6 Preclinical Models for Evaluating Topoisomerase I-Targeted Drugs

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1. INTRODUCTION

Type I and II DNA topoisomerases are the targets for numerous clinically efficacious antitumor agents. Over the last decade, considerable effort has been expended in developing camptothecin (CPT) derivatives that selectively target DNA topoisomerase I (TOP-I) *(1)*. The prodrug irinotecan (CPT-11) is approved for treatment of colon carcinoma and has demonstrated significant activity against numerous other cancers in adults and

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children. Topotecan is approved for treatment of platinum- or taxane-resistant ovarian carcinoma and has demonstrated broad-spectrum activity *(2)*. Other analogs are in clinical development, such as D5198f and the homocamptothecins and liposomal formulations of CPT derivatives, and offer the potential for prolonged plasma exposures.

Agents targeting TOP-I in clinical trials have proceeded through the preclinical stages of identifying cytotoxic potency and confirmation of in vivo antitumor activity. Acceptable toxicity in rodents and other species, as mandated by regulatory agencies, had been studied before clinical evaluation. CPTs have demonstrated remarkable activity against animal models *(3)*. However, less dramatic clinical activity has been reported, resulting in the discontinuation of at least one agent, 9-aminocamptothecin (9-AC).

In this review, we examine this preclinical-clinical interface with respect to understanding the value and limitations of preclinical models. Hopefully, lessons learned regarding development of camptothecins can be applied to the future development of drugs that induce cytotoxicity through their interactions with TOP-I. This article will focus on preclinical models used to assess antitumor activity and toxicity for TOP-I–targeted drugs and how information derived from valid models may be used to direct the design of clinical trials.

2. EARLY STUDIES

CPT was studied extensively in the Cancer Chemotherapy National Service Center of the National Cancer Institute during the 1960s. It was formulated in carboxymethylcellulose and administered by intraperitoneal (ip) injection using the Walker 256 rat carcinosarcoma model as the test system. Relative to other drugs evaluated, camptothecin had relatively poor activity *(4)*. However, the sodium salt of CPT demonstrated significant activity in increasing survival time in several lymphocytic leukemias *(5)*. Based on a lack of cross-resistance to dichloromethotrexate, BCNU, cytosine arabinoside, 6-mercaptopurine, and other agents, it was proposed that CPT had a novel mechanism of action. In contrast to the significant activity observed in these preclinical models, CPT, evaluated as the sodium salt, was found to be ineffective in patients with advanced disseminated melanoma or gastrointestinal malignancies *(6,7)*. Severe toxicities included myelo-suppression, vomiting, diarrhea, and hemorrhagic cystitis and resulted in the discontinuation of the clinical trial of sodium CPT. Other studies in China, however, demonstrated activity of 10-hydroxycamptothecin in treatment of head-and neck-and bladder cancers (reviewed in ref. *8*).

Studies by the Liu laboratory defined TOP-I as the target for CPT and the observation that the CPTs caused trapping of TOP-I on DNA and induced single-strand breaks *(9,10)* served as an impetus to reexplore this class of agent. Although CPT is frequently referred to as an "inhibitor" of TOP-I, it

actually functions to convert this cellular enzyme into a cellular toxin *(11)*. CPTs inhibit the religation step, effectively trapping covalently linked TOP-I on DNA after a single-strand nick has been made by the enzyme. In cells replicating DNA, this could result in a collision between an advancing replication fork and the covalently linked enzyme-DNA complex, leading to replication fork stalling or a double-strand DNA break. The mechanism leading to cell death remains to be characterized, although it is believed that double-strand DNA breaks can initiate a cascade leading to apoptosis. Thus increased levels of TOP-I would favor increased formation of DNA–TOP-I–drug complexes, which would increase the probability of a collision with the advancing replication fork and the generation of DNA damage. In the absence of DNA replication, the reversibly stabilized DNA–TOP-I covalent complexes are not toxic, unless suprapharmacological drug concentrations are used. Based on the mechanism of action, one would anticipate predominantly or exclusively S-phase cells would be sensitive to the CPTs *(12)*. Because many human cancers are characterized by having relatively low growth fractions, protracted infusions or repeated exposures to drug over a long period should optimize cell killing.

3. RODENT TUMOR MODELS

Syngenic transplanted rodent tumors have been used as the primary in vivo screen for the activity of the CPT analogs. For leukemic models, such as L1210 or P388, tumor cells are inoculated to the peritoneal cavity, and drugs are administered ip. End points for these experiments are the drug-induced increase in lifespan (ILS). Thus with increasing drug dose there is an increase in lifespan until ILS is reduced because of drug induced toxicity. These models are valuable in determining differences in efficacy between analogs. Although such tests have been described as “in vivo test tubes,” an objective of such screens is to avoid elimination of active compounds (i.e., false negatives).

In developing a CPT derivative at SmithKlineBeecham, several in vivo criteria were established for selecting analogs for further development. These included (1) being as active as CPT in a panel of preclinical models and (2) minimizing the requirements for camptothecin as a starting material—therefore, the analog was required to demonstrate potency in vivo (i.e., a maximally tolerated dose, MTD) at similar or lower levels than CPT *(8)*. The analog 9-dimethylaminomethyl, 10-hydroxycampto-thecin (topotecan) demonstrated superior ILS in mice bearing L1210 leukemia compared with that achieved by camptothecin at their respective MTD (173 ± 16 versus 118 ± 6% ILS).

For reasons given previously, protracted therapy with TOP-I inhibitors theoretically should prove most efficacious. Thus, assuming reasonable oral bioavailability, oral administration may prove to be most practical in

therapy of human cancer. Secondary evaluation of topotecan compared the efficacy of oral and intravenous (iv) administered drug in syngeneic mice bearing (1) advanced systemic (iv inoculated) L1210, (2) advanced systemic (iv inoculated) Lewis lung carcinoma,(3) subcutaneously implanted Lewis lung carcinoma, (4) systemic (iv) B16 melanoma, and (5) ip implanted M5076 reticulum cell sarcoma *(13)*. Drug was administered every 3 hours four times per day at 4- or 7-day intervals. Orally administered topotecan was comparable in efficacy to parenteral treatment in four of five tumor models tested. The M5076 sarcoma implanted ip responded to topotecan administered ip or subcutaneously, but not when given orally.

Irinotecan (CPT-11; 7-ethyl-10-(4-[1-piperidino]-1-piperidino)-carbonyloxy-(20S)camptothecin) is a prodrug activated in rodents by plasma carboxylesterases and has been extensively studied in syngenic tumors *(14–16)*. Irinotecan demonstrated significant activity by both parenteral and oral routes against disseminated models, including the intravenous inoculation of the highly metastatic B16-F10 melanoma and the spontaneous metastases from subcutaneous implants of murine colon 26. The most comprehensive study reported *(16)* evaluated irinotecan in 10 murine tumors and 1 human xenograft. All 11 tumors responded to irinotecan, with 8 of them being responsive at the Decision Network-2 level (in which treated/control volumes were <10%), the criteria used by the National Cancer Institute to justify further development. This work also showed no cross-resistance in vivo in P388/vincristine leukemic cells resistant to vincristine and in human breast carcinoma cells selected for resistance to docetaxel (Taxotere). Thus rodent models indicate that camptothecins have significant antitumor efficacy. However, rodents appear to be highly resistant to the toxic effects of CPTs. For example, pharmacokinetic data showed that plasma concentrations and exposures of SN-38 (7-ethyl,10-hydroxy-(20S)camptothecin), the active metabolite of irinotecan, were significantly higher in mice than can be achieved in patients. Although this problem is not unique to CPTs, it is particularly problematic to irinotecan, because metabolism in mice is very different from that in humans.

4. HUMAN XENOGRAFT MODELS

Since the early 1980s, human tumor xenografts grown in immune-incompetent mice have to a large extent replaced transplantable syngenic animal models. There remains debate over the predictive value of these tumor models, because there was poor correlation between drug activity against specific tumor types in mice and comparable tumor histologies in clinical trials *(17)*. In contrast, our experience using models of pediatric solid tumors have been highly predictive in identifying agents active against specific tumor types *(18,19)*. The minimum requirement for validation of

these models is that they should parallel the chemosensitivity-chemoresistance profile of the clinical disease. Thus one would anticipate colon carcinomas would be less sensitive to chemotherapeutic agents than, for example, pediatric rhabdomyosarcoma.

The most frequent approach taken is to heterograft surgical specimens of tumor into mice that are congenitally immune deficient (athymic nude mice or severe combined immunodeficiency mice) or mice that have been immune-deprived to prevent graft rejection. Alternatively, cells initially propagated in vitro from human tumors may be injected into these mice subcutaneously, or iv, if disseminated disease is required. In certain circumstances (for example, evaluation of brain tumors), it may be important to assess the preclinical activity of a new drug under conditions that closely mimic the clinical situation, in which case the development of orthotopic models can be attempted by injecting cells into the analogous site within the host. However, conditions for tumor growth in the mouse may differ from patients and differences in drug disposition and metabolism in the mouse may significantly influence tumor responses. Thus orthotopic models still have intrinsic limitations characteristic of other preclinical in vivo models. Several lines representing a tumor type are generally required to accurately recapitulate the clinical situation and to conduct "preclinical phase II evaluation." We have used six tumor lines per tumor "model" (e.g., rhabdomyosarcoma, neuroblastoma). However, the exact number that accurately predicts clinical response rates has not been determined and may require at least 10 tumors per histiotype. In developing topotecan for the treatment of neuroblastoma, we found that a daily systemic exposure to 100 ng/hour/mL topotecan lactone gave objective regressions in four of six neuroblastoma xenograft models. Interestingly, targeting the same exposure using the same schedule of drug administration in children with stage IV neuroblastoma yielded a response rate of approximately 60%.

The initial study by Giovanella and colleagues *(3)* demonstrating the curative activity of 9-AC in chemorefractory colon cancer xenografts served to focus considerable attention on this class of anticancer agent *(3)*. Subcutaneous administration of drug was highly active, whereas subsequent studies with iv administration were relatively disappointing. Significant antitumor activity of camptothecin analogs has been confirmed using an extensive panel of human tumor xenografts possessing a broad pattern of biological properties and chemosensitivities *(20–34)* (Table 1). In contrast, standard agents used for clinical treatment of the appropriate tumor type, showed considerably less activity. 9-AC induced complete remissions in mice bearing xenografts of colon adenocarcinoma and malignant melanoma BRO xenografts. 9-Nitrocamptothecin (rubitecan), is converted to 9-AC and is currently under clinical investigation. 9-Nitrocamptothecin demonstrated superior therapeutic efficacy compared with 9-AC and CPT

in a large number of human xenograft models *(21)*. Topotecan also demonstrated good antitumor activity when administered iv, ip, and orally against xenografts derived from various childhood solid tumors; of note topotecan induced a high percentage of complete regressions in rhabdomyosarcomas, neuroblastomas, and some brain tumors. Clinical studies support the predictive value of these models. Clinically, topotecan has elicited high response rates in rhabdomyosarcoma *(35)*, neuroblastoma (approximately 60%), and medulloblastoma (approximately 35%). In contrast, reduced activity was found against colon carcinoma xenografts *(22)*.

Irinotecan was a highly efficacious analog in preclinical studies and currently is the "gold standard" against which new analogs are compared. When administered by iv, ip, or oral routes, irinotecan showed substantial activity against a broad spectrum of human tumor xenografts, including human cancer xenograft lines unresponsive to many cytotoxic agents. High cure rates were obtained against MX-1 mammary tumor, rhabdomyosarcomas, neuroblastomas, colon cancers, and brain tumors. Activity was also retained against tumors selected for resistance to topotecan, vincristine, melphalan, busulphan, procarbazine, and cyclophosphamide. As mentioned previously, mice readily activate irinotecan to SN-38, and plasma systemic exposure to SN-38 in mice greatly exceeds that achieved in patients. Thus exposures to SN-38 associated with tumor regressions in mice may be far in excess of exposures achievable in patients (discussed in Section 5.).

Two water-soluble analogs of CPT, GI147211 and GI149893 (10,11-methylenedioxy, 7-substituted compounds), have been assessed in preclinical models of colon and mammary carcinoma. Antitumor effects were dose- and schedule-dependent, with a greater reduction in tumor volume achieved by protracted dosing. Concurrent experiments demonstrated that these agents were more effective than topotecan in suppressing tumor growth, although optimal schedules for topotecan were not compared in these studies *(27)*. As a liposomal formulation, GI147211 (designated NX211) has demonstrated good antitumor activity against more than 20 lines of tumor xenografts and yielded with minimal toxic effects.

Alternatively, it has been proposed that hydrophobic CPTs may have greater E-ring stability, and hence may exert longer plasma exposures of the lactone form. 7-[(2-trimethylsilyl)ethyl)]-20 (S)-camptothecin (Karenitecin) is under clinical development, as are other water-insoluble derivatives such as DB-67. Homocamptothecins with an expanded E-ring are also in clinical trials. Again, increased lactone stability was the rationale behind the synthesis of these E-ring-modified agents.

For many of the experiments reported in Table 1, systemic exposures in the mouse to lactone forms of the given CPT derivative far exceed exposures that can be achieved in patients at tolerated dose levels. The data presented demonstrate the relative sensitivity of a given tumor to a series of analogs

administered to their MTD levels in the mouse. Such data may overestimate the potential for activity in patients. Without knowing the relative toxicity–systemic exposure relationship in humans, such data may have limited predictive value for selecting analogs for further development. Although determination of the therapeutic efficacy in murine models serve as a potential criterion to select among analogs (assuming that mouse toxicity accurately reflects dose limiting toxicity in patients), it may have relatively little value in predicting clinical antitumor activity. For example, irinotecan administered daily for 10 days (MTD approximately 40 mg/kg/day) causes objective regressions in approximately 50% of colon carcinoma models *(24)*. However, we now know that a dose of 1.25 mg/kg to mice generates plasma SN-38 exposures that are tolerated in patients when irinotecan is administered on the same schedule. Thus evaluating the effect of camptothecin at the MTD in mice significantly overpredicts clinical activity.

5. SCHEDULE-DEPENDENT ANTITUMOR ACTIVITY

Animal models have been useful for examining alternative schedules of drug administration. Obtaining information about the schedule dependency in relation to both the antitumor activity and host toxicity of an agent is one of the goals of preclinical studies. TOP-I inhibitors are S-phase-specific cytotoxins. It is assumed therefore that after a cytotoxic threshold is achieved, exposure time, rather than further dose escalation, is the important parameter for determining the tumor response. Consequently, protracted drug administration could increase antitumor activity. Recent clinical data support schedule-dependent activity *(36)*, and additional clinical data, even in phase I trials, show greater antitumor activity is associated with protracted schedules of administration *(19,37)*.

The importance of scheduling was first reported by Kawato *(20)*. Additional testing confirmed this observation *(21–23)*. These studies showed that, for similar total dosages, protracted schedules were more effective than were more intense treatments of shorter duration. Several groups have reported schedule-dependent activity of camptothecin analogs, although this finding does not appear to have been used in design of the initial clinical trials *(38)*. Schedule-dependency is illustrated in Fig. 1, where the responses of individual rhabdomyosarcoma Rh30 xenografts have been measured in mice receiving drug vehicle (control) or topotecan treatment. Both treatment groups received the same total dose of drug, the only difference being that topotecan was either given over 5 days or 10 days. Clearly, topotecan administered over 10 days was significantly more active than the same dose given over 5 days. Also, in xenograft models drugs such as topotecan and irinotecan appear to be “self-limiting.” Above some dose level, further increases in dose per administration do not result in further antitumor activ-

Table 1
Responsiveness of Human Tumor Xenografts to Treatment With Camptothecin Analogs

Drug	*Xenograft tumor*	*Dose (mg/kg) and schedule*	*Comments*		*Reference*
9AC	Colon HT-29 Colon CASE Colon Sw48	10–12.5 mg/kg × 2 /week for 5–6 weeks SC	Highly effective	ADR, 5-FU, MTX nitrosoureas, ALK, less effective/ineffective	*3*
CPT11	Mammary MX-1 Gastric St-15 Gastric SC-6 Lung QG56 Colon Co-4	200 mg (TD) iv 400–00 mg (TD) q (4 days × 3) PO	Very significant antitumor activity against all tumors Curative against MX-1 CPT11 more effective as three injections than one single injection for same total dose.	ADR, 5FU, CDDP less effective	*20*
CAM 9AC 9NC	Melanoma BRO	4 mg × 2/week IM	Growth inhibition and tumor regression	BRO tumors unresponsive to ADR, 5-FU, VCR, VBL, MTX, nitrosourea, and ALK.	*21*
Topotecan	Six rhabdomyo-sarcoma lines Seven colon lines Three osteosar-coma lines	1.5–2.0 mg (days × 5)3 IV/PO 12.5 mg q (4 days × 4) IP	Complete regressions in rhabdomyosarcomas. Significant activity in osteosarcomas. Growth inhibition in several colon lines. Results suggest significant schedule dependency		*22*
9AC 9NC 9CL-CAM	Breast carcinoma		9AC effective. 9NC effective. 9CL-CAM not effective. Results were dose, schedule, and route of administration-dependent.	Short infusions (72 hours every 21 days) not effective. Long infusions (5 days every 7 days) very effective.	*23*

CPT11	Six rhabdoyosarcoma lines Seven colon lines	10–40 mg (days ×5)2 IV and [(days ×5)2]3 IV Complete responses in five	All tumors very sensitive Complete and partial regressions in five out of eight colon lines. resistant to VCR lines out of six rhabdomyosarocma	CPT 11 effective against two xenografts selected for resistance to topotecan and rhabdomyosarcoma lines and melphalan.	*24*
CPT11	TNB9 Neuroblastoma	15–59 mg q (4 days ×3) IP	Growth inhibition.	VCR, Aclarubicin, VP16, 5-FU, and THP-ADR, ineffective.	*25*
CPT11 Topotecan	Six rhabdomyosarcoma lines Eight colon lines Three brain tumor lines	CPT11: 2.5–10 mg[(days ×5)2]3 IV Topotecan: 0.5–1.5 mg (days ×5) 12 PO Concluded: Low-dose	CPT 11 highly active (complete regressions) against colon lines. Both drugs similar high activity against rhabdomyosarcomas and brain tumors. protracted scheduling of daily administration is equiefficacies than shorter, more intense, schedules	CPT 11 and topotecan active against tumors selected for resistance to VCR CPT11 active against tumor selected for resistance to melphalan	*26*
Topotecan G1147211 G1149893 Prostate PC-	Colon HT-79 Colon SW-48 Mammary MX-1	3 MTD divided into three doses infused q 4 hourly in 24 hours ×2/week for 5 weeks Topotecan:	G1147211: G1149893: Regressions >50% in HT79 and SW-48 Complete regressions in MX-1, growth inhibition in PC3. Growth inhibition only		*27*
CPT11	PNET SKNMC Neuroblastomas	27–40 mg (days ×5) IV and q (4 days ×3) IV	Very effective, high complete response rates.		*28*

(continued)

Table 1 (Continued)

Drug	*Xenograft tumor*	*Dose (mg/kg) and schedule*	*Comment*		*Reference*
	N835 NB8 NB3				
CPT11	Six neuroblastoma lines	10–40 mg (days ×5)2 IV 5–10 mg [(days ×5)2]3 IV 25–50 mg (days ×5)12PO	Highly efficacious Complete regression of all tumors on the protracted iv schedule and all tumors using oral schedule		*29,30*
CPT11	Nine Brain tumor lines Gliomas Ependymomas Medulloblastomas	40 mg (days ×5)2 IP	Tumor regression in every treated tumor line.	CPT11 active against tumors resistant to busulphan, procarbazine, cyclophos-phamide, and melphalan.	*31*
9AC	Prostate PC3	2 mg ×2/week for 3 weeks SC 0.35–1 mg (days ×5)3 PO	Inhibition and regression of tumor growth.	PC3 is a hormone refractory prostate line	*32*
Topotecan	Six neuroblastoma lines	0.36 2 mg [(days ×5)2]3 IV	Highly effective Complete regressions in all tumors.	Minimum daily systemic exposure related to objective response	*33*

CAM, camptothecin; 9AC, 9-aminocamptothecin; 9NC, 9-nitrocamptothecin; 9CL-CAM, 9-chlorocamptothecin; SC, subcutaneous; IM, intramuscular; IV, intravenous; PO, by mouth; IP, intraperitoneal.

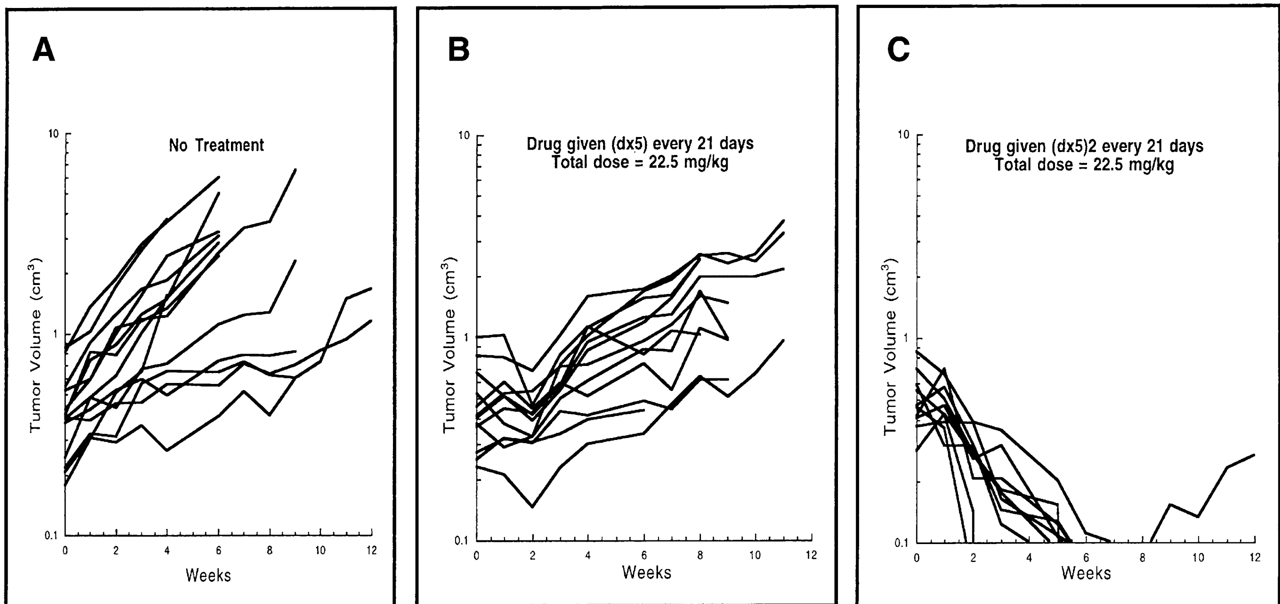

Fig. 1. Schedule-dependent antitumor activity of topotecan. Mice bearing Rh30 rhabdomyosarcoma xenografts were randomized into three groups of seven mice. Group A received no treatment (controls); group B received topotecan daily for 5 days at a dose of 1.5 mg/kg/day. Mice in group C received 0.75 mg/kg daily for 5 days on 2 consecutive weeks. Courses of treatment were repeated ever 21 days over 8 weeks for groups B and C. The total dose per 21-day course was 7.5 mg/kg for both treatment groups, and total drug per three cycles was 22.5 mg/kg. Each curve shows the growth of an individual tumor. In group B, topotecan caused disease stabilization, but most tumors progressed through treatment. In contrast, delivering the same dose of topotecan over 10 days induced complete responses with only a single tumor regrowth during the period of observation (12 weeks). (Houghton, unpublished data).

ity *(21)*. Similar results have been obtained with 9-AC and other CPT analoganalogs in various tumor models. Interestingly, administration of irinotecan using the (day × 5) 2 schedule every 21 days has demonstrated significant antitumor activity in a phase I clinical trial in children with tumors resistant to conventional therapy *(19,39)*.

6. MODELS OF RESISTANCE TO TOP-I INHIBITORS

Two CPT analogs, topotecan and irinotecan, are approved for treatment of refractory ovarian carcinoma and 5-fluorouracil refractory colon carcinoma, respectively. Thus new agents should demonstrate clear superiority over these established drugs to justify full development. Irinotecan is highly active against certain tumors that are intrinsically resistant to topotecan and against some xenografts selected *in situ* for acquired resistance to topotecan *(24)*. Several cell lines selected for resistance to camptothecin have been reported. In one line (CEM/C2), resistance is mediated by a mutation (Asn722Ser) in TOP-I *(39)*. In yeast, several mutations in TOP-I yield CPT resistance *(40)*. However, it is less certain in clinical tumors whether intrinsic or acquired resistance is the result of TOP-I mutations. Thus establishing xenograft models from cell lines in which resistance is caused by mutant enzyme may not necessarily recapitulate clinical resistance. At this time, mechanisms conferring CPT resistance in clinical cancers remain uncharacterized; however, the role of the breast cancer resistance–associated protein is associated in vitro with resistance to several camptothecin analogs *(41–44)*. Resistance to CPTs is undoubtedly complex, potentially analog-specific, and involves mechanisms proximal to DNA damage (i.e., accumulation/efflux), at the target level (mutation or activity of TOP-I) or distal to damage (repair processes). In several xenograft models, selection *in situ* for resistance to topotecan did not result in cross-resistance to irinotecan *(24)*. However, relatively few models of acquired resistance to CPT analogs have been reported. Relatively rapid development of an irinotecan-resistant neuroblastoma xenograft (NB-1691/CPT) has been reported *(45)*. Resistant tumors were derived after only four rounds of treatment/transplantation a stable irinotecan-resistant line was derived. This tumor is partially resistant to topotecan. Although the mechanism of resistance remains to be characterized, this tumor may represent a useful model for identifying novel TOP-I-targeted agents with characteristics significantly different from either irinotecan or topotecan.

7. TOXICITY

7.1. Hematopoietic Toxicity

The often dramatic preclinical activity of CPT analogs in xenograft models contrasts with the clinical activity observed in many phase II stud-

ies. As would be predicted from their S-phase activity, TOP-I inhibitors cause dose-limiting toxicity to rapidly renewing tissues such as hematopoietic tissues in humans and animals. Dose-limiting toxicity occurs at far lower systemic exposures in humans than in mice. Humans can tolerate only 11% as much topotecan per day as mice. This differential may be greater for irinotecan. Based on pharmacokinetic estimates of SN-38 systemic exposure at the MTD in patients receiving irinotecan every 7 days, it was estimated that the systemic exposure represented only 6% of the MTD in mice *(46)*. For myelosuppressive CPT analogs, failure to achieve drug exposures in patients that are curative in the murine models might be due to greater sensitivity of human myeloid progenitors. Using in vitro colony-forming assays, Erickson-Miller et al. *(47)* showed that hematopoietic progenitors of the myeloid lineage from humans, mice, and dogs exhibit differential sensitivity to the CPTs. The toxicity of CPT analogs to human and animal myeloid progenitors was quantified from the inhibition of marrow colony-forming unit–granulocyte macrophage (CFU-GM) colony. CPT lactone, topotecan, and 9-AC inhibited colony formation in a concentration-dependent manner. These results suggest that, because of greater sensitivity of the myelopoietic tissue, humans cannot tolerate exposures to CPTs that are curative in murine models. Relative to human myeloid progenitors, murine myeloid progenitors are relatively insensitive to all the compounds examined. The differences between mice and humans are large. For example, the concentration of topotecan causing a 50% reduction in CFUs was 46-fold lower for human cells when compared with murine progenitors. This differential was even greater (107-fold) for 9-AC *(47)*. The susceptibility of human CFU-GM to drug toxicity is more closely approximated by canine than by murine CFU-GM. This finding explains, in part, why even subcurative doses of CPTs may be severely myelotoxic in patients. The use of in vitro systems for predicting human tissue toxicity may have wider application to drug development *(48,49)*.

7.2. Gastrointestinal Toxicity

Irinotecan, administered on most of the schedules evaluated clinically, has been associated with an unanticipated and significant diarrhea. This is characterized by the early onset of symptoms and is probably a consequence of the acetylcholinergic activity of the bipiperidino side chain *(50)*. This toxicity is well controlled by atropine. However, delayed diarrhea is now recognized as a dose-limiting toxicity of this compound *(51)*. This toxicity was not anticipated from studies in rodents, in which diarrhea was not observed. Diarrhea may be caused by abnormalities of intestinal absorption or secretion, increased peristalsis, or drug-induced epithelial damage. The considerable interpatient variability in the severity of the diarrhea has made it difficult to explain the mechanism of irinotecan-associated diarrhea. This

toxicity, however, is not unique to irinotecan. Delayed-onset diarrhea is the dose-limiting toxicity of topotecan administered orally to patients for 21 days. The etiology of this side effect of the CPTs is not yet clear, although several animal models have been established that attempt to simulate irinotecan-induced diarrhea.

7.2.1. Mouse Models

Ikuno et al. *(52)* observed characteristic changes in the intestinal mucosa of irinotecan-treated mice, including villous atrophy characterized by marked shortening of the villi, epithelial vacuolation of the ileum (associated with increased apoptosis), and goblet cell hyperplasia in the cecum. These structural and functional effects were postulated as the main causes of irinotecan-induced diarrhea and resulted in malabsorption and hypersecretion of mucin. Malabsorption in irinotecan-treated mice was thought to be caused by villous atrophy after crypt damage and apoptosis of absorptive cells in the small intestine. The goblet cell hyperplasia associated with excessive production of mucin in the cecum could be another contributing factor to the cause of diarrhea with irinotecan. A model of intestinal toxicity has been developed in the mouse; this has been used to identify potential modulators of irinotecan-induced diarrhea. Daily administration of very high dose levels (100 mg/kg) of irinotecan to mice resulted in loss of villi, epithelial vacuolation, decreased numbers of S-phase cells in the crypts, increased apoptotic cells, and reduced numbers of lymphocytes in the lamina propria. Oral administration of a synthetic bacterial lipopeptide, JBT 3002, encapsulated in phospholipid liposomes prevented irinotecan-induced damage to the intestinal epithelium and lamina propria *(53)*. Similarly, dietary supplementation with fish oil reduced gastrointestinal damage induced by irinotecan *(54)*.

7.2.2. Rat Models

Frequently, diarrhea is caused by the active secretion of electrolytes, especially chloride ions, suggesting this toxicity is independent of the action of irinotecan or the active metabolite, SN-38, on DNA–TOP-I. Relatively high concentrations of irinotecan caused eicosanoid-mediated chloride secretion in isolated rat colon *(55)*. Irinotecan-induced diarrhea was characterized in rats by assessing the relationship between intestinal toxicity and the activity of enzymes involved in the major metabolic pathways of this drug *(56)*. In rodents, irinotecan is converted to its active metabolite SN-38 by carboxylesterase; one possible mechanism for the diarrhea might include the structural and functional injuries to the intestinal tract resulting from the direct cytotoxic activity of the SN-38. Detoxification of SN-38 occurs by liver glucoronidation and conjugated SN-38 is secreted into the bile and in the feces. Conjugated metabolites may be further converted or processed to

an active SN-38 by β-glucuronidase of the microflora resident in the large intestine. In this rat model, histological damage was most severe in the cecum, with a markedly decrease in the size and number of crypts and evidence of superficial mucosal erosion. The segmental differences in the degree of damage showed good correlation with the β-glucuronidase activity in the contents of the lumen, suggesting that this enzyme plays a key role in intestinal toxicity induced by irinotecan. Intestinal tissue carboxylesterase activity, which also converts irinotecan to its active form, showed poor correlation to the degree of tissue damage. Administration of antibiotics to sterilize the intestine exerted a protective effect against the diarrhea by completely inhibiting the β-glucuronidase activity of the intestinal flora and, accordingly, the formation of active SN-38. Rustum and colleagues *(57)* have also developed a rat model of irinotecan-induced gastrointestinal toxicity. In their study, very high dose levels of irinotecan (150–200 mg/kg daily × 3 iv) resulted in 86–100% lethality in treated animals and 93–100% incidence of severe diarrhea, which was associated with serious damage to the duodenal villi and colonic crypts. Interleukin-15 (100–400 μg/kg (3, 8, and 11 doses ip) completely protected against irinotecan-induced delayed diarrhea and lethality. The validity of these rodent models must, however, wait for confirmatory results in other models and, ultimately, clinical trials.

7.2.3. Hamster Models

The hamster has also been proposed as a model for irinotecan-induced intestinal toxicity *(58)*. Female Syrian hamsters were dosed ip with irinotecan (50 mg/kg/day) for 10 days and observed through day 20. By day 5, all treated animals had developed diarrhea and deaths occurred starting on day 7. Histological examination revealed a time-dependent loss of structural integrity in the jejunal and ileal mucosa; the typical columnar morphology of the epithelial cells was lost and the villi appeared corrugated. The epithelium was thinned and vacuolated in the colon within the first 5 days of treatment. Detection of proliferating cell nuclear antigen showed an increase in the number of labeled epithelial cells and labeling intensity in treated animals. The labeled cells were located further toward the tips of villi compared with control animals. Increased levels of proliferating cell nuclear antigen and loss of differentiation in cell morphology suggested that irinotecan induces a cell-cycle block in S-G2, with subsequent loss of physiologic function in hamster intestinal epithelium. Kobayashi et al. *(59)* have also studied the effect of pH on uptake of irinotecan, SN-38 lactone, and SN-38 carboxylate in isolated intestinal cells from Syrian hamsters. From these studies, it is proposed that uptake of lactone is by passive diffusion, whereas there may be an energy-dependent accumulation (transport) for carboxylate. Accumulation of irinotecan carboxylate showed saturation kinetics with apparent K_m approximately 50 μ*M* in jejunal and ileal cells.

8. INTERSPECIES DIFFERENCES THAT COMPLICATE TRANSLATION OF PRECLINICAL RESULTS

8.1. Interspecies Differences in Drug Metabolism and Disposition

CPTs have demonstrated greater activity against model tumors in rodents than against tumors in patients. In part, this appears to be a consequence of the greater tolerance of the toxic effects of these agents in mice than in humans. Analysis of data from mice and rats showed that predicting clinical maximally tolerated doses for eight TOP-I inhibitors from rodent data would result in starting clinical trials very close to, or at dose levels exceeding, the human MTD *(60)*. In contrast, initial starting doses based on canine data would be safe. The plasma systemic exposures, expressed as an area under the concentration-time curve, for irinotecan and its active metabolite SN-38 in mice *(16,61)* and patients *(62–64)* are presented in Table 2. To facilitate comparison between schedules, systemic exposure has been expressed for each course of therapy, usually in a 21-day time frame at the highest non-toxic dose for mice and the MTD for humans. Not all investigators report both the lactone and total drug, hence it is difficult to directly compare the systemic exposure of irinotecan and SN-38 between studies. However, when given once weekly in humans, the systemic exposure to irinotecan and, particularly SN-38, is significantly greater in mice than in humans.

This raises the concern that studies with syngenic tumors or human xenograft models in mice may overpredict the potential clinical utility of this and other classes of anticancer drugs. For CPTs, the reasons for the interspecies differences are not well understood. Rather than use the mouse model to predict systemic drug exposures associated with toxicity, we have determined the systemic exposure associated with antitumor effect against the human tumor xenograft models. For a series of neuroblastoma xenografts, the daily systemic exposure to topotecan that caused objective regressions was determined when the drug was administered 5 days per week for 2 consecutive weeks *(33)*. Partial responses were achieved in each of six independently derived neuroblastoma lines at a daily topotecan lactone systemic exposure of 100 ng/mL/hour, whereas complete responses were achieved in four tumor lines. The results of these studies define the effective antitumor systemic exposure to the camptothecin analog. Current data from our studies in children indicate that exposure of 100 ng/mL/hour (achieved after a dose of 0.61 mg/kg in mice) results from a daily dose of approximately 3 mg/m^2 in children. For irinotecan, dose levels of approximately 1.25 mg/kg in mice yield SN-38 plasma systemic exposures achieved at doses of 20–30 mg/m^2 administered to children *(19)*. This difference is a consequence of very efficient activation of irinotecan by plasma carboxylesterase in mice. In contrast, activation of irinotecan in humans is poor.

Table 2
Comparison of Irinotecan and SN-38 Systemic Exposure (AUC) Between Mice and Humans

Reference		*Lactone Irinotecan (mg/mL/hour)*	*Lactone SN-38 (mg/mL/hour)*	*Total Irinotecan (mg/mL/hour)*	*Total SN-38 (mg/mL/hour*
Mice					
16	Irinotecan 52.5 mg/kg IV	NA	NA	62.5	34.5
61	Irinotecan 10 mg/kg IV [(days ×5)2]3	13.0	5.3	NA	NA
Humans					
62	Irinotecan 350 mg/m^2 IV once every 3 weeks	NA	NA	34.0	0.45
63	Irinotecan 100 mg/m^2 IV for 3 consecutive days every 3 weeks	NA	NA	27.9	0.96
64	Irinotecan 150 mg/m^2 week IV for 4 of 6 weeks	5.6	0.24	16.8	0.82
19	Irinotecan 20 mg/m^2 IV [(days ×5)2]3	4.0	1.4	NA	NA

Note: Total and lactone AUC have been calculated for the cumulative exposure for a 21-day cycle of therapy. AUC, area under the curve; IV, intravenous; NA, not available.

Recently, a strain of mouse, designated *Es1^e*, deficient in plasma esterases, has been identified. Kinetic studies indicated that the activation of irinotecan to SN-38 by *Es1^e* mouse plasma in vitro is 600-fold less efficient, although extracts from organs indicated no difference in drug metabolism as compared with controls *(65)*. It is proposed that the *Es1^e* mouse may represent a more representative model of irinotecan drug activation *(66,67)*.

8.2. Protein Binding

Systemic exposure to CPT analogs represents total drug concentration, which consists of both drug bound to plasma protein and unbound drug. For drugs extensively bound to plasma proteins, such as SN-38, unbound drug concentrations correlate best with the indices of pharmacologic effect. Where there is significant interspecies variability in the plasma protein binding, comparison between unbound drug concentrations and toxicity in humans and animals may be more appropriate than the total drug concentration. Interspecies differences in drug protein binding are seen with the CPT analogs. CPT exists as a pentacyclic structure with a lactone moiety in the terminal E-ring. When used against purified TOP-I, the presence of a lactone ring is a structural requirement for activity. Factors influencing the lactone-carboxylate equilibrium may therefore be important determinants of drug activity. In addition to pH, presence of protein, particularly albumin, has been shown to be important to the stability of the lactone moiety *(68,69)*. Human serum albumin has a marked preference for the carboxylate form of CPT, greater than serum albumin from five other species. Thus binding of the carboxylate to albumin drives the equilibrium away from the active lactone form of the drug. Structural modifications to CPT, as seen with irinotecan, SN-38, and topotecan, diminish the interspecies differences in stabilization of the lactone. This is in contrast with 9-AC, in which the marked interspecies difference in stabilization of the carboxylate form was similar to that observed with CPT. Four hours after intragastric administration of camptothecin or 9-nitrocamptothecin, lactone forms compose 57–81% and 47–95% of the total drug, in mouse plasma, respectively. In contrast, the lactone composed only a minor component of total drug levels in plasma from humans treated orally with either drug *(70)*. This interspecies variability in protein stabilization of the carboxylate form is important for translation of data derived in rodents to clinical trials. These results also illustrate the importance of determining the systemic exposure to lactone forms of CPT analogs that induce objective regressions in xenograft models. This information may be valuable in understanding and designing phase II clinical trials *(71,72)*. Attempts to encapsulate and stabilize lactone forms of CPTs may also increase the therapeutic utility of drugs such as CPT or topotecan *(73,74)*.

9. FUTURE DIRECTIONS

Animal models, and human tumor xenografts in particular, have predicted dramatic clinical therapeutic activity of CPT drugs. Agents, such as topotecan and irinotecan, are clearly active in several adult and childhood cancers, as well as myelodysplastic syndromes. For analogs such as 9-AC, clinical results have been quite disappointing. Retrospective analysis of preclinical data for TOP-I inhibitors shows that such differences are caused by interspecies differences in drug disposition and host tolerance. For CPTs, mice tolerate significantly greater systemic exposure than can be achieved in patients at tolerated levels of toxicity. Further, the remarkable schedule dependency for antitumor activity seen in many preclinical models has not been adequately addressed in the design of clinical trials. When response rates for xenograft tumors are calculated for doses that yield clinically achievable systemic exposures, these models rather accurately predict the clinical results. Similarly, we would anticipate approximately 15–25% of colon carcinoma xenografts demonstrating objective responses (≥50% volume regression) using doses of irinotecan that in mice yield clinically achievable systemic exposures for SN-38. However, such information is not available when selecting between analogs at a relatively early stage in development. One way in which equi-efficacious analogs could be distinguished would be to introduce assays of differential species marrow toxicity at an early stage in development. This may allow identification of analogs with significant antitumor activity, but with little difference in species toxicity. Whether mouse transgenic models will obviate any of these issues remains to be determined. These models may have the advantage of development of spontaneous tumors, at more natural sites (e.g., medulloblastoma in the cerebellum). Thus transgenics may represent specialized models for drug evaluation. However, the problems of translating drug effects in rodent to human remain.

The TOP-I-targeted agents in clinical investigation are mainly based on a CPT structure. There are many more analogs in preclinical development. One focus in developing novel CPTs has been on stabilization of the E-ring lactone. This has been achieved by increasing the lipophilicity of CPTs by substitution on the 7-position with bulky alkyl, alkylamino, and alkylsilyl groups. A more novel approach has been synthesis of homocamptothecins, in which a seven-membered lactone E-ring has far greater stability. Interestingly, the homocamptothecin appears to change the sequence specificity of the drug-induced DNA cleavage by TOP-I *(75)*. However, differences in lipophilicity are more likely to result in alterations in the pharmaceutical properties of this class of agent, rendering longer plasma clearance times, and potentially allowing greater systemic exposure to active forms of these drugs. Because there are few clinical data to support the value of delivering

CPTs by prolonged continuous infusion, it is unclear if analogs with greater lactone stability will be more efficacious. Definitive activity in preclinical models with intrinsic or acquired resistance to current CPTs would be important in advancing such analogs to clinical testing. Perhaps of greater interest will be development of novel topoisomerase inhibitors based on the crystal structure of the DNA–TOP-I–DNA ternary complex.

New structures such as protoberberines *(76)*, indolocarbazoles (rebeccamycin analogs) *(77)*, and lipophilic epipodophyllotoxins have emerged as potential dual inhibitors of topoisomerases. Of particular interest is F11782, an ethylidene glucoside ester of epipodophyllotoxin that putatively inhibits the catalytic cycle of both type I and II enzymes, preventing their binding to DNA *(78)*. This agent has demonstrated significantly better activity against both syngenic and xenograft tumor models than etoposide *(79)*. However, direct comparison with irinotecan and topotecan has not been reported. Demonstration of activity of these newer inhibitors against CPT-resistant tumors would be an exciting development. Development and characterization of additional human tumor models resistant to CPTs would be valuable.

The full curative/therapeutic potential of these drugs will not be realized without compensating for the dose-limiting neutropenia and intestinal toxicity. Thus approaches to reducing myelosuppression, through use of hematopoietic growth factors, reconstitution with peripheral blood cell progenitors, or protecting marrow through transduction of CPT-resistant genes, appear rational. Attempts to modulate intestinal toxicity through administration of interleukin-15, or JBT 3002, or alkalinization of the intestinal lumen *(80)* may allow increased dose intensity, or (rationally) more protracted courses of treatment with these agents. Oral administration of topotecan and irinotecan is limited by poor bioavailability. In children and mice, approximately 24% of topotecan and approximately 9% of irinotecan is absorbed. Unabsorbed drug passing to the distal intestine may contribute to the greater gastrointestinal toxicity observed in patients treated with oral dosing. Recently, the use of agents that block the ABC-transporter, breast cancer resistance protein ABCG2, have been shown to increase oral bioavailability of CPTs *(81)*. Such modulators may allow effective oral therapy with these agents using protracted schedules of administration. Validation of animal models of intestinal toxicity is important. Design of clinical protocols that more accurately recapitulate optimal schedules and drug exposures determined in xenograft models also seems appropriate with these agents that are highly schedule-dependent in their antitumor activity. Clearly an understanding of the biochemical or molecular events that determine such dramatic schedule dependency will help in more effective clinical utilization of these agents, alone, or in combination with other cytotoxic agents.

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