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Pharmacodynamic and Pharmacokinetic Evaluation of Two Dosing Schedules of Indotecan (LMP400), a Novel Indenoisoquinoline, in Patients with Advanced Solid Tumors

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Abstract

Background—Indenoisoquinolines are non-camptothecin topoisomerase 1 (Top1) inhibitors with activity against camptothecin-resistant cell lines. Two dosing schedules of the novel indenoisoquinoline, indotecan (LMP400), were evaluated in patients with advanced solid tumors.

Methods—The maximum tolerated dose (MTD), toxicities, and pharmacokinetics of two indotecan drug administration schedules (daily for 5 days or weekly) were investigated. Modulation of Top1 and the phosphorylation of histone H2AX (γ H2AX) were assessed in tumor biopsies; γ H2AX levels were also evaluated in circulating tumor cells (CTCs) and hair follicles to assess DNA damage response.

Results—An MTD of 60 mg/m²/day was established for the daily regimen, compared to 90 mg/m² for the weekly regimen. The top1 response to drug showed target engagement in a subset of tumor biopsies. Dose-dependent decreases in total CTCs were measured in 7 patients. Formation of γ H2AX-positive foci in CTCs (day 3) and hair follicles (4–6 hours) was observed following treatment.

Conclusions—We established the MTD of 2 dosing schedules for a novel Top1 inhibitor, indotecan. No objective responses were observed on either schedule. The principal toxicity of both

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schedules, myelosuppression, prevented further dose escalation. Increased DNA damage response was observed in CTCs, hair follicles, and a subset of tumor biopsies.

Keywords

DNA Damage; DNA Topoisomerases; Type 1; TOP1 protein; human; NSC 724998; Isoquinolines; H2AFX protein; human; Neoplastic Cells; Circulating; Hair Follicle

INTRODUCTION

Topoisomerase 1 (Top1) generates transient single-strand breaks to relieve supercoiling during replication, and is essential for transcription, replication, recombination, and repair of DNA double-strand breaks.¹ Top1 inhibitors, such as the camptothecin derivatives irinotecan and topotecan, have demonstrated clinically significant antitumor activity in various tumor types.^{1–3} However, clinical use of camptothecins is limited by their inherent chemical instability (opening of the E-ring lactone and rapid inactivation in plasma), the short half-life and reversibility of Top1 cleavage complexes requiring prolonged exposure, drug efflux by ABCG2 reducing intracellular drug concentrations, dose-limiting bone marrow toxicity (topotecan), and gastrointestinal toxicities (irinotecan).

A new class of synthetic non-camptothecin Top1 inhibitors, indenoisoquinolines, cause the formation of stable DNA-Top1 cleavage complexes by binding at the intermolecular interface, target unique DNA cleavage sites, and have activity against camptothecin-resistant cell lines.⁴ Based on promising antitumor activity in preclinical models, we conducted a first-in-human trial of the indenoisoquinoline, indotecan (NSC 743400, LMP400⁵), on a daily schedule for 5 days (28-day cycles) in patients with advanced solid tumors (“daily” trial; ClinicalTrials.gov Identifier: NCT01051635). The objectives were to determine indotecan’s safety, tolerability, and maximum tolerated dose (MTD); to characterize its pharmacokinetic (PK) profile; and to measure changes in phosphorylation of histone H2AX (γH2AX) in tumor biopsies, circulating tumor cells (CTCs), and hair follicles following drug administration. Following interim analysis of the daily schedule, we initiated a second clinical trial to evaluate whether once-weekly administration (days 1, 8, and 15 in 28-day cycles) improved drug tolerability (“weekly” trial; ClinicalTrials.gov Identifier: NCT01794104).

PATIENTS AND METHODS

Eligibility criteria

Patients 18 years or older with pathologically confirmed metastatic solid tumors refractory to standard therapy were eligible. A Karnofsky performance status ≥ 60% and adequate liver, kidney, and marrow function defined as absolute neutrophil count ≥ 1,500/μL, platelets ≥ 100,000/μL, total bilirubin ≤ 1.5 X the upper limit of normal (ULN), aspartate aminotransferase and/or alanine aminotransferase ≤ 2.5 X ULN, and serum creatinine < 1.5 X ULN were required. Previous anticancer therapy or surgery must have been completed 4 weeks prior to enrollment; patients with known brain metastases were required to be stable off steroids or anti-seizure medications for 2 months prior to enrollment. Both trials were

conducted under a National Cancer Institute (NCI)-sponsored IND with institutional review board approval, and investigators obtained informed consent from each participant. Protocol designs and conduct followed all applicable regulations, guidances, and local policies.

Trial design

Indotecan was developed and supplied by the Division of Cancer Treatment and Diagnosis, National Cancer Institute. Indotecan was administered intravenously through a central line over 1 hour for the daily schedule (days 1–5, followed by 23 days without drug in 28-day cycles) and over 3 hours for the weekly schedule (days 1, 8, and 15 in 28-day cycles). For the daily schedule, the starting dose of 2.5 mg/m²/day was sequentially escalated by 100% up to 80 mg/m²/day following the accelerated titration design.⁶ For the weekly schedule, the starting dose of 60 mg/m²/day was escalated by 50% to 90 mg/m²/day and then 135 mg/m²/day. One patient was to be accrued per dose level (DL) until the first dose-limiting toxicity (DLT) or until two different patients at the same dose level experienced grade 2 toxicities during cycle 1. At the first grade 2 toxicity, two additional patients were to be treated at that dose; if no further grade 2 toxicities were observed, accelerated dose escalation could continue. After the accelerated phase, a traditional 3+3 design was utilized for further dose escalation.⁶ For the weekly administration trial, 10 additional patients were enrolled at the MTD to further define PK and PD parameters.

Toxicities were graded using CTCAE version 4.0. Toxicities were required to resolve to grade 1 or baseline prior to initiating the next cycle. Treatment could be delayed by a maximum of 2 weeks beyond the 28-day cycle length for resolution of toxicities. A DLT was defined as an adverse event that occurred during cycle 1, was thought to be related to study drug, and which met one of the following criteria: grade 3 non-hematologic toxicities (except diarrhea, nausea, vomiting without maximal supportive therapy; alopecia) and grade 4 hematologic toxicities (except lymphopenia). Occurrence of a DLT resulted in a dose reduction until resolution to grade 1 or baseline. Up to 2 dose reductions were allowed before taking the patient off study. The MTD was defined as the dose level at which no more than 1 in 6 patients experienced a DLT.

Safety and efficacy evaluations

History and physical examination, including performance status and vital signs, were performed at baseline and at the start of every cycle for both trials. Complete blood counts with differential and serum chemistries were performed at baseline, weekly during cycles 1 and 2, and at the start of every subsequent cycle. Radiographic evaluation was performed at baseline and every two cycles to assess tumor response based on the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0.

Pharmacokinetic evaluations

Blood samples for PK analysis were collected during cycle 1 of both trials. For the daily schedule, samples were collected before start of infusion, 2 minutes before the end of infusion, and at 0.5, 1, 2, 4, 6, 12, and 23 hours after the end of infusion. Samples were also obtained 4 hours after the start of infusion on day 3, before the start of the infusion and 2 min before the end of the infusion on day 5, and 24 (day 6) and 48 hours (day 7) after the

start of the day 5 infusion. For the weekly schedule, samples were collected before the first infusion; 0.5, 1, and 2 hours after the start of infusion; 2 minutes prior to the end of the infusion; and at 0.5, 1, 2, 4, 6, 12, 21, and 45 hours after start of infusion. A sample was also collected prior to the next infusion on day 8. All samples were centrifuged at $2000 \times g$ at 4°C for 10 min, and the resulting plasma was stored at -70°C until analysis. Urine for PK analysis was collected before treatment and every void post-treatment on day 1 of cycle 1, and pooled into 8-hour batches.

Indotecan was quantitated using a validated QuattroMicro LC-MS/MS assay.⁷ The maximum concentration (C_{max}) and time to reach C_{max} (T_{max}) were determined by visual inspection of the concentration versus time data. Other pharmacokinetic parameters were calculated non-compartmentally using PK Solutions 2.0 (Summit Research Services, Montrose, CO; www.summitPK.com). Descriptive statistics were calculated with Microsoft Excel 2010.

Topoisomerase 1 and γH2AX levels in paired biopsies and PBMCs

For the daily schedule, optional tumor biopsies collected at baseline and 2–4 hours after the start of drug infusion on day 3 of cycle 1 became mandatory for patients in the MTD expansion cohort. Peripheral blood mononuclear cells (PBMCs) were also collected prior to and during drug infusion simultaneously with PK collections during day 1 of cycle 1 and 4 hours after the start of drug infusion on days 3 and 5 of cycle 1. Levels of γH2AX and Top1 were measured in tumor tissue and PBMCs using validated immunofluorescence assays.^{8, 9} To be reportable, pre-dose Top1 values were required to exceed twice the assay performance lower level of quantification (LLQ) to allow measurement of a 50% reduction in target level; a post-dose increase two-fold above baseline was required to report γH2AX .

Circulating tumor cells

CTCs isolated from 7.5 mL whole blood collected at baseline and 2–4 hours after the start of drug infusion on cycle 1, day 3 of the daily trial only were processed on the CellTracks AutoPrep system (Veridex LLC) using the CellSearch CTC epithelial kit (Veridex LLC) per the manufacturer's protocol and analyzed by the CellTracks analyzer II (Veridex LLC). Samples with 6 or more CTCs were considered reportable. The γH2AX status of the CTCs was analyzed as previously described.¹⁰ To account for biological variability, a response was defined as a 3-fold increase in the ratio of γH2AX -positive cells to the total number of CTCs after treatment.

γH2AX in hair follicles

In the daily trial only, hair samples were collected prior to the start of infusion and approximately 4–6 hours after the end of infusion. Scalp hairs were plucked with forceps to obtain 5–10 anagen-phase hair bulbs. γH2AX was detected by immunohistochemistry as previously described.¹¹ Samples were imaged by laser scanning confocal microscopy (Nikon PCM 2000, Nikon, Inc.; Augusta GA, USA). Optical sections were combined in a maximum projection using Simple 32 software (Compix Inc.; Cranberry, PA, USA). Foci were visually quantified by eye in the extremity of the hair bulbs. Differences between pre-

and post-indotecan infusion, across the patients, were analyzed by the paired Student's t-test; a 1-sided p-value < 0.05 was considered significant.

RESULTS

Demographics

Twenty-one patients were enrolled on both the daily- and weekly- administration trials (Table 1). Most patients had good performance status and were heavily pretreated (median of 4 prior therapies).

Clinical Outcome

Number of cycles ranged from 1–4; no patient on either schedule had an objective response. Three patients withdrew from study following one cycle of therapy to pursue alternate treatments.

Toxicity

Daily Administration Schedule—The principal toxicity of the daily schedule was myelosuppression (Table 2). Two of the 3 patients on daily dose level 6 (DL-D6; 80 mg/m²/day) had DLTs. One, a 56-year old female with sigmoid adenocarcinoma whose disease had progressed following multiple lines of combination therapies (FOLFOX+Bevacizumab, FOLFIRI+Bevacizumab, TACE, and Panitumumab+Bevacizumab), was treated for 5 days but developed grade 4 thrombocytopenia with grade 3 neutropenia during cycle 1 at DL-D6. CT scans performed post-treatment on cycle 1 showed an improvement in lung lesions (data not shown); however, the patient refused further therapy and was taken off study. The second, a patient with colorectal cancer, developed grade 4 thrombocytopenia and neutropenia with grade 3 fatigue. A total of 6 patients were then enrolled on DL-D5 to establish the safety of 40 mg/m²/day for 5 days. Because DL-D5 was well tolerated and there was a suggestion of antitumor activity in one patient (shrinkage of lung lesions), the study was amended to interrogate an intermediate dose level of 60 mg/m²/day (DL-D5a). Of the 6 patients enrolled at this dose level, one developed grade 4 myelosuppression, establishing the MTD at 60 mg/m²/day for 5 days in 28-day cycles. Grade 2 myelosuppression, anorexia, and weight loss were the principal toxicities.

Weekly Administration Schedule—Given that administering topotecan on a weekly schedule improves its tolerability in terms of myelosuppression, thus allowing higher dose escalation,¹² we pursued a second trial with a weekly schedule of indotecan, starting at 60 mg/m² (weekly dose level 1; DL-W1) on days 1, 8, and 15 in 28-day cycles. Two patients on DL-W3 (135 mg/m²) developed DLTs: one had refractory grade 3 nausea and another had grade 4 thrombocytopenia (Table 3). Three additional patients were enrolled on DL-W2 (90 mg/m²) without DLT, establishing the MTD as 90 mg/m² on days 1, 8, and 15 in 28-day cycles.

Pharmacokinetics

PK data were obtained from 41 subjects. Representative examples from patients on both schedules are shown in Figure 1. Drug concentration-versus-time plots show multi-

compartmental behavior with a terminal half-life of 2 to 3 days, resulting in drug accumulation in plasma during the daily schedule. PK parameters are presented in Table 4. Calculation of all non-compartmental parameters was only possible for the weekly schedule because of the long terminal half-life. $C_{\max}$ and AUC values increased with dose; dose-normalized $C_{\max}$ values are presented in Figure 2. Renal excretion of unchanged compound was minimal (less than 0.24% of dose over the first 24 hours).

Top1 levels in tumor biopsies and PBMCs

Paired biopsy samples were collected from 9 patients on DL-D5 and DL-D5a; 2 additional patients provided baseline samples only. The mean Top1 level for all 11 baseline tumor biopsy samples was 15.0 pg/ μ g protein (SD, 12.5 pg/ μ g), a value within a 1-standard deviation of other samples collected at baseline from patients enrolled in clinical trials at the Developmental Therapeutics Clinic, NCI (mean, 21.2 pg/ μ g [SD, 30.5 pg/ μ g]). All but one of the baseline samples was above the assay performance LLQ of 2.4 pg/ μ g protein; tumor Top1 levels were too variable to assess treatment effect except in patients #0025 and #0029, which paradoxically showed an increase in total Top1 level (Figure 3), the opposite of the expected treatment results.¹³

Twenty patients on DL-D2 through DL-D6 provided baseline PBMC samples; all but one sample passed assay performance criteria. The mean Top1 level at baseline was 16406 pg/ 10^7 cells (SD, 8642 pg/ 10^7 cells). Baseline and post-dose sample values (4 hours after the start of drug infusion on cycle 1, days 1 and 3) are presented in Figures 4A and 4B, respectively. The effects of indotecan treatment on PBMC Top1 levels were inconclusive, although we observed > 70% decreases in patients #0003 and #0015.

γ H2AX levels in tumor biopsies, CTCs, and hair follicles

Two of the paired biopsy samples (patients #0026, #0029) had sufficient tumor content to allow measurement of γ H2AX. The baseline sample for patient #0026 (DL-D5) was below the assay performance LLQ of 1% nuclear area positive (NAP) but increased to 7.6% NAP in the day 3 post-dose sample; baseline and post-dose samples for patient #0029 (DL-D5a) were 9.0% NAP and 10.2% NAP, respectively (data not shown). Levels of γ H2AX in both patients were within 2 standard deviations of other baseline clinical samples collected at the Developmental Therapeutics Clinic (mean, 3.5% NAP [SD 4.5%]).

Twenty-one patients on the daily schedule provided baseline and post-dose samples for CTC isolation. Seven patients had more than 6 CTCs/sample; total CTCs dropped for all but one patient by the post-dose sample on day 3 (Figure 5A). Only one patient (#0016) on DL-D6 met the criterion for a γ H2AX drug response (3-fold increase in baseline ratio of γ H2AX-positive cells to total number of CTCs in the post-dose sample; Figure 5B), presumably due to the high number of apoptotic and thus γ H2AX-positive cells observed at baseline.

The quality of plucked hairs for both pre- and post-infusion time points was sufficient in 4 patients (patients #0009, #0024, #0029, and #0031 on the daily trial) to allow measurement of γ H2AX. Daily indotecan induced significant DNA damage, as assayed by γ H2AX, in all 4 patients, and the differences between pre- and post-infusion values, across the 4 patients,

was statistically significant ($p=0.027$, 1-sided; Figure 6). Induction of γ H2AX in hair bulbs indicated both drug permeation and Top1 inhibition *in vivo*.

DISCUSSION

Over 400 indenoisoquinolones have been screened for anticancer activity *in vitro* by the National Cancer Institute; three were further evaluated based on their ability to inhibit Top1 in camptothecin-refractory cell lines and to induce different patterns of DNA breaks compared to camptothecin (suggesting a different mechanism of action). These indenoisoquinolones are also not substrates of the plasma membrane drug efflux transporter ABCG2, a mechanism of camptothecin resistance.

In this study, we evaluated the safety and tolerability of daily and weekly dosing schedules for the indenoisoquinolone indotecan. The weekly administration schedule was evaluated to determine if less frequent administration obviated the myelosuppression observed on the daily schedule; however, unlike topotecan, we were unable to substantially increase the tolerated dose on a weekly schedule (MTD, 90 mg/m²/dose) compared to the daily dosing MTD (60 mg/m²/dose). This may be explained by the long half-life of indotecan relative to topotecan, which results in sustained exposure of the patient to drug despite increasing the dose interval from daily to weekly. No clinical activity was observed other than minor shrinkage of metastatic lung nodules in one patient with colorectal cancer on the daily schedule (DL-D6).

Dose-normalized $C_{\max}$ was lower in the patients administered indotecan in 3-hour weekly infusions than 1-hour daily infusions, as expected with a 3-fold longer infusion duration. Although dose-normalized $C_{\max}$ and AUC_{0-24h} appear to inversely correlate with increasing dose, this is limited to the highest dose-level within each cohort, and any effect is relatively small compared to the inter-patient variability (58% coefficient of variation in drug clearance). The distribution volume of indotecan is larger than body volume, suggesting preferential distribution into tissues. The long half-life and good tissue distribution suggest that indotecan is available for target inhibition for a prolonged period of time after a single dose, which may be an advantage over other Top1 inhibitors. The clearance of 1 L/h/m² is an order of magnitude lower than that observed in rats and dogs,¹⁴ and represents approximately 2% of human hepatic blood flow.¹⁵ Indotecan is tightly protein-bound with only approximately 2% of drug in the unbound state,⁷ suggesting that clearance is mostly due to metabolism or excretion of the complete unbound fraction that passes through the liver; the minimal excretion of unchanged indotecan in the urine (< 0.25% of the dose over the first 24 hours) is consistent with this hypothesis.

Top1 expression in primary colorectal cancer and liver and lymph node metastases has been assessed in patients treated with first-line irinotecan (FOLFIRI) chemotherapy regimen.¹⁶ Univariate analysis indicated Top1 expression did not correlate with overall survival or disease-free survival; however, with multivariate analysis, those patients who had expression of Top1 in their tumors and received irinotecan-containing chemotherapy had better overall survival (HR=0.47, 95% CI 0.23–0.94, $p=0.033$).² To demonstrate proof-of-mechanism, we therefore evaluated levels of Top1 in tumor biopsies and PBMCs utilizing a validated ELISA

assay developed within our group.⁹ We found that 3 of the 8 patients on the daily schedule experienced significant Top1 decreases in post-treatment biopsies, consistent with Top1 engagement by indotecan (Figure 3). However, biopsies from 4 additional patients unexpectedly showed significant increases in Top1 levels following daily indotecan administration. The surprisingly wide variation across the cohort could be due to specimen heterogeneity (tumor versus surrounding tissue), poor biopsy quality, tumor heterogeneity (particularly related to the requirement to biopsy different locations within a tested node pre- and post-dose), or a lack of correlation between Top1 levels and indotecan effect. The variability of baseline Top1 levels observed across subjects in PBMCs and tumor samples may also explain the variation in drug response, as only high baseline Top1 levels have been linked with responsiveness to drug.^{9, 13, 17} This high baseline variability may be due to differences in anatomic sites and tumor histologies across patients; hence the lack of statistical significance in the small, heterogeneous patient population presented here.

Top1 inhibitors trigger the rapid phosphorylation of H2AX (γ H2AX), a marker of DNA damage response.¹³ Because obtaining repeat solid tumor biopsies presents logistical and ethical challenges, we evaluated CTCs and hair follicles collected from patients receiving daily indotecan as surrogates for measuring drug-induced γ H2AX foci. After treatment, the number of CTCs per sample decreased in most patients (Figure 5A). One patient who received the highest dose (DL-D6) had an increase in total CTCs, which may be attributed to tumor shedding into the bloodstream. This phenomenon has been described previously as a potential early marker of apoptosis, consistent with target engagement and drug activity.¹⁸ Furthermore, increased numbers of γ H2AX foci were observed in the hair bulbs of all 4 evaluable patients (Figure 6).

We established the MTD of 2 dosing schedules for a novel, synthetic, Top1 inhibitor, indotecan, and demonstrated a DNA damage response post-drug administration in hair follicles. Further trials of indotecan in combination with other cytotoxic agents such as 5-FU and platinum are under consideration to develop treatment regimens against diseases such as refractory colorectal and ovarian cancer.

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Figure 1.

Representative concentration versus time profiles of the daily schedule 1-hour infusion (○), and the weekly schedule 3-hour infusion (Δ), both dosed at 60 mg/m² (A) over 5 days and (B) an expanded view of the first 24 hours.

Figure 2.

Dose normalized C_{max} plotted against dose for the daily schedule 1 hour infusion (○), and the weekly schedule 3 hour infusion (△).

Figure 3.

Levels of Top1 protein measured in tumor biopsies collected pre-dose (baseline) and 4 hours after the start of indotecan infusion on cycle 1 day 3. Percent changes from the baseline sample are indicated for each patient; DL-D5 and DL-D5a are indicated with black and purple circles, respectively. +No post-dose sample; ^pre-dose or #post-dose sample < LLQ.

Figure 4.

Changes in levels of Top1 protein measured in PBMC samples collected pre-dose (baseline) and (A) 4 hours after the start of indotecan infusion on day 1 or (B) day 3. Percent changes from baseline are indicated for each patient; dose levels are indicated by the different colored circles. +No post-dose sample; ^pre-dose or #post-dose sample < LLQ; ‡pre-dose or †post-dose sample failed assay performance quality control criteria.

Figure 5. Changes in **(A)** total number of CTCs and **(B)** percent of CTCs positive for γ H2AX in 7.5-mL blood samples collected pre-dose (baseline) and 2–4 hours after the start of indotecan infusion on day 3. Percent changes from baseline are indicated for each patient; DLs indicated by the differently colored circles. *The baseline proportion of γ H2AX-positive CTCs was too high to assess a 3-fold response over baseline.

Figure 6. γ H2AX formation in plucked hairs from patients receiving indotecan. Hair samples were collected prior to and 4 to 6 hours post-infusion. **(A)** On the left, individual patient data are plotted as percentage of γ H2AX-positive cells (defined as more than 4 γ H2AX foci per cell) $\pm$ standard errors. On the right, average grouped patient data plotted $\pm$ standard deviations indicate a significant change between pre- and post-treatment samples ($p=0.027$; $N=4$ individuals). Numbers in parentheses refer to the numbers of hairs analyzed for each patient. **(B)** Representative images of γ H2AX staining in plucked hair bulbs collected prior and after indotecan. The image in the right panel shows the regions of plucked hairs where both image capture and γ H2AX quantification were performed. Green, γ H2AX; red, DNA stained with propidium iodide; scale bar, 50 μ m.

Table 1**Demographics**

Parameter	Daily Indotecan	Weekly Indotecan
Number of patients enrolled	21	21
Male/Female	12/9	14/7
Median age, years (range)	57 (35–72)	63 (21–77)
ECOG Performance Status		
0	3	-
1	18	19
2	-	2
Median number of prior therapies (range)	4 (2–12)	4 (2–11)
Tumor type		
Adenoid cystic cancer	2	-
Adrenocortical cancer	-	1
Bladder cancer	1	1
Breast adenocarcinoma	1	-
Breast cancer	-	2
Colorectal cancer	13	3
Head and neck cancer	-	2
Melanoma	2	-
Non-small cell lung cancer	-	5
Ovarian cancer	-	3
Pancreas adenocarcinoma	-	1
Parotid cancer	1	-
Renal cell carcinoma	-	1
Sarcoma	-	1
Small cell lung cancer	-	1
Vaginal adenocarcinoma	1	-

ECOG=Eastern Cooperative Oncology Group

Table 2

Adverse events by patient; daily schedule

Worst grade (2) at least possibly related to indotecan for each patient

Adverse Event	Grade	DL-D1 (N=1)	DL-D2 (N=3)	DL-D3 (N=1)	DL-D4 (N=1)	DL-D5 (N=6)	DL-D5a (N=6)	DL-D6 (N=3)
Anemia	2			1		2	4	1
	3						1	1
Diarrhea	2						1	
Dyspepsia	2				1			
Fatigue	2						2	1
	3							2
Febrile Neutropenia	3							2
Hyperkalemia	2		1				1	
Hypophosphatemia	2							1
Lethargy	2							1
Leucopenia	2					1		
	3						1	
	4							2
Lymphopenia	2			1			3	
	3					1		2
Nausea	2					1		
Neutropenia	2					1		
	4						1	2
Serum bilirubin increased	2							2
Transaminases increased	2				1		1	1

Abbreviations: DL-D1=daily dose level 1; N=total number of patients per dose level

Table 3
Adverse Events by patient; weekly schedule

Worst grade (2) at least possibly related to indotecan for each patient.

Adverse Event	Grade	DL-W1 (N=6)	DL-W2 (N=8)	DL-W3 (N=7)
Abdominal pain	2		1	
Anemia	2	2	5	3
	3			3
Anorexia	2		1	
Diarrhea	2	1	1	
Fatigue	2		4	1
GE reflux	2		1	
Hypophosphatemia	2			1
	3	1		
Leucopenia	2			1
	3		2	1
Lymphopenia	2	2		3
	3	1	2	1
Nausea	2		3	
	3			1
Neutropenia	2		1	1
	3		1	1
Serum bilirubin increased	2	1		
Transaminases increased	4	1		
Thrombocytopenia	4			1
Weight loss	2	1		

Abbreviations: DL-W1=weekly dose level 1; N=total number of patients per dose level

Table 4

Indotecan non-compartmental pharmacokinetic parameters (standard deviation of the geometric mean in parenthesis).

Daily trial									
Dose Level	Dose (mg/m² over 1 h)	C_{max} (μg/mL)	AUC₀₋₂₄ (μg×h/mL)	AUC_{0-inf} (μg×h/mL)	AUC₀₋₂₄ per Dose ($\frac{\mu g \times h}{mL}$)	t_{1/2} (h)	V_{ss} (L/m²)	CL (L/h/m²)	Renal excretion 0- 24 h (% dose)
D1 (N=1)	2.5	0.0943	0.547	-	0.219	-	-	-	0.49%
D2 (N=3)	5	0.191 (0.123)	1.45 (0.66)	-	0.291 (0.133)	-	-	-	0.48% (0.21%)
D3 (N=1)	10	0.347	2.89	-	0.289	-	-	-	0.36%
D4 (N=1)	20	0.583	5.09	-	0.254	-	-	-	0.26%
D5 (N=5)		1.13 (0.26)	8.12 (1.14)	-	0.203 (0.029)	-	-	-	0.16% (0.09%)
D5a (N=6)	60	1.61 (0.51)	12.8 (2.5)	-	0.213 (0.042)	-	-	-	0.18% (0.17%)
D6 (N=3)	80	1.65 (0.05)	14.7 (1.4)	-	0.184 (0.018)	-	-	-	0.26% (0.03%)
Total (N=20)		-	-	-	0.221 (0.065)	-	-	-	0.24% (0.17%)
Weekly trial									
Dose Level	Dose (mg/m² over 3 h)	C_{max} (μg/mL)	AUC₀₋₂₄ (μg×h/mL)	AUC_{0-inf} (μg×h/mL)	AUC₀₋₂₄ per Dose ($\frac{\mu g \times h}{mL}$)	t_{1/2} (h)	V_{ss} (L/m²)	CL (L/h/m²)	Renal excretion 0- 24 h (% dose)
W1 (N=6)	60	1.27 (0.59)	13.7 (5.5)	56.4 (16.6)	0.228 (0.092)	89 (82)	116 (121)	1.06 (0.34)	0.21% (0.08%)
W2 (N=8)	90	1.69 (1.18)	18.7 (5.6)	88.6 (56.2)	0.208 (0.063)	74 (60)	99.3 (32.6)	1.02 (0.73)	0.15% (0.13%)
W3 (N=7)	135	1.93 (0.19)	21.5 (5.0)	70.8 (18.3)	0.159 (0.037)	50 (17)	114 (39)	1.91 (0.73)	0.22% (0.16%)
Total (N=21)		-	-	-	0.195 (0.070)	69 (60)	109 (69)	1.27 (0.74)	0.19% (0.13%)