Preclinical and Clinical Pharmacologic Studies of 9-Nitrocamptothecin and its 9-Aminocamptothecin Metabolite

by

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I would like to dedicate this dissertation to my Mom who passed away on January 31, 2002. She was always there for our family and she is truly missed more and more every day.

Peace…

DISSERTATION ABSTRACT

Preclinical and Clinical Pharmacologic Studies of

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University of Pittsburgh, 2005

The camptothecins are DNA topoisomerase I-interactive anticancer agents and have a wide range of antitumor activity. Currently approved camptothecin analogues (i.e., topotecan and irinotecan) are only available for IV administration. 9-Nitrocamptothecin (9NC) is administered orally and is partially metabolized to an active metabolite, 9-aminocamptothecin (9AC). As with other camptothecin analogues, 9NC and 9AC undergo a reversible, pH-dependent reaction between the active-lactone and inactive-hydroxy acid forms. *In vitro* and *in vivo* preclinical studies suggest that protracted administration of low doses of camptothecin analogues produces better antitumor activity than the less frequent administration of higher doses. Oral administration of 9NC could mimic the protracted schedule and maximize patient convenience. However, the optimal oral dose and schedule of 9NC and other camptothecin analogues are currently unclear. In addition, oral administration of camptothecin analogues has been characterized by extensive inter- and intra-patient variability in bioavailability. The primary goal of this dissertation research was to evaluate the pharmacokinetics and pharmacogenetics of 9NC and its 9AC metabolite in preclinical models and in patients as part of phase I and II trails.

Daily administration of 9NC orally for 5 days per week for two consecutive weeks repeated every four weeks is tolerable and may be an active regimen in patients with gastric or pancreatic cancers. The responses seen in xenografts models evaluating the same regimen of 9NC as evaluated in the phase I study occurred at systemic exposures that are tolerable in patients. There was significant inter- and intra-patient variability in the pharmacokinetics of 9NC and 9AC when 9NC was administered under fasting conditions. Most of the drug remained in the 9NC form with an overall ratio of 9NC to 9AC of 4:1. Co-administration of 9NC with food reduces the oral absorption of 9NC; however there was no difference in the exposure of 9AC. The functional consequences of known single nucleotide polymorphisms in genes of known ATP-binding cassette (ABC) transporters were evaluated as potential sources of the pharmacokinetic variability of 9NC and 9AC. Our findings suggest that the inter-individual variability in the disposition of 9AC, but not 9NC, may be influenced, in part, by *ABCG2* genotype. The factors associated with the high inter- and intra-patient variability remain unclear.

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1.0

CHAPTER 1:

Cellular, Pharmacokinetic, and Pharmacodynamic Aspects of Response to Camptothecins: Perspectives to Improve Response

1.1 INTRODUCTION

Camptothecins are a class of anticancer agents that are the prototypical DNA topoisomerase I (topo I) inhibitors and appear to be active in human cancers previously resistant to chemotherapy (1). Members of the camptothecins, such as topotecan, irinotecan, 9-aminocamptothecin (9-AC), and 9-nitrocamptothecin (9-NC), are analogs of the plant alkaloid 20(S)-camptothecin (Table 1) (2). Camptothecin analogs occur in equilibrium between the active-lactone and inactive-hydroxy acid forms (Figure 1) (3). These agents interact with topo I and DNA to form cleavage complexes, and prevent resealing of the topo I-mediated DNA single strand breaks (4,5). This interaction eventually leads to double-strand DNA breaks and apoptosis, or cell death.

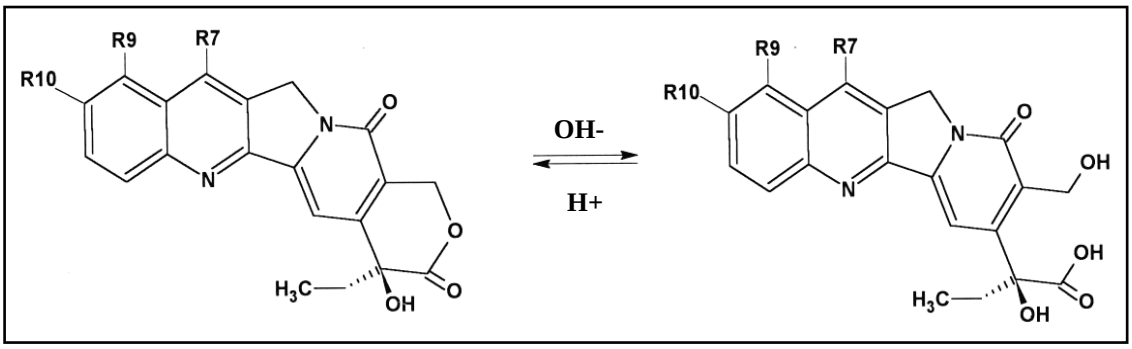
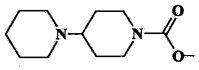


Figure 1. Chemical structure of camptothecin lactone and conversion to the hydroxy acid form. Locations of the structural modifications noted in Table 1 are indicated.

Table 1. Camptothecin Derivatives

Compound	R7	R9	R10
Camptothecin	H	H	H
9-AC	H	NH_2	H
9-NC	H	NO_2	H
Topotecan	H	$CH_2NH(CH_3)_2$	OH
Irinotecan	C_2H_5	H	
SN-38	C_2H_5	H	OH
DB-67	t-Bu$(CH_3)_2$Si	H	OH

The molecular mechanisms of selectivity and cellular resistance associated with camptothecins have been extensively reviewed (1). Factors influencing resistance include reduction in the formation of cleavage complexes due to mutations in topo I that render the enzyme drug-resistant and decreased topo I activity. Topo I protein levels are commonly decreased in camptothecin-resistant cell lines including those with well-characterized topo I mutations. In addition, certain factors following the formation of the cleavage complex, such as DNA repair, cell cycle check points, and apoptosis can produce resistance to the camptothecins. It is likely that the selectivity and effectiveness of camptothecin analogs depends on the deficiencies in DNA repair, cell cycle regulation, and apoptosis associated with topo I in cancer cells, whereas normal cells can adapt more effectively to topo I-mediated DNA damage (1).

Results of preclinical and clinical studies suggest that camptothecin analogs have a wide range of antitumor activity against adult and pediatric solid tumors and leukemias (6,7). Preclinical studies evaluating the antitumor response of camptothecin analogs have shown significant activity against solid tumor xenografts, with the greatest antitumor responses found with protracted continuous schedules (7-9). Preclinical studies also suggest a lack of cross-

resistance with other anticancer agents and that maintenance of an exposure threshold may be required to achieve optimal cytotoxicity (6,10-12).

Even though camptothecin analogs have a wide range of antitumor activity, this activity is not the same for each member of the class. Topotecan (Hycamptin®) is approved by the U.S. Food and Drug Administration (FDA) for the treatment of metastatic carcinoma of the ovary after failure of initial or subsequent chemotherapy and for the treatment of small cell lung cancer sensitive disease after failure of first-line therapy (13). Irinotecan (Camptosar®) is approved by the FDA as first-line therapy for the treatment of patients with metastatic colorectal cancer in conjunction with 5-fluorouracil and leucovorin (14).

9-NC is currently in Phase III trials for the treatment of newly diagnosed and refractory pancreatic cancer (15-16). Newer agents such as homocamptothecins and silatecans are now in preclinical and early clinical development (17-21). As with the differences in antitumor activity among camptothecin analogs, the toxicity profile is unique to each agent. The primary dose-limiting toxicities of topotecan are neutropenia and thrombocytopenia (3,22,23). Bone marrow suppression may occur with irinotecan and 9-NC; however, the primary toxicities associated with these agents are diarrhea and cystitis, respectively (24-27). The exact mechanisms for the differences in antitumor effect and toxicities of the camptothecin analogs are unknown.

In addition to the molecular factors associated with camptothecin-induced cytotoxicity, there are several pharmacokinetic and pharmacodynamic aspects of camptothecins that have been related to antitumor response and toxicity. These factors consist of cellular mechanisms of resistance including active cellular efflux, modulation of topo I and II; the influence of protein binding on lactone stability, structural modifications that have been developed to improve lactone stability; and alterations in the metabolism of camptothecins. Many of these factors have

been evaluated in the clinical setting to improve response. However, the optimal treatment schedule of camptothecins is currently unknown.

1.2 CELLULAR MECHANISMS OF RESISTANCE

Efflux

Resistance to chemotherapeutic agents is a major clinical complication in cancer therapy, in many cases resulting in treatment failure. One common mechanism of resistance is the reduction in cellular concentration of anticancer agents via efflux by multi-drug resistance pumps (MDR). Prior studies have shown insignificant differences in accumulation of camptothecins in P-glycoprotein (P-gp) positive and negative cells (28,29). In addition, when cell lines displayed a decrease in cellular accumulation, this decreased accumulation was not associated with P-gp or multi-drug resistance associated protein (MRP). However, new evidence suggests camptothecins undergo cellular efflux modulated by an alternative anionic pump (30,31). Gupta and colleagues reported the absorption of camptothecin across Caco-2 cells was mediated by passive diffusion and active transport (30). Although the active transporter was not identified, the authors speculated that the absorptive transport of camptothecin was mediated by common transporters such as organic anion, organic cation, and/or bile acid transporters. Vanhoefer and colleagues evaluated the effect of the dihydropyridine analogue, PAK-200S, on P-gp mediated resistance to topotecan in human breast cancer cells resistant to adriamycin (MCF-7/adr) and ovarian tumor cells resistant to doxorubicin, daunorubicin, idarubicin, and etoposide (A2780/Dx5) (32). Despite prior reports that camptothecins are not significantly affected by P-gp, cellular exposure to PAK-200S at a non-cytotoxic concentration almost completely reversed resistance to topotecan in both cell lines (28,29). Cellular topotecan lactone levels were about 2.5-fold lower

in the resistant breast cancer cell line compared to the wild type. Exposure to PAK-200S completely restored cellular topotecan concentrations in resistant cells as compared to the parenteral cells with no effect on the drug concentrations in parental MCF-7 cells lacking P-gp expression. Similar results were reported for the ovarian cell lines. Furthermore, the addition of PAK-200S in the presence of topotecan significantly increased the induction of protein linked DNA breaks. These results suggest that the reversal of P-gp mediated resistance to topotecan by PAK-200S is related to restoration of drug concentrations of topotecan rather than a direct effect on topoisomerases.

The efflux of camptothecins from cells has previously been reported to be associated with to the breast cancer resistance protein (BCRP/MXR/ABCG2). Topotecan and mitoxantrone resistant cell lines (T8 and MX3, respectively) both displayed a decreased accumulation of drug caused by an enhanced energy-dependent efflux (33). The authors reported overexpression of the breast cancer resistance protein/mitoxantrone resistance/placenta-specific ATP-binding cassette (BCRP/MXR/ABCG2). This plasma-membrane protein transported 70 - 80% of the intracellular topotecan out of the cell within 30 seconds (33,34). These results suggest that the efficient efflux of topotecan by BCRP/MXR/ABCG2 may have clinical relevance for patients being treated with topotecan, but extended studies will be required to analyze the possible contribution of BCRP to clinical multidrug resistance.

The ABC transporter superfamily contains membrane proteins that transport a wide variety of substrates across extra-and intracellular membranes. These substrates include metabolic products, lipids and sterols, and drugs (36,37). Over-expression of certain ABC transporters occurs in cancer cell lines and tumors that are multidrug resistant (38-42). Phylogenetic analysis has identified 48 human ABC transporters and assigned them to seven

distinct subfamilies of proteins (43). ABC transporters have been reported to alter the pharmacokinetics of camptothecin analogues and are associated with camptothecin resistance (44,45).

ABCB1 (PGP/MDR1) was the first human ABC transporter to be cloned and also to be identified as conferring an MDR phenotype to cancer cells (38-44). ABCB1 is expressed in the kidney, liver, intestine, adrenal gland, blood brain barrier and hematopoetic stem cells (42). *ABCB1* transports colchicine, etoposide, doxorubicin, and vinblastine, as well as lipids and steroids. The effects of three single nucleotide polymorphisms (SNPs) of *ABCB1* (1236C>T, 3435C>T, 2677G>A/T) have been evaluated in preclinical and clinical trials (43). *ABCC2* (MRP2/cMOAT) is expressed in canalicular cells in the liver (46,47). It functions as an important efflux pump that transports organic ions from the liver to the bile. The *ABCG2* gene (formally known as the MXR/BCRP/ABCP) encodes a half transporter with a nucleotide-binding fold-transmembrane domain orientation (38,46). *ABCG2* is expressed in stem cells, the placenta, and the intestine (48,49). *ABCG2*-associated proteins transport mitoxantrone, methotrexate, and camptothecin analogues such as topotecan, diflomotecan, and SN-38 (38,50,51).

To combat the efflux of camptothecins by BCRP, Erlichman and colleagues evaluated the effect of BCRP inhibition by CI1033 (PD 183805), a quinazoline-based HER family tyrosine kinase inhibitor, in combination with SN-38, topotecan, and camptothecin (35). The combination of CI1033 with SN-38 was synergistic in T98G glioblastoma cells and HCT8 colorectal carcinoma cells. The increased activity of SN-38 and topotecan in combinations with CI1033 was secondary to an increased intracellular accumulation of SN-38 and topotecan, coupled with an increase in the number of covalent topo I DNA complexes stabilized by SN-38 or topotecan. To address the possibility that CI1033 was inhibiting BCRP, drug accumulation

and cytotoxicity were examined in MDA-MB-231 cells transfected with DNA encoding BCRP. CI1033 increased the steady-state level of SN-38 and topotecan by 7.8 ± 1.0 and 2.2 ± 0.5-fold, respectively. The accumulation of CI1033 was 4.9 ± 0.1-fold lower in cells transfected with BCRP, suggesting that CI1033 is a substrate for BCRP (35). The results of this study indicate that CI1033 may modulate the accumulation and subsequent cytotoxicity of topo I inhibitors, but the clinical effect may vary depending on the camptothecin derivative analyzed.

The presence of P-gp in the luminal side of the intestinal epithelium may reduce the oral bioavailability of drugs. Jonker and colleagues evaluated the effect of P-gp on the oral bioavailability of topotecan in mice deficient for P-gp and wild type mice (52). A two-fold decrease in the AUC of topotecan, when administered orally, was observed in mice positive for P-gp in comparison to wild type mice. However, there was no significant difference in the pharmacokinetics of topotecan after IV administration in either mice group. These results suggest that P-gp is mainly important in modulating the oral uptake of topotecan (52). In addition, these results suggest the oral absorption and achievable AUC of topotecan may be influenced clinically by varying expression of P-gp in patients and that modulators of P-pg may increase the oral bioavailability of topotecan (53).

Modulation of anionic excretion or efflux may also alter the systemic, renal, and non-renal pharmacokinetics of camptothecins. Zamboni and colleagues[31] reported the inhibition of anionic renal tubular secretion of topotecan when administered with probenecid, an inhibitor of anionic tubular transport in the proximal tubule of the kidney (54). The co-administration of topotecan lactone or hydroxy acid with probenecid significantly decreased topotecan lactone, total, and hydroxy acid systemic clearance in mice ($P < 0.05$). In addition, probenecid decreased the renal and non-renal clearance of topotecan (31). Clinically, these results suggest the

combination of topotecan with agents that compete for anionic renal tubular secretion could increase systemic exposure to the active lactone moiety, with a subsequent increase in toxicity and or antitumor efficacy.

Modulation of topoisomerase I and II

As treatment of cancer continues to progress, many new compounds will be evaluated and studied in combination with other chemotherapeutic agents. The combination of various agents can lead to discoveries of additive, synergistic, or even antagonistic effects. Several preclinical studies have evaluated the ability of anticancer agents to modulate topo I expression and, therefore, affect the activity of camptothecins (55-60).

Kano and colleagues reported the additive effects of sequential paclitaxel and SN-38 in a series of human cancer cell lines (55). Interestingly, the simultaneous exposure of the cells to paclitaxel and SN-38 produced antagonistic effects in some cell lines while producing an additive effect in others. These results are similar to those published by Kaufmann and colleagues which showed simultaneous exposure to topotecan and vincristine or paclitaxel produced antagonistic effects against A549 human non-small cell lung cancer cells (57). Madden and colleagues reported the effects of low-level taxane exposure on the activity of topo I inhibitors in MCF-7 and MDAH B231 human breast cancer cells (58). Prior treatment with paclitaxel or docetaxel decreased the IC50 for 9-AC and topotecan by nearly 10-fold. No difference was reported from controls for the reverse sequence or simultaneous drug exposure. These results suggest taxane exposure prior to camptothecin exposure impacts the cytotoxicity by increasing topo I expression in a sequence-dependent manner. In addition, Hallin and colleagues reported the increase in expression and activity of topo I and II after taxane treatment

in human breast cancer cell lines (59). The increase in topo I was associated with a taxane-induced G2/M blockade as depicted by the increase percent of cells in G2 after taxane exposure.

Although cell culture studies have shown that the simultaneous exposure of cells to a topo I inhibitor and a topo II inhibitor can result in antagonism (56), Eder and colleagues reported no therapeutic antagonism with the combinations of irinotecan and doxorubicin or etoposide in mice bearing the EMT-6 mammary tumor (60). The administration of irinotecan alone increased the expression of topo II and decreased the expression of topo I while the administration of etoposide or doxorubicin alone increased the expression of topo I and decreased the expression of topo II. Levels of topo I and II were decreased with concomitant administration of irinotecan and etoposide or doxorubicin, while sequential administration reflected the effect of the last agent administered. These results suggest that combinations and sequences of topo I and topo II inhibitors may produce greater cytotoxicity than either agent alone.

The combination of topotecan and vincristine has demonstrated greater than additive effects in mice with pediatric solid tumor xenografts including neuroblastoma, rhabdomyosarcoma, and brain tumors (61). Topotecan was administered as an IV bolus daily for 5 days on 2 consecutive weeks with cycles repeated every 21 days over a period of 8 weeks. Vincristine was administered IV every 7 days at a fixed dose of 1 mg/kg. The therapeutic effect of combining topotecan with vincristine was greater than additive in most tumor models of childhood solid tumors. Toxicity data suggested that this regimen could be administered to mice with only moderate reductions in the dose levels for each agent. In addition, there was no pharmacokinetic interaction between topotecan and vincristine. The mechanism of this synergistic interaction is unknown suggesting a cellular effect (61). Several preclinical and

clinical studies evaluating the sequence of administration of camptothecins, and topo II inhibitors and taxanes are now being performed.

1.3 LACTONE STABILITY

Lactone stability of topotecan

The lactone ring is a structural requirement for effective biologic activity of camptothecins. Prior data indicate a closed α-hydroxylactone ring is important for passive diffusion of these drugs into cancer cells and for successful interaction with topo I (60-66). Therefore, factors that influence or shift the lactone-carboxylate equilibrium of camptothecins may alter the function of these agents.

One factor that clearly affects the camptothecin lactone-carboxylate equilibrium is protein binding. Early research found human serum albumin (HSA) to have a 200-fold binding preference for camptothecin carboxylate over the lactone form (57). In the presence of HSA, the carboxylate form was preferentially bound shifting the lactone-carboxylate equilibrium in favor of the carboxylate (Figure 2). This provided an explanation for the rapid hydrolysis of camptothecin in the presence of HSA. This effect appears to be limited to HSA, as rapid and complete ring opening was not reported in the presence of albumin or plasma from mouse, rat, dog, rabbit, or bovine sources. Thus, these species have higher equilibrium levels of camptothecin lactone relative to humans (68). While HSA has the ability to shift the camptothecin lactone-carboxylate equilibrium to the right, other components (low-density lipoprotein, α_1-acid glycoprotein, red blood cells) found in human whole blood have the ability to stabilize the lactone ring (68,69). The enhanced stability of the camptothecin lactone ring in whole blood may be attributed to its ability to partition into lipid bilayer structures, especially of

red blood cells (RBCs) (67,70,71). The intercalation of camptothecin within the acyl chain region of the bilayer protects the lactone ring from hydrolysis (67). Despite the RBCs protective effect of the camptothecin lactone form, a certain fraction of the administered drug will hydrolyze in human blood.

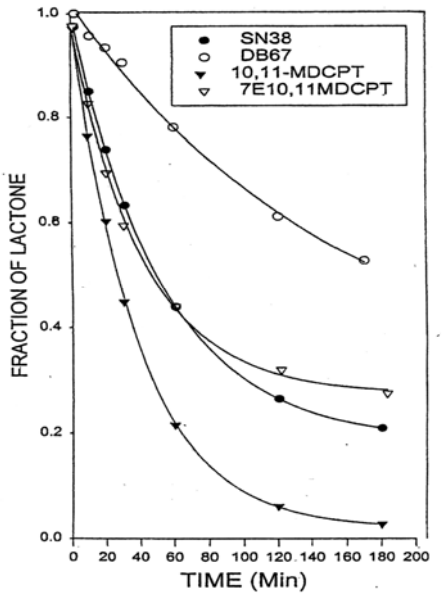


Figure 2. Depiction of the effects of protein binding in human blood on lactone stability. DB-67, a silatecan, displays improved lactone stability relative to SN-38 and the lipophilic experimental agents 10,11-methylenedioxycamptothecin (10,11-MDCPT) and 7-ethyl-10,11-methylenedioxycamptothecin (7-E-10,11-MDCPT). Stability profiles were determined using HPLC methods. All experiments were conducted at pH 7.4 and 37°C. Each data point represents the average of three or more determinations with an uncertainty of 10% or less. Reprinted with permission from reference (75).

Gender-dependent differences in the pharmacokinetics of topotecan in adults may result from the interaction between camptothecins and RBCs (72). Loos and colleagues reported a 1.4-fold higher clearance of topotecan lactone in males compared to females ($P = 0.0082$). After correction for body surface area, the apparent topotecan lactone clearance remained 1.3-fold higher in males compared to females ($P = 0.0076$). In addition, the lactone to total ratio of the AUC of topotecan was significantly higher in females compared to males. Individual hematocrit

values were consistently lower in females ($P < 0.023$) and were a significant predictor of topotecan lactone clearance. The authors concluded that there is a significant gender-dependent difference in the pharmacokinetics of topotecan as a result of physiologic differences in the hematocrit values between men and women.

Structural modifications

Since the closed, active lactone ring is a structural requirement for effective biologic activity of the camptothecins, many researchers have investigated various modifications of the camptothecins in an effort to promote lactone stability yet retain antitumor efficacy (62). An approach to a more stable topo I inhibitor has included structural modifications that eliminate the highly preferential binding of the carboxylate form to HSA and thus reduce the rate of hydrolysis. In addition, the discovery of lactone stabilization through lipid bilayer partitioning has led to the design of more lipophilic analogues in order to promote partitioning of these agents into the lipid bilayers of erythrocytes and protect the active lactone form from hydrolysis.

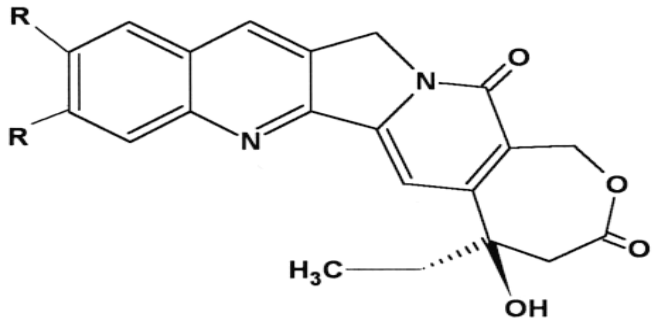
Silatecans

The synthesis of 7-silycamptothecins by adding a silyl group at position 7 with various substitutions at position 10 has demonstrated increased stability of the lactone ring (17,18). The addition of a silyl group may limit drug inactivation by both protein binding and hydrolysis of the lactone ring, and enhance lipophilicity, which would increase *in vivo* activity while possibly limiting toxicity (Table 1) (73). The majority of these new compounds demonstrate potencies comparable to or better than other camptothecin derivatives. Two of the leading members of this class, DB-67 and karenitecin, are in preclinical and phase II development, respectively.

Preclinical nonhuman primate and phase I clinical studies of karenitecin indicated the percent lactone was 104% and 87 ± 11%, respectively (19,74). DB-67 was found to have more potent antitumor activity than topotecan, and at least comparable to SN-38, against a panel of five high-grade glioma cell lines (75). Although the results of this study are promising for the future treatment of human gliomas, it is unclear if the increased stability of the lactone ring in human blood and its potentially increased lipophilicity will translate into increased activity in humans. One potential problem is that silatecans are highly protein bound and have low penetration into the cerebrospinal fluid (CSF) (19).

Homocamptothecins

To further stabilize the lactone ring, Lesueur-Ginot and colleagues expanded the E-ring of camptothecin to a seven member β-hydroxylactone ring with the insertion of a methylene spacer between 20-OH and the carboxyl moiety (Figure 3) (20). This new lactone E-ring modified compound has been termed a homocamptothecin and provides a less reactive lactone with enhanced stability and decreased protein binding in human plasma in comparison to camptothecin. In addition, i*n vitro* studies demonstrated greater antiproliferative effects and higher levels of DNA cleavage after exposure to homocamptothecin compared to camptothecin (20).



BN 80245: R = H
BN 80915: R = F

Figure 3. Chemical structures of the homocamptothecin, BN 80245, and the fluorinated homocamptothecin, BN 80915. Reproduced with permission from reference (21).

Philippart and colleagues reported two homocamptothecins, BN 80245 and BN 80915 exerted higher antiproliferative activity than camptothecin, topotecan, and SN-38 (21). Bailly and colleagues reported higher levels of protein-DNA complexes in P388 leukemia cells treated with homocamptothecin than those treated with camptothecin (76). The slow hydrolysis of homocamptothecin instead of the fast equilibrium between the lactone and carboxylate of camptothecin may account for its improved *in vivo* activity. Also, homocamptothecin has been found to induce DNA cleavage at T$^{\downarrow}$G sites more efficiently than camptothecin and induce DNA cleavage at additional sites unseen with camptothecin containing an AAC$^{\downarrow}$G motif. These observed differences could further stabilize the lactone moiety and broaden the cytotoxicity profile of these drugs (76).

Homosilatecans

Homocamptothecins have been further modified to form homosilatecans (18). In addition to the expanded β-hydroxylactone E-ring, each of the homosilatecans also contains a silylalkyl functionality at the 7-position. As with the silatecans, this functionality group increases the lipophilicity while reducing the strength of carboxylate interactions with HSA.

Two homosilatecans, DB-90 and DB-91, contain amino and hydroxyl groups at the 10-position, respectively, to further reduce binding of the carboxylate form to HSA. Homosilatecans display improved lipophilicty and stability in human whole blood compared to camptothecin and topotecan. Interestingly, homosilatecans display similar stabilities in human and mouse blood, which contrasts with the interspecies variations in blood stabilities observed for camptothecins (18). Thus, preclinical animal modeling and efficacy studies with the homosilatecans may be predictive of their use in a clinical setting.

1.4 METABOLISM AND EXCRETION

Oxidative metabolism

Initial preclinical studies suggested the camptothecins did not undergo significant oxidative metabolism. However, recent preclinical and clinical studies report clinically significant oxidative metabolism and drug interactions.

9-Aminocamptothecin

Grossman and colleagues reported an increased 9AC dose requirement in patients with newly diagnosed glioblastoma multiforme or recurrent high grade astrocytomas concomitantly receiving cytochrome P-450 (CYP) enzyme inducing anticonvulsants (EIA) (77). In addition, grade III or IV myelosuppression did not occur in 29 patients who received 9AC 850 mg/m^2/day continuous infusion for three days with co-administration of phenytoin, phenobarbtial, carbamazepine, and/or valproic acid. In contrast, two of three patients not taking EIAs developed grade IV myelosuppression. Steady state total 9AC plasma levels were 3-fold lower in patients on anticonvulsants than in patients receiving anticonvulsants. The maximum

tolerated dose (MTD) of 9AC in an additional 27 patients on EIAs was 1776 μg/m^2/day in patients with newly diagnosed tumors and 1611 μg/m^2/day for patients with recurrent disease. The exact mechanism of why patients receiving EIAs were able to tolerate significantly higher doses of 9AC is unclear; however, the EIAs in this study have a wide range of CYP activation.

Topotecan

The combination of other camptothecins with EIAs has also resulted in altered pharmacokinetic disposition of camptothecins. Topotecan is known to undergo oxidative metabolism to form the metabolite N-desmethyl topotecan. This reaction is presumably followed by two oxidation steps leading to a nitroso-derivative, which could potentially be a suicide inhibitor of CYP 3A (78). An increase in lactone and total topotecan clearance from 43.4 ± 1.9 L/h/m^2 to 62.9 ± 6.4 L/h/m^2, and 20.8 ± 2.8 L/h/m^2 to 30.6 ± 4.1 L/h/m^2, respectively ($P < 0.05$), has been reported in a pediatric patient receiving phenytoin (78). There was a 2-fold increase in the N-desmethyl oxidative metabolite of topotecan when co-administered with phenytoin. The concentration versus time profiles of topotecan and N-desmethyl topotecan with and without co-administration of phenytoin are presented in figure 4. In addition, a population pharmacokinetic study reported that co-administration of phenytoin was a significant covariate in predicting topotecan clearance (79). Ma and colleagues reported a tripling of the trough cyclosporine concentration during a 10 day course of topotecan with only a 30% increase in cyclosporine dose (80). In addition, on day 8 of the topotecan therapy, the N-desmethyl topotecan systemic exposure doubled despite a 20% reduction in topotecan dose compared with day 1. In addition, Goldwasser and colleagues reported a 3-fold increase in the Cmax of cyclosporine in a patient receiving concomitant topotecan (81). No change in plasma total or topotecan lactone was

reported. These results suggest that topotecan may undergo significant oxidative metabolism and may act as a CYP 3A inhibitor. However, studies using human liver microsomes are needed to fully elucidate potential drug interactions and toxicity.

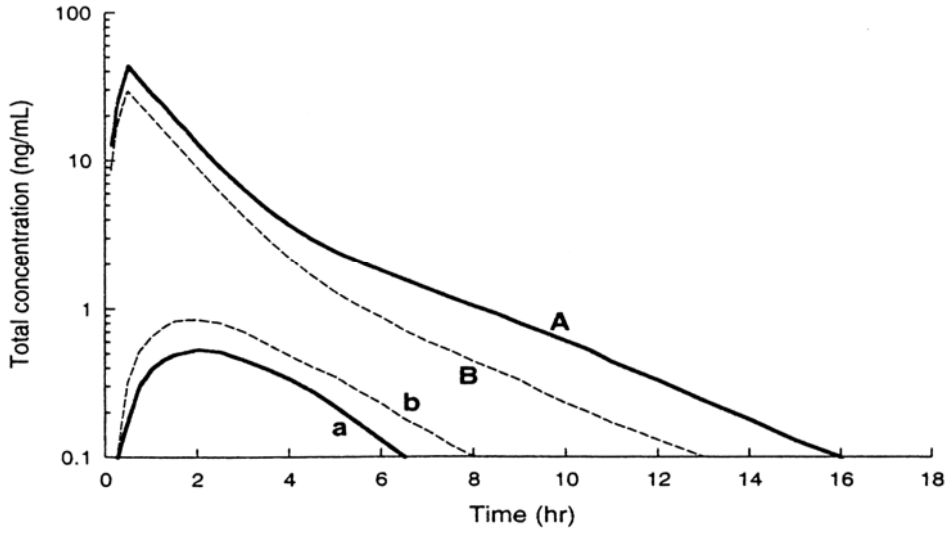


Figure 4. Implication of coadministration of topotecan with phenytoin. Depiction of total topotecan and total N-desmethyl topotecan concentration-time plots after a simulated dose of 2.0 mg/m^2 administered alone and in combination with phenytoin. Lines represent total topotecan (A, —) and total N-desmethyl topotecan (a, —) alone, and total topotecan (B, ---) and total N-desmethyl topotecan (b, ---) in combination with phenytoin. The plasma AUC of total topotecan after administration of topotecan alone and in combination with phenytoin was 81.3 and 52.4 ng/ml·hr, respectively. The plasma AUC of total N-desmethyl topotecan after administration of topotecan alone and in combination with phenytoin was 2.9 and 4.0 ng/ml·hr, respectively. Reprinted with permission from reference (78).

When camptothecin analogs are combined with other anticancer agents, the sequence of administration may influence potential metabolic interactions. Zamboni and colleagues evaluated the combination of topotecan and docetaxel administered in different sequences (82). On cycle 1, docetaxel was administered on day 1 and topotecan on days 1 to 4. On cycle 2, topotecan was administered on days 1 to 4 and docetaxel was administered on day 4. During cycle 2, the clearance of docetaxel was decreased by 50% (75.6 ± 79.6 L/h/m^2 vs 29.2 ± 17.3 L/h/m^2, P = 0.046). The mean absolute neutrophil count (ANC) nadir in cycle 2 was also lower

than in cycle 1, 2808 ± 4518/μL and 4857 ± 6738/μL, respectively (P = 0.02). Since docetaxel is metabolized by CYP 3A4, the results of this study suggest prior topotecan treatment decreased docetaxel clearance via inhibition of CYP 3A which was associated with an increase in the severity of neutropenia (83,84).

Irinotecan

Irinotecan undergoes oxidative metabolism to form 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino] carbonyloxycamptothecin (APC) (Figure 5) (95). In an effort to establish the oxidative enzymatic pathway involved in the formation of APC, Haaz and colleagues investigated the metabolism of irinotecan in human liver microsomes (95). The oxidation of the distal piperidine ring of APC was found to be catalyzed primarily by CYP3A. The CYP 3A selective inhibitors, ketoconazole and troleandomycin, inhibited APC formation by 98 and 100%, respectively. The clinical relevance of these findings is uncertain at this time because APC has 500-fold less antitumor activity compared to SN-38.

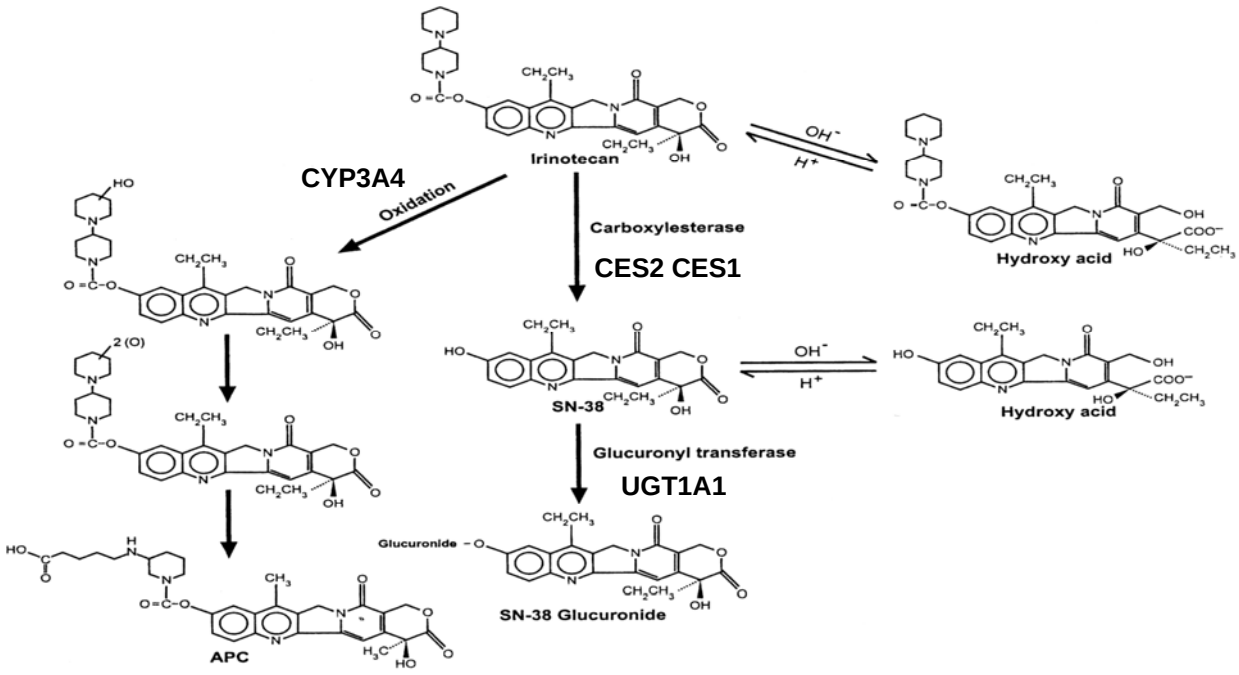


Figure 5. Metabolic schema for irinotecan. The enzymatic formation of the active metabolite SN-38 and hydrolysis of both irinotecan and SN-38 to their hydroxy acid forms are shown. Irinotecan also undergoes oxidative metabolism to form the APC metabolite, while SN-38 undergoes glucuronidation to form SN-38 glucuronide. Reprinted with permission from reference (93).

Clinical studies have identified drug interactions between irinotecan and EIAs. Gajjar and colleagues reported a 2-fold increase in irinotecan clearance and a 3-fold decrease in SN-38 area under the concentration time curve (AUC) in children receiving EIAs compared to those not taking EIAs (14.1 ± 6.6 vs 46.4 ± 14.0 ng·hr/mL, respectively; P = 0.007) (86). However, the mean APC AUC was not statistically different between the two groups (126.4 ± 47.9 vs 117.5 ± 26.0 ng·hr/mL, respectively; P = 0.73). Due to the complicated metabolic disposition and high intrapatient variability of irinotecan, controlled clinical trials are required to further elucidate clinically significant drug interactions.

Conversion of Irinotecan to SN-38

Irinotecan is a prodrug that is activated primarily in the liver by carboxylesterase (CE) enzymes to form an active metabolite, SN-38 (Figure 5) (87). Humerickhouse and colleagues evaluated the kinetics of irinotecan hydrolysis by the two human liver CE iso-forms (hCE-1 and hCE-2) using purified enzymes from human liver and the effect of these two enzymes on irinotecan cytotoxicity (88). The hCE-2 had a 12.5-fold higher affinity for irinotecan and a 5-fold higher maximal rate of irinotecan hydrolysis when compared with hCE-1. These data indicate that hCE-2 is a high-affinity, high-velocity enzyme with respect to irinotecan and is likely to play a substantial role in irinotecan activation in human liver and intestine. Also, CE isolated from human intestinal biopsies has demonstrated activation of irinotecan and may be responsible for gut toxicity associated from irinotecan (89).

Recently, a mouse strain Esl(e), with low levels of Es-1 RNA, has been identified that demonstrates a 3-fold decrease in total plasma esterase activity in comparison to control mice (74). These mice carry an allele of the esterase locus Es-1 that results in reduced plasma esterase enzyme activity. These mice demonstrated a 5-fold decrease in conversion of irinotecan to SN-38 as compared to control mice. These results suggest that the reduced levels of Es-1 esterase present in Esl(e) mice are due to down-regulation of gene transcription, and this plasma esterase is responsible for the majority of irinotecan metabolism in mice (90). However, enzymes other than CE may be involved in the activation of irinotecan. The conversion of irinotecan to SN-38 by butyrlcholinesterase isolated from humans, horses, and mice, has also been reported *in vitro* (91,92).

There is high interpatient variability in the pharmacokinetics of irinotecan and

SN-38. The expression of CE in tissue and tumor may account for this pharmacokinetic variability. Increases in irinotecan and SN-38 systemic exposures have been reported in mice bearing human neuroblastoma xenografts when compared to non-tumor bearing mice after both IV and oral administration (93). Guichard and colleagues reported significantly lower camptothecin CE activity and increased topo I activity in human primary colorectal tumors in comparison to normal tissue (94). The local activation of irinotecan is an important consideration secondary to different metabolic pathways between hepatocytes and tumor cells. In hepatocytes, irinotecan is converted to SN-38 which can then be glucuronidated to SN-38G, an inactive metabolite excreted in the bile (95). Also, irinotecan can be inactivated by hepatic CYP 3A to APC (85). In contrast, inactivation of irinotecan by CYP 3A in human colorectal tumors is unlikely as only low levels of this enzyme are expressed in this tissue (96). Therefore, the production of the active, cytotoxic metabolite, SN-38, may come from both local tumor as well as hepatic conversion from irinotecan.

Future investigations with irinotecan will involve producing tumor-specific cytotoxicity with various enzyme/prodrug combinations. Researchers have discovered rabbit CE is 100 to 1000 fold more efficient at converting irinotecan to SN-38 *in vitro* and 12 to 55 fold more efficient in sensitizing transfected cells to irinotecan *in vitro* than human CE (97). Irinotecan treatment of immune-deprived mice with Rh30 rhabdomyosarcoma cells expressing rabbit carboxylesterse resulted in regression and no recurrence of tumors after treatment whereas, tumor recurrence was noted in 7/7 mice bearing control Rh30 xenografts and in 2/7 mice bearing Rh30 xenografts that expressed the human enzyme. Therefore, the combination of irinotecan with rabbit carboxylase may be a beneficial combination. Future research is investigating the

adenoviral delivery of an ornithine decarboxylase promoter of rabbit CE cDNA cassette for potential virus-directed enzyme prodrug therapy applications (98).

Glucuronidation of SN-38

SN-38 undergoes glucuronidation by hepatic uridine diphosphate glucuronosyltransferases (UGTs) to form the inactive SN-38 glucuronide (SN-38G) metabolite (Figure 5). The glucuronidation of SN-38 to SN-38G is the major elimination pathway of SN-38 and is thought to protect against irinotecan-induced gastrointestinal toxicity. Lalitha and colleagues reported glucuronidation of SN-38 by the UGT-1A1 (99). Therefore, patients with low UGT-1A1 activity, such as those with Gilbert's syndrome and Crigler-Najjar syndromes I and II may be at an increased risk for irinotecan toxicity. SN-38 and SN-38G are primarily excreted in bile and emptied into the gastrointestinal tract (100). SN-38G can then be deconjugated by intestinal flora to SN-38, with accumulation of SN-38 in the intestine as a possible cause of diarrhea attributed to irinotecan administration (99).

Since a genetic predisposition for decreased glucuronidation of SN-38 may exist in patients with low UGT-1A1 activity, researchers have investigated a potential relationship between UGT-1A1 phenotypes with UGT-1A1 promoter polymorphism. Phenotypic measurements of SN-38 and bilirubin glucuronidation in human liver microsomes were performed *in vitro* (101). Based on the presence of an additional TA repeat [$(TA)_7TAA$] in the TATA sequence of UGT-1A1 in Gilbert's syndrome, genotypes were assigned as: 7/7 homozygous for the $(TA)_7TAA$ allele; 6/6, homozygous for the $(TA)_6TAA$ allele; and 6/7, heterozygous with 1 of each allele. Nine percent of screened liver samples were found to be homozygous for allele 7 (7/7), 43% were homozygous for allele 6 (6/6), and 48% were

heterozygous (6/7). SN-38 glucuronidation rates were significantly lower in homozygotes of $(TA)_7TAA$ allele (7/7) and heterozygotes (6/7) compared with homozygotes of $(TA)_6TAA$ allele ($P < 0.01$). Glucuronidation of SN-38 and bilirubin were significantly lower in the 7/7 and 6/7 groups compared with the 6/6 group. These results suggest that the UGT-1A1 promoter genotype would be a good predictor of SN-38 glucuronidation in an *in vitro* setting. However, it is unclear if such a phenotype-genotype correlation will exist *in vivo* and ultimately allow individualization of irinotecan therapy to improve its therapeutic index.

To evaluate the relationship between irinotecan disposition and toxicity, a biliary index was developed to estimate the relationship between gastrointestinal toxicity and SN-38 glucuronidation (95). The biliary index consisted of a biliary ratio (AUC_{SN-38}/ AUC_{SN-38G}), to express the free concentration of SN-38 in bile, multiplied by the AUC_{CPT-11} to control for individual variability in the amount of available drug. A significantly higher biliary index was observed in patients with grade 3 to 4 diarrhea compared to patients with grade 0 to 2 diarrhea ($P = 0.03$) (79). The elevated biliary indexes were possibly secondary to higher dose levels in some studies or possibly due to low glucuronidation rates. However, wide interpatient variability was noted, which may cause insufficient precision to warrant clinical application (102,103).

Studies have evaluated ways to decrease the diarrhea associated with irinotecan by modulating the metabolism of irinotecan and SN-38. In an effort to modulate the glucuronidation of SN-38, irinotecan has been administered with either valproic acid, an inhibitor of glucuronidation, or phenobarbital, an inducer of oxidative metabolism.[88] Pretreatment of Wistar rats with valproic acid caused a 99% inhibition in the formation of SN-38G leading to a 270% increase in the AUC of SN-38 compared with the control rats. The irinotecan estimations were unchanged in the two groups. Pretreatment with phenobarbital

resulted in a 1.7-fold increase in the AUC of SN-38G and a concomitant 31% and 59% reduction in the AUCs of SN-38 and irinotecan, respectively. It is currently unclear if the modulation of irinotecan, SN-38, and SN-38G disposition by valproic acid or phenobarbital can prevent the diarrhea associated with irinotecan.

In addition, modulation of irinotecan with cyclosporine has been reported in an effort to reduce biliary excretion of irinotecan and its metabolites and potentially reduce their gastrointestinal toxicity (105). Cyclosporine pretreatment resulted in an average increase of 339%, 361%, and 192% in the AUC of irinotecan, SN-38, and SN-38G, respectively. Analysis of clearance of irinotecan indicated a 55% and 81% reduction in the average renal and non-renal clearances, respectively. Although the exact mechanism of the decrease is not evident, cyclosporine related cholestasis could be one of the factors.

1.5 TREATMENT REGIMENS

Preclinical studies

The optimal treatment regimens for the camptothecins have not been identified. Moreover, the optimal schedule of administration of topotecan, irinotecan, and other camptothecin analogs may not be the same. Studies of camptothecin analogs in mice bearing human adult and pediatric tumors suggest they are among the most active agents evaluated (6-9). The antitumor activity of the camptothecins is highly schedule dependent, where daily low-dose protracted schedules (i.e., daily for 5 consecutive days) have greater efficacy than more intense shorter schedules of administration (i.e., once every three weeks) (7,9). However, the dose administered per day and subsequent plasma concentration must be above a critical threshold to produce tumor regression (6,7). For example, prolonged (i.e., daily for 5 days per week for eight

consecutive weeks) administration of doses below this critical threshold may not achieve antitumor response, whereas administration of camptothecins on an intermittent (i.e., daily for 5 days per week x 2 weeks repeated every 4 weeks) schedule may allow for administration of higher doses per day that achieve drug exposures above this critical threshold (7,16). This schedule specificity is based on the steep relationship between camptothecin exposure and response (i.e., antitumor or toxicity).

Preclinical studies suggest protracted schedules of administration produce greater antitumor effect than bolus administration (9,104,105). Studies evaluating colon and pediatric solid tumor xenografts demonstrated that administration of topotecan and irinotecan daily for 5 days for 2 consecutive weeks every 3 weeks was equally or more effective and less toxic than shorter treatment schedules with higher doses (9). In addition, preclinical studies with L1210 leukemia and several human tumor xenografts have demonstrated repeated intermittent treatment was superior to a single injection administration (106,107). Moreover, comparing the administration of irinotecan daily for 5 consecutive days for 1 week versus daily for 5 consecutive days for 2 weeks, both repeated every 21 days, and at the same cumulative dose per 21-day cycle, showed that the 2 week administration schedule produced significantly greater antitumor response (Figure 6) (7). These effects are consistent with the S-phase specific cytotoxic action of these agents (1).

There has been a shift from the use of syngeneic transplantable murine tumors to the use of human tumor xenografts for the identification and development of anticancer agents (6,79,108). However, there is limited data that human tumor xenografts are better in identifying agents that ultimately prove efficacious in human cancers or in the clinical setting. The failure of these models to predict the drug response may be due to species differences as mice may be able

to tolerate more or less of an agent compared to humans. The primary concern is that efficacy studies in xenografts are performed at dose levels that are several fold higher than doses tolerated in humans.

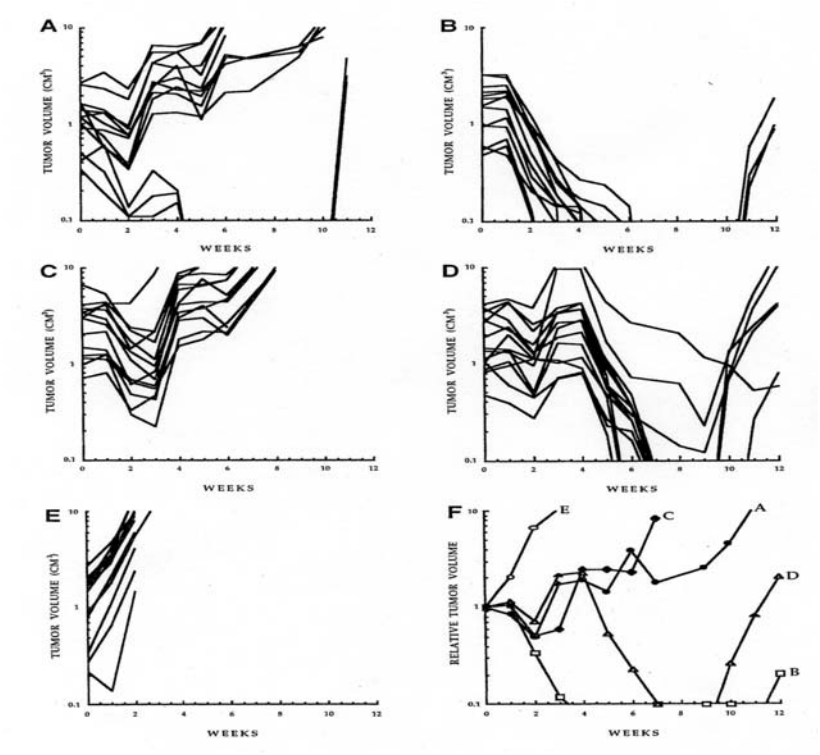


Figure 6. Improved antitumor activity of irinotecan against neuroblastoma xenografts with protracted schedule of administration. One 5-day cycle (A, C) or two 5-day cycles (B, D) repeated every 21 days over 8 weeks. Cumulative dose per cycle was 25 mg/kg (A, B) or 6.25 mg/kg (C, D), (E) control; (F) relative tumor volumes for treatment groups. Reprinted with permission from reference (7).

To address this issue, the minimum effective dose of topotecan and associated systemic exposure were determined against a panel of neuroblastoma xenografts and compared to the maximum tolerated dose in pediatric Phase I trials (6,23). The same topotecan regimen in a Phase I pediatric trial was used in the xenograft studies. Analysis of the topotecan lactone systemic exposures associated with antitumor activity in the xenograft model were tolerable and achieved clinical response in the Phase I study (23). As a result, the topotecan regimen used in the

preclinical and Phase I studies is currently being evaluated as a Phase II window study in patients with high risk neuroblastoma.

Alternatively, the comparison of xenograft models and early human trials can help to explain why a specific agent may not be effective in patients. 9AC is a topo I inhibitor with activity against xenografts from childhood solid tumors; however, clinical trials with this compound have been disappointing, resulting in discontinuation of further development. The protracted administration of IV and oral 9AC therapies, daily for 5 days for 1, 2, or 3 weeks and for 1 or 3 cycles was evaluated in mice bearing human solid tumor xenografts by Kirstein and colleagues (99). The tumors were found to be highly sensitive to 9-AC therapy, but the systemic exposure required for antitumor effect was in excess of that achievable in patients. These data support that for some anticancer agents, including camptothecins, more detailed preclinical studies evaluating the minimum effective dose and associated systemic exposure may be of value in the analysis of antitumor activity in clinical trials and in guiding clinical drug development.

Clinical Studies

As stated earlier, preclinical studies suggest protracted daily administration of camptothecins achieve the greatest antitumor activity (7,9). The FDA approved schedule for topotecan, 1.5 mg/m^2/day IV for 5 consecutive days repeated every 21 days, follows this theory. However the FDA approved treatment regimen for irinotecan, 125 mg/m^2 IV once a week for 4 weeks followed by a 2 week rest or 350 mg/m^2 IV once every 3 weeks, does not (14). To address these issues, alternative treatment regimens of irinotecan and topotecan are now being evaluated (7,25,110-112).

Single bolus administration if irinotecan may saturate the ability of CE to convert irinotecan to SN-38 and result in a lower formation of SN-38 (113). Thus, prolonged daily schedules of irinotecan are being evaluated by several groups (7,25,111). Blaney and colleagues evaluated irinotecan administered as a 60-minute infusion daily for 5 days every 21 days in children with refractory solid tumors (95). The recommended Phase II doses were 39 and 50 mg/m^2/day in heavily and less-heavily pretreated patients, respectively. Furman and colleagues directly translated a protracted irinotecan schedule from a xenograft model to a Phase I trial in children with refractory solid tumors.[7] Administration of irinotecan daily for 5 days per week for 2 consecutive weeks repeated every 21 days was well tolerated with an MTD of 20 mg/m^2/day and achieved significant antitumor response. This study implies that data obtained from xenograft models can be effectively translated into the design of clinical trials. In addition, the relative extent of conversion and SN-38 systemic exposure achieved with this schedule was much greater than reported from adults and children administered larger intermittent doses (113).

The optimal treatment regimen for topotecan is currently unclear (112,114). Hoskins and colleagues performed a randomized Phase II study of two schedules of topotecan in previously treated patients with ovarian cancer (114). Patients were randomized to either topotecan 1.5 mg/m^2/day as a 30-minute infusion for 5 days (qd x 5) repeated every 21 days or topotecan 1.75 mg/m^2 administered as a 24-hour infusion once a week for 4 weeks repeated every 6 weeks. The response rate in the qd x 5 arm was 22.6% (95% confidence interval [CI], 9.6% to 41.2%), which was significantly superior to 24-hour topotecan arm, 3.1% (95% CI, 0.1% to 16%) (P = 0.026). However, the qd x 5 arm was more toxic with 94% of patients experiencing grade 3 or 4 granulocytopenia as opposed to 52% in the other arm. The authors concluded that the qd x 5 schedule remains the schedule of choice. To simulate the xenograft data, topotecan is also being

evaluated on a daily for 5 days per week for 2 consecutive weeks repeated every 4 weeks schedule in Pediatric Phase II studies (115,116). Alternatively, administration of topotecan daily for 3 days repeated every 3 weeks is being evaluated to reduce the bone marrow suppression associated with topotecan and reduce the logistical difficulties associated with IV administration for 5 consecutive days (112).

Oral administration

Oral administration of camptothecins would mimic the protracted schedule used successfully in preclinical models, maximize patient convenience, minimize the use of clinical resources, and achieve a favorable pharmacokinetic profile based on low gastric pH, which may favor retention of the drug in the active-lactone form (7,117-121). Preliminary data suggest that the oral formulation of topotecan has efficacy similar to that of the IV formulation in patients with recurrent or refractory ovarian and small-cell lung cancer (122,123). However, oral absorption of anticancer agents, especially camptothecins, is characterized by extensive inter- and intrapatient variability in oral bioavailability (110,119,121,124). Given the high interpatient variability in topotecan exposure after oral administration, it is interesting to note that flat dosing (i.e., dose based on mg instead of mg/m^2) of topotecan resulted in the same systemic exposure compared with the more complex dosing per body surface area (125). Topotecan is presently supplied in gelatin capsules and is administered on an empty stomach, although administration of topotecan with a high fat meal only led to a small decrease in the rate of absorption but not the extent of absorption (126). Oral administration of topotecan is associated with lower peak concentration and longer mean residence time than IV topotecan with average oral bioavailability of 30% (range of 10% to 50%) (24,122). The lactone to carboxylate ratios of

topotecan after oral administration were higher or comparable after oral administration as compared to IV administration (24).

The chemical properties of camptothecins lend to the possibility that a decrease in the gastric pH could shift the lactone-carboxylate equilibrium towards the lactone form and increase oral absorption and bioavailability of the agents. Therefore, topotecan and other camptothecins are usually administered orally in conjunction with an acidic beverage, such as fruit juice or cola. However, it is currently unclear if this process affects bioavailability. The administration of ranitidine prior to oral topotecan resulted in a slightly faster rate of absorption, but the overall extent of absorption was not altered (127). Thus, in patients administered ranitidine, dosage adjustments of topotecan are not required. However, it is currently unknown if significant changes in gastric pH or co-administration with other agents will alter the oral bioavailability of topotecan.

Gerrits and colleagues evaluated the pharmacokinetic and pharmacodynamic relationships from four Phase I trials of oral topotecan using different treatment schedules (24). There was no difference in the pharmacokinetics of topotecan among the different regimens. Topotecan administered daily for 5 days repeated every 3 weeks was associated with limited interpatient variability and similar dose intensity as compared with other schedules of oral administration. However, there were differences in the toxicity profile associated with the different treatment regimens. Topotecan administered daily for 5 days produced uncomplicated and non-cumulative granulocytopenia as the primary toxicity, whereas the oral administration of topotecan for 10 and 21 consecutive days may result in unpredictable, severe, and uncontrollable diarrhea. Thus for these reasons and patient convenience, the authors recommended the single

daily administration of topotecan for 5 consecutive days repeated every 3 weeks for Phase II trials.

Oral administration of irinotecan may take advantage of several factors associated with its pharmacokinetics and metabolism (25). The high concentration of tissue carboxylesterases in the gut and liver may promote conversion of irinotecan to SN-38 (25,110,124). This, coupled with first pass effect after oral administration may increase the concentration of SN-38 systemically and in the liver, the primary site of colon cancer metastases (25,110). After oral administration of irinotecan, there was an association between the irinotecan dose and SN-38 systemic exposure, suggesting that there was no saturation in the conversion of irinotecan to SN-38 by CE (110,124). In addition, there was a high ratio of SN-38 to irinotecan systemic exposure suggesting that the oral administration resulted in significant presystemic formation of SN-38 (110,124). In a Phase I pediatric study, the ratio of SN-38 to irinotecan systemic exposure was approximately 3-fold higher after oral as compared to IV administration (124). However, the actual exposure of SN-38 after oral administration was significantly lower as compared to IV administration.

9NC is an oral camptothecin analog currently in Phase III studies for pancreatic carcinoma administered daily for 5 days per week for 8 consecutive weeks (128-130). The pharmacokinetic disposition of 9NC is relatively complicated. 9NC should be administered on an empty stomach, because administration with fatty food decreases oral bioavailability by 50% (131). 9NC undergoes metabolism to form an active-metabolite, 9-AC (130,132,133). However, approximately 80% of the drug remains in the 9-NC form (15,133). Repeated daily dosing of 9NC results in accumulation of all forms of the drug (15,133). In addition, 9NC undergoes

primarily non-renal elimination[117], thus there is the potential for drug interactions involving oxidative metabolism and biliary elimination.

In current Phase II and III trials, 9NC is administered at 1.25 to 1.5 mg/m^2/day for 5 days per week for 8 consecutive weeks (15,128,131,132). Dose-limiting toxicities (DLT) on the eight week schedule of 9NC are neutropenia and hematuria (15,128). To increase the dose administered per day and to avoid toxicities that occur in the 3rd and 4th weeks of the consecutive week schedule, a Phase I study of 9NC administered orally daily for 5 days per week for 2 consecutive weeks repeated every 4 weeks has potential clinical advantages compared to the continuous regimen (16,133).

Pharmacokinetically guided dosing

Most anticancer agents are characterized by extensive interpatient variability in systemic disposition (134). The standard doses derived from studies do not allow for this pharmacokinetic variability (134). Thus, when standard doses based on weight or body surface area are used, this variability leads to a wide range of systemic exposures and alterations in effect (ie, antitumor or toxicity). Previous studies with methotrexate and carboplatin have defined the relationships between drug exposure and effect (ie, antitumor effect and response), and have individualized treatment based on patient specific pharmacokinetic parameters (135,136). Pharmacokinetically guided dose adjustments may be used to account for pharmacokinetic variability achieve a target systemic exposure, and to optimize the therapeutic index of chemotherapeutic agents (99,137-139).

Pharmacokinetically guided dose adjustments have been used to achieve a maximum tolerated systemic exposure of topotecan and limit the interpatient variability in exposure of

topotecan (115,137). Preclinical *in vitro* and *in vivo* studies can be used to define the target concentration or AUC (6,11). Dose adjustments of topotecan administered as 30-minute and 120-hour infusions have been used to achieve target plasma exposures measured as AUC and steady-state concentration, respectively (115,137). In addition, individualizing topotecan doses to achieve target drug exposures in the CSF have been performed in patients with primary and metastatic central nervous system (CNS) tumors (140).

A Phase I trial of topotecan in pediatric patients with refractory solid tumors was performed to determine if the variability in topotecan systemic exposure could be reduced by using a pharmacokinetic-guided dosing strategy (115,116). The topotecan target AUC in plasma was defined by the AUC associated with antitumor activity in the xenograft models and was associated with clinical response in children with neuroblastoma in a prior Phase I trial (6,23). Topotecan was administered as a 30-minute infusion daily for 5 consecutive days for 2 weeks repeated every 4 weeks, and doses were individualized based on the patient's topotecan systemic clearance. The pharmacokinetically-guided dosing strategy adjusted topotecan doses to attain the desired plasma AUC in 75% of the targeting opportunities and significantly reduced the variability in topotecan systemic exposure.

Individualizing topotecan treatment has also been used to attain an exposure duration threshold (EDT) in the CSF (11,140,141). Embryonal CNS tumors such as medulloblastoma have a high propensity to disseminate throughout the subarachnoid space suggesting that the CSF is an important source of drug exposure (11). The target EDT in the CSF was determined by *in vitro* studies that defined the concentration and length of topotecan exposure associated with 99% inhibition of cell growth (11). These results defined the EDT as a topotecan lactone concentration of > 1 ng/mL for 8 hours. Studies in a nonhuman primate model reported

prolonging the topotecan IV infusion from the standard 30-minutes to 4-hours at a target plasma AUC achieved the EDT throughout the neuroaxis as presented in Figures 7a and 7b (11).

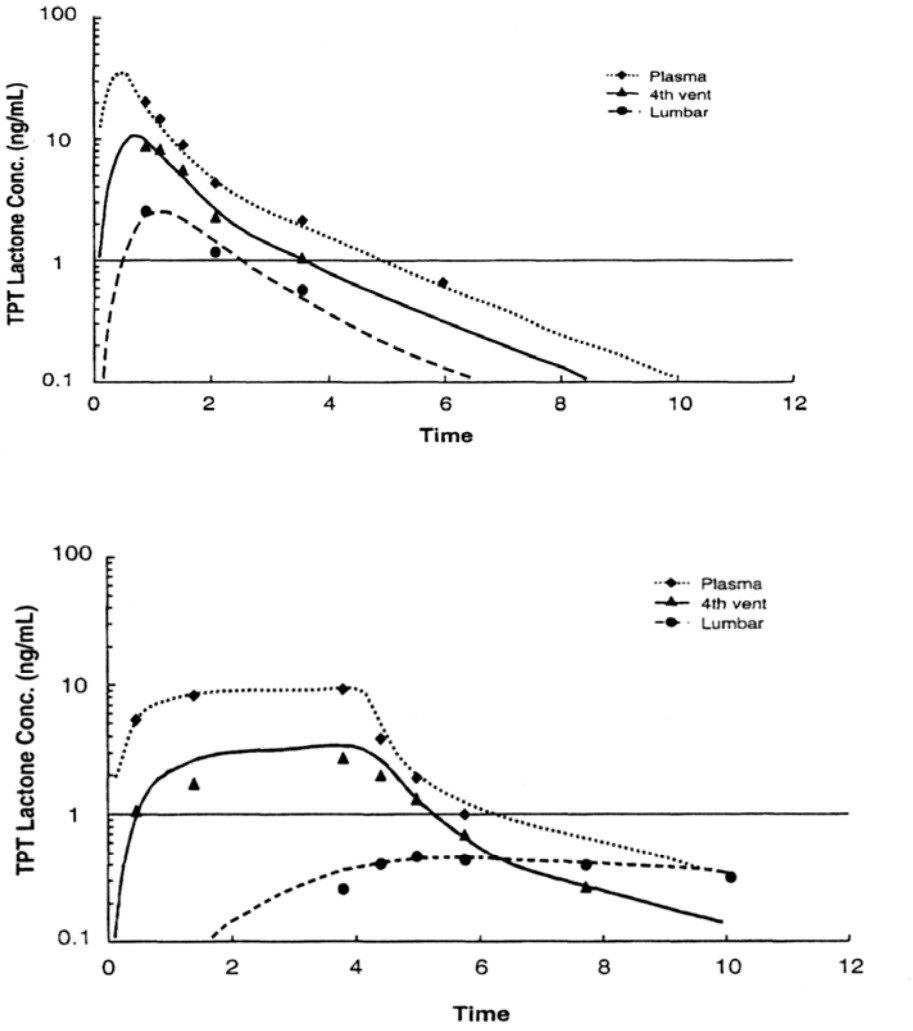


Figure 7a and 7b. Improved topotecan neuroaxis EDT in nonhuman primate model with prolonged IV infusion. Topotecan (TPT) lactone concentration-time profiles in the plasma, fourth ventricular CSF, and lumbar CSF after a TPT dose of 2.0 mg/m^2 administered over 30-minutes **(a)** and 4 hours **(b)**. Individual data points and best-fit line of the data are represented for TPT lactone concentrations in the plasma (♦, ---), fourth ventricular CSF (▲, —), and lumbar CSF (●, ---). Reprinted with permission from reference (11).

The ability of a 4-hour as compared to the standard 30-minute topotecan infusion to prolong the topotecan exposure in the CSF was confirmed in a patient with breast cancer metastatic to the

CNS (141). This approach has also been used to individualize doses to attain cytotoxic exposure of topotecan in the CSF of children with high-risk medulloblastoma (140). As with the Phase I study of topotecan in pediatric patients with refractory solid tumors, doses were individualized based on the patient's topotecan systemic clearance. As a result of dose individualization, 73% of studies achieved the target plasma AUC. Among those patients with CSF studies that attained the target plasma topotecan AUC, 90% achieved a putative cytotoxic exposure of topotecan in the CSF (i.e. 1 ng/mL for 8 hours). Thus, individualizing therapy based on patient specific pharmacokinetic parameters can be used to target drug concentrations at the site of the tumor. These studies demonstrate the ability to develop treatment strategies of systemically administered topotecan to enhance cytotoxic exposure in the CSF.

There are several logistical issues associated with individualizing anticancer therapy based on cytotoxic exposures of agents, such as the turn around time associated with obtaining blood samples, quantitation of drug concentrations using analytical chemistry methods, and performing pharmacokinetic analyses (137,138). However, studies using a maximum tolerated systemic exposure (MTSE) approach have the potential to determine the maximum tolerated plasma exposure of anticancer agents with fewer patients than more traditional Phase I designs. In addition, the results of these pharmacokinetic studies can greatly add to the understanding of the pharmacology and pharmacodynamics of anticancer agents. Moreover, individualizing therapy to achieve a target cytotoxic exposure may increase antitumor effect and decrease treatment related toxicity.

1.6 CONCLUSIONS

Camptothecin analogs are a class of anticancer agents with a wide range of antitumor activity, toxicities, and pharmacokinetic and pharmacodynamic features related to response. There are several cellular mechanisms which may alter the cytotoxicity of the camptothecins. The modulation of camptothecins by efflux pumps (P-gp, BCRP/ABC) may alter the cellular exposure of these agents and subsequent cytotoxicity. In addition, the presence of efflux pumps in the intestinal lining and kidney influence the oral bioavailibility and elimination pathways, respectively. Future studies are needed to investigate the combination of camptothecins with pump inhibitors and evaluate the antitumor efficacy, toxicity, and modulation of pharmacokinetics associated with these combinations. In addition, modulation of topo I and II may affect clinical activity of the camptothecins; however well designed clinical studies are needed to evaluate sequence and timing of administration of camptothecins and other drugs that can modulate topo I and II.

Preclinical studies suggest protracted schedules of administration achieve the greatest antitumor response; however the optimal schedule of administration may be unique to each camptothecin analog. Future preclinical and clinical studies for each camptothecin analog directly comparing alternative schedules of administration are required to address these issues. The issues related to the optimal schedule of administration of anticancer agents are not unique to the camptothecins (142). However, the optimal treatment regimens for camptothecins administered IV and orally are currently unknown. To adequately determine the optimal treatment schedule for each camptothecin, randomized Phase I and II trials may be required. Moreover, the questions regarding schedule of administration emphasizes the importance of Phase IV (post approval) studies in which the majority of clinical and pharmacologic drug development occurs (142).

The toxicities associated with the camptothecins are not uniform to the class. In general, the camptothecin analogs induce bone marrow suppression; however the dose limiting toxicities of topotecan, irinotecan, and 9NC are neutropenia, diarrhea, and cystitis, respectively. With the exception of irinotecan-induced diarrhea, the mechanisms associated with these differences in toxicities are currently unknown. The mechanism of camptothecin-induced cystitis is also currently unknown; however the incidence of cystitis is not associated with the percentage of drug excreted renally or the percent of drug in the lactone form in the urine (143). The clinical significance of the cellular and systemic pharmacokinetic and pharmacodynamic factors, such as modulation of topo I and cellular efflux, must be evaluated in clinical studies. In addition, phenotypic factors and drug interactions associated with the metabolism of camptothecins must be evaluated in clinical trials.

1.7 INTRODUCTION TO DISSERTATION

The primary objective of this dissertation research was to evaluate the pharmacokinetics, pharmacodynamics, and pharmacogenetics of 9NC and its 9AC metabolite in preclinical models and in patients as part of phase I and II trails. We hypothesized that pharmacokinetic, pharmacodynamic, and pharmacogenetic principles can be used in the optimization of the chemotherapeutic treatment of cancer, such as 9NC. Information obtained from preclinical and clinical translational studies can greatly add to the understanding of the pharmacology of 9NC and allow for the rational design of therapeutic regimens. The application of these principles have not been applied to the development of 9NC, especially the comparisons of drug exposures in preclinical models and in clinical trials and the evaluation of known SNP polymorphisms in the ABC transporter genes as related to the pharmacokinetic disposition of 9NC and 9AC. In

addition, previous pharmacokinetic studies of 9NC have not evaluated the disposition of the active-lactone forms of 9NC and 9AC.

Full descriptions of the study design and methods used in this research are outlined in each chapter. The aim of Chapter 2 was to compare the 9NC and 9AC systemic exposure associated with antitumor response in mice bearing human tumor xenografts to those reported in a phase I study of 9NC that used the s same intermittent schedule as in the xenografts studies and use allometric scaling analysis to normalize the data. The goal of Chapter 3 was to perform phase I studies of intermittent regimens of 9NC in order to determine the maximum tolerated dose (MTD) and toxicities associated with each regimen and evaluate the pharmacokinetics of 9NC and its 9AC metabolite. The purpose of Chapter 4 was to examine the inter- and intra-patient pharmacokinetic variability and the urine recovery of 9NC and 9AC. The aim of Chapter 5 was to evaluate the plasma disposition of 9NC and 9AC after administration of 9NC with and without food in a randomized cross over study. The goal of Chapter 6 was to evaluate the functional consequence of known SNP polymorphisms in the transporter genes *ABCB1, ABCC2,* and *ABCG2* on the pharmacokinetic disposition of 9NC and 9AC.

REFERENCES

1. Pommier Y, Pourquier P, Urasaki Y et al. Topoisomerase I inhibitors: selectivity and cellular resistance. Drug Resist Updates 1999; 2: 307-318.

2. Wall ME, Wani MC. Antineoplastic agents from plants. Annu Rev Pharmacol Toxicol 1997; 17: 177-132.

3. Stewart CF, Zamboni WC, Crom WR, et al. Topoisomerase I interactive drugs in children with cancer. Invest New Drugs 1996; 14: 37-47.

4. Urasaki Y, Laco G, Takebayashi Y et al. Use of camptothecin-resistant mammalian cell lines to evaluate the role of topoisomerase I in the antiproliferative activity of the indolocarbazole, NB-506, and its topoisomerase I binding site. Cancer Res 2001; 61: 504-508.

5. Gupta M, Fujimori A, Pommer Y. Eukaryotic DNA topoisomerase I. Biochim Biophys Acta 1995; 1262: 1-14.

6. Zamboni WC, Stewart CF, Thompson J et al. Relationship between topotecan systemic exposure and tumor response in human neuroblastoma xenografts. J Natl Cancer Inst 1998; 90: 505-511.

7. Furman WL, Stewart CF, Poquette CA et al. Direct translation of a protracted irinotecan schedule from a xenograft model to a phase I trial in children. J Clin Oncol 1999; 17: 1815-1824.

8. Thompson J, Stewart CF, Houghton PJ. Animal models for studying the action of topoisomerase I targeted drugs. Biochim Biophys Acta 1998; 1400: 301-319.

9. Houghton PJ, Cheshire PJ, Hallman JC et al. Efficacy of topoisomerase I inhibitors, topotecan and irinotecan administered at low dose levels in protracted schedules to mice bearing xenografts of human tumors. Cancer Chemother Pharmacol 1995;36:393-403.

10. Burris HA, III, Hanauske AR, Johnson RK et al. Activity of topotecan, a new topoisomerase I inhibitor, against human tumor colony-forming units *in vitro*. J Natl Cancer Inst 1992; 84: 1816-1820.

11. Zamboni WC, Gajjar AJ, Mandrell TD et al. A four-hour topotecan infusion achieves cytotoxic exposure throughout the neuraxis in the nonhuman primate model: implications for treatment of children with metastatic medulloblastoma. Clin Cancer Res 1998;4: 2537-2544.

12. Li M, Horn L, Firby PS et al. Pharmacological determinants of 9-aminocampothecin cytotoxicity. Clin Cancer Res 2001; 7: 168-174.

13. Hycamtin [package insert]. Philadelphia, PA: SmithKline Beecham Pharmaceuticals;1998.

14. Camptosar [package insert]. Kalamazoo, MI: Pharmacia and Upjohn Company; 2000.

15. Felton SA, Denis LJ, Rizzo J et al. Pharmacokinetic (PK) profile of 9-nitrocamptethecin (9-NC, RFS2000) following oral administration daily for 5 consecutive days every week. Proc Am Assoc Cancer Res 2000; 91.

16. Zamboni WC, Jung LL, Jin R et al. Phase I and pharmacokinetic (PK) study of intermittently administered 9-nitrocampothecin (9NC, Rubitecan) in patients with advanced malignancies. Proc Am Soc Clin Onc 2001;20;104a (abstract #411).

17. Josien H, Bom D, Curran DP. 7-Silylcamptothecins (silatecans): a new family of camptothecin antitumor agents. Bioorg Med Chem Lett 1997;7:3189-3194.

18. Bom D, Curran DP, Chavan AJ et al. Novel A, B, E-ring modified camptothecins displaying high lipophilicity and markedly improved human blood stabilities. J Med Chem 1999;42:3018-3022.

19. Kerr JZ, Aleksic A, McGuffey L et al. Plasma and cerebrospinal fluid pharmacokinetics of karenitecin (BNP-1350) in nonhuman primates. Proc Am Assoc Cancer Res 2000;41:815 (abstract #5173).

20. Lesueur-Ginot L, Demarquay D, Kiss R et al. Homocamptothecin, an E-ring modified camptothecin with enhanced lactone stability, retains topoisomerase I targeted activity and antitumor properties. Cancer Res 1999;59:2939-2943.

21. Philippart P, Harper L, Chaboteaux C et al. Homocamptothecin, an E-ring modified camptothecin, exerts more potent antiproliferative activity than other topoisomerase I inhibitors in human colon cancers obtained from surgery and maintained in vitro under histotypical culture conditions. Clin Cancer Res 2000;6:1557-1562.

22. Armstong D, O'Reilly S. Clinical guidelines for managing topotecan-related hematologic toxicity. The Oncologist 1998; 3: 4-10.

23. Tubergen DG, Stewart CF, Pratt CB et al. Phase I trial and pharmacokinetic (PK) and pharmacodynamic (PD) study of topotecan using a five-day course in children with refractory solid tumors: a pediatric oncology group study. J Pediatr Hematol Oncol 1996; 18: 352-61.

24. Gerrits CJH, Schellens JHM, Burris H et al. A comparison of clinical pharmacodynamics of different administration schedules of oral topotecan (Hycamtin). Clin Cancer Res 1999; 5: 69-75.

25. Rothenberg ML, Kuhn JG, Schaaf LJ et al. Alternative dosing schemes for irinotecan. Oncology (Huntingt) 1998; 12 (Suppl): 68-71.

26. Verschraegen CF, Gupta E, Loyer E et al. A phase II clinical and pharmacological study of oral 9-nitrocamptothecin in patients with refractory epithelial ovarian, tubal or peritoneal cancer. Anticancer Drugs 1999;10:375-83.

27. Stehlin JS, Giovanella BC, Natelson EA et al. A study of 9-nitrocamptothecin (RFS-2000) in patients with advanced pancreatic cancer. Int J Oncol 1999;14:821-831.

28. Mattern MR, Hofmann GA, Polsky RM et al. In vitro and in vivo effects of clinically important camptothecin analogues on multidrug-resistant cells. Oncol Res 1993;5:467-474.

29. Yang CHJ, Horton JK, Cowan KH et al. Cross-resistance to camptothecin analogues in a mitoxantrone-resistant human breast carcinoma cell line is not due to DNA topoisomerase I alterations. Cancer Res 1995;55:4004-4009.

30. Gupta E, Luo F, Lallo A et al. The intestinal absorption of camptothecin, a highly lipophilic drug, across Caco-2 cells is mediated by active transporter(s). Anticancer Res 2000;20:1013-1016.

31. Zamboni WC, Houghton PJ, Johnson RK et al. Probenecid alters topotecan systemic and renal disposition by inhibiting renal tubular secretion. J Pharmacol Exp Ther 1998;284(1):89-94.

32. Vanhoefer U, Muller MR, Hilger RA et al. Reversal of MDR-1 associated resistance to topotecan by PAK-200S, a new dihydropyridine analogue, in human cancer cell lines. Br J Cancer 1999:81:1304-1310.

33. Maliepaard M, van Gastelen MA, de Jong LA et al. Overexpression of the BCRP/MXR/ABCP gene in a topotecan selected ovarian tumor cell line. Cancer Res 1999;59:4559-4563.

34. Scheffer GL, Maliepaard M, Pijnenborg ACLM et al. Breast cancer resistance protein is localized at the plasma membrane in mitoxantrone and topotecan resistant cell lines. Cancer Res 2000;60:2589-2593.

35. Erlichman C, Boerner SA, Hallgren CG et al. The HER tyrosine kinase inhibitor CI1033 enhances cytotoxicity of 7-ehtyl-10-hydroxycamptothecin and topotecan by inhibiting breast cancer resistance protein-mediated drug efflux. Cancer Res 2001;61:739-748.

36. Bates SE, Robey R, Miyake K, Rao K, Ross DD, Litman T. The role of half-transporters in multidrug resistance. J Bioenerg Biomembr 2001;33:503-511.

37. Leonard GD, Polgar O, Bates SE. ABC transporters and inhibitors: new targets, new agents. Curr Opin Investig Drugs 2002;3:1652-1659.

38. Allikmets R, Schriml LM, Hutchinson A, Romano-Spica V, Dean M. A human placenta-specific ATP-binding cassette gene (ABCP) on chromosome 4q22 that is involved in multidrug resistance. Cancer Res 1998;58:5337-5339.

39. Doyle LA, Yang W, Abruzzo LV et al. A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci U S A 1998;95:15665-15670.

40. Miyake K, Mickley L, Litman T et al. Molecular cloning of cDNAs which are highly overexpressed in mitoxantrone-resistant cells: demonstration of homology to ABC transport genes. Cancer Res 1999;59:8-13.

41. Allen JD, Schinkel AH. Multidrug resistance and pharmacological protection mediated by the breast cancer resistance protein (BCRP/ABCG2). Mol Cancer Ther 2002;1:427-434.

42. Ross DD, Yang W, Abruzzo LV et al. Atypical multidrug resistance: breast cancer resistance protein messenger RNA expression in mitoxantrone-selected cell lines. J Natl Cancer Inst 1999;91:429-433.

43. Dean M, Allikmets R. Evolution of ATP-binding cassette transporter genes. Curr Opin Genet Dev 1995;5:779-785.

44. Brangi M, Litman T, Ciotti M et al. Camptothecin resistance: role of the ATP-binding cassette (ABC), mitoxantrone-resistance half-transporter (MXR), and potential for glucuronidation in MXR-expressing cells. Cancer Res 1999;59:5938-5946.

45. Miyake K, Litman T, Robey R, Bates SE, Brangi M. Camptothecin resistance: role of the ATP-binding cassette (ABC), mitoxantrone-resistance half-transporter (MXR), and potential for glucuronidation in MXR-expressing cells. Cancer Letters 1999;146:117-126.

46. Allikmets R, Gerrard B, Hutchinson A, Dean M. Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the expressed sequence tags database. Hum Mol Genet 1996;5:1649-1655.

47. Kool M, de Haas M, Scheffer GL et al. Analysis of expression of cMOAT (MRP2), MRP3, MRP4, and MRP5, homologues of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. Cancer Res 1997;57:3537-3547.

48. Rocchi E, Khodjakov A, Volk EL et al. The product of the ABC half-transporter gene ABCG2 (BCRP/MXR/ABCP) is expressed in the plasma membrane. Biochem Biophys Res Commun 2000;271:42-46.

49. Jonker JW, Smit JW, Brinkhuis RF et al. Role of breast cancer resistance protein in the bioavailability and fetal penetration of topotecan. J Natl Cancer Inst 2000;92:1651-1656.

50. Miyake K, Mickley L, Litman T et al. Molecular cloning of cDNAs which are highly overexpressed in mitoxantrone-resistant cells: demonstration of homology to ABC transport genes. Cancer Res 1999;59:8-13.

51. Doyle LA, Yang W, Abruzzo LV et al. A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci U S A 1998;95:15665-15670.

52. Jonker JW, Smit JW, Wagenaar E et al. P-glycoprotein reduces the oral availability of topotecan in mice. Proc Am Assoc Cancer Res 2000;41: 814-815 (abstract #5172).

53. Sparreboom A, Nooter K. Does P-glycoprotein play a role in anticancer drug pharmacokinetics? Drug Resistance Updates 2000; 3:357-363.

54. Cunningham RF, Israili ZH, Dayton PG. Clinical pharmacokinetics of probenecid. Clin Pharmacokinet 1981;6:135-151.

55. Kano Y, Akutsu M, Tsunoda S et al. In vitro schedule-dpepndent interaction between paclitaxel and SN-38 (the active metabolite of irinotecan) in human carcinoma cell lines. Cancer Chemother Pharmacol 1998;42:91-98.

56. Kaufmann SH. Antagonism between camptothecin and topoisomerase II directed chemotherapy agents in a human leukemia cell line. Cancer Res 1991;51:4213-4218.

57. Kaufmann SH, Peereboom D, Buckwalter et al. Cytotoxic effects of topotecan combined with various anticancer agents in human cancer cells lines. J Natl Cancer Inst 1996;88:734-741.

58. Madden T, Newman RA, Antoun G et al. Low-level taxane exposure increases the activity of topoisomerase I targeted agents. Proc Am Assoc Cancer Res 1998;39:527 (abstract #3583).

59. Hallin PA, Johansen M, Newman RA et al. Taxane effects on cell regulation and topoisomerase I activity. Proc Am Assoc Cancer Res 2000;41:143 (abstract #914).

60. Eder JP, Chan V, Wong J et al. Sequence effect of irinotecan (CPT-11) and topoisomerase II inhibitors in vivo. Cancer Chemother Pharmacol 1998;42:327-335.

61. Thompson J, George EO, Poquette CA et al. Synergy of topotecan in combination with vincristine for treatment of pediatric solid tumor xenografts. Clin Cancer Res 1999;5:3617-3631.

62. Hsiang YH, Hertzberg R, Hecht S et al. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J Biol Chem 1985;260:14873-14878.

63. Hertzberg RP, Caranfa MJ, Holden KG et al. Modification of the hydroxy lactone ring of camptothecin: inhibition of mammalian topoisomerase I and biological activity. J Med Chem 1989;32:715-20.

64. Slichenmyer WJ, Rowinsky EK, Donehower RC et al. The current status of camptothecin analogues as antitumor agents. J Natl Cancer Inst. 1993;85:271-291.

65. Potmesil M. Camptothecins: from bench research to hospital wards. Cancer Res 1994;54:1431-1439.

66. Wani MC, Nicholas AW, Manikumar G et al. Plant antitumor agents. 25. Total synthesis and antileukemic activity of ring A substituted camptothecin analogues. Structure-activity correlations. J Med Chem 1987;30:1774-79.

67. Zihou M, Burke TG. Differential interactions of camptothecin lactone and carboxylate forms with human blood components. Biochemistry 1994;33:10325-10336.

68. Zihou M, Burke TG. Marked interspecies variations concerning the interactions of camptothecin with serum albumin: a frequency-domain fluorescence spectroscopic study. Biochemistry 1994;33:12540-12545.

69. Schrijvers D, Higley MS, de Bruijn EA et al. Role of red blood cells in pharmacokinetics of chemotherapeutic agents. Anticancer Drugs 1999; 10: 147-153.

70. Cole DE, Blaney SM, Balis FM. Topotecan in physiologic fluids: depot of active drug in red blood cells. Proc Am Assoc Cancer Res 1995; 36: 363 (abstract #2161).

71. Loos WJ, Verweij J, Gelderblom HJ et al. Role of erythrocytes and serum proteins in the kinetic profile of total 9-amino-20-(S)-camptothecin in humans. Anticancer Drugs 1999; 10: 705-710.

72. Loos WJ, Gelderblom HJ, Verweij J et al. Gender-dependent pharmacokinetics of topotecan in adult patients. Anticancer Drugs 2000; 11: 673-680.

73. Hausheer F, Zhao M, Kochat H et al. Karenitecins: further developments with BNP1350, a novel, highly lipophilic, lactone stable camptothecin. Proc Am Assoc Can Res 2000;41:13(abstract #1360).

74. Schilsky RL, Hausheer FH, Bertucci D et al. Phase I trial of karenitecin administered intravenously daily for five consecutive days in patients with advanced solid tumors using accelerated dose titration. Proc Am Soc Clin Oncol 2000;19:195a (abstract #758).

75. Pollack IF, Erff M, Bom D et al. Potent topoisomerase I inhibition by novel silatecans eliminates glioma proliferation *in vitro* and *in vivo*. Cancer Res 1999;59:4898-4905.

76. Bailly C, Lansiaux A, Dassonneville L et al. Homocamptothecin, an E-ring modified camptothecin analogue, generates new topoisomerase I mediated DNA breaks. Biochemistry 1999;38:15556-15563.

77. Grossman SA, Hochberg F, Fisher J et al. Increased 9-aminocamptothecin dose requirements in patients on anticonvulsants. Cancer Chemother Pharmacol 1998;42:118-126.

78. Zamboni WC, Gajjar AJ, Heideman RL et al. Phenytoin alters the disposition of topotecan and N-desmethyl topotecan in a patient with medulloblastoma. Clin Cancer Res 1998;4:783-789.

79. Stewart CF, Liu CY, Zamboni WC et al. Population pharmacokinetics of topotecan in children and adolescents. Proc Am Soc Clin Oncol 2000; 19: 177a (abstract #687).

80. Ma MK, Turpen E, Stewart CF. Topotecan reaction phenotype provides insight into topotecan drug interactions in patients. Proc Amer Assoc Cancer Res 2000;41:538 (abstract #3429).

81. Goldwasser F, Lemoine A, Gross M et al. Topotecan is a cytochrome P-450 3A inhibitor: clinical and pharmacological evidence. Proc Am Assoc Cancer Res 1999;40:84 (abstract #559).

82. Zamboni WC, Egorin MJ, Van Echo DA et al. Pharmacokinetic and pharmacodynamic study of the combination of docetaxel and topotecan in patients with solid tumors. J Clin Oncol 2000;18:3288-3294.

83. Marre F. Sanderink GJ. de Sousa G et al. Hepatic biotransformation of docetaxel (Taxotere) in vitro: involvement of the CYP3A subfamily in humans. Cancer Res 1996;56:1296-302.

84. Royer I, Monsarrat B, Sonnier M et al. Metabolism of docetaxel by human cytochromes P450: interactions with paclitaxel and other antineoplastic drugs. Cancer Res 1996;56:58-65.

85. Haaz MC, Rivory L, Riche C et al. Metabolism of irinotecan (CPT-11) by human hepatic microsomes: participation of cytochrome P-450 3A and drug interactions. Cancer Res 1998;58:468-472.

86. Gajjar AJ, Radomski KM, Bowers DC et al. Pharmacokinetics of irinotecan and metabolites in pediatric high-grade glioma patients with and without co-administration of enzyme-inducing anticonvulsants. Proc Am Soc Clin Oncol 2000;19:162a (abstract #626).

87. Haaz MC, Rovory LP, Riche C et al. The transformation of irinotecan (CPT-11) to its active metabolite SN-38 by human liver microsomes. Differential hydrolysis for the lactone and carboxylate forms. Naunyn-Schmiedebergs Arch Pharmacol 1997;356:257-62.

88. Humerickhouse R, Lohrbach K, Li L et al. Characterization of CPT-11 hydrolysis by human liver carboxylesterase isoforms hCE-1 and hCE-2. Cancer Res 2000;60:1189-1192.

89. Khanna R, Morton CL, Danks MK et al. Proficient metabolism of irinotecan by a human intestinal carboxylesterase. Cancer Res 2000;60:4725-4728.

90. 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;60:4206-4210.

91. Morton CL, Wadkins RM, Danks MK et al. The anticancer prodrug CPT-11 is a potent inhibitor of acetylcholinesterase but is rapidly catalyzed to SN-38 by butyrylcholinesterase. Cancer Res 1999;59:1458-1463.

92. Wierdl M, Morton CL, Danks MK et al. Isolation and characterization of a cDNA encoding a horse liver butyrylcholinesterase. Evidence for CPT-11 drug activation. Biochem Pharmacol 2000;59:773-781.

93. Zamboni WC, Houghton PJ, Thompson J et al. Altered irinotecan and SN-38 disposition after intravenous and oral administration of irinotecan in mice bearing human neuroblastoma xenografts. Clin Cancer Res 1998;4:455-462.

94. Guichard S, Terret C, Hennebelle I et al. CPT-11 converting carboxylesterase and topoisomerase I activities in tumor and normal colon and liver tissues. Br J Cancer 1999;80:364-370.

95. Gupta E, Lestingi TM, Mick R et al. Metabolic fate of irinotecan in humans: correlation of glucuronidation with diarrhea. Cancer Res 1994;54:3723-3725.

96. Massaad L, de Waziers I, Ribrag V et al. Comparison of mouse and human colon tumors with regard to phase I and phase II drug metabolizing enzyme systems. Cancer Res 1992;52:6567-6575.

97. Danks MK, Morton CL, Durll EJ et al. Comparison of activation of CPT-11 by rabbit and human carboxylesterases for use in enzyme/prodrug therapy. Clin Cancer Res 1999;5:917-924.

98. Pawlik CA, Iyengar RV, Krull EJ et al. Use of the ornithine decarboxylase promoter to achieve N-MYC –mediated overexpression of a rabbit carboxylesterase to sensitize neuroblastoma cells to CPT-11. Mol Ther 2000;1:457-463.

99. Lalitha I, 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;101:847-854.

100. Kaneda N, Nagata H, Furuta T, Yokokura T. Metabolism and pharmacokinetics of camptothecin analogue CPT-11 in the mouse. Cancer Res 1990;50:1715-1720.

101. Lalitha I, Hall D, Das S et al. Phenotype-genotype correlation of in vitro SN-38 (active metabolite of irinotecan) and bilirubin glucuronidation in human liver tissue with UGT1A1 promoter polymorphism. Clin Pharmacol Ther 1999;65:576-582.

102. Mick R, Gupta E, Vokes EE et al. Limited-sampling models for irinotecan pharmacokinetics-pharmacodynamics: prediction of biliary index and intestinal toxicity. J Clin Oncol 1996;14:2012-2019.

103. Gupta E, Mick R, Ramirez J et al. Pharmacokinetic and pharmacodynamic evaluation of the topoisomerase inhibitor irinotecan in cancer patients. J Clin Oncol 1997;15:1502-1510.

104. Gupta E, Wang X, Ramirez J et al. Modulation of glucuronidation of SN-38, the active metabolite of irinotecan, by valproic acid and phenobarbital. Cancer Chemother Pharmacol 1997;39:440-444.

105. Gupta E, Safa AR, Wang X et al. Pharmacokinetic modulation of irinotecan and metabolites by cyclosporine A. Cancer Res 1996;56:1309-1314.

106. Kawato Y, Furuta T, Aonuma M et al. Antitumor activity of a camptothecin derivative CPT-11, against human tumor xenografts in nude mice. Cancer Chemother Pharmacol 1991; 28: 192.

107. Furuta T, Yokokura T. Effect of administration schedules on the antitumor activity of irinotecan, a camptothecin derivative. Gan To Kagaku Ryoho 1990; 17: 121.

108. Thompson J, Zamboni W, Cheshire P et al. Efficacy of systemic administration of irinotecan against neuroblastoma xenografts. Clin Can Res 1998; 3: 423-431.

109. Kirstein MN, Houghton PJ, Cheshire et al. Relation between 9-aminocamptothecin systemic exposure and tumor response in human solid tumor xenografts. Clin Cancer Res 2001;7:358-366.

110. Drengler RL, Kuhn JG, Schaaf LJ et al. Phase I and pharmacokinetic trial of oral irinotecan administered daily for 5 days every 3 weeks in patients with solid tumors. J Clin Oncol 1999; 17: 685-696.

111. Blaney S, Berg SL, Pratt C et al. A phase I study of irinotecan in pediatric patients: a pediatric oncology group study. Clin Cancer Res 2001; 7: 32-37.

112. Brown JV, Peters WA, Rettenmaier MA et al. A phase I trial of 3-day topotecan Q 21 days for recurrent epithelial cancers of the ovary, fallopian tube, and peritoneum. Gynecol Oncol 2000; 79: 495-498.

113. Ma MK, Zamboni WC, Radomski KM et al. Pharmacokinetics of irinotecan and its metabolites SN-38 and APC in children with recurrent solid tumors after protracted low-dose irinotecan. Clin Cancer Res 2000; 6: 813-819.

114. Hoskins P, Eisenhauer E, et al. Randomized phase II study of two schedules of topotecan in previously treated patients with ovarian cancer: a National Cancer Institute of Canada Clinical Trials Group study. J Clin Oncol 1998;16:2233-2237.

115. Zamboni WC, Santana V, Gajjar AJ et al. Pharmacokinetically guided dose adjustment reduces variability in topotecan (TPT) systemic exposure in children with solid tumors. Proc Am Soc Clin Oncol 1997; 16: 511a (abstract #1839).

116. Santana V, Zamboni W, Gajjar A et al. Pharmacokinetically guided use of topotecan (TPT) given (daily x 5) x2, in children with solid tumors. Proc Am Soc Clin Oncol 1997; 16: 205a (abstract #717).

117. Hardman WE, Moyer MP, Cameron IL. Efficacy of treatment of colon, lung and breast human carcinoma xenografts with: doxorubicin, cisplatin, irinotecan or topotecan. Cancer Res 1999; 19: 2269-2274.

118. De Cesare M, Zunino F, Pace S et al. Efficacy and toxicity profile of oral topotecan in a panel of human tumour xenografts. Eur J Cancer 2000; 36: 1558-1564.

119. Zamboni WC, Bowman LC, Tan M et al. Interpatient variability in bioavailability of the intravenous formulation of topotecan given orally to children with recurrent solid tumors. Cancer Chemother Pharmacol 1999; 43: 454-460.

120. Schellens JHM, Creemers GJ, Beijnen JH et al. Bioavailability of and pharmacokinetics of oral topotecan: a new topoisomerase I inhibitor. Br J Cancer 1996; 73: 1268.

121. Creemers GJ, Gerrits CJH, Eckhardt J et al. Phase I and pharmacologic study of oral topotecan administered twice daily for 21 days to adult patients with solid tumors. J Clin Oncol 1997; 15: 1087.

122. Burris HA. The evolving role of oral topotecan. Semin Hematol 1999; 36 Suppl:26-32.

123. Gelderblom HAJ, de Jonge MJA, Sparreboom A et al. Oral topoisomerase 1 inhibitors in adult patients: present and future. Invest New Drugs 1999; 17: 401-415.

124. Radomski KM, Stewart CF, Panetta JC. Phase I and pharmacokinetic study of oral irinotecan in pediatric patients with solid tumors. Proc Am Soc Clin Oncol 2000;19: 592a (abstract #2329).

125. Loos WJ, Gelderblom H, Sparreboom A et al. Inter- and intrapatient variability in oral topotecan pharmacokinetics: implications for body-surface area dosage regimens. Clin Cancer Res. 2000; 6: 2685-2689.

126. GeldHerben VMM, Rosing H, ten Bokkel Huinink WW et.al. A new oral dosage form of topotecan: Bioavailability and effect of food coadministration. Br J Cancer 1999; 80:1380-1386.

127. Akhtar S, Beckman RA, Mould DR et al. Pretreatment with ranitidine does not reduce the bioavailability of orally administered topotecan. Cancer Chemother Pharmacol 2000;46:204-210.

128. Rivkin S, Burris H, Gerstein H et al. A phase II study of rubitecan (RFS 2000, 9NC, 9-Nitro-20(S)-Camptothecin) in patients with refractory pancreatic cancer. Proc NCI-EORTC-AACR 2000; 91-92 (abstract #244).

129. Dennis LJ, Tolcher A, Rizzo J et al. Pharmacokinetic (PK) profile of 9-nitrocamptothecin (9-NC, RFS2000) following oral administration daily for 5 consecutive days every week. Proc Am Assoc Cancer Res 2000;41:538 (abstract #3428).

130. Natelson EA, Giovanella BC, Verschraegen CF et al. Phase I clinical and pharmacological studies of 20-(S)-camptothecin and 9-nitro-20(S)-camptothecin as anticancer agents. Ann N Y Acad Sci 1996; 13: 224-230.

131. Schöffski P, Herr A, Beijnen J et al. Effect of food intake on the pharmacokinetics of a single administration of oral Rubitecan (RFS-2000), a topoisomerase-1-inhibiting camptothecin analogue. Proc NCI-EORTC-AACR 2000; 91 (abstract #241).

132. Hinz HR, Harris NJ, Natelson EA et al. Pharmacokinetics of the in vivo and in vitro conversion of 9-nitro-20(S)-camptothecin to 9-amino-20(S)-camptothecin in humans, dogs, and mice. Cancer Res 1994; 54: 3096-100.

133. Zamboni WC, Jin R, Delauter BJ et al. Plasma & urine disposition of 9-nitrocamptothecin (9NC, rubitecan) & its 9-aminocamptothecin (9AC) metabolite as part of a phase I study

of intermittently administered rubitecan. Proc NCI-EORTC-AACR 2000; 91 (abstract #242).

134. Zamboni WC, Tonda ME. New designs of clinical trials. Highlights in Oncology Practice 2000; 18: 2-7.

135. Evans WE, Crom WR, Abromowitch M et al. Clinical pharmacodynamics of high-dose methotrexate in acute lymphocyte leukemia. Identification of a relation between concentration and effect. N Engl J Med. 1986; 314: 471-477.

136. Jodrell DL, Egorin MJ, Canetta RM et al. Relationships between carboplatin exposure and tumor response and toxicity in patients with ovarian cancer. J Clin Oncol. 1992; 10: 520-528.

137. Furman WL, Baker SD, Pratt CD et al. Escalating systemic exposure to topotecan following a 120-hr continuous infusion in children with relapsed acute leukemia. J Clin Oncol 1996; 14: 1504-1511.

138. Evans WE, Relling MV, Rodman JH et al. Conventional compared with individualized chemotherapy for childhood acute lymphoblastic leukemia. N Engl J Med. 1998; 338: 499-505.

139. Evans WE, Pui CH, Relling MV. Defining the optimal dosage of methotrexate for childhood acute lymphoblastic leukemia. New insights from the lab and clinic. Adv Exp Med Biol. 1999; 457: 537-541.

140. Ma MK, Gajjar AJ, Ashley DM et al. Individualized topotecan (TPT) dosing attains cytotoxic CSF TPT exposures in children with high-risk medulloblastoma. Neuro-Oncology 1999;1(4):323 (abstract #125).

141. Zamboni WC, Luftner D, Egorin M et.al. Increasing topotecan infusion from 30-minutes to 4-hours infusions prolongs the duration of exposure in the cerebrospinal fluid. Ann Oncol 2001;12:119-122.

142. O'Reilly S. Topotecan: what dose, what schedule, what route? Clin Cancer Res 1999; 5: 3-5.

143. Vozniak JM, Stewart CF, Ruzhi J et al. Lactone stability of camptothecin in urine: implications for the development of cystitis. Proc AACR 2001;42:935 (abstract #5021).

2.0

CHAPTER 2:

Relationship between Plasma Exposure of 9-Nitrocamptothecin and its 9-Aminocamptothecin Metabolite and Antitumor Response in Mice Bearing Human Colon Carcinoma Xenografts

2.1 ABSTRACT

Purpose: 9-nitrocamptothecin (9NC) has completed phase III studies in patients with newly diagnosed and refractory pancreatic cancer; however the optimal 9NC treatment regimen is unclear. We used an intermittent schedule of 9NC to evaluate the relationship between plasma exposure of 9NC and its 9-aminocamptothecin (9AC) metabolite and antitumor response in mice bearing human colon carcinoma xenografts.

Methods: 9NC was administered orally at 0.44, 0.67, or 1.0 mg/kg/d qd x 5d x 2 weeks repeated q 4 weeks for 2 cycles to female C.B-17 SCID mice bearing HT29 or ELC2 human colon xenografts. Pharmacokinetic studies were performed after oral administration of 0.67 mg/kg x 1. Serial samples were obtained and 9NC and 9AC lactone concentrations in plasma were determined by HPLC analysis with fluorescence detection. The areas under plasma concentration versus time curve (AUC) from 0 to infinity for 9NC and 9AC were calculated.

Results: The antitumor activity of 9NC was dose-dependent in both colon xenografts. At all doses, 9NC treatment resulted in significant antitumor activity in both xenografts as compared to vehicle-treated and control groups and achieved levels of tumor regression that met criteria (minimum %T/C $\leq$ 40%) for antitumor activity. In mice bearing HT29 xenografts, the 9NC and 9AC lactone AUCs after administration of 9NC at 0.67 mg/kg were 41.3 ng/ml•h and 5.7 ng/ml•h, respectively.

Conclusions: The responses seen in these xenograft models occurred at systemic exposures that are tolerable in adult patients. These results suggest that the intermittent schedule of 9NC may be an active-regimen in patients with colo-rectal cacinoma.

2.2 INTRODUCTION

9-nitrocamptothecin (9NC, rubitecan, RFS2000) has completed phase III studies in patients with newly diagnosed and refractory pancreatic cancer. However, the optimal dose, schedule, and route of administration of 9NC and other camptothecin analogues, such as topotecan and irinotecan, are currently unclear (1-5). *In vitro* and *in vivo* preclinical studies suggest protracted administration of low-doses of camptothecin analogues achieve better antitumor activity than does less frequent administration of higher doses (6-9). Repeated oral administration of 9NC could mimic the protracted schedule, maximize patient convenience, and minimize the use of health care resources. However, oral administration of camptothecin analogues has been associated with extensive inter- and intra-patient variability in bioavailability (10-13). In addition, the antitumor activity associated with camptothecin analogues, such as topotecan and irinotecan, may require a dose that achieves a systemic exposure above an exposure threshold (8,9,14,15). As a result, continuous and prolonged administration of low doses of camptothecins, which achieve systemic exposures below this threshold, may not produce an antitumor response. Thus, the highly schedule-dependent antitumor activity, steep relationship between systemic exposure and antitumor activity, and pharmacokinetic variability associated with oral absorption of camptothecin analogues, require studies evaluating the relationship between drug exposure and antitumor effect in preclinical and clinical studies. This process has been used in the development of topotecan, irinotecan, and 9-aminocamptothecin (9AC) (6,9,16).

9NC is administered orally and is partially metabolized to an active-metabolite, 9-aminocamptothecin (9AC) (5,13,17,18). As with other camptothecin analogues, 9NC and 9AC undergo a reversible, pH-dependent hydrolysis between the active lactone and inactive hydroxy

acid forms (19). At acidic pH, the lactone form predominates, and at physiologic pH, the hydroxy acid form predominates (19-21). However, changes in plasma pH, serum-albumin concentration, and route of administration may affect the conversion between the lactone and hydroxy acid forms (20,21). Thus, measurement of the active lactone form of the drug may be basic to understanding the clinical pharmacology of 9NC and other camptothecin analogues (21-24). Due to the differences in oral absorption, elimination, and percentage of 9NC and 9AC lactone in mice and humans, comparing the dose or total (sum of lactone plus hydroxyl acid) exposure that produces antitumor response in mice and humans would not be appropriate. We evaluated the relationship between plasma exposure of 9NC and 9AC lactone and antitumor response in mice bearing human colon carcinoma xenografts. In addition, we compared the 9NC and 9AC systemic exposures associated with antitumor response in the xenograft models to those reported in a Phase I study of 9NC that used the same intermittent schedule as in the xenograft studies and used allometric scaling analysis to normalize the data (1, 25).

2.3 METHODS

Mice

This study was approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh, and all animals were handled in accordance with the Guide to the Care and Use of Laboratory Animals (National Research Council, 1996). Mice (female C.B-17 SCID, 4-6 weeks of age, specific pathogen-free), were obtained from the National Cancer Institute (NCI) Animal Production Program (Frederick, MD), and were allowed to acclimate to the University of Pittsburgh Central Animal Facility for 1 week prior to initiation of study. Mice were housed in autoclaved micro isolator caging and were allowed Prolab ISOPRO RMH 3000

Irradiated Lab Diet (PMI Nutrition International, Brentwood, MO) and water *ad libitum*. Animal rooms were maintained at 22 ± 2°C on a 12-hour light and dark cycle with at least 12 air changes per hour. Tri-monthly analysis of sentinel mice (Assessment Plus, Charles River, Boston, MA) housed in 1/5 dirty bedding confirmed that the study mice remained murine antibody profile (MAP) negative throughout the study.

Tumor Lines, Implantation, and Measurements

HT29 and ELC2 human colon xenografts were obtained from the NCI Tumor Repository (Frederick, MD) and Dr. Janet Houghton at St. Jude Children's Research Hospital (Memphis, TN), respectively, and were MAP test-negative. HT29 and ELC2 cells were passaged in C.B-17 SCID female mice. When the tumors reached 500 to 1000 mm^3 (500 to 1000 mg), the tumors were harvested and approximately 25-mg fragments were implanted subcutaneously on the right flank of study mice using aseptic techniques. Mice were observed twice daily. Body weights and tumor measurements were recorded twice weekly. Tumor volumes were calculated from the formula: length x $(width)^2/2$, where length is the longest diameter, and width is the shortest diameter perpendicular to the length (25,26). Median days to one and two doublings of tumor volume and median optimal %T/C [(ratio of median tumor volume for treatment group (T) to median tumor volume for control group (C)) x 100] were calculated (26).

Efficacy Studies

Efficacy studies were performed in female C.B-17 SCID mice bearing HT29 or ELC2 human colon carcinoma xenografts. Mice bearing HT29 xenografts were stratified on day 19 post implantation when the tumor volumes were 26 to 71 mm^3. Mice bearing ELC2 xenografts

were stratified on day 17 post-implantation when the tumor volumes were 68 to 195 mm^3. Mice were stratified into groups of ten such that the mean body weight and tumor volumes for the groups were not statistically different. The stratification was performed 3 days prior to treatment (day –3).

9NC, obtained from Supergen, Dublin, CA, was prepared in 1 mM phosphoric acid:polyethylene glycol 400:2% N,N-dimethylacetamide acid (48:50:2, v/v/v). 9NC concentrations of 0.1 mg/ml, 0.067 mg/ml, or 0.044 mg/ml were prepared for the 1 mg/kg/d, 0.67 mg/kg/d, and 0.44 mg/kg/d doses, respectively. 9NC dosing solutions or vehicle were administered at 0.01 ml/g body weight using a curved, 20 gauge, oral gavage needle attached to a 1 ml syringe. Mice (n = 10 per group) bearing HT29 xenografts were treated with 9NC daily for 5 days per week for 2 consecutive weeks repeated every 4 weeks for a total of 2 cycles. To serve as a positive control, 5-fluorouracil (5-FU) was prepared at 2 mg/ml in 0.9% NaCl and administered at 0.01 ml/g body weight (27). Mice (n = 10 per group) bearing HT29 tumors were treated with 5FU intraperitoneal (IP) at 20 mg/kg/d for 5 days per week for 2 consecutive weeks repeated every 4 weeks for a total of 2 cycles (27). For mice bearing HT29 xenografts, treatment days during cycle 1 were days 0 to 4 and days 7 to 11, and treatment days during cycle 2 were days 28 to 32 and days 35 to 39. At the completion of the study, mice were euthanized by CO_2 inhalation, and complete necropsies were performed.

Mice (n = 10 per group) bearing ELC2 xenografts were treated with 9NC at the same doses and on the same schedules as mice bearing HT29 xenografts; however the mice bearing ELC2 tumors received only the first 5 days of the treatment of the second cycle. The study was terminated early because the tumor volumes in the control and vehicle-treated groups reached 2,000 m^3, and the mice in these groups had significant (> 10%) loss of body weight. Treatment

days during cycle 1 were days 0 to 4 and days 7 to 11, and treatment days during cycle 2 were days 28 to 32. At the completion of the study, mice were euthanized by CO_2 inhalation, and complete necropsies were performed.

Pharmacokinetic Studies

Plasma pharmacokinetic studies were performed in C.B-17 SCID mice bearing HT29 xenografts at 27 days post-tumor implantation and in non-tumor bearing mice. Mice were stratified into three mice per group such that the mean body weight and tumor volumes were not statistically different (28).

Animals were fasted overnight prior to dosing and 9NC was administered at 0.67 mg/kg as a single oral dose. After dosing, mice (n = 3 per time point) were euthanized by CO_2 inhalation, and blood (approximately 1 ml) was collected, by cardiac puncture using heparinized syringes, at 0.08, 0.25, 0.5, 1, 1.5, 2, 4, 6, 7, 18, 24, and 48 h after 9NC administration. In addition, vehicle-treated mice were euthanized at 5 min after administration using the same procedure as stated above. Blood was transferred to microcentrifuge tubes and immediately placed on ice until centrifuged.

Sample Preparation

Plasma was prepared by centrifuging blood samples at 13,000 x g at room temperature for 4 min. For analysis of 9NC lactone, solid phase extraction (SPE) was used to separate the lactone and hydroxy acid forms of 9NC and 9AC (13). Waters OASIS HLB columns (1 ml, 30 mg; Milford, MA) were used for the SPE. The columns were conditioned with 1 ml of methanol and equilibrated with 1 mL of water prior to loading plasma samples (0.2 ml) on the columns.

The columns were then washed with 1 ml of methanol:water (5:95, v/v) to remove the hydroxy acid forms of 9NC and 9AC. 9NC lactone was then eluted with 0.5 ml of methanol and stored at -80°C until analyzed. For analysis of 9AC lactone, plasma was processed using methanol extraction as previously described and stored at -80°C until analyzed (13).

High Performance Liquid Chromatography (HPLC) Analysis

The concentrations of 9NC and 9AC lactone in plasma were quantitated using an HPLC assay with fluorescence detection modified from that described in our previous pharmacokinetic studies in humans (13). Because 9NC is not highly fluorescent and 9AC is, 9NC lactone was measured by chemically reducing 9NC to 9AC (13,29,30). The concentration of 9NC was calculated by subtracting the concentration of 9AC from the concentration of 9NC plus 9AC after the conversion of 9NC to 9AC (13,30). The lower limit of quantitation (LLQ) for 9NC lactone was 0.5 ng/ml, and the assay was linear from 0.5 ng/ml to 100 ng/ml. The LLQ for 9AC lactone was 0.3 ng/ml, and the assay was linear from 0.3 ng/ml to 100 ng/ml. When expressed as a percentage coefficient of variation, the within-day and between day variation in 9NC and 9AC lactone triplicate standards in plasma were < 15%.

Pharmacokinetic Analysis

Compartmental pharmacokinetic methods were used to analyze the 9NC and 9AC lactone plasma concentration versus time data from mice bearing HT29 human colon tumors and non-tumor bearing mice. Compartmental modeling was performed with the ADAPT II computer program (31). The estimation procedure and variance model used in the compartmental

pharmacokinetic analysis was maximum likelihood estimation and linear models for the variance of the additive errors, respectively. Different pharmacokinetic model structures were considered to characterize the disposition of 9NC and 9AC in plasma. In the model development, one- and two-compartment models were evaluated to describe the systemic disposition of 9NC and 9AC. Akaike's Information Criteria (AIC), Schwartz Criteria, estimated error of the model parameters, and residual analysis were use to select the model structure that maximized the fit accuracy and simultaneously minimized the number of model parameters. The final model structure used for the pharmacokinetic analysis produced identifiable parameters in both groups.

A 4-compartment model with an oral absorption compartment was simultaneously fit to the mean plasma concentration versus time profiles of 9NC and 9AC (31). Individual parameters estimated by the model included: the rate constant describing oral absorption (k_a), intercompartmental rate constants for 9NC (k_{12}, k_{21}) and 9AC (k_{34}, k_{43}), the rate constant describing conversion of 9NC to 9AC (k_{13}), the elimination rate constants for 9NC (k_{10}) and 9AC (k_{30}), and the apparent volume of the central compartments for 9NC (Vc1/F)and 9AC (Vc3/F) The apparent clearance (CL/F) and half-life ($t_{1/2}$) of 9NC and 9AC lactone were calculated using standard equations (32). The areas under the 9NC and 9AC lactone plasma concentration versus time curves (AUC) from 0 to infinity were calculated using the log trapezoidal method by simulating the concentration versus time data based upon model-specific parameters (32).

Allometric Scaling

Allometric scaling was used to compare the disposition of 9NC and 9AC lactone in patients with refractory solid tumors and in mice bearing HT29 human colon xenografts (1,25). The 9NC and 9AC lactone concentration versus time data in patients was from a phase I study of

orally administered 9NC daily for 5 consecutive days per week for two weeks repeated every 4 weeks (1). Pharmacokinetic studies were performed on day 1 after a single dose of 9NC at 2.4 mg/m^2 (n = 9), which was the maximum tolerated dose (MTD). The mean 9NC and 9AC lactone concentration at each time point was used in the allometric calculations.

Allometric scaling is based on the power-law relationship between physiological and pharmacokinetic processes and body weight among mammals (25). Complex Dedrick Plots were used to normalize the concentration versus time profiles in patients and mice (25). Interspecies concentrations and time were normalized by dividing the plasma concentration (ng/ml) by the dose (mg/kg) and by dividing the time (h) by body $weight^{0.25}$ (kg). The 9NC and 9AC lactone AUCs from 0 to last measured concentration-time point for the Complex Dedrick Plots were calculated using the log trapezoidal method (32).

Statistics

Body weights and tumor volumes from both of the efficacy studies were analyzed for statistical significance using the statistical software package Minitab (Minitab Inc., State College PA) (28). Values were expressed as the mean ± SD and median. Mean data were analyzed by one-way ANOVA followed by pair-wise comparisons using Dunnett's test. Median data were analyzed using Kruskal-Wallis followed by pair-wise comparisons by Mann-Whitney test (28). The *a priori* level of significance was set at P = 0.05.

2.4 RESULTS

Efficacy and Toxicity

Tumor growth curves for HT29 and ELC2 xenografts are presented in Figure 1 and Figure 2, respectively. Days to 1 and 2 doublings of tumor volume and median optimal %T/C for HT29 and ELC2 xenografts are presented in Table 1 and Table 2, respectively. 9NC treatment resulted in significant antitumor activity in both human colon xenografts as compared to the vehicle-treated and control groups ($P < 0.05$) (26). All doses of 9NC in both xenografts lines achieved levels of activity that met criteria for antitumor activity (minimum %T/C $\leq$ 40%) (26).

Table 1. Doubling Times for Tumor Volume and Median Optimal % T/C for HT29 Human Colon Xenografts

Treatment Groups (n=10)	Days to 1 Doubling of Tumor Volume	Days to 2 Doublings of Tumor Volume	Median Optimal % T/C
	Mean ± SD (Median)	Mean ± SD (Median)	% (Day)
Control	5.3 ± 2.0 (5.0)	22.5 ± 7.3 (21.0)	---
5FU 20 mg/kg	8.8 ± 2.9 (10.0)	33.3 ± 14.0 (34.0)	44.7 (43)
9NC 1 mg/kg	48.7 ± 12.0[a,b,c] (52.5)	58.7 ± 5.1[a,b,c] (59.7)	9.8 (43)
9NC 0.67 mg/kg	28.9 ± 8.7[a] (29.0)[b]	46.9 ± 5.6[a] (47.9)[b]	23.0 (43)
9NC 0.44 mg/kg	25.5 ± 10.3[a] (26.6)[b]	42.0 ± 6.9[a] (41.5)[b]	34.7 (43)
Vehicle	6.9 ± 2.3 (6.5)	30.9 ± 6.7 (30.1)	73.1 (43)

T = median tumor volume in treated group; C = median tumor volume in control group.

[a]Significantly different as compared to control and vehicle at $P \leq 0.05$ as determined by Dunnett's Test.

[b]Significantly different as compared to control and vehicle at $P \leq 0.05$ as determined by Mann-Whitney Test.

[c]Significantly different as compared to 0.67 and 0.44 mg/kg at $P \leq 0.05$ as determined by Mann-Whitney Test.

Table 2. Doubling Times for Tumor Volume and Median Optimal % T/C for ELC2 Human Colon Xenografts

Treatment Groups (n=10)	Days to 1 Doubling of Tumor Volume	Days to 2 Doublings of Tumor Volume	Median Optimal % T/C
	Mean ± SD (Median)	Mean ± SD (Median)	% (Day)
Control	10.6 ± 3.7 (10.5)	24.1 ± 6.6 (26.0)	---
9NC 1 mg/kg	>35.0[a,b,c] (>35.0)	>35.0[a,b,c] (>35.0)	18.4 (35)
9NC 0.67 mg/kg	34.2 ± 2.5[a,b,c] (>35.0)	>35.0[a,b,c] (>35.0)	21.8 (35)
9NC 0.44 mg/kg	25.7 ± 8.9[a] (26.9)[b]	34.8 ± 4.2[a] (>35.0)[b]	31.1 (35)
Vehicle	12.2 ± 5.6 (13.3)	24.2 ± 5.7 (25.1)	111.8 (35)

T = median tumor volume in treated group; C = median tumor volume in control group.

[a]Significantly different as compared to control and vehicle at $P \leq 0.05$ as determined by Dunnett's Test.

[b]Significantly different as compared to control and vehicle at $P \leq 0.05$ as determined by Mann-Whitney Test.

[c]Significantly different as compared to 0.44 mg/kg at $P \leq 0.05$ as determined by Mann-Whitney Test.

The antitumor activity of 9NC in both xenografts was dose-dependent (Figure 1 and Figure 2). In mice bearing HT29 xenografts, the days to one and two tumor doublings were significantly longer after administration of 1 mg/kg as compared to 0.67 mg/kg and 0.44 mg/kg ($P < 0.05$). For HT29 xenografts, the median optimal % T/C after administration of 1 mg/kg, 0.67 mg/kg, and 0.44 mg/kg were 9.8 %, 23.0 %, and 34.7 %, respectively, as measured on day

43. In mice bearing ELC2 xenografts, the days to one and two tumor doublings were significantly longer after administration of 1 mg/kg and 0.67 mg/kg as compared to 0.44 mg/kg ($P < 0.05$). For ELC2 xenografts, the median optimal % T/C after administration of 1 mg/kg, 0.67 mg/kg, and 0.44 mg/kg were 18.4 %, 21.8 %, and 31.1 %, respectively, as measured on day 35.

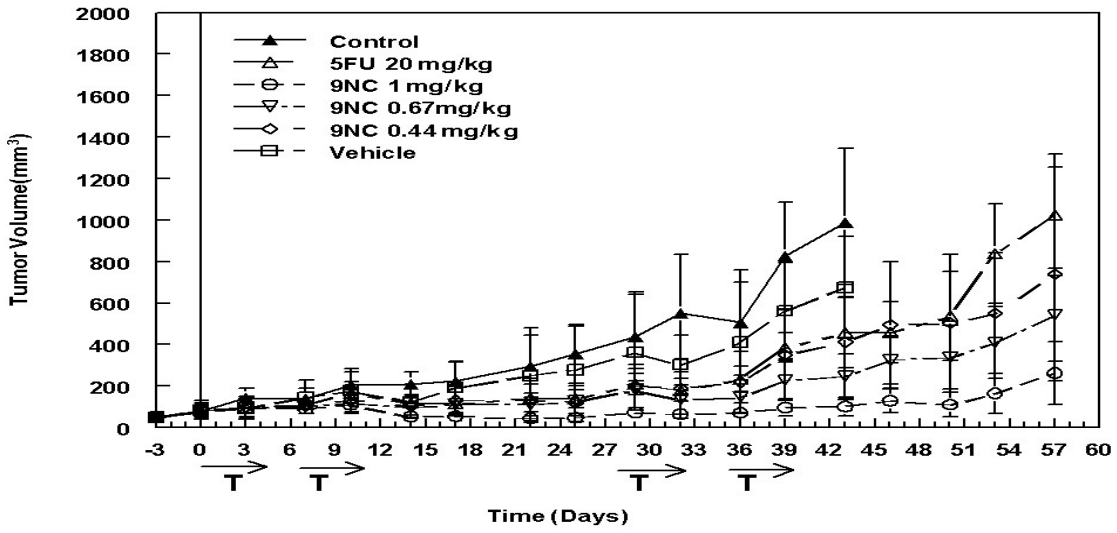


Figure 1. Tumor growth curves for mice (n = 10 per group) bearing HT29 human colon carcinoma xenografts. Mice were treated with 9NC at doses of 0.44, 0.67, or 1 mg/kg, or vehicle via oral gavage daily for 5 days per week for 2 weeks repeated every four weeks for 2 cycles. As a positive control, 5FU was administered IP at 20 mg/kg daily for 5 days per week for 2 consecutive weeks repeated every four weeks for 2 cycles. The control group received no treatment or vehicle and the vehicle group received only the vehicle used in the 9NC formulation. Treatment (T) days on cycle 1 were days 0 to 4 and days 7 to 11, and treatment days on cycle 2 were days 28 to 32 and days 35 to 39. The vertical line at day 0 represents the start of treatment. Results are expressed as the mean ± SD tumor volumes.

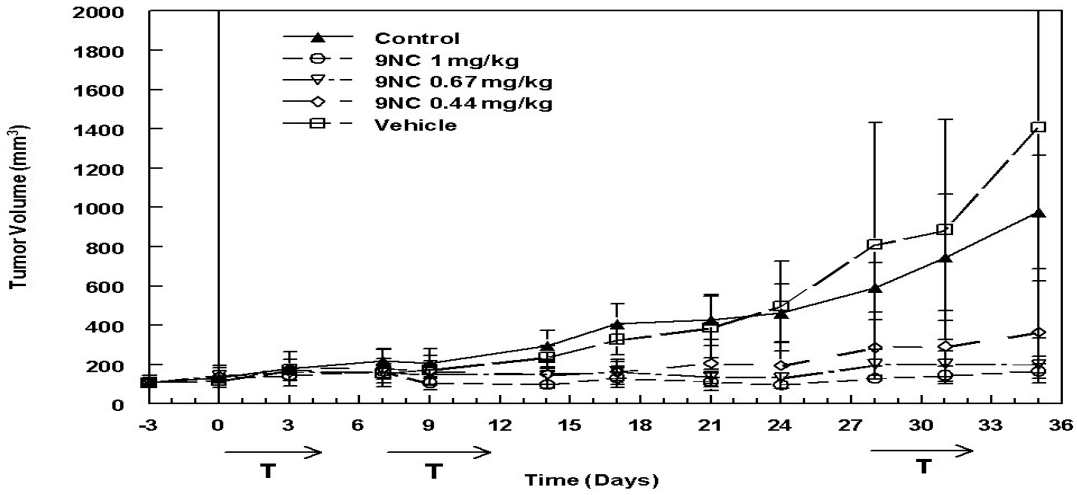


Figure 2. Tumor growth curves for mice (n = 10 mice per group) bearing ELC2 human colon carcinoma xenografts. Mice were treated with 9NC at doses of 0.44, 0.67, or 1 mg/kg, or vehicle via oral gavage daily for 5 days per week for 2 weeks repeated every four weeks for 2 cycles. The control group received no treatment or vehicle and the vehicle group received only the vehicle used in the 9NC formulation. Mice bearing ELC2 xenografts received only the first 5 days of the treatment of the second cycle. The study was terminated early because the tumor volumes in the control and vehicle-treated groups reached 2,000 mm^3, and mice in these groups had significant (> 10%) weight loss. In mice bearing ELC2 tumors, treatment (T) days on cycle 1 were days 0 to 4 and days 7 to 11, and treatment days on cycle 2 were days 28 to 32. The vertical line at day 0 represents the start of treatment. Results are expressed as the mean ± SD tumor volumes.

The primary toxicity associated with 9NC in mice bearing HT29 and ELC2 human colon xenografts was soft stools and diarrhea. After administration of 9NC at 1 mg/kg in mice bearing HT29 and ELC2 xenografts, all mice had soft stools by day 3 of treatment and developed diarrhea by day 7 of treatment. After administration of 9NC at 0.67 mg/kg in mice bearing HT29 and ELC2 xenografts, mice had soft stools by day 7 and developed diarrhea by day 9 to 10. After administration of 9NC at 0.44 mg/kg and vehicle in mice bearing both tumor xenografts, soft stools developed during the second week of treatment. During the two week break in treatment (days 13 to 28), stools became more solid. The pattern of soft stools and diarrhea was similar during cycles 1 and 2.

Pharmacokinetic Studies

Pharmacokinetic studies of 9NC and 9AC lactone were performed in non-tumor bearing mice and mice bearing HT29 xenografts after administration of a single dose of 9NC at 0.67 mg/kg. The 9NC and 9AC lactone concentration versus time profiles in mice bearing HT29 xenografts are presented in Figure 3. In mice bearing HT29 xenografts, peak plasma concentrations of 9NC and 9AC were detected at 15 minutes after administration. The mean ± SD peak plasma concentrations of 9NC and 9AC lactone were 45.4 ± 8.1 ng/ml and 4.2 ± 1.4 ng/ml, respectively. Pharmacokinetic parameters of 9NC and 9AC in mice bearing HT29 xenografts are summarized in Table 3. The 9NC and 9AC lactone AUCs were 41.3 ng/ml•hr and 5.7 ng/ml•hr, respectively.

The 9NC and 9AC lactone concentration versus time profiles in non-tumor bearing mice were similar to those in mice bearing HT29 colon xenografts and are presented in Figure 3. In non-tumor bearing mice, peak plasma concentrations of 9NC and 9AC lactone were detected at 15 minutes after administration. The mean ± SD peak plasma concentrations of 9NC and 9AC lactone were 46.8 ± 27.6 ng/ml and 3.3 ± 1.8 ng/ml, respectively. Pharmacokinetic parameters of 9NC and 9AC in non-tumor bearing mice are summarized in Table 3. The 9NC and 9AC lactone AUCs were 40.7 ng/ml•hr and 5.2 ng/ml•hr, respectively.

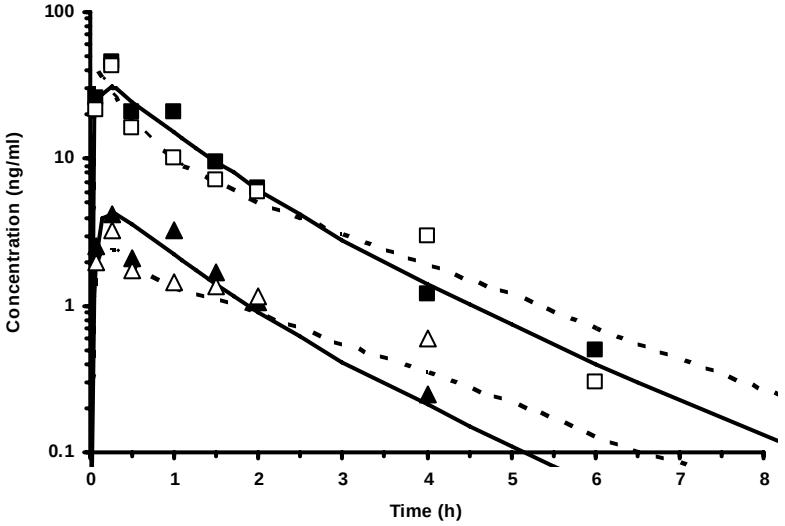


Figure 3. 9NC and 9AC lactone concentration versus time profiles in mice bearing HT29 human colon xenografts and non-tumor bearing mice after administration of 9NC at 0.67 mg/kg x 1 via oral gavage. For mice bearing HT29 tumors, individual data points and best fit line of the data for 9NC lactone (■, —) and 9AC lactone (▲, —) are presented. For non-tumor bearing mice, individual data points and best fit line of the data for 9NC lactone (□, ---) and 9AC lactone (Δ, ---) are presented. Individual concentration versus time points represent the average concentration of 9NC or 9AC from three mice at each time point.

Table 3. Pharmacokinetic Parameters of 9NC and 9AC in Mice Bearing HT29 Human Colon Xenografts and Non-tumor Bearing Mice

Parameters	Units	HT29 Bearing Mice	Non-Tumor Bearing Mice
9NC AUC	ng/mL●h	41.3	39.1
9NC CL/F	L/h/m^2	61.6	52.6
9NC $t_{1/2\,\beta}$	h	1.2	1.5
k_a	h^{-1}	1.2	3.3
Vc1/F	L/m^2	4.5	4.0
k_{10}	h^{-1}	10.8	12.9
k_{12}	h^{-1}	3.4	3.0
k_{21}	h^{-1}	0.8	1.2
k_{13}	h^{-1}	0.8	0.3
9AC AUC	ng/mL●h	5.7	5.2
9AC CL/F	L/h/m^2	26.3	9.6
9AC $t_{1/2\,\beta}$	h	0.6	1.0
Vc3/F	L/m^2	1.2	1.1
k_{34}	h^{-1}	2.8	7.4
k_{43}	h^{-1}	5.3	1.3
k_{30}	h^{-1}	21.8	8.8

Allometric Scaling Studies

Complex Dedrick Plots of 9NC and 9AC lactone in patients after administration of 9NC at 2.4 mg/m^2 (n = 9) and in mice bearing HT29 human colon xenografts after administration of 9NC at 0.67 mg/kg are presented in Figure 4. The 9NC and 9AC lactone AUCs for the Complex Dedrick Plots in the patients treated at 2.4 mg/m^2 were 393 and 60 (ng/ml / mg/kg)•h/kg$^{0.25}$, respectively. The 9NC and 9AC lactone AUCs for the Complex Dedrick Plots in mice bearing HT29 human colon xenografts were 193 and 30 (ng/ml / mg/kg)•h/kg$^{0.25}$, respectively.

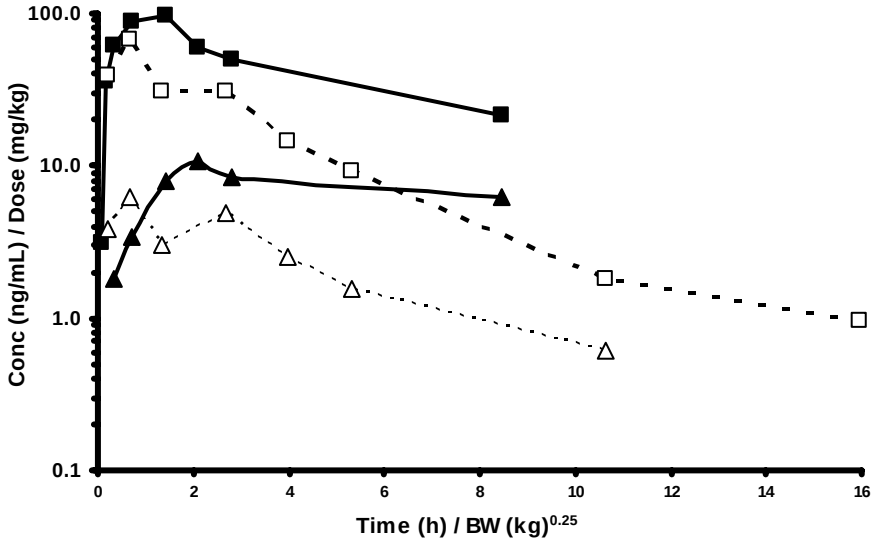


Figure 4. Complex Dedrick Plots of 9NC and 9AC lactone in patients after oral administration of 9NC at 2.4 mg/m^2/d and in mice bearing HT29 human colon xenografts after administration of 9NC at 0.67 mg/kg x 1 via oral gavage. Interspecies dose and time were normalized by dividing the plasma concentration (ng/mL) by the dose (mg/kg) and by dividing the time (h) by body weight$^{0.25}$ (kg). Data for 9NC lactone (■, —) and 9AC lactone (▲, —) in a patients and 9NC lactone (□, ---) and 9AC lactone (Δ, ---) in mice bearing HT29 human colon xenografts concentration versus time profiles are presented. The data presented at each data point are the average of nine patients and three mice.

2.5 DISCUSSION

It takes a considerable length of time for a drug to be approved for the treatment of cancer (33-36). Thus, there is a need to expedite the preclinical and clinical studies of anticancer agents and determine which agents should continue, or be stopped, in development (33,37,38).

During the past 25 years, there has been a progressive shift from syngeneic transplantable tumors to the use of human tumor xenografts for the identification and development of new anticancer agents (6-9,33). Support for these preclinical studies is based on the proposed improved ability of xenograft models to identify agents with clinical utility. The factors associated with correctly predicting the activity of anticancer agents in patients using xenograft models are currently unclear (7,9,16,19-21). Camptothecin analogues are some of the most highly active agents evaluated in xenograft models. However, mice are able to tolerate 10- to 20-fold greater doses and systemic exposures of camptothecin analogues (i.e., topotecan, irinotecan, 9AC) compared to humans (6,7,9,16). Therefore, the activity of camptothecins in xenograft models may not translate to clinical activity in humans. To address this issue as related to 9NC, we evaluated the relationship between plasma exposure of 9NC and 9AC lactone and antitumor response in mice bearing human colon xenografts and compared this to clinically relevant exposures in patients (1). Comparing the lactone forms of 9NC and 9AC overcomes potential differences in the lactone to hydroxyl acid ratio associated with protein binding and other factors between mice and humans (6). This process has previously been used in the development of topotecan, irinotecan, and 9AC (6,9,16).

9NC produced significant antitumor activity at all doses evaluated in both human colon xenografts. The 9NC and 9AC lactone AUCs after administration of 9NC at 0.67 mg/kg were 41.3 and 5.7 ng/mL•hr, respectively. The dose of 0.67 mg/kg evaluated in the pharmacokinetic study was selected based on the greater overall antitumor activity after administration of 0.67 mg/kg compared to 0.44 mg/kg and less diarrhea after administration of 0.67 mg/kg compared to 1 mg/kg. In the Phase I study using the same regimen as in the xenograft studies, 4 of 6 patients treated at the MTD (2.4 mg/m^2/day) had a 9NC AUC > 41 ng/mL·h (13). In addition, 5 of the 6

patients who responded had a 9NC AUC > 41 ng/mL·h. In our Phase II study of 9NC administered orally daily for 5 days per week for 8 weeks in patients with refractory colon cancer, 3 of 8 patients had a 9NC lactone AUC < 41 ng/mL·hr (13,39). Moreover, there were no responders in Phase II studies of 9NC in patients with colon cancer (2,39). The overall lack of 9NC response in patients with refractory colon carcinoma raises concerns about the ability of mice bearing human colon xenografts to predict 9NC response in humans (19-21). However, this lack of response in the phase II studies may be associated with the low daily exposures achieved with this regimen. The intermittent schedule of 9NC may be an active regimen because it achieves exposures that are tolerable in humans and above the exposure associated with antitumor response in the colon xenograft models.

Using the approach comparing the drug exposure associated with response in preclinical xenografts models and in patients may also explain the variable clinical response of camptothecin analogues (6,9,16). The topotecan systemic exposure associated with antitumor response in the xenograft models of neuroblastoma was tolerable and was associated with clinical responses in children with neuroblastoma (9). Kirstein and colleagues evaluated the relationship between 9AC systemic exposure and tumor response in human solid tumor xenografts (30). The systemic exposure of 9AC required for antitumor effect in the xenograft models was in excess of that achievable in patients, possibly explaining why 9AC has not produced significant antitumor activity in clinical trials (16,40,41). In our studies, the conversion of 9NC to 9AC in mice and patients was similar, with most of the drug remaining in the 9NC form. Thus, the antitumor activity associated with administration of 9NC is most likely due to the parent compound and not the 9AC metabolite.

Even though the exposures of 9NC associated with response in the xenograft models are tolerable in clinical trials, there are potential differences in the disposition of 9NC between mice and humans, and tumor-specific issues that must be addressed (6,9,13,16,20). The concentration versus time profiles of 9NC and 9AC in mice and humans are different. Thus, there may be differences in the duration of exposure that 9NC and 9AC are maintained above a potential threshold required for activity (13,17,18,42-44). However, the normalized concentration versus time profiles in the Complex Dedrick Plots were greater in patients as compared to mice. The relationship between 9NC and 9AC exposure and response may be different for specific tumors, and thus these types of studies may need to be performed for each tumor type (13,42-44). The tumor exposures of anticancer agents in xenografts located on the flank of mice may be different than the exposure in tumors of patients, and thus evaluating plasma exposures in mice and man may not be an accurate comparison (45,46).

The use of preclinical translational studies may be fundamental to the design and interpretation of clinical trials in humans. The direct comparison of drug exposures will remove variability associated with differences in metabolism or elimination and allometric scaling analysis can also be used to normalize the concentration versus time profiles in mice and patients (6,9,16,25,33-36). This information can then be used to make informed decisions in the development of new anticancer agents for the treatment of solid tumors and other malignancies (6,33). Based on these results presented in our study, we recommend performing phase II studies of 9NC using the intermittent schedule of administration.

2.6 REFERENCES

1. Zamboni WC, Jung LL, Egorin MJ, Jin R, Wong MMW, Potter D, Vozniak M, Sun S, Trump DL, Fakih M, Ramanathan R. Phase I and Pharmacologic studies of intermittently administered 9-nitrocamptothecin. Clin Cancer Res 2004;10(15): 5058-64.
2. Schoffski P, Herr A, Vermorken JB, Van den BJ, Beijnen JH, Rosing H, Volk J, Ganser A, Adank S, Botma HJ, Wanders J : Clinical phase II study and pharmacological evaluation of rubitecan in non-pretreated patients with metastatic colorectal cancer-significant effect of food intake on the bioavailability of the oral camptothecin analogue. Eur J Cancer 2002;38: 807-813.
3. Ebrahimi B, Phan A, Yao J, Paul MF, Pisters PW, Feig BW, Ajani JA: Phase II clinical trial of Rubitecan against advanced gastric adenocarcinoma. Proc Am Soc Clin Oncol 2002;21:596a.
4. Konstadoulakis MM, Antonakis PT, Tsibloulis BG, Stathopoulos GP, Manouras AP, Mylonaki DB, Golematis BX : A phase II study of 9-nitrocamptothecin in patients with advanced pancreatic adenocarcinoma. Cancer Chemother Pharmacol 2001;48: 417-420.
5. Natelson EA, Giovanella BC, Verschraegen CF, Fehir KM, De Ipolyi PD, Harris N, Stehlin JS. Phase I clinical and pharmacological studies of 20-(S)-camptothecin and 20-(S)-9-nitrocamptothecin as anticancer agents. Ann N Y Acad Sci 1996;803:224-230.
6. Furman WL, Stewart CF, Poquette CA, Pratt CB, Santana VM, Zamboni WC, Bowman LC, Ma MK, Hoffer FA, Meyer WH, Pappo AS, Walter AW, Houghton PJ. Direct translation of a protracted irinotecan schedule from a xenograft model to a phase I trial in children. J Clin Oncol 1999;17:1815-1824.
7. Thompson J, Stewart CF, Houghton PJ. Animal models for studying the action of

topoisomerase I targeted drugs. Biochim Biophys Acta 1998;1400:301-319.

8. Houghton PJ, Cheshire PJ, Hallman JD, Lutz L, Friedman HS, Danks MK, Houghton JA. Efficacy of topoisomerase I inhibitors, topotecan and irinotecan, administered at low dose levels in protracted schedules to mice bearing xenografts of human tumors. Cancer Chemother Pharmacol 1995;36:393-403.

9. Zamboni WC, Stewart CF, Thompson J, Santana VM, Cheshire PJ, Richmond LB, Luo X, Poquette C, Houghton JA, Houghton PJ. Relationship between topotecan systemic exposure and tumor response in human neuroblastoma xenografts. J Natl Cancer Inst 1998;90:505-511.

10. Zamboni WC, Bowman LC, Tan M., Santana, VM, Houghton PJ, Meyer WH, Pratt CB, Heideman RL, Gajjar AJ, Pappo AS, Stewart CF. Interpatient variability in bioavailability of the intravenous formulation of topotecan given orally to children with recurrent solid tumors. Cancer Chemother Pharmacol 1999;43:454-460.

11. Drengler RL, Kuhn JG, Schaaf LJ, Rodriguez GI, Villalona-Calero M.A., Hammond LA, Stephenson JA, Hodges S, Kraynak MA, Staton BA, Elfring GL, Locker PK, Miller LL, Von Hoff DD, Rothenberg ML. Phase I and pharmacokinetic trial of oral irinotecan administered daily for 5 days every 3 weeks in patients with solid tumors. J Clin Oncol 1999;17: 685-696.

12. Loos WJ, Gelderblom H, Sparreboom A, Verweij J, de Jonge MJ. Inter- and intrapatient variability in oral topotecan pharmacokinetics: implications for body-surface area dosage regimens. Clin Cancer Res 2000;6:2685-2689.

13. Jung LL, Ramanathan R, Egorin MJ, Jin R, Wong MMW, Potter D, Strychor S, Sun S, Trump DL, Fakih M, Zamboni WC. Pharmacokinetics studies of 9-nitrocamptothecin on

intermittent and continuous schedules in patients with advanced cancer. Cancer Chemother Pharmacol 2004;54(6): 487-96.

14. Stewart CF, Baker SD, Heideman RL, et al. Clinical pharmacodynamics of continuous infusion topotecan in children: systemic exposure predicts hematologic toxicity. J Clin Oncol 1994;12:1946-1954.

15. Zamboni WC, Gajjar AJ, Mandrell TD, et al. A four-hour topotecan infusion achieves cytotoxicity exposure throughout the neuraxis in the nonhuman primate model: implications for treatment of children with metastatic medulloblastoma. Clin Cancer Res 1998;4:2537-2544.

16. Kirstein MN, Houghton PJ, Cheshire PJ, Richmond LB, Smith AK, Hanna SK, Stewart CF. Relation between 9-aminocamptothecin systemic exposure and tumor response in human solid tumor xenografts. Clin Cancer Res 2001;7:358-366.

17. Gounder M, Saleem A, Roychowdhury M. Metabolism of 9-nitrocamptothecin (RFS2000/9NC) to 9-aminocamptothecin (9AC) in patients and in vitro. Proc Am Assoc Cancer Res 2001;42:537.

18. Miller KD, Soule SE, Haney LG, Guiney P, Murry DJ, Lenaz L, Sun SL, Sledge GW Jr. A phase II study of 9-nitro-camptothecin in patients with previously treated metastatic breast cancer. Invest New Drugs 2004;22(1):69-73.

19. Jaxel C, Kohn KW, Wani MC, Wall ME, Pommier Y. Structure-activity study of the actions of camptothecin derivatives on mammalian topoisomerase I: evidence for a specific receptor site and a relation to antitumor activity. Cancer Res 1989;49:1465-1469.

20. Stewart CF, Zamboni WC, Crom WR, Gajjar A, Heideman RL, Furman WL, Meyer WH,

Houghton PJ, Pratt CB. Topoisomerase I interactive drugs in children with cancer. Invest New Drugs 1996;14:37-47.

21. Mi Z, Burke TG. Differential interactions of camptothecin lactone and carboxylate forms with human blood components. Biochem 1994;33:10325-10336.

22. Hsiang YH, Hertzberg R, Hecht S, Liu LF. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J Biol Chem 1985;260:14873-14878.

23. Covey JM, Jaxel C, Kohn KW, Pommier Y. Protein-linked DNA strand breaks induced in mammalian cells by camptothecin, an inhibitor of topoisomerase I. Cancer Res 1989;49:5016-5022.

24. Jaxel C, Kohn KW, Wani MC, Wall ME, Pommier Y. Structure-activity study of the actions of camptothecin derivatives on mammalian topoisomerase I: evidence for a specific receptor site and a relation to antitumor activity. Cancer Res 1989;49:1465-1469.

25. Boxenbaum H. and Ronfeld R. Interspecies pharmacokinetic scaling and the Dedrick plots. Am J of Physiology 1983;245(6):R768-75.

26. Alley MC, Hollingshead MG, Pacula-Cox CM, Waud WR, Hartley JA, Howard PW, Gregson SJ, Thurston DE, Sausville EA. SJG-136 (NSC 694501), a novel rationally designed DNA minor groove interstrand cross-linking agent with potent and broad spectrum antitumor activity. Part 2: efficacy and evaluations. Cancer Res 2004;64: 6700-06.

27. Britten CD, Hilsenbeck SG, Eckhardt SG, Marty J, Mangold G, MacDonald JR, Rowinsky EK, Von Hoff DD, Weitman S. Enhanced antitumor activity of 6-

hydroxymethylfulvene in combination with irinotecan and 5-fluorouracil in HT29 human colon tumor xenograft model. Cancer Res 1999;59(5):1049-53.

28. Egorin MJ, Lagattuta TF, Hamburger DR, Covey JM, White KD, Musso SM, Eiseman JL. Pharmacokinetics, tissue distribution, and metabolism of 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (NSC-707545) in CD2F1 mice and Fischer 344 rats. Cancer Chemother Pharmacol 2002;9(1):7-19.

29. Supko JG, Malspeis L. Liquid chromatographic analysis of 9-aminocamptothecin in plasma monitored by fluorescence induced upon post-column acidification. J Liquid Chromatograph 1992;15:3261-64.

30. Gounder MK, Sun SL, Sands H, Lin Y, Shih WJ, Gu Z, Charles-Williams S, Roychowdhury M, Rajendra R, Rubin EH : Development of a bioanalytical liquid chromatography method for quantitation of 9-nitrocamptothecin in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 2004; 99:63-72.

31. D'Argenio DZ, Schmuitzky A ADAPT II user's guide: biomedical simualtions resource 1990.

32. Rowland M and Tozer T (eds). Clinical pharmacokinetics: concepts and applications. Lea and Febiger, Philadelphia 1999.

33. Zamboni WC, Tonda ME. New designs of clinical trials. Highlights in Oncology Practice 2000;18(1):2-7.

34. Grever MR and Chabner BA, Shoemaker D. Cancer Drug Discovery and Development. In: DeVita VT, Hellman S, and Rosenberg SA, eds. Cancer principles and practices of oncology, 5th Edition. Philadelphia, PA: Lippincott-Raven; 1997: 385-393.

35. Grever MR. Cancer drug screening. In: Schilsky RL, ed. Principles of antineoplastic drug development and pharmacology. New York, NY: Marcel Dekker, Inc; 1, 1996.

36. Grever MR, Schepartz S, Chabner BA. The National Cancer Institute: Cancer drug discovery and development program. Semin Oncol 1992;19:622-24.

37. Goodman SN, Zahurak ML, Piantadosi S. Some practical improvements in the continual reassessment method for phase I studies. Stat In Med 1995;14:1149-1161.

38. Korn EL, Midthune D, Chen TT, et al. A comparison of two phase 1 trial designs. Stat In Med 1994;13:1799-1806.

39. Patel H, Stoller R, Auber M, Potter D, Cai C, Zamboni W, Keifer G, Matin K, Schmotzer A, and Ramanathan R. Phase II and pharmacokinetic study of rubitecan (RFS 2000, Orathecin) in patients with advanced colorectal cancer who have failed previous 5-FU based chemotherapy. Submitted to Invest New Drugs in April 2005.

40. Pitot HC, Knost JA, Mahoney MR, Kugler J, Krook JE, Hatfield AK, Sargent DJ, Goldberg RM. A North Central Cancer Treatment Group Phase II trial of 9-aminocamptothecin in previously untreated patients with measurable metastatic colorectal carcinoma. Cancer 2000;89:1699-1705.

41. Vokes EE, Gordon GS, Rudin CM, Mauer AM, Watson S, Krauss S, Arrieta R, Golomb HM, Hoffman PC. A phase II trial of 9-aminocaptothecin (9-AC) as a 120-h infusion in patients with non-small cell lung cancer. Invest New Drugs 2001;19:329-333.

42. Hinz HR, Harris NJ, Natelson EA, Giovanella BC. Pharmacokinetics of the in vivo and in vitro conversion of 9-nitro-20(S)-camptothecin to 9-amino-20(S)-camptothecin in humans, dogs, and mice. Cancer Res 1994;54:3096-3103.

43. Schoemaker NE, Mathot RAA, Schoffski P, Rosing H, Schellens JHM, Beijnen JH. Development of an optimal pharmacokinetic sampling schedule for rubitecan administered orally in a daily times five schedule. Cancer Chemother Pharmacol 2002;50:514-517.

44. Raymond D, Campone M, Stupp R, Menten J, Chollet P, Lesimple T, Fety-Deporte R, Lacombe D, Paoletti X, Fumoleau P. Multicentre phase II and pharmacokinetic study of RFS2000 (9-nitrocamptothecin) administered orally 5 days a week in patients with glioblastoma multiforme. European Journal of Cancer 2002;38:1348-1350.

45. Zamboni WC, Eiseman JL, Muller M, Brunner M, Joseph E, Strychor S, Egorin MJ, Jung LL. Tumor disposition of carboplatin in mice bearing murine B16 melanoma is not indicative of the disposition of patients with cutaneous melanoma. Proceedings of AACR-Japanese Cancer Assoc 2004;B21.

46. Zamboni W. Use of microdialysis in preclinical and clinical development. In: Figg WD, McLeod H, editors. Handbook of Pharmacokinetics and Pharmacodynamics of Anti-Cancer Drugs, First edition. Humana Press. 2004.

3.0

CHAPTER 3:

Phase I and Pharmacologic Study of Intermittently Administered 9-Nitrocamptothecin in Patients with Advanced Solid Tumors

3.1 ABSTRACT

Purpose: 9-Nitrocamptothecin (9NC) is an oral camptothecin analogue currently administered at 1.5 mg/m^2/day x 5 days per week in Phase III studies for pancreatic carcinoma. In an effort to increase the dose administered per day and determine if the daily dose or number of days of treatment influence toxicity, we performed a Phase I study of 9NC using intermittent schedules of administration.

Methods: On schedule A, 9NC was administered orally daily x 5 days for 2 weeks every 4 weeks (1 cycle). On schedule B, 9NC was administered orally daily x 14 days every 4 weeks (1 cycle). Dose levels were determined by adaptive dose finding. Serial blood samples were obtained on day 1 of each schedule for pharmacokinetic studies of 9NC and its 9-aminocamptothecin (9AC) metabolite, and lactone forms were measured by HPLC.

Results: The recommended Phase II doses for schedules A and B were 2.43 mg/m^2/d and 1.70 mg/m^2/d, respectively, each providing the same dose intensity (i.e., 24 mg/m^2/cycle). The primary toxicities on schedules A and B were neutropenia, thrombocytopenia, and diarrhea. On schedule A, 2 patients with gastric cancer and 2 patients with pancreatic cancer had stable disease for > 6 cycles. On schedule B, 1 patient with pancreatic cancer had stable disease for > 6 cycles, and a patient with pancreatic cancer had a partial response. There was significant interpatient variability in the disposition of 9NC and 9AC. Most of the drug remained in the 9NC form with a ratio of 9NC to 9AC of approximately 4 to 1.

Conclusions: These studies suggest that 9NC administered on an intermittent schedule is tolerable and may be an active regimen in patients with gastric or pancreatic cancers. Dosing 9NC on a mg/m^2 basis does not reduce pharmacokinetic variability.

3.2 INTRODUCTION

The camptothecins are DNA topoisomerase I-interactive anticancer agents and have a wide range of antitumor activity (1-3). Currently approved camptothecin analogues (i.e., topotecan and irinotecan) are only available for IV administration (4,5). 9-Nitrocamptothecin (9NC) is administered orally and is partially metabolized to an active metabolite, 9-aminocamptothecin (9AC) (6,7). As with other camptothecin analogues, 9NC and 9AC undergo a reversible, pH-dependent hydrolysis between the active-lactone and inactive-hydroxy acid forms (8). *In vitro* and *in vivo* preclinical studies suggest that protracted administration of low doses of camptothecin analogues produces better antitumor activity than does less frequent administration of higher doses (9-12) Oral administration of 9NC could mimic the protracted schedule and maximize patient convenience (13). However, oral administration of camptothecin analogues has been characterized by extensive inter- and intra-patient variability in bioavailability (13-16)

In Phase II and III studies, 9NC is administered continuously at 1.25 to 1.5 mg/m^2/day for 5 days per week (17,18). On this schedule, dose reductions and delays in therapy frequently occur during weeks 3 to 5 and are due to myelosuppression, diarrhea, and hematuria. In xenograft studies, antitumor activity of camptothecin analogues requires a dose that produces a systemic exposure above a critical threshold (11,12). It is possible that administration of continuous, low dose 9NC might not produce a systemic exposure above this critical threshold and as a result might fail to produce an antitumor response (19). In contrast, administration of 9NC on an intermittent schedule (e.g., 2 weeks of treatment followed by 2 weeks off) may allow for the administration of a higher dose per day that would produce therapeutic drug concentrations, and also avoid toxicities in weeks 3 and 4.

Thus, we evaluated two intermittent schedules of 9NC administration with the goals of increasing the dose administered per day and the dose intensity and determining if the daily dose or number of days of treatment influence toxicity. The objectives of this study were to: 1) determine the maximum tolerated dose (MTD) and toxicities associated with two intermittent schedules of 9NC; 2) document any antitumor response to 9NC in patients with various solid tumors; and 3) determine the plasma pharmacokinetics of 9NC and 9AC.

3.3 PATIENTS AND METHODS

Patients

Patients 18 years of age or older with a histologically or cytologically confirmed malignancy for which no curative or effective therapy was available were eligible for this study. Other eligibility criteria included a Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, adequate bone marrow, hepatic, and renal function as evidenced by the following: absolute neutrophil count (ANC) $\geq$ 1500/μL, platelets $\geq$ 100,000/μL, total bilirubin $\leq$ 1.5 x upper limit of the institutional normal range (ULN), aspartate aminotransferase (AST) $\leq$ 1.5 x the ULN if liver metastases were not present and $\leq$ 4 x the ULN if liver metastases were present, and absence of microscopic hematuria. Prior irradiation to brain metastases was allowed if the patient's neurological status was stable 4 weeks after irradiation. Prior treatment with camptothecin analogues except 9NC was permitted. Written informed consent, approved by the Institutional Review board of the University of Pittsburgh Medical Center, was obtained from all patients before they entered the study.

Dosage and Administration

Two intermittent schedules of 9NC were evaluated. On schedule A, 9NC was administered orally daily for 5 days per week for 2 consecutive weeks and repeated every 4 weeks (one cycle). On schedule B, 9NC was administered orally daily for 14 days and repeated every 4 weeks (one cycle). The daily dose was rounded to the nearest 0.25 mg. On both schedules, 9NC was administered daily on an empty stomach and with an acidic beverage (e.g., orange juice or cola) (20,21). Patients were required to increase their oral hydration to at least 2 liters per day during 9NC treatment.

Dose levels for schedules A were determined by adaptive dose finding (22). During stage I of this two-stage method, doses were escalated by a factor of 1.5 until the first dose-limiting toxicity (DLT) occurred. Then, in stage II, escalation was switched to a model-guided mode similar to the continual reassessment method (CRM) (23). In these methods, the MTD is defined in terms of a fixed probability that patients in the population experience DLT. For our study, this probability was set to be 0.3. The DLT experience of all previous cohorts is used to select the dose for a new cohort that will have this probability of DLT. In stage II, the dose-response model is $\{\log [p/(1-p)] = A + B \log (\text{dose})\}$, where p is the DLT probability, and A and B are constants updated during the study. The two constants are initialized so that $p = 0.1$ at half the dose at which the first DLT is observed, and $p = 0.9$ at 5 times that dose. Six patients were to be entered in the first cohort in this trial; subsequent cohorts were to consist of 3 patients.

The initial dose level on schedule B was 30% lower than the MTD in schedule A. Due to relatively small dose changes and extensive pharmacokinetic variability in 9NC and 9AC, the adaptive dose finding procedure was not used to calculate dose levels for schedule B.

Response Assessment

After every 2 cycles of treatment, patients with measurable disease were assessed for response according to the World Health Organization Criteria (24). Toxicity was defined by the NCI Common Toxicity Criteria version 2.0 (25). Dose-limiting toxicities (DLT) were defined as platelets ≤ 25,000/μL during treatment, ANC ≤ 500/μL for more than 1 week, fever accompanied by ANC ≤ 1000/μL, a delay in re-treatment for ≥ 2 weeks for any reason, and all non-reversible grade III and IV non-hematologic toxicities except bone pain. These toxicities were considered DLTs only if they occurred during cycle 1.

Pharmacokinetic Sample Collection and Preparation

On schedules A and B, serial blood samples for pharmacokinetic analysis were obtained on day 1. Blood samples (5 mL) were obtained prior to administration of 9NC, and at 0.25, 0.5, 1, 2, 3, 6, 8, and 24 hours after administration. Blood was placed into heparinized tubes and centrifuged at 1200 x g at 4° C for 5 minutes. The resulting plasma samples were then processed immediately in order to measure the lactone forms of 9NC and 9AC.

The processing for the measurement of the 9NC lactone in plasma was performed using solid phase extraction (SPE) with Waters OASIS HLB columns (1 mL, 30 mg) to separate the lactone and hydroxy acid forms of 9NC. Columns were conditioned with 1 mL of methanol and equilibrated with 1 mL of water. Plasma (1 mL) was passed through the column, and the column was then washed with 1 mL of 5% methanol in water to remove the hydroxy acid forms of 9NC and 9AC. 9NC lactone was then eluted with 1 mL of methanol and stored at -80° C until analyzed.

For measurement of 9AC lactone, plasma was processed by methanolic extraction (13). A total of 600 μL of plasma was added to 1200 μL of cold (-20° C) methanol. The methanol was maintained at –20°C by placing the vials in an insulated cooler containing dry ice. The samples were vortexed and centrifuged at 10,000 x g for 5 min. The resulting supernatant was decanted and stored at -80°C until analyzed.

High-Performance Liquid Chromatography (HPLC) Analysis

9NC and 9AC lactone plasma concentrations were measured using an HPLC assay with fluorescence detection as described previously (26). Because 9NC is not highly fluorescent, 9NC lactone was measured by chemically reducing 9NC to 9AC. The concentration of 9NC was calculated by subtracting the concentration of 9AC from the concentration of 9NC plus 9AC after the conversion of 9NC to 9AC using iron reduction.

The HPLC system consisted of a Waters 2695 separation module (Waters, Inc. Milford, MA), a C18 reverse column [Ultrasphere 5 μm ODS 4.6 x 250 mm, Beckman Coulter, Inc., Fullerton, CA], and a C18 guard column (Brownlee C18 7 μm, 15 x 3.2 mm, PerkinElmer Corp., Norwalk, CT). Samples were injected by an autosampler set at 4° C. The isocratic mobile phase consisted of methanol, acetonitrile, and ammonium acetate (10:23:97, v/v/v), pH 5.5, at a flow rate of 1.0 mL/min. Post column acidification (pH 2 – 3) was performed using 0.3 M trifluoroacetic acid at 0.3 mL/min (26). 9AC was detected by a Waters #474 fluorescence detector with excitation wavelength of 365 nm and emission wavelength of 440 nm, 18-nm bandwidth, gain 1000, attenuation 16, with RC filter with fast response setting. MILLENIUM 32 software (Waters, Inc.) was used for data collection and analysis. All the glassware,

including the injection vials, were treated with 3% surfasil in toluene (Fisher Scientific Inc., Fair Lawn, NJ).

The lower limit of quantitation (LLQ) for 9NC lactone was 0.5 ng/mL, and the assay was linear from 0.5 ng/mL to 100 ng/mL. The LLQ for 9AC lactone was 0.3 ng/mL, and the assay was linear from 0.3 ng/mL to 100 ng/mL.

Pharmacokinetic Analysis

Compartmental pharmacokinetic analysis of 9NC and 9AC was performed using ADAPT II (27). A linear model with an oral absorption compartment and one-compartment each for 9NC and 9AC was simultaneously fit to 9NC and 9AC concentration versus time profiles. The area under the 9NC and 9AC plasma concentration versus time curves (9NC AUC and 9AC AUC) from zero to 24 hours were calculated using the log trapezoidal method by simulating the concentration versus time data for each patient using patient-specific parameters (27).

Urine Stability Studies

The stability of lactone and total forms of camptothecin (Supergen, Dublin, CA), 9NC, and topotecan (GlaxoSmithKline, Collegeville, PA) in urine at 37° C and at pH of 5 and 6 was evaluated over 2 hours. Camptothecin, 9NC, and topotecan were added to urine to achieve a final concentration of 100 ng/mL. Urinary pH was measured at baseline and after the addition of the drug. Hydrochloric acid and sodium hydroxide were used to adjust the urine to the desired pH. Triplicate urine samples (200 μL) were obtained at baseline, and 5 min, 30 min, 60 min, and 120 min. As appropriate, samples were processed immediately via solid phase extraction for analysis of 9NC lactone and via methanolic extraction for analysis of 9NC total or for

camptothecin and topotecan lactone and total as described previously (26). The percent lactone was calculated as (the ratio of lactone concentration to total concentration) x 100.

3.4 RESULTS

Patient Characteristics

Patient characteristics are summarized in Table 1. Twenty-three patients were enrolled on schedule A, and 9 patients were enrolled on schedule B. Six patients on schedule A were not evaluable; three patients developed elevated serum bilirubins during cycle 1 (2 due to biliary stent blockage); one patient developed increase an in AST; one patient had severe nausea and vomiting; and one patient was noncompliant with the oral therapy. All patients enrolled on schedule B were evaluable.

On schedule A, dose levels 1, 2, and 3 were 2.0 mg/m^2/d (n = 6 evaluable patients), 2.7 mg/m^2/d (n = 4 evaluable patients), and 2.4 mg/m^2/d (n = 7 evaluable patients), respectively. The starting dose of schedule B was 30% lower than the MTD (i.e., 2.4 mg/m^2/d) on schedule A. On schedule B, dose levels 1 and 2 were 1.7 mg/m^2/d (n = 6 evaluable patients) and 2.4 mg/m^2/d (n = 3 evaluable patients), respectively.

Toxicity and Tolerability

The toxicity profiles of schedules A and B were similar with diarrhea, nausea, neutropenia, and thrombocytopenia as the primary toxicities (7,17,18). A summary of toxicities for evaluable patients on schedule A during cycle 1 are included in Table 2. On schedule A, the most common toxicities for all dose levels were anemia, nausea, and vomiting. The majority of adverse effects reported during cycle 1 were grade 1 or 2. There was no DLT at 2.0 mg/m^2/d.

Table 1. Patient Characteristics

Characteristics	Schedule A	Schedule B
Male / Female Enrolled (N)	17 / 6	5 / 4
Male / Female Evaluable (N)	14 / 3	5 / 4
Age (yr) Median	55	54
Range (yr)	(37 to 69)	(40 to 68)
ECOG Performance Status (N)		
0	6	6
1	16	3
2	1	0
Diagnosis (N)		
Pancreatic	9	1
Gastric / Esophageal	2	1
Colo-rectal	4	5
NSCLC	2	1
Hepatocellular / Biliary Tree	3	1
Unknown Primary, Sarcoma, Thyroid	1 each	0
Prior Treatments		
Median (Range)		
Prior Chemotherapy	2 (0 to 9)	4 (0 to 8)
Camptothecin analogues		
Irinotecan (N)	3	6

Table 2. Toxicity Summary for Schedule A During Cycle 1

	2.0 mg/m^2/day (n = 6)		2.4 mg/m^2/day (n = 7)		2.7 mg/m^2/day (n = 4)	
	Grade 1 - 2	Grade 3 - 4	Grade 1 - 2	Grade 3 - 4	Grade 1 - 2	Grade 3 - 4
Anemia	6	---	4	1*	3	1
Neutropenia	2	1	1	1*	1	2
Thrombocytopenia	1	---	---	1*	2	1
Diarrhea	1	---	2	---	3	---
Nausea	2	---	6	---	2	---
Vomiting	---	---	5	---	1	---
Hematuria	1	---	1	---	2	---

* One patient at 2.4 mg/m2 developed grade 4 febrile neutropenia leading to sepsis and death.

Two of 4 evaluable patients at 2.7 mg/m^2/d experienced DLT of febrile neutropenia. The dose was then reduced to 2.4 mg/m^2/d. One of 7 patients at 2.4 mg/m^2/d developed DLT of febrile neutropenia, grade 4 thrombocytopenia, grade 4 anemia, and treatment-related death. Therefore, the recommended Phase II dose for schedule A was 2.4 mg/m^2/d. Six of 17 evaluable patients developed grade 1 or 2 hematuria during treatment with 9NC, and four of these patients developed hematuria during cycle 1. Of note, 6 patients were found to have grade 1 or 2 hematuria during screening and were considered ineligible.

A summary of toxicities on schedule B during cycle 1 is included in Table 3. On schedule B, the most common toxicities were anemia, diarrhea, thrombocytopenia, nausea, and vomiting. The majority of the adverse effects reported during cycle 1 were grade 1 or 2. No DLT occurred in patients receiving 1.7 mg/m^2/d. As a result the dose was escalated to 2.4 mg/m^2/d. During cycle 1, 2 of 3 patients treated at 2.4 mg/m^2/d developed DLT. One patient developed grade 3 diarrhea and the other patient developed febrile neutropenia with grade 4 thrombocytopenia. Therefore, the recommended Phase II dose for schedule B was 1.7 mg/m^2/d. No patients experienced hematuria during treatment on schedule B.

A total of 77 cycles were administered on schedules A and B. The occurrence of selected grade 3 or 4 toxicities on all cycles were as follows: anemia (n = 8), neutropenia (n = 9), thrombocytopenia (n = 5), diarrhea (n = 1), nausea (n = 1), vomiting (n = 1), and hematuria (n = 1). Increased neutropenia and thrombocytopenia were seen in patients with higher 9NC and 9AC total and lactone AUC, however there was no direct relationship between the percentage decrease in neutrophils or platelets and 9NC or 9AC exposure.

Table 3. Toxicity Summary for Schedule B During Cycle 1

	1.7 mg/m^2/day (n = 6)		2.4 mg/m^2/day (n = 3)	
	Grade 1 - 2	Grade 3 - 4	Grade 1 - 2	Grade 3 - 4
Anemia	4	---	2	1
Neutropenia	1	---	1	1
Thrombocytopenia	4	---	---	1
Diarrhea	4	---	2	1
Nausea	2	---	2	---
Vomiting	1	---	1	---
Hematuria	---	---	---	---

Clinical Activity

Of the 17 evaluable patients on schedule A, 4 had SD. Two of these patients had gastric cancer, and 2 had pancreatic cancer. The 2 patients with gastric cancer completed 8 cycles of treatment at 2.0 mg/m^2/d and 6 cycles of treatment at 2.7 mg/m^2/d, respectively. The 2 patients with pancreatic cancer completed 6 cycles of treatment at 2.0 mg/m^2/d and 16 cycles of treatment at 2.4 mg/m^2/d. On schedule B, one patient with pancreatic cancer had SD, and one patient with pancreatic cancer had a PR. The patient with SD received 4 cycles of treatment at 2.4 mg/m^2/d.

The patient with the PR received 8 cycles of treatment at 2.4 mg/m^2/d. None of the patients in schedule A or B with SD or PR had received a camptothecin analogue as prior therapy.

9NC and 9AC Pharmacokinetics

Pharmacokinetic studies were performed on day 1 of both schedules A and B. On schedule A, pharmacokinetic data were not available due to lack of intravenous access in two patients and patient refusal in two patients. On schedule B, pharmacokinetic data were available for all patients.

9NC and 9AC lactone AUCs on schedule A and B are presented in Figure 1 and Figure 2, respectively. There was significant interpatient variability in the exposure of 9NC and 9AC lactone. The 9NC lactone AUCs at individual doses varied from 6 to 16-fold. On schedule A, the mean ∀ SD 9NC lactone AUCs at 2.0, 2.4, and 2.7 mg/m^2 were 183 ± 119, 79 ± 54, and 89 ± 91 ng/mL·h, respectively. On schedule B, the mean ± SD 9NC lactone AUCs at 1.7 and 2.4 mg/m^2 were 156 ± 113 and 278 ± 218 ng/mL·h, respectively.

The 9AC lactone AUCs at individual doses ranged from 6 to 12-fold. On schedule A, the mean ± SD 9AC lactone AUCs at 2.0, 2.4, and 2.7 mg/m^2 were 34 ± 21, 17 ± 18, and 32 ± 16 ng/mL·h, respectively. On schedule B, the mean ∀ SD 9AC lactone AUCs at 1.7 and 2.4 mg/m^2 were 41 ± 17 and 75 ± 70 ng/mL·hr, respectively. Most of the drug remains in the 9NC form with ratios of 9NC to 9AC lactone or total of 4 to 1.

The relationships between dose expressed as mg/m^2 or mg, and drug exposure were evaluated (16,29). The following measures of drug exposure were evaluated; 9NC and 9AC lactone AUC, and the sum of 9NC lactone AUC plus 9AC lactone AUC. There was no relationship between 9NC and 9AC AUC and doses based on mg or mg/m^2. In addition, there

was no relationship between dose and Cmin or Cmax of 9NC or 9AC. Thus, administration of 9NC doses based on mg or mg/m^2 were equally poor predictors of exposure.

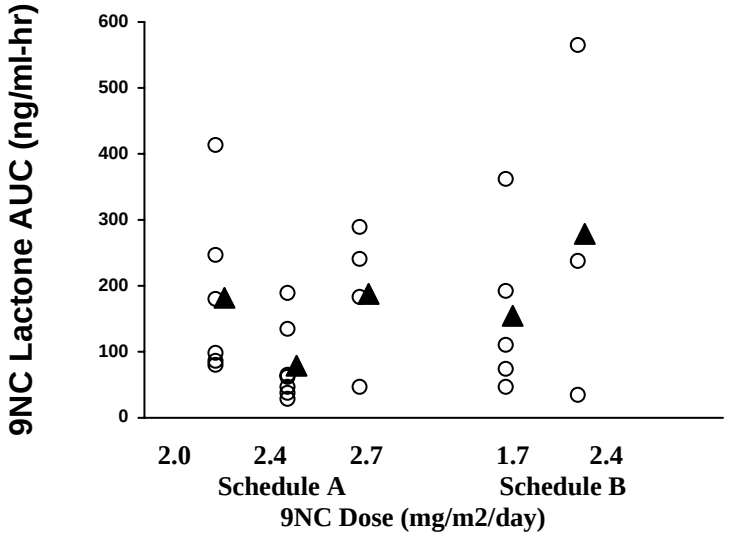


Figure 1. 9NC lactone AUC after administration of 2.0, 2.4, and 2.7 mg/m^2/d on schedule A and 1.7 and 2.4 mg/m^2/d on schedule B. Individual 9NC lactone AUC are represented by ○, and mean 9NC lactone AUC are represented by □.

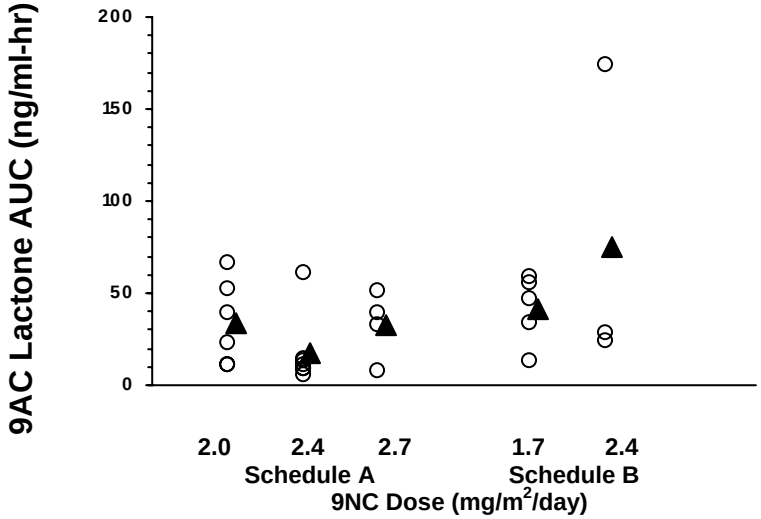


Figure 2. 9AC lactone AUC after administration of 2.0, 2.4, and 2.7 mg/m^2/d on schedule A and 1.7 and 2.4 mg/m^2/d on schedule B. Individual 9AC lactone AUC are represented by ○, and mean 9AC lactone AUC are represented by □.

Urine Stability

9NC, camptothecin, and topotecan lactone and total concentrations were evaluated in urine at pH 5 and 6. The mean ± SD percentage lactone of 9NC in urine at pH 5 at 5 min, 30 min, 60 min, and 120 min was 93 ± 6 %, 99 ± 3 %, 89 ± 3 %, and 94 ± 8 % respectively. The mean ± SD percentage lactone of camptothecin in urine at pH 5 at 5 min, 30 min, 60 min, and 120 min was 102 ± 7 %, 100 ± 9 %, 98 ± 6 %, and 99 ± 8 %, respectively. The mean ± SD percentage lactone of topotecan in urine at pH 5 at 5 min, 30 min, 60 min, and 120 min was 103 ± 5 %, 99 ± 8 %, 101 ± 5 %, and 101 ± 1 %, respectively.

The mean ± SD percentage lactone of 9NC in urine at pH 6 at 5 min, 30 min, 60 min, and 120 min was 108 ± 9 %, 79 ± 13 %, 94 ± 4 %, and 78 ± 14 %, respectively. The mean ± SD percentage lactone of camptothecin in urine at pH 6 at 5 min, 30 min, 60 min, and 120 min was 88 ± 4 %, 90 ± 3 %, 87 ± 3 %, and 94 ± 5 %, respectively. The mean ± SD percentage lactone of topotecan in urine at pH 6 at 5 min, 30 min, 60 min, and 120 min was 99 ± 6 %, 98 ± 6 %, 89 ± 14 %, and 91 ± 9 %, respectively.

3.5 DISCUSSION

In Phase II and III studies, 9NC was administered orally daily x 5 days per week for 8 weeks (1 cycle); however, the optimal schedule of administration of camptothecin analogues, especially 9NC, is unclear (7,17,18). As a result, we performed the first study evaluating intermittent schedules of 9NC and have shown that intermittent schedules of 9NC are tolerable and may be active. The MTD on schedules A and B were 2.4 and 1.7 mg/m^2/day, respectively, however the dose intensity (24 mg/m^2/cycle) was the same for both schedules. It is currently

unclear which of the intermittent schedules of administration is superior. Schedule A may prove to be the optimal intermittent regimen due to administration of a higher daily dose which may be above a critical threshold required for antitumor response (12,19). However, since the exposures of 9NC and 9AC are similar due to the high interpatient variability in 9NC and 9AC disposition at the MTD of each regimen, administration of 9NC daily for 14 days every 4 weeks (schedule B) may be the most appropriate regimen based on the ability to administer treatment for 14 consecutive days. The dose intensity achieved on either of the intermittent schedules on our study appear less than the dose intensity achieved with the continuous schedule at 1.5 mg/m^2 (30 mg/m^2/4 weeks). However, due to the relatively high pharmacokinetic variability the exposures of 9NC and 9AC achieved with both regimens are similar (17,18). Moreover, recent studies using the continuous schedule have required dose reductions and delays during weeks 3 to 5, thus the dose intensity over 4 weeks is less than the intermittent schedules evaluated in our study (17,18). In our Phase I studies, there were 5 patients with prolonged (i.e., > 6 cycles) stable disease, and one patient with pancreatic cancer achieved a PR. The DLT were neutropenia, thrombocytopenia, and diarrhea. There was significant interpatient variability in the disposition of 9NC and 9AC. Most of the drug remained in the 9NC form with an average 9NC to 9AC ratio of 4 to 1. The fact that most of the administered drug remains in the 9NC form is significant because the development of 9AC was stopped due to lack of efficacy (30-32).

As part of our drug development program of camptothecin analogues, we determined the minimum effective dose of 9NC and associated drug exposure in mice bearing human tumor xenografts and compared this to the MTD and associated drug exposure in a Phase I trial using the same regimen (19). In the preclinical studies, the 9NC and 9AC lactone AUCs associated with the minimum dose that produced a response were 43 and 6 ng/mL·h, respectively. Of the 6

patients with pharmacokinetic studies at 2.4 mg/m^2/day, only 2 (i.e., 27.3 and 37.4 ng/mL·h) had 9NC AUC < 43 ng/mL·h. In addition, 5 of the 6 responders on schedule A or B had 9NC AUC > 43 ng/mL·h. In our Phase II study of 9NC administered orally daily for 5 days per week for 8 weeks in patients with refractory colon cancer, 3 of 8 patients had a 9NC lactone AUC < 43 ng/mL·h (26,33). Moreover, there were no responders in the Phase II study of 9NC in patients with colon cancer. This lack of response may be associated with the low daily exposures achieved with this regimen. Thus, the intermittent schedule may be more appropriate than the continuous 8-week schedule because it can achieve exposures that are tolerable in humans and above the target threshold defined in xenograft models. This information and study design can be used to make informed decisions regarding the most appropriate dose and schedule of administration of 9NC and other anticancer agents.

Several studies have reported significant interpatient variability in the pharmacokinetics of some orally administered drugs, especially camptothecin analogues (11,13-16,36,37). In this study, at any given dose of 9NC there was 4 to 16-fold variability in 9NC and 9AC exposure, and there was no relationship between dose and AUC. The method of dose calculation (i.e., mg versus mg/m^2) did not reduce the variability in drug exposure. Thus, there is no evidence that dosing 9NC on a mg/m^2 basis is warranted to reduce the pharmacokinetic variability. This is an important question for future studies to address because the most appropriate method to use for calculation of doses for oral administration of 9NC and other anticancer agents is unclear (29). It is currently unclear if the pharmacokinetic variability of 9NC and 9AC are due to variable gastrointestinal absorption, hepatic metabolism, and/or biliary elimination.

The minimal hematuria observed in schedule A and lack of hematuria observed in schedule B are in contrast to the 14% of patients that developed grade 3 hemorrhagic cystitis on

the prior Phase I study of 9NC, which used an 8-week continuous dosing schedule (7,17). This decreased hematuria may be associated with the extensive oral hydration and/or the break in therapy during weeks 3 and 4 used in the intermittent study. In addition, 6 patients had grade 1 or 2 hematuria during screening and were ineligible for this study. Thus, subclinical hematuria may occur patients with cancer, and the occurrence of grade 1 or 2 hematuria may not be clinically relevant or drug-induced.

The percent of the 9NC dose recovered in urine as 9NC or 9AC is relatively low (< 15%) (26). However, as stated above, 9NC-induced cystitis has required dose reductions and breaks in therapy (7,17,18). Currently, patients are instructed to consume 2 to 3 L of fluid per day, which becomes problematic in patients with gastrointestinal cancers and in patients with cardiac conditions. Thus, it is critical to determine the mechanism of camptothecin analogue-induced cystitis and develop feasible treatment options. Camptothecin and 9NC produce cystitis, however the development of cystitis has not been associated with topotecan (7,17,18,34-37). The differential of cystitis induced by camptothecin, 9NC, and topotecan is not associated with the percentage of drug renally eliminated (13,33,35). The pH of normal urine ranges from 4.5 to 6.3 (38), however it is unclear whether the cystitis associated with camptothecin analogues is due to the lactone or hydroxy acid forms. Because of this, we evaluated the stability of total and lactone forms of 9NC, camptothecin, and topotecan in urine at pH 5 and 6. In our study, the percentage lactone and equilibrium between lactone and hydroxy acid forms of 9NC, camptothecin, and topotecan were similar in urine at pH 5 and 6. Thus, the relative exposure of the lactone form of the camptothecin analogues may not be associated with the development of cystitis, and the conversion of lactone to hydroxy acid form via alkalization of the urine may not be a feasible treatment option in the prevention of 9NC-induced cystitis. Alternatively, the

lipophilicity and bladder penetration of camptothecin analogues may be related to the development of cystitis (41,42).

The clinical importance of this study is underscored by the need to evaluate alternate schedules of administration prior to the initiation of Phase II and III studies (43,44). Moreover, well designed translational studies, such as those used in the development of 9NC on the intermittent schedule and previously used to develop topotecan and irinotecan in pediatric solid tumors, can be highly informative when determining the most appropriate regimen to take forward in development (11,12,19). The intermittent regimen of 9NC evaluated in this study has preclinical rationale, acceptable toxicity, and should be evaluated in Phase II trials, especially in patients with pancreatic and gastric cancer.

3.6 REFERENCES

1. Hsiang, Y.H., Hertzberg, R., Hecht, S., Liu, L.F. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J Biol Chem 1985;*260:* 14873-14878.
2. Covey, J.M., Jaxel, C., Kohn, K.W., Pommier, Y. Protein-linked DNA strand breaks induced in mammalian cells by camptothecin, an inhibitor of topoisomerase I. Cancer Res 1989;*49:* 5016-5022.
3. Jaxel, C., Kohn, K.W., Wani, M.C., Wall, M.E., Pommier, Y. Structure-activity study of the actions of camptothecin derivatives on mammalian topoisomerase I: evidence for a specific receptor site and a relation to antitumor activity. Cancer Res 1989;*49:* 1465-1469.
4. Bookman, M.A., Malmstrom, H., Bolis, G., Gordon, A., Lissoni, A., Krebs, J.B., Fields, S.Z. Topotecan for the treatment of advanced epithelial ovarian cancer: an open-label phase II study in patients treated after prior chemotherapy that contained cisplatin or carboplatin and paclitaxel. J Clin Oncol 1998;*16:* 3345-3352.
5. Rougier, P., Bugat, R., Douillard, J.Y., Culine, S., Suc, E., Brunet, P., Becouarn,Y., Ychou, M., Marty, M., Extra, J.M., Bonneterre, J., Adenis, A., Seitz, J.F., Ganem, G., Namer, M., Conroy, T., Negrier, S., Merrouche, Y., Burki, F., Mousseau, M., Herait, P., Mahjoubi, M. Phase II study of irinotecan in the treatment of advanced colorectal cancer in chemotherapy-naive patients and patients pretreated with fluorouracil-based chemotherapy. J Clin Oncol 1997;*15:* 251-260.
6. Gounder, M., Saleem, A., Roychowdhury, M. Metabolism of 9-nitrocamptothecin (RFS2000/9NC) to 9-aminocamptothecin (9AC) in patients and *in vitro*. Proc Am Assoc Cancer Res 2001;*42:* #537.

7. Natelson, E.A., Giovanella, B.C., Verschraegen, C.F., Fehir, K.M., De Ipolyi, P.D., Harris, N., Stehlin, J.S. Phase I clinical and pharmacological studies of 20-(S)-camptothecin and 20-(S)-9-nitrocamptothecin as anticancer agents. Ann N Y Acad Sci., *803:* 224-230, 1996.
8. Stewart, C.F., Zamboni, W.C., Crom, W.R., Gajjar, A., Heideman, R.L., Furman, W.L., Meyer, W.H., Houghton, P.J., Pratt, C.B. Topoisomerase I interactive drugs in children with cancer. Invest New Drugs 1996;*14:* 37-47.
9. Furman, W.L., Stewart, C.F., Poquette, C.A., Pratt, C.B., Santana, V.M., Zamboni, W.C., Bowman, L.C., Ma, M.K., Hoffer, F.A., Meyer, W.H., Pappo, A.S., Walter, A.W., Houghton, P.J. Direct translation of a protracted irinotecan schedule from a xenograft model to a phase I trial in children. J Clin Oncol 1999;*17:* 1815-1824.
10. Thompson, J., Stewart, C.F., Houghton, P.J. Animal models for studying the action of topoisomerase I targeted drugs. Biochim Biophys Acta 1998;*1400:* 301-319.
11. Houghton, P.J., Cheshire, P.J., Hallman, J.D., Lutz, L., Friedman, H.S., Danks, M.K., Houghton, J.A. Efficacy of topoisomerase I inhibitors, topotecan and irinotecan, administered at low dose levels in protracted schedules to mice bearing xenografts of human tumors. Cancer Chemother Pharmacol 1995;*36:* 393-403.
12. Zamboni, W.C., Stewart, C.F., Thompson, J., Santana, V.M., Cheshire, P.J., Richmond, L.B., Luo, X., Poquette, C., Houghton, J.A., Houghton, P.J. Relationship between topotecan systemic exposure and tumor response in human neuroblastoma xenografts. J Natl Cancer Inst 1998;*90:* 505-511.
13. Zamboni, W.C., Bowman, L.C., Tan, M., Santana, V.M., Houghton, P.J., Meyer, W.H., Pratt, C.B., Heideman, R.L., Gajjar, A.J., Pappo, A.S., Stewart, C.F. Interpatient variability

in bioavailability of the intravenous formulation of topotecan given orally to children with recurrent solid tumors. Cancer Chemother Pharmacol 1999;*43:* 454-460.

14. Gupta, E., Toppmeyer, D., Zamek, R., Much, J., Ibrahim, N., Goodin, S., Kane, M., Aisner, J., Li, X., Haluska, P., Meidna, M., Corfield, A., Vyas, V., Natelson, E., Giovanella, B., Saleem, A., Rubin, E. Clinical evaluation of sequential topoisomerase targeting in the treatment of advanced malignancy. Cancer Ther 1998;*1:* 292-301.
15. Drengler, R.L., Kuhn, J.G., Schaaf, L.J., Rodriguez, G.I., Villalona-Calero, M.A., Hammond, L.A., Stephenson, J.A., Jr., Hodges, S., Kraynak, M.A., Staton, B.A., Elfring, G.L., Locker, P.K., Miller, L.L., Von Hoff, D.D., Rothenberg, M.L. Phase I and pharmacokinetic trial of oral irinotecan administered daily for 5 days every 3 weeks in patients with solid tumors. J Clin Oncol 1999;*17:* 685-696.
16. Loos, W.J., Gelderblom, H., Sparreboom, A., Verweij, J., de Jonge, M.J. Inter- and intrapatient variability in oral topotecan pharmacokinetics: implications for body-surface area dosage regimens. Clin Cancer Res 2000;*6:* 2685-2689.
17. Verschraegen, C.F., Natelson, E.A., Giovanella, B.C., Kavanagh, J.J., Kudelka, A.P., Freedman, R.S., Edwards, C.L., Ende, K., Stehlin, J.S. A phase I clinical and pharmacological study of oral 9-nitrocamptothecin, a novel water-insoluble topoisomerase I inhibitor. Anticancer Drugs 1998;*9:* 36-44.
18. Schoffski, P., Herr, A., Vermorken, J.B., Van den, B.J., Beijnen, J.H., Rosing, H., Volk, J., Ganser, A., Adank, S., Botma, H.J., Wanders, J. Clinical phase II study and pharmacological evaluation of rubitecan in non-pretreated patients with metastatic colorectal cancer-significant effect of food intake on the bioavailability of the oral camptothecin analogue. Eur J Cancer 2002;*38:* 807-813.

19. Zamboni, W.C., Hamburger, D.R., Jung, L.L., Jin, R., Joseph, E., Strychor, S., Sun, S.L., Ramanathan, R.K., Egorin, M.J., Eiseman, J.L. Relationship between systemic exposure of 9-nitrocamptothecin (9NC, Rubitecan, RFS2000) & its 9-aminocamptothecin (9AC) metabolite & response in human colon cancer xenografts. Proc of AACR-NCI-EORTC Meeting 2001, #400.

20. Schoffski, P., Herr, A., Beijnen, J.H., Rosing, H., Reinke, F., Assochert, L., Botma, H.J., Ganser, A., Wanders, J. Effect of food intake on the pharmacokinetics of a single administration of oral rubitecan (RFS-2000), a topoisomerase-I-inhibiting camptothecin analogue. Proc of AACR-NCI-EORTC Meeting., 2000; #2525.

21. Akhtar, S., Beckman, R.A., Mould, D.R., Doyle, E., Fields, S.Z., Wright, J. Pretreatment with ranitidine does not reduce the bioavailability of orally administered topotecan. Cancer Chemother Pharmacol 2000;*46:* 204-210.

22. Potter, D.M. Adaptive dose finding for phase I clinical trials of drugs used for chemotherapy of cancer. Statistics in Medicine 2002;*21:* 1805-1823.

23. O'Quigley, J., Pepe, M., Fisher, L. Continual reassessment method: A practical design for phase I clinical trials in cancer. Biometrics 1990;*46:* 33-48.

24. Miller, A.B., Hoogstraten, B., Staquet, M., Winkler, A. Reporting results of cancer treatment. Cancer 1981;*47:* 207-214.

25. National Cancer Institute Common Toxicity Criteria, Version 2.0. 2002.

26. Jung LL, Ramanathan RK, Egorin MJ, Jin R, Belani CP, Potter DM, Strychor S, Trump DL, Walko C, Fakih M, Zamboni WC. Pharmacokinetic Studies of 9-Nitrocamptothecin on Intermittent and Continuous Schedules of Administration in Patients with Solid Tumors. Cancer Chemotherapy and Pharmacol 2004;54(6):487-96.

27. D'Argenio, D.Z., Schmuitzky, A. ADAPT II user's guide: biomedical simulations resource, 1990.

28. Rowland, M., Tozer, T. Clinical pharmacokinetics: concepts and applications, pp 9-32. Philadelphia: Lea and Febiger, 1989.

29. de Jongh, F.E., Verweij, J., Loos, W.J., de Wit, R., de Jonge, M.J., Planting, A.S., Nooter, K., Stoter, G., Sparreboom, A. Body-surface area-based dosing does not increase accuracy of predicting cisplatin exposure. J Clin Oncol 2001;*19:* 3733-3739.

30. Kirstein, M.N., Houghton, P.J., Cheshire, P.J., Richmond, L.B., Smith, A.K., Hanna, S.K., Stewart, C.F. Relation between 9-aminocamptothecin systemic exposure and tumor response in human solid tumor xenografts. Clin Cancer Res 2001;*7:* 358-366.

31. Pitot, H.C., Knost, J.A., Mahoney, M.R., Kugler, J., Krook, J.E., Hatfield, A.K., Sargent, D.J., Goldberg, R.M. A North Central Cancer Treatment Group Phase II trial of 9-aminocamptothecin in previously untreated patients with measurable metastatic colorectal carcinoma. Cancer 2000;*89:* 1699-1705.

32. Vokes, E.E., Gordon, G.S., Rudin, C.M., Mauer, A.M., Watson, S., Krauss, S., Arrieta, R., Golomb, H.M., Hoffman, P.C. A phase II trial of 9-aminocaptothecin (9-AC) as a 120-h infusion in patients with non-small cell lung cancer. Invest New Drugs 2001;*19:* 329-333.

33. Zamboni, W.C., Jung, L.L., Egorin, M.J., Jin, R., Wong, M.K., Fakih, M., Belani, C.P., Potter, D.M., Walko, C.W., Tauch, J.S., Strychor, S., Sun, S.L., Trump, D.L., Ramanathan, R.K. Phase I studies of intermittently administered 9-nitrocamptothecin (9NC): relationship between daily dose and toxicity. Proc Am Soc Clin Oncol., 2002, #3523..

34. Wall, M.E., Wani, M.C. Camptothecin and taxol: discovery to clinic--thirteenth Bruce F. Cain Memorial Award Lecture. Cancer Res 1995;*55:* 753-760.

35. Moertel, C.G., Schutt, A.J., Reitemeier, R.J., Hahn, R.G. Phase II study of camptothecin (NSC-100880) in the treatment of advanced gastrointestinal cancer. Cancer Chemother Rep 1972;*56:* 95-101.

36. Rowinsky, E.K., Verweij, J. Review of phase I clinical studies with topotecan. Semin Oncol., *24:* S20, 1997 (suppl 6).

37. Rowinsky, E.K., Kaufmann, S.H., Baker, S.D., Miller, C.B., Sartorius, S.E., Bowling, M.K., Chen, T.L., Donehower, R.C., Gore, S.D. A phase I and pharmacological study of topotecan infused over 30 minutes for five days in patients with refractory acute leukemia. Clin Cancer Res 1996;*2:* 1921-1930.

38. Greischar, A., Nakagawa, Y., Coe, F.L. Influence of urine pH and citrate concentration on the upper limit of metastability for calcium phosphate. J Urol 2003;*169:* 867-870.

39. Mi, Z., Burke, T.G. Differential interactions of camptothecin lactone and carboxylate forms with human blood components. Biochem 1994;*33:* 10325-10336.

40. Mi, Z., Burke, T.G. Marked interspecies variations concerning the interactions of camptothecin with serum albumins: a frequency-domain fluorescence spectroscopic study. Biochem 1994;*33:* 12540-12545.

41. Jung, L.L., Zamboni, W.C. Cellular, pharmacokinetic, and pharmacodynamic aspects of response to camptothecins: can we improve it? Drug Resist Update 2001;*4:* 273-288.

42. Schellens, J.H., Maliepaard, M., Scheper, R.J., Scheffer, G.L., Jonker, J.W., Smit, J.W., Beijnen, J.H., Schinkel, A.H. Transport of topoisomerase I inhibitors by the breast cancer resistance protein. Potential clinical implications. Ann N Y Acad Sci 2000;*922:* 188-194.

43. Fabbro, D., Ruetz, S., Buchdunger, E., Cowan-Jacob, S.W., Fendrich, G., Liebetanz, J., Mestan, J., O'Reilly, T., Traxler, P., Chaudhuri, B., Fretz, H., Zimmermann, J., Meyer, T.,

Caravatti, G., Furet, P., Manley, P.W. Protein kinases as targets for anticancer agents: from inhibitors to useful drugs. Pharmacol Ther 2002;*93:* 79-98.

44. Zamboni, W.C., Tonda, M.E. New designs of clinical trials. Highlights in Oncol Practice 2002;*18:* 2-7.

4.0

CHAPTER 4:

Pharmacokinetic Studies of 9-Nitrocamptothecin on Intermittent and Continuous Schedules of Administration in Patients with Solid Tumors

4.1 ABSTRACT

Purpose: Oral administration of 9-nitrocamptothecin (9NC), and the formation of its metabolite 9-aminocamptothecin (9AC), may be associated with high inter- and intra-patient variability. Therefore, we evaluated the plasma pharmacokinetics and urine recovery of 9NC administered on 3 different schedules as part of Phase I and Phase II studies.

Methods: In Phase I schedule A, 9NC was administered orally qd x 5d x 2 wks q 4 weeks (one cycle). On Phase I schedule B, 9NC was administered qd x 14d q 4 weeks (one cycle). In Phase II, 9NC was administered qd x 5d x 8 weeks (one cycle). Serial blood samples were obtained on day 1 and day 10 or 11 for Phase I studies, and day 1 and day 50 for the Phase II study. Recovery of 9NC and 9AC in urine was evaluated on day 1 in the Phase I study. Area under the 9NC and 9AC plasma concentration versus time curves from 0 to 24 hours ($AUC_{0\text{-}24h}$) were calculated using compartmental analysis.

Results: Mean ± SD 9NC lactone $AUC_{0\text{-}24h}$ on day 1 at the maximum tolerated dose of schedules A and B (2.43 and 1.70 mg/m^2, respectively) and the Phase II dose (1.5 mg/m^2) were 78.9 ± 54.4, 155.7 ± 112.8, and 48.3 ± 17.5 ng/mL·h, respectively. Mean ± SD 9AC lactone $AUC_{0\text{-}24h}$ at these same doses of 9NC were 17.3 ±17.9, 41.3 ± 16.6, and 31.3 ± 12.8 ng/mL·h, respectively. Mean ± SD ratios of 9NC lactone $AUC_{0\text{-}24h}$ on day 10 or 11 to day 1 on Phase I A and B were 1.27 ± 0.68 and 1.73 ± 1.56, respectively. Mean ± SD ratios 9AC lactone $AUC_{0\text{-}24h}$ on day 10 or 11 to day 1 on Phase I A and B were 2.23 ± 1.02 and 1.65 ± 0.97, respectively. The recovery of 9NC and 9AC in the urine was < 15%.

Conclusions: There is significant inter-and intra-patient variability in the disposition of 9NC and 9AC. 9NC and 9AC undergo primarily non-renal elimination.

4.2 INTRODUCTION

The camptothecin analogues are topoisomerase I-interactive anticancer agents (1-3). 9-Nitrocamptothecin (9NC) is an orally administered camptothecin analogue (4-6). *In vitro* and *in vivo* preclinical studies suggest protracted administration of low doses of camptothecin analogues produce greater anti-tumor activity than do less frequent administration of higher doses [7,8]. Consistent with the mechanism of action of camptothecin analogues being cell-cycle-specific, prolonged exposures may be more effective than shorter exposures (9-11). Daily oral administration of 9NC may mimic a protracted parenteral schedule, achieve prolonged exposure, and maximize patient convenience. However, oral administration of camptothecin analogues, has been characterized by extensive inter-and intra-patient variability in bioavailability (12-16).

9NC is partially converted to the equipotent metabolite, 9-aminocamptothecin (9AC) (7,12,20). The terminal lactone ring is a key structural feature for the antitumor activity of camptothecin analogues (17-19). The conversion between lactone and hydroxy acid is reversible and is driven by pH and protein binding (17-19). Acidic conditions favor the lactone whereas basic conditions favor the hydroxy acid form. Pharmacokinetic data for 9NC and its conversion to 9AC are limited (7,12,20,33,34). Therefore, we evaluated the plasma and urinary disposition of 9NC and 9AC as part of Phase I and Phase II studies investigating 3 different schedules of administration.

In Phase II and III studies, 9NC is administered continuously at 1.25 to 1.5 mg/m^2/day for 5 days per week (21,27). On this schedule, dose reductions and delays in therapy frequently occur during weeks 3 to 5 and are due to myelosuppression, diarrhea, and hematuria. In xenograft studies, antitumor activity of camptothecin analogues requires a dose that produces a

systemic exposure above a critical threshold (7,8,11). It is possible that administration of continuous, low dose 9NC might not produce a systemic exposure above this critical threshold and as a result might fail to produce an antitumor response. In contrast, administration of 9NC on an intermittent schedule (e.g., 2 weeks of treatment followed by 2 weeks off) may allow for the administration of a higher dose per day that would produce therapeutic drug concentrations, and also avoid toxicities in weeks 3 and 4. In addition, there is no scientific basis for the administration of 9NC or other camptothecins for 5 days per week and then give a drug holiday for 2 days (7,8,11, 21,27). Thus, we evaluated 9NC administered daily for 5 days per week for 2 weeks repeated every 4 weeks to 9NC administered daily for 14 day repeated every 4 weeks to determine if the two day drug holiday affects the deliverable dose or pharmacokinetics. The pharmacokinetics of 9NC and its 9AC metabolite after administration of 9NC on the two intermittent schedules and the eight-week schedule are presented in this manuscript. The clinical evaluation from these studies have been described previously (36).

4.3 PATIENTS AND METHODS

Patients

Written informed consent, approved by the Institutional Review board of the University of Pittsburgh Medical Center, was obtained from all patients before they entered the study. The eligible patients for the Phase I study were 18 years of age or older and had a histologically or cytologically confirmed malignancy for which no curative or effective therapy was available. Other eligibility criteria included an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 and adequate bone marrow, hepatic, and renal function defined as: absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L, platelets ≥ 100 x 10^9/L, total bilirubin ≤ 1.5 x upper limit of the

institutional normal (ULN) range, aspartate aminotransferase (AST) ≤ 1.5 x ULN if liver metastases were not present and ≤ 4 x ULN if liver metastases were present, and the absence of microscopic hematuria. Prior treatment with camptothecin analogues, except 9NC, was permitted.

Patients eligible for the Phase II study had histologically or cytologically confirmed advanced colon carcinoma with measurable disease that had failed to respond or relapsed after receiving at least one prior 5-fluorouracil-based chemotherapy regimen for advanced disease or had evidence of metastatic disease within 6 months of completion of adjuvant therapy. Other eligibility criteria included an ECOG performance status ≤ 2 and adequate bone marrow, hepatic, and renal function defined as: absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L, platelets ≥ 100 x 10^9/L, hemoglobin > 10 g/dl, total bilirubin ≤ 2.0 mg/dL, AST and ALT ≤ 3 x ULN if liver metastases were not present and ≤ 5 x ULN if liver metastases were present, and serum creatinine ≤ 2.0 mg/dL. Patients with hematuria and those that had previously been treated with 9NC or any other camptothecin analogue were excluded from the Phase II study.

Dosage and Administration

9NC was supplied by the manufacturer, Supergen Inc. (Dublin, CA), as a crystalline powder in hard gelatin capsules that contained active drug and lactose excipient. The capsule strengths were 0.5 mg and 1.25 mg. All doses of 9NC were rounded to the nearest 0.25 mg. 9NC was administered orally on an empty stomach with an acidic beverage (i.e. orange juice, cola) (20,21). Patients were also instructed to drink 2 to 3 L of fluid per day.

In the phase II study, 9NC was administered at 1.5 mg/m^2/day x 5 days per week. In the Phase I study, two intermittent schedules of 9NC were evaluated. The phase I study evaluating

schedule A was completed prior to the initiation of schedule B. On schedule A, 9NC was administered orally daily for 5 days per week for 2 consecutive weeks and repeated every 4 weeks (one cycle). On schedule B, 9NC was administered orally daily for 14 days and repeated every 4 weeks (one cycle). On the Phase II study, 9NC was administered for 5 consecutive days per week for 8 weeks with no dose escalation. Dose levels for schedules A were determined by adaptive dose finding (22). In this two-stage method, doses are escalated during stage I by a factor of 1.5 until the first dose-limiting toxicity (DLT) occurred. Then, in stage II, escalation switched to a model-guided mode similar to the continual reassessment method (CRM) (23). Based on the number of days of treatment per cycle in schedule A and B, the initial dose level on schedule B was 30% lower than the MTD in schedule A. The adaptive dose finding procedure was not used to calculate dose levels for schedule B due to high pharmacokinetic variability and small changes in doses calculated by the adaptive dosing finding method. For all studies, patient diaries and interviews were used as documentation of 9NC administration.

Sample Collection and Preparation

On schedules A and B of the Phase I study, serial blood samples for pharmacokinetic analysis were obtained on day 1 and day 10 or 11. Due to scheduling issues, the second pharmacokinetic study was performed on day 10 or 11. On the Phase II study, serial blood samples were obtained on days 1 and 50. For each pharmacokinetic study day, blood samples (5 mL) were obtained prior to administration of 9NC, and 0.25, 0.5, 1, 2, 3, 6, 8, and 24 h after administration. Blood was placed into heparinized tubes and immediately centrifuged at 1200 x g at 4ºC for 5 min. The resulting plasma sample was then processed immediately in order to separate the lactone and hydroxy acid forms of 9NC and 9AC.

The processing for the measurement of the 9NC lactone in plasma used solid phase extraction (SPE) with Waters OASIS HLB columns (1 mL, 30 mg). Columns were conditioned with 1 mL of methanol and equilibrated with 1 mL of water. Plasma (1 mL) was passed through the column, and the column was then washed with 1 mL of 5% methanol in water to remove the hydroxy acid forms of 9NC and 9AC. 9NC lactone was then eluted with 1 mL of methanol and stored at -80°C until analyzed.

For measurement of 9AC lactone, 9NC total (sum of lactone and hydroxy acid) and 9AC total, the plasma was processed by methanolic extraction. A total of 600 μL of plasma was added to 1200 μL of methanol on dry ice. The samples were vortexed and centrifuged at 10,000 x g for 5 min. The supernatant was decanted and stored at -80°C until analyzed.

On day 1 and day 10 or 11 of the Phase I studies, 24-h urine collections were performed. Total urine volume was measured and a 600 μL sample was processed by the same methanolic extraction described above for measurement of 9NC and 9AC total.

High-Performance Liquid Chromatography (HPLC) Analysis

Because 9NC is not highly fluorescent, 9NC lactone and 9NC total were measured by chemically reducing 9NC to 9AC (23). Reduction of the 9NC to 9AC was accomplished by mixing 500 μL of the 9NC sample solution (methanolic extraction for measurement of 9NC total or the SPE elution for measurement of 9NC lactone) and 25 μL of 12N HCL. Then 50 μL of the Fe-reduction reagent (25 mg reduced Pentacarbonyl Iron/ml H_2O, Sigma Chemical Co., St. Louis, MO) was added and the mixture was sonicated for 30 min at 70°C, and then centrifuged at 13,400 x g for 5 min at 5°C. For analysis of 9NC lactone, 150 μL of the supernatant was added to 100 μL of 0.5 M ammonium acetate (pH 5.5), vortexed, and 100 μL was injected into the

HPLC. For analysis of 9NC total in plasma or urine, 150 μL supernatant was added to 75 μl of 0.5 M ammonium acetate (pH 5.5), vortexed, and 100 μL was injected into the HPLC.

The concentration of 9NC was calculated by subtracting the concentration of 9AC present before conversion of 9NC to 9AC from the concentration of 9AC after the conversion of 9NC to 9AC. For analysis of 9AC lactone, 150 μL of the methanolic extract was added to 10 μL of 0.5 M ammonium acetate. A total volume of 100 μL was then injected into the HPLC. For analysis of 9AC total, 150 μL of the methanolic extract was added to 15 μL of 20% phosphoric acid and vortexed. Then, 10 μL of 0.5 M ammonium acetate (pH 5.5) was added and vortexed, and a total volume of 100 μL was injected into the HPLC.

The HPLC system consisted of a Waters 2695 separation module (Waters Corp., Milford, MA), a C18 reverse phase column [Ultrasphere 5 μm ODS 4.6 x 250 mm, Beckman Coulter, Inc., Fullerton, CA], and a C18 guard column (Brownlee C18 7 μm, 15 x 3.2 mm, PerkinElmer Corp., Norwalk, CT). Samples were injected by an autosampler set at 4°C. The isocratic mobile phase consisted of methanol:acetonitrile:ammonium acetate (10:23:97, v/v/v), pH 5.5, pumped at a flow rate of 1.0 mL/min. Post column acidification (pH 2 – 3) was performed using 0.3 M trifluoroacetic acid at 0.3 mL/min (24). 9AC was detected by a Waters 474 fluorescence detector with excitation wavelength of 365 nm and emission wavelength of 440 nm. MILLENIUM 32 software (Waters Corporation) was used for data collection and analysis. All glassware, including the injection vials, was treated with 3% surfasil in toluene (Fisher Scientific Inc., Fair Lawn, NJ).

Pharmacokinetic Analysis

Compartmental pharmacokinetic analysis of 9NC and 9AC was performed using ADAPT II (25). The estimation procedure and variance model used in the compartmental

pharmacokinetic analysis was maximum likelihood estimation and linear models for the variance of the additive errors, respectively. Different pharmacokinetic model structures were considered to characterize the disposition of 9NC and 9AC in plasma. In the model development, one- and two-compartments models were evaluated to describe the systemic disposition of 9NC and 9AC. In addition, we evaluated the use of single and separate apparent volumes of the central compartments for 9NC and 9AC. Akaike's Information Criteria (AIC), Schwartz Criteria, estimated error of the model parameters, and residual analysis were use to select the model structure maximizing the fit accuracy while simultaneously minimizing the number of model parameters. The final model structure used for the pharmacokinetic analysis produced identifiable parameters in all patients.

A linear PK model describing oral administration of 9NC was simultaneously fit to 9NC and 9AC concentration versus time profiles. The model contained one-compartment for 9NC systemic disposition, subsequent conversion of 9NC to 9AC, and one-compartment for 9AC systemic disposition. Individual parameters estimated were the absorption rate constant (k_a), the lag time prior to absorption (τ), the apparent volume of the central compartment (V_c/F), the rate constant describing conversion of 9NC to 9AC (k_{12}), and the elimination rate constants for 9NC (k_{10}) and 9AC (k_{20}). The apparent clearance of 9NC (9NC CL/F) and 9AC (9AC CL/F) total and lactone were calculated using standard equations [i.e. $V_c/F \times (k_{10} + k_{12})$ and $V_c/F \times k_{20}$, respectively] (25,26). The area under the 9NC and 9AC plasma concentration versus time curves (9NC $AUC_{0\text{-}24\ h}$ and 9AC $AUC_{0\text{-}24\ h}$) from zero to 24 hours were calculated using the log trapezoidal method by simulating the concentration versus time data from each patient using patient-specific parameters (25). Intra-patient variability of 9NC and 9AC on the Phase I study was estimated by the ratio of $AUC_{0\text{-}24\ h}$ on day 10 or 11 to $AUC_{0\text{-}24\ h}$ on day 1. Intra-patient

variability of 9NC and 9AC on the Phase II study was estimated as the ratio of $AUC_{0\text{-}24\ h}$ on day 50 to the $AUC_{0\text{-}24\ h}$ on day 1.

In another attempt to evaluate the intra-patient variability in the disposition of 9NC and 9AC, patient-specific pharmacokinetic parameters describing the disposition of 9NC and 9AC on day 1 were used to simulate the 9NC and 9AC $AUC_{0\text{-}24\ h}$ expected in each patient on day 10, and the simulated $AUC_{0\text{-}24\ h}$ were compared with the 9NC and 9AC $AUC_{0\text{-}24\ h}$ actually measured in each patient on day 10.

Statistical Analysis

Statistical analysis was performed using the two-sided Wilcoxon signed rank test. The level of significance was set at $P < 0.05$.

4.4 RESULTS

High-Performance Liquid Chromatography (HPLC) Analysis

As stated in the methods section, 9NC was measured indirectly as 9AC. There was no endogenous materials in plasma and urine that interfered with measurement of 9AC. With the chromatography conditions described, 9AC eluted at 7.1 min. The sample preparation described results in > 90% recovery of 9AC when compared to the direct injection of an equivalent amount of 9AC in mobile phase. The conversion of 9NC to 9AC via the reduction method described was 54 ± 4.6%. When stored at – 80EC the percentage change in measured concentration of 9NC and 9AC lactone and total from baseline was < 10% at 2 months. The stability of 9NC and 9AC lactone and total on the autosampler at 24 h was > 90%.

The lower limit of quantitation (LLQ) for 9NC lactone in plasma and 9NC total in plasma and urine was 0.5 ng/mL, and the assay was linear from 0.5 ng/mL to 100 ng/mL. The correlation coefficients for three successive 9NC lactone triplicate standard curves in plasma and 9NC total triplicate standard curves in plasma or urine were > 0.99. When expressed as a percentage coefficient of variation, the within-day and between day variation in 9NC lactone in plasma and total in plasma and urine triplicate standards was always < 15%.

The LLQ for 9AC lactone and 9AC total was 0.3 ng/mL, and the assay was linear from 0.3 ng/mL to 30 ng/mL. The average ± SD correlation coefficients for three successive 9AC lactone triplicate standard curves in plasma and 9AC total triplicate standard in plasma or ruine curves was > 0.99. When expressed as a percentage coefficient of variation, the within-day and between day variation in 9AC lactone triplicate standards in plasma and total triplicate standards in plasma or urine was always < 15%.

Phase I Study 9NC and 9AC Lactone Pharmacokinetics

A summary of 9NC and 9AC lactone pharmacokinetic parameters from the Phase I study is presented in Table 1, and $AUC_{0\text{-}24h}$ are presented in Table 2 and Table 3. There was no difference ($P > 0.05$) in the pharmacokinetic parameters after administration of different doses of 9NC, thus data from all doses were pooled on day 1 or day 10 and 11. Representative concentration versus time profiles of 9NC and 9AC total and lactone after administration of 9NC 2.43 mg/m^2/d on day 1 and day 11 of schedule A of the Phase I study within the same patient are presented in Figure 1 and Figure 2, respectively. The 9NC and 9AC lactone $AUC_{0\text{-}24h}$ on day 1 and day 10 or 11 of schedules A and B are represented in Figure 3 and Figure 4, respectively.

Table 1. 9NC and 9AC Lactone Pharmacokinetic Parameters for Phase I Schedules A and B and Phase II

Parameter	Units	Schedules A, B, & Phase II Day 1 Mean ± SD	Schedule A Day 10 or 11 Mean ± SD	Schedule B Day 10 or 11 Mean ± SD	Phase II Week 8 Day 1 Mean ± SD
Lactone		(n = 34)	(n = 11)	(n = 9)	(n = 4)
9NC CL/F	$L/h/m^2$	27.0 ± 33.7	9.5 ± 16.2	16.5 ± 32.5	59.8 ± 45.8
9AC CL/F	$L/h/m^2$	4.0 ± 8.8	0.4 ± 0.4	2.8 ± 5.5	1.2 ± 0.9
k_a	h^{-1}	0.3 ± 0.4	0.04 ± 0.04	0.1 ± 0.1[a]	0.3 ± 0.2
τ	h	0.5 ± 0.3	0.8 ± 1.3	0.2 ± 0.2	0.4 ± 0.1
k_{12}	h^{-1}	0.04 ± 0.03	0.09 ± 0.08	0.3 ± 0.3[a]	0.1 ± 0.02
V_c/F	L/m^2	37.5 ± 46.2	10.0 ± 0.1	4.9 ± 7.0	99.9 ± 98.4
Total		(n = 39)	(n = 13)	(n = 9)	(n = 4)
9NC CL/F	$L/h/m^2$	6.3 ± 6.2	2.0 ± 3.1[a]	1.4 ± 1.1[a]	4.6 ± 6.6
9AC CL/F	$L/h/m^2$	2.2 ± 3.6	0.3 ± 0.3[a]	0.2 ± 0.1	1.0 ± 1.5
k_a	h^{-1}	0.4 ± 0.7	0.02 ± 0.02[a]	0.03 ± 0.04[a]	0.1 ± 0.1
τ	h	0.4 ± 0.2	0.7 ± 0.6	0.2 ± 0.1	0.3 ± 0.1
k_{12}	h^{-1}	0.04 ± 0.03	0.1 ± 0.1[a]	0.1 ± 0.1	0.1 ± 0.1
V_c/F	L/m^2	27.5 ± 35.4	11.0 ± 10.0	10.2 ± 1.2	8.1 ± 12.4

[a] P-values < 0.05 as determined by two-sided Wilcoxon signed rank test.

Table 2. 9NC and 9AC Lactone and Total AUCs on Phase I Schedule A

	Units	Day 1 2.00 mg/m^2 Mean ± SD (Range)	Day 1 2.43 mg/m^2 Mean ± SD (Range)	Day 1 2.68 mg/m^2 Mean ± SD (Range)	Day 10 or 11 2.00 mg/m^2 Mean ± SD (Range)	Day 10 or 11 2.43 mg/m^2 Mean ± SD (Range)	Day 10 or 11 2.68 mg/m^2 Mean ± SD (Range)
Lactone		(n = 6)	(n = 7)	(n = 4)	(n = 6)	(n = 7)	(n = 3)
9NC $AUC_{0\text{-}24\ h}$	ng/mL·h	183.1 ± 119.0 (78.4 – 412.7)	78.9 ± 54.4 (27.0 – 187.2)	89.2 ± 90.9 (45.8 – 287.9)	254.7 ± 190.0 (110.4 – 671.9)	102.8 ± 121.0 (11.0 – 360.7)	229.6 ± 66.8 (154.6 – 316.8)
9AC $AUC_{0\text{-}24\ h}$	ng/mL·h	33.6 ± 20.7 (10.81 – 66.14)	17.3 ± 17.9 (5.2 – 60.6)	32.2 ± 16.1 (7.3 – 51.5)	45.1 ± 23.4 (20.1 – 80.4)	43.7 ± 42.5 (8.3 – 136.1)	88.8 ± 34.6 (42.0 – 124.5)
Ratio of 9AC $AUC_{0\text{-}24\ h}$ to 9NC $AUC_{0\text{-}24\ h}$		0.19 ± 0.07 (0.11 – 0.29)	0.23 ± 0.13 (0.06 – 0.41)	0.18 ± 0.04 (0.11 – 0.21)	0.21 ± 0.13 (0.11 – 0.48)	0.70 ± 0.66 (0.14 – 2.06)	0.41 ± 0.19 (0.19 – 0.65)
Total		(n = 6)	(n = 7)	(n = 4)	(n = 6)	(n = 7)	(n = 3)
9NC $AUC_{0\text{-}24\ h}$	ng/mL·h	647.4 ± 294.5 (136.3 – 1037.0)	482.1 ± 597.6 (109.0 – 1936.0)	942.0 ± 443.6 (292.0 – 1474.0)	1075.3 ± 811.3 (408.4 – 2646.0)	651.6 ± 774.7 (62.8 – 2487.0)	1663.3 ± 556.8 (1115.0 – 2427.0)
9AC $AUC_{0\text{-}24\ h}$	ng/mL·h	196.4 ± 134.1 (54.5 – 441.2)	83.5 ± 78.9 (31.1 – 274.0)	245.5 ± 122.4 (49.7 – 382.0)	304.9 ± 197.1 (112.9 – 668.0)	178.3 ± 179.8 (23.4 – 582.0)	537.4 ± 238.9 (201.9 – 739.4)
Ratio of 9AC $AUC_{0\text{-}24\ h}$ to 9NC $AUC_{0\text{-}24\ h}$		0.30 ± 0.10 (0.14 – 0.43)	0.21 ± 0.05 (0.14 – 0.29)	0.25 ± 0.05 (0.17 – 0.31)	0.23 ± 0.05 (0.15 – 0.29)	0.33 ± 0.12 (0.14 – 0.50)	0.32 ± 0.12 (0.18 – 0.46)

* Area under the plasma concentration versus time curves were calculated from 0 to 24 h ($AUC_{0\text{-}24\ h}$).

Table 3. 9NC and 9AC Lactone and Total AUCs on Phase I Schedule B

		Day 1		Day 10 or 11	
		1.70 mg/m^2	2.40 mg/m^2	1.70 mg/m^2	2.40 mg/m^2
	Units	Mean ± SD (Range)	Mean ± SD (Range)	Mean ± SD (Range)	Mean ± SD (Range)
Lactone		(n = 5)	(n = 3)	(n = 6)	(n = 3)
9NC $AUC_{0\text{-}24\ h}$	ng/mL·h	155.7 ± 112.8 (45.2 – 359.2)	277.9 ± 218.4 (34.1 – 563.9)	188.1 ± 112.3 (15.8 – 338.5)	182.7 ± 25.1 (152.8 – 241.3)
9AC $AUC_{0\text{-}24\ h}$	ng/mL·h	41.3 ± 16.6 (13.1 – 58.3)	75.4 ± 69.6 (23.6 – 173.8)	51.8 ± 21.2 (22.9 – 74.9)	58.4 ± 17.7 (40.3 – 82.5)
Ratio of 9AC $AUC_{0\text{-}24\ h}$ to 9NC $AUC_{0\text{-}24\ h}$		0.38 ± 0.24 (0.13 – 0.79)	0.37 ± 0.24 (0.12 – 0.69)	0.62 ± 0.78 (0.10 – 2.36)	0.32 ± 0.10 (0.25 – 0.46)
Total		(n = 5)	(n = 3)	(n = 6)	(n = 3)
9NC $AUC_{0\text{-}24\ h}$	ng/mL·h	431.0 ± 213.7 (91.0 – 737.4)	289.5 ± 92.5 (161.8 – 377.7)	534.3 ± 339.7 (83.6 – 1065.0)	337.4 ± 22.2 (321.7 – 368.8)
9AC $AUC_{0\text{-}24\ h}$	ng/mL·h	183.8 ± 109.8 (31.6 – 345.2)	281.7 ± 198.3 (114.1 – 560.2)	233.6 ± 155.8 (73.6 – 446.9)	306.6 ± 123.3 (218.7 – 481.0)
Ratio of 9AC $AUC_{0\text{-}24\ h}$ to 9NC $AUC_{0\text{-}24\ h}$		0.46 ± 0.25 (0.15 – 0.90)	0.95 ± 0.54 (0.45 – 1.70)	0.51 ± 0.23 (0.23 – 0.88)	0.92 ± 0.41 (0.59 – 1.49)

* Area under the plasma concentration versus time curves were calculated from 0 to 24 h ($AUC_{0\text{-}24\ h}$).

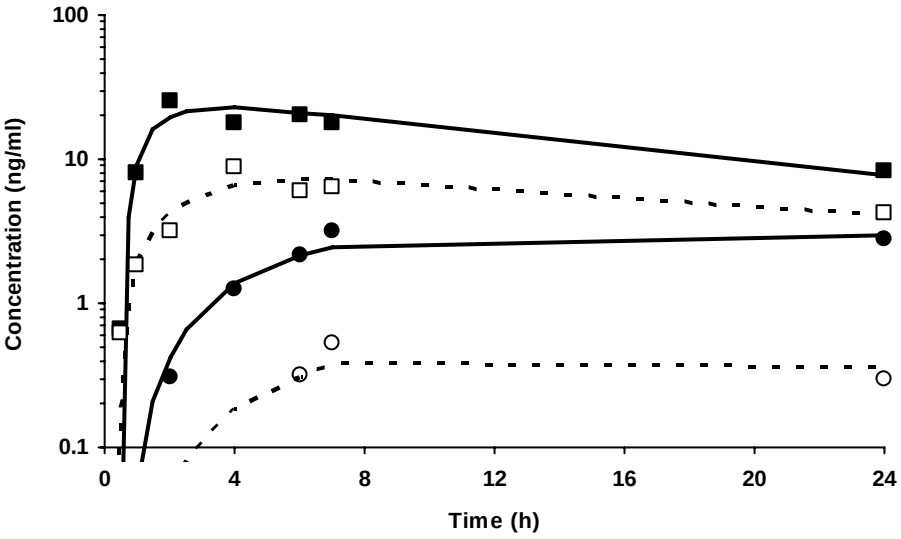


Figure 1. Representative concentration versus time profiles of 9NC and 9AC total and lactone profiles in a single patient after oral administration of 9NC 2.43 mg/m^2/d on day 1 of schedule A of the Phase I study. The concentration versus time profiles in Figure 1 and 2 are from the same patient. Individual data and best fit line for 9NC total (■, —) and lactone (□, ---) concentration versus time profiles are presented. Individual data and best fit line for 9AC total (● ,—) and lactone (○,---) concentration versus time profiles are also presented.

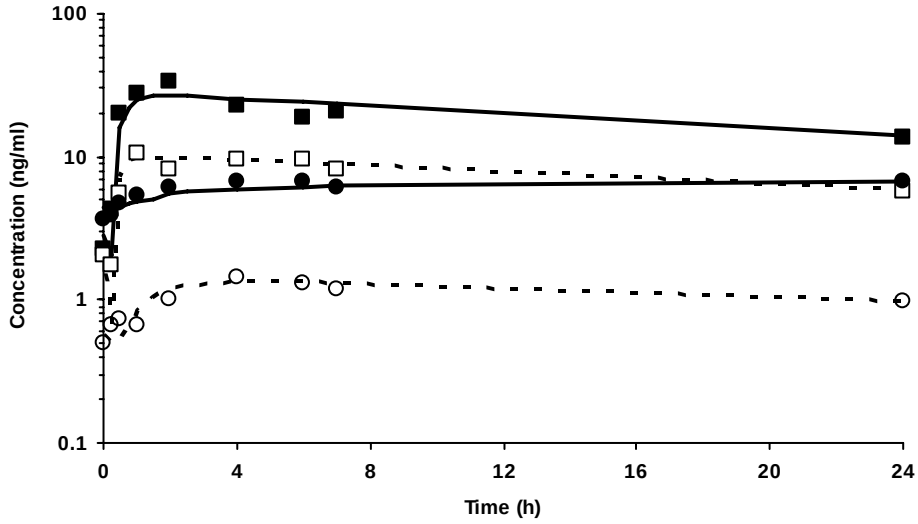


Figure 2. Representative concentration versus time profiles of 9NC and 9AC total and lactone profiles in a single patient after oral administration of 9NC 2.43 mg/m^2/d on day 10 of schedule A of the Phase I study. The concentration versus time profiles in Figure 1 and 2 are from the same patient. Individual data and best fit line for 9NC total (■, —) and lactone (□, ---) concentration versus time profiles are presented. Individual data and best fit line for 9AC total (●,—) and lactone (○,---) concentration versus time profiles are also presented.

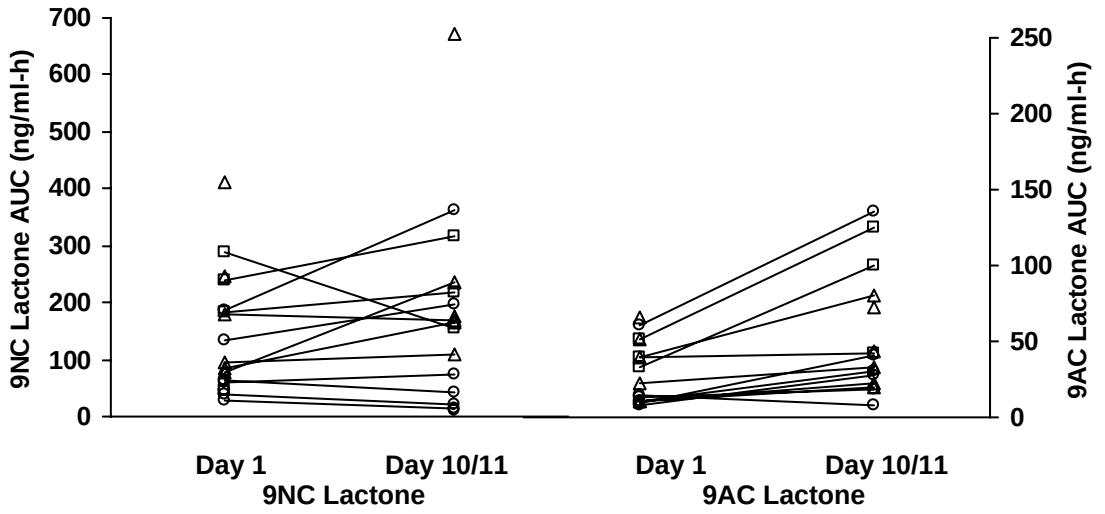


Figure 3. Inter- and intra-patient variability in 9NC and 9AC lactone AUCs on Schedule A of the Phase I study. Individual 9NC and 9AC lactone AUC values for patients administered 2.00 mg/m^2 (Δ), 2.43 mg/m^2 (○), and 2.68 mg/m^2 (□) are presented. Data within the same patient from day 1 to day 10 or 11 are connected by solid lines.

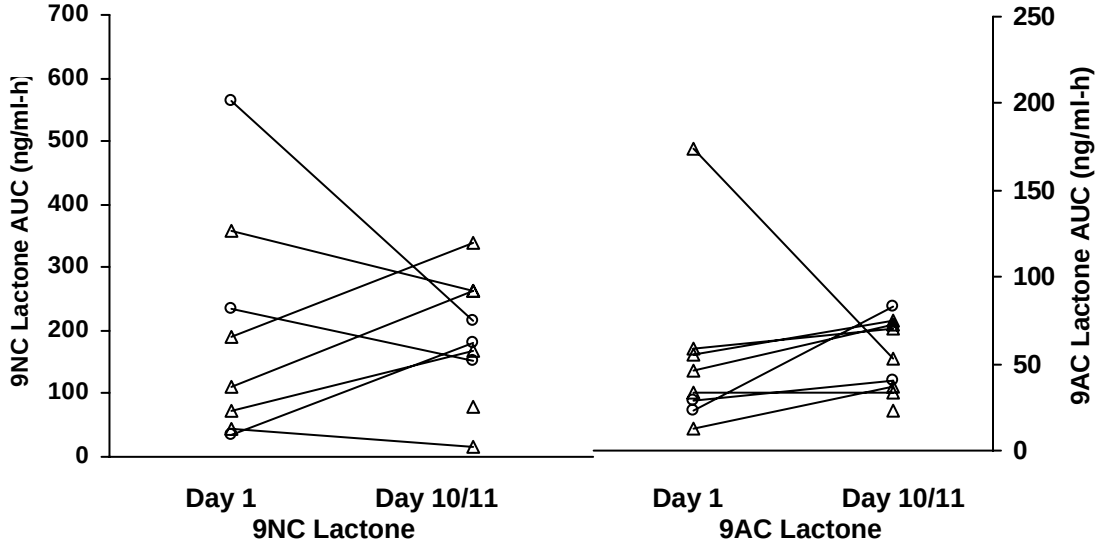


Figure 4. Inter- and intra-patient variability in 9NC and 9AC lactone AUCs on Schedule B of the Phase I study. Individual 9NC and 9AC lactone AUC values for patients administered 1.70 mg/m^2 (Δ) and 2.40 mg/m^2 (○) are presented. Data within the same patient from day 1 to day 10 or 11 are connected by solid lines.

On schedule A, the mean ± SD ratio of 9NC lactone $AUC_{0\text{-}24\ h}$ on day 10 or 11 to day 1 was 1.27 ± 0.68. On schedule A, the mean ± SD ratio of 9AC lactone $AUC_{0\text{-}24\ h}$ on day 10 or 11 to day 1 was 2.23 ± 1.02. On schedule B, the mean ± SD ratio of 9NC lactone $AUC_{0\text{-}24\ h}$ on day 10 to day 1 was 1.73 ± 1.56. In addition, on schedule B the mean ± SD ratio of 9NC lactone $AUC_{0\text{-}24\ h}$ simulated on day 10 to the $AUC_{0\text{-}24\ h}$ measured on day 10 was 1.6 ± 1.1. On schedule B, the mean ± SD ratio of 9AC lactone $AUC_{0\text{-}24\ h}$ on day 10 to day 1 was 1.65 ± 0.97. In addition, on schedule B the mean ± SD ratio of 9AC lactone $AUC_{0\text{-}24\ h}$ simulated on day 10 to the $AUC_{0\text{-}24\ h}$ measured on day 10 was 3.7 ± 4.2.

Phase I Study 9NC and 9AC Total Pharmacokinetics

A summary of 9NC and 9AC total pharmacokinetic parameters is presented in Table 1 and $AUC_{0\text{-}24\ h}$ are presented in Table 2 and Table 3. There was no difference ($P > 0.05$) in the pharmacokinetic parameters after administration of different doses of 9NC, thus data from all doses were pooled on day 1 or day 10 and 11. On schedule A, the mean ± SD ratio of 9NC total $AUC_{0\text{-}24\ h}$ from day 10 or 11 to day 1 was 1.62 ± 0.57. On schedule A, the mean ± SD ratio of 9AC total $AUC_{0\text{-}24\ h}$ from day 10 or 11 to day 1 was 2.08 ± 0.75. On schedule B, the mean ± SD ratio of 9NC total $AUC_{0\text{-}24\ h}$ from day 10 to day 1 was 1.40 ± 0.61. In addition, on schedule B the mean ± SD ratio of 9NC total $AUC_{0\text{-}24\ h}$ simulated on day 10 to the $AUC_{0\text{-}24\ h}$ measured on day 10 was 1.7 ± 1.0. On schedule B, the mean ± SD ratio of 9AC total $AUC_{0\text{-}24\ h}$ from day 10 to day 1 was 1.50 ± 0.45. In addition, on schedule B the mean ± SD ratio of 9AC total $AUC_{0\text{-}24\ h}$ simulated on day 10 to the $AUC_{0\text{-}24\ h}$ measured on day 10 was 6.5 ± 4.4.

Phase II 9NC and 9AC Lactone and Total Pharmacokinetics

Summaries of 9NC and 9AC pharmacokinetic parameters from the Phase II study are presented in Table 1 and Table 4. The mean ± SD ratios of 9NC lactone $AUC_{0\text{-}24\ h}$ on day 50 to day 1 was 1.16 ± 0.89. The mean ± SD ratios of 9AC lactone $AUC_{0\text{-}24\ h}$ on day 50 to day 1 was 1.74 ± 0.13. The mean ± SD ratio of 9NC total $AUC_{0\text{-}24\ h}$ on day 50 to day 1 was 1.68 ± 0.61. The mean ± SD ratio of 9AC total $AUC_{0\text{-}24\ h}$ on day 50 to day 1 was 1.50 ± 0.76.

Table 4. 9NC and 9AC Lactone and Total AUCs on the Phase II Study

	Units	Day 1 1.50 mg/m^2 Mean ± SD (Range)	Day 50 1.50 mg/m^2 Mean ± SD (Range)
Lactone		(n = 5)	(n = 3)
9NC $AUC_{0\text{-}24\ h}$	ng/mL·h	48.3 ± 17.5 (23.4 – 69.3)	51.9 ± 31.1 (11.9 – 87.7)
9AC $AUC_{0\text{-}24\ h}$	ng/mL·h	31.3 ± 12.8 (10.2 – 44.8)	38.8 ± 19.7 (19.1 – 58.5)
9AC $AUC_{0\text{-}24\ h}$ to 9NC $AUC_{0\text{-}24\ h}$		0.81 ± 0.65 (0.28 – 1.92)	0.63 ± 0.41 (0.22 – 1.04)
Total		(n = 9)	(n = 3)
9NC $AUC_{0\text{-}24\ h}$	ng/mL·h	377.0 ± 221.2 (90.7 – 798.1)	310.7 ± 178.2 (89.8 – 526.1)
9AC $AUC_{0\text{-}24\ h}$	ng/mL·h	88.5 ± 41.32 (27.0 – 141.8)	97.8 ± 79.5 (16.1 – 205.5)
9AC $AUC_{0\text{-}24\ h}$ to 9NC $AUC_{0\text{-}24\ h}$		0.31 ± 0.21 (0.10 – 0.83)	0.27 ± 0.09 (0.18 – 0.39)

* Area under the plasma concentration versus time curves were calculated from 0 to 24 h ($AUC_{0\text{-}24\ h}$).

Renal Elimination

On schedule A of the Phase I study, the percentage of the dose renally eliminated on day 1 as 9NC total and 9AC total were 5.9 ± 4.3 % and 3.5 ± 1.7 %, respectively. On schedule A of the Phase I study, the percentage of the dose renally eliminated as 9NC total and 9AC total on day 10 or 11 was 5.6 ± 3.8 % and 4.2 ± 2.2 %, respectively. On schedule B of the Phase I study, the percentage of the dose renally eliminated on day 1 as 9NC total and 9AC total was 11.3 ± 3.8 % and 5.0 ± 2.6 %, respectively. On schedule B of the Phase I study, the percentage of the dose renally eliminated as 9NC total and 9AC total on day 10 or 11 was 9.0% ± 6.2 and 5.5% ± 1.9, respectively.

4.5 DISCUSSION

Although Phase III trials of 9NC have been completed, there is limited pharmacokinetic data on 9NC and its conversion to 9AC. Prior studies have reported the pharmacokinetics of 9NC after oral and inhaled administration (21,27-30,33,34). However, this is the first pharmacokinetic study evaluating the inter- and intra-patient variability of 9NC and its 9AC metabolite on continuous and intermittent schedules of administration. There was high inter-and intra-patient pharmacokinetic variability of 9NC and 9AC regardless of the schedule of administration (12-16). The clinical importance of this study is underscored by the need to evaluate the pharmacokinetics of anticancer agents that are orally administered and have a steep relationship between exposure and response (ie, antitumor or toxicity).

Preliminary evidence suggests 9NC is metabolized extensively in the liver by an NADPH-dependent system, probably involving a cytochrome P450 isotype (30). Previous *in*

vitro studies have demonstrated the conversion of 9NC to 9AC was relatively minor (i.e. 12% - 25%), but highly variable (30-32). In our current study, most of the drug remained in the 9NC form with a mean ratio of 9NC to 9AC of 4 to 1, which is consistent with the previous studies. The fact that most of the administered drug remains in the 9NC form is significant because the development of 9AC was stopped due to lack efficacy (4-6). However, our studies also demonstrated highly variable conversion of 9NC to 9AC, as 2 patients had undetectable 9AC concentrations and 4 patients had higher exposures of 9AC compared to 9NC. In addition, from day 1 to day 10 in the Phase I study or day 1 to day 50 in the Phase II study some patients had greater accumulation of 9NC compared to 9AC, whereas others had greater accumulation of 9AC.

Although the complete metabolic pathway of 9NC and 9AC is unclear, our study suggests that the clearance of 9NC appears to be non-renal with possible biliary elimination. The percentage of the dose recovered in the urine was low (i.e., < 15 %). However, if the oral bioavailability, which is currently unknown because no IV formulation exists, is only 30 % then the percentage of the dose recovered in the urine would be 50 %. Patients with who had elevated bilirubin levels related to biliary stent blocks or disease had the highest 9NC and 9AC exposures. A plateau of 9NC and 9AC concentrations from 6 to 24 hours, and T_{max} which ranged from 0.5 to 24 hr, suggests 9NC and 9AC may undergo enterohepatic circulation (28). Similar results have been reported for 9NC and with other camptothecin analogues. However, the long duration of 9NC exposure was much greater than reported for other orally administered camptothecin analogues (12-16).

Several studies have reported significant interpatient variability in the pharmacokinetics of orally administered camptothecin analogues (11,13,14,15,16,31,32). Our study also

demonstrated relatively high inter- and intra-patient pharmacokinetic variability of 9NC and 9AC. At individual doses there was 4 to 16-fold variability in 9NC and 9AC exposure among different patients and there was no relationship between dose and $AUC_{0\text{-}24\ h}$. The ratio of 9NC or 9AC $AUC_{0\text{-}24\ h}$ from day 1 to day 10 in the Phase I study or day 50 in the Phase II study ranged from 0.6 to 4. Some accumulation of 9NC was seen on both the intermittent and continuous schedules of administration. However, the accumulation of 9NC or 9AC from day 1 to day 10 or 11 in the Phase I studies or from day 1 to day 50 on the Phase II study was not as great as predicted by the pharmacokinetics on day 1. This also suggests the disposition of 9NC and 9AC is not consistent over time.

The high inter- and intra-patient variability in 9NC and 9AC disposition could be due to variability in oral absorption, the affect of food, pH of the gastric tract, and hepatic and extra-hepatic conversion of 9NC to 9AC (7,12-16,20,21). The presence of a nitro group at the 9-position makes 9NC water-insoluble and an intravenous formulation has not been developed. Thus, the absolute oral bioavailability of 9NC can not be determined in patients and the influence of oral absorption and systemic metabolism on the overall variability in 9NC and 9AC disposition can not be determined. Schoffski and colleagues demonstrated that a meal consisting of 58% fat, 15% protein, and 27% carbohydrate, consumed 30 min prior to drug administration, led to a 50% reduction in the oral absorption of 9NC (21). In our study 9NC was administered on an empty stomach. In our study, 9NC was administered with an acidic beverage, however, it is still unclear if co-administration with an acidic beverage improves oral absorption and reduces variability (20,21). Patients in this study were allowed to continue any routine medications, including histamine-2 blockers and proton pump inhibitors. There was no relationship between the administration of these agents. In addition, there was no relationship between the level of

compliance as documented by patient diaries and interviews and the exposure of 9NC and 9AC. Similar results have been seen with topotecan after administration of ranitidine (20). Thus, the factors associated with the extensive variability in the disposition of 9NC and 9AC are unknown.

The results from our study are similar to the results of the studies by Schoemaker et.al. and Raymond et.al (33,34). For example, the k_a in our study and the study by Schoemaker et.al. were 0.4 ± 0.7 h^{-1} and 0.8 h^{-1}, respectively. In addition, the CL/F of 9NC in our study and the study by Schoemaker et.al. were 6.3 ± 6.2 L/h/m2 and 1.7 L/h, respectively. The slight difference in CL/F may be explained by administration of different doses and slightly different model framework used between the 2 studies, and additional samples and evaluation of more patients in our study. In the study by Raymond et.al., administration on 9NC at 1.5 mg/m^2 x 1, the 9NC and 9AC $AUC_{0\text{-}24\ h}$ were 231 ± 137 ng/mL·hr and 36.9 ± 28.5 ng/mL·hr, respectively, which are similar to the $AUC_{0\text{-}24\ h}$ in our studies after administration of 9NC at 2.0 mg/m^2. In addition, the concentration versus time profiles of 9NC and 9AC, delayed absorption, increases in 9AC concentrations over the 24 hour period, and no drug accumulation over the 21 days of treatment in the study by Raymond et.al. are very similar to our study.

We used an HPLC assay with fluorescence detection to measure 9NC and 9AC. As stated previously, 9NC was measured indirectly by chemically reducing 9NC to 9AC using an Fe-reduction reagent (23). The need to convert 9NC to 9AC via Fe-reduction is highly complicated and logistically difficulty. Moreover, the conversion of 9NC to 9AC is only approximately 54% which requires the indirect estimation of 9NC concentrations by subtracting the concentration of 9AC present before conversion of 9NC to 9AC from the concentration of 9AC after the conversion of 9NC to 9AC. The use of an LC/MS or LC/MS/MS assay for 9NC

would not require the conversion of 9NC to 9AC and may allow for the simultaneous determination of lactone and hydroxy acid forms of 9NC and 9AC.

4.6 REFERENCES

1. Hsiang YH, Hertzberg R, Hecht S, Liu LF. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J Biol Chem 1985;260:14873.
2. Covey JM, Jaxel C, Kohn KW, Pommier Y. Protein-linked DNA strand breaks induced in mammalian cells by camptothecin, an inhibitor of topoisomerase I. Cancer Res 1989; 49:5016.
3. Jaxel C, Kohn KW, Wani MC, Wall ME, Pommier Y. Structure-activity study of the actions of camptothecin derivatives on mammalian topoisomerase I: evidence for a specific receptor site and a relation to antitumor activity. Cancer Res 1989;49:1465.
4. Kirstein MN, Houghton PJ, Cheshire PJ, Richmond LB, Smith AK, Hanna SK, Stewart CF. Relation between 9-aminocamptothecin systemic exposure and tumor response in human solid tumor xenografts. Clin Cancer Res 2001;7:358.
5. Pazdur R, Diaz-Canton E, Ballard WP, Bradof JE, Graham S, Arbuck SG, Abbruzzese JL, Winn R. Phase II trial of 9-aminocamptothecin administered as a 72-hour continuous infusion in metastatic colorectal carcinoma. J Clin Oncol 1997;15:2905.
6. Takimoto CH, Thomas R. The clinical development of 9-aminocamptothecin. Ann N Y Acad Sci 2000;922:224.
7. Furman WL, Stewart CF, Poquette CA, Pratt CB, Santana VM, Zamboni WC, Bowman LC, Ma MK, Hoffer FA, Meyer WH, Pappo AS, Walter AW, Houghton

PJ. Direct translation of a protracted irinotecan schedule from a xenograft model to a phase I trial in children. J Clin Oncol 1999;17:1815.

8. Thompson J, Zamboni WC, Cheshire PJ, Lutz L, Luo X, Li Y, Houghton JA, Stewart CF, Houghton PJ. Efficacy of systemic administration of irinotecan against neuroblastoma xenografts. Clin Cancer Res 1997;3:423.
9. Hsiang YH, Lihou MG, Liu LF. Arrest of replication forks by drug-stabilized topoisomerase I-DNA cleavable complexes as a mechanism of cell killing by camptothecin. Cancer Res 1989;49:5077.
10. Del Bino G, Darzynkiewicz Z. Camptothecin, teniposide, or 4'-(9-acridinylamino)-3-methanesulfon-m-anisidide, but not mitoxantrone or doxorubicin, induces degradation of nuclear DNA in the S phase of HL-60 cells. Cancer Res 1991;51:1165.
11. Houghton PJ, Cheshire PJ, Hallman JD, Lutz L, Friedman HS, Danks MK, Houghton. Efficacy of topoisomerase I inhibitors, topotecan and irinotecan, administered at low dose levels in protracted schedules to mice bearing xenografts of human tumors. Cancer Chemother Pharmacol 1995;36:393.
12. Zamboni WC, Bowman LC, Tan M, Santana VM, Houghton PJ, Meyer WH, Pratt CB, Heideman RL, Gajjar AJ, Pappo AS, Stewart CF. Interpatient variability in bioavailability of the intravenous formulation of topotecan given orally to children with recurrent solid tumors. Cancer Chemother Pharmacol 1999;43:454.

13. Gupta E, Luo F, Lallo A, Ramanathan S, Vyas V, Rubin E, Sinko P. The intestinal absorption of camptothecin, a highly lipophilic drug, across Caco-2 cells is mediated by active transporter(s). Anticancer Res 2000;20:1013.

14. Drengler RL, Kuhn JG, Schaaf LJ, Rodriguez GI, Villalona-Calero MA, Hammond LA, Stephenson JA, Jr., Hodges S, Kraynak MA, Staton BA, Elfring GL, Locker PK, Miller LL, Von Hoff DD, Rothenberg ML. Phase I and pharmacokinetic trial of oral irinotecan administered daily for 5 days every 3 weeks in patients with solid tumors. J Clin Oncol 1999;17:685.

15. Loos WJ, Gelderblom H, Sparreboom A, Verweij J, de Jonge MJ. Inter- and intrapatient variability in oral topotecan pharmacokinetics: implications for body-surface area dosage regimens. Clin Cancer Res 2000;6:2685.

16. Kruijtzer CM, Beijnen JH, Rosing H, Bokkel Huinink WW, Schot M, Jewell RC, Paul EM, Schellens JH. Increased oral bioavailability of topotecan in combination with the breast cancer resistance protein and P-glycoprotein inhibitor GF120918. J Clin Oncol 2002;20:2943.

17. Mi Z, Burke TG. Differential interactions of camptothecin lactone and carboxylate forms with human blood components. Biochemistry 1994;33:10325.

18. Mi Z, Burke TG. Marked interspecies variations concerning the interactions of camptothecin with serum albumins: a frequency-domain fluorescence spectroscopic study. Biochemistry 1994;33:12540.

19. Schrijvers D, Highley M, De Bruyn E, Van Oosterom AT, Vermorken JB (1999) Role of red blood cells in pharmacokinetics of chemotherapeutic agents. Anticancer Drugs 10:147

20. Akhtar S, Beckman RA, Mould DR, Doyle E, Fields SZ, Wright J. Pretreatment with ranitidine does not reduce the bioavailability of orally administered topotecan. Cancer Chemother Pharmacol 2000;46:204.

21. Schoffski P, Herr A, Vermorken JB, Van den BJ, Beijnen JH, Rosing H, Volk J, Ganser A, Adank S, Botma HJ, Wanders J. Clinical phase II study and pharmacological evaluation of rubitecan in non-pretreated patients with metastatic colorectal cancer-significant effect of food intake on the bioavailability of the oral camptothecin analogue. Eur J Cancer 2002;38:807.

22. Potter DM. Adaptive dose finding for phase I clinical trials of drugs used for chemotherapy of cancer. Stat Med 2002;21:1805.

23. O'Quigley J, Pepe M, Fisher L. Continual reassessment method: a practical design for phase 1 clinical trials in cancer. Biometrics 1990;46:33.

24. Supko JG, Malspeis L. Liquid chromatographic analysis of 9-aminocamptothecin in plasma monitored by fluorescence induced upon post-column acidification. J Liquid Chromatograph 1992;15:3261.

25. D'Argenio DZ, Schmuitzky A. ADAPT II user's guide: biomedical simualtions resouce. 1990.

26. Rowland M, Tozer T (eds). Clinical pharmacokinetics: concepts and applications. Lea and Febiger, Philadelphia, 1990.

27. Natelson EA, Giovanella BC, Verschraegen CF, Fehir KM, De Ipolyi PD, Harris N, Stehlin JS. Phase I clinical and pharmacological studies of 20-(S)-camptothecin and 20-(S)-9-nitrocamptothecin as anticancer agents. Ann N Y Acad Sci 1996;803:224.

28. Verschraegen CF, Gilbert BE, Huaringa AJ, Newman R, Harris N, Leyva FJ, Keus L, Campbell K, Nelson-Taylor T, Knight V. Feasibility, phase I, and pharmacological study of aerosolized liposomal 9-nitro-20(S)-camptothecin in patients with advanced malignancies in the lungs. Ann N Y Acad Sci 2000;922:352.

29. Gilbert BE, Newman R, Zamboni WC, Knight V, Verschraegen CF. Pharmacokinetics of multiple 9-nitro-camptothecin (9NC) liposome aerosols during a Phase I study: levels of total and lactone forms, and its conversion to 9-amino-camptothecin (9AC). Proc Am Soc Clin Onc 2001;20:90b.

30. Gounder M, Saleem A, Roychowdhury M. Metabolism of 9-nitrocamptothecin (RFS2000/9NC) to 9-aminocamptothecin (9AC) in patients and in vitro. Proc Am Assoc Cancer Res 2002;42.

31. Pantazis P, Harris N, Mendoza J, Giovanella B. The role of pH and serum albumin in the metabolic conversion of 9-nitrocamptothecin to 9-aminocamptothecin by human hematopoietic and other cells. Eur J Haematol 1995;55:211.

32. Hinz HR, Harris NJ, Natelson EA, Giovanella BC. Pharmacokinetics of the in vivo and in vitro conversion of 9-nitro-20(S)-camptothecin to 9-amino-20(S)-camptothecin in humans, dogs, and mice. Cancer Res 1994;54:3096.

33. Schoemaker NE, Mathot RAA, Schoffski P, Rosing H, Schellens JHM, Beijnen JH. Development of an optimal pharmacokinetic sampling schedule for rubitecan administered orally in a daily times five schedule. Cancer Chemother Pharmacol 2002;50:514-517.

34. Raymond D, Campone M, Stupp R, Menten J, Chollet P, Lesimple T, Fety-Deporte R, Lacombe D, Paoletti X, Fumoleau P. Multicentre phase II and pharmacokinetic study of RFS2000 (9-nitrocamptothecin) administered orally 5 days a week in patients with glioblastoma multiforme. European Journal of Cancer 2002;38:1348-1350.

5.0

CHAPTER 5:

Clinical and Pharmacokinetic Study Evaluating the Effect of Food on the Disposition of 9-Nitrocamptothecin and its 9-Aminocamptothecin Metabolite in Patients with Solid Tumors

5.1 ABSTRACT

Purpose: 9-nitrocamptothecin (NC) is an orally administered camptothecin analogue that has completed phase III trials for pancreatic cancer. In biological matrices, camptothecin analogues exist in equilibrium between the active-lactone (LAC) and inactive-hydroxy acid (HA) forms. 9NC has been administered on an empty stomach; however it is unclear if food alters the absorption and disposition of 9NC and its 9-aminocamptothecin (9AC) active-metabolite. Thus, we evaluated the disposition of 9NC and 9AC after administration of 9NC under fasting conditions and after a standard meal.

Methods: Patients were randomized to receive 9NC as a single oral dose at 1.5 mg/m^2 with 8 ounces of an acidic beverage under fasting conditions, or after a meal consisting of 2 eggs, 8 ounces (oz) of orange juice, buttered toast, 8 oz of milk, and 4 oz of hash brown potatoes. Following a 72-h washout period, 9NC was administered with the alternative condition (i.e., with food or fasting). 9NC was then continued for five days of every week. Serial blood samples were obtained prior to and from 0.25 h to 24 h after administration of 9NC. 9NC and 9AC total (sum of LAC + HA) were measured by an LC-MS/MS assay. Area under the plasma concentration versus time curve (AUC) for 9NC and 9AC total were calculated. After the pharmacokinetic section of the study, patients received 9NC 1.5 mg/m^2 orally under fasting conditions daily for 5 days per week for 8 weeks.

Results: Sixteen patients with median (range) age 62 (47-83) years, diagnoses of colorectal (6 patients), lung (2 patients), and other (8 patients) malignancies, received 83 [median (range) 4 (2-9)] weeks of therapy. Patients with toxicities greater than grade 2 were diarrhea (1), nausea (2), vomiting (2), fatigue (2), anemia (3), neutropenia (3), and

febrile neutropenia (2). Three patients (lung, unknown primary, and colon) had stable disease for eight weeks. The mean ± SD of 9NC AUC_{food} and 9NC AUC_{fast} were 300 ± 182 and 569 ± 362 ng/ml·h, respectively ($P < 0.05$). The mean ± SD of 9AC AUC_{food} and 9AC AUC_{fast} were 244 ± 60 and 291 ± 195 ng/ml·h, respectively ($P > 0.05$). The mean ± SD ratio of 9NC AUC_{food} to $AUC_{fasting}$ in individual patients was 0.67 ± 0.22. The mean ± SD ratio of 9AC AUC_{food} to $AUC_{fasting}$ in individual patients was 1.14 ± 0.61.

Conclusions: Co-administration of 9NC with food reduces the oral absorption of 9NC; however there was no difference in the exposure of 9AC. The effect of food on the absorption of 9NC is highly variable and is less consistent for 9AC compared to 9NC.

5.2 INTRODUCTION

The camptothecin analogues are topoisomerase I-interactive anticancer agents (16, 5, 19). 9-Nitrocamptothecin (9NC) is an orally administered camptothecin analogue (21, 35, 48). *In vitro* and *in vivo* preclinical studies suggest protracted administration of low doses of camptothecin analogues produce greater anti-tumor activity than does less frequent administration of higher doses (9, 49). Consistent with the mechanism of action of camptothecin analogues being cell-cycle-specific, prolonged exposures may be more effective than shorter exposures (17, 7, 15). Daily oral administration of 9NC may mimic a protracted parenteral schedule, achieve prolonged exposure, and maximize patient convenience. However, oral administration of camptothecin analogues, has been characterized by extensive inter-and intra-patient variability in bioavailability and may be clinically significant (51, 12, 8, 24, 23).

9NC is partially converted to a 9-aminocamptothecin (9AC) active-metabolite (9, 51, 1). 9NC and 9AC both occur in equilibrium between the active-lactone and inactive-hydroxy acid forms (28, 29, 44). In our previous pharmacokinetic studies of 9NC and its 9AC metabolite, we found at any given dose of 9NC there was 4 to 16-fold variability in 9NC and 9AC exposure (33). The method of dose calculation (i.e., mg versus mg/m^2) did not reduce the variability in drug exposure. It is currently unclear whether the pharmacokinetic variability of 9NC and 9AC is due to variable gastrointestinal absorption, hepatic metabolism, and/or biliary elimination.

As is standard for orally administered drugs, the effect of food on the oral absorption and bioavailability of drugs should be evaluated (51, 12, 8, 24, 23). Schoffski and colleagues evaluated the effect of food intake on the bioavailability of orally

administered 9NC in a randomized crossover study in which patients were administered 9NC with and without food (43). Because administration of 9NC with food resulted in a 50% reduction in the exposure of 9NC, the authors concluded that 9NC should be administered under fasting conditions. However, the study did not evaluate the disposition of 9AC which has ranged from 20% to 200% of the 9NC exposure in patients. Thus, we evaluated the disposition of 9NC and 9AC after administration of 9NC with and without a standard meal in a randomized cross over study.

5.3 PATIENTS AND METHODS

Patient Selection

Eligible patients were required to have an advanced solid malignancy confirmed on histology and refractory to standard treatment; age greater than or equal to 18 years; measurable or evaluable disease; ECOG performance status (PS) $\leq$ 2; and adequate organ function as defined by absolute neutrophil count (ANC) $\geq$1500/mm^3, platelet count $\geq$100,000/mm^3, hemoglobin > 9 gm/dl; creatinine clearance $\geq$ 50 ml/minute; serum bilirubin within 1.5 X institutional normal limits (INL); and ALT and AST $\leq$ 3 times INL. In addition, patients were required to be able to swallow oral medication, and have adequate venous access to permit repeat blood sampling. Patients with malabsorption syndrome or gastric or small bowel resection were excluded, as were pregnant or nursing women. Patients of childbearing age were required to practice contraception. Patients were required to have at least a 4-week interval from all previous surgery, chemotherapy, and radiotherapy prior to study initiation. All patients gave written informed consent approved by the Institutional Review Boards at the Albert Einstein College of Medicine (AECOM) and Montefiore Medical Center (MMC).

Study Design

This trial was a two-period crossover design with patients randomized to receive the study medication either in the "fasting" or the "fed" state (period 1). They received one dose of the drug on the first day. After a 72-hour washout, the patients were crossed over to the other arm (period 2). After a repeat washout period of at least 72 hours, patients were entered on to period 3 of the study. This entailed ingestion of 9NC for 5 days on a weekly basis for a period of eight weeks. It was an open-label, single-arm trial conducted at the AECOM and MMC from December 2002 to May 2004.

Study Drugs and Administration

9NC was supplied by Supergen, Inc (Dublin, CA) as 1.25 and 0.5 mg tablets. The drug was stored in the outpatient pharmacy in a secure, limited access area at 15-25°C (59-77ºF) in a dry place protected from light. The dose was calculated as 1.5 mg/m^2 and rounded to the closest multiple of 0.25 mg. 9NC was administered by the study staff under supervision during periods 1 and 2. The standard meal consisted of 2 eggs, 8 ounces (oz) of orange juice, buttered toast, 8 oz of milk, and 4 oz of hash brown potatoes. During period 3, the patient was given a five-day supply each week and the drug was self administered at home. Each dose was administered with six to eight oz of an acidic beverage, such as orange juice (51). During the "fasting" period and in period 3, the dose of 9-NC was preceded and followed by a 2-hour fasting period. During the "fed" period, the 9-NC was swallowed within 5 minutes of ingestion of the standard meal. The study drug administration was repeated every 7 days, until disease progression or development of intolerable toxicities. Objective disease evaluations were performed at the end of eight weeks of period 3.

Patient Evaluation and Follow-up

All patients underwent a complete medical history, physical examination, and performance status evaluation within 2 weeks of entry into the study, prior to drug administration for periods 1 and 2, and at the start of every week of therapy during period 3. Complete blood count with differential (CBC), a complete chemistry profile, and a urinalysis were obtained with each physical examination. Imaging studies of chest, abdomen and pelvis were performed as indicated for tumor measurements within 4 weeks of initiation of study drugs. Toxicity assessments were performed weekly. Tumor assessment was performed after every eight weeks of weekly therapy using the World Health Organization criteria.

A complete response (CR) was defined as disappearance of all measurable lesions at two examinations at least 4 weeks apart. Partial response (PR), stable disease (SD), and progressive disease (PD) were defined as $\geq$50% decrease, <25% decrease or <25% increase, and $\geq$25% increase in the sum of bi-dimensional measurements of tumor burden, respectively. Appearance of any new lesion constituted disease progression. Response duration was the time from documentation of first response to the first date of objective progression of disease. After removal from study, all patients underwent follow-up examinations and toxicity assessments every 2 months until death.

Study Endpoints and Dose Modifications

The primary and secondary endpoints of the study were to determine the effect of food on the oral absorption of 9NC and 9AC and to characterize toxicities, respectively. The starting dose was 1.5 mg/m^2 for all patients and for all periods 1-3. If a patient tolerated the first four weeks of study drug without any grade 2 toxicities, the dose could be escalated to 2 mg/m^2 for all subsequent administrations. Appearance of grade 2 thrombocytopenia, grade 3 neutropenia, grade 3 non-hematologic toxicity, or grades1-2 chemical cystitis, necessitated dose reduction by one level, i.e. 1.5 mg/m^2 administered

for 4 days every week. Appearance of grade 3 thrombocytopenia, grade 4 neutropenia, grade 4 non-hematologic toxicity, or grade 3 chemical cystitis, necessitated dose reduction by 2 levels, i.e., 1 mg/m^2 administered for 4 days every week. Grade 4 chemical cystitis required discontinuation of protocol therapy. All toxicities were graded based on the Expanded NCI CTC version 2.0.

Sample Collection and Preparation

Blood samples (5 mL) were obtained after administration of 9NC, on an empty stomach or with a standard meal, prior to administration and at 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, and 24 h after administration. Blood was placed into heparinized tubes and immediately centrifuged at 1200 x g at 4ºC for 5 min. The resulting plasma was processed via methanolic extraction to measure 9NC and 9AC total as previously described (51, 8, 33).

Mass Spectrometric Assay

In previous studies, 9NC was measured indirectly by conversion to 9AC via a logistically difficult iron-reduction process [33, 47]. To overcome these issues, we developed a highly sensitive and specific HPLC-tandem mass spectrometric assay to simultaneously and directly quantitate 9NC and 9AC total concentrations in human plasma. Our assay was modified from the assay developed by Xenobiotics Laboratories, Inc. (Plainsboro, NJ) (32).

Chromatography was performed on a Waters Alliance 2695 system (Milford, MA, USA) and a Phenomenex Luna C_{18} (5 μm, 150 x 2 mm) analytical column (Torrance, CA). The isocratic mobile phase, consisting of 0.1% formic acid in acetonitrile: 50 μM

ammonium acetate buffer (40:60, v/v), was pumped at 0.2 mL/min, and the run time was 7 min. Mass detection was carried out using a Waters Quattro micro triple-stage, bench top quadrupole mass spectrometer with electrospray ionization in positive-ion, multiple reaction mode (MRM). The settings of the mass spectrometer were as follows: capillary voltage 4 kV; cone voltage 40 V; source temperature 120°C; and desolvation temperature 450°C. The cone and desolvation gas flows were 110 and 550 L/h, respectively. The collision voltage was 30 V. 9NC, 9AC, and camptothecin ions monitored in MRM scans were m/z 394 > 350, 364 > 320, and 349 > 304, respectively. The LC system and mass spectrometer were controlled by Waters Mass Lynx software (version 4.0), and data were collected with the same software. 9NC, 9AC, and camptothecin eluted at 5.04, 2.70, and 3.83 min, respectively. The assay was linear from 0.5 ng/mL to 1000 ng/mL for both 9NC and 9AC.

Pharmacokinetic Analysis

Compartmental pharmacokinetic analysis of 9NC and 9AC was performed using ADAPT II (6). The estimation procedure and variance model used in the compartmental pharmacokinetic analysis was maximum likelihood estimation and linear models for the variance of the additive errors, respectively. Different pharmacokinetic model structures were considered to characterize the disposition of 9NC and 9AC in plasma. In the model development, one- and two-compartments models were evaluated to describe the systemic disposition of 9NC and 9AC. In addition, we evaluated the use of single and separate apparent volumes of the central compartments for 9NC and 9AC. Akaike's Information Criteria (AIC), estimated error of the model parameters, and residual

analysis were used to select the model structure maximizing the fit accuracy while simultaneously minimizing the number of model parameters. The final model structure used for the pharmacokinetic analysis produced identifiable parameters in all patients as described below.

A linear PK model describing oral administration of 9NC was simultaneously fit to 9NC and 9AC concentration versus time profiles. The model contained one-compartment for 9NC systemic disposition, subsequent conversion of 9NC to 9AC, and one-compartment for 9AC systemic disposition. Individual parameters estimated were the absorption rate constant (k_a), the lag time prior to absorption (τ), the apparent volume of the central compartment (V_c/F), the rate constant describing conversion of 9NC to 9AC (k_{12}), and the elimination rate constants for 9NC (k_{10}) and 9AC (k_{20}). The apparent clearance of 9NC (9NC CL/F) and 9AC (9AC CL/F) total were calculated using standard equations [i.e. $V_c/F \times (k_{10} + k_{12})$ and $V_c/F \times k_{20}$, respectively] [6, 40]. The area under the 9NC and 9AC plasma concentration versus time curves (9NC $AUC_{0\text{-}24\ h}$ and 9AC $AUC_{0\text{-}24\ h}$) from zero to 24 hours were calculated using the log trapezoidal method by simulating the concentration versus time data from each patient using patient-specific parameters (6). Effect of food on the disposition of 9NC and 9AC was evaluated as the ratio of AUC with food (AUC_{food}) to AUC without food ($AUC_{fasting}$).

Statistical Analysis

Statistical analysis was performed comparing all parameters for 9NC and 9AC after administration with and without food. The mean and standard deviation for all parameters were calculated and the values were evaluated for normalcy. The log of the

values were used for the non-parametric Wilcoxon signed rank test. All analysis was performed using the SPSS version 10.0 (Chicago, IL).

5.4 RESULTS

Patient characteristics

Sixteen patients with a median (range) age of 62 (29-85) years and ECOG PS of 1 (15 pts) or 2 (1 pt) were enrolled onto this trial (Table 1). All patients had received prior treatment with 3(1-4) [median (range)] chemotherapy regimens. The primary sites included patients with colorectal (n = 6), lung (n = 2), and others (n = 8).

Table 1: Patient Characteristics

Patient characteristics	# of patients
Patients enrolled	16
Males	5
Females	11
Age - median (range), years	62 (29-85)
ECOG performance status	
1	15
2	1
# Prior chemotherapy regimens	
1	2
2	3
3	8
4	3
Primary site	
Colorectal	6
NSCLC	2
Others*	8
Evaluable for PK	12

*Other sites (8): pancreas (1), esophageal (1), ovarian (1), cervix (1), endometrial (1), adenocarcinoma of unknown primary (1), thyroid (1) and head and neck (1)
Abbreviation: ECOG, Eastern Cooperative Oncology Group; #, number; PK, pharmacokinetics

Pharmacokinetic studies were performed in 16 patients. Samples from the first five patients were inadequate for analysis due to a storage malfunction. One patient had pharmacokinetic studies performed after administration of 9NC with food and then withdrew from the study prior to the crossover to administration of 9NC without food. One patient only had blood samples obtained from 0 to 6 h after administration of 9NC with and without food and thus an AUC from 0 to 24 h could not be determined. In 2 patients administered 9NC with food, AUC from 0 to 24 h could not be estimated due to a limited number of samples from 1 to 6 h after administration. Thus, complete sets of plasma samples were available for 9 and 11 patients after administration of 9NC with food and without food, respectively.

Pharmacokinetics

Pharmacokinetics studies of 9NC and 9AC were available for 9 and 11 patients after administration of 9NC with food and without food, respectively. 9NC and 9AC pharmacokinetic parameters after administration of 9NC with a standard meal and under fasting conditions are presented in Table 2. 9NC and 9AC AUCs after administration of 9NC with food and under fasting conditions are presented in Figure 1 and Figure 2. The ratio of 9AC AUC to 9NC AUC after administration of 9NC with food and under fasting conditions is presented in Figure 3.

The mean ± SD of the ratio of 9NC AUC_{food} to 9NC $AUC_{fasting}$ in individual patients was 0.67 ± 0.22. The mean ± SD of the ratio of 9AC AUC_{food} to 9AC $AUC_{fasting}$ in individual patients was 1.14 ± 0.61. The mean ± SD of the ratio of 9AC AUC_{food} / $AUC_{fasting}$ to 9NC AUC_{food} / $AUC_{fasting}$ in individual patients was 1.80 ± 0.99.

Table 2. 9NC and 9AC Pharmacokinetic Parameters after Administration of 9NC with Food and Under Fasting Conditions

Parameters	Units	Food Mean ± SD (n = 9)	Fasting Mean ± SD (n = 11)
9NC			
k_a	h^{-1}	0.12 ± 0.11[a]	1.45 ± 1.25[a]
τ	h	0.26 ± 0.31	0.14 ± 0.27
k_{13}	h^{-1}	0.21 ± 0.17	0.12 ± 0.18
k_e	h^{-1}	0.23 ± 0.38	0.26 ± 212
Vd	L	16.0 ± 12.5	26.7 ± 23.9
CL/F	$L/h/m^2$	2.1 ± 1.9	1.6 ± 1.3
C_{max}	ng/mL	28.3 ± 14.1[a]	54.7 ± 27.2[a]
T_{max}	h	5.47 ± 1.45[a]	3.61 ± 2.95[a]
AUC_{0-24}	ng/ml·h	330 ± 182[a]	569 ± 362[a]
9AC			
k_{30}	h^{-1}	0.33 ± 0.56	0.11 ± 0.11
CL/F	$L/h/m^2$	2.2 ± 1.6	2.6 ± 2.5
C_{max}	ng/mL	15.1 ± 5.6	18.8 ± 11.9
T_{max}	h	13.3 ± 6.4	12.3 ± 6.6
AUC_{0-24}	ng/ml·h	244 ± 60	291 ± 195

[a] Values for 9NC administered under fasting conditions are significantly different ($P < 0.05$) than for 9NC administered with food.

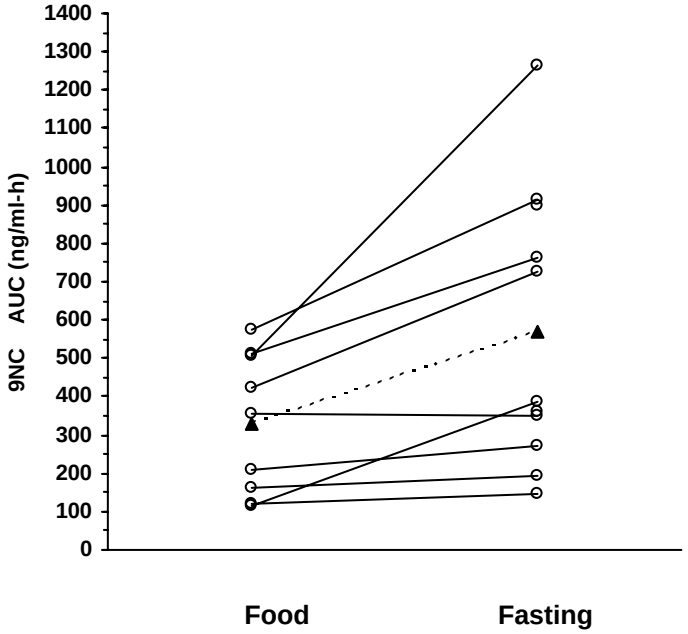


Figure 1. 9NC AUCs in individual patients when 9NC was administered with food (n = 9) and under fasting conditions (n = 11). Individual data points are presented by ○ and data within a single patient are connected by a solid line. The mean value for each group is presented by ▲ and connected by a dashed line.

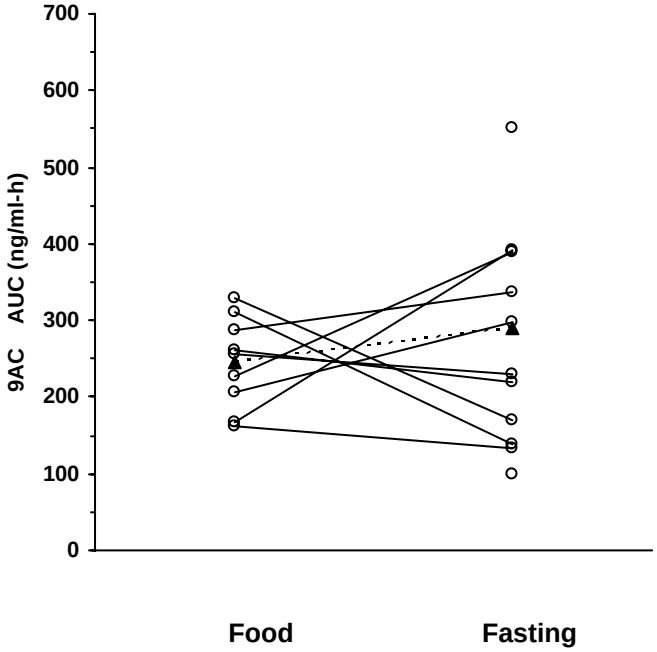


Figure 2. 9AC AUCs in individual patients when 9NC was administered with food (n = 9) and under fasting conditions (n = 11). Individual data points are presented by ○ and data within a single patient are connected by a solid line. The mean value for each group is presented by ▲ and connected by a dashed line.

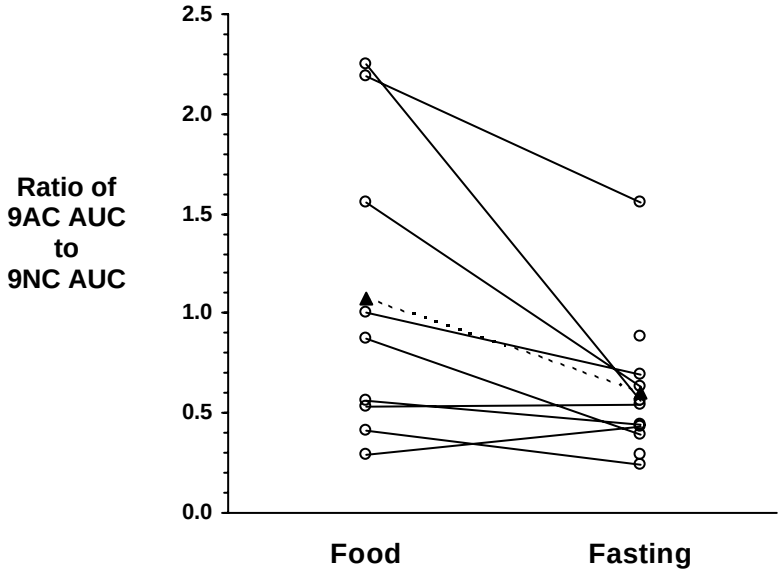


Figure 3. The ratio of 9AC AUC to 9NC AUC patients when 9NC was administered with food (n = 9) and under fasting conditions (n = 11). Individual data points are presented by ○ and data within a single patient are connected by a solid line. The mean value for each group is presented by ▲ and connected by a dashed line.

Toxicities

Overall, non-hematologic toxicity was more common than hematologic toxicity (Table 3 and Table 4). The most common non-hematologic toxicity observed was nausea, which occurred in 8 patients. Other grade 2 toxicities included vomiting, diarrhea, fatigue, urinary infection (UTI), and anorexia. Skin rash was observed in 2 patients and resulted in interruption of therapy for a week until the spontaneous resolution of the rash. This was a diffuse, macular-papular rash, more prominent on the trunk and the back, associated with itching, and resolved within 1-2 weeks, and did not reappear with the reintroduction of the drug at a lower dose. Also observed were renal changes as evidenced by hematuria, proteinurea, dysuria and UTI. One patient, a 64-year woman with metastatic colon cancer, had grade 3 diabetic nephropathy-induced proteinurea at baseline, which did not appear to worsen during administration of 9NC.

Table 3. Non-hematologic toxicities (by number of patients)

Toxicity (CTC)	Grade 1	Grade 2	Grade 3	Grade 4
Nausea	5	1	2	
Vomiting	2		2	
Diarrhea	1	3	2	
Fatigue	5	2	2	
Anorexia	3		1	
Mucositis		1		
Dysuria		2		
Proteinuria	4			
Hematuria	4	3		
UTI		2	1	
Skin rash		2		

Abbreviation: UTI, urinary tract infection; CTC, common toxicity criteria version 2.0

Table 4. Hematologic toxicities (by number of patients)

Toxicity (CTC)	Grade 1	Grade 2	Grade 3	Grade 4
Neutropenia	2	1	1	2
Febrile neutropenia			2	
Anemia	3	6	3	
Thrombocytopenia	3	1		

Abbreviation: CTC, common toxicity criteria version 2.0

Grade 3-4 neutropenia was observed in 3 patients (Table 4). Two patients experienced febrile neutropenia. Both patients recovered with appropriate antibiotics and without any sequelae. The median (interquartile range) of the nadir neutrophil count was 2400/mm^3 (1200-3600/mm^3). One patient experienced grade 2 thrombocytopenia. Grade 3 anemia, requiring transfusion, occurred in 3 patients. One of these patients was a

patient with metastatic colon cancer that developed episodes of gastrointestinal bleeding which contributed to the anemia.

Antitumor Response

Although response evaluation was not the primary end point of this study, 13 patients were evaluable for objective response. Of the 3 patients not evaluable for response, one was withdrawn from study after only 4 weeks of therapy because of cystitis, and 2 patients withdrew consent and did not complete the planned 8 weeks of therapy. There was no objective tumor response observed. Three patients (lung, unknown primary, and colon) had stable disease for a period of 8 weeks.

5.5 DISCUSSION

Previous studies evaluated the oral bioavailability of camptothecin analogues administered with and without food (24, 23, 31, 22, 13). Schoffski and colleagues reported that the oral bioavailability of 9NC was found to be strongly dependent on the timing of food intake (43). The plasma exposure of 9NC was approximately half when administered with food as compared to fasting conditions. However, that study did not evaluate the disposition of 9AC, the major metabolite of 9NC, which has ranged from 20% to 200% of the 9NC exposure in patients (33, 10, 34, 13). Thus, our study is the first report evaluating the disposition of 9NC and 9AC when administered with and without food. In our study, food inhibited the oral absorption of 9NC with a mean ± SD ratio of 9NC AUC_{food} to 9NC $AUC_{fasting}$ of 0.67 ± 0.22. Administration of 9NC with food did not alter the exposure of 9AC with a mean ± SD ratio of 9AC AUC_{food} to 9AC

$AUC_{fasting}$ of 1.14 ± 0.61. However, the effect of food on the absorption of 9NC is highly variable and is less consistent for 9AC compared to 9NC. The mechanism associated with differential effects of food on the exposure of 9NC and 9AC are currently unclear. One potential explanation is that 9NC is converted to 9AC in the gastrointestinal tract, in addition to the liver, and food does not alter the absorption of 9AC (10, 34, 11). The differential effects of food on 9NC and 9AC is significant because the development of 9AC was stopped due to lack of efficacy and significant toxicity in clinical trials (21, 36, 50). Thus, to maximize the exposure of 9NC relative to 9AC, 9NC should be administered orally without food (38).

The results from our study are similar to previous studies evaluating the pharmacokinetics of 9NC when administered under fasting conditions (43, 10, 34, 14, 42, 38). For example, the k_a in our study and the study by Schoemaker et.al. were 1.4 ± 1.2 h^{-1} and 0.8 h^{-1}, respectively (42). In addition, the CL/F of 9NC in our study and the study by Schoemaker et.al. were 1.6 ± 1.4 L/h/m^2 and 1.7 L/h, respectively. In the study by Raymond et.al., in which 9NC was administered at 1.5 mg/m^2 x 1, the 9NC and 9AC $AUC_{0\text{-}24\ h}$ were 231 ± 137 ng/mL·hr and 36.9 ± 28.5 ng/mL·hr, respectively, which are similar to the 9NC and 9AC $AUC_{0\text{-}24\ h}$ in this current study and our prior phase I study after administration of 9NC at 2.0 mg/m^2 (38). In addition, the concentration versus time profiles of 9NC and 9AC were representative of delayed absorption, and the 9AC concentrations increase over the 24-hour period.

9NC is an orally administered camptothecin analogue with significant interpatient variability in drug disposition (33, 34, 14, 42, 38). The interpatient variability in 9NC was similar when administered with (8.0-fold) and without food (6.2-fold). In addition,

the interpatient variability in 9AC was similar when administered with (3.9-fold) and without (6.4-fold) food. Thus, there is still a need to identify factors responsible for the variability and develop techniques to reduce the interpatient variability in the exposure of 9NC and 9AC (23, 33, 43,20). The interpatient variability in the exposure of 9NC and 9AC may be explained by variability in the expression and function of the ATP-binding cassette (ABC) transporters (23, 46,37,30,26,3,18). The ABC transporters are membrane proteins that modulate a wide variety of substrates, including metabolic products, lipids and sterols, and drugs, across extra-and intracellular membranes, such as the gastrointestinal tract (25,45). ABC transporters have been reported to modulate camptothecin analogues and are associated with camptothecin resistance in cancer cell lines (46,37,30,26,3,18). Fumitremorgin C, a potent and specific inhibitor of ABCG2, has been evaluated as a way to increase the absolute oral bioavailability and reduce interpatient variability in oral absorption of camptothecins analogues (2). Future studies evaluating the effects of fumitremorgin C on 9NC should be performed.

5.6 REFERENCES

1. Akhtar S, Beckman RA, Mould DR, Doyle E, Fields SZ, Wright J. Pretreatment with ranitidine does not reduce the bioavailability of orally administered topotecan. Cancer Chemother Pharmacol 2000;46:204.
2. Allen JD, van Loevezijn A, Lakhai JM, van der Valk M, van Tellingen C, Reid G, Schellens JH, Koomen GJ, Schinkel AH. Potent and specific inhibition of the breast cancer resistance protein multidrug transporter in vitro and in mouse intestine by a novel analogue of fumitremorgin C. Mol Cancer Ther 2002;1(6):417-425.
3. Bates SE, Medina Perez W, Kohlhagen G, Pommier Y. BCRP/MXR/ABCG2-mediated resistance to homocamptothecins. Proc Am Assoc Cancer Res 2003;44:113,#595.
4. Chasseaud LF, Taylor T. Bioavailability of drugs from formulations after oral administration. Ann Reviews 1974;6579:35-46.
5. Covey JM, Jaxel C, Kohn KW, Pommier Y. Protein-linked DNA strand breaks induced in mammalian cells by camptothecin, an inhibitor of topoisomerase I. Cancer Res 1989;49:5016.
6. D'Argenio DZ, Schmuitzky A. ADAPT II user's guide: biomedical simualtions resouce. 1990.
7. Del Bino G, Darzynkiewicz Z. Camptothecin, teniposide, or 4'-(9-acridinylamino)-3-methanesulfon-m-anisidide, but not mitoxantrone or

doxorubicin, induces degradation of nuclear DNA in the S phase of HL-60 cells. Cancer Res 1991;51:1165.

8. Drengler RL, Kuhn JG, Schaaf LJ, Rodriguez GI, Villalona-Calero MA, Hammond LA, Stephenson JA, Jr., Hodges S, Kraynak MA, Staton BA, Elfring GL, Locker PK, Miller LL, Von Hoff DD, Rothenberg ML. Phase I and pharmacokinetic trial of oral irinotecan administered daily for 5 days every 3 weeks in patients with solid tumors. J Clin Oncol 1999;17:685.

9. Furman WL, Stewart CF, Poquette CA, Pratt CB, Santana VM, Zamboni WC, Bowman LC, Ma MK, Hoffer FA, Meyer WH, Pappo AS, Walter AW, Houghton PJ. Direct translation of a protracted irinotecan schedule from a xenograft model to a phase I trial in children. J Clin Oncol 1999;17:1815.

10. Gounder M, Saleem A, Roychowdhury M. Metabolism of 9-nitrocamptothecin (RFS2000/9NC) to 9-aminocamptothecin (9AC) in patients and in vitro. Proc Am Assoc Cancer Res 2002;42:#1554.

11. Gounder MK, Sun SL, Sands H, Lin Y, Shih WJ, Gu Z, Charles-Williams S, Roychowdhury M, Rajendra R, Rubin EH. Development of a bioanalytical liquid chromatography method for quantitation in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 2004;799(1):63-72.

12. Gupta E, Luo F, Lallo A, Ramanathan S, Vyas V, Rubin E, Sinko P. The intestinal absorption of camptothecin, a highly lipophilic drug, across Caco-2 cells is mediated by active transporter(s). Anticancer Res 2000;20:1013.

13. Herben VM, Rosing H, ten Bokkel Huinink WW, van Zomeren DM, Batchelor D, Doyle E, Beusenberg FD, Beijen JH, Schellens JH. Oral topotecan:

bioavailability and effect of food co-administration. Br J Cancer 1999;80(9):1380-1386.

14. Hinz HR, Harris NJ, Natelson EA, Giovanella BC. Pharmacokinetics of the in vivo and in vitro conversion of 9-nitro-20(S)-camptothecin to 9-amino-20(S)-camptothecin in humans, dogs, and mice. Cancer Res 1994;54:3096.

15. Houghton PJ, Cheshire PJ, Hallman JD, Lutz L, Friedman HS, Danks MK, Houghton JA. Efficacy of topoisomerase I inhibitors, topotecan and irinotecan, administered at low dose levels in protracted schedules to mice bearing xenografts of human tumors. Cancer Chemother Pharmacol 1995;36:393.

16. Hsiang YH, Hertzberg R, Hecht S, Liu LF. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J Biol Chem 1985;260:14873.

17. Hsiang YH, Lihou MG, Liu LF. Arrest of replication forks by drug-stabilized topoisomerase I-DNA cleavable complexes as a mechanism of cell killing by camptothecin. Cancer Res 1989;49:5077.

18. Iida A, Saito S, Sekine A, Mishima C, Kitamura Y, Kondo K. Catalog of 605 single-nucleotide polymorphisms (SNPs) among 13 genes encoding human ATP-binding cassette transporters: ABCA4, ABCA7, ABCA8, ABCD1, ABCD3, ABCD4, ABCE1, ABCF1, ABCG1, ABCG2, ABCG4, ABCG5, and ABCG8. J Hum Genet 2002;47:285-310.

19. Jaxel C, Kohn KW, Wani MC, Wall ME, Pommier Y. Structure-activity study of the actions of camptothecin derivatives on mammalian topoisomerase I: evidence

for a specific receptor site and a relation to antitumor activity. Cancer Res 1989;49:1465.

20. Jung, LL, Zamboni, WC. Cellular, pharmacokinetic, and pharmacodynamic aspects of response to camptothecins: can we improve it? Drug Resist Update 2001;4: 273-288.

21. Kirstein MN, Houghton PJ, Cheshire PJ, Richmond LB, Smith AK, Hanna SK, Stewart CF. Relation between 9-aminocamptothecin systemic exposure and tumor response in human solid tumor xenografts. Clin Cancer Res 2001;7:358.

22. Kuppens IE, Beijnen J, and Schellens JH. Topoisomerase I inhibitors in the treatment of gastrointestinal cancer: from intravenous to oral administration. Clin Colorectal Cancer 2004;4(3):163-180.

23. Kruijtzer CM, Beijnen JH, Rosing H, Bokkel Huinink WW, Schot M, Jewell RC, Paul EM, Schellens JH. Increased oral bioavailability of topotecan in combination with the breast cancer resistance protein and P-glycoprotein inhibitor GF120918. J Clin Oncol 2002;20:2943.

24. Loos WJ, Gelderblom H, Sparreboom A, Verweij J, de Jonge MJ. Inter- and intrapatient variability in oral topotecan pharmacokinetics: implications for body-surface area dosage regimens. Clin Cancer Res 2000;6:2685.

25. Lockhart AC, Tirona RG, Kim RB. Pharmacogenetics of ATP-binding cassette transporters in cancer and chemotherapy. Mol Cancer Ther 2003;2:685-698.

26. Maliepaard M, van Gastelen MA, de Jong LA, Pluim D, van Waardenburg RC, Ruevekamp-Helmers MC. Overexpression of the BCRP/MXR/ABCP gene in a topotecan-selected ovarian tumor cell line. Cancer Res 1999;59:4559-4563.

27. Martinez MN, Amidon GL. A mechanistic approach to understanding the factors affecting drug absorption: a review of fundamentals. J Clin Pharmacol 2002;42(6):620-643.

28. Mi Z, Burke TG. Differential interactions of camptothecin lactone and carboxylate forms with human blood components. Biochemistry 1994;33:10325.

29. Mi Z, Burke TG. Marked interspecies variations concerning the interactions of camptothecin with serum albumins: a frequency-domain fluorescence spectroscopic study. Biochemistry 1994;33:12540.

30. Nakatomi K, Yoshikawa M, Oka M, Ikegami Y, Hayasaka S, Sano K. Transport of 7-ethyl-10-hydroxycamptothecin (SN-38) by breast cancer resistance protein ABCG2 in human lung cancer cells. Biochem Biophys Res Commun 2001;288:827-832.

31. Natelson EA, Giovanella BC, Verschraegen CF, Fehir KM, De Ipolyi PD, Harris N, Stehlin JS. Phase I clinical and pharmacological studies of 20-(S)-camptothecin and 20-(S)-9-nitrocamptothecin as anticancer agents. Ann N Y Acad Sci 1996;803:224.

32. Gu Z, Zhou D, Wu J. Bioanalytical method for the determination of 9-nitrocamptothecin (9NC) and 9-aminocamptothecin (9AC) levels in human

plasma. XenoBiotic Laboratories, Inc., Plainsboro, NJ. Personal Communication 2003.

33. Jung LL, Ramanathan RK, Egorin MJ, Jin R, Wong MMW, Potter D, Strychor S, Sun S, Trump DL, Fakih M, Zamboni WC. Pharmacokinetic studies of 9-nitrocamptothecin on intermittent and continuous schedules in patients with advanced cancer. Cancer Chemother Pharmacol 2004;54(6):487-96.

34. Pantazis P, Harris N, Mendoza J, Giovanella B. The role of pH and serum albumin in the metabolic conversion of 9-nitrocamptothecin to 9-aminocamptothecin by human hematopoietic and other cells. Eur J Haematol 1995;55:211.

35. Pazdur R, Diaz-Canton E, Ballard WP, Bradof JE, Graham S, Arbuck SG, Abbruzzese JL, Winn R. Phase II trial of 9-aminocamptothecin administered as a 72-hour continuous infusion in metastatic colorectal carcinoma. J Clin Oncol 1997;15:2905.

36. Pitot, H.C., Knost, J.A., Mahoney, M.R., Kugler, J., Krook, J.E., Hatfield, A.K., Sargent, D.J., Goldberg, R.M. A North Central Cancer Treatment Group Phase II trial of 9-aminocamptothecin in previously untreated patients with measurable metastatic colorectal carcinoma. Cancer 2000;89:1699-1705.

37. Rajendra R, Gounder MK, Saleem A, Schellens JH, Ross DD, Bates SE. Differential effects of the breast cancer resistance protein on the cellular accumulation and cytotoxicity of 9-aminocamptothecin and 9-nitrocamptothecin. Cancer Res 2003;63:3228-33.

38. Raymond D, Campone M, Stupp R, Menten J, Chollet P, Lesimple T, Fety-Deporte R, Lacombe D, Paoletti X, Fumoleau P. Multicentre phase II and pharmacokinetic study of RFS2000 (9-nitrocamptothecin) administered orally 5 days a week in patients with glioblastoma multiforme. European Journal of Cancer 2002;38:1348-1350.

39. Reddy, GK. Efficacy of adjuvant capecitabine compared with bolus 5-fluorouracil/leucovorin regimen in dukes C colon cancer: results from the X-ACT trial. Clin Colorectal Cancer 2004;4(2):87-8.

40. Rowland M, Tozer T (eds). Clinical pharmacokinetics: concepts and applications. Lea and Febiger, Philadelphia, 1999.

41. Scheithauer W, McKendrick J, Begbie S, Borner M, Burns WI, Burris HA, Cassidy J, Jodrell D, Koralewski P, Levine EL, Marschner N, Maroun J, Garcia-Alfonso P, Tujakowski J, Van Hazel G, Wong A, Zaluski J, Twelves C. X-ACT Study Group. Oral capecitabine as an alternative to i.v. 5-fluorouracil-based adjuvant therapy for colon cancer: safety results of a randomized, phase III trial. Ann Oncol 2003;14(12):1735-43.

42. Schoemaker NE, Mathot RAA, Schoffski P, Rosing H, Schellens JHM, Beijnen JH. Development of an optimal pharmacokinetic sampling schedule for rubitecan administered orally in a daily times five schedule. Cancer Chemother Pharmacol 2002;50:514-517.

43. Schoffski P, Herr A, Vermorken JB, Van den BJ, Beijnen JH, Rosing H, Volk J, Ganser A, Adank S, Botma HJ, Wanders J. Clinical phase II study and

pharmacological evaluation of rubitecan in non-pretreated patients with metastatic colorectal cancer-significant effect of food intake on the bioavailability of the oral camptothecin analogue. Eur J Cancer 2002;38:807.

44. Schrijvers D, Highley M, De Bruyn E, Van Oosterom AT, Vermorken JB. Role of red blood cells in pharmacokinetics of chemotherapeutic agents. Anticancer Drugs 1999;10:147.

45. Sparreboom A, Danesi R, Ando Y, Chan J, Figg WD: Pharmacogenomics of ABC transporters and its role in cancer chemotherapy. Drug Resist Updat 2003;6:71-84.

46. Sparreboom A, Gelderblom H, Marsh S, Ahluwalia R, Obach R, Principe P, Twelves C, Verweij J, McLeod HL. Diflomotecan pharmacokinetics in relation to ABCG2 421C>A genotype. Clin Pharmacol Ther 2004;76(1):38-44.

47. Supko JG, Malspeis L. Liquid chromatographic analysis of 9-aminocamptothecin in plasma monitored by fluorescence induced upon post-column acidification. J Liquid Chromatograph 1992;15:3261.

48. Takimoto CH, Thomas R. The clinical development of 9-aminocamptothecin. Ann N Y Acad Sci 2000;922:224.

49. Thompson J, Zamboni WC, Cheshire PJ, Lutz L, Luo X, Li Y, Houghton JA, Stewart CF, Houghton PJ. Efficacy of systemic administration of irinotecan against neuroblastoma xenografts. Clin Cancer Res 1997;3:423.

50. Vokes, EE, Gordon, GS, Rudin, CM, Mauer, AM, Watson, S, Krauss, S, Arrieta, R, Golomb, HM, Hoffman, PC. A phase II trial of 9-aminocaptothecin (9-AC)

as a 120-h infusion in patients with non-small cell lung cancer. Invest New Drugs 2001;19: 329-333.

51. Zamboni WC, Bowman LC, Tan M, Santana VM, Houghton PJ, Meyer WH, Pratt CB, Heideman RL, Gajjar AJ, Pappo AS, Stewart CF. Interpatient variability in bioavailability of the intravenous formulation of topotecan given orally to children with recurrent solid tumors. Cancer Chemother Pharmacol 1999;43:454.

6.0

CHAPTER 6:

Disposition of 9-Nitrocamptothecin and its 9-Aminocamptothecin Metabolite

In relation to ABC Transporter Genotypes

6.1 ABSTRACT

Purpose: The source of the pharmacokinetic variability of 9-nitrocamptothecin (9NC) and its 9-aminocamptothecin (9AC) metabolite is unknown. ATP-binding cassette (ABC) transporters have been reported to modulate camptothecin analogues, are associated with camptothecin resistance, and might also affect 9NC and 9AC pharmacokinetics. The aim of this study was to evaluate the functional consequence of known single nucleotide polymorphisms in the transporter genes *ABCB1*, *ABCC2*, and *ABCG2* on the pharmacokinetic disposition of 9NC and 9AC.

Methods: Pharmacokinetic and genotyping studies were performed in 55 patients as part of two phase I studies of 9NC in patients with refractory solid tumors, a phase II study of 9NC in patients with advanced colon cancer, and a study evaluating the disposition of 9NC after administration of a single dose under fasting conditions. DNA was isolated from plasma and analyzed for variants in *ABCB1, ABCC2,* and *ABCG2* genes. The *ABCB1* 1236C>T, *ABCB1* 2677G>T/A, *ABCB1* 3435C>T, *ABCC2* 3972C>T, and *ABCG2* 421C>A variants were analyzed using Pyrosequencing.

Results: The *ABCG2* 421C>A genotype significantly affected the pharmacokinetics of 9AC. The mean 9AC lactone AUC/dose for wild-type (n = 25) and heterozygous (n = 2) patients were 14.3 ng/mL·h and 51.1 ng/mL·h, respectively (P = 0.032). The mean ± SD 9AC total AUC/dose for wild-type (n = 39) and heterozygous (n = 3) patients were 91.9 ± 78.3 ng/mL·h and 129.0 ± 90.5 ng/mL·h, respectively (P = 0.40). 9NC and 9AC disposition were not significantly influenced by variants in *ABCB1*, *ABCC2*, and *ABCG2*, and *ABCB1* and *ABCC2*, respectively (P > 0.05).

Conclusion: These findings suggest that inter-individual variability in 9AC disposition, but not 9NC, may be influenced, in part, by *ABCG2* genotype.

6.2 INTRODUCTION

Several studies have reported significant interpatient variability in the pharmacokinetics of some orally administered drugs, including camptothecin analogues (1-7). In our previous pharmacokinetic studies of 9-nitrocamptothecin (9NC, rubitecan, RFS2000) and its 9-aminocamptothecin (9AC) metabolite, we found that at any given dose of 9NC there was 4 to 16-fold variability in 9NC and 9AC exposure, and that no relationship between dose and AUC was detectable (8). The method of dose calculation (i.e., mg versus mg/m^2) did not affect the variability in 9NC and 9AC exposure after administration of 9NC at doses ranging from 1.7 to 2.7 mg/m^2. It is currently unclear whether the pharmacokinetic variability of 9NC and 9AC is due to variable gastrointestinal absorption, hepatic metabolism, and/or biliary elimination (8-10).

In our previous studies with 9NC, most of the drug remained in the 9NC form, with a mean 9NC to 9AC ratio of 4 to 1, which is consistent with the previous studies (6-8). The fact that most of the administered drug remains in the 9NC form is important because the development of 9AC was stopped due to lack of efficacy (11-13). However, our studies also demonstrated highly variable conversion of 9NC to 9AC, as 2 patients had undetectable 9AC concentrations and 4 patients had higher exposures to 9AC than to 9NC (8).

The ATP-binding cassette (ABC) transporter superfamily contains membrane proteins that transport a wide variety of substrates across extra-and intracellular membranes. These substrates include metabolic products, lipids and sterols, and drugs (14,15). Over-expression of certain ABC transporters occurs in cancer cell lines and tumors that are multidrug resistant (16-20). Phylogenetic analysis has identified 48

human ABC transporters and assigned them to seven distinct subfamilies of proteins (21). ABC transporters have been reported to alter the pharmacokinetics of camptothecin analogues and are associated with camptothecin resistance (22,23).

ABCB1 (PGP/MDR1) was the first human ABC transporter to be cloned and also to be identified as conferring an MDR phenotype to cancer cells (16-22). ABCB1 is expressed in the kidney, liver, intestine, adrenal gland, blood brain barrier and hematopoetic stem cells (20). *ABCB1* transports colchicine, etoposide, doxorubicin, and vinblastine, as well as lipids and steroids. The effects of three single nucleotide polymorphisms (SNPs) of *ABCB1* (1236C>T, 3435C>T, 2677G>A/T) have been evaluated in preclinical and clinical trials (23). *ABCC2* (MRP2/cMOAT) is expressed in canalicular cells in the liver (24,25). It functions as an important efflux pump that transports organic ions from the liver to the bile. This may especially important for drugs, such as 9NC, that undergo primarily biliary elimination (8). The *ABCG2* gene (formally known as the MXR/BCRP/ABCP) encodes a half transporter with a nucleotide-binding fold-transmembrane domain orientation (16,24). *ABCG2* is expressed in stem cells, the placenta, and the intestine (26,27). *ABCG2*-associated proteins transport mitoxantrone, methotrexate, and camptothecin analogues such as topotecan, diflomotecan, and SN-38 (16,28,29).

The aim of this study was to perform an exploratory and retrospective evaluation of the functional consequence of known SNPs in the *ABCB1*, *ABCC2*, and *ABCG2* genes on the pharmacokinetic disposition of 9NC and its 9AC metabolite. Pharmacokinetic studies of 9NC and 9AC were carried out and DNA samples were obtained in two phase I studies of 9NC in patients with refractory solid tumors, a phase II study of 9NC in

patients with advanced colon cancer, and a study evaluating the disposition of 9NC after administration of a single-dose under fasting conditions.

6.3 PATIENTS AND METHODS

Patients and Treatment

All patients were deceased prior to the initiation of the genotyping studies. Thus, the genotyping studies were approved by the Committee for Oversight of Research Involving the Dead at UPMC. For the pharmacokinetic and clinical studies written informed consent, approved by the Institutional Review board of the University of Pittsburgh Medical Center (UMPC), was obtained from all patients before they entered each study. The eligible patients for the Phase I studies and single dose pharmacokinetic study were 18 years of age or older and had a histologically or cytologically confirmed malignancy for which no curative or effective therapy was available. Other eligibility criteria included an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 and adequate bone marrow, hepatic, and renal function defined as: absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L; platelets ≥ 100 x 10^9/L; total bilirubin ≤ 1.5 x upper limit of the institutional normal (ULN) range; aspartate aminotransferase (AST) ≤ 1.5 x ULN if liver metastases were not present and ≤ 4 x ULN if liver metastases were present; and the absence of microscopic hematuria. Prior treatment with camptothecin analogues, except 9NC, was permitted.

Patients eligible for the phase II study had histologically or cytologically confirmed advanced colon carcinoma with measurable disease that had failed to respond or relapsed after receiving at least one prior 5-fluorouracil-based chemotherapy regimen

for advanced disease or had evidence of metastatic disease within 6 months of completion of adjuvant therapy. Other eligibility criteria included an ECOG performance status ≤ 2 and adequate bone marrow, hepatic, and renal function defined as: absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L; platelets ≥ 100 x 10^9/L; hemoglobin > 10 g/dl; total bilirubin ≤ 2.0 mg/dL; AST and ALT ≤ 3 x ULN if liver metastases were not present and ≤ 5 x ULN if liver metastases were present; and serum creatinine ≤ 2.0 mg/dL. Patients with hematuria and those that had previously been treated with 9NC or any other camptothecin analogue were excluded from the Phase II study.

Dosage and Administration

9NC was supplied by the manufacturer, Supergen Inc. (Dublin, CA), as a crystalline powder in hard gelatin capsules that contained active drug and lactose excipient. The capsule strengths were 0.5 mg and 1.25 mg. All doses of 9NC were rounded to the nearest 0.25 mg. 9NC was administered orally on an empty stomach with an acidic beverage (e.g. orange juice, cola) (20,21). Patients were also instructed to drink 2 to 3 L of fluid per day.

Pharmacokinetic studies of 9NC and 9AC were performed as part each study. In the phase I studies, intermittent schedules of 9NC were evaluated. The phase I study evaluating schedule A was completed prior to the initiation of schedule B. On schedule A, 9NC was administered orally at 2.0, 2.4, and 2.7 mg/m^2/d for 5 days per week for 2 consecutive weeks and repeated every 4 weeks (one cycle). On schedule B, 9NC was administered orally at 1.7 and 2.4 mg/m^2/d for 14 days and repeated every 4 weeks (one cycle). In the phase II study, 9NC was administered at 1.5 mg/m^2/d for 5 days per week

for 8 weeks (one cycle). In the single-dose study, the pharmacokinetics of 9NC and 9AC were also evaluated after administration of 9NC at 1.5 mg/m^2 under fasting conditions. For all studies, patient diaries and interviews were used as documentation of 9NC administration.

Sample Collection and Preparation.

On schedules A and B of the phase I studies and the phase II study, serial blood samples for pharmacokinetic analysis were obtained on day 1. For each pharmacokinetic study day, blood samples (5 mL) were obtained before administration of 9NC, and 0.25, 0.5, 1, 2, 3, 6, 8, and 24 h after administration. On the single-dose pharmacokinetic study, blood samples (5 mL) were obtained before administration of 9NC, and 5 min, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12 h, 24, and 48 h after administration.

In all studies, blood was collected in heparinized tubes and centrifuged within 5 min at 1200 x g at 4ºC for 5 min. In the Phase I and II studies, the resulting plasma sample was then processed immediately using solid phase extraction in order to separate the lactone and hydroxy acid forms of 9NC and 9AC as previously described (8). In the single-dose pharmacokinetic study, the plasma was processed via methanolic extraction to measure 9NC and 9AC total as previously described (6,7,8,13).

Analytical Assays

In the phase I and II studies, the 9NC and 9AC lactone and total (sum of lactone plus hydroxyl acid) plasma concentrations were measured using an HPLC assay with fluorescence detection as described previously (8). Because 9NC lactone and hydroxy

acid are not highly fluorescent, 9NC lactone and total concentrations were measured by chemically reducing 9NC to 9AC. The concentration of 9NC was calculated by subtracting the concentration of 9AC from the concentration of 9NC plus 9AC after the conversion of 9NC to 9AC using iron reduction.

The HPLC system consisted of a Waters 2695 separation module (Waters, Inc. Milford, MA), a C18 reverse column (Ultrasphere 5 μm ODS 4.6 x 250 mm, Beckman Coulter, Inc., Fullerton, CA), and a C18 guard column (Brownlee C18 7 μm, 15 x 3.2 mm, PerkinElmer Corp., Norwalk, CT). Samples were injected by an autosampler set at 4° C. The isocratic mobile phase consisted of methanol; acetonitrile; ammonium acetate (10:23:97, v/v/v), pH 5.5, at a flow rate of 1.0 mL/min. Post-column acidification (pH 2 – 3) was performed using 0.3 M trifluoroacetic acid at 0.3 mL/min (8). 9AC was detected by a Waters 474 fluorescence detector with excitation wavelength of 365 nm and emission wavelength of 440 nm, 18-nm bandwidth, gain 1000, attenuation 16, with RC filter with fast response setting. MILLENIUM 32 software (Waters, Inc.) was used for data collection and analysis. All the glassware, including the injection vials, was treated with 3% surfasil in toluene (Fisher Scientific Inc., Fair Lawn, NJ). The lower limit of quantitation (LLQ) for 9NC lactone and total was 0.5 ng/mL, and the assays were linear from 0.5 ng/mL to 100 ng/mL. The LLQ for 9AC lactone and total was 0.3 ng/mL, and the assays were linear from 0.3 ng/mL to 100 ng/mL.

9NC and 9AC total plasma concentrations from the single-dose pharmacokinetic study were simultaneously and directly quantitated using a highly sensitive and specific HPLC-tandem mass spectrometric assay (LC-MS) that we developed (30). Chromatography was performed on a Waters Alliance 2695 system (Milford, MA, USA)

and a Luna C_{18} (5 µm, 150 x 2 mm) analytical column (Phenomenex, Torrance, CA). The isocratic mobile phase, consisting of 0.1% formic acid in acetonitrile:50 µM ammonium acetate buffer (40:60, v/v), was pumped at 0.2 mL/min, and the run time was 7 min. Mass detection was carried out using a Waters Quattro micro triple-stage, bench top quadrupole mass spectrometer with electrospray ionization in positive-ion, multiple reaction mode (MRM). The settings of the mass spectrometer were as follows: capillary voltage 4 kV; cone voltage 40 V; source temperature 120°C; and desolvation temperature 450°C. The cone and desolvation gas flows were 110 and 550 L/h, respectively. The collision voltage was 30 V. 9NC, 9AC, and camptothecin internal standard ions monitored in MRM scans were *m/z* 394 > 350, 364 > 320, and 349 > 304, respectively. The LC system and mass spectrometer were controlled by Waters MassLynx software (version 4.0), and data were collected with the same software. 9NC, 9AC, and camptothecin eluted at 5.04, 2.70, and 3.83 min, respectively. The assay was linear from 0.5 ng/mL to 1000 ng/mL for both 9NC and 9AC. The concentrations of 9NC and 9AC in plasma as estimated by the HPLC and LC-MS were not directly compared in the same sample from this study. However, in a prior study the plasma concentration of 9NC and 9AC as estimated by our HPLC and an LC-MS assay developed by Xenobiotics were < 15% different for all samples.

Pharmacokinetic Analysis

Compartmental pharmacokinetic analysis of 9NC and 9AC was performed using ADAPT II (31). Parameters were estimated using maximum likelihood estimation. Different pharmacokinetic model structures were considered to characterize the

disposition of 9NC and 9AC in plasma. In the model development, one- and two-compartments models were evaluated to describe the plasma disposition of 9NC and 9AC lactone and total. In addition, we evaluated the use of single and separate apparent volumes of the central compartments for 9NC and 9AC. Akaike's Information Criteria was used to select the model. The final model structure used for the pharmacokinetic analysis produced identifiable parameters in all patients.

A linear pharmacokinetic model describing oral administration of 9NC was simultaneously fit to 9NC and 9AC plasma concentration versus time profiles (31). The model contained one-compartment for 9NC plasma disposition, subsequent conversion of 9NC to 9AC, and one-compartment for 9AC plasma disposition. The individual parameters estimated were the absorption rate constant (k_a), the lag time prior to absorption (τ), the apparent volume of the central compartment (V_c/F), the rate constant describing conversion of 9NC to 9AC (k_{12}), and the elimination rate constants for 9NC (k_{10}) and 9AC (k_{20}). The apparent clearances of 9NC (9NC CL/F) and 9AC (9AC CL/F) total and lactone were calculated using standard equations [i.e. $V_c/F \times (k_{10} + k_{12})$ and $V_c/F \times k_{20}$, respectively] (31,32). The area under the 9NC and 9AC plasma concentration versus time curves (9NC $AUC_{0\text{-}24\ h}$ and 9AC $AUC_{0\text{-}24\ h}$) from zero to 24 hours were calculated using the log trapezoidal method by simulating the concentration versus time data from each patient using patient-specific parameters (31).

Analysis of Genotypes

DNA was isolated from plasma using the QIAamp DNA Blood midi kit (Qiagen Inc, Valencia, CA, USA) following the manufacturers instructions, and analyzed for

variants in *ABCB1, ABCC2,* and *ABCG2* genes. The PCR reactions for *ABCB1* 1236C>T, *ABCB1* 2677G>T/A, *ABCB1* 3435C>T, *ABCC2* 3972C>T, and *ABCG2* 421C>A were performed using primers and conditions as previously described (33,35). Pyrosequencing for all variants was carried out using the Pyrosequencing PSQ hs96A instrument and software (Biotage, Uppsala, Sweden), as previously described (27).

Statistical Analysis

Hardy-Weinberg equilibrium was determined using HWSIM (http://krunch.med.yale.edu/hwsim). Haplotype for *ABCB1* was inferred using the Polymorphism and Haplotype Analysis Suite (34). For each of the 3 genes, association between genotype (or haplotype pair, in the case of *ABCB1*) and pharmacokinetic variables were evaluated with the exact Kruskal-Wallis test. Each genotype or haplotype pair was treated as a distinct group. The *a priori* level of significance was $P < 0.05$. Because this was an exploratory study intended to be hypothesis-generating, no corrections for multiple comparisons were made.

6.4 RESULTS

Patient Characteristics

Pharmacokinetic and genotyping studies were performed on 55 patients. Of the patients, 33 and 22 were male and female, respectively. The median (range) for age was 57 yrs (29 to 85 yrs). There were 43 Caucasians, 7 African Americans, and 5 Hispanics. There was no relationship between genotype and sex or race.

Genotype Analysis

The allele frequencies for *ABCB1* 1236C>T, *ABCC2* 3972C>T, and *ABCG2* 421C>A are presented in Table 1, and are similar to those previouly reported (35). No patient was homozygous for a mutant allele in *ABCG2*, which was expected based upon previously published frequency data (35,36). All of the observed allele frequencies were in Hardy-Weinberg equilibrium.

Table 1. Frequencies for studied variant genes and genotype-phenotype relationships

Polymorphism p>q	Allele Frequencies p	q
ABCB1 1236 C>T	0.56	0.44
ABCB1 2677 G>A/T	0.60	0.01/0.39
ABCB1 3435 C>T	0.49	0.51
ABCC2 3972 C>T	0.71	0.29
ABCG2 421 C>A	0.97	0.03

ABCB1 haplotypes for the 1236C>T, 3435C>T, and 2677G>A/T SNPs were defined using standard criteria (34). The *ABCB1* A/A haplotype consisted of 1236C/C, 3435C/C, and 2677G/G. The *ABCB1* A/B haplotype consisted of 1236C/T, 3435C/T, and 2677G/T. The *ABCB1* A/C haplotype consisted of 1236C/C, 3435C/T, and 2677G/G. The *ABCB1* B/B haplotype consisted of 1236T/T, 3435T/T, and 2677T/T. All remaining combinations were combined and listed as miscellaneous.

Functional Significance of Variant Allele

Summaries for 9NC and 9AC lactone pharmacokinetics as a function of *ABCB1* haplotype, *ABCC2* genotype and *ABCG2* genotype are presented in Table 2, Table 3, and Table 4 respectively. We also evaluated the relationship between 9NC and 9AC disposition and *ABCB1* 3435C>T genotype; however, there were no significant differences in the pharmacokinetic parameters for this SNP, and thus, only the summaries of 9NC and 9AC pharmacokinetics by *ABCB* haplotype are presented. The relationship between 9NC lactone AUC/Dose and 9NC total AUC/Dose and *ABCG2* genotype are presented in Figure 1 and Figure 2, respectively. The relationship between 9AC lactone AUC/Dose and 9AC total AUC/Dose and *ABCG2* genotype are presented in Figure 3 and Figure 4, respectively.

Among the genetic variants, only the *ABCG2* 421C>A genotype significantly affected the disposition of 9AC lactone. For *ABCG2* 421C>A, the mean 9AC lactone AUC/dose for wild-type (n = 25) and heterozygous (n = 2) patients were 14.3 ng/mL·h and 51.1 ng/mL·h, respectively (P = 0.032). For *ABCG2* 421C>A, the mean ± SD 9AC total AUC/dose for wild-type (n = 39) and heterozygous (n = 3) patients were 91.9 ± 78.3 ng/mL·h and 129.0 ± 90.5 ng/mL·h, respectively (P = 0.40). The disposition of 9AC was not significantly influenced by variants in *ABCB1* or *ABCC2* (P > 0.05). The disposition of 9NC was not significantly influenced by variants in *ABCB1*, *ABCC2*, and *ABCG2* (P > 0.05).

In the phase I study, there was no relationship between response and *ABCB1* haplotype, *ABCC2* genotype and *ABCG2* genotype or a relationship between toxicity and *ABCC2* and *ABCG2* genotypes. However, four of the five patients who experienced

dose-limiting toxicities (DLT) and also had genotyping studies performed were heterozygous for the three SNPs of *ABCB1* (1236C>T, 3435C>T, 2677G>A/T) evaluated in this study. The DLT in these four patients were grade 4 neutropenia and thrombocytopenia (n = 3) and grade 3 diarrhea (n = 1). The fifth patient who experienced DLT (grade 4 neutropenia and thrombocytopenia) was homozygous wild-type for the three SNPs of *ABCB1*.

Table 2. 9NC and 9AC pharmacokinetics as a function of *ABCB1* haplotype

Parameter	Units	Haplotype A/A (Mean ± SD)	Haplotype A/B (Mean ± SD)	Haplotype A/C (Mean ± SD)	Haplotype B/B (Mean ± SD)	Haplotype Miscellaneous (Mean ± SD)
Lactone		(n = 6)	(n = 9)	(n = 4)	(n = 5)	(n = 6)
9NC AUC/Dose	ng/mL·h / mg/m^2	148.0 ± 82.5	79.0 ± 70.5	34.5 ± 36.9	60.7 ± 84.3	61.6 ± 35.9
9NC CL/F	L/h/m^2	29.3 ± 41.1	22.8 ± 25.4	29.2 ± 26.4	36.6 ± 21.8	0.05 ± 0.03
k_a	h^{-1}	0.40 ± 0.28	0.17 ± 0.21	0.55 ± 0.87	0.17 ± 0.08	0.18 ± 0.23
k_{13}	h^{-1}	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.02	0.06 ± 0.06	0.05 ± 0.03
9AC AUC/Dose	ng/mL·h / mg/m^2	22.7 ± 4.6	22.2 ± 22.6	11.8 ± 11.4	14.8 ± 12.8	12.8 ± 12.4
9AC CL/F	L/h/m^2	1.8 ± 2.0	2.7 ± 4.1	10.4 ± 20.1	2.7 ± 3.5	6.8 ± 11.9
Total		(n = 11)	(n = 10)	(n = 5)	(n = 7)	(n = 10)
9NC AUC/Dose	ng/mL·h / mg/m^2	291.2 ± 184.2	252.4 ± 192.5	322.5 ± 328.8	238.7 ± 207.7	236.2 ± 107.0
9NC CL/F	L/h/m^2	81.9 ± 88.9	8.1 ± 8.4	5.3 ± 3.9	0.05 ± 0.03	10.0 ± 18.4
k_a	h^{-1}	0.42 ± 0.61	0.23 ± 0.27	1.1 ± 1.7	0.48 ± 0.45	0.27 ± 0.21
k_{13}	h^{-1}	0.04 ± 0.03	0.05 ± 0.05	0.04 ± 0.03	0.05 ± 0.03	0.09 ± 0.01
9AC AUC/Dose	ng/mL·h / mg/m^2	94.5 ± 67.8	100.7 ± 78.7	54.9 ± 42.3	84.6 ± 91.6	77.6 ± 40.3
9AC CL/F	L/h/m^2	1.7 ± 2.4	0.97 ± 0.79	3.4 ± 3.7	1.8 ± 1.6	2.2 ± 2.5

Table 3. 9NC and 9AC pharmacokinetics as a function of *ABCC2* genotype

Parameter	Units	Wt Homozygote (Mean ± SD)	Heterozygote (Mean ± SD)	Variant Homozygote (Mean ± SD)
Lactone		(n = 18)	(n = 14)	(n = 1)
9NC AUC/Dose	ng/mL·h / mg/m^2	77.5 ± 72.4	55.9 ± 31.7	15.6
9NC CL/F	L/h/m^2	24.6 ± 26.8	17.9 ± 17.2	63.5
k_a	h^{-1}	0.22 ± 0.25	0.31 ± 0.49	0.20
k_{13}	h^{-1}	0.04 ± 0.02	0.04 ± 0.02	0.17
9AC AUC/Dose	ng/mL·h / mg/m^2	16.2 ± 17.2	17.6 ± 11.4	30.0
9AC CL/F	L/h/m^2	3.5 ± 7.4	4.6 ± 10.9	0.13
Total		(n = 22)	(n = 14)	(n = 3)
9NC AUC/Dose	ng/mL·h / mg/m^2	226.3 ± 148.0	320.0 ± 222.1	190.6 ± 96.4
9NC CL/F	L/h/m^2	10.8 ± 16.0	7.7 ± 7.7	25.8 ± 30.8
k_a	h^{-1}	0.39 ± 0.52	0.56 ± 0.95	0.57 ± 0.65
k_{13}	h^{-1}	0.04 ± 0.04	0.04 ± 0.01	0.24 ± 0.35
9AC AUC/Dose	ng/mL·h / mg/m^2	82.5 ± 68.6	95.3 ± 74.2	109.2 ± 46.9
9AC CL/F	L/h/m^2	1.3 ± 2.0	3.2 ± 4.9	4.1 ± 3.5

Table 4. 9NC and 9AC pharmacokinetics as a function of *ABCG2* genotype

Parameter	Units	Wt Homozygote (Mean ± SD)	Heterzygote (Mean ± SD)
Lactone		(n = 26)	(n = 2)
9NC AUC/Dose	ng/mL·h / mg/m^2	58.6 ± 45.1	125.3
9NC CL/F	L/h/m^2	22.9 ± 23.3	33.3
k_a	h^{-1}	0.26 ± 0.39	0.22
k_{13}	h^{-1}	0.04 ± 0.02	0.11
9AC AUC/Dose	ng/mL·h / mg/m^2	14.3 ± 10.4 [a]	51.2 [a]
9AC CL/F	L/h/m^2	3.8 ± 9.3	2.7
Total		(n = 39)	(n = 3)
9NC AUC/Dose	ng/mL·h / mg/m^2	267.8 ± 185.4	201.3 ± 130.3
9NC CL/F	L/h/m^2	11.2 ± 15.0	4.1 ± 4.3
k_a	h^{-1}	0.44 ± 0.71	0.25 ± 0.34
k_{13}	h^{-1}	0.05 ± 0.09	0.12 ± 0.09
9AC AUC/Dose	ng/mL·h / mg/m^2	91.9 ± 78.3	129.0 ± 90.5
9AC CL/F	L/h/m^2	2.2 ± 3.5	2.3 ± 3.0

[a] P = 0.032

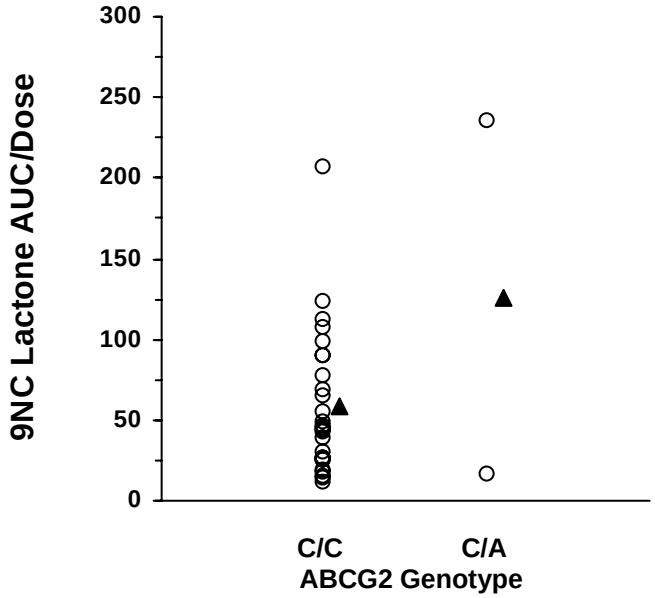


Figure 1. Relationship between 9NC lactone AUC divided by dose and *ABCG2* genotype. Data are presented for C/C (n = 26) and C/A (n = 2) genotyptes. Individual 9NC lactone AUC data are listed for each patient are represented by ○. Mean data for each group is represented by ▲.

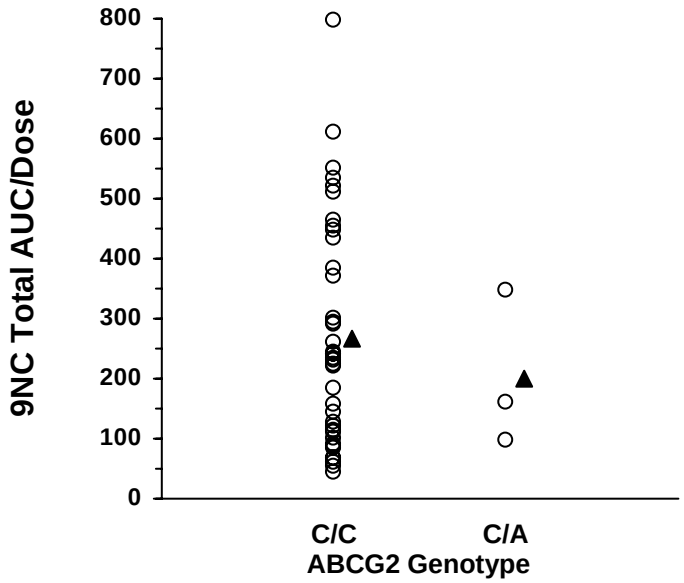


Figure 2. Relationship between 9NC total AUC divided by dose and *ABCG2* genotype. Data are presented for C/C (n = 39) and C/A (n = 3) genotyptes. Individual 9NC lactone AUC data are listed for each patient are represented by ○. Mean data for each group is represented by ▲.

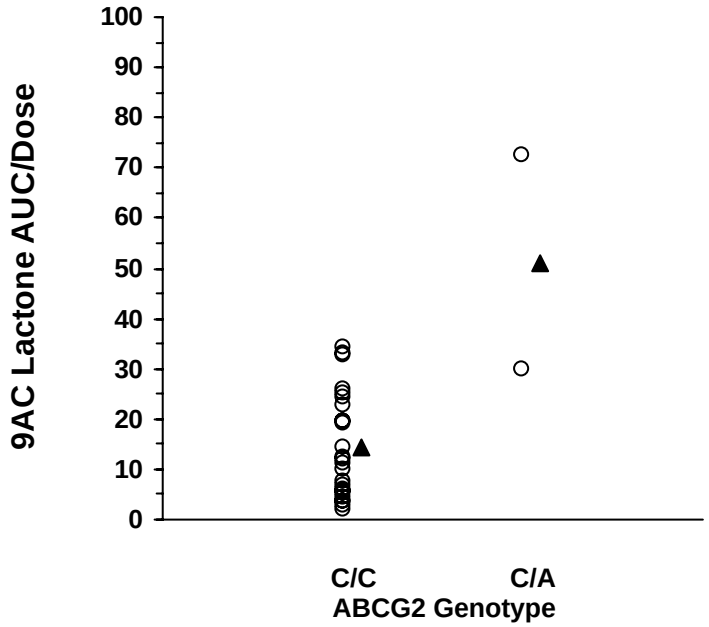


Figure 3. Relationship between 9AC lactone AUC divided by dose and *ABCG2* genotype. Data are presented for C/C (n = 26) and C/A (n = 2) genotyptes. Individual 9NC lactone AUC data are listed for each patient are represented by ○. Mean data for each group is represented by ▲. The 9AC lactone AUC was significantly higher for the C/A genotype compared to the C/C genotype (P = 0.032).

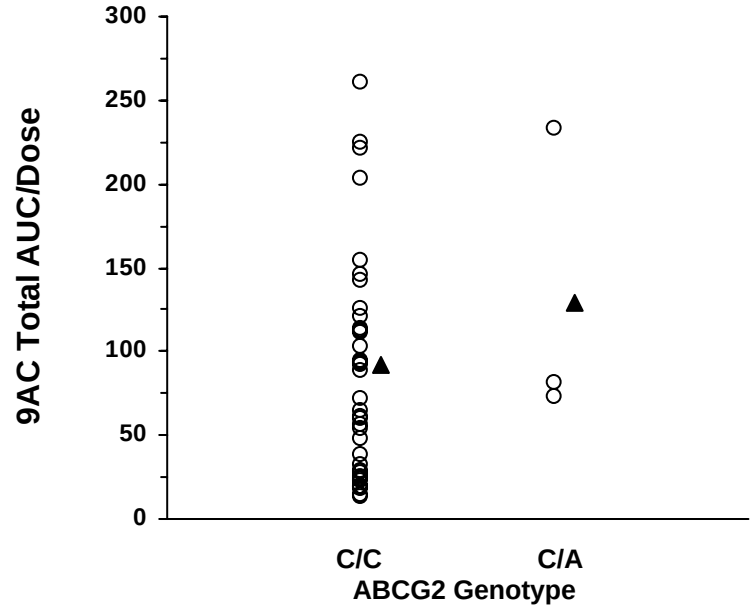


Figure 4. Relationship between 9AC total AUC divided by dose and *ABCG2* genotype. Data are presented for C/C (n = 39) and C/A (n = 3) genotyptes. Individual 9NC lactone AUC data are listed for each patient are represented by ○. Mean data for each group is represented by ▲.

6.5 DISCUSSION

Relatively high pharmacokinetic variability has been reported after intravenous and oral administration of camptothecins analogues (1-7,35,36). Several studies have evaluated the relationship between ABC genotypes and disposition of camptothecin analogues (5,22,23,35-39). 9NC is an orally administered camptothecin analogue with significant interpatient variability in drug disposition (6-10). This is the first study that compares the disposition of 9NC and its 9AC metabolite to the *ABCB1*, *ABCC2*, and *ABCG2* genotypes in patients. Our findings suggest that interpatient variability in 9AC disposition is influenced, in part, by *ABCG2*; however, in this exploratory study we performed many statistical tests, and thus can not rule out the possibility that this association is due to chance. In contrast, there was no evidence for a relationship between *ABCG2* and the disposition of 9NC, or for relationships between *ABCB1* and *ABCC2* genotypes and the disposition of 9NC or 9AC. The effects of *ABCG2* SNPs on the disposition of 9NC and 9AC are consistent with the previous *in vitro* study reporting that wild-type forms of *ABCG2* mediates the cellular efflux of 9AC, but not 9NC (37). The results of the *in vitro* study and our clinical study suggest that *ABCG2* genetic variants may explain the variable ratio of 9NC to 9AC exposure. However as related to the *ABCG2* 421C>A genotype, it is possible that in patients with the variant allele there was also a reduction in the excretion of 9NC that was not detected because 9NC was subsequently converted to 9AC. There was no relationship between ABC genotypes and response; however, 4 of 5 patients developing DLTs were heterozygous for the three SNPs of *ABCB1*.

The study by Sparreboom and colleagues evaluated the relationship between ABC genotypes and diflomotecan pharmacokinetics after IV and oral administration (35). This was the first reported evidence linking the variant *ABCG2* 421C>A allele to altered drug exposure and suggested that interpatient variability in substrate drug effects might be influenced, in part, by *ABCG2* genotype. These results are consistent with the results of our 9NC study. Among the genetic variants studied by Sparreboom, only the *ABCG2* 421C>A genotype significantly affected the pharmacokinetics of diflomotecan after IV, but not oral administration. In addition, there was no relationship between *ABCB1* and *ABCC2* genotypes and the disposition of diflomotecan after IV or oral administration.

de Jong and colleagues evaluated ethnic differences in *ABCG2* allele frequency and the influence on irinotecan disposition (38). *ABCG2* genotyping was performed on 88 Caucasian Americans, 94 African Americans, 938 African, and 95 Han Chinese, as well as in 84 European Caucasians who were treated with irinotecan and underwent blood sampling as part of pharmacokinetic studies. There were significant differences in allele frequencies among the populations. The variant allele was most common in the Han Chinese population with a frequency as high as 34%. In addition, the frequency of the variant allele (10.7%) was in line with results in American Caucasians. The variation in allele frequency in various ethnic populations is very interesting, and should be evaluated in future studies of 9NC, especially in Han Chinese populations. The authors concluded that the *ABCG2* 421C>A polymorphism appears to play a limited role in the disposition of irinotecan in European Caucasians and that the contribution of this genetic variant is obscured by a functional role of other polymorphic proteins. There was no

relationship between allele frequency and race in our study. However, 43 of the 55 patients in our study were Caucasian and no patients were Chinese.

ABCB1 is a known transporter of hydrophobic substrates including drugs such as colchicine, etoposide, adriamycin, and vincristine (19-22). However, consistent with the result of our 9NC study, there have been no reports of a functional consequence of *ABCB1* as related to the disposition of camptothecin analogues (35,38). Innocenti and colleagues reported the first evidence for a functional variant of *ABCC2* and its influence on interindividual irinotecan pharmacokinetic variability (39). That study identified 3972C>T as a variant potentially affecting *ABCC2* activity and suggested that *ABCC2* may have a significant impact on clearance of irinotecan. There was no relationship between *ABCC2* variant alleles and the disposition of 9NC and 9AC in our study.

There is a need to perform pharmacogenetic studies of anticancer agents that are orally administered and have a steep relationship between exposure and response (i.e., antitumor or toxicity) so that factors related to the pharmacokinetic variability may be determined (8,36). It is likely that the contributions of the genetic variants evaluated in this study are obscured by the functional role of an undiscovered or unevaluated polymorphic protein with a transport or metabolic function (e.g., cytochrome P450) (40). This is especially important for 9NC because the enzyme that converts 9NC to its major metabolite, 9AC, has not been identified (41,42). In addition, as new transporters and associated genes are discovered, future studies should evaluate the influences of variant alleles of these genes and the disposition of 9NC and 9AC and other camptothecin analogues, especially in various ethnic populations (38). Moreover, as number of patients undergoing pharmacogenetic studies of 9NC and ABC transporters expands,

there is a greater likelihood that patients will be discovered who are homozygous for the variant allele and thus may be more likely to experience an alteration in the disposition of 9NC and 9AC and toxicity and response (35,38,39). These studies may also explain the highly variable response and toxicity associated with 9NC treatment of patients with advanced pancreatic cancer and with other camptothecin analogues used in a wide variety of tumor types (43,44).

6.6 REFERENCES

1. Zamboni WC, Bowman LC, Tan M et al. Interpatient variability in bioavailability of the intravenous formulation of topotecan given orally to children with recurrent solid tumors. Cancer Chemother Pharmacol 1999;43:454-460.

2. Gupta E, Luo F, Lallo A et al. The intestinal absorption of camptothecin, a highly lipophilic drug, across Caco-2 cells is mediated by active transporter(s). Anticancer Res 2000;20:1013-1016.

3. Drengler RL, Kuhn JG, Schaaf LJ et al. Phase I and pharmacokinetic trial of oral irinotecan administered daily for 5 days every 3 weeks in patients with solid tumors. J Clin Oncol 1999;17:685-696.

4. Loos WJ, Gelderblom H, Sparreboom A, Verweij J, de Jonge MJ. Inter- and intrapatient variability in oral topotecan pharmacokinetics: implications for body-surface area dosage regimens. Clin Cancer Res 2000;6:2685-2689.

5. Kruijtzer CM, Beijnen JH, Rosing H et al. Increased oral bioavailability of topotecan in combination with the breast cancer resistance protein and P-glycoprotein inhibitor GF120918. J Clin Oncol 2002;20:2943-2950.

6. Schoemaker NE, Mathot RA, Schoffski P, Rosing H, Schellens JH, Beijnen JH. Development of an optimal pharmacokinetic sampling schedule for rubitecan administered orally in a daily times five schedule. Cancer Chemother Pharmacol 2002;50:514-517.

7. Raymond E, Campone M, Stupp R et al. Multicentre phase II and pharmacokinetic study of RFS2000 (9-nitro-camptothecin) administered orally 5 days a week in patients with glioblastoma multiforme. Eur J Cancer 2002;38:1348-1350.

8. Jung LL, Ramanathan RK, Egorin MJ et al. Pharmacokinetic studies of 9-nitrocamptothecin on intermittent and continuous schedules of administration in patients with solid tumors. Cancer Chemother Pharmacol 2004;54:487-496.

9. Akhtar S, Beckman RA, Mould DR, Doyle E, Fields SZ, Wright J. Pretreatment with ranitidine does not reduce the bioavailability of orally administered topotecan. Cancer Chemother Pharmacol 2000;46:204-210.

10. Allen JD, van Loevezijn A, Lakhai JM et al. Potent and specific inhibition of the breast cancer resistance protein multidrug transporter in vitro and in mouse intestine by a novel analogue of fumitremorgin C. Mol Cancer Ther 2002;1:417-425.

11. Pazdur R, Diaz-Canton E, Ballard WP et al. Phase II trial of 9-aminocamptothecin administered as a 72-hour continuous infusion in metastatic colorectal carcinoma. J Clin Oncol 1997;15:2905-2909.

12. Takimoto CH, Thomas R. The clinical development of 9-aminocamptothecin. Ann N Y Acad Sci 2000;922:224-236.

13. Kirstein MN, Houghton PJ, Cheshire PJ et al. Relation between 9-aminocamptothecin systemic exposure and tumor response in human solid tumor xenografts. Clin Cancer Res 2001;7:358-366.

14. Bates SE, Robey R, Miyake K, Rao K, Ross DD, Litman T. The role of half-transporters in multidrug resistance. J Bioenerg Biomembr 2001;33:503-511.

15. Leonard GD, Polgar O, Bates SE. ABC transporters and inhibitors: new targets, new agents. Curr Opin Investig Drugs 2002;3:1652-1659.

16. Allikmets R, Schriml LM, Hutchinson A, Romano-Spica V, Dean M. A human placenta-specific ATP-binding cassette gene (ABCP) on chromosome 4q22 that is involved in multidrug resistance. Cancer Res 1998;58:5337-5339.

17. Doyle LA, Yang W, Abruzzo LV et al. A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci U S A 1998;95:15665-15670.

18. Miyake K, Mickley L, Litman T et al. Molecular cloning of cDNAs which are highly overexpressed in mitoxantrone-resistant cells: demonstration of homology to ABC transport genes. Cancer Res 1999;59:8-13.

19. Allen JD, Schinkel AH. Multidrug resistance and pharmacological protection mediated by the breast cancer resistance protein (BCRP/ABCG2). Mol Cancer Ther 2002;1:427-434.

20. Ross DD, Yang W, Abruzzo LV et al. Atypical multidrug resistance: breast cancer resistance protein messenger RNA expression in mitoxantrone-selected cell lines. J Natl Cancer Inst 1999;91:429-433.

21. Dean M, Allikmets R. Evolution of ATP-binding cassette transporter genes. Curr Opin Genet Dev 1995;5:779-785.

22. Brangi M, Litman T, Ciotti M et al. Camptothecin resistance: role of the ATP-binding cassette (ABC), mitoxantrone-resistance half-transporter (MXR), and potential for glucuronidation in MXR-expressing cells. Cancer Res 1999;59:5938-5946.

23. Miyake K, Litman T, Robey R, Bates SE, Brangi M. Camptothecin resistance: role of the ATP-binding cassette (ABC), mitoxantrone-resistance half-transporter (MXR), and potential for glucuronidation in MXR-expressing cells. Cancer Letters 1999;146:117-126.

24. Allikmets R, Gerrard B, Hutchinson A, Dean M. Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the expressed sequence tags database. Hum Mol Genet 1996;5:1649-1655.

25. Kool M, de Haas M, Scheffer GL et al. Analysis of expression of cMOAT (MRP2), MRP3, MRP4, and MRP5, homologues of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. Cancer Res 1997;57:3537-3547.

26. Rocchi E, Khodjakov A, Volk EL et al. The product of the ABC half-transporter gene ABCG2 (BCRP/MXR/ABCP) is expressed in the plasma membrane. Biochem Biophys Res Commun 2000;271:42-46.

27. Jonker JW, Smit JW, Brinkhuis RF et al. Role of breast cancer resistance protein in the bioavailability and fetal penetration of topotecan. J Natl Cancer Inst 2000;92:1651-1656.

28. Miyake K, Mickley L, Litman T et al. Molecular cloning of cDNAs which are highly overexpressed in mitoxantrone-resistant cells: demonstration of homology to ABC transport genes. Cancer Res 1999;59:8-13.

29. Doyle LA, Yang W, Abruzzo LV et al. A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci U S A 1998;95:15665-15670.

30. Zamboni WC, Goel S, Iqbal T, Parise RA, Strychor S, Repinski TVW, Egorin MJ, Mani S. Clinical and pharmacokinetic study evaluating the effect of food on the disposition of 9-nitrocamptothecin and its 9-aminocamptothecin metabolite in patients with solid tumors. Submitted to Cancer Chemother Pharmacol in April 2005.

31. D'Argenio DZ, Schmuitzky A. ADAPT II user's guide: biomedical simulations resource; 1990.

32. Rowland M, Tozer T, editors. Clinical pharmacokinetics: concepts and applications. Philadelphia: Lea and Febiger; 1999.

33. Mathijssen RH, Marsh S, Karlsson MO et al. Irinotecan pathway genotype analysis to predict pharmacokinetics. Clin Cancer Res 2003;9:3246-3253.

34. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. Am J Hum Genet 2002;70:425-434.

35. Sparreboom A, Gelderblom H, Marsh S et al. Diflomotecan pharmacokinetics in relation to ABCG2 421C>A genotype. Clin Pharmacol Ther 2004;76:38-44.

36. Santana VM, Zamboni WC, Kirstein MN et al. A pilot study of protracted topotecan dosing using a pharmacokinetically guided dosing approach in children with solid tumors. Clin Cancer Res 2003;9:633-640.

37. Rajendra R, Gounder MK, Saleem A et al. Differential effects of the breast cancer resistance protein on the cellular accumulation and cytotoxicity of 9-aminocamptothecin and 9-nitrocamptothecin. Cancer Res 2003;63:3228-3233.

38. de Jong FA, Marsh S, Mathijssen RH et al. ABCG2 pharmacogenetics: ethnic differences in allele frequency and assessment of influence on irinotecan disposition. Clin Cancer Res 2004;10:5889-5894.

39. Innocenti F, Undevia SD, Chen X, Das S, Ramirex J, Dolan ME, Relling MV, Kroetz DL, Ratain MJ. Pharmacogenetic analysis of interindividual irinotecan (CPT-11) pharmacokinetics (PK) variability: Evidence for a functional variant of ABCC2. Proceedings of ASCO 2004: #2010.

40. Mathijssen RH, de Jong FA, van Schaik RH et al. Prediction of irinotecan pharmacokinetics by use of cytochrome P450 3A4 phenotyping probes. J Natl Cancer Inst 2004;96:1585-1592.

41. Pantazis P, Harris N, Mendoza J, Giovanella B. The role of pH and serum albumin in the metabolic conversion of 9-nitrocamptothecin to 9-aminocamptothecin by human hematopoietic and other cells. Eur J Haematol 1995;55:211-213.

42. Hinz HR, Harris NJ, Natelson EA, Giovanella BC. Pharmacokinetics of the in vivo and in vitro conversion of 9-nitro-20(S)-camptothecin to 9-amino-20(S)-camptothecin in humans, dogs, and mice. Cancer Res 1994;54:3096-3100.

43. Schoffski P, Herr A, Vermorken JB et al. Clinical phase II study and pharmacological evaluation of rubitecan in non-pretreated patients with metastatic colorectal cancer-significant effect of food intake on the bioavailability of the oral camptothecin analogue. Eur J Cancer 2002;38:807-813.

44. Natelson EA, Giovanella BC, Verschraegen CF et al. Phase I clinical and pharmacological studies of 20-(S)-camptothecin and 20-(S)-9-nitrocamptothecin as anticancer agents. Ann N Y Acad Sci 1996;803:224-230.

7.0

CHAPTER 7:

Summary and Future Directions for the Development of 9-Nitrocamptothecin

7.1 SUMMARY

9-nitrocamptothecin (9NC) has completed phase III studies in patients with newly diagnosed and refractory pancreatic cancer, however the optimal 9NC regimen is unclear. The primary objective of this dissertation research was to evaluate the pharmacokinetics, pharmacodynamics, and pharmacogenetics of 9NC and its 9AC metabolite in preclinical models and in patients as part of phase I and II trails. Information obtained from preclinical and clinical translational studies can greatly add to the understanding of the pharmacology of 9NC and allow for the rational design of therapeutic regimens. To address these issues in the development of 9NC we performed preclinical and clinical pharmacologic studies of 9NC

The aim of Chapter 2 was to compare the 9NC and 9AC systemic exposure associated with antitumor response in mice bearing human tumor xenografts to those reported in a phase I study of 9NC that used the same intermittent schedule as in the xenografts studies and use allometric scaling analysis to normalize the data. The responses seen in these xenograft models occurred at systemic exposures that are tolerable in adult patients. These results suggest that the intermittent schedule of 9NC may be an active-regimen in patients with colo-rectal cacinoma. However, there are several potential limitations to this study. The overall lack of 9NC response in patients with refractory colon carcinoma raises concerns about the ability of mice bearing human colon xenografts to predict 9NC response in humans. In addition, the relationship between 9NC and 9AC exposure and response may be different for specific tumors, and thus these types of studies may need to be performed for each tumor type, especially pancreatic cancer. The tumor exposures of anticancer agents in xenografts located on the

flank of mice may be different than the exposure in tumors of patients, and thus evaluating plasma exposures in mice and man may not be an accurate comparison (45,46).

The pharmacologic goals of Chapters 3 and 4 were to examine the inter- and intra-patient pharmacokinetic variability of 9NC and 9AC. There was significant inter- and intra-patient variability in the disposition of 9NC and 9AC. Dosing 9NC on a mg/m^2 basis does not reduce pharmacokinetic variability. Because no IV formulation of 9NC is available it is unclear if the inter- and intra-patient variability in the pharmacokinetics of 9NC or 9AC is due to gastrointestinal and absorption related factors and/or systemic disposition and elimination.

The aim of Chapter 5 was to evaluate the plasma disposition of 9NC and 9AC after administration of 9NC with and without food in a randomized cross over study. Co-administration of 9NC with food reduces the oral absorption of 9NC; however there was no difference in the exposure of 9AC. The effect of food on the absorption of 9NC is highly variable and is less consistent for 9AC compared to 9NC. The mechanism associated with differential effects of food on the exposure of 9NC and 9AC are currently unclear. One potential explanation is that 9NC is converted to 9AC in the gastrointestinal tract, in addition to the liver, and food does not alter the absorption of 9AC. Additional preclinical and clinical studies are needed to identify the mechanism associated with the differential effects of food on the disposition of 9NC and 9AC, especially regarding the conversion of 9NC to 9AC in the gastrointestinal tract and to identify the location of the absorption of 9NC and 9AC.

The goal of Chapter 6 was to evaluate the functional consequence of known SNP polymorphisms in the transporter genes *ABCB1, ABCC2,* and *ABCG2* on the pharmacokinetic disposition of 9NC and 9AC. 9NC and 9AC disposition were not significantly influenced by variants in *ABCB1*, *ABCC2*, and *ABCG2*, and *ABCB1* and *ABCC2*, respectively ($P > 0.05$). These findings suggest that inter-individual variability in 9AC disposition, but not 9NC, may be influenced, in part, by *ABCG2* genotype. Because all patients did not have studies evaluating the pharmacokinetics of the lactone and total forms of 9NC and 9AC, it is unclear if the lactone and hydoxyacid forms are handled differently by ABC transporters. It is likely that the contributions of the genetic variants evaluated in this study are obscured by the functional role of an undiscovered or unevaluated polymorphic protein with a transport or metabolic function (e.g., cytochrome P450). This is especially important for 9NC because the enzyme that converts 9NC to its major metabolite, 9AC, has not been identifie. In addition, as new transporters and associated genes are discovered, future studies should evaluate the influences of variant alleles of these genes and the disposition of 9NC and 9AC and other camptothecin analogues. The primary limitation of this study and most pharmacogenetic studies is a limited sample size and a low frequency of the variant allele in the study population (e.g., the predominantly Caucasian population in our study). For example, studies evaluating the relationship between ABC genotypes and pharmacokinetic variability of camptothecins required approximately 100 to 120 patients, where as our study only included 55 patients. In addition, because this pharmacogenetic study was performed on patients enrolled on 2 phase I studies, a phase II study, and a single dose pharmacokinetic study, the relationship between ABC genotypes and response (i.e., toxicity or antitumor)

could not be made. Ideally, effective pharmacogenetic studies will need to be performed in large phase II studies or even as part of phase III studies,

7.2 FUTURE DIRECTIONS

The NDA filed at the US FDA for 9NC (Orathecin, rubitecan) has been withdrawn by SuperGen, Inc. This NDA included studies evaluating 9NC for the treatment of patients with newly diagnosed pancreatic cancer and in patients with pancreatic cancer who failed first line and second line therapy. The regimen used in these studies was 9NC administered orally daily for 5 days per week for 8 consecutive weeks. The results of our studies suggest that the intermittent regimen of 9NC (i.e., orally daily for 5 days per week for 2 weeks repeated every 4 weeks) may be more effective than the 8 week continuous schedule of regimen. SuperGen is currently reviewing the possibility of performing a phase II study of 9NC in patients with refractory pancreatic cancer using the intermittent regimen evaluated in our preclinical and clinical studies. If this phase II study is performed, my research group will be involved in pharmacologic studies associated with this study. In addition, a randomized phase II study 9NC in combination with gemcitabine compared to gemcitabine alone is currently being performed in patients with newly diagnosed pancreatic cancer. Thus, the clinical development of 9NC in pancreatic cancer continues.

As described in our studies, the factors associated with the high inter- and intra-patient variability in the disposition of 9NC and 9AC are currently unclear. Future studies evaluating the effect of CYP3A4/5 and additional ABC genotypes on the pharmacokinetics of 9NC and 9AC are planned. The effect of *ABCG2* genotypes should

be further evaluated in Chinese patients. In addition, studies evaluating the ability of fumitremorgin-C, an inhibitor of efflux by ABC transporters, to reduce the pharmacokinetic variability of 9NC and 9AC in patients are also being planned. These studies will compare the pharmacokinetics of 9NC and 9AC when 9NC is administered alone and in combination with fumitremorgin C. The studies described here for the preclinical and clinical development of 9NC can also be used for other camptothecin analogues.