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Experimental antitumor activity and pharmacokinetics of the camptothecin analog irinotecan (CPT-11) in mice

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Irinotecan (CPT-11) is a semi-synthetic derivative of camptothecin currently in clinical trials. *In vitro*, CPT-11 presented preferential cytotoxicity toward some solid tumor cells (mouse colon 38 and pancreas 03; human pancreas MIA PaCa-2) as compared to leukemia cells (L1210), whereas SN-38, a metabolite of CPT-11, was not solid tumor selective. *In vivo*, schedule of administration studies in P388 leukemia and mammary adenocarcinoma 16/C (MA16/C) showed that CPT-11 was not markedly schedule dependent. In order to determine its spectrum of anticancer activity, CPT-11 was evaluated against a variety of mouse and human tumors. The end points used were total log cell kill (Lck) for solid tumors and increase in life span (% ILS) for leukemia. Intravenous CPT-11 was found highly active against both early and advanced stage pancreatic ductal adenocarcinoma 03 (P03), with 60% long-term survivors and 100% complete regressions, respectively. Other responsive tumors included: colon adenocarcinomas 38 and 51 (both 1.0 Lck); MA16/C (3.4 Lck); MA13/C (1.0 Lck); human Calc18 breast adenocarcinoma (2.8 Lck); Glasgow osteogenic sarcoma (1.8 Lck); Lewis lung carcinoma (1.4 Lck); B16 melanoma (1.4 Lck); P388 leukemia (170% ILS) and L1210 leukemia (64% ILS). Of interest, CPT-11 was active against tumors with acquired resistance to vincristine (P388/Vcr), to doxorubicin (P388/Dox) and to docetaxel (Calc18/TXT). CPT-11 was also found highly active after oral administration in mice bearing P03 and MA16/C tumors. Pharmacokinetic evaluations performed i.v. at the highest non-toxic dosage in mice bearing P03 tumors revealed CPT-11 peak plasma concentrations (C_{max}) of 8.9 μg/ml and a terminal half-life of 0.6 h. The metabolite SN-38 plasma concentrations presented a C_{max} of 1.6 μg/ml and a terminal half-life of 7.4 h. Although the CPT-11 tumor levels were similar to the plasma concentrations for early time points, drug levels decreased more slowly in the tumor compared to plasma (half-life, 5.0 h). SN-38 tumor levels reached concentrations in the range of 0.32–0.34 μg/g and decayed with a half-life of 6.9 h. No significant difference in plasma or tumor pharmacokinetics of either CPT-11 or SN-38 were noted after one or five daily i.v. injections. Overall, these data show that CPT-11 has good activity in experimental models, when administered both by the i.v. and the oral routes. Compared to humans, a similar schedule of administration independence was observed and similar CPT-11 levels could be reached at efficacious dosages although metabolite SN-38 levels were found higher in mice.

Key words: Antitumor activity, CPT-11, camptothecin analog, irinotecan, pharmacokinetics, mice.

Introduction

Irinotecan [Campto®, CPT-11, (+)-7-ethyl-10-hydroxy-camptothecin 10-1,4′-bipiperidine-1′-carboxylate, hydrochloride, trihydrate] is a new semi-synthetic derivative of the plant alkaloid camptothecin, isolated by Wall and colleagues in 1966[1] from the stemwood of the *Camptotheca acuminata* tree (Figure 1). In the late 1960s, clinical studies of camptothecin were carried out in the US through the National Cancer Institute but development of the compound was stopped because of severe and unpredictable side effects.[2]

Semi-synthesis of soluble derivatives was undertaken at Yakult Honsha Co. Ltd in Japan since 1978 and CPT-11 was chosen as a candidate for clinical studies. This compound was found active *in vitro*

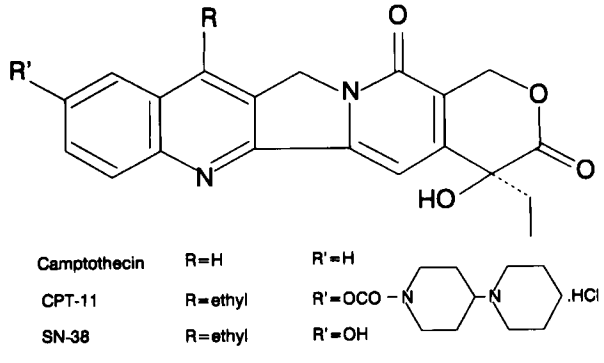

Figure 1. Structures of camptothecin, irinotecan (CPT-11) and metabolite SN-38.

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and *in vivo* against murine and human tumor cells.[3–7] In addition, it was found active against pleiotropic drug-resistant tumors.[6,8] As with the parent compound camptothecin[9,10] (reviewed in Slichenmyer[11]), the mechanism of action of CPT-11 involves topoisomerase I inhibition, specific of the camptothecin family.[12,13] CPT-11 is metabolized to SN-38, a metabolite (Figure 1) which is formed *in vivo*, in man and mouse, by a carboxyesterase.[14,15] Compared to CPT-11, the metabolite SN-38 is more potent *in vitro* against tumor cells and is thought to play an important role in the antitumor effect of CPT-11 *in vivo*.[15] SN-38 is also 1000-fold more potent *in vitro* with respect to the inhibition of topoisomerase I compared to CPT-11.[16]

Prior phase I and II studies have shown encouraging responses in a variety of human malignancies including colon, lung, refractory leukemia, and lymphoma and gynecological cancers.[17–27] Human pharmacokinetics have shown a relatively large inter-patient variability[17–28] with some relationships with the pharmacodynamics of CPT-11 and metabolite SN- 38.[28]

This report presents our contribution to the evaluation of the *in vivo* antitumor activity of i.v. CPT-11 against murine solid tumors and leukemias, the determination of optimal schedule of administration, the efficacy against tumors selected for resistance to vincristine, doxorubicin and docetaxel (Taxotere®), and the pharmacokinetics of CPT-11 and SN-38 in both plasma and tumors. Lastly, we also present the efficacy and toxicity of CPT-11 administered orally.

Materials and methods

Drugs and administration

CPT-11 was formulated by Yakult Honsha Co. Ltd (Tokyo, Japan), at a concentration of 20 mg/ml (2 ml vials), and was further diluted in 5% glucose in water for most of the experiments or with sodium chloride 0.9% (pH 5). Other compounds were obtained from various suppliers: doxorubicin and cisplatin (Laboratoire Bellon, Neuilly-sur-Seine, France), cyclophosphamide (Laboratoire Lucien, Colombes, France), SN-38 (Yakult Honsha, Tokyo, Japan), camptothecin (Sigma, St Louis, MO), and docetaxel (Taxotere®, Rhône-Poulenc Rorer SA, Vitry-sur-Seine, France). Drug solutions were kept on ice and injected within 20 min after dilution. The volume of injection per mouse was mostly 0.4 ml for i.v. bolus injection and 0.1 ml for oral administration.

For the i.v. infusion trials, CPT-11 was infused over 4 h under a volume of 1 ml. Briefly, the lateral tail vein was cannulated with an heparinized 24-gauge Teflon catheter (Becton Dickinson, Grenoble, France).[29] When blood return was noted, the guide was removed, and the extension tubing (20-in plastic tubing, 0.6 ml volume; Medex, Hilliard, OH) was attached. The drug was infused using a Harvard Pump (Ealing, Les Ulis, France).

Mice

Male and female DBA/2, C57BL/6 and B6D2F$_1$ (C57BL/6 females × DBA/2 males) and Swiss-nu were bred at IFFA CREDO (L'Arbresle, France) from strains obtained from Jackson Laboratories (Bar Harbor, ME). Male and female C3H/HeN and BALB/c were bred at Charles Rivers (Cléon, France) from strains obtained from Charles Rivers Laboratories (Wilmington, MA). Mice were over 18 g at the start of chemotherapy. They were supplied food (UAR reference 113, Epinay sur Orge, France) and water *ad libitum*.

Tumor models

The tumors used for *in vivo* evaluation were: colon adenocarcinomas 38 (C38) and 51 (C51),[30] pancreatic ductal adenocarcinoma 03 (P03),[31] mammary adenocarcinoma 13/C (MA13/C) and 16/C (MA16/C),[32] human breast adenocarcinoma Calc18,[33] Glasgow Osteogenic sarcoma (GOS),[34] Lewis lung carcinoma (3LL),[35] B16 melanoma (B16),[36] P388 lymphocytic leukemia (P388) and L1210 lymphoid leukemia (L1210). The tumors used for cross- resistance evaluation were: P388 leukemia resistant to doxorubicin (P388/Dox), P388 leukemia resistant to vincristine (P388/Vcr),[37] and the human Calc18 resistant to docetaxel (Calc18/TXT).[38] These tumors are in the National Cancer Institute frozen tumor repository, maintained at the Frederick Cancer Research Facility (Frederick, MD), and have a code identification number, a detailed description and a list of references, except for Calc18/TXT.

Tumors were maintained in the mouse strain of origin, i.e. C57BL/6 (C38, P03, B16, 3LL, GOS), BALB/c (C51), C3H/HeN (MA16/C, MA13/C), DBA2 (P388, P388/Dox, P388/Vcr, L1210) and xenografts on Swiss-nu (Calc18, Calc18/TXT). Solid tumors were transplanted as s.c. fragments. Leuke-

mias were passaged as weekly i.p. implants. For chemotherapy trials, tumors were transplanted in the strain of origin or in the appropriate F_1 hybrid.

In vitro disk diffusion assay for the determination of selective cytotoxicity

The assay is a variation of the Kirby–Bauer disk diffusion antibiotic sensitivity technique used in microbiology. For this assay, the cells were prepared from freshly excised tissue for the murine tumors, from normal mouse femurs for the bone marrow cells and from cell culture for the human tumors. The mouse leukemia (L1210), the solid tumor cells (pancreatic ductal adenocarcinoma 03 and colon adenocarcinoma 38), the bone marrow cells (CFU-GM), the human colon tumors HCT-116 and HCT-8, and pancreatic tumor MIA PaCa-2 (from the American Type Culture Collection) were then plated in soft agar to determine the selective cellular cytotoxicity of CPT-11 for the different tumor types. The technique was similar to the human tumor stem cell assay[39–41] with the exception that the drug was not added to the cells. Instead, the drug was placed on a filter-paper disk, which was then placed on top of the soft agar containing the tumor cells.[42] A hard bottom layer [containing tryptic soy broth (0.8%), Difco noble agar (0.8%), media (CMRL/Fishers, 1/1, v/v) and horse serum (11%) at 48°C] was poured into 60 mm plastic dishes (3 ml in each), allowed to solidify and stored at 37°C in 5% CO_2. A soft agar top layer, containing Difco noble agar media (0.44%), media (CMRL 1066/Fisher, 50%/50%, v/v), serum (10%) and titered tumor cells was poured on top and allowed to solidify. The cells were prepared from 800–1500 mg fresh tumors (except for human tumor cells that came from cell culture), cut into fragments in 10–15 ml cold Hank's basal salt solution media containing 10% horse serum. The tumor was disrupted using a Stomacher 80 for 10–15 s. This material was then poured through a 100 mesh sieve. Residual material was forced through (by finger with a sterile glove) and the sieve rinsed with cold media. The cell suspension was centrifuged twice at 150 *g* for 5 min in cold CMRL/Fisher media with 10% horse serum. Titers were adjusted to produce 300–600 colonies per dish. For the assessement of the CFU-GM, the femoral marrow was flushed out using a 23G needle. Cells were counted and 2×10^6 cells were plated per dish. In the case of human tumors, cells were mechanically dispersed, counted and plated (2×10^5 cells per plate). A volume of 0.05 ml of each drug dilution in distilled water was added to 6.5 mm diameter paper filter disks (Whatman #1 filter paper). The disks were allowed to dry and placed at the edge of the 60 mm tumor-containing dish. The plates were incubated in the conditions described above for 6–13 days and examined on an inverted microscope (×40–60 magnification). Depending upon the innate sensitivity of the cells for the drug (and the concentration of the drug), a zone of inhibition of colony formation occurred. The zone of inhibition measured from the edge of the paper disk to the first colonies, was determined in zone units (200 zu = 6.5 mm, the size of the filter paper disk, i.e. 1 zu = 33 μm). A zone differential of 300 units or more was necessary to indicate a solid tumor selective agent.

In vivo solid tumor evaluation

Chemotherapy. The methods of protocol design, chemotherapy techniques and data analysis have been presented in detail.[43,44] The number of animals necessary to begin a given experiment were pooled and implanted s.c. bilaterally with 30–60 mg tumor fragments on day 0 with a 12 g trocar. Bilateral implants were used to ensure a more uniform tumor burden per mouse. For an early stage tumor treatment, the animals were again pooled before distribution to the various treatment and control groups. For an advanced stage treatment, tumors were allowed to grow to the desired size range (animals with tumors not in the desired range were excluded). The mice were distributed to the various treatment and control groups. Non-tumor-bearing animals were matched to tumor-bearing groups and given the same agents by the same route, dose and schedules. In this way, drug-induced toxicity could be clearly separated from the toxic effects of tumors. Chemotherapy was started 3–14 days after tumor transplantation. Each group of mice was treated on the basis of group average weight. Mice were checked daily and adverse clinical reactions were noted. Each group of mice was weighed three to five times weekly until the weight nadir was reached. Then, groups were weighed once or twice weekly until the end of the experiment. Body weight change data were reported as the maximum treatment-related weight loss. Tumors were measured with a caliper twice or three times weekly, depending on the tumor growth rate, until the tumor reached 2000 mg or until the animal died. Solid tumor weights (mg) were estimated from two-dimensional tumor measurements (mm):

$$\text{tumor weight} = (\text{length} \times \text{width}^2)/2$$

The day of death was recorded, and macroscopic examination of the thoracic and abdominal cavities was carried out to assess the probable cause of death. In some cases, tissue samples were submitted for histological evaluation. A reference drug known to be active for the tumor under investigation, was included at two or more dose levels in the majority of the trials to ensure proper tumor chemosensitivity.

End points for assessing solid tumor activity. In each trial, three or four dose levels were evaluated including at least one dose level that was frankly toxic. Antitumor activity was evaluated at the highest non-toxic dosage (HNTD), which is the highest dosage that can be administered without causing death or undue toxicity. A dosage producing a weight loss nadir or drug-related deaths of 20% or above was considered as excessively toxic. Animal body weights included tumor weights.

Tumor growth inhibition (T/C value). For early stage disease, this is the most widely used criterion for the determination of antitumor activity. The tumor weight was determined simultaneously for the treated and the control groups. When the median tumor weight of the control (C) reached approximately 750–1500 mg, the median tumor weight of each treated group (T) were determined, including zeros. The T/C value in percent is calculated as follows:

$$\text{T/C (\%)} = \frac{\text{median tumor weight of treated}}{\text{median tumor weight of control}} \times 100$$

According to the National Cancer Institute (NCI) standards, a T/C $\leq$42% is the minimum level for activity.[45] A T/C $<$ 10% is considered as a high antitumor activity level which justifies further development (Decision Network-2 level, DN-2).

Tumor growth delay (T – C value). The tumor growth delay assays are based on the median time (in days) required for the treatment group (T) and the control group (C) tumors to reach a predetermined size (usually 750– 1000 mg). Tumor-free survivors are excluded from these calculations and tabulated separately. This value is very useful as it allows the quantitation of the tumor cell kill.

Determination of the tumor doubling time (T_d). T_d is estimated from the best fit straight line from a log linear growth plot of the control group tumors in exponential growth (100– 1000 mg range).

Calculation of tumor cell kill. For s.c. growing tumors, the total cell kill is calculated from the following formula:

$$\text{log cell kill (gross or total)} = \frac{\text{T} - \text{C value in days}}{3.32 \times T_d}$$

where T – C is the tumor growth delay in days as defined above and T_d is the tumor volume doubling time in days. The conversion of the tumor growth delay values to log cell kill is possible because the T_d of tumors regrowing after treatment approximated the T_d values of tumors in the control mice.

Regression of advanced stage primary site tumor. This criteria is used for advanced stage trials. A complete regression is a regression below the limit of palpation. A partial regression is a regression greater than 50% reduction in tumor mass. In the tables, the partial regression column includes complete regressions.

In vivo antileukemic activity evaluation

Chemotherapy. Hemocytometer counted cells [10^6 cells per mouse for P388, P388/Dox or P388/Vcr, or 10^5 cells per mouse for L1210, suspended in Hank's medium (Gibco, Cergy-Pontoise, France)] were implanted i.p. (0.5 ml per mice) in $B6D2F_1$ mice after randomization on day 0. The mice were again randomized into treatment cages. The test agents were injected i.v. starting day 1, on the basis of average group weight. The body weight change was recorded daily during therapy and once a week thereafter. Cause of death was determined by examination of spleen size, liver involvement and presence or absence of ascites. This visual examination allowed us to discriminate between delayed drug deaths and tumor deaths. Non tumor-bearing animals were often matched to tumor-bearing groups and received the same agents by the same route, dose and schedules, to clearly separate drug-induced toxicity from leukemia effects.

End points for assessing anti-leukemic activity. The percent increase in life span (% ILS) was determined as follows:

$$\text{\% ILS} = 100 \times \frac{\text{MDD treated} - \text{MDD control}}{\text{MDD control}}$$

where MDD is the median day of death. % ILS was only used for assays of antileukemic activity.

According to NCI standards for i.p. implanted tumor/i.p. administered drug, an ILS $\geq 25\%$ for L1210 and $\geq 27\%$ for P388 is the minimum level for activity. An ILS $\geq 50\%$ for L1210 and $\geq 75\%$ for P388 is considered a high level of antileukemic activity (DN-2 level).[36] However, the model used in this evaluation is a more difficult one (i.p. tumor, i.v. drug) and any ILS over 40% is considered good leukemia activity.

Pharmacokinetic studies

Drug administration. The required CPT-11 dosages were prepared from the stock solution (20 mg/ml) by further diluting in 0.9% sodium chloride solution to a final volume of 0.4 ml per mouse. The drug was administered as an i.v. bolus into a lateral tail vein in pancreatic adenocarcinoma 03 (P03)-bearing mice ($B6D2F_1$), either as a single injection or as five consecutive daily administrations of 52.5 mg/kg per injection.

Plasma and tumor collection. Pharmacokinetics was determined using two groups of tumored mice (33 mice per group): one group after a single CPT-11 treatment and one group after a 5 day treatment). Heparinized blood samples and tumors (three mice per time point) were harvested at the following time points after drug administration: 5, 10, 15, 30 and 45 min, 1, 2, 4, 6, 12 and 24 h. Blood was immediately centrifuged at 2000 *g* for 15 min at 4°C, and the plasma was transferred to a polypropylene tube and stored immediately at −80°C until HPLC analysis. Tumors were quickly excised, weighed and stored at −80°C until HPLC analysis.

HPLC determination of CPT-11 and SN-38 levels. CPT-11 and SN-38 were assayed by reversed-phase HPLC with fluorescence detection using a procedure that allows the simultaneous determination of both compounds.[46] This assay measures total CPT-11 and SN-38, i.e. the addition of the lactone and the carboxylate forms of either compound. Briefly, after addition of camptothecin as the internal standard, plasma samples (100 μl) were extracted using a solid-phase (C18) extraction step. The extracts were then chromatographed on a C18 reversed-phase analytical column using a mobile phase (1 ml/min) composed of 34% acetonitrile and 66% potassium dihydrogen phosphate (0.1 M) containing 3 mM sodium heptanesulfonic acid (pH 4). The fluorescence detector wavelengths were set at 380 nm (excitation) and 500 nm (emission). The calibration curves were linear over a wide range of concentrations (1 ng/ml to 10 μg/ml), and the lower limit of quantification was 1 ng/ml for both CPT-11 and SN-38. The concentrations of CPT-11 and SN-38 were determined from peak area ratios of either compounds to the internal standard, by reference to a calibration curve run daily. Tumors were homogenized in 4 volumes of 0.01 N HCl using a Polytron homogenizer and 100 μl of the homogenate was extracted on a C18 column as described above for the plasma samples. The calibration curve for tumors were prepared using control tumor samples added with known concentrations of CPT-11 and SN-38 as described above for plasma samples.

Pharmacokinetic parameters. Model-independent parameters included the following:[47] the actual concentration at the end of i.v. infusion (C_{max}); the total area under the plasma concentration versus time curve ($AUC_{0-\infty}$) determined by the trapezoidal method to infinity; the terminal apparent half-life determined by the linear regression analysis of the last three to four aligned concentration time points from a semi-logarithmic plot; the volume of distribution at steady state (V_{dss}) calculated according to the statistical moment theory; and the total body clearance (CL) calculated as the dose divided by the total AUC.

Statistical Analysis. Possible difference between day 1 and day 5 tumor volume or pharmacokinetic parameters, was evaluated using the Student's *t*-test. Significance was reached at $p < 0.05$.

Results

In vitro solid tumor specificity

In order to determine if CPT-11 was preferentially cytotoxic toward leukemia or solid tumor cells, it was evaluated in a soft agar disk diffusion assay.[42] In this assay, CPT-11 was found preferentially cytotoxic toward two mouse solid tumors, colon adenocarcinoma 38 and pancreatic ductal adenocarcinoma 03, as compared with the L1210 leukemia (Table 1).

We also evaluated camptothecin and SN-38, the major metabolite of CPT-11. Clearly, the metabolite SN-38 was the most potent compound in the case of murine tumors with a 19- and 4.8-fold greater cytotoxicity compared with CPT- 11 and camptothecin,

respectively. Both CPT-11 and camptothecin were found murine solid tumor selective, whereas SN-38 was not.

Using normal mouse bone marrow cells, SN-38 was found more myelotoxic than CPT-11.

Three human tumors were also evaluated in this assay, HCT-8, HCT-116 and MIA PaCa-2. Interestingly, the three compounds were found equitoxic on the two colon tumors compared with the mouse L1210 leukemia whereas they all presented solid tumor selectivity toward MIA PaCa-2.

In vivo antitumor efficacy

Optimal schedule determination. To determine optimal administration schedules, CPT-11 was administered i.v. with various schedules to mice bearing i.p. P388 leukemia and s.c. MA16/C.

P388 leukemia. Mice bearing early stage tumor were treated with five schedules: experiment 1: schedule A, i.v. single bolus; schedule B, i.v. bolus for 4 days; schedule C, twice daily i.v. bolus for 4 days; experiment 2: schedule D, i.v. 4 h infusion every 4 days for 2 treatments; schedule E, i.v. bolus every 4 days for two treatments (Table 2).

With a single i.v. bolus (schedule A), dosages equal to 150 mg/kg and above caused immediate deaths. In this trial, the highest single i.v. bolus that could be administered was 93 mg/kg. The corresponding increase in life span (ILS) was 77%.

Using the daily day 1–4 schedule, CPT-11 was found toxic at the two top dosages evaluated, 93 and 57 mg/kg per injection. The HNTD of 35 mg/kg per injection (total dose of 140 mg/kg) was found highly active with a 115% ILS.

With the split dose schedule, twice daily on days 1–4 (schedule C), the HNTD was 17.5 mg/kg per injection (total dose of 140 mg/kg) and the corresponding ILS was 92%, with 1/5 tumor-free survivors on day 94. Higher dosages caused an important body weight loss or drug death.

In the second set of P388 experiments, CPT-11 was infused over 4 h on days 1 and 4 (schedule D) to avoid immediate toxicity observed with single i.v. bolus. The highest dosages evaluated 492 and 297 mg/kg per infusion were found toxic. The HNTD of 179 mg/kg per infusion (total dose 358 mg/kg) was found active with 71% ILS. The lowest dosages retained efficacy.

Lastly, using the intermittent i.v. bolus schedule days 1 and 4 (schedule E), immediate toxicity occurred at a dosage of 179 mg/kg. The highest dose tested with this schedule was 108 mg/kg per injection (total dose 216 mg/kg). It was found active with a 71% ILS. The lowest dosages retained efficacy.

Mammary adenocarcinoma 16/C. Mice bearing early stage tumor were treated with four different schedules: schedule A, i.v. bolus injection; schedule B, 4 h i.v. infusion; schedule C, i.v. bolus every 3 days for two injections; schedule D, i.v. bolus for 4 days (Table 3).

With a single i.v. bolus (schedule A), the highest dose tested (180 mg/kg) caused immediate toxicity. The highest dose tested was 108 mg/kg corresponding to a 11% T/C and to a 1.2 log cell kill. The dosage below retained a good activity.

Using the 4 h infusion schedule on day 4 (schedule B), true cumulative toxicity was reached with drug death occurring 5–6 days post-treatment at the 500 mg/kg dosage and with an excessive body weight loss at the 300 mg/kg dosage. The HNTD was 180 mg/kg corresponding to a 11% T/C and to a 1.5 log cell kill. The dosage below 108 mg/kg retained activity with a 1.2 log cell kill although it was administered on day 5 due to pump failure.

With the intermittent schedule days 4 and 7 (schedule C), immediate toxicity was obtained with the highest dosage tested (180 mg/kg per injection). The highest dosage tested was 108 mg/kg per injection (total dose of 216 mg/kg) with a 6% T/C and a 2.0 log cell kill. The lowest dosages were found active with a 1.6 log cell kill.

With schedule D, daily on days 4–7, the optimal dosage was 23.4 mg/kg per injection (total dose of 93.6 mg/kg) and the corresponding T/C was 6% with a 1.8 log cell kill. The dosage below retained activity with a 1.5 log cell kill.

Antitumor efficacy of i.v. CPT-11 against solid tumors and leukemias. Taking into account the above determined best administration schedules, we next evaluated the breadth of CPT-11 activity against tumors of various tissue origin.

Pancreatic ductal adenocarcinoma 03. CPT-11 was evaluated i.v. against both early (treatment starting day 3) and advanced (treatment starting day 14) stage disease (Table 4). Treated at an early stage, P03 was found highly sensitive to CPT-11. The optimal dosage was 52.5 mg/kg per injection on days 3–7 (total dose of 262.5 mg/kg). The corresponding T/C was 0% and there were 3/5 tumor-free survivors on day 179. The dosage below was also highly active with 4/5 tumor-free survivors. Against a measurable disease (> 100 mg), CPT-11 retained mod-

Table 1. Cytotoxicity of CPT-11 and related compounds in the disk diffusion assay

	Dose (μg/disk)	Tumor cell growth inhibition (zu)[a]							
		Mouse tumors				Human tumors			
		L1210	C38	P03	(CFU-GM)[d]	L1210	HCT-8	HCT-116	MIA PaCa-2
CPT-11	25	630 (450–750)	>1000 (975–>1000)	>1000 (975– >1000)	160	450	600	150	800
	12.5	150	–	–	75	150	300	50	600
	2.5	0 (0–0)	550 (450–760)	616 (450–825)	0	0	0	0	75
	0.5	0 (0–0)	0 (0–0)	0 (0–0)	–	–	–	–	–
SN-38	0.5	600	>1000	>1000	590	750	750	300	>1000
	0.13	550	600	640	310	250	100	0	>1000
	0.06	300	300	450	20	0	75	0	975
	0.03	–	–	–	–	0	0	0	600
Camptothecin	2.5	725 (700–750)	>1000 (>1000)	>1000 (>1000– >1000)	–	–	–	–	–
	0.63	50 (0–100)	647 (620–675)	675 (675–675)	290	600[b]	700	900	>1000
	0.15	0	360	510	225	0[c]	0	150	600

[a] One zone unit (zu) = 33 μm indicates the zone of inhibition between the edge of the filter paper disk containing the drug to the first colonies. Number in parenthesis indicates the range of values in separate experiments.
[b] Tested at 0.5 μg/disk.
[c] Tested at 0.13 μg/disk.
[d] CFU–GM, normal bone marrow cells.

Table 2. Evaluation of CPT-11 against P388 leukemia on B6D2F$_1$ male mice: efficacy and schedule of administration studies

i.v. route	CPT-11 dosage (mg/kg per injection)	Schedule (days)	Total dose (mg/kg)	Mortality (day of death)	Mean body weight change (g) day 1–day 7	Therapeutic response: Median day of death	Therapeutic response: ILS %	Comments
Experiment 1								
Schedule A								
bolus	241	1	241	5/5 (3d1, 21, 23)[a]				toxic
0.4 ml	150		150	5/5 (1, 17, 18, 20, 21)				toxic
	93		93	5/5 (17, 20, 23, 28, 30)	+1.58	23	77	HNTD[b] highly active
	57		57	5/5 (3d17, 18, 19)	+1.96	17	31	minor activity
Schedule B								
bolus	93	1, 2, 3, 4	372	5/5 (3, 2d8, 2d9)				toxic
0.4 ml	57		228	5/5 (3, 23, 24, 27, 30)				toxic
	35		140	5/5 (22, 27, 28, 29, 33)	−0.28	28	115	HNTD highly active
Schedule C								
bolus	46.5	1, 2, 3, 4	372	5/5 (3d7, 2d8)				toxic
0.4 ml	28.5	(2 × /day)	228	5/5 (21, 23, 2d25, 40)	−4.56			toxic 22.3% bwl[b]
	17.5		140	4/5 (22, 23, 27, 29)	+1.40	25	92	HNTD highly active 1/5 TFS[b]
	control			10/10 (11, 2d12, 3d13, 2d14, 16, 17)	+3.02	13		
Experiment 2								
Schedule D								
infusion	492	1, 4	984	8/8 (2d7, 6d8)[a]				toxic
1 ml/4 h	297	inf. 4 h	594	6/6 (21, 2d24, 2d26, 29)	−3.97			toxic 21% bwl on day 6
	179		358	10/10 (21, 22, 2d23, 2d24, 25, 26, 27, 36)	−1.30	24	71	HNTD active
	108		216	10/10 (2d21, 2d22, 4d23, 25, 29)	−0.29	23	64	active
	65		130	8/8 (20, 3d21, 22, 2d23, 24)	−0.43	21.5	54	active
Schedule E								
bolus	179	1	179	5/5 (5d1)				immediate toxicity
0.4 ml	108	1, 4	216	5/5 (21, 22, 2d24, 26)	+0.08	24	71	HDT[b] active
	65		130	5/5 (3d21, 23, 25)	+0.75	21	50	active
	39		78	5/5 (20, 21, 2d22, 23)	+0.17	22	57	active
	control			9/9 (3d12, 5d14, 17)	+1.93	14		

[a] Mortality: underlined numbers correspond to drug death determined at necropsy.
[b] Abbreviations: HNTD, highest non-toxic dose; bwl, body weight loss; HDT, highest dose tested; TFS, tumor-free survivors on day 94.

Table 3. Evaluation of CPT-11 against early stage mammary adenocarcinoma MA16/C on C3H/HeN female mice: efficacy and schedule of administration studies

i.v. route	CPT-11 dosage (mg/kg per injection)	Schedule (days)	Total dose (mg/kg)	Drug death (day of death)	Mean body weight change (g) (day of nadir)	T/C (%) day 12	T − C (days)	Log cell kill total	Comments
Schedule A									
bolus	180	4	180	5/5 (5d4)[a]					immediate toxicity
0.4 ml	108		108	0/5	− 1.21 (21)	11	6.1	1.2	HDT[b] active
	65		65	0/5	− 1.07 (21)	17	6.1	1.2	active
Schedule B									
infusion	500	4	500	5/6 (3d9, 2d10)	− 3.84 (8)				toxic
1 ml/4 h	300	4	300	1/6 (21)	− 4.21 (8)				toxic 20.5% bwl[b]
	180	4	180	0/6	− 1.64 (8)	11	7.5	1.5	HNTD[b] active
	108	5[c]	108	0/6	− 0.97 (19)	14	5.9	1.2	active
Schedule C									
bolus	180	4	180	5/5 (5d4)					immediate toxicity
0.4 ml	108	4, 7	216	0/5	− 1.55 (9)	6	9.8	2.0	HDT active
	65		130	0/5	− 1.29 (9)	6	8.2	1.6	active
	39		78	0/5	− 1.14 (21)	9	8.2	1.6	active
Schedule D									
bolus	65	4–7	260	4/5 (2d11, 2d12)					toxic
0.4 ml	39		156	5/5 (3d11, 12, 13)					toxic
	23.4		93.6	0/5	− 2.66 (10)	6	9.2	1.8	HNTD active
	14		56	0/5	− 0.86 (8)	10	7.3	1.5	active

[a] 5d4: 5 deaths on day 4.
[b] Abbreviations: HDT, highest dose tested; bwl, body weight loss, HNTD, highest non-toxic dose.
[c] On day 4, a technical problem occurred with the pump. The 108 mg/kg dosage was infused on day 5.
[d] Tumor doubling time = 1.5 days. The T/C was determined on day 12 (control = 874 mg). The T − C was determined when the tumors reached 750 mg (11.7 days for the control).

Table 4. Evaluation of CPT-11 against early and advanced stage pancreatic ductal adenocarcinoma 03

i.v. CPT-11 dosage (mg/kg per injection)	Schedule (days)	Total dose (mg/kg)	Drug death (day of death)	Mean body weight change (g) (day of nadir)	T/C (%) day 39	T − C (days)	Log cell kill total	Regressions PR[a]	Regressions CR[a]	Tumor-free survivors on day 179	Comments
Early stage tumor[b]											
137.5	3–7	687.5	5/5 (3, 7, 8, 2d11)	toxic							toxic
85		425	1/5 (11)	toxic							toxic
52.5		262.5	0/5	− 2.99 (9)	0					3/5	HNTD[a] highly active
32.5		162.5	0/5	− 1.68 (8)	0					4/5	highly active
Advanced stage tumor[c]											
140	14–17	560	3/5 (3d14)	− 3.54 (20)							immediate toxicity
84		336	0/5	− 2.34 (19)		7.5	0.8	5/5	5/5	0/5	HNTD modest activity
50.4		201.6	0/5	− 1.86 (17)		3.8	0.4	1/5	0/5	0/5	inactive
30.2		120.8	0/5	− 0.82 (17)		0	0	0/5	0/5	0/5	inactive

[a] Abbreviations: PR, partial regressions: CR, complete regressions; HNTD, highest non-toxic dose.
[b] Tumor doubling time = 3 days; $B6D2F_1$ female mice. The T/C was determined on day 39 (control = 1422 mg).
[c] Tumor doubling time = 3 days; C57BL/6 male mice. The T − C was determined when the tumors reached 1000 mg (27.5 days for the control).

est activity at the optimal dosage 84 mg/kg per injection on days 14–17 (total dose of 336 mg/kg). The corresponding log cell kill was 0.8 with 5/5 complete regressions.

Colon adenocarcinoma 38. CPT-11 was administered i.v. using a daily schedule on days 3–7 (Table 5). At the HNTD of 43 mg/kg per injection (total dose of 215 mg/kg), it was found modestly active, with a T/C of 25% and a 1.0 log cell kill. The dosage below retained a similar activity.

Colon adenocarcinoma 51. Mice bearing early stage C51 were administered using an intermittent schedule on days 4, 6 and 8 with i.v. CPT-11 (Table 5). The highest dose tested, 62 mg/kg per injection (total dose of 186 mg/kg), was active with a 9% T/C and a 1.0 log cell kill. The lowest dosages were found inactive. Cisplatin, a positive control, was found highly active.

Mammary adenocarcinoma 16/C. Mice bearing early stage MA16/C were treated i.v. using an intermittent schedule on days 4, 7, 10 and 13 (Table 6). At the HNTD (52.5 mg/kg per injection, i.e. 210 mg/kg total dose), CPT-11 was found highly active with a 0% T/C corresponding to a 3.4 log cell kill. The two dosages below 32.5 and 20.2 mg/kg per injection retained activity with 2.2 and 1.3 log cell kill, respectively. Doxorubicin was found highly active in this trial.

Mammary adenocarcinoma 13/C. Using this model, CPT-11 was evaluated i.v. using a daily schedule on days 3–7 (Table 6). The HNTD (211 mg/kg total dose) was found active with a 9% T/C and a 1.0 log cell kill. Modest activity was retained at the two lower dosages.

Human Calc18 breast adenocarcinoma. This tumor xenografted in nude mice was treated i.v. using an intermittent schedule on days 5, 7, 9, 13 and 14 (Table 6). At the optimal dosage 67 mg/kg per injection, representing a total dose of 335 mg/kg, CPT-11 was found highly active with a 1% T/C and a 2.8 log cell kill. Doxorubicin was found inactive.

Glasgow osteogenic sarcoma. CPT-11 was tested i.v. using an intermittent schedule on days 5, 8 and 11 (Table 7). At the optimal dosage of 100 mg/kg per injection, CPT-11 was found active with 9.8 days tumor growth delay corresponding to a 1.8 log cell kill. The dosage below, 66 mg/kg per injection retained activity with a 1.4 total log cell kill. The two lowest dosages were found inactive. Cisplatin, a positive control, was found highly active at the highest dose evaluated.

Lewis lung carcinoma. CPT-11 was administered i.v. on days 3, 5 and 7 to mice bearing Lewis lung carcinoma (Table 7). The HNTD was 66 mg/kg per injection (total dose of 198 mg/kg) and was found active with a 4% T/C and a 1.4 log cell kill. The dosage below retained activity with a 0.8 log cell kill. Cyclophosphamide, a positive control, was found highly active at the highest dose evaluated.

B16 melanoma. Mice bearing B16 melanoma were administered i.v. with CPT-11 using a daily schedule on days 3–7 (Table 7). The highest dose tested (100 mg/kg per injection) produced a 0% T/C and a 1.4 log cell kill. The two dosages below retained activity.

P388 leukemia. CPT-11 evaluation was also performed against i.p. P388 leukemia using a day 1–4 schedule (Table 8). At the highest dose tested, 80 mg/kg per injection (total dose 320 mg/kg), CPT-11 was highly active against P388, with an ILS of 170% and 1/5 tumor-free survivors on day 85. The three dosages below were highly active. Doxorubicin, a positive control, was found active at the highest dose tested given once on day 1.

L1210 leukemia. Mice bearing i.p. L1210 leukemia were treated with i.v. CPT-11 using a day 1–4 schedule (Table 8). A 64% ILS was obtained at the highest dose tested (total dose of 320 mg/kg) and the three dosages below retained activity. Cyclophosphamide, a positive control, was found active against L1210 at the HNTD.

CPT-11 antitumor efficacy against tumors with acquired resistance

P388 resistant to vincristine (P388/Vcr). Using a daily schedule, days 1–4, CPT-11 was found highly active at the optimal dosage of 80 mg/kg per injection, a total dose of 320 mg/kg against i.p. P388/Vcr (Table 9). The ILS was 159% with 1/5 tumor-free survivors. The three dosages below were highly active.

P388 resistant to doxorubicin (P388/Dox). Using the i.p. P388/Dox model, CPT-11 administered i.v. using a day 1–4 schedule was found modestly active (Table 9). At the highest dose tested, 80 mg/kg per injection (total dose of 320 mg/kg), CPT-11 produced 18% body weight loss and a 45% ILS. The dosages below retained activity but with a poor dose–response relationship.

Human Calc18 breast carcinoma resistant to docetaxel (Calc18/TXT). Nude mice bearing early stage disease (treatment starting day 5) were administered CPT-11 as five i.v. boluses over 9 days (Table 10). At the highest dose administered, CPT-11 was found active with a 0% T/C and a 1.5 log cell kill. The dosage below retained activity.

Table 5. Evaluation of CPT-11 against colon tumors

i.v. agents	Dosage (mg/kg per injection)	Schedule (days)	Total dose (mg/kg)	Drug death (day of death)	Mean body weight change (g) (day of nadir)	T/C (%)	T − C (days)	Log cell kill total	Comments
Colon adenocarcinoma 38[a]									
CPT-11	120.0	3–7	600.0	5/5 (3, 4d10)					toxic
	72.0		360.0	4/5 (2d12, 2d13)	−5.21 (10)				toxic
	43.0		215.0	0/5	−1.95 (8)	25	11.2	1.0	HNTD[b] active
	26.0		130.0	0/5	−0.49 (7)	25	10.0	0.9	active
Colon adenocarcinoma 51[c]									
CPT-11	62.0	4, 6, 8	186.0	0/5	−1.21 (10)	9	10.0	1.0	HDT[b] active
	38.4		115.2	0/5	−0.98 (11)	23	6.4	0.6	inactive
	23.8		71.4	0/5	−0.60 (7)	33	5.8	0.6	inactive
	14.8		44.4	0/5	−0.57 (10)	23	6.9	0.7	inactive
cisplatin	3.0	4, 6, 8	9.0	0/5	−3.32 (11)	0	28.6	2.9	HNTD highly active

[a] C38; tumor doubling time = 3.5 days; C67BL/6 male mice; the T/C calculation was determined on day 34 (control = 999 mg). The T − C was determined when the tumors reached 1000 mg (34 days in the control).
[b] Abbreviations: HNTD, highest non-toxic dose; HDT, highest dose tested.
[c] C51; tumor doubling time = 3 days; BALB/c female mice; the T/C calculation was determined on day 19 (control = 917 mg). The T − C was determined when the tumors reached 1000 mg (20 days in the control).

Table 6. Evaluation of CPT-11 against mammary tumors

i.v. agents	Dosage (mg/kg per injection)	Schedule (days)	Total dose (mg/kg)	Drug death (days of death)	Mean body weight change (g) (day of nadir)	T/C (%)	T − C (days)	Log cell kill total	Comments
Mammary adenocarcinoma 16/C[a]									
CPT-11	84.5	4, 7, 10, 13	338.0	3/5 (3d4)	− 4.06 (16)		NTBA		immediate toxicity
	52.5		210.0	0/5	− 1.93 (17)	0	13.5	3.4	HNTD[b] highly active
	32.5		130.0	0/5	− 1.26 (16)	6	8.7	2.2	active
	20.2		80.8	0/5	− 1.10 (7)	28	5.1	1.3	active
doxorubicin	5.0	4, 7, 10, 13	20.0	0/5	− 2.36 (17)	0	21.7	5.4	HNTD highly active
Mammary adenocarcinoma 13/C[c]									
CPT-11	68.0	3–7	340.0	5/5 (2d10, 11, 2d13)	− 4.96 (9)				toxic
	42.2		211.0	0/5	− 2.75 (10)	9	8.3	1.0	HNTD active
	26.1		130.5	0/5	− 0.57 (8)	21	4.9	0.6	inactive
	16.2		81.0	0/5	− 0.15 (6)	16	7.2	0.9	active
Human Calc18 breast adenocarcinoma[d]									
CPT-11	87.0	5, 7, 9,	435.0	4/5 (3d5, 7)					immediate toxicity
	67.0	13, 14	335.0	0/5	− 0.84 (16)	1	38.3	2.8	HTD[b] highly active
	41.0		205.0	0/5	− 0.91 (14)	15	21.5	1.6	active
	26.0		130.0	0/5	− 0.53 (6)	53			inactive
doxorubicin	7.5	5, 9	15.0	0/5	− 0.83 (14)	81			HDT inactive

[a] MA16/C; tumor doubling time = 1.2 days; C3H/HeN female mice. The T/C calculation was determined on day 10 (control = 1227 mg). The T − C was determined when the tumors reached 1000 mg (9.7 days for the control).

[b] Abbreviations: NTBA, non-tumor bearing animals; HNTD, highest non-toxic dose; HDT, highest dose tested.

[c] MA13/C; tumor doubling time = 2.4 days; C3H/HeN female mice. The T/C calculation was determined on day 21 (control = 824 mg). The T − C was determined when the tumors reached 1000 mg (21.9 days for the control).

[d] Calc18; tumor doubling time = 4.1 days; Swiss-nu female mice. The T/C calculation was determined on day 40 (control = 1257 mg). The T − C was determined when the tumors reached 1000 mg (38 days for the control).

Table 7. Evaluation of CPT-11 against sarcoma, lung and melanoma tumors

i.v. agents	Dosage (mg/kg per injection)	Schedule (days)	Total dose dose (mg/kg)	Drug death (day of death)	Mean body weight change (g) (day of nadir)	T/C (%)	T – C (days)	Log cell kill total	Comments
Glasgow osteogenic sarcoma[a]									
CPT-11	100	5, 8, 11	300	0/5	– 1.10 (9)	34	9.8	1.8	HDT[b] active
	66		198	0/6	– 0.68 (6)	33	7.3	1.4	active
	44		132	0/6	– 0.57 (9)	43	3.3	0.6	inactive
	29		87	0/6	– 0.53 (6)	68			inactive
cisplatin	5	5, 11	10	0/6		7	16.6	3.1	HDT highly active
Lewis lung carcinoma[c]									
CPT-11	100	3, 5, 7	300	2/6 (6, 8)					toxic
	66		198	0/6	– 0.82 (8)	4	6.8	1.4	HNTD[b] active
	44		132	0/6	– 0.90 (8)	3	3.9	0.8	active
	29		87	0/6	– 0.22 (8)	29	2.1	0.4	inactive
cyclophosphamide	148	3, 5, 7	444	0/5		0			HDT highly active 5/5 TFS[b]
B16 Melanoma[d]									
CPT-11	100	3–7	500	0/5	– 3.30 (10)	0	8.6	1.4	HDT active
	60		300	0/5	– 3.76 (10)	0	10.7	1.8	active
	36		180	0/5	– 0.49 (9)	0	6.5	1.1	active

[a] Glasgow osteogenic sarcoma; tumor doubling time = 1.6 days; $B6D2F_1$ male mice. The T/C was determined on day 13 (control = 938 mg). The T – C was determined when the tumors reached 100 mg (13.2 days for the control).

[b] Abbreviations: HDT, highest dose tested; HNTD, highest non-toxic dose; TFS, tumor-free survivors on day 34.

[c] Lewis lung carcinoma; tumor doubling time = 1.5 days; C57BL/6 male mice. The T/C was determined on day 10 (control = 1595 mg). The T – C was determined when the tumors reached 1000 mg (9.1 days for the control).

[d] B16 melanoma; tumor doubling time = 1.8 days; $B6D2F_1$ female mice. The T/C was determined on day 14 (control = 844 mg). The T – C was determined when the tumors reached 1000 mg (15 days for the control).

Table 8. Evaluation of CPT-11 against murine leukemia in B6D2F$_1$ female mice

i.v. agents	Implant cells	Dosage (mg/kg per injection)	Schedule (days)	Total dose (mg/kg)	Mortality (days of death)	Mean body weight change (g) between day 1 and 7	Tumor-free survivors day 85	Median day of death (range)	ILS (%)	Comments
P388 Leukemia										
CPT-11	10^6	80.0	1–4	320.0	4/5 (25, 2d31, 35)	− 2.84	1/5	31 (25–35)	170	HDT[a] highly active
		49.6		198.4	6/6 (22, 23, 25, 2d27, 28)	− 1.88	0/6	26 (22–28)	126	highly active
		30.8		123.2	5/6 (2d23, 2d27, 28)	− 0.06	1/6	27 (23–28)	135	highly active
		19.0		76.0	6/6 (2d21, 23, 2d24, 25)	+0.10	0/6	23.5 (21–25)	104	highly active
doxorubicin	10^6	10.0	1	10.0	5/5 (2d19, 21, 22, 26)	− 0.29	0/5	21 (19–26)	83	HDT highly active
control	10^6				10/10 (3d10, 2d11, 3d12, 16, 20)			11.5 (10–20)		
L1210 Leukemia										
CPT-11	10^5	80.0	1–4	320.0	5/5 (16, 2d18, 22, 27)	− 3.00	0/5	18 (16–27)	64	HDT highly active
		49.6		198.4	6/6 (2d15, 16, 18, 19, 20)	− 0.73	0/6	17 (15–20)	55	highly active
		30.8		123.2	5/6 (3d15, 16, 54)	− 0.01	1/6	15 (15–54)	36	active
		19.0		76.0	6/6 (14, 2d15, 16, 18, 20)	+0.71	0/6	15.5 (14–20)	41	active
cyclophosphamide	10^5	155.0	1, 4	310.0	3/5 (19, 21, 35)	− 0.38	2/5[b]	21 (19–35)	91	HNTD[a] highly active
control	10^5				9/9 (9, 10, 4d11, 12, 2d13)	+2.26		11 (9–13)		

[a] Abbreviations: HDT, highest dose tested; HNTD, highest non-toxic dose.
[b] At necroscopy, 2/5 without ascite.

Table 9. Evaluation of CPT-11 against resistant leukemia in B6D2F$_1$ female mice

Agents	Implant cells	Dosage (mg/kg per injection)	Schedule (days)	Total dose (mg/kg)	Mortality (days of death)	Mean body weight change (g) between day 1 and 7	Median day of death (range)	ILS (%)	Comments
P388 Leukemia resistant to vincristine									
CPT-11	10^6	80.0	1–4	320.0	4/5 (24, 28, 29, 35)	−1.67	28.5 (24–35)	159	HDT[a] highly active 1/5 TFS[a]
		49.6		198.4	6/6 (21, 23, 25, 26, 28, 29)	+0.81	25.5 (21–29)	132	highly active
		30.8		123.2	6/6 (24, 25, 26, 2d27, 32)	+1.09	26.5 (24–32)	141	highly active
		19.0		76.0	6/6 (21, 22, 23, 24, 31, 34)	+1.61	23.5 (21–34)	114	highly active
control	10^6				9/9 (8, 10, 3d11, 12, 3d13)	+4.28	11 (8–13)		
P388 Leukemia resistant to doxorubicin									
CPT-11	10^6	80.0	1–4	320.0	5/5 (15, 2d16, 17, 20)	− 3.46	16 (15–20)	45	HDT active (18% bwl[a])
		49.6		198.4	6/6 (15, 17, 3d18, 20)	− 0.55	18 (15–20)	64	active
		30.8		123.2	6/6 (13, 15, 2d16, 17, 18)	+0.61	16 (13–18)	45	active
		19.0		76.0	6/6 (13, 14, 16, 2d17, 20)	+1.02	16.5 (13–20)	50	active
control	10^6				10/10 (4d10, 3d11, 2d12, 14)		11 (10–14)		

[a] Abbreviations: HDT, highest dose tested; TFS, tumor-free survivors on day 85; bwl, body weight loss.

CPT-11 antitumor efficacy using oral administration

Pancreatic ductal adenocarcinoma 03. CPT-11 was administered orally using a split dose schedule over 5 days (Table 11). The optimal dosage of 60.76 mg/kg per administration (total dose of 729.12 mg/kg) was found highly active with a 0% T/C and 4/5 tumor-free survivors on day 119. The 37.67 mg/kg per administration dosage was found also highly active with 2/5 tumor-free survivors, and a 1.6 log cell kill on the three mice the tumors of which regrew. The lowest dosage tested (23.35 mg/kg per administration) retained efficacy with 1.5 log cell kill and no tumor-free survivors.

Mammary adenocarcinoma 16/C. Against this tumor treated at an early stage, orally administered CPT-11 demonstrated a good level of activity with a 0% T/C at the HNTD (372 mg/kg total dose) and a 3% T/C at the dosage below, with 1.9 and 1.7 log cell kill, respectively (Table 11).

Toxicity and host recovery. Dosages equal to 150 mg/kg and above administered i.v. as a bolus injection produced immediate toxicity with drug death occurring as early as 5 min post-injection after hyperpnae, tachycardia, tremors, occasional convulsions, followed by lethargy. This phenomena disappeared when CPT-11 was infused over 4 h or when the compound was administered orally.

Dosages below 150 mg/kg could be administered successfully and repeatedly to reach true cumulative toxicity. At lethal dose levels where true cumulative toxicity was reached, most drug death occurred between 3 and 6 days post last treatment. Necropsy and histological evaluation revealed mostly small spleen and small rounded liver. In addition, gastrointestinal toxicity was noted when the compound was administered i.v. or orally. Evidence of diarrhea was seen with the presence of intestines dilated with orange to brown fluid. The C3H/HeN mice were far more sensitive to the gastrointestinal toxicity than the C57BL/6 or the $B6D2F_1$ mice regardless of the route of administration.

At the HNTD, a review of all the i.v. efficacy trials performed using solid tumors showed that the mean optimal total dose was approximately 235 mg/kg.

Using the bolus schedule, a 8% body weight loss nadir occurred usually 2 days post last treatment (range 0–4 days) and full host recovery was seen 6 days post last therapy. Using a 4 h infusion schedule, 8–14% body weight loss occurred 3–4 days post single infusion or between the two infusions. Full host recovery was obtained 5–7 days post last infusion. Using oral administration, the optimal dosage was two to three times higher than the i.v. optimal dosage. The total body weight loss at nadir was 2–11% occurring 3 days post last treatment and the host recovery was obtained 6 days post last therapy.

Pharmacokinetics of CPT-11 and metabolite SN-38. Pharmacokinetic evaluations were performed at the HNTD determined above in $B6D2F_1$ mice bearing pancreatic P03 tumors, i.e. at 52.5 mg/kg per injection. Two groups of tumor-bearing mice (33 mice per group) received either a single i.v. CPT-11 treatment and one group received a 5 day treatment (total dose of 262.5 mg/kg). The average tumor burden per mouse on the first day of treatment was 0.62 ± 0.04 g ($N = 66$).

Plasma pharmacokinetics. CPT-11 plasma concentrations after the i.v. administration of 52.5 mg/kg in the tumor-bearing mice are presented in Figure 2. Maximum CPT-11 concentrations (C_{max}) at 5 min after the i.v. bolus reached 8.9 μg/ml and the terminal half-life was 0.6 h. Other pharmacokinetic parameters are presented in Table 12. The metabolite SN-38 plasma concentrations following the administration of CPT-11 in tumor-bearing mice (Figure 2) presented a C_{max} of 1.6 μg/ml and a terminal half-life of 7.4 h (Table 12), which is significantly longer than the CPT-11 half-life in the plasma. A comparison of the plasma pharmacokinetics of the two groups receiving the drug either as a single or as a daily × 5 schedule did not disclose any significant difference in their parameters for CPT-11 and SN-38, indicating that the number of treatments, within this time window, does not alter their pharmacokinetics (Table 12).

Tumor pharmacokinetics. CPT-11 and SN-38 tumor levels after i.v. administration of CPT-11 are presented in Figure 2. Although the maximum CPT-11 tumor concentrations were similar to the plasma concentrations for the first time points, drug levels decreased more slowly in the tumor than in the plasma with half-lives of 5.0 h (Table 12) in the tumors compared to plasma CPT-11 half-life (0.6 h) in tumor-bearing animals. The tumor CPT-11 AUCs were consequently about 10 times higher than the plasma AUCs. Metabolite SN-38 levels in tumors reached concentrations in the range of 0.32–0.34 μg/g and decayed with half-lives of 6.9– 6.7 h for the 1 and 5 day i.v. schedule, respectively (Table 12). No significant differences in tumor pharmacokinetics of either CPT-11 or SN-38 were noted between the two groups of tumor-bearing mice receiving the drug as a single i.v. or as daily

Table 10. Evaluation of CPT-11 against human mammary adenocarcinoma Calc18 resistant to docetaxel (Calc18/TXT)

i.v. agents	Dosage (mg/kg per injection)	Schedule (days)	Total dose (mg/kg)	Drug death (day of death)	Mean body weight change (g) (day of nadir)	T/C (%) day 30	T − C (days)	Log cell kill total	Comments
CPT-11	108	5	108	4/5 (4d5)					immediate toxicity
	87	5, 7, 9, 12, 14	428.9	2/5 (7, 9)	−1.47 (12)				immediate toxicity
	67		335	0/5	−1.98 (11)	0	25.1	1.5	HDT[a] active
	41		205	0/5	−0.07 (8)	6	17.1	1.0	active
	26		130	0/5	+1.94 (15)	30	10.4	0.6	inactive
Doxorubicin	7.5	5, 9	15.6	0/5	−0.81 (14)	118			HDT inactive

Calc18/TXT; tumor doubling time = 5 days; Swiss-nu female mice. The T/C was determined on day 30 (control = 1121 mg). The T − C was determined when the tumors reached 1000 mg (28.4 days for the control).

[a] Abbreviation: HDT, highest dose tested.

Table 11. Evaluation of CPT-11 efficacy following oral administration

Agent	Dosage (mg/kg per administration)	Schedule (days)	Total dose (mg/kg)	Drug death (day of death)	Mean body weight change (g) (day of nadir)	T/C (%)	T – C (days)	Log cell kill total	Tumor-free survivors day 119	Comments
Pancreatic ductal adenocarcinoma 03[a]										
CPT-11	98.00	3–7[b]	1176.00	0/5	– 3.83 (12)					toxic 19% bwl[c]
	60.76		729.12	0/5	– 0.48 (10)	0			4/5	HNTD[c] highly active
	37.67		452.04	0/5	– 0.34 (7)	0	18.8	1.6	2/5	active
	23.35		280.20	0/5	– 0.43 (4)	0	17.8	1.5	0/5	active
Mammary adenocarcinoma 16/C[d]										
CPT-11	150.00	2–4, 7	600.00	2/5 (10, 12)	– 6.72 (10)					toxic
	93.00		372.00	0/5	– 2.57 (9)	0	8.3	1.9	0/5	HNTD active
	57.70		230.80	0/5	– 0.47 (8)	3	7.2	1.7	0/5	active

[a] P03; tumor doubling time = 3.5 days; $B6D2F_1$ female mice; the T/C was determined on day 31 (control = 664 mg). The T – C was determined when the tumors reached 750 mg (32.1 days for the control).
[b] Schedule: two administrations per day on days 3–5 and three administrations per day on days 6 and 7.
[c] Abbreviations: HNTD, highest non-toxic dose; bwl, body weight loss.
[d] MA16/C; tumor doubling time = 1.3 days; C3H/HeN female mice. The T/C was determined on day 11 (control = 1586 mg). The T – C was determined when the tumors reached 1000 mg (10.2 days for the control).

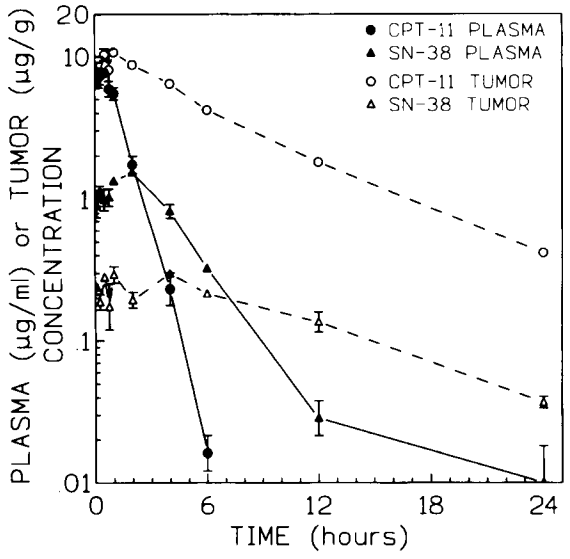

Figure 2. CPT-11 and metabolite SN-38 pharmacokinetics in plasma and tumor of B6D2F$_1$ mice bearing pancreatic adenocarcinoma 03 tumors (P03). Mice received a single i.v. bolus administration of CPT-11 (52.5 mg/kg) and drug or metabolite levels were assayed by HPLC. Solid lines, plasma levels of either CPT-11 (filled circles) or SN-38 (filled triangles); dashed lines, tumor levels of CPT-11 (open circles) or SN-38 (open triangles). Each point represents the mean ± SEM of three mice.

× 5 i.v. schedule (Table 12). These data indicate that there was no accumulation of either compounds in tumors following the daily × 5 schedule. The P03 tumors were weighed 0.62 ± 0.04 g (average weight) in the tumor groups and there was a significant reduction in tumor mass (29%) on day 5 compared with day 1 ($p < 0.001$, Student's paired t-test) in the group that received five i.v. treatments.

Discussion

In this paper, we have presented the *in vitro* and *in vivo* anticancer properties of CPT-11 in a variety of models, and we also determined the pharmacokinetics of the parent compound and its metabolite SN-38 in tumor-bearing mice.

Using an *in vitro* tumor soft agar disk diffusion assay, CPT-11 was found to be solid tumor selective with significant cytotoxicity for murine colon adenocarcinoma 38, pancreatic ductal adenocarcinoma 03 and human pancreas MIA PaCa-2, compared with L1210 leukemia and normal murine bone marrow cells. These results are in agreement with those published using human fresh tumor biopsies.[48] Our results also confirm that SN-38, one of the CPT-11 metabolites, is more potent than CPT-11 and, in addition, they indicate that qualitative differences exist between the two compounds as SN-38 does not present the solid tumor selectivity of CPT-11. In fact, SN-38 was equally toxic toward the majority of cell types, including normal bone marrow cells, murine and human tumor cells, except for MIA PaCa-2 that was found to be the most SN-38-sensitive cell line. The bone marrow results are truly interesting because they do confirm that SN-38 is more cytotoxic than CPT-11 and they also show that CPT-11 is more selective toward solid tumor cells than toward a normal cell population, which also clearly differs from SN-38. These observations seem to indicate that CPT-11 may not only be a prodrug of SN-38 and could exert antitumor activity by so far unidentified other mechanisms. This is also supported by Kunimoto *et al.* that clearly stated that CPT- 11 was more active *in vivo* than SN-38, which should not be the case if CPT-11 was only a prodrug.[3] On the other hand, it could be also proposed that L1210 leukemia cells are less able to hydrolyze CPT-11 to the metabolite SN-38 *in vitro*.

Optimal schedule of administration and antitumor efficacy of CPT-11 were evaluated *in vivo*. The schedule of administration was determined on the basis of CPT-11 toxicity using various schedules with short duration of treatment (less than 10 days), in which only minimal host recovery time from toxicity can occur. In this setting, if a drug is schedule dependent, the schedule will markedly influence the total dosage than can be administered and the HNTD will markedly decrease as the number of injections increase within the treatment period.[44] A schedule-dependent agent, e.g. arabinosylcytosine (ara-C), would have at least a 10-fold lower dosage on the twice daily schedule compared with the intermittent schedule.[44] This is opposed to the schedule-independent drugs where the schedule does not markedly influence the total dosage that can be administered even in the cases of a multiple administration schedule within a given time period.

In the studies presented, the optimal schedule was determined by comparing several administration schedules using two different murine tumors, a leukemia P388 and a solid tumor mammary adenocarcinoma MA16/C. With the two types of tumor tested, immediate toxicity often occurred with dosages equal to 150 mg/kg and above. These dosages could not be used for the determination of the HNTD. It could only be determined with schedules using lower dosage levels (below the dosage causing immediate death) for a prolonged period of time in order to obtain true cumulative toxicity. Of inter-

Table 12. Plasma and tumor pharmacokinetic parameters of CPT-11 and active metabolite SN-38 in tumor-bearing mice receiving CPT-11 at 52.5 mg/kg i.v. as a single dose or as a daily × 5 schedule[a]

Pharmacokinetic parameter	Plasma		Tumor	
	1 × i.v.	5 × i.v.	1 × i.v.	5 × i.v.
CPT-11				
$AUC_{0-\infty}$ (μg.h/ml or g)	12.7 ± 0.6[b]	10.3 ± 0.6	75.2 ± 3.9	106.1 ± 19
C_{max} (μg/ml or g)	8.9 ± 0.5	7.8 ± 0.8	11.8 ± 0.3	13.5 ± 0.3
Half-life (h)	0.6 ± 0.03	0.5 ± 0.05	5.0 ± 0.1	4.5 ± 0.1
V_{dss} (l/kg)	6.8 ± 0.2	7.2 ± 0.4	–	- -
CL (l/kg/h)	4.2 ± 0.2	5.1 ± 0.3	–	–
T_{max} (h)	–	–	0.8 ± 0.1	1.3 ± 0.3
SN-38				
$AUC_{0-\infty}$ (μg.h/ml or g)	6.9 ± 0.7	7.4 ± 0.4	3.6 ± 0.4	3.2 ± 0.3
C_{max} (μg/ml or g)	1.6 ± 0.1	2.0 ± 0.02	0.32 ± 0.04	0.34 ± 0.4
Half-life (h)	7.4 ± 3.7	4.8 ± 0.6	6.9 ± 0.4	6.7 ± 0.7
T_{max} (h)	≈2	≈2	1.9 ± 1.0	0.4 ± 0.2

[a] Two groups of 33 mice (12 week old male B6D2F$_1$) received CPT-11 (52.5 mg/kg) as an i.v. bolus and were sampled over 24 h (11 time points). The P03 tumors weights were 0.62 ± 0.04 g at the start of therapy.
[b] Mean ± SEM.

est is that infusing the compound over 4 h circumvented the immediate toxicity, indicating that this type of toxicity is probably due to a high initial plasma drug concentration.

The comparison between the different schedules did show that the schedule did not markedly influence the total dosage that could be administered over the same treatment duration. The HNTD varied 1.5-fold between the intermittent (number of injections $n = 2$) and the split dose schedule ($n = 8$) in the case of P388, and 2.3-fold between the intermittent ($n = 2$) and the daily schedule ($n = 4$) in the case of MA16/C. In addition, the infusion trial allowed us to administer a 2.6- and a 1.9-fold higher total dose for P388- and MA16/C-bearing mice, respectively, in comparison with the intermittent schedule. Taking into account the above results, CPT-11 appears to behave in mice more like a schedule category III agent, i.e. a schedule-independent agent causing intolerance at high peak plasma levels.[44] Of interest, this non-schedule dependency observed in mice is in agreement with the results obtained in phase I clinical trials. Using three different schedules, a similar total phase II recommended dose per cycle could be administered, i.e. 350 mg/m^2, once every 3 weeks;[23] 115 mg/m^2 once weekly × 3 (345 mg/m^2 total dose per cycle);[21] 100 mg/m^2 day 1–3, every 3 weeks (300 mg/m^2 total dose per cycle).[20] These observations clearly indicate that CPT-11 is also schedule independent in man, at least in terms of toxicity, in both mouse and man.

In vivo, CPT-11 was further tested against tumors with different biologic properties and chemosensitivities (10 transplantable murine tumors and one human tumor xenograft). It was clear that this compound had broad antitumor activity at maximum tolerated i.v. dosages. The 11 tumors evaluated responded to CPT-11, eight of them being responsive at the Decision Network-2 level (DN-2, i.e. T/C < 10%), which is the level used by the NCI to justify further development.[36] Overall, the most sensitive solid tumors evaluated were the pancreatic ductal adenocarcinoma 03, mammary adenocarcinomas (MA16/C; human Calc18) and the Glasgow osteogenic sarcoma. Using daily schedules, it was possible to obtain cures with early stage P03 tumor and, most importantly, complete regressions of advanced stage disease. In the case of mammary tumors, greater than 3 logs of cell kill could be achieved in mice bearing MA16/C. The other murine mammary tumor evaluated, MA13/C, responded to a lesser extent to CPT-11 but still at the DN-2 level. Of interest the human mammary adenocarcinoma Calc18 did show a good level of sensitivity. CPT-11 was found modestly active against the two colon tumors tested: colon adenocarcinoma 38, one of the *in vitro* sensitive tumors, and colon adenocarcinoma 51. Finally, CPT-11 was active against B16 melanoma and Lewis lung carcinoma. CPT-

11 was also found more active on early stage P388 leukemia than on L1210 leukemia.

Using *in vivo* multidrug-resistant tumors, CPT-11 was found as active against P388 resistant to vincristine, as against the sensitive parental line, but less active against P388 resistant to doxorubicin indicating a minimal recognition by the multidrug phenotype as described previously.[6,8] Interestingly, there was no cross-resistance with docetaxel (Taxotere®), a new anticancer agent that promotes tubulin polymerization and stabilizes microtubules, using a human mammary adenocarcinoma Calc18/TXT xenografted in nude mice, that present the multidrug-resistant phenotype.[38] This information is noteworthy should combination with these agents be performed.

The administration of CPT-11 by the oral route was also investigated. The HNTD for the oral route was three to four times higher than the HNTD for the i.v. route. Clearly CPT-11 retained the level of efficacy that was obtained using the i.v. route. An 80% cure rate of early stage disease was obtained using the pancreatic ductal adenocarcinoma 03 and a 1.9 log cell kill was obtained with early stage mammary adenocarcinoma 16/C. The overall tolerance was similar to that of the i.v. formulation. This information is clearly of importance for administration in humans, if similar absorption is achieved.

It should be noted that an attempt was made to administer SN-38 orally. However, 1.5 g/kg per dose for 5 days in C3H/HeN did not cause any body weight loss. Using a similar strain of mice, a total dosage of 600 mg of CPT-11 was an LD_{40}. These data indicate that the compound was probably poorly or not absorbed (data not shown).

Since pharmacokinetic studies in humans have shown a relatively important inter-patient pharmacokinetic variability,[17–28] it was of interest to determine CPT-11 and SN-38 pharmacokinetics at an efficacious dose in mice, and compare these data to the human situation. The pancreatic ductal adenocarcinoma 03 (P03) was chosen as it was found sensitive to CPT-11 both at an early stage and at an advanced stage, when administered as a daily × 5 schedule. The mouse tumor burden used for these experiments is not irrelevant to the clinical situation, since the mean tumor weight in our experiments (0.6 g) represents about 2% of the body weight, a ratio which could be found in humans with advanced disease, i.e. 1.4 kg total tumoral mass (including metastases) for a 70 kg human. CPT-11 and SN-38 pharmacokinetic parameters were found comparable to previously reported data at similar dose levels with regard to CPT-11 AUC and clearance values.[15] Also of interest was the similar CPT-11 plasma AUCs and maximum concentrations observed in the mouse at the HNTD compared to humans at near toxic dosages.[20,21,23] However, for the maximum concentrations of the metabolite SN-38, the mouse values were about 20 times higher compared with human values,[20,21,23] which indicates that the mouse metabolizes CPT-11 to SN-38 to a much greater extent than in man. A comparison of the pharmacokinetics of the two tumor-bearing groups of mice receiving the drug either as a single i.v. or as a daily × 5 i.v. did not disclose any difference in their pharmacokinetic parameters for either CPT-11 or SN-38. This indicates that there was no accumulation of drug nor enzyme induction within the 5 day treatment. This is similar to humans, where no change in pharmacokinetics was observed after repetitive treatments with this drug.[17–28]

In conclusion, the data presented here confirm and extend that CPT-11 has activity against fresh tumor cells *in vitro*, and against murine and human transplantable tumors when CPT-11 was administered by the i.v. and the oral routes. The *in vitro* solid tumor selectivity suggested that CPT-11 has activity on its own, not only through SN-38. Compared with humans, schedule of administration independence was observed and similar CPT-11 levels could be reached at efficacious dosages, although metabolite SN-38 levels were found to be higher in mice. Finally, absence or limited cross-resistance was found *in vivo* with docetaxel, vincristine and doxorubicin which is important when considering clinical combination trials. Based on these results, the evaluation of CPT-11 and Taxotere® in combination appears worthwhile.

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References

1. Wall ME, Wani MC, Cook CE, *et al.* Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata. J Am Chem Soc* 1966; **88**: 3888–90.

2. Muggia FM, Creaven PJ, Hansen, *et al.* Phase I clinical trial of weekly and daily treatment with camptothecin (NSC 100880): correlation with preclinical studies. *Cancer Chemother Rep* 1972; **56**: 515–21.
3. Kunimoto T, Nitta K, Tanaka T, *et al.* Antitumor activity of 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxy -camptothecin, a novel water-soluble derivative of camptothecin, against murine tumors. *Cancer Res* 1987; **47**: 5944–7.
4. Bissery MC, Mathieu-Boué A, Lavelle F. Experimental antitumor activity of CPT-11 *in vitro* and *in vivo*. *Ann Oncol* 1992; **3**: 82, abstr 93.
5. Kawato Y, Furuta T, Aonuma M, *et al.* Antitumor activity of a camptothecin derivative, CPT-11, against human tumor xenografts in nude mice. *Cancer Chemother Pharmacol* 1991; **28**: 192–8.
6. Houghton PJ, Cheshire PJ, Hallman JC, *et al.* Therapeutic efficacy of the topoisomerase I inhibitor 7-ethyl-10-(4-[1-piperidino]-1-piperidino)-carbonyloxy-camptothecin against human tumor xenografts: lack of cross-resistance *in vivo* in tumors with acquired resistance to the topoisomerase I inhibitor 9-dimethylaminomethyl-10-hydroxy-camptothecin. *Cancer Res* 1993; **53**: 2823–9.
7. Vassal G, Terrier-Lacombe MJ, Bissery MC, *et al.* Therapeutic activity of CPT-11, a DNA-topoisomerase I inhibitor, against peripheral PNET and neuroblastoma xenografts. *Br J Cancer*, 1996; in press.
8. Tsuruo T, Matsuzaki T, Matsushita M, *et al.* Antitumor effect of CPT-11, a new derivative of camptothecin, against pleiotropic drug-resistant tumors *in vitro* and *in vivo*. *Cancer Chemother Pharmacol* 1988; **21**: 71–4.
9. Hsiang YH, Hertzberg R, Hecht S, *et al.* Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J Biol Chem* 1985; **260**: 14873–8.
10. Hsiang YH, Liu LF. Identification of mammalian DNA topoisomerase I as an intracellular target of the anticancer drug camptothecin. *Cancer Res* 1988; **48**: 1722–6.
11. Slichenmyer WJ, Rowinsky EK, Donehower RC, *et al.* The current status of camptothecin analogues as antitumor agents (review). *J Natl Cancer Inst* 1993; **85**: 271–91.
12. Hsiang YH, Liu LF, Wall ME, *et al.* DNA topoisomerase I mediated DNA cleavage and cytotoxicity of camptothecin analogues. *Cancer Res* 1989; **49**: 4835–9.
13. Sugimoto Y, Tsukahara S, Oh-Hara T, *et al.* Decreased expression of DNA topoisomerase I in camptothecin-resistant tumor cell lines as determined by a monoclonal antibody. *Cancer Res* 1990; **50**: 6925–30.
14. Tsuji T, Kaneda N, Kado K, *et al.* CPT-11 converting enzyme from rat serum: purification and some properties. *J Pharmacobiodyn* 1991; **14**: 341–49.
15. Kaneda N, Nagata H, Furuta T, *et al.* Metabolism and pharmacokinetics of the camptothecin analogue CPT-11 in the mouse. *Cancer Res* 1990; **50**: 1715–20.
16. Kawato Y, Aonuma M, Hirota Y, *et al.* Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. *Cancer Res* 1991; **51**: 4187–91.
17. Ohno R, Okada K, Masaoka T, *et al.* An early phase II study of CPT- 11: a new derivative of camptothecin for the treatment of leukemia and lymphoma. *J Clin Oncol* 1990; **8**: 1907–12.
18. Taguchi T, Wakui A, Hasegawa K. Phase I clinical study of CPT-11. Research Group of CPT-11. *Jpn J Cancer Chemother* 1990; **17**: 115– 20.
19. Negoro S, Fukuoka M, Masuda N, *et al.* Phase I study of weekly intravenous infusion of CPT-11, a new derivative of camptothecin, in the treatment of advanced non-small cell lung cancer. *J Natl Cancer Inst* 1991; **83**: 1164–8.
20. Catimel G, Chabot GG, Guastalla JP, *et al.* Phase I and pharmacokinetic study of irinotecan (CPT-11) administered daily for three consecutive days every three weeks in patients with advanced solid tumors. *Ann Oncol* 1995; **6**: 133–40.
21. de Forni M, Bugat R, Chabot GG, *et al.* Phase I and pharmacokinetic study of the camptothecin derivative Irinotecan administered on a weekly schedule in cancer patients. *Cancer Res* 1994; **54**: 4347–54.
22. Fukuoka M, Miitani H, Suzuki A, *et al.* A Phase II study of CPT-11, a new derivative of camptothecin, for previously untreated non-small cell lung cancer. *J Clin Oncol* 1992; **10**: 16–20.
23. Abigerges D, Chabot GG, Armand JP, *et al.* Phase I and pharmacologic studies of the camptothecin analogue irinotecan (CPT-11) administered every three weeks in cancer patient. *J Clin Oncol* 1995; **13**: 210–21.
24. Ohe Y, Sasaki Y, Shinkai T, *et al.* Phase I study and pharmacokinetics of CPT-11 with 5-day continuous infusion. *J Natl Cancer Inst* 1992; **84**: 972–4.
25. Shimada Y, Yoshino M, Wakui A, *et al.* Phase II study of CPT-11, a new camptothecin derivative in metastatic colorectal cancer. *J Clin Oncol* 1993; **11**: 909–13.
26. Rothenberg ML, Kuhn JG, Burris III HA, *et al.* Phase I and pharmacokinetic trial of weekly CPT-11. *J Clin Oncol* 1993; **11**: 2194–204.
27. Rowinsky EK, Grochow L, Ettinger DS, *et al.* Phase I and pharmacological study of the novel topoisomerase I inhibitor 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxy-camptothecin (CPT-11) administered as a ninety-minute infusion every 3 weeks. *Cancer Res* 1994; **54**: 427– 36.
28. Chabot GG, Abigerges D, Catimel G, *et al.* Population pharmacokinetics and pharmacodynamics of irinotecan (CPT-11) and active metabolite SN-38 during phase I trials. *Ann Oncol* 1995; **6**: 141–51.
29. Lorusso P, Wozniak AJ, Polin L, *et al.* Antitumor efficacy of PD115934 (NSC 366140) against solid tumors of mice. *Cancer Res* 1990; **50**: 4900–5.
30. Corbett TH, Griswold DP Jr, Roberts BJ, *et al.* Evaluation of single agents and combinations of chemotherapeutic agents in mouse colon carcinomas. *Cancer* 1977; **40**: 2660–80.
31. Corbett TH, Roberts BJ, Leopold WR, *et al.* Induction and chemotherapeutic response of two transplantable ductal adenocarcinomas of the pancreas in C57Bl/6 mice. *Cancer Res* 1984; **44**: 717–26.
32. Corbett TH, Griswold DP Jr, Roberts BJ, *et al.* Biology and therapeutic response of a mouse mammary adenocarcinoma (16/C) and its potential as a model for surgical adjuvant chemotherapy. *Cancer Treat Rep* 1978; **62**: 1471–99.
33. Gioanni J, Courdi A, Lalanne CM, *et al.* Establishment, characterization, chemosensitivity and radiosensitivity of two different cell lines derived from a human breast cancer biopsy. *Cancer Res* 1985; **45**: 1246–58.
34. Glasgow LA, Crane JL Jr, Kern ER. Antitumor activity of interferon against murine osteogenic sarcoma cells *in*

vitro. J Natl Cancer Inst 1978; **60**: 659–63.
35. Mayo JG. Biologic characterization of the subcutaneously implanted Lewis lung tumor. *Cancer Chemother Rep* 1972; **3**: 325–30.
36. Geran RI, Greenberg NH, Macdonald MM, *et al.* Protocols for screening chemical agents and natural products against animal tumors and other biological systems (third edition). *Cancer Chemother Rep* 1972; **3**: 1-103.
37. Schabel FM Jr, Skipper HE, Trader MW, *et al.* Establishment of cross-resistance profiles for new agents. *Cancer Treat Rep* 1983; **67**: 905–22.
38. Riou JF, Petitgenet O, Aynié I, Lavelle F. Establishment and characterization of docetaxel (Taxotere®) resistant human breast carcinoma (Calc18/TXT) and murine leukemic (P388/TXT) cell lines (Abstract). *Proc Am Ass Cancer Res* 1994, **35**: 339.
39. Hamburger AW, Salmon SE. Primary bioassay of human tumor stem cells. *Science* 1977; **197**: 461–3.
40. Salmon SE, Hamburger AW, Soehnlen B, *et al.* Quantitation of differential sensitivity of human-tumor stem cells to anticancer drugs. *New Eng J Med* 1978; **298**: 1321–7.
41. Alberts DS, Salmon SE, Chen HSG, *et al.* Pharmacologic studies of anticancer drugs with the human tumor stem cell assay. *Cancer Chemother Pharmacol* 1981; **6**: 253–64.
42. Corbett TH, Wozniak A, Gerpheide S, Hanka L. A selective two-tumor soft agar assay for drug discovery In: White E, ed. In vitro *and* in vivo *models for detection of new antitumor drugs (14th International Congress of Chemotherapy).* Tokyo: University of Tokyo Press 1985: 5–14.
43. Corbett TH, Roberts BJ, Trader MW, *et al.* Response of transplantable tumors of mice to anthracenedione derivatives alone and in combination with clinically useful agents. *Cancer Treat Rep* 1982; **66**: 1187–200.
44. Corbett TH, Leopold WR, Dykes DJ, *et al.* Toxicity and anticancer activity of a new triazine antifolate (NSC 127755). *Cancer Res* 1982; **42**: 1701–15.
45. Slichenmyer WJ, Rowinski EK, Donehower RC, Kaufmann SH. The current status of camptothecin analogues as antitumor agents. *J Natl Cancer Inst* 1993; **85**: 271–91.
46. Barilero I, Gandia D, Armand JP, *et al.* Simultaneous determination of the camptothecin analogue CPT-11 and its active metabolite SN-38 by high-performance liquid chromatography—application to plasma pharmacokinetic studies in cancer patients. *J Chromatogr (Biomed Appl)* 1992; **575**: 275–80.
47. Gibaldi M, Perrier D. *Pharmacokinetics*, 2nd edn. New York: Marcel Dekker 1992.
48. Shimada Y, Rothenberg M, Hilsenbeck SG, Burris III HA, Degen D, Von Hoff DD. Activity of CPT-11 (irinotecan hydrochloride), a topoisomerase I inhibitor, against human tumor colony-forming units. *Anti-Cancer Drugs* 1994; **5**: 202–6.

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