DTS-108, A Novel Peptidic Prodrug of SN38: *In vivo* Efficacy and Toxicokinetic Studies

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Abstract

Purpose: Irinotecan is a prodrug converted to the active cytotoxic molecule SN38 predominantly by the action of liver carboxylesterases. The efficacy of irinotecan is limited by this hepatic activation that results in a low conversion rate, high interpatient variability, and dose-limiting gastrointestinal toxicity. The purpose of this study was to evaluate a novel peptidic prodrug of SN38 (DTS-108) developed to bypass this hepatic activation and thus reduce the gastrointestinal toxicity and interpatient variability compared with irinotecan.

Experimental Design: SN38 was conjugated to a cationic peptide (Vectocell) via an esterase cleavable linker. The preclinical development plan consisted of toxicity and efficacy evaluation in a number of different models and species.

Results: The conjugate (DTS-108) is highly soluble, with a human plasma half-life of 400 minutes *in vitro*. Studies in the dog showed that DTS-108 liberates significantly higher levels of free SN38 than irinotecan without causing gastrointestinal toxicity. In addition, the ratio of the inactive SN38-glucuronide metabolite compared with the active SN38 metabolite is significantly lower following DTS-108 administration, compared with irinotecan, which is consistent with reduced hepatic metabolism. *In vivo* efficacy studies showed that DTS-108 has improved activity compared with irinotecan. A significant dose-dependent antitumoral efficacy was observed in all models tested and DTS-108 showed synergistic effects in combination with other clinically relevant therapeutic agents.

Conclusions: DTS-108 is able to deliver significantly higher levels of SN38 than irinotecan, without the associated toxicity of irinotecan, resulting in an increased therapeutic window for DTS-108 in preclinical models. These encouraging data merit further preclinical and clinical investigation.

Irinotecan is an effective chemotherapeutic agent that is widely prescribed for advanced colorectal cancer as a first- or second-line treatment. Irinotecan has also been shown to be active in gastric cancer, non–small cell lung cancer, and small-cell lung cancer, alone or in combination with other cytotoxic agents (1). Currently, irinotecan is used in combination with 5-fluorouracil (5-FU) in first-line treatment for metastatic colorectal cancer (2). Irinotecan is also used with other agents, including the anti–vascular endothelial growth factor antibody bevacizumab (Avastin; refs. 3, 4). Following administration of irinotecan, the active metabolite SN38 (7-ethyl-10-hydroxycamptothecin) is formed by the action of carboxylesterases that are predominantly present in the liver (5, 6). SN38 is a topoisomerase I inhibitor with an activity a thousand times greater than irinotecan, but that cannot be administered directly as it is highly insoluble (1).

Several limitations to the clinical use of irinotecan arise due to its mechanism of activation, metabolism, and elimination. The first limitation is caused by the complexity of irinotecan metabolism, which results in high interpatient variability in both efficacy and toxicity, with only 2% to 8% of the administered dose of irinotecan converted into SN38 in man (7). Three potential irinotecan-activating carboxylesterases have been identified (CES1, CES2, and CES3) of which CES2 shows the greatest affinity for irinotecan (8). Studies have shown that genetic and environmental factors influence carboxylesterase enzyme activity by up to 10-fold (9). The detoxification of SN38 to the inactive SN38-glucuronide (SN38-G) by hepatic UDP-glucuronosyltransferases is also complex and variable. Seventeen human UDP-glucuronosyltransferases have been identified, the majority of which exhibits polymorphisms that could affect their activity (10). In addition, cytochrome CYP3A4–dependent irinotecan metabolism results in the formation of the inactive oxidation products 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]-carbonyloxy camptothecin and 7-ethyl-10-[4-amino-1-piperidino]-carbonyloxy

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camptothecin (NPC), from which NPC can be further converted to SN38 by carboxylesterase (6).

The second limitation of irinotecan is the emergence of delayed, severe, and potentially life-threatening diarrhea, due to an accumulation of SN38 in the intestine resulting from bile excretion of the parent molecule and its metabolites. Once in the intestine, SN38 is formed either from irinotecan by intestinal carboxylesterase or from SN38-G by β-glucuronidase produced by intestinal bacteria (11, 12). Such late-onset grade 3 to 4 diarrhea is observed in 30% to 40% of treated patients (1, 13, 14). It is also a major limitation because the dose has to be reduced for further treatment cycles, and dose intensification of irinotecan in colon cancer patients is known to improve the antitumoral response (15–17).

A significant clinical advantage could therefore be gained from the direct administration of the active metabolite, SN38, using a drug delivery technology that is not reliant on hepatic activation and metabolism. This would allow higher plasma and tissular levels of SN38 with a consequential increase in antitumoral efficacy while limiting the gastrointestinal toxicity and interpatient variability. Based on this rationale, a novel water-soluble conjugate (DTS-108) was generated by linking SN38 to a highly charged oligopeptide of human origin (Vectocell Technology; refs. 18, 19). This technology has already been successfully used to enhance both the *in vitro* and *in vivo* efficacy of doxorubicin (20) by altering its physicochemical, pharmacokinetic, and cellular delivery properties, with no evidence of additional toxicity.

This report describes the application of the Vectocell technology to SN38. Solubility, stability, and *in vitro* cytotoxicity assays have been undertaken to evaluate this novel SN38-peptide (DTS-108) conjugate. Toxicokinetic studies in the dog were done to quantify the potential benefit of DTS-108 administration, whereas studies in both mice and rats were used to evaluate the antitumoral efficacy of this novel conjugate. Results show that conjugation of SN38 to a cationic peptide renders it soluble for i.v. administration in purely aqueous vehicles at high dose levels. DTS-108 permits significantly higher plasma levels of the active drug SN38 to be reached with consequential improved efficacy without increasing gastrointestinal toxicity compared with irinotecan administration.

Materials and Methods

Drugs. Irinotecan (677 g/mol) was obtained as a ready-to-use clinical formulation at 20 mg/mL (Campto, Pfizer). Clinical grade 5-FU (Fluorouracil, Teva, Pharma, 130 g/mol), at 50 mg/mL, and bevacizumab (Avastin, Roche, 149 kDa at 25 mg/mL) were used in *in vivo* combination studies. These reference drugs as well as DTS-108 (3170 g/mol) were diluted in sterile 0.9% NaCl (w/v) for *in vivo* administration. SN38 (392.3 g/mol), purchased from Abatra Technology, was first dissolved in DMSO and then diluted in culture medium for *in vitro* cytotoxicity assays. SN38-G was synthesized from SN38 using the UDP-glucuronosyltransferase UGT1A1 (Sigma) and the method described by Jinno et al. (21). In Results, doses and concentrations are given in milligrams or nanograms. To determine the amount of equivalent SN38 between DTS-108 and irinotecan, the molar ratio DTS-108/irinotecan (4.7) should be taken into account.

Synthesis of DTS-108. The 20-amino-acid DPV1047 Vectocell peptide (CVKRGLKLRHVRPRVTRMDV) was synthesized as a trifluoroacetate salt by NeoMPS.

The 4-{4-[(*N*-maleimydomethyl)cyclohexanecarboxamido]methyl} cyclohexane-1-carboxylic acid linker (BCH) was synthesized as follows: succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (11 g, SMCC; Pierce Biotechnology) was dissolved in 220 mL of acetonitrile and 55 mL of water. *Trans*-4-(aminomethyl)cyclohexane-1-carboxylic acid (16.7 g, Sigma-Aldrich) was dissolved in 125 mL of water and 20 mL of *N*,*N*′-diisopropylethylamine (DIPEA; Sigma-Aldrich) and added to the SMCC solution. The reaction mixture was stirred at ambient temperature for 1 h before the addition of dichloromethane (170 mL, SDS) and methanol (50 mL, SDS). The organic layer containing BCH was then collected and dried by rotary evaporation. The crude BCH linker was dissolved in 60 mL of dichloromethane and 30 mL of methanol and added slowly to methyl *tert*-butyl ether (400 mL, SDS) at 0°C. The resulting white precipitate (BCH) was filtered and dried under vacuum.

Five grams of SN38 (Abatra Technology) were added to BCH (5.8 g) and *O*-(1*H*-6-chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium (3.1 g, SDS), previously dissolved in *N*-methyl-2-pyrrolydone (50 mL, Sigma-Aldrich), followed by DIPEA (5.4 mL). The mixture was stirred for 4 h at 0°C and was then taken up in 125 mL of dichloromethane. The organic layer was washed thrice with 130 mL of 1 mol/L NaCl and thrice with 130 mL of 5% (w/v) aqueous citric acid and was then added slowly to 300 mL of methyl *tert*-butyl ether at 0°C. The yellow precipitate (10-*O*-BCH-SN38) was recovered by filtration and dried under vacuum.

Five grams of 10-*O*-BCH-SN38 were added to a solution containing DPV1047 (21.5 g) in 200 mL of *N*,*N*-dimethylformamide (Sigma-Aldrich) and stirred for 3 h at room temperature. Water (200 mL) was added and the aqueous layer was extracted thrice with 200 mL of dichloromethane and freeze dried.

DTS-108 hydrochloride (DTS-108·HCl) was generated by ion exchange chromatography of DTS-108·trifluoroacetate using Amberlite IRA-410 resin (Sigma-Aldrich) conditioned with 2 N HCl. Product-containing fractions were pooled, frozen, and lyophilized (typical purity/potency ≈95%).

Solubility studies. Solubility studies were performed as previously described in the Organisation for Economic Cooperation and Development Guidelines for testing chemicals (22). The solubility of irinotecan, SN38, and DTS-108 was measured in water at 20°C. Solubility was initially evaluated by visual assessment at 50% (w/v); the different molecules were allowed to dissolve for 5 min with agitation. The quantity of water was then increased in increments of 5% (v/v) until the compound was completely dissolved.

Stability studies. The stability of the conjugates was tested in human, male beagle dog, and male CD1 mouse plasma. Mouse, dog, and human plasma were purchased from Charles River Laboratories and from EFS, respectively. DTS-108 (2.55 μmol/L) or irinotecan (2.55 μmol/L) were incubated at 37°C in citrated plasma and samples were harvested at 0, 3, 6, 20, 60, 180, and 360 min and at 24 and 48 h followed by analysis using the high-performance liquid chromatography fluorescence method described below (see Chromatographic equipment and conditions) following trifluoroacetate/methanol deproteination.

In vitro *cytotoxicity.* Viability assays were performed with DTS-108 or irinotecan on five human cancer cell lines treated with drugs for 48 h using the WST-1 assay (Roche). Three colon adenocarcinomas, HCT 116, HT-29, LS 174T; a lung adenocarcinoma, NCI-H460; and a mammary adenocarcinoma, MDA-MB-231 (all from American Type Culture Collection) were used in this study. Molar concentrations that inhibit 50% of cell viability (IC_{50} values) were calculated from sigmoid regressions, using the Graph Pad Prism 3.02 software.

Preliminary toxicity and toxicokinetic studies in the dog. Adult beagle dogs (1 dog for each drug and dose) were infused i.v. through the cephalic or saphenous vein. DTS-108 was administered at 5, 10, or 20 mg/kg as a 45-min infusion (0.4 mL/min). Irinotecan was injected at its maximum tolerated dose (MTD), that is, 30 mg/kg (23) as a 20-min infusion (0.4 mL/min). Clinical signs and hematologic toxicities were monitored for 15 days after infusion.

The body weight of each animal was recorded on days -1, 4, 10, and 13. Peripheral blood was sampled using EDTA tubes (Sarstedt) on days -1, 5, 11, and 14 for hematologic analyses. In addition, peripheral blood samples were taken at different times (up to 8 h) following infusion for pharmacokinetic analysis using 5 mL trisodium citrate tubes (Sarstedt S-Monovette 9NC) containing 2.5 mL of 5% trifluoroacetate (v/v). The area under the blood concentraion versus time curve (AUC) of the different compounds and their metabolites was measured up to the limit of quantification (7.8 ng/mL equivalent SN38). For irinotecan, blood was sampled at time 0; at the end of the infusion (20 min); and 10, 20, 40, 220, and 460 min after infusion. For DTS-108, blood was sampled at time 0; at the end of the infusion (45 min); and 10, 20, 40, 120, and 220 min after infusion. AUC values were calculated using the trapezoid summation rule from zero until the last sampling time point or until the limit of quantification was reached.

In vivo *efficacy studies in human tumor xenograft models.* All *in vivo* studies were performed in accordance with the guidelines set out by the European Economic Union and the United Kingdom's Co-ordinating Committee on Cancer Research (2nd edition; ref. 24). Human colon, lung, and mammary tumors were established by an intradermal or s.c. implantation of cells (1×10^7 HCT 116, 1×10^7 HT-29, 2×10^7 LS 174T, 3×10^6 NCI-H460, and 3×10^6 MDA-MB-231 cells injected) in the right flank of 7-wk-old female NMRI nude mice, or, for the LS 174T model, in Rh *rnu/rnu* nude rats (Harlan). Treatments were initiated when the tumors reached a size of ~100 mm^3 in mice or 1,000 mm^3 in rats [calculated using the following formula: (length × $width^2$) / 2]. The day of the first injection animals were randomized into different groups (6 or 8 animals per group) and were treated by bolus i.v. injection (in the lateral tail vein) or i.p. injection (only for bevacizumab) of the different drugs dissolved in saline in a volume of 10 μL/g following the indicated administration schedule. During and following treatment, clinical signs, body weight, and tumor size were recorded regularly to evaluate the efficacy and toxicity of the injected drugs. The ratio (T/C, %) of the mean tumor volumes of treated (T) versus control (C) groups was used to evaluate treatment efficacy. The minimal T/C reflects the maximal tumor growth inhibition achieved.

For combination studies, suboptimal doses of DTS-108, 5-FU, and bevacizumab were administered (25, 26). The T/C ratio was calculated for each individual compound (bevacizumab, 5-FU, or DTS-108). The combination index of each pair of drugs was calculated by dividing the expected T/C (T/C of DTS-108 × T/C of bevacizumab or 5-FU) by the observed T/C. An index >1 shows a synergistic effect; an index of ~1 indicates an additive effect; and an index <1 indicates an antagonistic effect (27).

Chromatographic equipment and conditions. Blood samples (500 μL) were immediately diluted using an equal volume of trifluoroacetate 2.5% (v/v) to stabilize DTS-108 and prevent further cleavage of the conjugate. Each sample was placed in a 2 mL microcentrifuge tube and centrifuged at 16,000 × *g* for 3 min at +4°C. Twenty μL of a 1.12 μg/mL freshly prepared camptothecin (Sigma) solution (internal standard, +4°C) were added to 100 μL of the supernatant. Methanol (100 μL) was then immediately added and the mixture was vortexed for 5 s. Samples were then centrifuged at 16,000 × *g*, for 3 min at room temperature. The supernatant (150 μL) was recovered for high-performance liquid chromatography analysis. Drugs and metabolites were separated by high-performance liquid chromatography (Agilent 1100 series equipped with a fluorescence detector) using a 4.6 × 100 mm (3 μm particle size) Luna C18(2) column (Phenomenex ref. 00D-4251-E0). The aqueous component of the mobile phase (A) was 0.1% (v/v) trifluoroacetate in water. The organic modifier (B) was acetonitrile containing 0.1% (v/v) trifluoroacetate. For DTS-108 and its metabolites, an elution gradient was applied with a constant flow rate of 1.2 mL/min, increasing linearly the proportion of B from 15% to 37% in 2 min, from 37% to 47% in 6 min, followed by 2 min at 90%. The proportion of B was then returned to the initial condition for 3 min. For irinotecan and its metabolites, an elution gradient was applied with a constant flow rate at 1.2 mL/min, increasing linearly the proportion of B from 15% to 50% in 10 min, from 50% to 90% in 0.5 min, followed by 2 min at 90%. The proportion of B was then returned to the initial condition for 3 min. Fluorescence detection ($\lambda_{exc.}$, 375 nm; $\lambda_{em.}$, 560 nm) was used, and drugs and metabolites were identified according to their relative retention times as determined from a set of standards ($Rt_{DTS\text{-}108}$ = 4.7 min; Rt_{SN38} = 5.3 min; $Rt_{internal\ standard\ camptothecin}$ = 5.7 min).

The method was validated in terms of specificity, linearity, imprecision/inaccuracy, carryover, recovery, dilution effect, and stability (with a lower limit of quantification of 7.8 ng/mL equivalent SN38 and an upper limit of quantification of 1,000 ng/mL equivalent SN38). Extraction recoveries of 67 ± 10% were obtained for DTS-108 and SN38.

Results

Peptide-SN38 conjugation, solubility and* in vitro *stability. SN38 is an efficient antitumoral agent. However, it is highly insoluble and therefore cannot be administered in pharmaceutically acceptable vehicles. The DPV1047 peptide was chemically conjugated to the 10-hydroxyl group of SN38 via a heterobifunctional cross-linker (BCH) with an ester bond (Fig. 1A). The solubility of the SN38 conjugate DTS-108 in water (>500 mg/mL; 317.4 mmol/L) was found to be much greater than that of either irinotecan (<2.5 mg/mL; 3.7 mmol/L) or SN38 (<5 μg/mL; 0.013 mmol/L).

The BCH cross-linker was specifically designed for optimal stability of the conjugate in plasma, allowing SN38 release upon cleavage of the ester bond (28). This is contrary to irinotecan activation that requires hepatic carboxylesterase-mediated hydrolysis of a carbamate bond (5, 6). The *in vitro* stability of DTS-108 is species dependent, being superior in human and dog plasma (with half lives of 400 and 290 minutes, respectively) than in mouse plasma (where the half

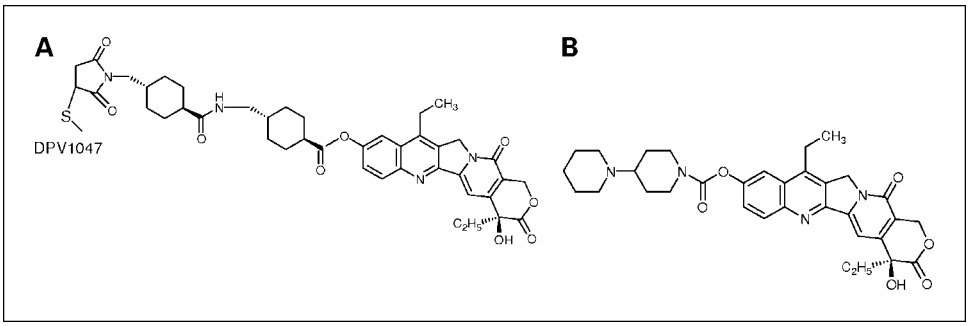


Fig. 1. The structure of DTS-108 in comparison with irinotecan. DTS-108 (*A*) is a Vectocell peptide – SN38 prodrug generated by chemical coupling of the 10-hydroxyl group of SN38 via a heterobifunctional cross-linker (BCH) to the Vectocell peptide DPV1047 (CVKRGLKLR-HVRPRVTRMDV). This linker results in the generation of an ester bond between the peptide and SN38. Irinotecan (*B*) is also an SN38 prodrug with a dipiperidine moiety coupled to the 10-hydroxyl group of SN38. For irinotecan, SN38 is linked via a carbamate bond to the dipiperidine moiety.

life is <3 minutes). In contrast, irinotecan is highly stable in human and dog plasma, showing only minimal release of SN38 (with a half life of >4,300 minutes in both human and dog plasma compared with 20 minutes in mouse plasma).

In vitro *cytotoxicity of DTS-108.* *In vitro* cytotoxicity experiments were performed to test whether the intact DTS-108 or its metabolites showed cytotoxic activity that was independent of the liver-specific activation of irinotecan. DTS-108 was shown to be cytotoxic *in vitro* in a panel of colon (HCT 116, HT-29, and LS 174T), lung (NCI-H460), and breast (MDA-MB-231) cancer cell lines after a 48-hour incubation. In these cell lines, the DTS-108 IC_{50} was 24, 94, 2, 40, and 834 nmol/L, respectively. In contrast, the prodrug irinotecan showed a very low cytotoxic activity (IC_{50} of 6, 50, 35, 11, and 85 μmol/L, respectively) as previously documented (29), with a 2 to 4 log decrease in activity compared with DTS-108.

Preliminary toxicity and toxicokinetic studies in the dog. The dog was selected as the most appropriate model for preliminary toxicity and toxicokinetic studies for DTS-108 because DTS-108 stability in dog plasma is similar to that observed in human plasma, and because, contrary to rodents, the dog is a relevant model that mirrors the human pharmacokinetics of irinotecan and the typical early- and late-stage diarrhea observed in man (30).

DTS-108 was administered at three doses (5, 10, and 20 mg/kg). No clinical signs of toxicity were observed at either 5 or 10 mg/kg, except for a moderate but non–dose-dependent decrease in WBC counts (Table 1). Administration of DTS-108 at 20 mg/kg induced toxicity, including a significant decrease in WBC counts (-93%), together with a decrease in food intake and late-stage diarrhea, suggesting that the MTD had been reached (Table 1). No early-stage diarrhea was observed following DTS-108 administration. Irinotecan was administered at its MTD (30 mg/kg), which resulted in the classic clinical signs (23), including early-stage (day 1) and late-stage (days 4-6) diarrhea, body weight loss, emesis, and a severe but reversible leucopenia on day 5 (Table 1).

Typical blood concentration versus time curves following DTS-108 and irinotecan administration in the dog are shown in Fig. 2. In both cases, the parent compound, SN38 and the glucuronoconjugate SN38-G were observed. The C_{max} of DTS-108 is observed, as for irinotecan, at the end of the infusion. DTS-108 concentration then decreases much more rapidly than that of irinotecan. SN38 concentrations were significantly lower after irinotecan administration than after DTS-108. After treatment with DTS-108, the AUC of DTS-108 and SN38 increased in a dose-dependent manner in the tested range (5-20 mg/kg of DTS-108; Table 1). At the lowest dose of DTS-108 tested (5 mg/kg), the SN38 AUC was 920 ng × h/mL, whereas at its MTD (20 mg/kg) the SN38 AUC reached 4,756 ng × h/mL. Following irinotecan administration at its MTD, the AUC of SN38 was 18 ng × h/mL. This result is consistent with the low level of irinotecan reactivation observed previously in the dog (30). At all three doses of DTS-108, a significantly higher SN38 AUC than that observed following administration of irinotecan at its MTD was therefore achieved.

The AUC of the inactive metabolite SN38-G was also determined. Following DTS-108 (20 mg/kg) or irinotecan (30 mg/kg) administration, it was 1083 and 432 ng × h/mL, respectively. The ratio of the AUCs of SN38-G versus SN38 were therefore 0.23 for DTS-108 and 24 for irinotecan, suggesting a dramatic reduction in glucuronidation with the DTS-108 peptidic prodrug.

In vivo *efficacy of DTS-108 in human tumor xenograft models.* The *in vivo* antitumoral efficacy of DTS-108 was first characterized in mice bearing HCT 116 human colorectal tumors (Fig. 3A). DTS-108 showed a significant dose-dependent effect in this model with transient tumor regressions and a minimal T/C ratio of 10% at the highest, well-tolerated dose level tested (95 mg/kg). Comparative studies between DTS-108 and irinotecan were also done, despite the fact that the activity of the latter is significantly overestimated in rodents compared with man due to its much higher conversion rate and the resulting increased exposure to SN38 (31, 32). Compared with irinotecan (Fig. 3B), an equimolar dose of DTS-108 results in improved antitumoral activity on HCT 116 tumors with a mean tumor volume of 813 ± 405 mm^3 for the group treated with DTS-108 compared with 1,228 ± 247 mm^3 for the group treated with irinotecan 28 days after treatment termination, although statistical significance was not reached.

DTS-108 was then evaluated in a panel of human tumor models including colon (HCT 116), lung (NCI-H460), and

Table 1. Clinical and toxicokinetic comparison of DTS-108 and irinotecan following i.v. infusion in the dog

Compound	Dose (mg/kg)	C_{max} (ng eq SN38)		AUC (ng eq SN38 × h/mL)			Clinical signs
		Prodrug	SN38	Prodrug	SN38	SN38-G	
Irinotecan	30	8,947	36	36,320	18	432	BWL (-6%) Strong reduction in WBC counts (-92%) Diarrhea (early and delayed) and vomiting
DTS-108	5	1,309	377	1,739	920	ND	No BWL Moderate reduction in WBC counts (-60%) No intestinal toxicity, diarrhea, or vomiting
	10	2,632	585	2,933	1,420	ND	No BWL Moderate reduction in WBC counts (-57%) No intestinal toxicity, diarrhea, or vomiting
	20	3,302	1,490	3,893	4,756	1083	No BWL Strong reduction in WBC counts (-93%) Diarrhea (delayed) and vomiting

Abbreviations: BWL, body weight loss; ND, not detected, below the limit of quantification; WBC, white blood cells.

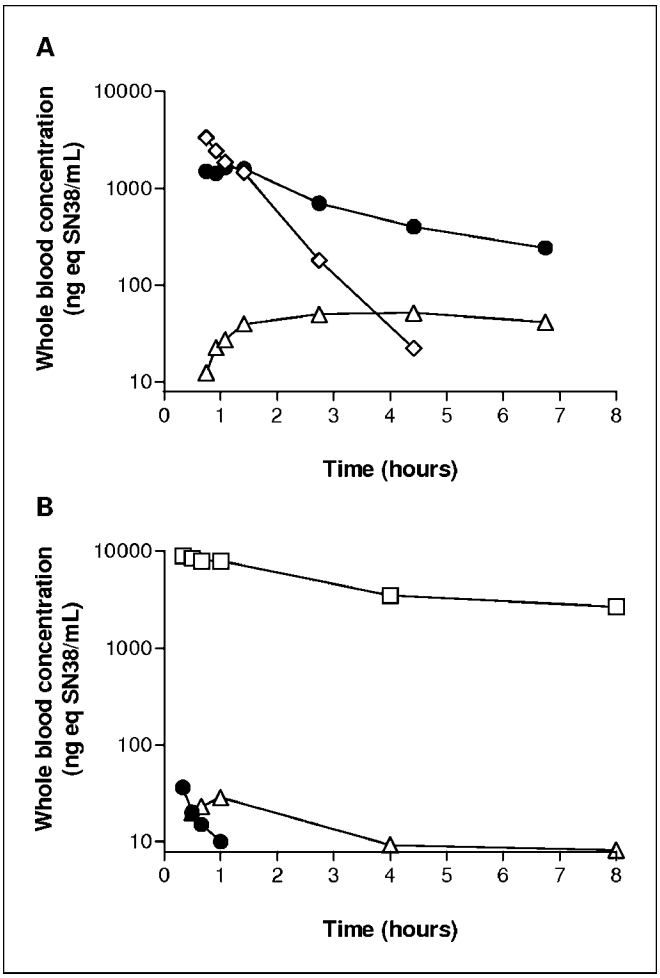


Fig. 2. Blood concentration versus time curves following DTS-108 (*A*, 20 mg/kg) or irinotecan (*B*, 30 mg/kg) administration in the dog. The concentration of the parent compounds and the major metabolites, SN38 and SN38-G, were evaluated. DTS-108 (◇), irinotecan (□), SN38 (●), and SN38-G (△).

breast (MDA-MB-231) carcinomas implanted into mice, using another injection schedule. These experiments confirmed that DTS-108 is extremely active with minimal T/C ratios of 3%, 23%, and 29% in the HCT 116, NCI-H460, and MDA-MB-231 tumor models, respectively (Fig. 4A-C). Significant antitumoral efficacy (with a minimal T/C ratio of 44%) was also observed in rats bearing LS 174T colon tumors (Fig. 4D).

In preparation for future preclinical and clinical development, the trifluoroacetate salt was exchanged for HCl and no difference in efficacy or solubility was observed between the two salt forms of DTS-108. *In vivo* efficacy studies using the NCI-H460 lung adenocarcinoma model confirmed that DTS-108·HCl has a similar activity with an *in vivo* minimal T/C of 18% compared with 23% for DTS-108·trifluoroacetate.

In vivo studies were performed using mice bearing human HT-29 colon carcinomas to evaluate whether synergistic or additive effects are observed when DTS-108 is combined with the cytotoxic agent 5-FU or the anti–vascular endothelial growth factor antibody bevacizumab. The results (Fig. 5) show that DTS-108 alone is active in this model (even at the suboptimal dose tested) and that increased antitumoral efficacy is observed in combination with either 5-FU or bevacizumab. For 5-FU, the combination effect was synergistic (combination index >1), whereas for bevacizumab the combination effect was additive (combination index is ~1).

Discussion

Irinotecan is a widely prescribed and effective antitumoral agent for the treatment of colorectal cancer. It is a prodrug of SN38 that was designed to improve the pharmaceutical properties, particularly water solubility, of this potent topoisomerase I inhibitor. However, the irinotecan therapeutic index in humans is much lower than expected based on preclinical rodent studies and is subject to significant interpatient

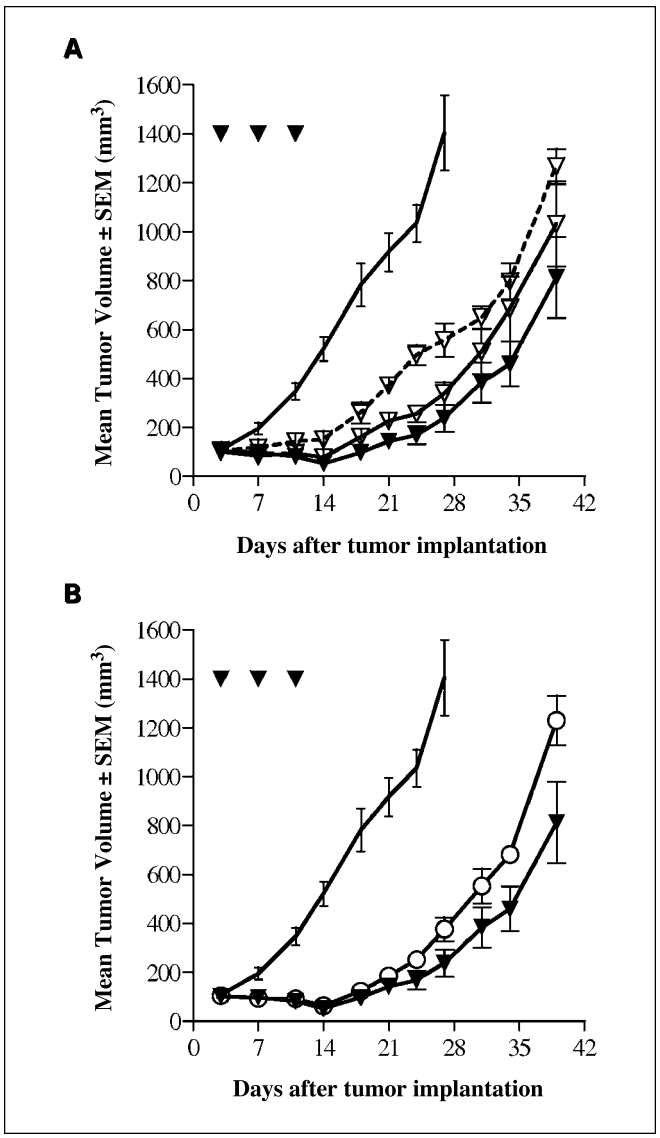


Fig. 3. Comparative *in vivo* efficacy study of DTS-108 in the human HCT 116 colorectal carcinoma model implanted intradermally into nude mice. Treatments were administered by the i.v. injection route on days 3, 7, and 11 (*arrowheads*). *A,* mice received either saline (—) or DTS-108 at 32 mg/kg (- -▽- -), 62 mg/kg (–▽–), or 95 mg/kg (–▼–). *B,* mice received either saline (—), DTS-108 (–▼–) at 95 mg/kg or irinotecan (–○–) at 20 mg/kg per injection (an equimolar dose of 30 μmol/kg). Points, mean tumor volume evolution; bars, SEM.

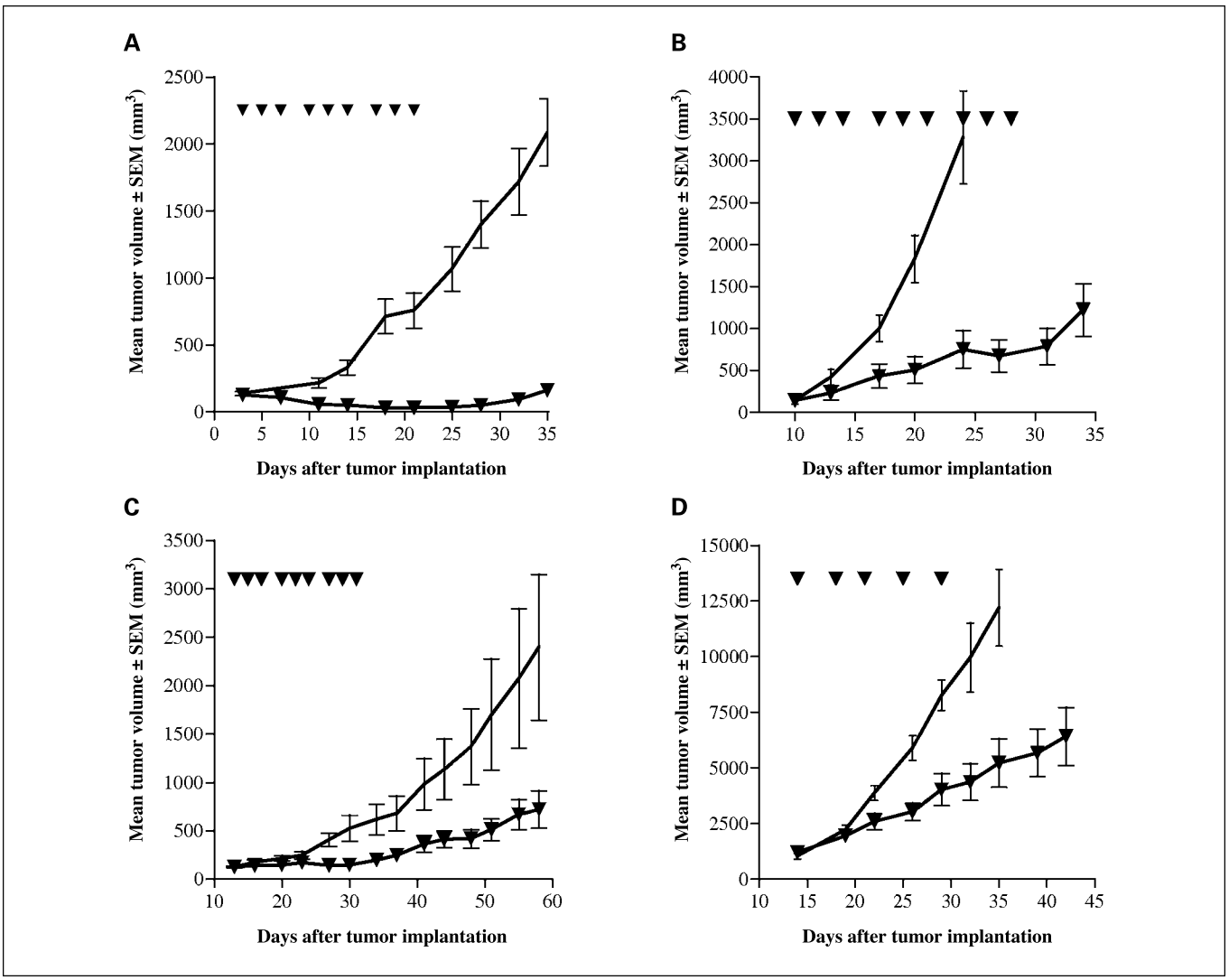


Fig. 4. *In vivo* efficacy study of DTS-108 in human HCT 116 colorectal (*A*), NCI-H460 lung (*B*), MDA-MB-231 mammary (*C*), and LS 174T colorectal (*D*) carcinomas implanted into nude mice (*A-C*) or rats (*D*). Treatments were administered by i.v. injection on days 3, 5, 7, 10, 12, 14, 17, 19, and 21 (*arrowheads*) in mice or on days 14, 18, 21, 25, and 29 (*arrowheads*) in rats. Animals received either saline (—) or DTS-108 (–▼–) at 80 mg/kg in mice or 63 mg/kg in the rat. Points, mean tumor volume; bars, SEM.

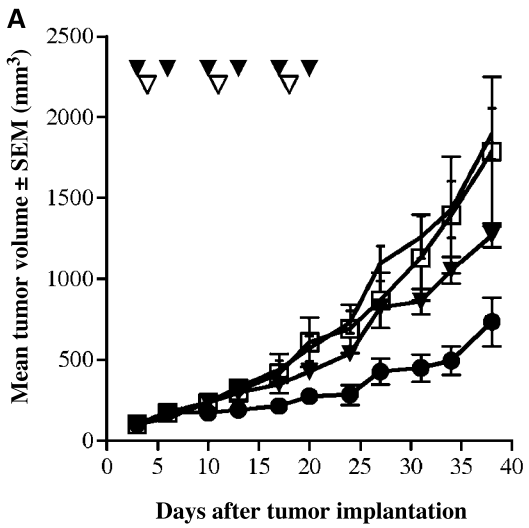
variability. This is mainly due to significant interspecies differences in its metabolism.

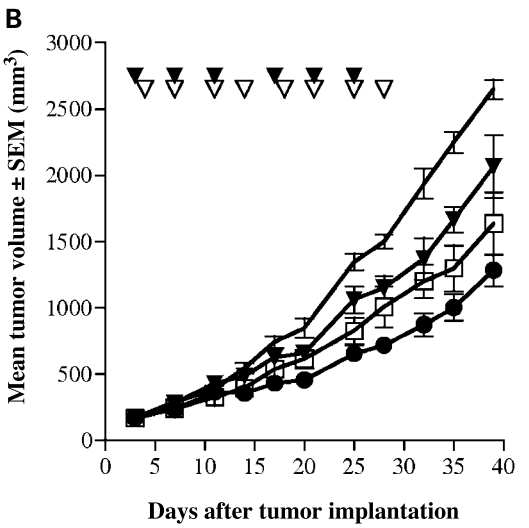
In humans, the metabolism of irinotecan is extremely complex and predominantly mediated by hepatic specific enzymes, active transport proteins regulating intestinal absorption, and hepatobiliary secretion mechanisms. Irinotecan is converted to SN38 by hepatic carboxylesterase-specific cleavage of a carbamate bond between the 10-hydroxyl group of SN38 and the dipiperidine promoiety (7). This is in contrast to what is observed in rodents in which high levels of tissue and circulating esterases other than carboxylesterase convert irinotecan into SN38 to a much greater extent (32). Furthermore, the activity of carboxylesterases from animal species has been compared with the human enzymes and the latter show consistently lower activity than those of the other species (33). According to pharmacokinetic data, only 2% to 8% of irinotecan is converted into SN38 in humans with significant interpatient variability, whereas a 50% conversion rate is observed in rodents (7, 32).

The generation of SN38 in the liver favors its metabolism to the inactive glucurono-conjugate SN38-G by the enzyme UGT1A1. As a consequence, the plasma concentration of this glucuronyl metabolite is around 7-fold higher than that of SN38 (34). This also results in the accumulation of high levels of SN38 in the intestine through biliary excretion, which is the root cause of the late-onset diarrheas affecting 30% to 40% of treated patients (1, 15, 16). In addition, considerable variation in the conversion of SN38 into SN38-G in humans has been observed (35, 36) and genetic polymorphism of the gene encoding UGT1A1 has been reported in cancer patients. Recently, the labeling information for irinotecan was modified to indicate the role of *UGT1A1*28* polymorphism in the metabolism of irinotecan and its effect on safety, and a lower dose is recommended for homozygous *UGT1A1*28* patients. In August 2005, the Food and Drug Administration approved the marketing of a *UGT1A1* molecular assay for use as an *in vitro* diagnostic test for detecting the **1* (wild-type) and **28* (variant) alleles of the gene (37, 38).

Several approaches have been considered to solubilize SN38, improve its pharmacokinetic properties, and increase its therapeutic efficacy, including liposomal or nanoparticle formulations, water-soluble polymer conjugates, vitamin E conjugates, or other derivatives (39–43). Very little data is, however, available to show that these approaches improve the therapeutic index of irinotecan. The present work was therefore undertaken to design a novel SN38 prodrug that would be water soluble and allow a rapid and efficient, non–hepatic release of the active drug. This, by avoiding the intervention of liver carboxylesterases and UDP-glucuronosyltransferases, should result in a higher and more reproducible exposure to SN38 compared with irinotecan, with only minimal gastrointestinal toxicity. Solubilization of SN38 was achieved through chemical conjugation with a cationic peptide via an optimized linker and an ester bond, which allows i.v. administration of the selected peptide-SN38 conjugate (DTS-108) using a purely aqueous vehicle.

As already discussed, rodents are not a good model for irinotecan pharmacokinetics or toxicity. DTS-108 and irinotecan pharmacokinetics and toxicity were therefore compared in the dog. Dogs and humans have similar rates of conversion of irinotecan into SN38 and both species develop the same gastrointestinal toxicity (23, 30). Furthermore, the kinetics of the *in vitro* release of SN38 from DTS-108 is also very close in dog and human plasma. In preliminary studies in the dog, the MTD of DTS-108 was found to be 20 mg/kg (6.3 μmol/kg) compared with 30 mg/kg (45 μmol/kg) for irinotecan (23). The dose-limiting toxicities were, as expected, hemotoxicity and late-onset diarrhea for both drugs. Irinotecan, contrary to DTS-108, also caused early-onset diarrhea. This was expected because such early-onset diarrhea is part of a cholinergic syndrome caused by the direct interaction of irinotecan with acetylcholine esterase and is often associated with lacrimation, hypersalivation, bradycardia, and abdominal cramps (44). Importantly, the SN38 plasma AUC values observed after DTS-108 treatment at 5, 10, and 20 mg/kg were 50-, 80-, and 270-fold higher than that observed with irinotecan at its MTD. Clearly, with DTS-108, much higher circulating levels of SN38 can be achieved without the intestinal toxicity associated with irinotecan administration. These improved pharmacokinetic and pharmacodynamic properties of DTS-108 are consistent with its anticipated non–hepatic mode of activation, the nature and the distribution of the esterases involved being different





Single drug treatment				Combination treatment		
DTS-108		Reference drug				
Doses (μmol/kg)	T/C % (Day)	Drug (dose, mg/kg)	T/C %	Expected T/C % *	Observed T/C %	Combination index[$]
12.5	68 (31)	5-FU (40)	90	61	36	1.69
12.5	77 (28)	bevacizumab (2)	67	52	48	1.08

*Expected T/C = DTS-108 T/C x reference drug T/C; [$]Combination index = expected T/C / observed T/C

Fig. 5. *In vivo* efficacy study of DTS-108 alone or in combination with either 5-FU or bevacizumab in the HT-29 human colorectal carcinoma tumor model. *A,* DTS-108 was administered by i.v. injections on days 3, 6, 10, 13, 17, and 20 (*closed arrowheads*) and 5-FU was administered on days 4, 11, and 18 (*open arrowheads*). The mice received either saline (—), DTS-108 at 40 mg/kg (–▼–), 5-FU at 40 mg/kg (–□–), or DTS-108 at 40 mg/kg + 5-FU at 40 mg/kg (–●–). *B,* DTS-108 was administered by i.v. injections on days 3, 7, 11, 17, 21, and 25 (*closed arrowheads*) and bevacizumab was administered by i.p. injections on days 4, 7, 11, 14, 18, 21, 25, and 28 (*open arrowheads*). The mice received either saline (—), DTS-108 at 40 mg/kg (–▼–), bevacizumab at 2 mg/kg (–□–), or DTS-108 at 40 mg/kg + bevacizumab at 2 mg/kg (–●–). Points, mean tumor volume; bars, SEM.

from that of the hepatic carboxylesterases that activate irinotecan (31). Also consistent with a reduction of the hepatic metabolism of DTS-108 compared with that of irinotecan is the observation of a significantly lower ratio of the AUCs of SN38-G versus SN38 (0.05 and 5.83, respectively).

Interestingly, optimization of both the peptide structure and linker stability was required to obtain an optimal therapeutic index (28), confirming that the benefit of the lead compound DTS-108 results from other specific properties, possibly tissue distribution characteristics and optimal stability of the conjugate, in addition to the ability of the peptide to solubilize SN38.

It is important to note that dose intensification with irinotecan in patients with metastatic colorectal cancer results in improved therapeutic responses (15–17), suggesting that increasing the circulating concentration of SN38 further, without the gastrointestinal toxicity, would be beneficial from an efficacy perspective. The results of the *in vivo* efficacy studies presented here confirm that DTS-108 has a significant, dose-dependent antitumoral activity in human colon, lung, and breast carcinoma models. DTS-108 was also shown to be more active than an equimolar dose of irinotecan, although rodent models overestimate irinotecan activity, as its conversion rate to SN38 in these species is much greater than in humans (32). Finally, as for irinotecan, DTS-108 has been shown to have synergistic or additive antitumoral effects in combination with either 5-FU or bevacizumab, which suggests that DTS-108 can be used in similar clinical settings.

Taken together, these results show that the i.v. injection of a new peptidic conjugate of SN38 improves antitumoral efficacy and safety compared with irinotecan as a result of a non–hepatic and efficient mode of activation. As the role of the two hepatic enzymes responsible for the variability of irinotecan metabolism (carboxylesterases and UDP-glucuronosyltransferases) is significantly reduced, the interpatient variability following DTS-108 administration is expected to be much lower. These results are highly encouraging and support the therapeutic potential of DTS-108. A phase I clinical study in cancer patients is planned to allow DTS-108 to be characterized further.

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References

1. Hartmann JT, Lipp H-P. Camptothecin and podophyllotoxin derivatives: inhibitors of topoisomerase I and II—mechanisms of action, pharmacokinetics and toxicity profile. Drug Saf 2006;29:209–30.
2. Pessino A, Sobrero A. Optimal treatment of metastatic colorectal cancer. Expert Rev Anticancer Ther 2006;6:801–12.
3. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004; 350:2335–42.
4. Vanhoefer U, Rougier P, Borner M, Munoz A, Van Laethem J-L, Sobrero A. Irinotecan in combination with new agents. Second Clinical Investigators Update Meeting on Gastrointestinal Cancer. Eur J Cancer Suppl 2004;2:14–20.
5. Mathijssen RHJ, Loos WJ, Verweij J, Sparreboom A. Pharmacology of topoisomerase I inhibitors irinotecan (CPT-11) and topotecan. Curr Cancer Drug Targets 2002;2:103–23.
6. Smith NF, Figg WD, Sparreboom A. Pharmacogenetics of irinotecan metabolism and transport: an update. Toxicol In Vitro 2006;20:163–75.
7. Senter PD, Beam KS, Mixan B, Wahl AF. Identification and activities of human carboxylesterases for the activation of CPT-11, a clinically approved anticancer drug. Bioconjug Chem 2001;12:1074–80.
8. Humerickhouse R, Lohrbach K, Li L, Bosron WF, Dolan ME. Characterization of CPT-11 hydrolysis by human liver carboxylesterase isoforms hCE-1 and hCE-2. Cancer Res 2000;60:1189–92.
9. Charasson V, Haaz M-C, Robert J. Determination of drug interactions occurring with the metabolic pathways of irinotecan. Drug Metab Dispos 2002;30: 731–3.
10. de Jong FA, de Jonge MJA, Verweij J, Mathijssen RHJ. Role of pharmacogenetics in irinotecan therapy. Cancer Lett 2006;234:90–106.
11. Treinen-Moslen M, Kanz MF. Intestinal tract injury by drugs: Importance of metabolite delivery by yellow bile road. Pharmacol Ther 2006;112:649–67.
12. Horikawa M, Kato Y, Sugiyama Y. Reduced gastrointestinal toxicity following inhibition of the biliary excretion of irinotecan and its metabolites by probenecid in rats. Pharm Res 2002;19:1345–53.
13. Alimonti A, Gelibter A, Pavese I, et al. New approaches to prevent intestinal toxicity of irinotecan-based regimens. Cancer Treat Rev 2004;30: 555–62.
14. Xie R, Mathijssen RH, Sparreboom A, Verweij J, Karlsson MO. Clinical pharmacokinetics of irinotecan and its metabolites in relation with diarrhea. Clin Pharmacol Ther 2002;72:265–75.
15. Ychou M, Raoul JL, Desseigne F, et al. High-dose, single-agent irinotecan as first-line therapy in the treatment of metastatic colorectal cancer. Cancer Chemother Pharmacol 2002;50:383–91.
16. Saigi E, Salut A, Campos JM, et al. Phase II study of irinotecan (CPT-11) administered every 2 weeks as treatment for patients with colorectal cancer resistant to previous treatment with 5-fluorouracil-based therapies: comparison of two different dose schedules (250 and 200 mg/m^2) according to toxicity prognostic factors. Anticancer Drugs 2004;15: 835–41.
17. Van Cutsem E, Dirix L, Van Laethem JL, et al. Optimisation of irinotecan dose in the treatment of patients with metastatic colorectal cancer after 5-FU failure: results from a multinational, randomised phase II study. Br J Cancer 2005;92:1055–62.
18. De Coupade C, Fittipaldi A, Chagnas V, et al. Novel human-derived cell-penetrating peptides for specific subcellular delivery of therapeutic biomolecules. Biochem J 2005;390:407–18.
19. Kearsey J. Strategies for highly targeted intracellular drug delivery. Drug Deliv 2004;3:17–9.
20. Meyer-Losic F, Quinonero J, Dubois V, et al. Improved therapeutic efficacy of doxorubicin through conjugation with a novel peptide drug delivery technology (Vectocell). J Med Chem 2006;49: 6908–16.
21. Jinno H, Tanaka-Kagawa T, Hanioka N, et al. Glucuronidation of 7-ethyl-10-hydroxycamptothecin (SN-38), an active metabolite of irinotecan (CPT-11), by human UGT1A1 variants, G71R, P229Q, and Y486D. Drug Metab Dispos 2003;31:108–13.
22. OECD guideline: Guideline for the testing of chemicals. Section 1: Physical chemical properties. 105 Water solubility. Adopted 27.07.95.
23. Lavelle F, Bissery MC, Andre S, Roquet F, Riou JF. Preclinical evaluation of CPT-11 and its active metabolite SN-38. Semin Oncol 1996;23:11–20.
24. United Kingdom Co-ordinating Committee on Cancer Research (UKCCCR) guidelines for the welfare of animals in experimental neoplasia. 2nd ed. Br J Cancer 1998;77:1–10.
25. Azrak RG, Cao S, Slocum HK, et al. Therapeutic synergy between irinotecan and 5-fluorouracil against human tumor xenografts. Clin Cancer Res 2004;10: 1121–9.
26. Prewett MC, Hooper AT, Bassi R, Ellis LM, Waksal HW, Hicklin DJ. Enhanced antitumor activity of anti-epidermal growth factor receptor monoclonal antibody IMC-C225 in combination with irinotecan (CPT-11) against human colorectal tumor xenografts. Clin Cancer Res 2002;8:994–1003.
27. Yokoyama Y, Dhanabal M, Griffioen AW, Sukhatme VP, Ramakrishnan S. Synergy between angiostatin and endostatin: inhibition of ovarian cancer growth. Cancer Res 2000;60:2190–6.
28. Michel M, Ravel D, Ribes F, Tranchant I. Camptothecin-peptide conjugates and pharmaceutical compositions containing the same. PCT WO 2007/ 113687,2007.
29. Pavillard V, Agostini C, Richard S, Charasson V, Montaudon D, Robert J. Determinants of the cytotoxicity of irinotecan in two human colorectal tumor cell lines. Cancer Chemother Pharmacol 2002;49: 329–35.
30. Inaba M, Ohnishi Y, Ishii H, et al. Pharmacokinetics of CPT-11 in rhesus monkeys. Cancer Chemother Pharmacol 1998;41:103–8.
31. Li B, Sedlacek M, Manoharan I, et al. Butyrylcholinesterase, paraoxonase, and albumin esterase, but not carboxylesterase, are present in human plasma. Biochem Pharmacol 2005;70:1673–84.
32. Morton CL, Wierdl M, Oliver L, et al. Activation of CPT-11 in mice: identification and analysis of a highly effective plasma esterase. Cancer Res 2000;60: 4206–10.
33. Satoh T, Hosokawa M, Atsumi R, Suzuki W, Hakusui H, Nagai E. Metabolic activation of CPT-11, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin, a novel antitumor agent, by carboxylesterase. Biol Pharm Bull 1994;17:662–4.
34. Sparreboom A, de Jonge MJ, de Bruijn P, et al. Irinotecan (CPT-11) metabolism and disposition in cancer patients. Clin Cancer Res 1998;4:2747–54.

35. Iyer L, King CD, Whitington PF, et al. Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. J Clin Invest 1998;101:847–54.

36. Mick R, Gupta E, Vokes EE, Ratain MJ. Limited-sampling models for irinotecan pharmacokinetics-pharmacodynamics: prediction of biliary index and intestinal toxicity. J Clin Oncol 1996;14:2012–9.

37. Hahn KK, Wolff JJ, Kolesar JM. Pharmacogenetics and irinotecan therapy. Am J Health Syst Pharm 2006;63:2211–7.

38. Marsh S. Impact of pharmacogenomics on clinical practice in oncology. Mol Diagn Ther 2007;11:79–82.

39. Kawano K, Watanabe M, Yamamoto T, et al. Enhanced antitumor effect of camptothecin loaded in long-circulating polymeric micelles. J Control Release 2006;112:329–32.

40. Schluep T, Hwang J, Cheng J, et al. Preclinical efficacy of the camptothecin-polymer conjugate IT-101 in multiple cancer models. Clin Cancer Res 2006;12:1606–14.

41. Sadzuka Y, Takabe H, Sonobe T. Liposomalization of SN-38 as active metabolite of CPT-11. J Control Release 2005;108:453–9.

42. Bhatt R, de Vries P, Tulinsky J, et al. Synthesis and in vivo antitumor activity of poly(L-glutamic acid) conjugates of 20S-camptothecin. J Med Chem 2003;46:190–3.

43. Tatalick LM, Hughes M, Spurgeon S, et al. Preclinical results with SN2310 emulsion indicates improved pharmacokinetic and pharmacodynamic profiles compared to irinotecan. Proceedings of AACR-NCI-EORTC international conference 2005. Session A (abstracts no A220); 2005.

44. Gandia D, Abigerges D, Armand JP, et al. CPT-11-induced cholinergic effects in cancer patients. J Clin Oncol 1993;11:196–7.

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DTS-108, A Novel Peptidic Prodrug of SN38: *In vivo* Efficacy and Toxicokinetic Studies

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