Pharmacokinetics and Antitumor Efficacy of XMT-1001, a Novel, Polymeric Topoisomerase I Inhibitor, in Mice Bearing HT-29 Human Colon Carcinoma Xenografts

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A Novel Polymer-Conjugated Topoisomerase I Inhibitor in Human Cancer Xenografts

XMT-1001, CPT, camptothecin, topoisomerase I inhibitor, pharmacokinetic, xenograft

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Polymer-drug conjugates, the subclass of carrier-mediated therapeutic agents, offer several advantages over small molecule drugs, including increased aqueous solubility, prolonged blood circulation time, enhanced tumor delivery and an improved toxicity profile. Camptothecin (CPT), a DNA topoisomerase I inhibitor, is a potent antineoplastic agent and an attractive target for conjugation with hydrophilic polymer carriers capable of improving its solubility, rapid plasma inactivation, and toxicity, which hindered the therapeutic application of the drug.

XMT-1001 is a novel macromolecular CPT conjugate prepared based on a biodegradable hydrophilic stealth polyacetal carrier. XMT-1001 is designed to produce prolonged tumor exposure to conjugated drug and its active release products to improve efficacy and tolerability in comparison to CPT. The improved distribution of XMT-1001 to the tumor and extended localized release of the novel CPT derivative, CPT-20-O-\((N\text{-succinimido-glycinate})\) (CPT-SI), as well as CPT itself, are hypothesized to improve safety and efficacy over existing drugs in this class.
Abstract (not to exceed 250 words): current word count = 241 words

**Purpose**: To evaluate the pharmacokinetics (PK) and tissue disposition of macromolecular camptothecin (CPT) drug conjugate, XMT-1001, and irinotecan (CPT-11) in mice bearing HT-29 xenograft tumors.

**Experimental Design**: The antitumor efficacy of XMT-1001 was evaluated in the mouse HT-29 human colon carcinoma xenograft model. XMT-1001 was administered IV to female athymic nude (nul/nul) mice bearing established HT-29 xenograft tumors (n=10) at 15, 30, and 60 mg CPT equivalents/kg on weekly or biweekly schedules. The tumor growth inhibition and tumor growth delay endpoints were used for efficacy evaluation. In the PK study, XMT-1001 was administered IV at a pharmacologically relevant dose of 60 mg CPT equivalents/kg × 1 via tail vein or an equimolar dose of CPT-11 at 100 mg/kg IP × 1. Mice (n = 3 per time point) were euthanized from 0.083 to 336 h after XMT-1001 administration and from 0.083 to 24 h after CPT-11. Plasma, tumor and tissues were collected from all animals. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay was used to measure XMT-1001, conjugate release products, CPT-20-O-(N-succinimido-glycinate) (CPT-SI) and CPT-20-O-(N-succinamidoyl-glycinate) (CPT-SA), and CPT.

**Results**: After XMT-1001 administration, the majority of the plasma exposure is accounted for by conjugated CPT. XMT-1001 exhibited a prolonged exposure of conjugated drug, active conjugate primary release products, CPT-SI and CPT-SA, and active-CPT, which was associated with greater antitumor response compared to CPT-11.

**Conclusions**: XMT-1001 provides an extended systemic and tumor exposure of conjugated drug and demonstrates improved antitumor effect compared with CPT-11.
Introduction

Camptothecin (CPT) was discovered from the leaf extracts of the *Camptotheca acuminata* tree (1). In 1965, Wall et al. successfully isolated and characterized the structure of CPT and its analogues (2). The pentacyclic structure of CPT contains unique properties, such as an α-hydroxy lactone system in the E-ring and an unsaturated conjugated pyridone in the D-ring. The aqueous solubility of CPT is very low and will only dissolve in a few organic solvents (3). At physiologic pH (pH = 7.4), the E-ring opens rapidly forming the inactive-hydroxy acid. On acidification, the hydroxy acid converts back to the active-lactone form (2, 4-6).

CPT has significant antitumor efficacy across a broad spectrum of human tumor xenograft models (2, 7, 8). While antitumor activity was evident in Phase I trials with the sodium salt of camptothecin, which was used to overcome the insolubility of the lactone form of CPT, only modest response rates and severe toxicities were observed in Phase II trials (9, 10). Clinical development of the drug was discontinued because of severe hemorrhagic cystitis (11-14). Rapid renal clearance of water soluble CPT-carboxylate from plasma and its conversion, in the acid milieu of the urine, to the active closed lactone form of CPT, was likely the cause of severe bladder toxicity.

More water soluble CPT derivatives, such as irinotecan and topotecan, that were developed subsequently, comprise the important class of antineoplastic agents. Irinotecan and topotecan are used to treat patients with broad spectrum of solid tumors. Although efficacious, these agents have significant toxicity. Topotecan treatment is commonly associated with severe neutropenia. Irinotecan treatment often causes both neutropenia and acute or delayed diarrhea, which can be life threatening in patient populations with genetic polymorphisms that reduce glucuronidation of active irinotecan metabolite SN-38 to SN-38-G.

Conjugation of CPT and other camptothecins, including irinotecan and SN-38, to polymer carriers have also been explored with the theoretical advantages of greater solubility, longer duration of exposure, selective delivery of entrapped drug to the site of action, superior...
therapeutic index, and the potential to overcome resistance associated with the small molecule anticancer agent(15, 16). The pharmacokinetics of these agents is dependent upon the carrier and not the parent drug until the drug is released from the carrier. The drug that remains linked to a conjugate or polymer or encapsulated in liposomes or nanoparticles is an inactive prodrug, and thus the drug must be released from the carrier to be active. Whether the drug needs to be released outside the cell in the tumor extracellular fluid or within the cell depends on the formulation of the carrier and the mechanism of release. After the drug is released from the carrier, the pharmacokinetics of the drug are the same as after administration of the non-carrier form of the drug. Thus, the pharmacology and pharmacokinetics of these agents are complex, and detailed studies must be performed to evaluate the disposition of the conjugated or encapsulated form of the drug and the released active drug in plasma, tumor and tissue(15).

Conventional small molecule anticancer agents have a low molecular weight resulting in rapid whole-body tissue distribution following systemic administration, as well as rapid diffusion out of these tissues, resulting in a relatively short residence time of the drug at the targeted site of action (i.e., tumor). In addition, small molecule drugs have the potential for exerting a pharmacological toxic effect in normal tissues. The development of polymer conjugates as a method for drug delivery was based on the discovery that macromolecular molecules >40 kDa produced prolonged plasma and tumor exposures and were able to evade renal clearance(17-21). The plasma, tumor and tissue pharmacokinetics of conjugated drug is dictated by the characteristics of the conjugate, such as size, surface charge, architecture, dose and route of administration(15, 16). Conjugates, liposomes and nanoparticles attain high intratumoral concentrations due to the enhanced permeability and retention (EPR) effect in tumors and tumor vasculature(18-22). For conjugate, liposomal or nanoparticle anticancer agents that do not contain an active targeting moiety (monoclonal antibody, ligand, etc.) on the surface of the carrier to be effective for the treatment of solid tumors, they must extravasate through the leaky vasculature of the tumor and release active drug into the extracellular matrix. Once inside the
tumor, small molecule drug is released from the conjugate, liposome or nanoparticle and diffuses into the cell to elicit a cytotoxic response.

Several macromolecular CPT prodrugs tested in Phase I/II trials have shown improvement in systemic exposure to CPT and provided the evidence of significant antitumor activity. Clinical development of some of these conjugates were not pursued further, partially due to bladder toxicity, which was likely related to high levels of excretion of CPT in the urine(23). The conjugates of irinotecan and SN-38, while not associated with bladder toxicity, have other toxicities, such as diarrhea(24-27).

XMT-1001 is designed to improve the safety profile compared to other camptothecin analogues by combining a passive tumor targeting of macromolecular prodrug with a hydrolytic dual phase drug release mechanism leading to distribution of the drug in a low soluble, less toxic and more stable prodrug form, which can potentially reduce bladder and gastrointestinal toxicities while enhancing efficacy. Unlike many conjugated CPT analogs which rely upon slow hydrolysis or enzymatic cleavage of the CPT-20-O-ester bond to release CPT in one step, XMT-1001 utilizes a CPT-20-O-(N-succinimidoyl-glycinate) linkage, which generates CPT in two steps via well-defined small molecule drug intermediates (Figure 1). XMT-1001 is a water soluble macromolecular conjugate of CPT. In this novel polymer prodrug, CPT is conjugated with a ~60 kDa biodegradable hydrophilic polyacetal, poly (1-hydroxymethylethylene hydroxymethylformal) (PHF)(28). In contrast to other polymer-camptothecin conjugates, such as IT-101 (or CRLX-101) or pegamotecan (EZN-246)(29), which directly release CPT from the conjugate, XMT-1001 was specifically designed to release a novel, active camptothecin analog, CPT-20-O-(N-succinimidoyl-glycinate) (CPT-SI), as its primary release product. In this first step, intramolecular cyclization occurs to release the highly lipophilic, lactone stabilized CPT-SI (ED50 in HT-29 cells 30 nM), which can be further hydrolyzed to the more soluble CPT-20-O-(N-succinamido-glycinate) (CPT-SA). In these two small molecule prodrugs, the CPT-20-glycine-ester bond can be further hydrolyzed to provide the active lactone form of CPT (ED50 in...
HT-29 cells = 15 nM). The intent of designing XMT-1001 to release primarily the lipophilic, lactone-stabilized CPT-SI, as opposed to CPT itself, was to minimize the plasma levels of CPT, and thereby avoid the renal clearance, bladder accumulation and associated severe bladder toxicity of CPT itself.

The early PK and biodistribution studies conducted with dual-labeled PHF-CPT conjugate (¹¹¹In-labeled PHF and ³H-labeled CPT) confirmed improved plasma exposure to conjugated drug and preferential tumor and tissue accumulation of polymer carrier and CPT. The level of tumor accumulation of CPT reported in this study was significantly higher than was expected based on prior studies using polymer carrier accumulation, indicating that both conjugated drug and drug released in circulation as lipophilic prodrugs CPT-SI and CPT-SA, and CPT contribute to intratumoral drug accumulation. Preclinical XMT-1001 pharmacokinetic studies in rats and dogs confirmed a prolonged plasma exposure of XMT-1001, CPT-SI, CPT-SA and CPT(30). However, the PK disposition of XMT-1001, CPT-SI, CPT-SA and CPT in tumor and tissues have not been evaluated.

The objectives of this study were to evaluate the plasma, tumor and tissue pharmacokinetics and antitumor response of XMT-1001 in female nu/nu mice bearing HT-29 human colon carcinoma xenografts. New sample processing methods were developed for quantitative determination of conjugated CPT (XMT-1001) and primary conjugate release products, CPT-SI and CPT-SA; and CPT in tumor and tissues.

Materials and Methods

**Chemicals and reagents.** XMT-1001 (6% weight/weight of CPT), CPT-20-O-(N-succinimido-glycinate) (CPT-SI) and CPT-20-O-(N-succinamidoyl-glycinate) (CPT-SA) were supplied by Mersana Therapeutics, Inc (Cambridge, MA, USA). CPT was purchased from Boehringer Ingelheim (Barcelona, Spain). CPT-11 and SN-38 were purchased from Sigma-
Aldrich (St. Louis, MO, USA). Topotecan was supplied by Dr. Reddy’s Laboratories (Andhra Pradesh, India).

Animal care. All mice were handled in accordance with animal care and use procedures set forth in the Guide for the Care and Use of Laboratory Animals with respect to restraint, husbandry, surgical procedures, feed and fluid regulation, and veterinary care(31). Animals were fed an irradiated NIH-31 Modified 6% Mouse/Rat Sterilizable Diet® (Teklad/Harlan Laboratories, Inc.) consisting of 18.0% crude protein, 6.0% crude fat and 5.0% crude fiber and had access to water ad libitum (reverse osmosis, 1 ppm Cl). They were housed on irradiated Enrich-o’cobs™ laboratory enrichment bedding (bed-o’cobs®/The Andersons, Inc., Maumee, OH, USA) in static microisolators on an alternating 12-h light/dark cycle at 21-22°C and 40-60% humidity.

HT-29 human colon carcinoma tumor xenografts. HT-29 human colon carcinoma cell lines (American Type Culture Collection, Manassas, VA, USA) were propagated in culture and harvested in log-phase growth. Cells (1 × 10⁷ cells per mouse) were implanted SC into the flank of female athymic nude (nu/nu) mice (Harlan Laboratories, Inc., Indianapolis, IN, USA). The HT-29 tumors were harvested when they reached 500 to 1,000 mm³ and were implanted as ~1 mm³ fragments SC in the right flank of female athymic nude (nu/nu) mice by aseptic techniques. The target tumor volumes of the pharmacokinetic and efficacy studies were 150 to 300 mm³ and 100 to 150 mm³, respectively. Tumor volume was calculated using the following formula: tumor volume (mm³) = (w² × l)/2, where w = width and l = length in mm of the tumor(32, 33).

Efficacy study. On day 22 following SC tumor implantation, mice (approximately 10 to 11 weeks of age) were sorted according to tumor volume into ten groups (n = 10). Individual tumor volumes ranged from 63.0 to 172.0 mm³ and group mean tumor volumes ranged from 112.2 to 113.8 mm³. Tumor volumes were measured using calipers twice weekly until study completion on day 100. Treatments were administered either once weekly for five weeks (qwk ×
5) or twice weekly for five weeks (qbiwk × 5). XMT-1001 was administered at 30 mg/kg and 60 mg/kg (qwkw × 5) and 15 mg/kg and 30 mg/kg (qbiwk × 5). All XMT-1001 dose levels are reported in CPT equivalents. The comparator, CPT-11, was administered at the highest equimolar to XMT-1001 dose, 100 mg/kg (qwkw × 5) and 50 mg/kg (qbiwk × 5), respectively, following the same administration schedule. Each dose was given in a volume of 0.2 mL per 20 g body weight (10 mL/kg) and was adjusted to the body weight of the individual mouse. Group 10 received XMT-1001 administered at 60 mg/kg IV as two consecutive 30 mg/kg dosages (separated by no more than two min) in a volume of 0.4 mL per 20 g body weight, adjusted to the body weight of the individual mouse. Mice were observed for overt signs of treatment-related side effects.

Body weight was measured daily for five days for the first week, then twice weekly until study completion. An acceptable side effect profile for the maximum tolerated dose (MTD) was defined as a group mean body weight loss of ≤20% and ≤10% of the deaths in that group determined to be treatment-related.

Mice were monitored individually and euthanized when tumor volume reached the endpoint volume of 1,000 mm$^3$ or on day 100, whichever came first. Treatment outcome is assessed as median tumor volume (MTV), regression response, tumor growth delay (TGD), tumor growth inhibition (TGI) and time to endpoint (TTE) (34). MTV is defined as the MTV ($n$) on day 100 in the number of mice remaining in the study, $n$, whose tumors have not attained the endpoint volume. Regression response is defined as a complete regression (CR) or partial regression (PR). CR is defined as tumor volume <13.5 mm$^3$ for three consecutive measurements. PR is defined as tumor volume ≤50% as measured on day 1 for three consecutive measurements and ≥13.5 mm$^3$ for one or more of these three measurements.

Pharmacokinetic study. On day 29 following SC tumor implantation, mice (approximately 10 weeks of age) were pair matched according to tumor volume into two treatment groups. Individual tumor volumes ranged from 172.0 to 352.0 mm$^3$ and group mean
tumor volumes ranged from 233.5 to 237.3 mm$^3$. Body weight was measured daily for five days for the first two weeks, then twice weekly until study completion. Tumor volumes were measured using calipers twice weekly until study completion. Group 1 (vehicle control, n = 3) received 0.9% NaCl administered IV × 1 via tail vein. Group 2 (n = 39) received XMT-1001 (dissolved in water) administered at 60 mg/kg (CPT equivalents) IV × 1 using a slow push over 30 seconds (sec) via tail vein. Group 3 (n = 27) received CPT-11 (dissolved in D$_2$W) administered at 100 mg/kg IP × 1. Each dose of XMT-1001 and CPT-11 was given in a volume of 0.2 mL per 20 g body weight (10 mL/kg) and was adjusted to the body weight of the individual mouse.

Mice (n = 3) were sacrificed at 5 minutes (min) after administration of 0.9% NaCl (vehicle control). Mice (n = 3 per time point) were sacrificed at 0.083, 0.25, 0.5, 1, 2, 4, 6, 16, 24, 48, 72, 168 and 336 hours (h) after administration of XMT-1001. Mice (n = 3 time point) were sacrificed at 0.083, 0.25, 0.5, 1, 2, 4, 6, 16 and 24 h after administration of CPT-11. Blood (~1 mL) was collected via terminal cardiac puncture using lithium heparin as an anticoagulant under CO$_2$ anesthesia and processed for plasma by centrifugation (1,500 × g for 5 min). A volume of 0.1 mL of 3M citric acid in water was added per 0.9 mL of plasma to prevent ex vivo conversion of XMT-1001 to the conjugate-release products CPT-SI, CPT-SA and CPT. Tumor, liver, skeletal muscle, spleen, brain, pancreas, kidney, lung and peritoneal fat tissues were collected from each mouse. Plasma, tumor and tissues were placed in cryopreservation vials and preserved by snap freezing using liquid nitrogen. They were stored at -80°C until analysis.

**Tissue homogenization procedure.** Tumor and tissue samples from female nude (nu/nu) mice bearing HT-29 human colon carcinoma xenografts were removed from the -80°C freezer and thawed on ice. Standard 2 mL screw-top tubes were filled with fifteen 1.4 mm and two 2.8 mm ceramic zirconium oxide beads (Omni International, Inc., Kennesaw, GA). Tumor and tissues were weighed in these 2 mL tubes. A volume of 3 mL 10% 3M citric acid in phosphate-buffered solution (pH 5) was added to the tubes for every 1 g of tumor or tissue.
weighed. The tubes were placed in a Precellys® 24 (13-RD000) bead mill homogenizer (Omni International, Inc., Kennesaw, GA) and homogenized at 6,000 rpm for 20 seconds. Once homogenized, tumor and tissue homogenates were placed in the -80°C freezer until extraction and analysis.

**Sample processing and liquid chromatography and tandem mass spectroscopy (LC-MS/MS) assay for XMT-1001.** Two separate assays were developed to measure XMT-1001, and CPT-SI, CPT-SA and CPT in plasma, tumor and tissue based on previous studies (30). SN-38 was used as an internal standard (IS) for both assays. For XMT-1001, 100 μL of acidified plasma, tumor or tissue homogenate was protein precipitated using 300 μL of acetonitrile. The samples were centrifuged and the acetonitrile supernatant containing CPT-SI, CPT-SA and CPT was discarded. The pellet samples were then centrifuged and the supernatant was decanted into clean tubes and evaporated under nitrogen in TurboVap. Samples were reconstituted in 100 μL of acidic mobile phase containing acetonitrile:methanol:water:formic acid (25:25:50:0.1, v:v:v) and analyzed by LC-MS/MS.

CPT-SI, CPT-SA and CPT, and IS were extracted from 100 μL of acidified mouse plasma, tumor or tissue homogenate by protein precipitation with 300 μL of acetonitrile. The sample was centrifuged and the acetonitrile supernatant containing CPT-SI, CPT-SA, CPT and IS was removed. The acetonitrile supernatant was evaporated, reconstituted with 100 μL of acidic mobile phase containing acetonitrile:methanol:water:formic acid (25:25:50:0.1, v:v:v:v) and analyzed by LC-MS/MS.

Briefly, the LC-MS/MS method consisted of reversed phase chromatography with triple quadrupole detection. The injection volume was 10 μL and solvent flow rate was 0.7 mL/min on a Chromolith® SpeedROD RP-18e (50 × 4.6 mm, 2 μm particle size) analytical column. Mobile phase A consisted of water and formic acid (100:0.1;v:v) and mobile phase B consisted of acetonitrile, methanol and formic acid (50:50:0.1;v:v:v). Peak detection was achieved using a Thermo TSQ Quantum Ultra mass spectrometer operating in electron spray positive ion (ESI+) mode.
mode (Spray voltage: 3,000V, Vaporizer temperature: 500°C). XMT-1001, CPT-SI, CPT-SA and CPT were detected using selected reaction monitoring (SRM) using a transition of 349.156 → 305.200, 488.210 → 331.150, 506.216 → 331.130 and 349.156 → 305.200, respectively. The retention times for XMT-1001, CPT-SI, CPT-SA, CPT and SN-38 (internal standard) under the above conditions were 3.44, 3.61, 3.37, 3.44 and 3.36 minutes, respectively. Quantitation was achieved by comparing the observed peak area ratios of analyte (XMT-1001, CPT-SI, CPT-SA or CPT) and IS (SN-38) of the samples to a regression curve determined from drug-fortified plasma, tumor or tissue standards. The standard curves of XMT-1001 and CPT-SI, CPT-SA and CPT in plasma, tumor and tissue homogenates showed linearity over the concentration ranges of 3 to 900 ng/mL and 3 to 3,000 ng/mL, respectively. Obtained XMT-1001 plasma and tissue concentrations were reported in CPT equivalents. The acceptable criterion for precision and accuracy was 15% deviation from the nominal, except at the lower limit of quantitation (LLOQ) where 20% deviation was acceptable, in accordance with the FDA Guidance for Industry on Bioanalytical Method Validation.

**Sample processing and high-performance liquid chromatography (HPLC) assay for CPT-11.** Based on our prior studies, an HPLC with fluorescence detection assay was used to measure total (lactone + hydroxy acid) form of CPT-11 and SN-38(35, 36). Topotecan (TPT) was used as the IS. A volume of 100 μL of acidified plasma, tumor or tissue homogenate was protein precipitated using methanol. A volume of 30 μL of supernatant was directly injected on the HPLC. Quantitation was achieved by comparing the observed peak area ratios of analyte (CPT-11 and SN-38) and IS (TPT) of the samples to a regression curve determined from drug-fortified plasma, tumor or tissue standards. The standard curve of CPT-11 and SN-38 in plasma, tumor and tissue homogenates showed linearity over the concentration range of 3 to 1,000 ng/mL.
Pharmacokinetic analysis. The pharmacokinetics of XMT-1001 and CPT-11 in plasma, tumor and tissue were analyzed by noncompartmental analysis using WinNonlin Professional Edition version 5.2.1 (Pharsight Corp., Cary, NC, USA). The area under the concentration versus time curve (AUC) was calculated using the linear up/log down rule. AUC from 0 to t (AUC0-t) and AUC from 0 to ∞ (AUC0-∞) were calculated. AUC0-∞ was reported if the percent extrapolation was ≤ 20%. The maximum concentration (Cmax), time of Cmax (tmax), last measured concentration (Clast) and time of Clast (tlast) were determined by visual inspection of the concentration versus time curve. Distribution half-life (t ½ α) and elimination half-life (t ½ β), time it takes for concentration to reach one-half of its original value during drug distribution and elimination phases, respectively, were calculated for XMT-1001.

Statistical analysis. Statistical analyses and graphical presentations of statistical analyses were performed using Prism for Windows version 3.03 (GraphPad Software, Inc., La Jolla, CA, USA). MTV was assessed between treatment and control groups using the Mann-Whitney U test. The log-rank test was used to compare the TTE of individual mice in each group (except those deaths determined to be NTR) between treatment and control groups. Statistical significance was determined using two-tailed tests with α = 0.05. OS curves showing the percentage of mice remaining in the study as a function of time were constructed using TTE data by the Kaplan-Meier method.

Results

Efficacy and toxicity of XMT-1001 and CPT-11. Tumor response parameters are presented in Table 1. Mean tumor growth curves and Kaplan-Meier plot are presented in Figure 2. All XMT-1001 and CPT-11 regimens and schedules were well tolerated with no significant weight loss observed even at the highest dose level. In each dose regimen and schedule examined, XMT-1001 exhibited statistically significant, greater antitumor activity in comparison
to equimolar regimens of CPT-11. Within the dose level range tested, XMT-1001 demonstrated
dose-dependent antitumor effect both at weekly and biweekly administration schedules, while
CPT-11 was therapeutically active only at a more frequent (biweekly) administration schedule.
Antitumor treatment efficacy determined as %TGI (day 27) indicated that XMT-1001 was
efficacious (%TGI > 60%) at the 15 mg/kg and 30 mg/kg qbiwk × 5, as well as at the 60 mg/kg
qwk × 5, while CPT-11 had significant antitumor effect only at the 50 mg/kg qbiwk × 5. The
comparison of TGD/TTE endpoint parameters for the biweekly (qbiwk × 5) administration
schedule has shown that mice treated with XMT-1001 at the 30 mg/kg had a superior TGD/TTE
as compared to mice treated with CPT-11 at 50 mg/kg (TGD, 147% vs. 115%; median TTE,
99.7 days vs. 86.7 days; log-rank, P=0.0089).

Tumor regression data at the end of the study (day 100) observed for the most
efficacious regimen (qbiwk × 5) also showed better response with XMT-1001 at 30 mg/kg (2
TFS, 5 CR and 5 PR) compared with CPT-11 at 50 mg/kg (9 PR) (Table 1).

**Plasma, tumor and tissue disposition of XMT-1001, CPT-SI, CPT-SA and CPT.**

Plasma, tumor and tissue concentration profiles of XMT-1001, CPT-SI, CPT-SA and CPT are
presented in Figure 3. Pharmacokinetic parameters of XMT-1001, CPT-SI, CPT-SA and CPT
are presented in Table 2.

Following administration of XMT-1001, conjugated CPT plasma concentration reached
C_{max} within 15 min and then declined biexponentially over 7 days. The majority of conjugated
CPT, responsible for >95% of XMT-1001 exposure, was cleared from the plasma in the first 48
h post administration with an t_{1/2a} of 4.0 h; the rest of the exposure (~5%) was cleared with an t_{1/2b}
of 30.9 h.

The plasma concentrations of CPT-SI, CPT-SA and CPT following administration of
XMT-1001 reached C_{max} within 15 min, and declined over 24 h. Estimated t_{1/2} values for CPT-SI,
CPT-SA and CPT were comparable with t_{1/2a} values for conjugated CPT ranging from 2.1 to 3.0
h. The AUC_{0-t} for CPT-SI, CPT-SA and CPT were relatively minor components in plasma as
compared with AUC values observed for conjugated CPT (1.48%, 1.27% and 0.24% for CPT-SI, CPT-SA and CPT, respectively).

The tumor concentration profiles for conjugated CPT and release product showed gradual accumulation of conjugated CPT, and the low water soluble CPT-SI and CPT and rapid accumulation of water soluble CPT-SA. The distribution phase for all CPT derivatives was completed by 24 h. Intratumoral concentrations of drug-related compounds declined gradually over a two-week period. High intratumoral concentrations of conjugated CPT (~1,000 ng/mL) and CPT-SI (30 ng/mL) were observed at the last sample time at 336 h.

Relative tumor exposure to lipophilic CPT-SI and CPT was 4.2- and 2.5-fold higher than their relative plasma exposure, indicating longer retention of the low water soluble prodrug CPT-SI in the tumor. The ratio of tumor AUC to plasma AUC for conjugated CPT, and conjugated release products, CPT-SI, CPT-SA and CPT were 15%, 65%, 14% and 38%, respectively. This indicates significant tumor distribution and accumulation of CPT prodrugs and, especially, for therapeutically active lipophilic CPT-SI and CPT.

The extended tissue retention and high exposure of XMT-1001 and conjugated drug release products, CPT-SI, CPT-SA and CPT was also observed in the liver, spleen, kidney, muscle, fat, lung and pancreas. The lowest exposure of CPT and its prodrugs were observed in the brain.

*Plasma, tumor and tissue disposition of CPT-11 and SN-38.* Plasma, tumor and tissue concentration versus time profiles of CPT-11 and SN-38 are depicted in Figure 4. Following administration of CPT-11, plasma CPT-11 and SN-38 concentrations reached $C_{max}$ within 30 min. CPT-11 and SN-38 plasma concentrations rapidly declined over 24 h. Pharmacokinetic parameters of CPT-11 and SN-38 are presented in Table 2. There were high exposures of CPT-11 and SN-38 observed in the plasma, tumor, liver, spleen, kidney, muscle, fat, lung and pancreas. The lowest exposure of CPT-11 and SN-38 was observed in the brain.
Discussion

The optimal pharmacokinetic characteristics of polymer-conjugate nanoparticle anticancer agents includes an extended blood or plasma circulation time, enhanced tumor delivery and release of active drug from the carrier into the tumor extracellular matrix (15, 16, 22, 37, 38). Preliminary pharmacokinetics of XMT-1001 in rats and dogs reported an extended plasma circulation time of conjugated drug, yet the full extent of tumor and tissue disposition of XMT-1001 had not been evaluated. This is the first study evaluating the plasma, tumor and tissue pharmacokinetics and antitumor efficacy of XMT-1001 and its release products CPT-SI, CPT-SA and CPT, in mice bearing HT-29 human colon carcinoma xenografts. The results of this study suggest that XMT-1001 provides pharmacokinetic advantages in plasma and tumors when compared to CPT-11. In addition, the results of our study are consistent with the improved antitumor efficacy and therapeutic index of XMT-1001 compared to CPT-11.

The theory behind the improved tumor localization of XMT-1001 revolves around the phenomenon known as enhanced permeability and retention (EPR) (39). It is believed that polymer-conjugate nanoparticles utilize the EPR effect of the tumor microenvironment as a means of passive tumor targeting (17-21). The vasculature of tumors, unlike normal vasculature, is comprised of poorly aligned and defective endothelial cells lacking innervation and smooth muscle cells, as well as upregulated vascular permeability enhancing factors, each facilitating the transport of the polymer-conjugate nanoparticle across tumor capillaries (18). Additionally, tumors also exhibit an impaired lymphatic drainage, which is the mechanism by which small molecule and nanoparticle drugs are cleared from normal tissues. The decreased clearance of polymer-conjugates by impaired lymphatic drainage in tumor results in the retention of these formulations at high concentrations for extended periods of time in the tumor (18). Normal tissue vasculature without the abnormal and disorganized qualities of tumor vasculature selectively excludes polymer-conjugates from penetration and accumulation. This reduces localization of these drugs in normal tissue and the associated toxicities.
Another primary driving force for the accumulation of polymer-conjugate nanoparticles in the tumor is the prolonged plasma circulation time exhibited by macromolecular drugs larger than 40 kDa (39-41). This size is sufficient for macromolecular drugs to escape the threshold for renal clearance and to remain in the plasma compartment until further distribution or metabolism. Factors that affect the EPR effect include vehicle-related (plasma residence time, conjugate or liposome size, conjugate or liposome architecture), tumor-related (size, type, microenvironment) and external mediators (radiation, bradykinin antagonist, cyclooxygenase inhibitor, and nitric oxide scavenger) (17-21).

Following the administration of XMT-1001 at 60 mg/kg IV × 1 or CPT-11 at 100 mg/kg IP × 1 to female nude (nu/nu) mice bearing HT-29 human colon carcinoma xenografts, the plasma exposure of XMT-1001 was 27.5-fold greater than CPT-11 at the equimolar dose. XMT-1001 exhibited a 7-fold increase in duration of exposure in plasma (168 h) compared with CPT-11 (24 h). After administration of XMT-1001, 97.0% of CPT remained conjugated to the polymer in plasma. Duration of exposure of the lipophilic, active primary release product of XMT-1001, CPT-SI, as well as the secondary release product, CPT-SA, and active drug CPT in plasma were 24 h. The duration of exposure of the active metabolite of CPT-11, SN-38, was also 24 h. The tumor exposure of XMT-1001 was 2.7-fold greater than that of CPT-11. Moreover, the duration of exposure of XMT-1001 (336 h) was 14-fold greater than that of CPT-11 (24 h). CPT-SI, CPT-SA and CPT (6.4%, 1.2% and 0.6%, respectively) represented 8.2% of the exposure in the tumor following XMT-1001 administration; whereas, SN-38 represented 12.3% of the exposure following CPT-11 administration. However, released CPT from XMT-1001 resulted in a 1.6-fold higher exposure in tumor than that of SN-38 from CPT-11. XMT-1001 release products and SN-38 had similar durations of exposure (24 h) in the tumor. The overall degree of tumor penetration and exposure is best described by the ratio of tumor to plasma exposures of XMT-1001, CPT-SI, CPT-SA and CPT. The ratio of tumor to plasma exposure of conjugate and conjugate release products following the administration of XMT-1001 is 0.157.
XMT-1001 distributed to the liver, spleen and kidney with resulting exposures of CPT-SI, CPT-SA and CPT. This tissue distribution corresponds to the known mechanisms of clearance of conjugates, liposomes and nanoparticles(12). The liver and spleen are part of the mononuclear phagocyte system (MPS) and their role is to filter large particles from the blood. Monocytes, macrophages and dendritic cells serve as scavengers of the MPS removing circulating large particles from the blood and as well as in other tissues and facilitate their transport to end organs of the MPS for removal(16).

Previously, XMT-1001 showed effectiveness against human colon (LS174T) and ovarian (A2780) carcinoma cell xenografts(42). In our current study, the antitumor response of XMT-1001 appears to be dose and schedule dependent. The best overall response was observed in the treatment group that received XMT-1001 at 30 mg/kg IV twice weekly × 5. This regimen resulted in 5 CR and 5 PR at day 100. XMT-1001 (60 mg/kg) administered once weekly was also more effective in controlling tumor growth compared to higher doses of CPT-11 (100 mg/kg once weekly) on the same regimen.

Results from the Phase I clinical trial of XMT-1001 also support the design of XMT-1001 to release primarily the lipophilic, active lactone-stabilized analog CPT-SI and avoid the severe bladder toxicity associated with CPT. In contrast to clinical results with alternative camptothecin-polymer-conjugates, such as pegamotecan, which release CPT directly and are associated with this toxicity(43, 23), results from clinical studies have shown that XMT-1001 is not associated with hemorrhagic cystitis(44). Compared to CPT and irinotecan, XMT-1001 also has an improved therapeutic index in human tumor xenograft models (45). In addition, conjugates of irinotecan and SN-38 commonly cause significant diarrhea, while not causing hemorrhagic cystitis (23,46,47).

The development of conjugate nanoparticles to improve the pharmacokinetic and pharmacodynamic disposition of small molecule anticancer agents is a promising technique to circumvent the less than ideal chemical properties of some small molecule agents. This
technique also helps to improve the delivery of active drug to tumors where it can exert its cytotoxic activity, as well as preventing the broad distribution of chemotherapy to normal tissues, where toxicity can occur. XMT-1001, a polymer conjugate of CPT, exhibits prolonged plasma and tumor exposure and improved antitumor efficacy compared to CPT-11 in HT-29 human colon carcinoma xenografts. The results of this study suggest that XMT-1001 has pharmacologic properties that may make it superior to other small molecule camptothecin analogues. XMT-1001 is currently being evaluated in a phase I clinical trial in patients with advanced stage solid tumors (48).

References


Figure 1. Dual-phase release of camptothecin (CPT) from XMT-1001 occurs via lipophilic intermediates, CPT-20-O-(N-succinimido-glycinate) (CPT-SI) and CPT-20-O-(N-succinamidoylglycinate) (CPT-SA). Hydrolysis of intermediates results in formation of an active-lactone form of CPT.

Figure 2. A, mean tumor growth curves for mice (n=10 mice per group) bearing HT-29 human colon carcinoma xenografts, and B, Kaplan-Meier plot comparing saline (control), CPT-11 and XMT-1001. Mice were treated with saline (×) i.v. qwk × 5; CPT-11 (▲) 50 mg/kg i.p. biwk × 5, (▼) 100 mg/kg i.p. qwk × 5, or (▽) 50 mg/kg i.p. qwk × 5; or XMT-1001 (○) 15 mg/kg i.v. biwk × 5, (●) 30 mg/kg i.v. biwk × 5, (□) 15 mg/kg i.v. qwk × 5, (□) 30 mg/kg i.v. qwk × 5, (■) 60 mg/kg i.v. qwk × 5, or (◆) 60 mg/kg i.v. qwk × 5.

Figure 3. Individual and mean concentration versus time profiles of: XMT-1001 (A), CPT-SI (B), CPT-SA (C) and CPT (D) in plasma, tumor and tissue of female nude (nu/nu) mice bearing HT-29 human colon carcinoma xenografts following administration of XMT-1001 at 60 mg/kg (CPT equivalents) IV × 1 via tail vein. Samples (n = 3 mice at each time point) were obtained at 0.083, 0.25, 0.5, 1, 2, 4, 6, 16, 24, 48, 72, 168 and 336 h following administration of XMT-1001. Concentration in a single mouse is represented by a symbol other than a (−). Mean concentration of at each time point is represented by a (−).

Figure 4. Individual and mean concentration versus time profiles of: CPT-11 (A) and SN-38 (B) in plasma, tumor and tissue of female nude (nu/nu) mice bearing HT-29 human colon carcinoma xenografts following administration of CPT-11 100 mg/kg IP × 1. Samples (n = 3 mice at each time point) were obtained at 0.083, 0.25, 0.5, 1, 2, 4, 6, 16 and 24 h following administration of CPT-11. Concentration in a single mouse is represented by a symbol other than a (−). Mean concentration of at each time point is represented by a (−).
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Table 1. Tumor response parameters of female nude (nu/nu) mice bearing HT-29 human colon carcinoma xenografts following administration of CPT-11 and XMT-1001.

Abbreviations: CR: complete regression; MTV (n): median tumor volume (number of animals assessed); PR: partial regression; TFS: tumor-free survivor; TGD: tumor growth delay; TGI: tumor growth inhibition; TTE: time to endpoint.

* Mann-Whitney U test.
† Log-rank test.
‡ CPT-11 50 mg/kg i.p. qbiwk × 5 vs XMT-1001 30 mg/kg i.v. qbiwk × 5; log-rank, P = 0.0089.
§ CPT-11 100 mg/kg i.p. qwk × 5 vs XMT-1001 60 mg/kg i.v. qwk × 5; Mann-Whitney U test, P = 0.0147.
¶ CPT-11 100 mg/kg i.p. qwk × 5 vs XMT-1001 60 mg/kg i.v. qwk × 5; log-rank, P = 0.0229.
†† XMT-1001 15 mg/kg i.v. qbiwk × 5 vs XMT-1001 30 mg/kg i.v. qwk × 5; Mann-Whitney U test, P = 0.0115.
‡‡ XMT-1001 15 mg/kg i.v. qbiwk × 5 vs XMT-1001 30 mg/kg i.v. qwk × 5; log-rank, P = 0.0007.
§§ XMT-1001 30 mg/kg i.v. qbiwk × 5 vs XMT-1001 60 mg/kg i.v. qwk × 5; Mann-Whitney U test, P = 0.0003.
*** XMT-1001 30 mg/kg i.v. qbiwk × 5 vs XMT-1001 60 mg/kg i.v. qwk × 5; log-rank, P = 0.0002.
††† XMT-1001 30 mg/kg i.v. qbiwk × 5 vs XMT-1001 60 mg/kg i.v. qwk × 5; Mann-Whitney U test, P < 0.0001.
‡‡‡ XMT-1001 30 mg/kg i.v. qbiwk × 5 vs XMT-1001 60 mg/kg i.v. qwk × 5; log-rank, P = 0.0064.
Figure 2. A, mean tumor growth curves for mice (n=10 mice per group) bearing HT-29 human colon carcinoma xenografts, and B, Kaplan-Meier plot comparing saline (control), CPT-11 and XMT-1001. Mice were treated with saline (×) i.v. qwk × 5; CPT-11 (▲) 50 mg/kg i.p. biwk × 5, (▼) 100 mg/kg i.p. qwk × 5, or (△) 50 mg/kg i.p. qwk × 5; or XMT-1001 (○) 15 mg/kg i.v. biwk × 5, (●) 30 mg/kg i.v. biwk × 5, (◇) 15 mg/kg i.v. qwk × 5, (□) 30 mg/kg i.v. qwk × 5, (■) 60 mg/kg i.v. qwk × 5, or ( وعد ) 60 mg/kg i.v. qwk × 5.
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Table 2. Noncompartmental pharmacokinetic parameters following administration of XMT-1001 and CPT-11 in mice bearing HT-29 human colon carcinoma xenografts. Pharmacokinetic parameters of XMT-1001, CPT-SI, CPT-SA and CPT in female nude (nu/nu) mice bearing HT-29 human colon carcinoma xenografts following the administration of XMT-1001 at 60 mg/kg (CPT equivalents) IV × 1 via tail vein, (A). Pharmacokinetic parameters of CPT-11 and SN-38 in female nude (nu/nu) mice bearing HT-29 human colon carcinoma xenografts following the administration of CPT-11 at 100 mg/kg IP × 1, (B).
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* AUC$_{0-t}$
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