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ORIGINAL ARTICLE

Clinical and pharmacologic evaluation of two dosing schedules of indotecan (LMP400), a novel indenoisoquinoline, in patients with advanced solid tumors

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Abstract
Purpose Indenoisoquinolines are non-camptothecin topoisomerase I (TopI) inhibitors that overcome the limitations of camptothecins: chemical instability and camptothecin resistance. 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 TopI and the phosphorylation of histone H2AX (γH2AX) were assayed in tumor biopsies; γH2AX levels were also evaluated in circulating tumor cells (CTCs) and hair follicles to assess DNA damage response.
Results An MTD of 60 mg/m^2/day was established for the daily regimen, compared to 90 mg/m^2 for the weekly regimen. The TopI response to drug showed target engagement in a subset of tumor biopsies. Pharmacokinetics profiles demonstrated a prolonged terminal half-life and tissue accumulation compared to topotecan. Dose-dependent decreases in total CTCs were measured in seven patients. Formation of γH2AX-positive foci in CTCs (day 3) and hair follicles (4–6 h) was observed following treatment.
Conclusions We established the MTD of two dosing schedules for a novel TopI inhibitor, indotecan. Target engagement was demonstrated as Top1 downregulation and γH2AX response. No objective responses were observed on either schedule in this small patient cohort. The principal toxicity of both schedules was myelosuppression; no significant gastrointestinal problems were observed. Increased DNA damage response was observed in CTCs, hair follicles, and a subset of tumor biopsies.

Keywords DNA damage · DNA topoisomerase I · NSC 743400 · Indenoisoquinolines · H2AX protein · Hair follicle

Introduction

Topoisomerase I (TopI) generates transient single-strand breaks to relieve DNA supercoiling during transcription, replication, recombination, and repair of DNA double-strand breaks [1]. TopI inhibitors such as the camptothecin derivatives irinotecan, topotecan, and belotecan 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 TopI cleavage complexes requiring prolonged exposure, drug efflux by ABCG2 reducing intracellular drug concentrations, dose-limiting bone marrow toxicity (topotecan), and gastrointestinal toxicities (irinotecan).

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A new class of synthetic non-camptothecin TopI inhibitors, indenoisoquinolines, causes the formation of stable DNA-TopI cleavage complexes by binding at the intermolecular interface, targeting unique DNA cleavage sites, and they 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, LMP400 [5]), 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 pharmacodynamic (PD) 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 ≥1500/μL, platelets ≥100,000/μL, total bilirubin ≤1.5× the upper limit of normal (ULN), aspartate aminotransferase and/or alanine aminotransferase ≤2.5× ULN, and serum creatinine <1.5× 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, NCI. Indotecan was administered intravenously through a central line over 1 h for the daily schedule (days 1–5, followed by 23 days without drug in 28-day cycles) and over 3 h for the weekly schedule (days 1, 8, and 15 in 28-day cycles). For the daily schedule, the starting dose of 2.5 mg/m^2/day was sequentially escalated by 100 % up to 80 mg/m^2/day following the accelerated titration design [6]. For the weekly schedule, the starting dose of 60 mg/m^2/day was escalated by 50 %–90 mg/m^2/day and then 135 mg/m^2/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 DL 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, ten 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 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 two dose reductions were allowed before taking the patient off study. The MTD was defined as the DL at which no more than one in six 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 min before the end of infusion, and at 0.5, 1, 2, 4, 6, 12, and 23 h after the end of infusion. Samples were also obtained 4 h 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 h (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 h after the start of infusion; 2 min prior to the end of the

infusion; and at 0.5, 1, 2, 4, 6, 12, 21, and 45 h after start of infusion. A sample was also collected prior to the next infusion on day 8. All samples were centrifuged at 2000×*g* at 4 °C for 10 min, and the resulting plasma was stored at less than or equal to −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-h 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, USA; www.summitPK.com). Descriptive statistics were calculated with Microsoft Excel 2010.

Topoisomerase I and γH2AX levels in paired biopsies and PBMCs

For the daily schedule, optional tumor biopsies collected at baseline and 2–4 h 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 h after the start of drug infusion on days 3 and 5 of cycle 1. Levels of γH2AX and TopI were measured in tumor tissue and PBMCs using validated immunofluorescence assays [8, 9]. To be reportable, pre-dose TopI 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 twofold above baseline was required to report γH2AX.

Circulating tumor cells

CTCs isolated from 7.5 mL whole blood collected at baseline and 2–4 h 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 six 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 greater than or equal to threefold 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 h 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 one-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 four prior therapies).

Table 1 Demographics

Parameters	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

Clinical outcome

The number of cycles ranged from 1 to 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 three patients on daily DL 6 (DL-D6; 80 mg/m^2/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 six patients were then enrolled on DL-D5 to establish the safety of 40 mg/m^2/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 DL of 60 mg/m^2/day (DL-D5a). Of the six patients enrolled at this DL, one developed grade 4 myelosuppression, establishing the MTD at 60 mg/m^2/day for 5 days in 28-day cycles. Grade 2 myelosuppression, anorexia, and weight loss were the principal toxicities.

Table 2 Adverse events by patient; daily schedule

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
Thrombocytopenia	4						1	2
Weight loss	2						1	

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

DL-D1 daily dose level 1, *DL-D2* daily dose level 2, *DL-D3* daily dose level 3, *DL-D4* daily dose level 4, *DL5* daily dose level 5, *DL5a* daily dose level 5a, *DL-D6* daily dose level 6, *N* total number of patients per dose level

Table 3 Adverse events by patient; weekly schedule

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		

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

DL-W1 weekly dose level 1, *N* total number of patients per dose level

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/m^2 (weekly DL 1; DL-W1) on days 1, 8, and 15 in 28-day cycles. Two patients on DL-W3 (135 mg/m^2) 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/m^2) without DLT, establishing the MTD as 90 mg/m^2 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 Fig. 1a, b. Drug concentration-versus-time plots show multicompartmental behavior with a terminal half-life of 2–3 days, resulting in drug accumulation in plasma during the daily schedule. PK parameters are presented in Supplementary Table 1. 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 Fig. 1c. Renal excretion of unchanged compound was minimal (<0.24 % of dose over the first 24 h).

TopI levels in tumor biopsies and PBMCs

Paired biopsy samples were collected from nine patients on DL-D5 and DL-D5a; two additional patients provided baseline samples only. The mean TopI level for all 11 baseline tumor biopsy samples was 15.0 pg/μg protein (SD, 12.5 pg/μg), a value within a one SD of other samples collected at baseline from patients enrolled in clinical trials at the Developmental Therapeutics Clinic, NCI (mean, 21.2 pg/μg [SD, 30.5 pg/μg]). All but one of the baseline samples were above the assay performance LLQ of 2.4 pg/μg protein; tumor TopI levels were too variable to assess treatment effect except in patients #0025 and #0029, which paradoxically showed an increase in total TopI level (Fig. 2a), 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 TopI level at baseline was 16,406 pg/10^7 cells (SD, 8642 pg/10^7 cells). Baseline and post-dose sample values (4 h after the start of drug infusion on cycle 1, days 1 and 3) are presented in Fig. 2b, c, respectively. The effects of indotecan treatment on PBMC TopI

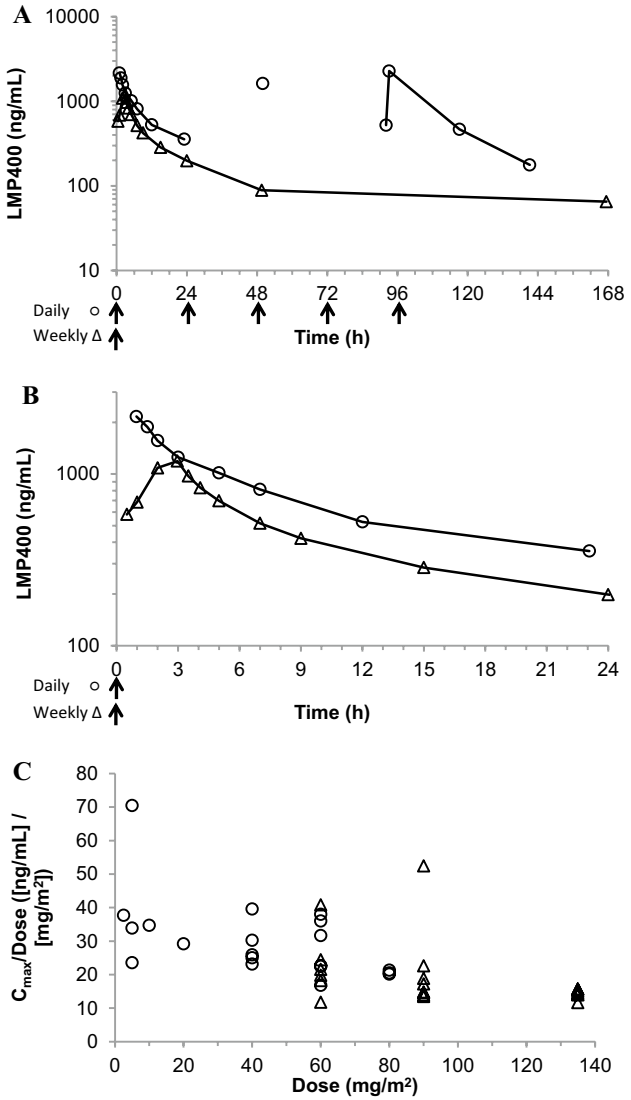

Fig. 1 Representative concentration versus time profiles of the daily schedule 1-h infusion (*circle*), and the weekly schedule 3-h infusion (*triangle*), both dosed at 60 mg/m^2: **a** over 5 days and **b** an expanded view of the first 24 h. **c** Dose-normalized C_{max} plotted against dose for the daily schedule 1 h infusion (*circle*), and the weekly schedule 3-h infusion (*triangle*). *Upward arrows* denote infusions

levels were inconclusive, although we observed >70 % decreases in patients #0003 and #0015.

γH2AX 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 γH2AX. 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 γH2AX in both patients were within two SDs 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 six CTCs/sample; total CTCs dropped for all but one patient by the post-dose sample on day 3 (Supplemental Figure 1a). Only one patient (#0016) on DL-D6 met the criterion for a γH2AX drug response (greater than or equal to threefold increase in baseline ratio of γH2AX-positive cells to total number of CTCs in the post-dose sample; Supplemental Figure 1b), presumably due to the high number of apoptotic and thus γH2AX-positive cells observed at baseline.

The quality of plucked hairs for both pre- and post-infusion time points was sufficient in four patients (patients #0009, #0024, #0029, and #0031 on the daily trial) to allow measurement of γH2AX. Daily indotecan induced significant DNA damage, as assayed by γH2AX, in all four patients, and the difference between pre- and post-infusion values, across the four patients, was statistically significant ($p = 0.027$, one-sided; Fig. 3). Induction of γH2AX in hair bulbs indicated both drug permeation and TopI inhibition in vivo.

Discussion

Over 400 indenoisoquinolines have been screened for anticancer activity in vitro by the NCI; three were further evaluated based on their selectivity and potency against human TopI and their ability to inhibit TopI in camptothecin-refractory cell lines and to induce different patterns of DNA breaks compared to camptothecin (suggesting a different spectrum of activity). These indenoisoquinolines 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 indenoisoquinoline indotecan (LMP400). The weekly administration schedule was evaluated to determine whether 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/m^2/dose) compared to the daily dosing MTD (60 mg/m^2/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

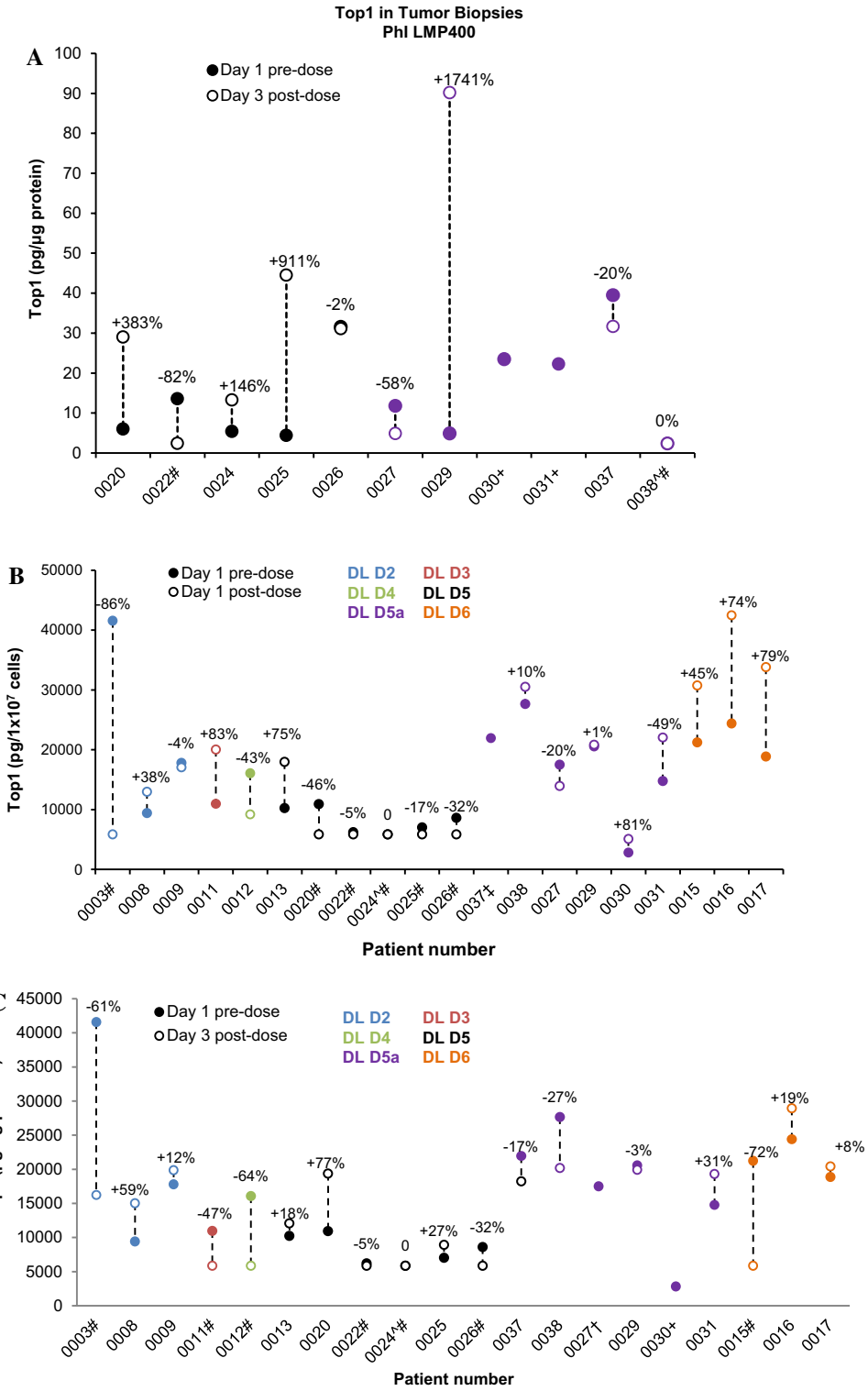

Fig. 2 **a** Levels of TopI protein measured in tumor biopsies collected pre-dose (baseline) and 4 h after the start of indotecan infusion on cycle 1 day 3. Percent changes from the baseline sample are indicated for each patient; DL-D5 and DL-D5a are indicated with *black* and *purple circles*, respectively; and changes in levels of TopI protein measured in PBMC samples collected pre-dose (baseline) and **b** 4 h after the start of indotecan infusion on day 1 or **c** day 3. Percent changes from baseline are indicated for each patient; dose levels are indicated by the different *colored circles*. $^{+}$No post-dose sample, $^{\wedge}$pre-dose or $^{\#}$post-dose sample <LLQ, ‡pre-dose or †post-dose sample failed assay performance quality control criteria

shrinkage of metastatic lung nodules in one patient with colorectal cancer on the daily schedule (DL-D6). Clinical trial NCT01051635 is currently ongoing to establish the MTD for LMP776, an analogue.

Dose-normalized C_{max} was lower in the patients administered indotecan in 3-h weekly infusions than 1-h daily infusions, as expected with threefold 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

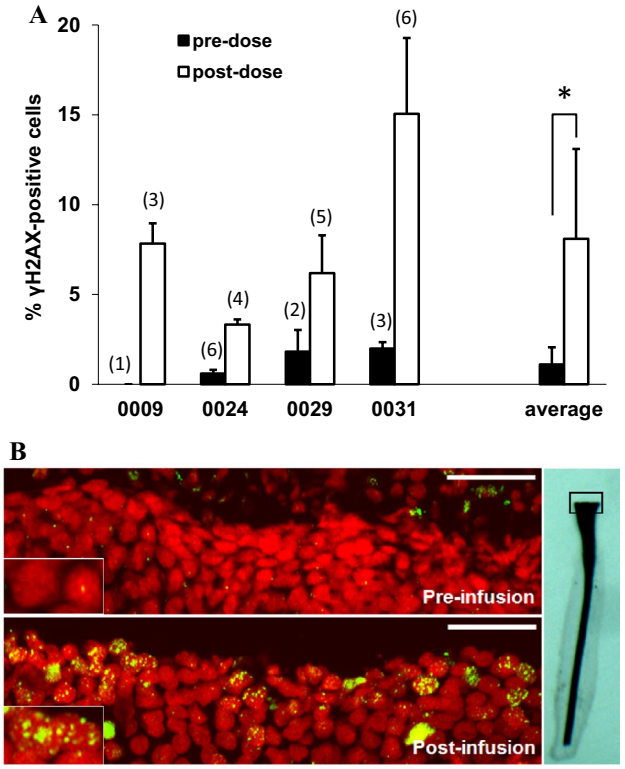

Fig. 3 γH2AX formation in plucked hairs from patients receiving indotecan. Hair samples were collected prior to and 4–6 h post-infusion. **a** On the *left*, individual patient data are plotted as percentage of γH2AX-positive cells (defined as more than four γH2AX foci per cell) ± SE. On the *right*, average grouped patient data plotted ± SDs 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 γH2AX 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 γH2AX quantification were performed. *Green* γH2AX, *red* DNA stained with propidium iodide, *scale bar* 50 μm

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 after a single dose, which may be an advantage over other TopI inhibitors. The clearance of 1 L/h/m^2 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 h) is consistent with this hypothesis.

TopI 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 TopI expression did not correlate with overall survival or disease-free survival; however, with multivariate analysis, those patients who had expression of TopI 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 TopI in tumor biopsies and PBMCs utilizing a validated ELISA developed within our group [9]. We found that three of the eight patients on the daily schedule experienced significant TopI decreases in post-treatment biopsies, consistent with TopI engagement by indotecan (Fig. 2a). However, biopsies from four additional patients unexpectedly showed significant increases in TopI levels following daily indotecan administration. The surprisingly wide variation across the cohort could be due to specimen heterogeneity (tumor vs. 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 TopI levels and any indotecan effect. The variability of baseline TopI levels observed across subjects in PBMCs and tumor samples may explain the variation in drug response, as only high baseline TopI 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.

TopI inhibitors trigger the rapid phosphorylation of H2AX (γH2AX), 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 γH2AX foci. After treatment, the number of CTCs per sample decreased in most patients (Supplementary Figure 1a). 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 γH2AX foci were observed in the hair bulbs of all four evaluable patients (Fig. 3).

We established the MTD of two dosing schedules for a novel, synthetic, TopI 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, platinum, ATR, CHK1, and PARP inhibitors are under consideration to

develop treatment regimens against diseases such as refractory colorectal and ovarian cancer.

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Compliance with ethical standards

Conflict of interest None.

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