Anti-Cancer Drugs 2001, **12,** pp. 89–105

Review paper

Modulation of camptothecin analogs in the treatment of cancer: a review

Diederik FS Kehrer,[1] Otto Soepenberg,[1] Walter J Loos,[1] Jaap Verweij[1] and Alex Sparreboom[1]
[1]Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital, Groene Hilledijk 301, 3075 EA Rotterdam, The Netherlands.

The topoisomerase I inhibitors reviewed in this paper are all semisynthetic analogs of camptothecin (CPT). Modulation of this intranuclear enzyme translates clinically in to antitumor activity against a broad spectrum of tumors and is therefore the subject of numerous investigations. We present preclinical and clinical data on CPT analogs that are already being used in clinical practice [i.e. topotecan and irinotecan (CPT-11)] or are currently in clinical development (e.g. 9-aminocamptothecin, 9-nitrocamptotecin, lurtotecan, DX 8951f and BN 80915), as well as drugs that are still only developed in a preclinical setting (silatecans, polymer-bound derivates). A variety of different strategies is being used to modulate the systemic delivery of this class of agents, frequently in order to increase antitumor activity and/or reduce experienced side effects. Three principal approaches are discussed, including: (i) pharmaceutical modulation of formulation vehicles, structural alterations and the search for more water-soluble prodrugs, (ii) modulation of routes of administration and considerations on infusion duration, and (iii) both pharmacodynamic and pharmacokinetic biomodulation. [© 2001 Lippincott Williams & Wilkins.]

Key words: Camptothecin analogs, modulation, pharmacokinetics, topoisomerase I.

Introduction

Camptothecin (CPT), a plant alkaloid isolated from *Camptotheca acuminata*, was first identified in the late 1950s.[1] Due to severe and unpredictable toxic side effects in early clinical studies, including myelosuppression, severe diarrhea and hemorrhagic cystitis, the clinical development of CPT was halted in the 1970s.[2–5] In the early 1980s several important events occurred that resulted in renewed interest in this agent. (i) The molecular target of CPT, i.e. the nuclear enzyme topoisomerase I, was identified. This topoisomerase I was described as an enzyme involved in transient scission and relegation of DNA during the replication and transcription phases. Binding of CPT to the topoisomerase I–DNA complex (cleavable complex) and interference with the relegation step of this process was recognized as the primary mechanism of action of CPT, finally leading to double-stranded DNA breaks and, ultimately, cell death.[6–8] Subsequent investigations indicated overexpression of this topoisomerase I enzyme in various types of solid tumors, including ovarian and colon cancer.[9,10] (ii) At the same time, it was shown that the failures encountered in the clinical development of CPT were related, at least partially, to the drug's poor water solubility, which necessitated pharmaceutical formulation in alkaline solutions for i.v. administration. This not only led to chemical modification of the original structure into an entity lacking antitumor activity, but also induced profound alterations in the toxicological behavior of the agent.[11]

These two key findings then boosted drug research efforts aimed at identifying and developing new [(semi-)synthetic] analogs with improved aqueous solubility while maintaining CPT's unique mechanism of action (Figure 1). Some of these agents are currently in clinical development, whilst irinotecan (CPT-11) and topotecan are now registered for use in colorectal cancer,[12] and ovarian and lung cancer,[13] respectively. Both topotecan and CPT-11 underwent extensive clinical evaluation in phase II and III trials, and data suggested that both drugs are also active in various other tumor types in addition to the indications mentioned.[14,15]

Unlike most other CPT analogs, CPT-11 is a prodrug with very little inherent antitumor activity. To form the active metabolite SN-38, which is 100- to 1000-fold more active than the parent compound,[16] CPT-11 is hydrolyzed by a carboxylesterase.[17] SN-38 in its turn

Correspondence to DFS Kehrer, Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital, Groene Hilledijk 301, 3075 EA Rotterdam, The Netherlands.
Tel. (+31) 10 4391733; Fax: (+31) 10 4391003;
E-mail: kehrer@vvdh.azr.nl

can be metabolized further by UDP glucuronosyltransferase 1A1 to form an inactive β-glucuronide derivative (SN-38-G).[18] In *in vitro* studies, topoisomerase I inhibitors showed more pronounced antitumor efficacy with protracted exposure to low concentrations. In animal models, low-dose prolonged exposure also resulted in less toxicity.[19–23] It should be noted that for several reasons, including species differences in drug disposition and tolerability as well as intrinsic differences in tumor sensitivity, *in vitro* and animal models have been shown to be poor predictors of clinical efficacy and toxicity. Nonetheless, most clinical studies have focused on low-dose exposure to topoisomerase I inhibitors in cancer patients.[24–29] Meanwhile, numerous researchers are unravelling the clinical pharmacodynamics and pharmacokinetics of the different analogs. This has led to an explosion in publications on this subject. The present review is focused on chemical and pharmacologic aspects of CPT analog development, with special emphasis on the choice of routes of delivery and on intrinsic differences in toxicity profiles of the various analogs, and possible ways to modulate these either pharmacodynamically or -kinetically.

Chemical properties

Structure–activity relationships

Most of the currently known CPT analogs share a basic five-ring structure with a chiral center located at C20 in the terminal E-ring. Extensive studies on the synthesis of CPT analogs and the development of structure–activity relationships have been carried out over recent years and some important general relationships have emerged.[30] While these relationships will clearly be refined in the years to come, current knowledge is potentially adequate for the design of improved analogs of CPT. This current knowledge is summarized in Figure 2 rather than being described exhaustively. Structure–activity studies have also shown a close correlation between the ability to inhibit topoisomerase I and overall cytotoxic potency.[31] For the purpose of this review, a number of regions in the CPT structure are particularly relevant:

(i) It has been shown that the topoisomerase I inhibitory activity of these agents is stereospecific, with the naturally occurring (*S*)-isomer being many-fold more potent that the (*R*)-isomer.[7,9]

(ii) In general, substitutions at C7, C9 and C10 tend to increase topoisomerase I inhibition and sometimes increase water solubility, whereas substitutions at C12 decrease antitumor activity.[16]

(iii) Similarly, the formation of certain additional ring structures, e.g. between C7 and C9 or C10 and C11, increases activity.[32–35]

(iv) One of the principal chemical features of this class of agents is the presence of a lactone functionality in the E-ring, which is not only essential for antitumor activity, but it also confers a degree of instability to these agents in aqueous solutions.[36] All known camptothecins can undergo a pH-dependent reversible interconversion between this lactone form and a ring-opened carboxylate (or hydroxy acid) form (Figure 1), of which only the lactone form is able to diffuse across cell membranes and exert the characteristic topoisomerase I inhibitory activity. At neutral or physiologic pH, the equilibrium between the two species favors the carboxylate form for all the CPTs. As outlined, an understanding of this hydrolysis reaction helps to explain several observations in the early development of these agents. Because CPT was administered as the more water-soluble sodium salt, patients were exposed to high concentrations of the relatively inactive carboxylate species, whereas large amounts of drug were excreted in urine, where the low pH favored closure of the lactone ring with resulting hemorrhagic cystitis.[2,4] The equilibrium between the lactone moiety ring and the carboxylate form of the CPTs is not solely dependent on the pH, but also on the presence of specific binding proteins in the biological matrix, most notably human serum albumin (HSA). Following establishment of equilibration at 37°C in phosphate-buffered saline (PBS), equal amounts of the various CPT analogs are present in the pharmacologically active lactone form, with values of 17, 19, 15, 13 and 15% for CPT, 9-aminocamptothecin (9-AC), topotecan, CPT-11 and SN-38, respectively.[37,37] Addition of 40 mg/ml HSA shifts the equilibrium for CPT and 9-AC towards the carboxylate form, with approximately 1% present in the lactone form at equilibrium.[37,38] In contrast to HSA, addition of murine serum albumin to 9-AC leads to approximately 35% existing in the lactone form at equilibrium.[38] As opposed to CPT and 9-AC, HSA actually stabilizes the lactone moiety of CPT-11 and SN-38, with values of 30 and 39%, respectively, present in the lactone form at equilibrium, while almost no effect was seen for topotecan.[37] It has been proposed that the differences in the percentiles present in the lactone form at equilibrium is related to sterical considerations of the various

substituents at the R_1 and R_2 positions (Figure 1A). For some of the more recently developed CPT analogs, the substituents cause sterical hindrance and prevent binding of the carboxylate forms to HSA, and so drive the equilibrium towards the lactone species.

Conventional drug formulations

The inherent instability of the lactone form and the inactivity of the carboxylate form have posed a specific challenge to the development of a suitable dosage form of CPT analogs. Since the lactone form is strongly

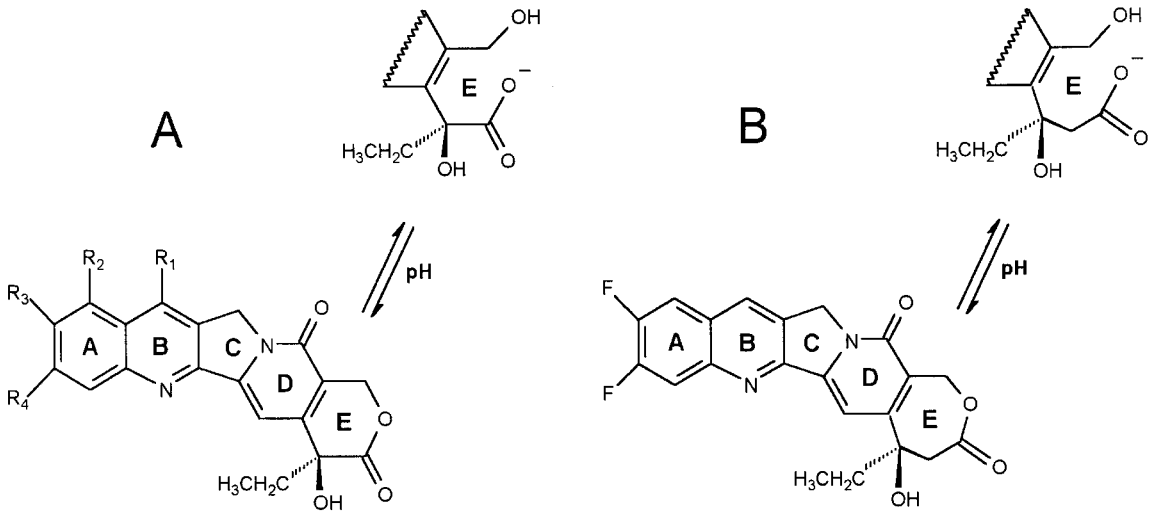


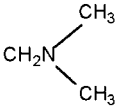
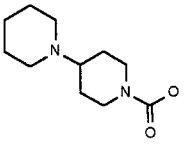
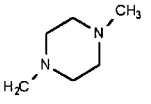
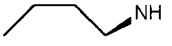
FIGURE A	R_1	R_2	R_3	R_4
CPT	H	H	H	H
9NC	H	NO_2	H	H
9AC	H	NH_2	H	H
TPT	H		H	H
CPT-11	CH_2CH_3	H		H
SN-38	CH_2CH_3	H	OH	H
LRT		H		
DX-8951			CH_3	F

Figure 1. Chemical structures of CPT analogs with a 6-numbered (A) or 7-numbered (B) E-ring.

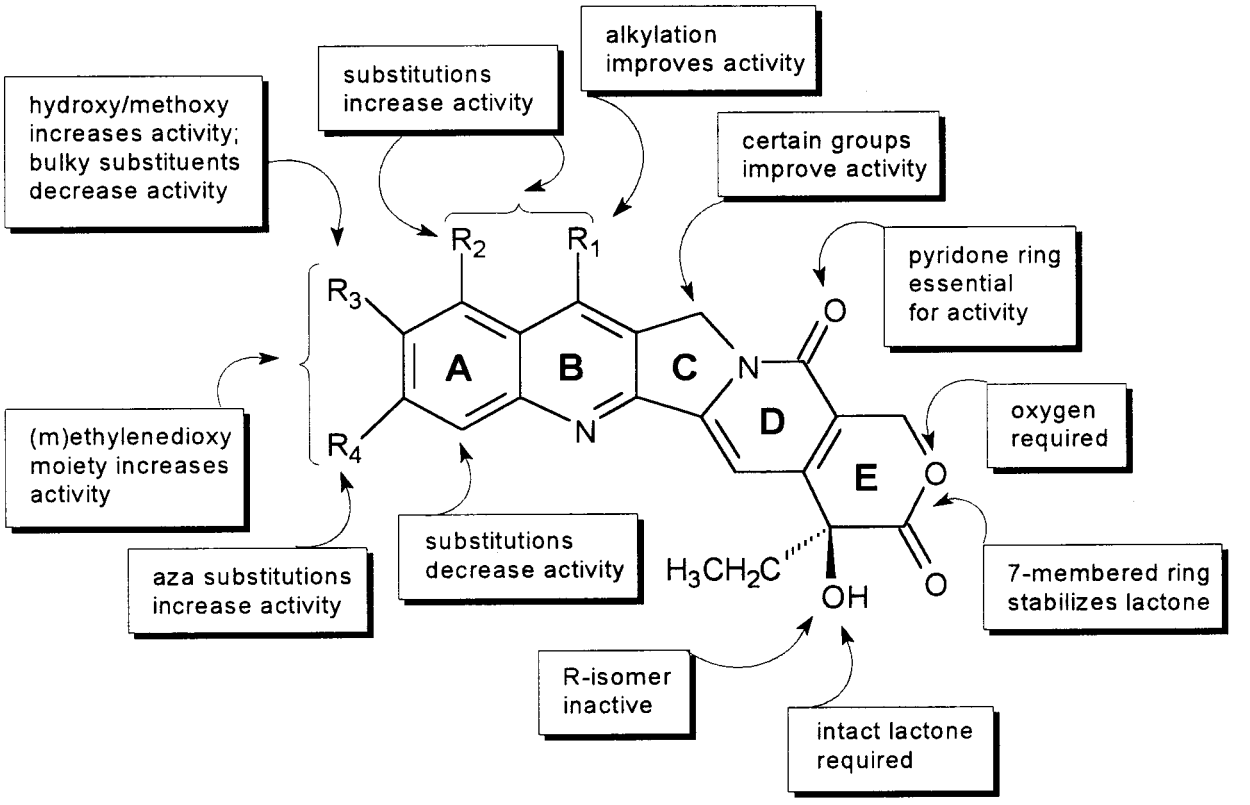


Figure 2. Structure–activity relationships of CPTs.

favored by an acidic pH, as outlined above, the currently registered agents topotecan and CPT-11 have both been formulated in buffered dosage forms. Topotecan is available as a powder containing topotecan hydrochloride and tartaric acid, yielding an aqueous solution for infusion of pH 2.5–3.5 after reconstitution. This solution is stable for at least 12 h at room temperature, whereas the dry powder is stable for at least 2 years at room temperature. The pharmaceutical dosage form of CPT-11 is similarly based on formulation of the hydrochloride form of the drug in an aqueous solution, containing a lactic acid–sodium hydroxide buffer system of pH 3.5–4.5. Current shelf-life studies have shown that the injection concentrate is stable for at least 3 years at room temperature when protected from light.

Pharmaceutical modulation

Alternative formulations

In recent years, a variety of alternative pharmaceutical formulations have been or are currently being evaluated. Important properties of these alternatives will be that they allow drug doses to be delivered at levels (at least) similar to those achieved with the conventional formulations and that the drugs should be stable for several hours in order to be handled in a clinical setting. Despite the tremendous efforts invested so far, only very few of the alternatives have fulfilled the requirements to justify clinical testing. The rationale for re-formulation of CPT or its analogs has been either to stabilize the lactone moieties or to induce sustained release combined with specific tumor targeting of these agents. An example of the first approach has been the formulation of CPT, CPT-11 or 10-hydroxy-CPT in microspheres composed of poly-D,L-lactic acid or poly(D,L-lactic-co-glycolic acid).[39–42] The influence of various encapsulation procedures on the release of these agents has been extensively examined and has shown stabilization of the lactone form due to an acidic microclimate of the microspheres combined with enhanced pharmacokinetic characteristics in animal models.[43] Although from a theoretical perspective the modulation of CPT in this prolonged release system could be attractive by reducing local toxicity and improving therapeutic efficacy, no phase I studies have been performed so far.

Alternatively, substantial progress has been made recently toward liposomal formulation of a number of

important CPT analogs. Liposomes are microparticulate carriers that consist of one or more lipid bilayer membranes enclosing an internal aqueous phase. The most common constituents are synthetic or naturally occurring phospholipids and cholesterol. Although several reports address the considerations in choosing the specific liposome constituents and their physical properties (e.g. for CPT,[21] topotecan[44,45] and CPT-11[46,47]), relatively little information has been presented on toxicity profiles and antitumor activity. A recent study reported, however, that liposomal topotecan, encapsulated in sphingomyelin/cholesterol liposomes using an ionophore-generated proton gradient, was eliminated from the plasma much more slowly than the free drug, resulting in a 400-fold increase in systemic exposure.[48] The liposomal preparation also protected topotecan from lactonolysis, and increased antitumor activity in both murine and human tumor models. Likewise, data have been generated demonstrating that unilamellar liposomal formulations of lurtotecan (NX 211[49] and SPI-355[50]) have significant therapeutic advantage over free drug, and that the increased activity is consistent with increased systemic exposure and enhanced tumor-specific delivery of the drug. Based on these exciting data, several phase I clinical trials have been initiated with NX 211 given to cancer patients in either weekly or 3-weekly regimens and preliminary findings corroborate the preclinical pharmacological profile of this agent.[51]

CPT and a number of analogs, notably topotecan, have also been formulated in solid-lipid nanoparticles [composed of stearic acid, soybean lecithin and polyoxyethylene–polypropylene copolymer (Poloxamer 188)][52] and dimethyl-β-cyclodextrin to stabilize the lactone form.[53] Although these systems are promising sustained release and drug-targeting systems in various preclinical models, their clinical merit has not yet been evaluated.

Synthetic derivatives

9-AC. 9-AC is a semisynthetic CPT derivative which showed outstanding preclinical activity against a wide spectrum of tumor types, including those of breast, colon, lung and prostate and melanoma.[54] In clinical trials, the drug has been very extensively studied using two different formulations based on the use of dimethylacetamide/polyethylene glycol 400 or a colloidal dispersion preparation, which enhances solubility and stability. Clinical phase I investigations have been conducted using a variety of i.v. administration schedules, including a 30-min infusion given daily for 5 days every 3 weeks, and more prolonged infusion schedules using 24-h, 72-h, 96-h and 7- or 21-day continuous dosing repeated every 4 weeks.[55–61] All of the studies report neutropenia as the dose-limiting toxicity, while thrombocytopenia is also frequent and sometimes severe. Gastrointestinal toxicity is the second most reported, though not dose limiting. Other toxicities are considered mild to moderate. Numerous multi-institutional phase II studies have been conducted in several disease types, and overall, 9-AC shows only very modest single-agent activity and its further evaluation does not seem indicated.[59,62–64] It has been suggested that the lack of clinically relevant antitumor efficacy relates to substantial inactivation of the agent due to the unfavorable lactone/carboxylate ratio in patients.[38]

Homocamptothecins (hCPTs). In the search for more stabile CPT analogs, the synthetic preparation of derivatives bearing a seven-membered E-ring, the so-called hCPTs, has been described (Figure 1B).[65] The lactone ring is stabilized by modification of the naturally occurring six-membered α-hydroxylactone ring into a seven-membered β-hydroxylactone ring by insertion of a methylene spacer between the alcohol and the carboxyl moiety. The lead compound in this series, i.e. hCPT, has been shown to be more stable than CPT, remains a highly potent inhibitor of both cell growth with superior topoisomerase I inhibitory activity as compared to CPT[66–68] and, most interestingly, changes the sequence–specificity of the drug-induced DNA cleavage by topoisomerase I.[69] Indeed, in contrast to CPT which shows a rapid hydrolysis of the lactone moiety until a pH- and protein-dependent equilibrium has been reached, hCPT displays a slow and irreversible hydrolytic lactone ring opening.[70] After a 3 h incubation of CPT and hCPT in human whole blood at 37°C, the fraction present in the lactone form was 6% in the case of CPT and 80% in the case of hCPT. This remarkable difference is not only due to the slower ring opening of hCPT, but also to a higher affinity of hCPT for red blood cells.[71] Based upon this promising feature of hCPT, a series of derivatives of this agent was developed that combine enhanced plasma stability and potent topoisomerase I-mediated cytotoxicity. Various fluorinated analogs were subsequently found to have potent cytotoxic activity against several cell lines, including those overexpressing a functionally active P-glycoprotein.[72] Figure 1(B) shows the chemical structure of BN 80915, one of the most promising fluorinated hCPT analogs, which recently has entered clinical phase I testing.

Silatecans. Using a cascade radical annulation route to the CPT family, a novel series of CPT analogs, i.e. 7-silylcamptothecins or silatecans, have been synthe-

sized that exhibited potent inhibition of topoisomerase I, dramatically improved blood stability and sufficient lipophilicity to favor blood–brain barrier transit.[73] Preliminary evaluation in preclinical mouse models indicate that silatecans may hold significant promise for the treatment of high-grade gliomas and provide a rationale for proceeding with further (pre)clinical evaluation of their efficacy and safety versus commercially available CPT derivatives, including topotecan and CPT-11.

Hexacyclic CPTs. Two representative agents of the hexacyclic CPT analogs are currently under investigation. The first of these, lurtotecan (also known as GI147211 or GG211), is a water-soluble, totally synthetic derivative with a dioxalane moiety between C10 and C11.[74] This agent has been evaluated clinically in various phase I and phase II trials using a 30-min i.v. infusion given daily for 5 consecutive days or as a 72-h continuous i.v. infusion.[75–77] The dose-limiting toxicity in both schedules was myelosuppression, including severe neutropenia and thrombocytopenia. Non-hematological toxicities were various and only mild to moderate. Because the oral bioavailability was highly variable and as low as 10%,[78] alternative ways of drug administration are currently being developed, including a new liposomal formulation (NX 211; see above).

The second agent, DX-8951f or exatecan mesylate, is a new water-soluble, CPT analog with an amino group at C1 and a fluorine at C5.[79] DX-8951f showed superior and a broader spectrum of antitumor activity *in vitro* and *in vivo* in comparison with the other CPT analogs tested.[80,81] Recently, the results of a phase I evaluation of DX-8951f have become available, with the drug administered as a 30-min i.v. infusion given daily for 5 days every 3 weeks.[82,83] Brief, non-cumulative neutropenia was the most common toxicity and was seen consistently at doses greater than 0.5 mg/m^2/day. Other non-hematologic toxicities were mild to moderate in severity. Various other schedules, including a once every 3 weeks regimen with 30-min i.v. infusions[84,85] and one based on continuous i.v. infusions of 5–21 days are presently under investigation.[86] The 30-min infusion regimen with daily administration for 5 consecutive days is now being tested in clinical phase II trials in various disease types, including non-small cell lung cancer,[87] pancreatic cancer,[88] ovarian cancer[89] and colorectal cancer.[90]

Prodrugs

Because of the poor aqueous solubility of some CPT analogs, some major efforts have been put into the design and synthesis of more water-soluble prodrugs that could be more readily formulated than the parent drug. Many of the synthesized compounds have shown only marginal improvements in solubility or are too unstable to allow administration in a clinical setting. The instability of prodrug forms of, for example, CPT is particularly problematic, since the product of degradation (generally the parent drug) is insoluble and precipitates in aqueous solutions. Other synthetic approaches have produced fairly stable prodrugs, but the rate of active drug liberation proceeds at a too slow and variable rate. To date, two approaches in prodrug design have yielded agents that have progressed to clinical evaluation, i.e. the 9-nitro derivative of CPT and polymer-coupled derivatives of CPT and DX-8951f.

C9-substituted derivatives. One of the most extensively studied agents of this class is 9-nitrocamptothecin (9-NC), which acts as a partial prodrug of 9-AC.[91,92] 9-NC has a nitro radical in the C9 position and is highly insoluble in water, and was initially identified as a precursor in the semi-synthetic production of 9-AC. Since nearly all human cells are able to convert 9-NC to 9-AC, including tumor cells, it has been proven difficult to identify whether 9-NC-mediated antitumor activity is directly associated with the parent drug alone or with 9-AC alone, or the combination of both.[93] Preliminary evidence generated in clinical phase II trials suggests that 9-NC may be potentially useful in the treatment of advanced pancreatic cancer and refractory ovarian cancer using a daily times 4 or 5 per week schedule with the drug given orally.[94] An extensive clinical phase II program is currently being conducted to test the efficacy of this agent against various malignant diseases.[95,96]

Polymer-bound derivatives. One of the possible ways to modulate anticancer agents is the use of their attachment to macromolecules because these high molecular weight prodrug carriers can lead to reductions in systemic toxicity, longer retention time within the body, alterations in biological distributions and possible improvements in therapeutic efficacy.[97,98] Its use is dependent on the concept of the enhanced permeability and retention (EPR) effect in solid tumors.[99] This EPR effect is based on four general characteristics of tumor tissues in comparison with normal tissues: (i) hypervascularity, (ii) hyperpermeability, (iii) defective vascular architecture and (iv) less efficacious drainage due to a hypoplastic or minimally effective lymphatic system.[99] The pharmacokinetic characteristics of CPT were the starting point to modulate its structure in this way. As indicated, CPT is highly insoluble in water and by converting the C20-

OH moiety into an ester coupled to an amino acid spacer to allow better solubilization in aqueous environments, several macromolecular prodrugs of CPT have been generated in recent years.[100] Two of these, MAG-CPT and PEG-CPT, have progressed to clinical evaluation.[97,101] The former consists of CPT covalently bound to a soluble polymer [*N*-(2-hydroxypropyl) methacrylamide (HPMA)] through a glycyl-aminohexanoyl-glycyl spacer, whereas the latter is a macromolecule derived through conjugation of chemically modified polyethyleneglycol with CPT at the C20-OH position. A variety of preclinical studies with MAG-CPT and PEG-CPT have shown stabilization and sustained release of CPT, and also prolonged drug retention within experimental tumors.[97,101] Recently, the preliminary results of clinical phase I studies of PEG-CPT[102] and MAG-CPT (also referred to as PNU 166148)[103] have been reported, and indicate substantially prolonged exposure times to the active species as compared to administration schedules of the free drugs.

In an effort to prolong exposure times of DX-8951f to tumor tissues that might increase cytotoxic properties and antitumor efficacy, a prodrug has recently been generated by linking the agent to a biodegradable carrier via a peptide spacer (DE-310). Clinical evaluation of this prodrug to examine this hypothesis is currently being conducted.

Routes of delivery

Intravenous dosing and considerations of infusion duration

Intravenous administration of CPT analogs is currently the most commonly used route of delivery. The advantages of this route are many, including the fact that the total amount and duration of the administered drug can be controlled. As mentioned earlier, this has the advantage of controlled prolonged delivery of the drug. In this regard, most of the studies have been done with topotecan,[5] and several of the most promising regimens have been selected to enter phase II testing, i.e. a daily times 5 every 3 weeks 30-min schedule,[104,105] weekly or 3-weekly 24-h infusion[106,107] and a 21-day continuous low-dose infusion administered every 4 weeks.[13,25,27,29,108] Besides proposed schedule dependency on antitumor activity, toxicity also appears to vary considerably. Overall, the dose-limiting toxicity is myelosuppression, consisting primarily of neutropenia, whereas in some continuous infusion schedules thrombocytopenia was more pronounced.[25,104] Anemia and non-hematological side effects, including nausea, vomiting, diarrhea, fatigue, asthenia, alopecia and mucositis, are common but usually mild, and do not seem to be schedule dependent. Most of the studies showed that prolonged exposure to i.v. administered topotecan is feasible. Randomized comparison of the daily times 5 schedule every 3 weeks and 24-h infusion once a week for 4 weeks repeated every 6 weeks in patients with ovarian cancer suggests that the daily times 5 topotecan regimen was significantly superior with respect to response rate.[109,110] Randomized studies between the 5-day schedule and the 21-day continuous infusion schedule are not yet available.[111]

Regarding the administration of CPT-11, different schedules are currently being used in Europe (350 mg/m^2 as a 90-min infusion once every 3 weeks),[112] the USA (125 mg/m^2 given weekly as a 90-min infusion for 4 or 6 weeks)[113] and Japan (100 mg/m^2 given weekly as a 30-min infusion).[114] In all these dosing schedules, the total amount of CPT-11 that can be tolerated in any time period is similar.[115] Schedules with protracted infusions that have been investigated vary between 96 h weekly to 14-day continuous infusion.[116,27,117] The maximum tolerated dose in these studies (10–30 mg/m^2/day) is much lower then for the short duration schedules. Surprisingly, the AUC of the active metabolite SN-38 reaches comparable levels as reported for the short-duration regimens, which is not completely understood. One possible explanation would be that the enhanced metabolism of CPT-11 relates to saturation of carboxylesterase-mediated conversion of CPT-11 with (high-dose) short infusion schedules. As for toxicity, myelosuppression and diarrhea are the dose-limiting events in all tested regimens. Like with topotecan, protracted low-dose schedules give rise to more thrombocytopenia,[116] while in shorter schedules neutropenia is more prominent. The influence of infusion duration on antitumor activity has not yet been evaluated in a randomized setting.

Oral dosing

As indicated, the high specificity in the mechanism of action of CPT analogs for the S-phase in the cell cycle has led to the recognition that the compounds may require prolonged exposure to maximize the fractional cell kill. In this regard, the availability of clinically useful oral formulations of currently available CPT analogs would provide increased convenience for the administration of chronic dosing regimens and the opportunity for cost-effective outpatient therapy.[94] Since most of the CPT analogs have relatively short terminal disposition half-lives, the use of protracted oral dosing is not necessarily the same as continuous i.v. infusion,

although if the postulated concept of time over threshold concentration is a valid indication of both toxicity and efficacy, oral dosing can mimic continuous infusion regimens. Formal oral bioavailability studies have been conducted for several agents, and have yielded bioavailability values for topotecan of 30–44%,[118–120] for 9-AC of about 50% depending on the formulation applied[121,122] and for lurtotecan of 11%.[78] Clinical data are not yet available for CPT-11, although murine data show an oral bioavailability of between 10 and 20%, depending on the dose administered.[123]

The development toward suitable oral regimens for CPT analogs has to date been most extensively studied for topotecan using daily or bi-daily administration of 5-,[124] 10-,[125] or 21-day schedules.[126] A variety of clinical studies have shown that with an increase in prolonged topotecan administration by this route, a shift occurs in dose-limiting toxicity from hematological toxicity (mainly granulocytopenia) toward severe gastrointestinal side effects, most notably diarrhea.[127] These investigations further indicated that the schedule applied, rather than the applied systemic exposure per course seemed to be related to the type of experienced toxicity.[127] Based on these considerations, the daily times 5 schedule has been recommended for future studies. The need for further clinical development of the oral topotecan formulation became even more important in view of recent findings that the oral formulation has similar efficacy in the treatment of advanced ovarian and small-cell lung cancers as compared to the i.v. formulation, while less hematological toxicity was observed.[128,129]

Based on theoretical considerations, including the fact that carboxylesterases are highly expressed in human liver and the gastrointestinal tract that could result in presystemic metabolism to SN-38,[130] it appears particularly attractive to deliver CPT-11 by the oral route. Indeed, the preliminary findings of substantially increased SN-38 to CPT-11 concentration ratios with oral CPT-11 administration as compared to i.v. administration seem to sustain this notion.[131,132] In addition, oral drug administration was associated with increased persistence of circulating levels of the lactone form of SN-38, which might be an additional advantage with potential pharmacodynamic importance.[133] The clinical utility of oral CPT-11 administration is currently under further investigation.

Local drug administration

Hepatic arterial dosing The narrow therapeutic window of systemic administration of CPT and its analogs has prompted a search toward local drug administration, with the rationale to obtain selectively higher activity against locally confined tumors and/or lower systemic toxicity without loss of antitumor activity. The pharmacokinetic behavior of CPT-11 was recently compared during 5-day hepatic arterial and i.v. infusion in a group a cancer patients.[134] These findings indicated that arterial drug administration leads to significantly higher conversion of CPT-11 into the active metabolite as compared to the i.v. administration, although the clinical relevance of this observation is, as yet, unknown.[135] In recent years, various agents have also been used for arterial embolization in an attempt to encapsulate the concomitantly administered chemotherapeutic agent and, thereby, further enhance the local drug concentration. This concept has been tested with CPT-11 administered with hepatic arterial chemoembolization to patients with primary and metastatic hepatic malignancies.[134] Further studies are clearly required to confirm efficacy of this treatment, and should aim at measuring systemic and tumor tissue concentrations.

Pulmonary delivery Liposomal aerosol formulations of CPT and 9-NC have recently been developed for nasal inhalation treatment of experimental lung tumors xenografted in nude mice. It was found that this preparation was strikingly effective in the treatment of these xenografts growing s.c. over the thorax at doses much lower than those traditionally used in preclinical models administered by other routes.[136] Interestingly, 9-NC aerosol therapy was also effective against established melanoma and osteosarcoma lung metastases.[137] Concurrent pharmacokinetic studies showed that this type of treatment results in a prompt pulmonary distribution at substantial levels that could not be achieved with conventional routes of delivery, including oral and intramuscular.[138,139] Overall, these data suggest that local delivery of CPT analogs to the respiratory tract by liposome aerosol treatment might offer advantages over existing methods in the treatment of some diseases.

Intraperitoneal dosing Intraperitoneal administration has been used as a strategy for increasing total drug delivery to ovarian cancers confined to the peritoneal cavity. The pharmacokinetic behavior of topotecan suggests that a substantial pharmacokinetic advantage might be obtained following i.p. injection. Indeed, recent clinical evidence suggests very slow peritoneal clearance of topotecan and high peritoneal:plasma concentration ratios of >10 after i.p. drug administration.[140] Intraperitoneal administration of CPT-11 has also been studied recently in animal models and showed some potential advantages over the i.v. route. It appeared that the therapy was more

efficient with an increase in life span and was less toxic as compared to the i.v. route in mice bearing C26 colon tumors. In addition, substantially elevated AUCs of CPT-11 and SN-38 were found in the peritoneum, although plasma levels were comparable to i.v. dosing.[140] The clinical implications of these observations have not yet been evaluated.

Biomodulation

Pharmacodynamic alterations

The use of biomodulators to increase the therapeutic index of chemotherapeutic treatment has made a significant impact on certain diseases.[141] A number of cancer chemotherapy biomodulators have been approved for clinical use in humans and these agents can modulate anticancer drugs through either pharmacodynamic or -kinetic modulations. Among extensively studied biomodulating agents are the class of hematopoietic growth factors to decrease chemotherapy-induced neutropenia and anemia. Initial attempts to increase topotecan dose intensity with the use of granulocyte colony stimulating factor (G-CSF) failed, since thrombocytopenia and fatigue rapidly emerged as dose-limiting effects with the daily times 5 schedule.[142,143] On the other hand, G-CSF administered after five daily infusions of topotecan permitted a 2.3-fold dose escalation above the maximum-tolerated dose,[144,145] although it was concluded that the substantial toxicity, inconvenience and costs associated with this high-dose topotecan/G-CSF regimen does not warrant further development. Similarly disappointing results have been obtained with topotecan or 9-AC administered by prolonged continuous infusions.[146,147] The addition of G-CSF with CPT-11 administration has also been advocated by some investigators;[148,149] evidence for increased dose intensity or clearly improved chemotherapy based on G-CSF support is still lacking. Therefore, the use of G-CSF outside clinical trials to support chemotherapeutic treatment is not recommended.

The principal non-hematologic toxicity for all topoisomerase I inhibitors is gastrointestinal. Nausea and vomiting are frequent[56,75,104,112] but, with the introduction of selective serotonine antagonist of the 5-HT_3 -receptor, this side effect is adequately manageable. Diarrhea is also frequent and mild to moderate in severity with i.v. administration schedules. It appears to be unrelated to the schedule used,[56,75,104,112] except in the case of CPT-11, where with oral administration diarrhea becomes the prime dose-limiting toxicity especially when using the prolonged schedules.[24,122,125,126,150]

In the treatment with CPT-11 two types of diarrhea can be distinguished, i.e. an early- and a late-onset form. The early-onset diarrhea is part of a cholinergic-like syndrome and manifests in sweating, salivation, abdominal cramping and diarrhea.[151] Interestingly, this cholinergic syndrome has not been described for other CPT analogs. It has been argued that the unique structural features of CPT-11, including a bipiperidino group that shows similarity with a known stimulant of nicotine receptors of autonomic ganglia, dimethylphenylpiperazinium iodide, is responsible for this phenomenon.[152] More recently, it has been found that the mechanism behind the transient cholinerigic reaction observed clinically is more likely mediated through a rapid reversible inhibition of acetylcholinesterase by the lactone form of CPT-11.[153] Clinical evidence indicates that this side effect can be adequately treated in the acute phase as well as prophylactically with the use of i.v. atropine.

CPT-11-induced delayed-type diarrhea has been reported to be severe (NCI-CTC grade 3–4) in 11–23% of patients,[113,154] but even the less severe diarrhea still might influence continuation of therapy. Moreover, diarrhea related to drug-induced colon-mucosal damage, as observed in both rodents[155] and humans,[156] can cause severe and potentially lethal illness, especially during concomitant occurrence of neutropenia. Once delayed-type diarrhea has occurred, high-dose regimen loperamide renders the diarrhea manageable.[157]

Prophylactic treatment of this frequently observed side effect has been investigated in numerous studies. Potential modulation of delayed-type diarrhea has been examined clinically with several agents, including an enkephalinase inhibitor [158] and glutamine,[159] and in animal models with a lipopeptide[160] and interleukin-15,[161] with different results. A recent preliminary report on co-treatment with thalidomide claims a good protection against diarrhea, but we still have to await pharmacokinetic results to weigh this study on its proper value.[162] To explain the mechanism by which CPT-11-mediated delayed-type diarrhea is triggered, many pharmacokinetic analyses in humans have been performed in order to predict the incidence of this diarrhea, with conflicting results. Some studies described a correlation between late-onset diarrhea and the systemic glucuronidation rates of SN-38 (i.e. the biliary index).[163] Recently, it was suggested from animal models that β-glucuronidase activity from the microflora in the large intestines may play a major role in the development of CPT-11-induced diarrhea, by mediating the hydrolysis of biliary secreted SN-38G, thereby causing local accumulation of SN-38, which subsequently causes damage to the intestinal epithe-

lium.[164] This observation has led to experiments in which antibiotic treatment inhibited the β-glucuronidase activity from the intestinal microflora, thereby decreasing the luminal SN-38 concentration, and subsequently reducing intestinal damage and ameliorating diarrhea.[155] A recent study in humans showed that antibiotic treatment with neomycin did not alter SN-38 pharmacokinetics in plasma and gave protection to recurrent diarrhea in over 85% of the patients experiencing diarrhea in the first course (unpublished data, DK and AS). The expected mechanism of blocking bacterial β-glucuronidase activity causing a subsequent rise in the SN-38/SN-38G concentration ratio has recently been confirmed. It would be even more attractive to use an agent that specifically inhibits the microbial β-glucuronidase activity. Hange-shasin-to (also referred to as TJ14), a herbal medicine that contains the β-glucuronidase inhibitor baicalin, has recently been described to be a potent inhibitor of delayed-type diarrhea caused by CPT-11 in a rat model[165] as well as in humans.[166] Unfortunately there is no information yet on possible changes in the systemic disposition of CPT-11 and its metabolites which are likely to occur due to inhibition of plasma β-glucuronidase activity by this agent—information of vital importance in view of antitumor activity.

It has also been speculated based on *in vitro* studies that raising pH values in the intestines by intestinal alkalization might decrease reabsorption of biliary secreted SN-38 after i.v. CPT-11 administration and, as a result, lowers intestinal side effects.[167] Again, demonstration of unaltered pharmacokinetics of SN-38 in the presence of intestinal alkalization is of crucial importance. Thus, although this approach might show reduced CPT-11-mediated intestinal toxicity, this may be a phyrric victory if a simultaneously altered metabolic clearance (by way of a decreased enterohepatic recirculation of SN-38) results in reduced antitumor activity.

Pharmacokinetic alterations

Intestinal metabolic systems and drug efflux pumps located in the intestinal mucosa represent a major limitation in the bioavailability of orally delivered drugs.[168] Several enzymes located in the enterocyte, like the cytochrome P450-3A4 isozyme (CYP3A4), are involved in the presystemic metabolism of many anticancer agents, including etoposide and paclitaxel, thereby limiting the oral absorption of these drugs. Since CYP3A4 is involved in the metabolism of CPT-11, it has been proposed that the bioavailability of this agent might be substantially enhanced by pharmacokinetic modulation of enteric CYP3A4 activity, e.g. by concomitant administration of ketoconazole, erythromycin or quinidine.[169] Similarly, P-glycoprotein and the Breast Cancer Resistance Protein (BCRP), which are abundantly present in the gastrointestinal tract, have been shown recently to limit the intestinal absorption of various agents, including topotecan.[170] Combined inhibition of intestinal P-glycoprotein and BCRP by the investigational agent GF120918 was shown recently to increase the systemic exposure to topotecan in both animals and patients with the bioavailability increasing from a mere 30–44 to >90%, suggesting that modulation of these transporters simultaneously could be considered in the development of substrate anticancer agents given by the oral route.[171]

As indicated, several preclinical studies have identified CYP3A4 to form two pharmacologically inactive oxidation products known as APC and NPC, as one of the principal enzymes involved in CPT-11. In addition, it was shown that ketoconazole, a synthetic imidazole-type broad-spectrum antifungal agent as well as a potent inhibitor of CYP3A4, inhibited APC and NPC formation by 98 and 99%, respectively, at tested concentrations as low as 1 μM.[172,173] Previous investigations indicated that with standard oral doses of ketoconazole (200–400 mg/day), peak plasma concentrations are in the range of 4–20 μM, suggesting that concomitant treatment of ketoconazole is likely to substantially alter the disposition of CPT-11 administered i.v. to cancer patients. Indeed, a recent pilot pharmacokinetic study in a cancer patient receiving CPT-11 with or without ketoconazole indicated a substantial pharmacokinetic interaction between the two drugs at the level of drug metabolism, and indicated that these agents cannot be administered together without dose adjustments (unpublished data, AS and JV). If confirmed in a larger group of patients, the concomitant administration of ketoconazole might enable CPT-11 dose reductions without affecting systemic exposure to the active metabolite, SN-38, and potentially eliminates interpatient variability in CPT-11 pharmacokinetics that arise as a result of (genetically defined) patient differences in CYP3A4 expression levels.[174]

There are many other potential approaches to improve the therapeutic index of CPT-11 through pharmacokinetic biomodulation, including modulation with inhibitors of biliary secretion processes mediated by P-glycoprotein and/or cMOAT (e.g. cyclosporin A) and with inducers of UDP glucuronosyltransferase isoforms involved in SN-38 glucuronidation (e.g. phenobarbital).[175,176] A clinical trial to evaluate the pharmacological and toxicological implications of a combination regimen of CPT-11, cyclosporin A and

phenobarbital is currently in progress, and preliminary findings indicate substantial antitumor activity with this combination without the occurrence of significant diarrhea despite a very low systemic exposure to SN-38.[177] This finding is supportive of the conjecture that activation of CPT-11 by intratumoral carboxylesterases might be even more important that circulating SN-38 concentrations.[178,179]

Conclusions and future perspectives

CPTs are among the most promising new anticancer drugs that have been developed in recent years. Topotecan and CPT-11 are now registered for the treatment of ovarian and colon cancer, respectively. The unique mechanism of action on topoisomerase I and activity against a broad spectrum of other malignancies are an ongoing stimulus for further clinical development. With the growing knowledge on pharmacodynamics and pharmacokinetics of the different CPT analogs, the poor water solubility and pH-dependent reversible interconvention between the active lactone and inactive carboxylate form, as well as increase in activity or stability by substitutions to specific sites on the molecule, much effort has and will be invested to increase the antitumor activity of this group of drugs. Meanwhile pharmacological modulation, particularly of CPT-11, can be of interest to reduce toxicity and to influence metabolic pathways. Although much effort is being put into development of new analogs, the question to answer remains if drugs under development will ultimately lead to the theoretically expected higher activity and/or reduced toxicity. Last but not least, optimization of schedules, routes of administration and combination therapies will lead to numerous new studies in different tumor types. It is to be expected that in the future many new formulations and/or combinations will be developed. In contrast to other previously registered anticancer drugs, the pharmacological knowledge on how CPT analogs behave in humans will lead to a more logical and quicker development of these agents.

References

1. Wall ME. Camptothecin and taxol: discovery to clinic. *Med Res Rev* 1998; **18**: 299–314.
2. Muggia FM, Creaven PJ, Hansen HH, Cohen MH, Selawry OS. Phase I clinical trial of weekly and daily treatment with camptothecin (NSC-100880): correlation with preclinical studies. *Cancer Chemother Rep* 1972; **56**: 515–21.
3. Schaeppi U, Fleischman RW, Cooney DA. Toxicity of camptothecin (NSC-100880). *Cancer Chemother Rep [3]* 1974; **5**: 25–36.
4. Moertel CG, Schutt AJ, Reitemeier RJ, Hahn RG. Phase II study of camptothecin (NSC-100880) in the treatment of advanced gastrointestinal cancer. *Cancer Chemother Rep* 1972; **56**: 95–101.
5. Creemers GJ, Lund B, Verweij J. Topoisomerase I inhibitors: topotecan and irenotecan. *Cancer Treat Rev* 1994; **20**: 73–96.
6. 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**: 5077–82.
7. Hsiang YH, Hertzberg R, Hecht S, Liu LF. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J Biol Chem* 1985; **260**: 14873–8.
8. Hsiang YH, Liu LF. Identification of mammalian DNA topoisomerase I as an intracellular target of the anticancer drug camptothecin. *Cancer Res* 1988; **48**: 1722–6.
9. Giovanella BC, Stehlin JS, Wall ME, *et al*. DNA topoisomerase I—targeted chemotherapy of human colon cancer in xenografts. *Science* 1989; **246**: 1046–8.
10. Van der Zee AG, Hollema H, de Jong S, *et al*. P-glycoprotein expression and DNA topoisomerase I and II activity in benign tumors of the ovary and in malignant tumors of the ovary, before and after platinum/cyclophosphamide chemotherapy. *Cancer Res* 1991; **51**: 5915–20.
11. Potmesil M. Camptothecins: from bench research to hospital wards. *Cancer Res* 1994; **54**: 1431–9.
12. Van Cutsem E, Cunningham D, ten Bokkel Huinink WW, *et al*. Clinical activity and benefit of irinotecan (CPT-11) in patients with colorectal cancer truly resistant to 5-fluorouracil (5-FU). *Eur J Cancer* 1999; **35**: 54–9.
13. ten Bokkel Huinink WW, Gore M, Carmichael J, *et al*. Topotecan versus paclitaxel for the treatment of recurrent epithelial ovarian cancer [see Comments]. *J Clin Oncol* 1997; **15**: 2183–93.
14. Shimada Y, Rothenberg M, Hilsenbeck SG, Burris III HA, Degen D, Von Hoff DD. Activity of CPT-11 (irinotecan hydrochloride), a topoisomerase I inhibitor, against human tumor colony-forming units. *Anti-Cancer Drugs* 1994; **5**: 202–6.
15. Hare CB, Elion GB, Houghton PJ, *et al*. Therapeutic efficacy of the topoisomerase I inhibitor 7-ethyl-10-(4-[1-piperidino]-1-piperidino)-carbonyloxy-camptothecin against pediatric and adult central nervous system tumor xenografts. *Cancer Chemother Pharmacol* 1997; **39**: 187–91.
16. Tanizawa A, Fujimori A, Fujimori Y, Pommier Y. Comparison of topoisomerase I inhibition, DNA damage, and cytotoxicity of camptothecin derivatives presently in clinical trials. *J Natl Cancer Inst* 1994; **86**: 836–42.
17. Humerickhouse R, Lohrbach K, Li L, Bosron WF, Dolan ME. Characterization of CPT-11 hydrolysis by human liver carboxylesterase isoforms hCE-1 and hCE-2. *Cancer Res* 2000; **60**: 1189–92.
18. Iyer L, King CD, Whitington PF, *et al*. Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. *J Clin Invest* 1998; **101**: 847–54.

19. Burris III HA, 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**: 1816–20.
20. Houghton PJ, Cheshire PJ, Hallman JD, *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.
21. Daoud SS, Fetouh MI, Giovanella BC. Antitumor effect of liposome-incorporated camptothecin in human malignant xenografts. *Anti-Cancer Drugs* 1995; **6**: 83–93.
22. Giovanella BC, Hinz HR, Kozielski AJ, Stehlin Jr JS, Silber R, Potmesil M. Complete growth inhibition of human cancer xenografts in nude mice by treatment with 20-(*S*)-camptothecin. *Cancer Res* 1991; **51**: 3052–5.
23. Pantazis P, Hinz HR, Mendoza JT, *et al*. Complete inhibition of growth followed by death of human malignant melanoma cells *in vitro* and regression of human melanoma xenografts in immunodeficient mice induced by camptothecins. *Cancer Res* 1992; **52**: 3980–7.
24. de Jonge MJ, Punt CJ, Gelderblom AH, *et al*. Phase I and pharmacologic study of oral (PEG-1000) 9-aminocamptothecin in adult patients with solid tumors. *J Clin Oncol* 1999; **17**: 2219–26.
25. Hochster H, Liebes L, Speyer J, *et al*. Phase I trial of low-dose continuous topotecan infusion in patients with cancer: an active and well-tolerated regimen. *J Clin Oncol* 1994; **12**: 553–9.
26. Herben VM, Schellens JH, Swart M, *et al*. Phase I and pharmacokinetic study of irinotecan administered as a low-dose, continuous intravenous infusion over 14 days in patients with malignant solid tumors. *J Clin Oncol* 1999; **17**: 1897–905.
27. Herben VM, ten Bokkel Huinink WW, Schot ME, Hudson I, Beijnen JH. Continuous infusion of low-dose topotecan: pharmacokinetics and pharmacodynamics during a phase II study in patients with small cell lung cancer. *Anti-Cancer Drugs* 1998; **9**: 411–8.
28. Kindler HL, Kris MG, Smith IE, *et al*. Phase II trial of topotecan administered as a 21-day continuous infusion in previously untreated patients with stage IIIB and IV non-small-cell lung cancer. *Am J Clin Oncol* 1998; **21**: 438–41.
29. Creemers GJ, Gerrits CJ, Schellens JH, *et al*. Phase II and pharmacologic study of topotecan administered as a 21-day continuous infusion to patients with colorectal cancer. *J Clin Oncol* 1996; **14**: 2540–5.
30. Chourpa I, Beljebbar A, Sockalingum GD, Riou JF, Manfait M. Structure–activity relation in camptothecin antitumor drugs: why a detailed molecular characterisation of their lactone and carboxylate forms by Raman and SERS spectroscopies? *Biochim Biophys Acta* 1997; **1334**: 349–60.
31. Takimoto CH, Wright J, Arbuck SG. Clinical applications of the camptothecins. *Biochim Biophys Acta* 1998; **1400**: 107–19.
32. Sugimori M, Ejima A, Ohsuki S, *et al*. Synthesis and antitumor activity of ring A- and F-modified hexacyclic camptothecin analogues. *J Med Chem* 1998; **41**: 2308–18.
33. Luzzio MJ, Besterman JM, Emerson DL, *et al*. Synthesis and antitumor activity of novel water soluble derivatives of camptothecin as specific inhibitors of topoisomerase I. *J Med Chem* 1995; **38**: 395–401.
34. Uehling DE, Nanthakumar SS, Croom D, *et al*. Synthesis, topoisomerase I inhibitory activity, and *in vivo* evaluation of 11-azacamptothecin analogs. *J Med Chem* 1995; **38**: 1106–18.
35. Vladu B, Woynarowski JM, Manikumar G, *et al*. 7- and 10-substituted camptothecins: dependence of topoisomerase I–DNA cleavable complex formation and stability on the 7- and 10-substituents. *Mol Pharmacol* 2000; **57**: 243–51.
36. Chourpa I, Riou JF, Millot JM, Pommier Y, Manfait M. Modulation in kinetics of lactone ring hydrolysis of camptothecins upon interaction with topoisomerase I cleavage sites on DNA. *Biochemistry* 1998; **37**: 7284–91.
37. Burke TG, Munshi CB, Mi Z, Jiang Y. The important role of albumin in determining the relative human blood stabilities of the camptothecin anticancer drugs [Letter] [published erratum appears in *J Pharm Sci* 1995; **84**: 1492]. *J Pharm Sci* 1995; **84**: 518–9.
38. 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. *Anti-Cancer Drugs* 1999; **10**: 705–10.
39. Machida Y, Onishi H, Kurita A, Hata H, Morikawa A, Machida Y. Pharmacokinetics of prolonged-release CPT-11-loaded microspheres in rats. *J Control Rel* 2000; **66**: 159–75.
40. Shenderova A, Burke TG, Schwendeman SP. The acidic microclimate in poly(lactide-co-glycolide) microspheres stabilizes camptothecins. *Pharmac Res* 1999; **16**: 241–8.
41. Shenderova A, Burke TG, Schwendeman SP. Stabilization of 10-hydroxycamptothecin in poly(lactide-co-glycolide) microsphere delivery vehicles. *Pharmac Res* 1997; **14**: 1406–14.
42. Ertl B, Platzer P, Wirth M, Gabor F. Poly(D,L-lactic-co-glycolic acid) microspheres for sustained delivery and stabilization of camptothecin. *J Control Rel* 1999; **61**: 305–17.
43. Shenderova A, Burke TG, Schwendeman SP. The acidic microclimate in poly(lactide-co-glycolide) microspheres stabilizes camptothecins. *Pharmac Res* 1999; **16**: 241–8.
44. Subramanian D, Muller MT. Liposomal encapsulation increases the activity of the topoisomerase I inhibitor topotecan. *Oncol Res* 1995; **7**: 461–9.
45. Burke TG, Gao X. Stabilization of topotecan in low pH liposomes composed of distearoylphosphatidylcholine. *J Pharmac Sci* 1994; **83**: 967–9.
46. Sadzuka Y, Hirotsu S, Hirota S. Effect of liposomalization on the antitumor activity, side-effects and tissue distribution of CPT-11. *Cancer Lett* 1998; **127**: 99–106.
47. Sadzuka Y, Hirotsu S, Hirota S. Effective irinotecan (CPT-11)-containing liposomes: intraliposomal conversion to the active metabolite SN-38. *Jpn J Cancer Res* 1999; **90**: 226–32.
48. Tardi P, Choice E, Masin D, Redelmeier T, Bally M, Madden TD. Liposomal encapsulation of topotecan enhances anticancer efficacy in murine and human xenograft models. *Cancer Res* 2000; **60**: 3389–93.
49. Emerson DL, Bendele R, Brown E, *et al*. Antitumor efficacy, pharmacokinetics, and biodistribution of NX 211: a low-clearance liposomal formulation of lurtotecan [In process citation]. *Clin Cancer Res* 2000; **6**: 2903–12.

50. Colbern GT, Dykes DJ, Engbers C, *et al*. Encapsulation of the topoisomerase I inhibitor GL147211C in pegylated (STEALTH) liposomes: pharmacokinetics and antitumor activity in HT29 colon tumor xenografts. *Clin Cancer Res* 1998; **4**: 3077–82.
51. Loos WJ, Kehrer D, Brouwer E, *et al*. Liposomal lurtotecan (NX211): determination of total drug levels in human plasma and urine by reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 2000; **738**: 155–63.
52. Yang SC, Lu LF, Cai Y, Zhu JB, Liang BW, Yang CZ. Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. *J Control Rel* 1999; **59**: 299–307.
53. Underberg WJ, Goossen RM, Smith BR, Beijnen JH. Equilibrium kinetics of the new experimental antitumour compound SKF 104864-A in aqueous solution. *J Pharm Biomed Anal* 1990; **8**: 681–3.
54. Saltz LB, Kemeny NE, Tong W, Harrison J, Berkery R, Kelsen DP. 9-Aminocamptothecin by 72-hour continuous intravenous infusion is inactive in the treatment of patients with 5-fluorouracil-refractory colorectal carcinoma. *Cancer* 1997; **80**: 1727–32.
55. Rubin E, Wood V, Bharti A, *et al*. A phase I and pharmacokinetic study of a new camptothecin derivative, 9-aminocamptothecin. *Clin Cancer Res* 1995; **1**: 269–76.
56. Dahut W, Harold N, Takimoto C, *et al*. Phase I and pharmacologic study of 9-aminocamptothecin given by 72-hour infusion in adult cancer patients. *J Clin Oncol* 1996; **14**: 1236–44.
57. Takimoto CH, Dahut W, Marino MT. Pharmacodynamics and pharmacokinetics of a 72-hour infusion of 9-aminocamptothecin in adult cancer patients. *J Clin Oncol* 1997; **15**: 1492–501.
58. Siu LL, Oza AM, Eisenhauer EA, *et al*. Phase I and pharmacologic study of 9-aminocamptothecin colloidal dispersion formulation given as a 24-hour continuous infusion weekly times four every 5 weeks. *J Clin Oncol* 1998; **16**: 1122–30.
59. Kraut EH, Balcerzak SP, Young D, *et al*. A phase II study of 9-aminocamptothecin in patients with refractory breast cancer. *Cancer Invest* 2000; **18**: 28–31.
60. Vey N, Kantarjian H, Tran H, *et al*. Phase I and pharmacologic study of 9-aminocamptothecin colloidal dispersion formulation in patients with refractory or relapsed acute leukemia. *Ann Oncol* 1999; **10**: 577–83.
61. Herben VM, van Gijn R, Schellens JH, *et al*. Phase I and pharmacokinetic study of a daily times 5 short intravenous infusion schedule of 9-aminocamptothecin in a colloidal dispersion formulation in patients with advanced solid tumors. *J Clin Oncol* 1999; **17**: 1906–14.
62. 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**: 2905–9.
63. Pazdur R, Medgyesy DC, Winn RJ, *et al*. Phase II trial of 9-aminocamptothecin (NSC 603071) administered as a 120-hr continuous infusion weekly for three weeks in metastatic colorectal carcinoma. *Invest New Drugs* 1998; **16**: 341–6.
64. Vokes EE, Ansari RH, Masters GA, *et al*. A phase II study of 9-aminocamptothecin in advanced non-small-cell lung cancer [see Comments]. *Ann Oncol* 1998; **9**: 1085–90.
65. Lavergne O, Lesueur-Ginot L, Pla RF, *et al*. Homocamptothecins: synthesis and antitumor activity of novel E-ring- modified camptothecin analogues. *J Med Chem* 1998; **41**: 5410–9.
66. Lavergne O, Demarquay D, Bailly C, *et al*. Topoisomerase I-mediated antiproliferative activity of enantiomerically pure fluorinated homocamptothecins. *J Med Chem* 2000; **43**: 2285–9.
67. 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**: 2939–43.
68. 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**: 1557–62.
69. 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**: 15556–63.
70. 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**: 2939–43.
71. Lavergne O, Bigg DC. [The other camptothecins: recent advances with camptothecin analogues other than irinotecan and topotecan]. *Bull Cancer* 1998; spec no: 51–8.
72. Lavergne O, Harnett J, Rolland A, *et al*. BN 80927: a novel homocamptothecin with inhibitory activities on both topoisomerase I and topoisomerase II. *Bioorg Med Chem Lett* 1999; **9**: 2599–602.
73. Pollack IF, Erff M, Bom D, Burke TG, Strode JT, Curran DP. Potent topoisomerase I inhibition by novel silatecans eliminates glioma proliferation *in vitro* and *in vivo*. *Cancer Res* 1999; **59**: 4898–905.
74. Emerson DL, Besterman JM, Brown HR, *et al*. *In vivo* antitumor activity of two new seven-substituted water-soluble camptothecin analogues. *Cancer Res* 1995; **55**: 603–9.
75. Gerrits CJ, Creemers GJ, Schellens JH, *et al*. Phase I and pharmacological study of the new topoisomerase I inhibitor GI147211, using a daily $\times 5$ intravenous administration. *Br J Cancer* 1996; **73**: 744–50.
76. Eckhardt SG, Baker SD, Eckardt JR, *et al*. Phase I and pharmacokinetic study of GI147211, a water-soluble camptothecin analogue, administered for five consecutive days every three weeks. *Clin Cancer Res* 1998; **4**: 595–604.
77. Paz-Ares L, Kunka R, DeMaria D, *et al*. A phase I clinical and pharmacokinetic study of the new topoisomerase inhibitor GI147211 given as a 72-h continuous infusion. *Br J Cancer* 1998; **78**: 1329–36.
78. Gerrits CJ, Schellens JH, Creemers GJ, *et al*. The bioavailability of oral GI147211 (GG211), a new topoisomerase I inhibitor. *Br J Cancer* 1997; **76**: 946–51.
79. Mitsui I, Kumazawa E, Hirota Y, *et al*. A new water-soluble camptothecin derivative, DX-8951f, exhibits potent antitumor activity against human tumors *in vitro* and *in vivo*. *Jpn J Cancer Res* 1995; **86**: 776–82.

80. Lawrence RA, Izbicka E, De Jager RL, *et al*. Comparison of DX-8951f and topotecan effects on tumor colony formation from freshly explanted adult and pediatric human tumor cells. *Anti-Cancer Drugs* 1999; **10**: 655–61.
81. Vey N, Giles FJ, Kantarjian H, Smith TL, Beran M, Jeha S. The topoisomerase I inhibitor DX-8951f is active in a severe combined immunodeficient mouse model of human acute myelogenous leukemia. *Clin Cancer Res* 2000; **6**: 731–6.
82. Rowinsky EK, Johnson TR, Geyer Jr CE, *et al*. DX-8951f, a hexacyclic camptothecin analog, on a daily-times-five schedule: a phase I and pharmacokinetic study in patients with advanced solid malignancies. *J Clin Oncol* 2000; **18**: 3151–63.
83. Kamiya Y, Yamamoto N, Yamada Y, *et al*. Phase I and pharmacokinetic (PK) study of DX-8951f, a novel camptothecin analog, given as a 30 minute infusion daily for 5 days. *Clin Cancer Res* 2000; suppl 5: 327.
84. Boige V, Raymond E, Gatineau M, *et al*. Final results of a phase I and pharmacokinetic study of DX-8951f in patients with advanced tumors. *Proc Am Ass Cancer Res* 2000; **40**: 754.
85. Minami H, Sasaki Y, Shigeoka Y, *et al*. Phase I study and pharmacology of DX-8951f, a new camptothecin derivative administered over 30 minutes every 3 weeks. *Clin Cancer Res* 2000; suppl 5: 326.
86. Garrison M, Hammond L, Geyer C, *et al*. A phase I and pharmacokinetic study of the camptothecin (CPT) analog DX-8951f (exetacan mesylate): escalating infusion duration and dose. *Proc Am Soc Clin Oncol* 2000; **19**: 765.
87. Talbot DC, White S, Jones P, *et al*. Phase II study of exatecan mesylate (DX-8951f) in advanced NSCLC. *Proc Am Soc Clin Oncol* 2000; **19**: 2166.
88. O'Reilly EM, Hammond L, Sharma S, *et al*. A phase II study of exatecan mesylate (DX-8951f, DX) in advanced pancreatic cancer. *Proc Am Soc Clin Oncol* 2000; **19**: 1170.
89. Kudelka AP, Verschraegen C, Vincent MD, *et al*. Phase II study of intravenous DX-8951f in patients (Pts) with advanced ovarian, tubal, or peritoneal cancer refractory to platinum, taxane, and topotecan. *Proc Am Soc Clin Oncol* 2000; **19**: 1550.
90. Royce M, Saltz L, Rowinsky EK, *et al*. A phase II study of intravenous exatecan mesylate (Dx-8951f, Dx) administered daily for five days every three weeks to patients with advanced or metastatic adenocarcinoma of the colon or rectum. *Proc Am Soc Clin Oncol* 2000; **19**: 1129.
91. Karaberis E, Mourelatos D. Enhanced cytogenetic and antitumor effects by 9-nitrocamptothecin and antineoplastics. *Teratogen Carcinogen Mutagen* 2000; **20**: 141–6.
92. Pantazis P, Early JA, Mendoza JT, DeJesus AR, Giovanella BC. Cytotoxic efficacy of 9-nitrocamptothecin in the treatment of human malignant melanoma cells *in vitro*. *Cancer Res* 1994; **54**: 771–6.
93. 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**: 3096–100.
94. Gelderblom HA, de Jonge MJ, Sparreboom A, Verweij J. Oral topoisomerase 1 inhibitors in adult patients: present and future. *Invest New Drugs* 1999; **17**: 401–15.
95. 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**: 821–31.
96. 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. *Anti-Cancer Drugs* 1999; **10**: 375–83.
97. Conover CD, Greenwald RB, Pendri A, Shum KL. Camptothecin delivery systems: the utility of amino acid spacers for the conjugation of camptothecin with polyethylene glycol to create prodrugs. *Anticancer Drug Des* 1999; **14**: 499–506.
98. Conover CD, Pendri A, Lee C, Gilbert CW, Shum KL, Greenwald RB. Camptothecin delivery systems: the antitumor activity of a camptothecin- 20-0-polyethylene glycol ester transport form. *Anticancer Res* 1997; **17**: 3361–8.
99. Maeda H, Matsumura Y. Tumoritropic and lymphotropic principles of macromolecular drugs. *Crit Rev Ther Drug Carrier Syst* 1989; **6**: 193–210.
100. Okuno S, Harada M, Yano T, *et al*. Complete regression of xenografted human carcinomas by camptothecin analogue-carboxymethyl dextran conjugate (T-0128). *Cancer Res* 2000; **60**: 2988–95.
101. Caiolfa VR, Zamai M, Fiorino A, *et al*. Polymer-bound camptothecin: initial biodistribution and antitumour activity studies. *J Control Rel* 2000; **65**: 105–19.
102. Ochoa L, Tolcher A, Rizzo J, *et al*. A phase I study of PEG–camptothecin (PEG–CPT) in patients with advanced solid tumors: a novel formulation for an insoluble but active agent. *Proc Am Soc Clin Oncol* 2000; **19**: 198a.
103. de Bono JS, Bisset D, Twelves C, *et al*. Phase I pharmacokinetic (PK) study of MAG-CPT (PNU 166148), a polymeric derivative of camptothecin (CPT). *Proc Am Soc Clin Oncol* 2000; **19**: 198a.
104. Verweij J, Lund B, Beijnen J, *et al*. Phase I and pharmacokinetics study of topotecan, a new topoisomerase I inhibitor. *Ann Oncol* 1993; **4**: 673–8.
105. Rowinsky EK, Kaufmann SH, Baker SD, *et al*. 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**: 1921–30.
106. Haas NB, LaCreta FP, Walczak J, *et al*. Phase I/ pharmacokinetic study of topotecan by 24-hour continuous infusion weekly. *Cancer Res* 1994; **54**: 1220–6.
107. van Warmerdam LJ, ten Bokkel Huinink WW, Rodenhuis S, *et al*. Phase I clinical and pharmacokinetic study of topotecan administered by a 24-hour continuous infusion. *J Clin Oncol* 1995; **13**: 1768–76.
108. Hochster H, Wadler S, Runowicz C, *et al*. Activity and pharmacodynamics of 21-day topotecan infusion in patients with ovarian cancer previously treated with platinum-based chemotherapy. New York Gynecologic Oncology Group. *J Clin Oncol* 1999; **17**: 2553–61.

109. Hoskins P, Eisenhauer E, Beare S, *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**: 2233–7.
110. Mainwaring PN, Nicolson MC, Hickish T, *et al*. Continuous infusional topotecan in advanced breast and non-small-cell lung cancer: no evidence of increased efficacy. *Br J Cancer* 1997; **76**: 1636–9.
111. Kollmannsberger C, Mross K, Jakob A, Kanz L, Bokemeyer C. Topote. *Oncology* 1999; **56**: 1–12.
112. Abigerges D, Chabot GG, Armand JP, Herait P, Gouyette A, Gandia D. Phase I and pharmacologic studies of the camptothecin analog irinotecan administered every 3 weeks in cancer patients. *J Clin Oncol* 1995; **13**: 210–21.
113. Rothenberg ML, Eckardt JR, Kuhn JG, *et al*. Phase II trial of irinotecan in patients with progressive or rapidly recurrent colorectal cancer. *J Clin Oncol* 1996; **14**: 1128–35.
114. de Forni M, Bugat R, Chabot GG, *et al*. Phase I and pharmacokinetic study of the camptothecin derivative irinotecan, administered on a weekly schedule in cancer patients. *Cancer Res* 1994; **54**: 4347–54.
115. Armand JP. CPT-11: clinical experience in phase I studies. *Semin Oncol* 1996; **23**: 27–33.
116. Takimoto CH, Morrison G, Harold N, *et al*. Phase I and pharmacologic study of irinotecan administered as a 96-hour infusion weekly to adult cancer patients. *J Clin Oncol* 2000; **18**: 659–67.
117. Ohe Y, Sasaki Y, Shinkai T, *et al*. Phase I study and pharmacokinetics of CPT-11 with 5-day continuous infusion. *J Natl Cancer Inst* 1992; **84**: 972–4.
118. Schellens JH, Creemers GJ, Beijnen JH, *et al*. Bioavailability and pharmacokinetics of oral topotecan: a new topoisomerase I inhibitor. *Br J Cancer* 1996; **73**: 1268–71.
119. Herben VM, Rosing H, ten Bokkel Huinink WW, *et al*. Oral topotecan: bioavailablity and effect of food co-administration. *Br J Cancer* 1999; **80**: 1380–6.
120. 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**: 454–60.
121. Sparreboom A, de Jonge MJ, Punt CJ, *et al*. Pharmacokinetics and bioavailability of oral 9-aminocamptothecin capsules in adult patients with solid tumors. *Clin Cancer Res* 1998; **4**: 1915–9.
122. Mani S, Iyer L, Janisch L, *et al*. Phase I clinical and pharmacokinetic study of oral 9-aminocamptothecin (NSC-603071). *Cancer Chemother Pharmacol* 1998; **42**: 84–7.
123. Stewart CF, Zamboni WC, Crom WR, Houghton PJ. Disposition of irinotecan and SN-38 following oral and intravenous irinotecan dosing in mice. *Cancer Chemother Pharmacol* 1997; **40**: 259–65.
124. Gerrits CJ, Burris H, Schellens JH, *et al*. Five days of oral topotecan (Hycamtin), a phase I and pharmacological study in adult patients with solid tumours. *Eur J Cancer* 1998; **34**: 1030–5.
125. Gerrits CJ, Burris H, Schellens JH, *et al*. Oral topotecan given once or twice daily for ten days: a phase I pharmacology study in adult patients with solid tumors. *Clin Cancer Res* 1998; **4**: 1153–8.
126. Creemers GJ, Gerrits CJ, Eckardt JR, *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**: 1087–93.
127. Gerrits CJ, Schellens JH, Burris H, *et al*. A comparison of clinical pharmacodynamics of different administration schedules of oral topotecan (Hycamtin) [see Comments]. *Clin Cancer Res* 1999; **5**: 69–75.
128. von Pawel J, Gatzemeier U, Harstrick A, *et al*. A multicentre randomised phase II study of oral topotecan versuis IV topotecan for second line therapy i sensitive patients with small cell lung cancer. *Proc Am Soc Clin Oncol* 1998; **17**: 1816.
129. Gore M, Rustin G, Calvert AH, *et al*. A multicentre, randomised, phase III study of topotecan (T) administered intravenously or orally for advanced epithelial ovarian carcinoma. *Proc Am Soc Clin Oncol* 1998; **17**: 1346.
130. Guichard S, Terret C, Hennebelle I, *et al*. CPT-11 converting carboxylesterase and topoisomerase activities in tumour and normal colon and liver tissues. *Br J Cancer* 1999; **80**: 364–70.
131. Thompson J, Zamboni WC, Cheshire PJ, *et al*. Efficacy of oral irinotecan against neuroblastoma xenografts. *Anti-Cancer Drugs* 1997; **8**: 313–22.
132. 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**: 455–62.
133. 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**: 685–96.
134. Guthrie TH, Sanal SM, Agaliotis DP, Cristea MC, Khan W, Swischuk PN. Hepatic artery chemoembolization (CE) with irinotecan (I) for primary and metstatic liver cancer; Preliminary results. *Proc Am Soc Clin Oncol* 2000; **19**: 305a.
135. Van der Vijgh WJ, Van Groeningen C, Kedde MA, *et al*. Pharmacokinetic and pharmacodynamics of CPT-11 during 5-day hepatic arterial (HAI) and intravenous (i.v.) infusion. *Proc Am Soc Clin Oncol* 2000; **39**: 600.
136. Knight V, Koshkina NV, Waldrep JC, Giovanella BC, Gilbert BE. Anticancer effect of 9-nitrocamptothecin liposome aerosol on human cancer xenografts in nude mice. *Cancer Chemother Pharmacol* 1999; **44**: 177–86.
137. Koshkina NV, Kleinerman ES, Waidrep C, *et al*. 9-Nitrocamptothecin liposome aerosol treatment of melanoma and osteosarcoma lung metastases in mice. *Clin Cancer Res* 2000; **6**: 2876–80.
138. Koshkina NV, Gilbert BE, Waldrep JC, Seryshev A, Knight V. Distribution of camptothecin after delivery as a liposome aerosol or following intramuscular injection in mice. *Cancer Chemother Pharmacol* 1999; **44**: 187–92.
139. Knight V, Koshkina N, Waldrep C, Giovanella BC, Kleinerman E, Gilbert B. Anti-cancer activity of 9-nitrocamptothecin liposome aerosol in mice. *Trans Am Clin Climatol Ass* 2000; **111**: 135–45.
140. Plaxe SC, Christen RD, O'Quigley J, *et al*. Phase I and pharmacokinetic study of intraperitoneal topotecan. *Invest New Drugs* 1998; **16**: 147–53.

141. Masson E, Zamboni WC. Pharmacokinetic optimisation of cancer chemotherapy. Effect on outcomes. *Clin Pharmacokinet* 1997; **32**: 324–43.
142. Saltz L, Janik JE. Topotecan and the treatment of recurrent ovarian cancer: is there a role for granulocyte colony-stimulating factor? *Semin Oncol* 1997; **24**: S5.
143. Saltz L, Sirott M, Young C, *et al*. Phase I clinical and pharmacology study of topotecan given daily for 5 consecutive days to patients with advanced solid tumors, with attempt at dose intensification using recombinant granulocyte colony- stimulating factor [published erratum appears in *J Natl Cancer Inst* 1993; **85**: 1777]. *J Natl Cancer Inst* 1993; **85**: 1499–507.
144. Rowinsky EK, Baker SD, Burks K, O'Reilly S, Donehower RC, Grochow LB. High-dose topotecan with granulocyte-colony stimulating factor in fluoropyrimidine-refractory colorectal cancer: a phase II and pharmacodynamic study. *Ann Oncol* 1998; **9**: 173–80.
145. Rowinsky EK, Grochow LB, Sartorius SE, *et al*. Phase I and pharmacologic study of high doses of the topoisomerase I inhibitor topotecan with granulocyte colony-stimulating factor in patients with solid tumors. *J Clin Oncol* 1996; **14**: 1224–35.
146. Abbruzzese JL, Madden T, Sugarman SM, *et al*. Phase I clinical and plasma and cellular pharmacological study of topotecan without and with granulocyte colony-stimulating factor. *Clin Cancer Res* 1996; **2**: 1489–97.
147. Wilson WH, Little R, Pearson D, *et al*. Phase II and dose-escalation with or without granulocyte colony-stimulating factor study of 9-aminocamptothecin in relapsed and refractory lymphomas [published errata appear in *J Clin Oncol* 1998; **16**: 2895 and 1999; **17**: 1964]. *J Clin Oncol* 1998; **16**: 2345–51.
148. Masuda N, Fukuoka M, Kudoh S, *et al*. Phase I and pharmacologic study of irinotecan and etoposide with recombinant human granulocyte colony-stimulating factor support for advanced lung cancer. *J Clin Oncol* 1994; **12**: 1833–41.
149. Fujita A, Takabatake H, Tagaki S, Sekine K. Phase I/II study of cisplatin, ifosfamide and irinotecan with rhG-CSF support in patients with stage IIIB and IV non-small-cell lung cancer. *Cancer Chemother Pharmacol* 2000; **45**: 279–83.
150. Verschraegen CF, Natelson EA, Giovanella BC, *et al*. A phase I clinical and pharmacological study of oral 9-nitrocamptothecin, a novel water-insoluble topoisomerase I inhibitor. *Anti-Cancer Drugs* 1998; **9**: 36–44.
151. Bleiberg H, Cvitkovic E. Characterisation and clinical management of CPT-11 (irinotecan)-induced adverse events: the European perspective. *Eur J Cancer* 1996; **32A**(suppl 3): S18–23.
152. Gandia D, Abigerges D, Armand JP, *et al*. CPT-11-induced cholinergic effects in cancer patients [Letter]. *J Clin Oncol* 1993; **11**: 196–7.
153. Dodds HM, Rivory LP. The mechanism for the inhibition of acetylcholinesterases by irinotecan (CPT-11). *Mol Pharmacol* 1999; **56**: 1346–53.
154. Rougier P, Bugat R, Douillard JY, *et al*. 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**: 251–60.
155. Takasuna K, Hagiwara T, Hirohashi M, *et al*. Inhibition of intestinal microflora beta-glucuronidase modifies the distribution of the active metabolite of the antitumor agent, irinotecan hydrochloride (CPT-11) in rats. *Cancer Chemother Pharmacol* 1998; **42**: 280–6.
156. Van Huyen JP, Bloch F, Attar A, *et al*. Diffuse mucosal damage in the large intestine associated with Irinotecan (CPT-11). *Dig Dis Sci* 1998; **43**: 2649–51.
157. Abigerges D, Armand JP, Chabot GG, *et al*. Irinotecan (CPT-11) high-dose escalation using intensive high-dose loperamide to control diarrhea. *J Natl Cancer Inst* 1994; **86**: 446–9.
158. Ychou M, Douillard JY, Rougier P, *et al*. Randomized comparison of prophylactic antidiarrheal treatment versus no prophylactic antidiarrheal treatment in patients receiving CPT-11 (irinotecan) for advanced 5-FU-resistant colorectal cancer: an open-label multicenter phase II study. *Am J Clin Oncol* 2000; **23**: 143–8.
159. Savarese D, Al Zoubi A, Boucher J. Glutamine for irinotecan diarrhea [Letter]. *J Clin Oncol* 2000; **18**: 450–1.
160. Shinohara H, Killion JJ, Kuniyasu H, Kumar R, Fidler IJ. Prevention of intestinal toxic effects and intensification of irinotecan's therapeutic efficacy against murine colon cancer liver metastases by oral administration of the lipopeptide JBT 3002. *Clin Cancer Res* 1998; **4**: 2053–63.
161. Cao S, Black JD, Troutt AB, Rustum YM. Interleukin 15 offers selective protection from irinotecan-induced intestinal toxicity in a preclinical animal model. *Cancer Res* 1998; **58**: 3270–4.
162. Govindarajan R, Heaton KM, Broadwater R, Zeitlin A, Lang NP, Hauer-Jensen M. Effect of thalidomide on gastrointestinal toxic effects of irinotecan [Letter]. *Lancet* 2000; **356**: 566–7.
163. Gupta E, Lestingi TM, Mick R, Ramirez J, Vokes EE, Ratain MJ. Metabolic fate of irinotecan in humans: correlation of glucuronidation with diarrhea. *Cancer Res* 1994; **54**: 3723–5.
164. Takasuna K, Hagiwara T, Hirohashi M, *et al*. Involvement of beta-glucuronidase in intestinal microflora in the intestinal toxicity of the antitumor camptothecin derivative irinotecan hydrochloride (CPT-11) in rats. *Cancer Res* 1996; **56**: 3752–7.
165. Takasuna K, Kasai Y, Kitano Y, *et al*. Protective effects of kampo medicines and baicalin against intestinal toxicity of a new anticancer camptothecin derivative, irinotecan hydrochloride (CPT-11), in rats. *Jpn J Cancer Res* 1995; **86**: 978–84.
166. Mori K, Hirose T, Machida S, Tominaga K. [Kampo medicines for the prevention of irinotecan-induced diarrhea in advanced non-small cell lung cancer]. *Gan To Kagaku Ryoho* 1998; **25**: 1159–63.
167. Kobayashi K, Bouscarel B, Matsuzaki Y, Ceryak S, Kudoh S, Fromm H. pH-dependent uptake of irinotecan and its active metabolite, SN-38, by intestinal cells. *Int J Cancer* 1999; **83**: 491–6.
168. Benet LZ, Izumi T, Zhang Y, Silverman JA, Wacher VJ. Intestinal MDR transport proteins and P-450 enzymes as barriers to oral drug delivery. *J Control Rel* 1999; **62**: 25–31.

169. Kobayashi K, Jodrell DI, Ratain MJ. Pharmacodynamic-pharmacokinetic relationships and therapeutic drug monitoring. *Cancer Surv* 1993; **17**: 51–78.
170. 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**: 4559–63.
171. Scheffer GL, Maliepaard M, Pijnenborg AC, *et al*. Breast cancer resistance protein is localized at the plasma membrane in mitoxant. *Cancer Res* 2000; **60**: 2589–93.
172. Dodds HM, Haaz MC, Riou JF, Robert J, Rivory LP. Identification of a new metabolite of CPT-11 (irinotecan): pharmacological properties and activation to SN-38. *J Pharmacol Exp Ther* 1998; **286**: 578–83.
173. Haaz MC, Rivory L, Riche C, Vernillet L, Robert J. Metabolism of irinotecan (CPT-11) by human hepatic microsomes: participation of cytochrome P-450 3A and drug interactions. *Cancer Res* 1998; **58**: 468–72.
174. Santos A, Zanetta S, Cresteil T, *et al*. Metabolism of irinotecan (CPT-11) by CYP3A4 and CYP3A5 in humans [In process citation]. *Clin Cancer Res* 2000; **6**: 2012–20.
175. Gupta E, Safa AR, Wang X, Ratain MJ. Pharmacokinetic modulation of irinotecan and metabolites by cyclosporin A. *Cancer Res* 1996; **56**: 1309–14.
176. Gupta E, Wang X, Ramirez J, Ratain MJ. Modulation of glucuronidation of SN-38, the active metabolite of irinotecan, by valproic acid and phenobarbital. *Cancer Chemother Pharmacol* 1997; **39**: 440–4.
177. Iyer L, Ratain MJ. Clinical pharmacology of camptothecins. *Cancer Chemother Pharmacol* 1998; **42**(suppl): S31–43.
178. Danks MK, Morton CL, Pawlik CA, Potter PM. Overexpression of a rabbit liver carboxylesterase sensitizes human tumor cells to CPT-11. *Cancer Res* 1998; **58**: 20–2.
179. Danks MK, Morton CL, Krull 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**: 917–24.

(Received 14 November 2000; accepted 20 November 2000)