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Afshin Dowlati · Nathan Levitan · Nahida H. Gordon
Charles L. Hoppel · David M. Gosky
Scot C. Remick · Stephen T. Ingalls
Sossamma J. Berger · Nathan A. Berger

Phase II and pharmacokinetic/pharmacodynamic trial of sequential topoisomerase I and II inhibition with topotecan and etoposide in advanced non-small-cell lung cancer

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Abstract *Purpose*: In vitro and in vivo preclinical models have demonstrated synergistic activity when topoisomerase I and II inhibitors are administered sequentially. Topoisomerase I inhibitors increase topoisomerase II levels and increase cell kill induced by topoisomerase II poisons. We evaluated this hypothesis in a cohort of patients with advanced non-small-cell lung cancer (NSCLC). *Methods*: A group of 19 patients with advanced NSCLC (70% adenocarcinoma) received topotecan at a dose of 0.85 mg/m^2 per day as a continuous 72-h infusion from days 1 to 3. Etoposide was administered orally at a dose of 100 mg twice daily for 3 days on days 7–9 (schedule and dose derived from prior phase I trials). Total and lactone topotecan concentrations were measured at the end of the 72-h infusion. Blood samples were obtained immediately after each 72-h topotecan infusion in order to measure the mutational frequency at the hypoxanthine phosphoribosyl transferase (HPRT) locus in peripheral lymphocytes. *Results*: A total of 55 cycles were administered. Toxicity was mainly hematologic with grade 4 neutropenia occurring in 7% of courses. Only one partial response and two stable diseases were observed. The 1-year survival rate was 33%. There was a statistically significant difference between steady-state lactone concentrations between cycle 1 and cycle 2 with decreasing concentrations with cycle 2 ($P = 0.02$). This was explained by a statistically significant increase in the clearance of topotecan lactone during cycle 2 ($P = 0.03$). Total but not lactone concentrations correlated with nadir WBC, ANC and platelet levels. Steady-state plasma lactone levels correlated with the mutational frequency at the HPRT locus ($P = 0.06$). In the one patient with a partial response a sixfold increase in HPRT mutational frequency was observed, which was not seen in patients with progressive disease. *Conclusion*: The combination of topotecan and etoposide in this schedule of administration has minimal activity in adenocarcinoma of the lung. This lack of activity may be due to the delay in administration of etoposide after the topotecan as studies have shown that the compensatory increase in topoisomerase II levels after treatment with topoisomerase I inhibitors is shortlived (< 24 h). The HPRT mutational frequency results suggest that the lack of clinical response may be associated with failure to achieve sufficient cytotoxic dose as indicated by a lack of increase in mutational frequency in those patients with progressive disease. HPRT mutational frequency may correlate with plasma steady-state topotecan lactone levels. Future studies should be directed toward earlier administration of topoisomerase II inhibitors after topoisomerase I inhibition.

Key words Topoisomerase I and II · HPRT mutation · Clinical trial · Non-small-cell lung cancer · Pharmacokinetics

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N. Levitan · N. H. Gordon · D. M. Gosky · S. C. Remick
S. J. Berger · N. A. Berger
Ireland Cancer Center at University Hospitals of Cleveland
and Case Western Reserve University, Cleveland, Ohio, USA

C. L. Hoppel · S. T. Ingalls
Veterans Administration Medical Center, Cleveland, Ohio, USA

A. Dowlati (✉)
Division of Hematology/Oncology,
Case Western Reserve University
and University Hospitals of Cleveland,
11100 Euclid Avenue, Cleveland, Ohio 44106, USA
e-mail: axd44@po.cwru.edu
Tel.: +1-216-8445181; Fax: +1-216-8445234

Introduction

The topoisomerases are DNA enzymes involved in controlling the topology of the supercoiled DNA double helix during cellular functions, namely transcription and

replication of cellular genetic materials. The mechanisms of action of these enzymes involve DNA cleavage and strand passage through the break, followed by religation of the cleaved DNA. Following induction of DNA strand breaks and formation of covalent "cleavable" topo-DNA complexes, the torsional strain in DNA is alleviated via strand passage, and enzymatic reunion occurs. There is evidence that topoisomerase II can function like topoisomerase I, albeit less efficiently [2].

Because topoisomerase I- and topoisomerase II-targeting agents exert their principal effects on two major classes of enzymes involved in regulating DNA topology, which may overlap functionally, there has been considerable interest in combining these two classes of agents. In preclinical studies, depending on the timing of administration of these agents, mixed results have been observed when these agents are combined. When topoisomerase I agents and etoposide are given simultaneously in vitro a less than additive effect has been seen in a variety of cell lines, including hamster lung fibroblasts, HT-29 human colon carcinoma, and leukemia cell lines [12, 23]. One possible explanation for this antagonism is that topoisomerase II-targeting agents inhibit nucleic acid synthesis that is required to convert topoisomerase I-DNA adducts to cytotoxic lesions [6, 10, 23]. However, when topoisomerase II inhibitors and either camptothecin or its hydrophilic analog, topotecan (9-dimethylaminomethyl-10-hydroxycamptothecin) are administered sequentially, at least an additive and sometimes a synergistic effect has been seen in hamster fibroblasts, as well as cell lines derived from human leukemia, colon carcinoma and a variety of other tumor types [3, 6, 10].

Similar schedule-dependent effects have been noted when camptothecin analogues are combined with topoisomerase II inhibitors in vivo [21, 37]. For example, simultaneous administration of topotecan and etoposide is no more effective in a murine leukemia model than either drug alone, whereas sequential administration is synergistic [21]. Similarly, combinations of the topoisomerase I inhibitor irinotecan and the topoisomerase II-directed agent doxorubicin fail to demonstrate synergy when the two agents are administered simultaneously, but the administration of irinotecan prior to doxorubicin is synergistic [24, 36]. It has been proposed that this synergy might be due to compensatory upregulation of topoisomerase II levels and, therefore, enhanced cytotoxicity of topoisomerase II inhibitors in cells treated initially with topoisomerase I inhibitors [35]. Indeed it has been shown, in vitro, that topotecan increases topoisomerase IIα levels and sensitivity to treatment with etoposide in a schedule-dependent manner [37]. In humans, Gupta et al. have shown induction of topoisomerase II by camptothecin in peripheral blood mononuclear cells from patients undergoing a phase I clinical trial of sequential treatment with camptothecin and etoposide [14].

The cytotoxicity of single-agent topotecan, in advanced non-small-cell lung cancer (NSCLC), was assessed by Perez-Solar et al. in 40 patients who were chemotherapy-naive [27]. Topotecan was given at a dose of 1.5 mg/m^2 for five consecutive days every 21 days. Six patients (15%) with stage IV disease had a partial response. Another 35% of patients had either a minor response or stable disease. Squamous cell carcinoma had a higher response rate of 36% versus 4% for other histologies. Although the response rate was low, an impressive 30% 1-year survival rate and a median survival of 38 weeks was seen. Perez et al. expanded their initial study and treated a total of 29 evaluable patients with squamous cell carcinoma and obtained a 24% response rate. In another trial in which the majority (85%) had adenocarcinoma of the lung, no partial responses were seen [26]. Another randomized phase II trial compared the efficacy of two schedules (1.5 mg/m^2 per day for 5 days versus 1.3 mg/m^2 per day continuous infusion for 3 days) of topotecan in advanced untreated NSCLC [39]. Patients with the daily ×5 schedule had a higher response rate (18%) compared to the 72-h continuous infusion schedule (8%). In terms of combination therapy, cisplatin and topotecan have been combined in a phase II trial [38]. A median survival of 32 weeks and a 1-year survival of 25% were seen. Other trials combining topotecan with non-platinum agents in NSCLC are ongoing.

Based on the preclinical data described above demonstrating potential clinical interest in sequential topoisomerase I and II inhibition and the initial data on single-agent topotecan in NSCLC, we undertook a phase II trial of topotecan treatment followed by etoposide in advanced NSCLC. The dose and schedule chosen were based on the results of prior phase I trials of this regimen [16, 25].

Because of the possible mutagenic activity of topoisomerase poisons, the mutational frequency at the hypoxanthine phosphoribosyl transferase (HPRT) locus was examined in peripheral blood lymphocytes obtained before and during treatment. Furthermore, preclinical data suggested that mutational frequency correlates with cell kill and may be a surrogate marker for clinical efficacy [9]. Given the difficulty in obtaining serial tumor tissue biopsies from lung cancer patients, lymphocytes were used as a surrogate tissue for this purpose. The use of surrogate tissue rather than tumor tissue for pharmacodynamic analyses, however, has recently been criticized [33].

Patients and methods

Patients

Between June 1995 and December 1996, 19 patients with stage IIIB or IV NSCLC were enrolled in this phase II single-institution study. In order to be eligible for protocol enrollment, patients were required to have histologically confirmed, inoperable, stage IIIB or IV NSCLC. No prior cytotoxic chemotherapy was allowed. Bidimensionally measurable disease was required. All patients were ≥18 years of age, with an ECOG performance status of 0–2, and a life expectancy ≥12 weeks. Required laboratory parameters

included serum creatinine ≤1.5 mg/dl, WBC ≥3500/μl, granulocytes ≥1500/μl, platelets ≥100,000/μl, total bilirubin ≤1.5 mg/dl, SGOT and alkaline phosphatase less than three times the upper limit of normal. Patients with brain metastases were ineligible.

Chemotherapy

Topotecan was supplied as a lyophilized formulation by the NCI (Bethesda, Md.) in vials that contained 4 mg topotecan as the base. The contents of each 4-mg vial were reconstituted with 4 ml sterile water for injection (USP) yielding a 1 mg/ml solution of topotecan AS. The appropriate amount of drug was diluted in bacteriostatic water for injection (USP) to a volume suitable for the pump (within the concentration range 0.02–0.1 mg/ml). Topotecan AS was administered as a continuous infusion at a dose of 0.85 mg/m^2 per 24 h for 72 h from days 1 to 3 of each cycle. Etoposide was administered orally at a dose of 100 mg twice daily for 3 days on days 7–9. Cycles were repeated every 21 days. This dose of topotecan was chosen because it has been previously shown to be the maximum tolerated dose (MTD) in a phase I trial [25]. Furthermore, a study by Hammond et al. has demonstrated the MTD of topotecan and etoposide in chemonaive patients (in the same schedule of administration) to be topotecan 0.85 mg/m^2 per 24 h for 72 h from days 1–3 and etoposide 100 mg/m^2 per day for 3 days on days 7–9 [16]. Topotecan was infused via a central venous access device using an ambulatory infusion pump.

Pharmacokinetic monitoring

Blood (5 ml) was drawn into methanol tubes for determination of topotecan concentrations before and at the completion of the 72-h topotecan infusion during cycles 1 and 2. A 2.5-ml portion of the drug was obtained from the drug cassette for determination of topotecan concentrations before and at the completion of the 72-h topotecan infusion during cycles 1 and 2 only. Specimen processing, extraction, and chromatographic quantitation of both the lactone and open-ring species of topotecan were performed using a modified method that was originally developed by Beijnen et al. [5]. These modifications enabled measurement of very low plasma topotecan concentrations observed in some of the topotecan long-duration, low infusion rate topotecan protocols.

Mutational frequency at the HPRT locus

Blood samples were obtained immediately prior to starting treatment and after each 72-h infusion of topotecan in order to measure the mutational frequency at the HPRT locus in peripheral lymphocytes. Lymphocytes were isolated from freshly obtained heparinized blood samples and grown in the presence of 10 μ*M* 6-thioguanine (6-TG) with controls grown in absence of drug [17]. After a 14-day exposure to 6-TG, the numbers of colonies containing 50 cells or more in control and 6-TG-treated plates were counted microscopically to determine the clonal presence of HPRT-negative mutants. Mutant frequency (Mf) was calculated as follows:

$$\mathrm{Mf} = \frac{\text{Cloning efficiency in the presence of } 6-\mathrm{TG}}{\text{Cloning efficiency in the absence of } 6-\mathrm{TG}}$$

Cloning efficiency was determined by Poisson statistics as previously described by Henderson et al. [18]. The HPRT Mf assay was also performed on normal lymphocytes donated by healthy donors to determine a baseline for the mutation study (data not shown).

Outcome end-points and statistical analyses

ECOG criteria were used to determine performance status and response. Partial remission was defined as a greater than 50% reduction in the area of measurable disease, as determined by calculating the products of two perpendicular dimensions, of at least 4 weeks duration. Progressive disease was defined as an increase in the size of initial disease by 25% or more, or the development of new lesions. Toxicity is reported using the guidelines of the National Cancer Institute common toxicity criteria. The primary objective of this trial was to determine whether topotecan given as a 72-h infusion followed by oral etoposide is an effective regimen (response rate) in the treatment of NSCLC. A two-stage approach for patient accrual was used in this study to allow for early closing if the regimen proved to be ineffective.

The statistical design was a minimax as proposed by Simon [31] and is based on testing the null hypothesis that the true response rate is ≤20%. Therefore if the major response rate (CR + PR) to this regimen is ≤20% (the null hypothesis) then there is a maximum probability of 0.10 of concluding that the chemotherapy regimen is sufficiently promising that it should be accepted for further study. Thus an initial 19 patients were enrolled on this trial. If there were three or fewer major responses (CR + PR) the trial would be discontinued, and the treatment would be considered to be ≤20% effective. If there turned out to be four or more responses in the initial 19 patients, an additional 17 patients would be accrued to this phase II trial. A Kaplan-Meier analysis was used to analyze the survival data. All patients were evaluable for toxicity and response. Correlation between pharmacokinetic and pharmacodynamic parameters was sought using linear regression statistics and the Spearman correlation coefficient.

Results

Patient characteristics are shown in Table 1. A total of 19 patients were enrolled. All but one patient had stage IV disease and 70% had adenocarcinoma as the histology. A total of 55 cycles were administered. All patients were evaluable for both toxicity and response.

Toxicity

Toxicity is shown in Table 2. This regimen had manageable toxicity. There were no treatment-related deaths. Grade 4 neutropenia occurred in 4 of 55 cycles (7%). Neutropenic fever occurred during only two cycles (4%). One of these was associated with sepsis. Grade 3/4

Table 1 Patient characteristics (values are number of patients, except age in years)

Characteristic	
Age (years)	
Median	60
Range	42–76
Sex	
Male	15
Female	4
Performance status	
0	2
1	10
2	7
Histology	
Adenocarcinoma	13
Squamous cell carcinoma	3
Large cell carcinoma	1
Stage	
IIIB	1
IV	18

Table 2 Toxicities

	Highest NCI toxicity grade in each patient (no. of patients $n=19$)				
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Anemia	2	5	8	3	1
Leukopenia	7	4	3	3	2
Neutropenia	9	4	2	0	4
Platelets	10	4	1	3	1
Infection	17	0	1	0	1
Nausea	12	7	0	0	0
Vomiting	16	3	0	0	0
Diarrhea	17	1	0	1	0
Stomatitis	15	4	0	0	0
Renal	17	1	1	0	0
Pulmonary	8	4	3	3	1
Cardiac	18	0	0	0	1
Fatigue	9	7	2	1	0
Neurosensory	13	3	3	0	0
SGOT	16	1	1	0	1
Calcium	15	3	1	0	0
Magnesium	18	0	1	0	0
Hypotension	17	0	1	1	0

thrombocytopenia occurred during 7% of cycles. Grade 3/4 anemia occurred in 7% of cycles. Grade 2 renal failure occurred in one patient. One patient developed a grade 4 increase in transaminases thought to be due to disease progression. Grade 2/3 pulmonary toxicities were all dyspnea and thought to be due to underlying cancer with severe chronic obstructive pulmonary disease. One patient developed pulmonary embolism accounting for the one incident of grade 4 pulmonary toxicity. The grade 4 cardiac toxicity consisted of rapid atrial fibrillation associated with hypotension.

Therapeutic course, response and survival

One patient achieved a partial response and received a total of ten cycles. Two other patients had stable disease and each received six cycles of therapy. There was no disease progression at the time their chemotherapy was stopped. All other patients experienced progressive disease. Three of these patients received four cycles of treatment and then experienced progressive disease. Eight patients received two cycles and five patients received only one cycle of treatment. Median survival of all patients was 7 months (Fig. 1). The 1-year survival rate was 33%. Since only one objective response was identified in the initial stage of the study, the trial was stopped.

Pharmacokinetics

The mean total topotecan concentration after the 72-h infusion of the first cycle was 3.5 ± 1.4 ng/ml ($\pm$SD) and the mean total topotecan concentration at the end of cycle 2 infusion was 3.0 ± 1.6 ng/ml. There was no significant difference, in paired comparisons, in the end of infusion topotecan plasma concentrations between cycle 1 and cycle 2 ($P=0.12$). The mean lactone topotecan concentration at the end of the cycle 1 infusion was 2.3 ± 1.0 ng/ml and for cycle 2 this was 1.5 ± 0.7 ng/ml. Of the19 patients, 17 had a decrease in lactone concentration at the end of infusion of cycle 2 compared to the end of cycle 1. There was a significant difference in paired comparisons between the end of infusion lactone concentrations of cycle 1 and cycle 2 ($P=0.02$) with decreased levels of plasma lactone drug with cycle 2 compared to cycle 1. This decrease in steady-state lactone levels with cycle 2 was explained by an increase in the lactone clearance.

In patients who received at least two cycles (and thus had pharmacokinetic evaluation during both cycles), the mean clearance during cycle 1 for topotecan lactone was 33 ± 12 l/h and for cycle 2 was 52 ± 23 l/h (Fig. 2). Paired analysis of clearances between the two cycles showed a

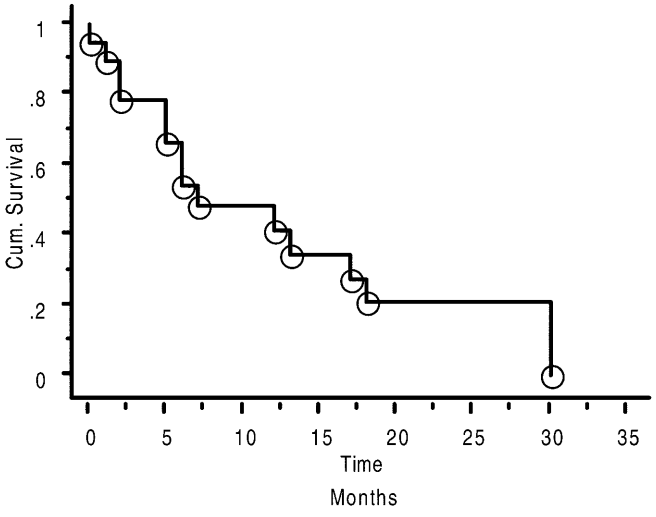

Fig. 1 Survival for all patients

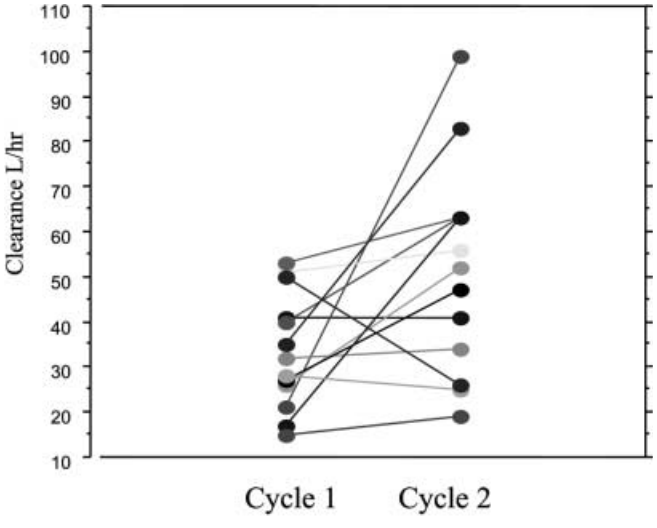

Fig. 2 Changes in clearance (l/h) of topotecan lactone in individual patients. Paired statistical analysis showed a significant increase in clearance with cycle 2 ($P=0.03$)

significantly higher clearance for cycle 2 ($P = 0.03$). No change in clearance in the total topotecan level was seen. No difference in serum creatinine or calculated creatinine clearance was observed between any of the cycles (data not shown). The end of infusion total topotecan concentration correlated with the nadir absolute neutrophil count (ANC) (coefficient −0.474, $P = 0.009$) while the nadir ANC did not correlate with the lactone concentrations ($P = 0.13$). Similarly, nadir WBC counts correlated with total (coefficient −0.495, $P = 0.004$) but not lactone levels. Nadir platelet counts also correlated with total topotecan concentrations (coefficient −0.369, $P = 0.03$).

Mutational frequency of the HPRT locus (pharmacodynamics)

The mutational frequency at the HPRT locus was examined in peripheral blood lymphocytes obtained from patients prior to starting treatment and after each 72-h infusion of topotecan. Before starting treatment, 17 samples were analyzed, and 14 were available after the first infusion of topotecan. For cycles 2, 3, 4, and 5 there were 7, 6, 3, and 2 samples collected for analysis, respectively. The patient receiving up to ten cycles had samples available after each topotecan infusion. Prior to therapy the mean HPRT mutational frequency was $2.6 \pm 1.3 \times 10^{-6}$, after cycle 1 infusion $3.9 \pm 2.1 \times 10^{-6}$, after cycle 2 infusion $4.7 \pm 2.7 \times 10^{-6}$, and after cycle 3 infusion $4.3 \pm 3.2 \times 10^{-6}$. There was no significant difference in paired t comparisons between each cycle of the HPRT mutational frequency.

In the one patient with a partial response, the HPRT mutational frequency fluctuated during chemotherapy cycles but had an overall trend to increase. The pretreatment value for this patient was 1.7×10^{-6} which increased to 10.7×10^{-6} after cycle 10 (Fig. 3). In addition, the topotecan lactone steady-state levels during cycle 2 correlated with the mutational frequency at the HPRT locus at the end of the cycle 2 topotecan infusion ($P = 0.06$, coefficient 0.612; Fig. 4). Total topotecan levels did not correlate with the HPRT mutational frequency. In addition, no correlation was seen between the mutational frequency and parameters of myelosuppression (nadir ANC or percentage decrease in ANC, data not shown).

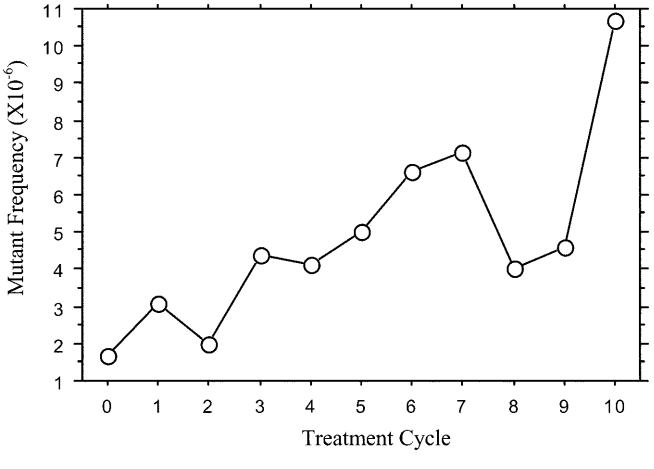

Fig. 3 Examination of mutational frequency at the HPRT locus in peripheral blood lymphocytes before treatment and after each 72-h infusion of topotecan in a patient achieving a partial response. This graph demonstrates a sixfold increase in the mutation frequency between pretreatment values and those after the tenth cycle

Discussion

In initial preclinical studies, the optimal administration schedule of topotecan varied depending on the model under investigation. Consequently, a number of schedules have been investigated. Based on preclinical xenograft studies indicating that topotecan efficacy is schedule-dependent, that prolonged administration is more effective [11, 20], and because of pharmacokinetic data indicating the short half-life of the topotecan lactone [29], we chose to study a continuous 72-h infusion as the treatment schedule for topotecan (at the time of this study oral topotecan was not available) [30]. Several initial trials have demonstrated only modest activity for single-agent topotecan in NSCLC, with a higher degree of activity in squamous cell histologies. In a combination of five trials involving single-agent topotecan in NSCLC, a total of 119 patients were treated with a 13% overall response rate [8]. A significant number of patients had stable disease. Despite relatively low response rates, single-agent topotecan demonstrated an impressive median survival of 38 weeks and a 1-year survival of 35%. It was thus logical to combine topotecan with agents with which preclinical data had demonstrated additive or synergistic effects and test them in NSCLC. Furthermore, the recent demonstration of significant activity in small-cell lung carcinoma also led to increased enthusiasm for testing this agent in NSCLC [4].

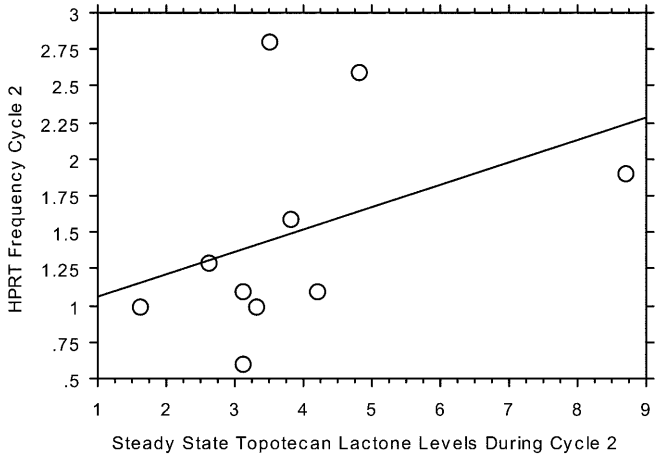

Fig. 4 Correlation between cycle 2 steady-state topotecan lactone levels (ng/ml) and mutational frequency at the HPRT locus ($\times 10^{-6}$; $P = 0.06$)

Our trial failed to show clinical value in terms of a meaningful objective response rate with this regimen in this schedule of administration. One possible explanation may be that 16 of 19 patients (70%) in our trial had adenocarcinoma or large-cell carcinoma while Perez-Solar et al. demonstrated preferential activity of this agent in squamous cell cancers [27]. A more attractive explanation for the lack of clinical efficacy may be the timing of administration of the topoisomerase II inhibitor. Preclinical data suggest significant synergism with topoisomerase I and II agents when given sequentially (with topoisomerase I agents given before topoisomerase II inhibition). It is thought that this may be due to increases in topoisomerase II levels with the topoisomerase I agents, thus rendering the cells susceptible to topoisomerase II poisons [37]. The lack of clinical effectiveness in our study and one other may be due to problems with the scheduling of etoposide after the topoisomerase I inhibition. In the study by Gupta et al., camptothecin was administered as a 14-day infusion, with a concomitant increase in peripheral blood mononuclear cell topoisomerase II levels [14]. Unfortunately, in that study also, for clinical safety reasons, etoposide was delayed until day 20 by which time the topoisomerase II levels had returned to baseline levels. In our study etoposide was given on days 7 through 9 by which time topoisomerase II levels increased by topotecan may have returned to baseline levels as found in the study by Gupta et al.

In a phase I and translational study by Hammond et al. in which tumor biopsies were done before and after topotecan (72-h infusion), no increase was seen in topoisomerase II levels in tumor specimens 24 h after the completion of the topotecan infusion [16]. However, posttreatment biopsies were available from only four patients, making a definite conclusion difficult. Furthermore, assessing for topoisomerase II levels in tumor tissue even only 24 h after completion of topoisomerase I inhibition may be too late. This is suggested by Gupta et al. who observed a very rapid (24-h) return of topoisomerase II levels in a surrogate tissue (peripheral blood mononuclear cells) back to baseline levels after topoisomerase I inhibition with camptothecin lactone [14]. Thus, the timing of etoposide or other topoisomerase II inhibitors after a topoisomerase I inhibitor must be addressed prospectively so as to optimize the potential synergy between these two classes of drugs. Furthermore, results reported after the design of this study have indicated that perhaps the daily ×5 schedule for topotecan may be superior to the 72-h infusion schedule in advanced NSCLC [39].

Pharmacodynamic correlations between parameters of systemic drug exposure and topotecan drug effects have been inconsistently reported. In a pediatric study, Stewart et al. [34] noted that both the total plasma (lactone plus carboxylate) AUC and topotecan lactone AUC correlated with the percentage change in platelet count and the granulocyte count following a 72-h infusion. Similar correlations have been reported for the total topotecan AUC following a 30-min infusion of topotecan given daily for 5 days [13, 29], and for a 24-h continuous infusion schedule [15]. In each case the total topotecan levels were as good as or better than the lactone drug levels as predictors of drug effects.

Our study is in concordance with the above studies and demonstrates that total but not lactone topotecan plasma levels at the end of the 72-h infusion correlate with nadir ANC. In contrast, no correlation between any drug level and toxicity was observed in one study of 24-h topotecan infusion [7] or during a prolonged 21-day continuous infusion [19]. Further studies are necessary to clarify these issues. Our study also demonstrated that the total topotecan level did not change between cycles 1 and 2; however, the lactone level decreased significantly with the 2nd cycle. This can be explained by an increase in the clearance of topotecan lactone but not of total topotecan thus suggesting an increased hydrolysis of the lactone form to the carboxylate form during cycle 2 compared to cycle 1. Since we did not determine the pharmacokinetics after cycle 2 it is unclear what happens to the drug after cycle 2. Previous studies have not looked at the pharmacokinetics of multiple cycles of topotecan.

This study also incorporated measurements of HPRT locus mutations in peripheral blood lymphocytes in order to assess the potential mutagenicity and the corresponding theoretical risk of secondary malignancies associated with topotecan therapy. The baseline HPRT mutational frequency rates obtained in our study are in concordance with published data from other laboratories showing a mutational frequency varying between 2.6×10^{-6} and 5×10^{-6} in normal subjects as well as pretreated lung cancer patients [1, 22, 28, 32]. Topotecan has been shown to produce qualitatively the same type of chromosomal damage as specific topoisomerase II-targeting agents, such as etoposide, which are known to be leukemogenic. Incorporating measurements of this kind into phase II drug trials is an attempt to obtain an early indication of the potential mutagenic or oncogenic risks of these new therapies. No statistically significant increase in the mean HPRT mutational frequency was seen during treatment with topotecan in the overall patient population. However, the single patient who had a partial response had a fluctuating but progressively increasing level of HPRT Mf during his ten cycles. We have previously shown that cell killing by topoisomerase poisons is proportional to the frequency of drug-induced sister chromatid exchanges or to the mutagenic frequency [9]. These observations suggest that lack of clinical response in these patients may be associated with failure to achieve a sufficient cytotoxic dose as indicated by lack of increase in mutation frequency.

Whether these measurements will ultimately be of value for predicting pharmacodynamic drug effects such as antitumor response or the risk of secondary malignancies must be evaluated in larger trials. The role of etoposide on the HPRT mutational frequency cannot not be assessed based on the design of this study, but a

previous study of single-agent etoposide in small-cell lung cancer has shown no effect of this agent on the mutational frequency at the HPRT locus of peripheral blood lymphocytes. Our trial is the first trial demonstrating that a pharmacokinetic parameter of topotecan infusion (steady-state lactone levels) correlates with the mutational frequency at the HPRT locus.

In conclusion, the combination of topotecan and etoposide in this schedule of administration has minimal activity in adenocarcinoma of the lung. Our results also suggest the value of additional studies: (1) To improve the scheduling of topoisomerase II inhibitor after inhibition of topoisomerase I during treatment of solid cancers, (2) to evaluate the differences among camptothecin analogs in their ability to induce topoisomerase II, and (3) to analyze whether response to treatment is dependent on the type of tumor, as suggested by differences in cell lines as well as the clinical observation of greater activity in squamous cell cancer of the lung.

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