and one patient died as a result of toxicity. Therefore, this dose/schedule was considered intolerable, and we decided to modify the treatment to a 2-weeks-on/1-week-off schedule.

The 2-weeks-on/1-week-off schedule was associated with a lower toxicity rate, enabling escalation of etoposide from 50 mg/m² to 75 mg/m² (Tables 2 and 3). One patient treated at the 75 mg/m² dose level had doses held on days 8 and 22 because of grade 3 neutropenia. This occurrence was defined as a DLT by the protocol and resulted in enrollment of six patients at this dose level. None of the other five patients experienced cycle-1 dose holds or DLTs. Escalation of the etoposide dose to 100

Table 2 Severe hematologic toxicities

Etoposide (mg/m ²)	Cohort	Number of Patients	Number of Cycles	Neutropenia			Thrombocytopenia		Anemia	
				Grade 3 or 4 < 7 Days	Grade 3 or 4 with Fever	Grade 4 > 7 Days	Grade 3	Grade 4	Grade 3	Grade 4
50	1A ^a	10	16	5/11 ^b	0/0	0/1	0/2	0/0	2/4	0/0
50	1B	3	20	1/2	0/0	0/0	0/0	0/0	0/1	0/0
75	2	6	27	1/7	0/0	0/0	0/0	0/0	0/0	0/0
100	3	1	0	0/0	1/1	1 ^c /1	0/0	0/0	0/0	0/1
150 (oral)	4	7	23	2/5	1/1	1 /1	0/0	0/0	0/1	1/1
110 (oral)	5	7	10	2/3	1/1	0/0	1/1	0/0	2/3	0/0
85 (oral)	6	6	26	0/1	0/0	0/0	0/0	0/0	1/2	0/0

^a Patients in cohort 1A were treated with a 4-weeks-on/2-weeks-off schedule, whereas patients in cohort 1B and the other cohorts were treated with a 2-weeks-on/1-week-off schedule.

^b Numbers indicate occurrences in first cycle/occurrences in any cycle.

^c Bold indicates that this toxicity was a dose limiting toxicity.

Table 3 Severe nonhematologic toxicities

Etoposide (mg/m ²)	Cohort	Number of Patients	Number of Cycles	Nausea		Vomiting		Diarrhea	
				Grade 3	Grade 4	Grade 3	Grade 4	Grade 3	Grade 4
50	1A ^a	10	16	0/0 ^b	0/0	0/0	0/0	0/0	1 ^c /2
50	1B	3	20	0/0	0/0	0/0	0/0	0/0	0/0
75	2	6	27	0/0	0/0	0/0	0/0	0/0	0/0
100	3	1	0	0/0	0/0	0/0	0/0	1 /1	0/0
150 (oral)	4	7	23	0/0	0/0	0/0	0/0	1 /1	0/0
110 (oral)	5	7	10	0/0	0/0	1 /1	0/0	3 /4	0/0
85 (oral)	6	6	26	0/0	0/0	0/0	0/0	0/0	0/0

^a Patients in cohort 1A were treated with a 4-weeks-on/2-weeks-off schedule, whereas patients in cohort 1B and the other cohorts were treated with a 2-weeks-on/1-week-off schedule.

^b Numbers indicate occurrences in first cycle/occurrences in any cycle.

^c Bold in indicates that this toxicity was a dose-limiting toxicity.

mg/m² was associated with both hematologic and nonhematologic DLTs (febrile, prolonged grade 4 neutropenia, and grade 3 diarrhea, respectively) in the first patient enrolled in this cohort. Because of the severity of the toxicity experienced by this patient and the occurrence of missed doses during cycles 1 and 2 because of neutropenia at the previous dose level, additional patients were not enrolled at the 100 mg/m² etoposide dose level, and 75 mg/m² was defined as the MTD.

Next, i.v. etoposide was replaced with oral etoposide to facilitate patient convenience. A tolerable oral etoposide dose was estimated to be 150 mg/m², based on an MTD of 75 mg/m² for i.v. etoposide and a reported bioavailability of near 50% for oral etoposide (13). However, two of six patients who received 150 mg/m² etoposide p.o. experienced DLTs (prolonged grade 4 neutropenia and grade 3 diarrhea, respectively), which resulted in de-escalation of oral etoposide to 110 mg/m². At this dose level, three of five patients experienced DLTs (all grade 3 diarrhea), which resulted in further de-escalation of etoposide to 85 mg/m². Because there were no DLTs among six patients who were treated with this dose, 85 mg/m² was defined as the MTD for oral etoposide combined with irinotecan/cisplatin using a 2-weeks-on/1-week-off schedule.

Antitumor Activity. A partial response occurred in a 54-year-old male patient with gastric cancer and extensive hepatic metastases. This patient was treated previously with 5-

fluorouracil/leucovorin/etoposide. After four cycles of PIE therapy administered using the 2-weeks-on/1-week-off schedule (etoposide dose 50 mg/m²), there was a 65% reduction in measurable disease and normalization of carcinoembryonic antigen from 18.9 to 2.0 ng/ml. This patient remained on the protocol for 36 weeks until his disease progressed.

Three other patients had detectable decreases in measurable or evaluable disease that did not meet criteria for partial response. The etoposide dose was 50 mg/m² in each of these patients. In a patient with mesothelioma, who had received docetaxel and doxorubicin/LY335979 (a P-glycoprotein inhibitor) previously, there was a 36% reduction in measurable disease after two cycles of therapy using the 4-weeks-on/2-weeks-off schedule. Treatment continued for 32 weeks before disease progression. A 34% reduction in measurable disease occurred in a patient with ovarian cancer who was treated previously with a combination of paclitaxel and carboplatin. This patient received one cycle of PIE therapy using the 4-weeks-on/2-weeks-off schedule, with subsequent cycles administered using the 2-weeks-on/1-week-off schedule. There was also a reduction in serum CA-125 in this patient from 44.8 to 11.6 units/ml after two cycles of therapy. This patient remained on study for 24 weeks until disease progression. There was a significant improvement in nonmeasurable pulmonary infiltrates in a patient with breast cancer who was treated previously with a combina-

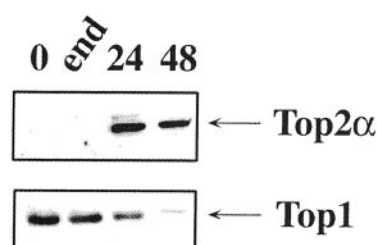


Fig. 1 Topoisomerase protein levels in PBMNCs obtained from patient 14. The panels represent sequential immunoblotting of a single nitrocellulose filter. 0 and end refer to samples obtained immediately before and after the irinotecan infusion, respectively. 24 and 48 indicate hours after the end of the irinotecan infusion. Note that the 48-hour time point corresponds to a sample obtained just before the etoposide infusion.

tion of cyclophosphamide, methotrexate, and 5-fluorouracil, as well as paclitaxel and doxorubicin. This patient had undergone wedge resection of a pulmonary metastasis in the past. Her serum carcinoembryonic antigen level also decreased from 75.5 to 24.5 ng/ml after two cycles of therapy administered using the 4-weeks-on/2-weeks-off schedule. Therapy was discontinued in this patient after an additional two cycles because of prolonged neutropenia.

In addition to these responses, prolonged stable disease occurred in a patient with non-small cell lung cancer (who received 8 cycles), a patient with sarcoma (who received 8 cycles), and a patient with mesothelioma (who received 11 cycles). With the exception of the patient with mesothelioma, these patients had received at least one prior chemotherapy regimen.

Pharmacokinetics/Pharmacodynamics. Increases in PBMNC Top2 α levels were observed in a previous study involving oral camptothecin administered daily for 14 days, with the increases typically occurring on day 3 (8). These increases in Top2 α were often accompanied by decreases in Top1 PBMNC protein levels (8). Similar assays were performed in the current study. Although decreases in Top1 PBMNC protein levels after the irinotecan infusion were observed in a few patients (Fig. 1), Top1 protein degradation was present in many samples and precluded a more comprehensive assessment. By contrast, serial Top2 α PBMNC levels were quantifiable in six patients. At the time of the first dose of etoposide, Top2 α levels were increased significantly relative to baseline in two of the six patients (Fig. 1 and 2). Both patients received 50 mg/m² etoposide i.v. One patient had gastric cancer and had a partial response to treatment that lasted for eight cycles. The other patient had colon cancer and had disease progression after the second cycle of therapy.

Plasma concentrations of irinotecan, SN-38, and glucuronidated SN-38 (SN-38G) were measured in 15 patients, including the six patients for whom PBMNC Top2 α data were available. The pharmacokinetic parameters obtained from this analysis (Table 4) were similar to those reported previously in a trial involving weekly administration of irinotecan/cisplatin (12). Furthermore, similar to this previous trial, there was no detectable relationship between neutrophil nadirs and any of the irinotecan/metabolite pharmacokinetic parameters. In addition, irinotecan and SN-38 C_{max} and AUC values in the two patients

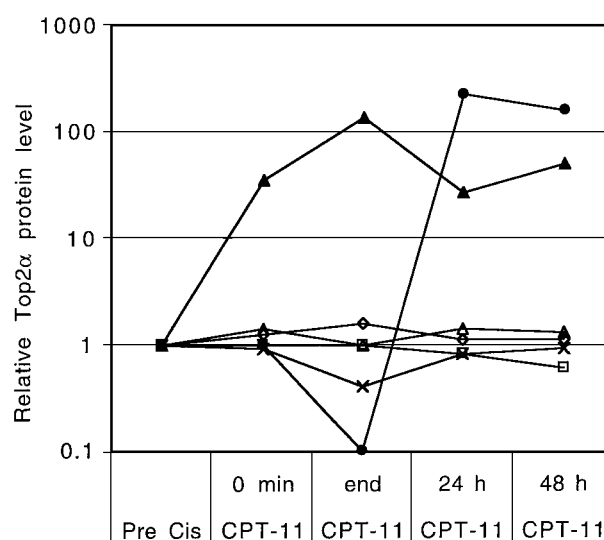


Fig. 2 Fractional change in Top2 α protein levels in PBMNCs obtained from six patients after treatment with cisplatin/irinotecan. PBMNCs were collected and analyzed for Top2 α protein levels as described in "Patients and Methods." Pre Cis refers to a baseline level obtained just before the cisplatin infusion. 0 min CPT-11 refers to a sample obtained after administration of cisplatin but immediately before initiation of the irinotecan infusion. end CPT-11 refers to a sample obtained immediately after the infusion, whereas 24 h CPT-11 and 48 h CPT-11 refer to time intervals after the end of the infusion. Note that the 48 h time point corresponds to a sample obtained just before the etoposide infusion. For each patient, Top2 α protein levels are expressed relative to the value obtained before the cisplatin infusion.

with increases in Top2 α PBMNC levels were similar to values observed in patients in whom Top2 α levels did not change after the irinotecan infusion (Tables 4 and 5). However, the two patients with increases in Top2 α PBMNC protein levels had the highest SN-38 glucuronide C_{max} and AUC values among the 15 patients with available pharmacokinetic data (Table 5).

DISCUSSION

Top1-targeting is a relatively new anticancer strategy, and there is considerable interest in combining Top1-targeting drugs such as the camptothecins with other anticancer therapies. Combinations that have received notable attention include irinotecan with 5-fluorouracil in the treatment of patients with colon cancer (16) and irinotecan with cisplatin in the treatment of patients with small cell lung cancer (17). Combinations of camptothecins with Top2-targeting drugs have also been pursued (8, 18–23) and are based on strong supportive preclinical data indicating that simultaneous or sequential topoisomerase-targeting yields additive or synergistic cytotoxicity (9, 24, 25). However, in none of these clinical trials was the timing of etoposide administration based on studies of Top2 protein modulation in patient tissues. By contrast, the administration of etoposide in this study was based on previous data indicating that peak PBMNC Top2 α protein levels usually occurred 2 to 3 days after initiation of daily oral camptothecin (8).

Although this trial included concurrent administration of cisplatin with irinotecan, Top2 α PBMNC levels were increased

Table 4 Pharmacokinetic parameters for irinotecan, SN-38, and SN-38 glucuronide in 15 patients.

	Irinotecan	SN-38	SN-38 glucuronide
C_{\max} (ng/mL)	612 ± 237 ^a	12.9 ± 4.3	47 ± 14.2
AUC _{0-24h} (ng × h/mL)	3094 ± 879	108 ± 31	551 ± 277
CL (L/h/m ²)	14.5 ± 4.5	ND ^b	ND

^a Values are expressed as mean ± SD.

^b ND, not determined.

Table 5 Pharmacokinetic parameters for irinotecan, SN-38, and SN-38 glucuronide in two patients with increases in Top2 α PBMNC levels after cisplatin/irinotecan treatment.

	Irinotecan		SN-38		SN-38 glucuronide	
	Pt. ^a 13	Pt. 14	Pt. 13	Pt. 14	Pt. 13	Pt. 14
C_{\max} (ng/mL)	536	634	9.71	17.4	75.8	78.3
AUC _{0-24h} (ng × h/mL)	4020	4477	123	150	1231	1057

^a Pt., patient.

at the time of the first etoposide dose in two of six evaluable patients. The finding that these two patients had the highest SN-38 glucuronide C_{\max} and AUC values among evaluated patients is intriguing. Glucuronidation of SN-38 is involved in hepatic clearance of this compound (26), and is implicated in cellular resistance (27). It is possible that metabolism of camptothecins, including glucuronidation, is important in the modulation of tissue Top2 α protein levels by these drugs. However, it is not yet clear whether treatment with camptothecins increases Top2 protein levels in tumor tissues and whether the timing of these increases would be similar to that observed in PBMNCs. In one study of patients with nonhematologic malignancies, a slight increase in tumor Top2 α protein levels was observed in only one of six patients (7 days after topotecan administration; see Ref. 22). Nevertheless, these kinds of studies are hampered by difficulties in obtaining sequential tumor biopsies from patients with nonhematologic malignancies. By contrast, studies of circulating blasts in nine leukemia patients treated with topotecan indicated transient increases in blast cell Top2 α protein levels in most of the patients (28). Furthermore, results from a recent study indicated that patients with a $\geq 40\%$ increase in leukemic blast Top2 α protein levels on day 4 were more likely to respond to treatment consisting of topotecan administration on days 1–3, followed by a combination of etoposide/mitoxantrone on days 4, 5, 9, and 10 (29).

The results of the current trial indicate that the addition of etoposide on day 3 to a weekly irinotecan/cisplatin schedule is tolerable, with the MTD of etoposide 75 mg/m² for i.v. administration and 85 mg/m² for oral administration. The finding that the MTD of oral etoposide is 85 mg/m² is somewhat surprising, given a predicted bioavailability of $\sim 50\%$ for oral administration of this drug (13). It is possible that oral administration of etoposide results in greater intestinal tissue concentrations of this drug, which augment the intestinal toxicities of irinotecan. Another possibility is that irinotecan-induced alterations in the intestinal mucosa result in increased etoposide absorption.

With regard to antitumor activity, it is not clear from this trial that a strategy of sequential topoisomerase-targeting combined with cisplatin is effective in overcoming resistance to

camptothecins such as irinotecan. Nevertheless, several previously treated patients experienced objective responses or prolonged disease stabilization while receiving PIE therapy. In addition, the best response observed in the trial occurred in one of the two patients with increased Top2 α PBMNC levels at the time of the first administration of etoposide, which is consistent with the idea that camptothecin-induced increases in Top2 protein expression will augment responses to Top2-targeting drugs. Phase II clinical trials are in progress to evaluate the PIE regimen in the treatment of patients with gastric and small cell lung cancer. Additional studies are necessary to evaluate mechanisms of resistance to camptothecins and whether sequential topoisomerase-targeting strategies are capable of overcoming this resistance. We are currently conducting a study of sequential topoisomerase-targeting in patients with leukemia, in which Top2 protein levels and activity will be quantified in blasts obtained from patients receiving 1- β -D-arabinofuranosylcytosine/topotecan induction therapy. The timing of peak Top2 alterations will be evaluated to determine an optimal time for administration of etoposide. In addition, we are evaluating possible mechanisms of resistance to topotecan, including analyses of intracellular topotecan accumulation, BCRP expression, topoisomerase I mutations, and topoisomerase I ubiquitination and degradation.

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