Topoisomerase I inhibitors and drug resistance

Ralph E. Parchment¹ & Augusto Pessina²

¹ Division of Hematology-Oncology, The Barbara Ann Karmanos Cancer Institute, Wayne State University, 3900 John R., Detroit, MI, U.S.A.

² Institute of Microbiology, University of Milan, Via Pascal 36, 20133 Milan, Italy

Received 25 May 1998; accepted 25 May 1998

Key words: camptothecin, drug resistance, topo I inhibitors, topoisomerase I

Abstract

DNA topoisomerase I is a nuclear enzyme which catalyzes the conversion of the DNA topology by introducing single-strand breaks into the DNA molecule. This enzyme represents a novel and distinct molecule target for cancer therapy by antitopoisomerase drugs belonging to the camptothecin series of antineoplastics. As many tumors can acquire resistance to drug treatment and become refractory to the chemotherapy it is very important to investigate the mechanisms involved in such a drug resistance for circumventing the phenomenon.

This article describes the role of topoisomerase I in cell functions and the methods used to assess its *in vitro* catalytic activity. It reviews the mechanisms of cytotoxicity of the most specific antitopoisomerase I drugs by considering also the phenomenon of drug resistance. Some factors useful to drive the future perspectives in the development of new topoisomerase I inhibitors are also evidenced and discussed.

Introduction

The success of anticancer chemotherapy is basically due to a cytotoxic mechanism in combination with a selective capacity of the drug to distinguish tumor cells from normal host cells. Thus, resistance to drug toxicity in tumor cells is the major problem for successful cancer treatment because it eliminates therapeutic selectivity.

Generally, metastatic cancers are intrinsically resistant to drug treatment or, in spite of a first promising response, can acquire resistance to multiple drugs (Multiple Drug Resistance) and become refractory at time of relapse or progression. Therefore, it is very important to know the mechanisms involved in drug resistance in order to attempt to circumvent the phenomenon and make the therapy more effective.

Among the cytotoxic drugs currently used in cancer chemotherapy the chemicals which inhibit DNA topoisomerases have a relevant role both for their wide use and for their important implication in the phenomena of resistance. Recently, topoisomerase I inhibitors have received approval for the treatment of human

cancers. HycamtinTM is an oncology product containing topotecan, whereas CytosarTM is the formulated product of CPT-11. In fact, the function of Topoisomerases is to modulate the topology of DNA by complex processes of strand cleavage, passage and following religation. They have an important function in chromosome condensation and segregation other than transposition, recombination, replication, transcription and DNA repair processes (Vinograd *et al.*, 1965; Liu, 1983; Kafani *et al.*, 1986; Zijlstra *et al.*, 1990; Hsiang *et al.*, 1992; Cobb *et al.*, 1997) and for this reason the topoisomerase inhibitors play a decisive role in controlling cell proliferation.

Two types of topoisomerases (Topo I and Topo II) have been distinguished and characterized. Topoisomerase I catalyzes the conversion of the DNA topology by introducing single-strand breaks into the DNA molecule. Topoisomerase II (two mammalian isoforms have been purified: II-alpha of p170 kDa and II-beta of p180 kDa) are able to cleave double-stranded DNA and can support more complex interconversions of the DNA topology by an ATP-dependent strand passage process (Wang, 1985; D'Arpa, 1989).

This paper considers only the function of eukaryotic Topoisomerase I and describes the mechanism of Topoisomerase I inhibitors by discussing the possible ways by which tumoral cells may become resistant to these drugs.

A particular attention is given to the known mechanisms of multi-drug resistance, the '*in vitro*' systems developed for assaying Topoisomerase I activity, and the methods for assessing and investigating drug resistance of primary and established tumor cell lines to topoisomerase I poisons.

Mechanism of Action of Topoisomerase I

Human DNA topoisomerase I is a monomeric protein of 100 kDa (Gellert, 1981) encoded by a single copy gene located on chromosome 20q12-13.2 (Juan *et al.*, 1988; Zhou *et al.*, 1989). Eukaryotic DNA topoisomerase I catalyzes the relaxation of positively and negatively supercoiled DNA acting as a swivel for removing torsional strains from DNA helix. It nicks one DNA strand (cleavage step) and then reseals the break (religation step) after a single rotation event. The whole process is an ATP-independent mechanism which does not require Mg^{2+} (the reaction is stimulated by Mg^{2+} ions and other polycations while it is inhibited by polyanions).

DNA topoisomerase I binds preferentially at the nodes created by the crossing of two duplex elices (Madden *et al.*, 1995) and is assumed to form two different complexes with DNA: a non-cleavable complex and a cleavable complex. The cleavable complex represents a transient covalent intermediate formed by DNA-topoisomerase I which induces a single strand nick. The covalent linking of Topo I to the 3'-phosphoryl end of the broken DNA strand is made possible by the presence of a tyrosine (Champoux, 1981). The consequence of this break is the rotation of the two broken ends of DNA leading to relaxation of the supercoiled DNA. If this complex is treated with a strong protein denaturant (such as Sodium Dodecyl Sulphate or alkali) a protein-linked single strand DNA break is produced (See Figure 1). Also many drugs (as topoisomerase I inhibitors) can stabilize this DNA-Topo I complex.

The enzyme is present in the nucleolous and nucleoplasm of cells (Muller *et al.*, 1985) and the content doubles as the cells progress from G1 to G2/M phases of the cell cycle remaining almost constant when the

cells are normalized for nuclear size (Baker *et al.*, 1995).

As evidenced by many studies, several leukemias and lymphomas (Potmesil *et al.*, 1988), colon adenocarcinomas (Giovannella *et al.*, 1989; Husain, 1994) as well as many different types of human malignancies (Masin *et al.*, 1995; Bronstein *et al.*, 1996; McLeod *et al.*, 1994) express higher levels of topoisomerase I than normal tissues.

The role of topoisomerase I has not been completely clarified even for the multiplicity of biochemical functions described. It seems to be very important in the transcription processes (Zhang *et al.*, 1988; Liu LF, 1989) by promoting both the separation of the two DNA strands (for receiving the RNA-polymerase complex) and the subsequent rapid rotation of DNA (that allows copying of the base sequence into the newly formed RNA strand) (Rose *et al.*, 1988). As suggested by Wang (1996) the topoisomerase system (type I and II) may also prevent excessive supercoiling of DNA generated by a number of processes involving the movement of macromolecular assemblies along DNA, and human topoisomerase I is probably a part of a nucleoprotein complex structure which is involved in the regulation of many genes. Recently Topo I has been identified as specific kinase for phosphorylation of SR protein splicing factors (Rossi *et al.*, 1996) and furthermore there are evidences that it can form molecular complexes with p53 both '*in vitro*' and '*in vivo*' suggesting that p53 mediated response to DNA damage may, at least in part, involve activation of topo I (Gobert *et al.*, 1996). Topoisomerase I appears to be essential also for the genomic insertion of viral DNA (Wang *et al.*, 1991) and its activity is enhanced by phosphorylation and is inactivated by poly-ADP-ribosylation (Pommier, 1990; Turman, 1993). A list of the most important functions attributed to the eukaryotic DNA topoisomerase I is reported in Table I. For a complete review on topoisomerases see Wang, 1996.

In Vitro Assay of Topoisomerase I Activity

Many methods have been developed for quantifying topoisomerase I activity. One strategy is based on the measurement of the damage produced by the enzyme on the DNA molecules after incubation of whole cells or nuclei in the presence of drugs able to stabilize the cleavable complex. These methods are based on the alkaline and neutral elution assay (Khon *et al.*, 1981) that measure strand breaks in DNA as DNA-

Figure 1. Schematic representation of Topo I mechanism. Exposure of the cleavable complex (in equilibrium with non-cleavable) to strong protein denaturant produces a single strand DNA breaks linked to topoisomerase I. After digestion of the topo I with proteinase K the relative quantity of supercoiled and relaxed DNA may be assessed by agarose gel electrophoresis.

DNA cross links by means of radiolabeled thymidine or by detecting differences in the fluorescence of DNA (Kanter and Schwartz, 1982). By similar techniques (as SDS sedimentation or filter binding assay) DNA-bound protein can also be measured, assuming that the majority of the linked protein is topoisomerase (Trask *et al.*, 1984; Liu *et al.*, 1983). All these systems give quantitative but non-specific data owing to the indirect nature of the measurement.

A second group is represented by more specific assays which measure the direct '*in vitro*' relaxing activity of topoisomerase I on supercoiled DNA as substrate (e.g.: pBR322, ColE1, pUC18, PC15, pAO3, OX174). This tests, first described by Wang 1971, measures the catalytic activity of Topo I in the absence of ATP and Mg^{2+} by detecting the nicked intermediate complex after protein denaturation with SDS. Following digestion by proteinase K, the relaxed product (nicked open circular DNA) may be quantified on agarose gel electrophoresis by ethidium bromide staining. The test works well also with crude nuclear extract and supercoiled substrate. This relaxation test has been widely used to study specific '*in vitro*' effect of drugs and chemical on topoisomerase I either for evaluating the capacity of a compound to stabilize the nicked intermediate or to inhibit catalytic activity of topoisomerase I (Hussy *et al.*, 1986).

Another technique is based on the detection of the enzyme by means of specific anti-topo I antibodies directly performed on whole cells (immunocytochemistry) or by Western blot analysis of the nuclear extract. This type of assays is highly specific but does not seem to be predictive of the biological activity of the enzyme (Tsuruo *et al.*, 1988).

A quantitative polymerase chain reaction (PCR) method to determine DNA topoisomerase I in cell lines and tumor samples has been developed and reported by Yang *et al.*, 1993. For a detailed review concerning '*in vitro*' assays used to measure the activity of topoisomerases see Barrett *et al.*, 1990. Many of these powerful new technologies are available commercially as an assay kit by Topogen inc. (Ohio, U.S.A.).

Topoisomerase I Inhibitors

Intercalator drugs of the acridine group (Pommier, 1985), actinomycin-D (Trask *et al.*, 1988) distamycin (Smith, 1990) and also heparin have evidenced the capacity to inhibit *in vitro* the topoisomerase I activity present in nuclear extract (Ishii, 1987). Recently Chen *et al.* (1993) pointed out the capacity of Hoechst dye 33342 to interrupt the breakage/reunion reac-

Table 1. Functions of eukaryotic DNA topoisomerase I

Function	Reference
RNA transcription	Javaherian <i>et al.</i> , Nucl. Acids Res. 11: 461, 1983. Egyhazi and Durban, Mol. Cell Biol. 12: 4308, 1983. Garg <i>et al.</i> , PNAS 84: 3185, 1987. Stewart and Schutz, Cell 50: 1109, 1987. Zhang <i>et al.</i> , PNAS 85: 1060, 1989. Camilloni <i>et al.</i> , PNAS 86: 3080, 1989.
RNA splicing	Rossi <i>et al.</i> , Nature 381: 80, 1996.
RNA polymerase II cofactor	Merino <i>et al.</i> , Nature 365: 227, 1993. Kretschmar <i>et al.</i> , PNAS 90:11508, 1993.
Nucleolin interaction	Bharti <i>et al.</i> , J. Biol. Chem. 271, 1993, 1996.
SV40 DNA replication	Brill <i>et al.</i> , Nature 326: 414, 1987. Yang <i>et al.</i> , PNAS 84: 950, 1987.
Genomic insertion of viral DNA	Wang <i>et al.</i> , J. Virol. 65: 2381, 1991.
Recombination	Eng <i>et al.</i> , Mol. Pharmacol. 34: 755, 1989. Hsiang <i>et al.</i> , J. Biol. Chem. 260: 14873, 1985. Nitiss <i>et al.</i> , PNAS 85: 7501, 1988. Bae <i>et al.</i> , Proc Natl Acad Sci 85: 2076, 1988. Christman <i>et al.</i> , Cell 55: 413, 1988. Sekiguki and Kmiec, Mol. Cell. Biol. 83: 195, 1988.
DNA repair	Musk and Steel, Br. J. Cancer 62: 364, 1990. Wang <i>et al.</i> , Cell 62: 403, 1990. Stevnsner and Bohr, Carcinogenesis 14: 1841, 1993. Gangloff <i>et al.</i> , Experientia 50: 261, 1994.
p53 association	Gobert <i>et al.</i> , Biochemistry 35: 5778, 1996.

tion of mammalian DNA topoisomerase I by trapping reversible topo I cleavable complexes.

The most specific drug family of topoisomerase I inhibitors is the camptothecin series (Figure 2), named for the prototype natural product camptothecin, a plant alkaloid isolated from *Camptotheca acuminata* that was developed as an antitumor drug (Wall *et al.*, 1966; see Horwitz, 1975). In recent studies it has been identified as a specific topoisomerase I inhibitor. In fact, by studying the products of topo I cleavable complexes it has been proved that the protein linked to broken DNA in detergent lysates of camptothecin treated cells was topo I (Hsiang, 1985; Hsiang, 1988).

Camptothecin is a lactone that equilibrates with the opened-ring carboxylate form under physiologi-

cal conditions. Because of its greater solubility, the sodium salt of camptothecin carboxylate was the first of the series to be evaluated clinically. However, clinical development was halted because of life-threatening myelosuppression, diarrhea, and hemorrhagic cystitis (Gottlieb *et al.*, 1970; Muggia *et al.*, 1972).

Clinical interest in the camptothecin series was renewed based on findings that the lactone form is the active species (Eng *et al.*, 1988) and is less toxic to the urinary system (Slichenmyer *et al.*, 1993). Formulation of camptothecins have been improved by the development of second generation analogs with greater water solubility such as the lactone forms of topotecan (9-(dimethylamino)methyl-10-hydroxycamptothecin) and Irinotecan (7-thyl-10-(4-(1-pi-

	R ₁	R ₂	R ₃
Camptothecin	-H	-H	-H
Topotecan	-OH	-CH ₂ N(CH ₃) ₂	-H
SN-38 [from CPT-11]	-OH	-H	-CH ₂ CH ₃
9-Aminocamptothecin	-H	-NH ₂	-H
9-Nitrocamptothecin	-H	-NO ₂	-H

Figure 2. Camptothecin series of topoisomerase I inhibitors.

peridino)-1-piperidino)carbonyloxycamptothecin). The products based on these camptothecins, HycamtinTM and CamptosarTM, have been approved for oncology indications in the United States and Japan.

In Europe the procedure (concerning Campto-R) is ongoing and approval is pending.

Other camptothecins are under development, including 9-aminocamptothecin and 9-nitrocamptothecin (Wani *et al.*, 1980; Kingsbury, 1991; Pantazis *et al.*, 1992) and other analogs (Wang *et al.*, 1994; Valenti *et al.*, 1997; Zhao *et al.*, 1997). Some authors have described lipid formulations of camptothecin which display *in vivo* the capacity to concentrate in the gastrointestinal tract (Sugarman, 1996). It is important to realize that 9-nitro-camptothecin is converted by human cells to 9-amino-camptothecin, even by cells cultured *in vitro* (Pantazis *et al.*, 1994b). Until there are inhibitors of this metabolism, it cannot be deter-

mined if 9-nitro-camptothecin is active itself, or serves as a pro-drug for delivering the relatively unstable 9-amino-camptothecin to the target cells.

The camptothecins cure nude mice harboring xenografts from a variety of human cancers (Giovannella *et al.*, 1989, 1991; Pantazis *et al.*, 1994a; Friedman *et al.*, 1994). However, clinical studies demonstrated that continuous infusion and multi-consecutive day schedules were limited by severe neutropenia that occurred at dosages 3–15% of the murine MTD (Rowinsky *et al.*, 1992; Kantarjian *et al.*, 1993; Verweij *et al.*, 1993; Dahut *et al.*, 1994; Hochster *et al.*, 1994; Pratt *et al.*, 1994), even with G-CSF support (Saltz *et al.*, 1993; Slichenmyer *et al.*, 1993). These doses are well below the curative doses in the nude mouse models. The human MTD is 1.4 mg m⁻² day⁻¹ on a qdx5 schedule without cytokine support (Rowinsky *et al.*, 1992; Saltz *et al.*, 1993; Verweij *et*

al., 1993), and the mouse LD10 on the same schedule is $14 \text{ mg m}^{-2} \text{ day}^{-1}$ (Grochow *et al.*, 1992). Obviously the full curative potential of these drugs will not be realized without the development of approaches, in addition to cytokine support, to compensate for the dose limiting neutropenia. The decreased drug tolerance of human compared to mouse is unlikely to be due to pharmacokinetic differences, since plasma clearance rate of topotecan is similar in humans and mice (Grochow *et al.*, 1992).

An important aspect of the pharmacology of the camptothecin compounds is the equilibrium between hydroxy acid (hydrolyzed A ring) and lactone forms, the latter being responsible for cytotoxicity (Eng *et al.*, 1988). Mi and Burke have shown that 13–18% of total TPT exists in the lactone form at equilibrium regardless of the protein concentration or the species of origin of the plasma, and there are no differences between mouse and human in the plasma equilibrium levels of TPT lactone (Burke and Mi, 1993, 1994; Mi and Burke, 1994a, b). However, other members of the family do show dramatic inter-species differences in the percent lactone at equilibrium. For example, the equilibrium is shifted more toward the hydroxy acid of CAM by human serum albumin than by murine, and 9-amino-camptothecin shows large differences in percent lactone at equilibrium between dog (25%) and human (<0.5%) (Burke and Mi, 1993, 1994; Mi and Burke 1994a, b).

Nevertheless, the clinical success of the camptothecin series has established topoisomerase I as an important and commercially viable pharmacological target in cancer treatment. Thus, there is an active effort to develop additional topoisomerase I inhibitors with structures and properties distinct from the camptothecins, such as benzophenanthridine alkaloids (nitidine and fagaronine) (Wang LK, 1993), coralyne and its analogues (Wang LK, 1996), intoplicine (Abigerges *et al.*, 1996), GI147211C (Besterman *et al.*, 1996; Gerrits *et al.*, 1996), quinolines and quinoxalines (Deady *et al.*, 1997), benzimidazoles (Kim *et al.*, 1996), an ellipticine-distamycin hybrid (Riou *et al.*, 1995), and others (Funabashi *et al.*, 1994; Gupta *et al.*, 1995; Meikle *et al.*, 1995; Boege *et al.*, 1996; Cummings *et al.*, 1996; Funayama *et al.*, 1996; Makhey *et al.*, 1996; Ray *et al.*, 1996, 1997; Rodriguez-Campos *et al.*, 1996; Xie *et al.*, 1996; Spicer *et al.*, 1997). This promising class of anticancer drugs also exhibits antiviral activity (Priel *et al.*, 1991).

Obviously, the complicated nature of the reaction

catalyzed by topoisomerase I suggests several mechanisms by which it can be inhibited, and it seems likely that several mechanistic classes of topoisomerase inhibitors will emerge. This may be of some benefit clinically as acquired resistance to one mechanism via mutation may not necessarily confer cross-resistance to the other mechanisms.

Cytotoxicity of Topoisomerase I Inhibitors

Topoisomerase I inhibitors are drugs able to bind the cleavable complex Topo I-DNA resulting in single strand DNA breaks that in the presence of drug cannot be religated (Creemers *et al.*, 1994). Although the steps by which the formation of cleavable complex by camptothecin occurs is not fully clarified, it seems that camptothecin complexes at sites having a G at their 5'-DNA terminus and non-contiguous domains of Topo I with the consequent block of the resealing step of strand-passing reaction (Hsiang, 1985; Jaxel *et al.*, 1991; Rubin *et al.*, 1994). DNA cleavage pattern observed after denaturation shows wide variation in cleavage sites intensity and moreover the cleavages occurs at sites that were not detectably cleaved in the absence of drug (Perez-Stable *et al.*, 1988). Since cleavages should occur at sequences with similar twist angle variation it has been proposed that camptothecin produces alteration of the topo I cleavage pattern as a consequence of alteration of the enzyme's normal recognition of twist angle variations (Shen, 1990).

The cytotoxicity of camptothecins is cell cycle phase-specific, specifically in S-phase (Li *et al.*, 1972), and it has been calculated that a cell in S-phase is about 1000 times more sensitive to the effect of camptothecin than cells in G1 or G2 phase (Horwitz, 1975). However, if the toxicity is measured as damage produced by camptothecin on DNA the result is very similar either in S-phase cells and in cells in G1-phase (Horwitz, 1973). This apparent discrepancy suggests that the cause of cell death by camptothecin is probably due to the collision between the cleavage complex and the replication forks and that the entering in S-phase converts a reversible damage of DNA into a lethal lesion. This hypothesis of cell kill is also supported by studies on *in vitro* models with SV-40 virus replicating in cell free systems (Hsiang *et al.*, 1989) even if it is also possible that a part of the lethal effects may be due to the stabilization of cleavable complexes in genomic regions essential for cell viability (Hsiang *et al.*, 1989).

The capacity of camptothecin and other topo I inhibitors to kill cells is considered a very interesting perspective for the treatment of tumors with elevated Topo I level or activity (Houghton *et al.*, 1992; Bronstein *et al.*, 1996). The progressive knowledge that the mechanism of action of Camptothecin and its derivatives was directed against topo I allowed to better clarify some effects on cellular functions but does not explain completely the mechanism of cell killing. The inhibition of RNA synthesis is a reversible phenomenon probably due to the Camptothecin altered cleavable complex which blocks the elongation process by preventing the progression of RNA polymerase molecules along the transcription unit (Zhang, 1990). If camptothecin is removed from cells the phenomenon reverses (probably because the topo I cleavable complex dissociates from the transcription unit) and RNA synthesis restarts.

Multiple Drug Resistance

Since for an antitumor drug, the selectivity of action is fundamental, many studies were directed to investigate the mechanisms by which cells become resistant to drug treatment (Clynes, 1993). For drugs acting on topoisomerases it has been reported that irreversible damages occur with a higher frequency in cells containing high levels of these enzymes (that is the case of many tumoral cells) (Van der Zee *et al.*, 1991). When a tumor cell population has a low content of topoisomerases it becomes refractory to the treatment and this type of resistance is called primary resistance. Different mechanisms of resistance have been characterized in many different experimental systems and currently they may be classified into three basic groups according to the mechanisms involved: drug transport (Endicott *et al.*, 1989), drug-target interaction (Beck, 1987) and drug detoxification (Deffie *et al.*, 1988).

The most important phenomenon of resistance made evident was the so called 'multiple drug resistance' (MDR) that is caused by the drug transportation made by a 'pump' system requiring energy and able to throw the drug out of the cell (Endicott *et al.*, 1989; Roninson, 1992). The best example of MDR is represented by a 170 kDa P-glycoprotein (P-gp), a member of a family of proteins acting as membrane carriers of different chemical compounds such as sugar, ions, hormones and proteins and by multi-resistance associated proteins of 190 kDa (MRP) (these systems have been reviewed by Clynes *et al.*, 1990; Germann,

1996; Loe, 1996). The P-glycoprotein is present in normal tissues in which it protects the cells against toxic compounds providing an important factor of selectivity (Fojo *et al.*, 1987) that during chemotherapy protects normal cells whereas it does not defend neoplastic cells which do not contain P-gp. Unfortunately, this selectivity is lost when tumoral cells express P-glycoprotein by acquiring MDR phenotype and worst when the P-gp is associated with the expression of 'ras' and 'p53' oncogenes then an acceleration of tumor growth occurs (Chin *et al.*, 1992). P-glycoprotein is encoded by MDR-1 gene and may be the result both of a gene expression as well as of a gene amplification process. The main part of the studies (performed on topoisomerases II poisons) pointed out that MDR cells are very resistant to Vinca alkaloids such as vincristine or vinblastine, moderately resistant to doxorubicin, etoposide and teniposide while maintaining a relative sensitivity to amsacrine (Qian *et al.*, 1990; Baguley *et al.*, 1990). However, it must be considered that some MDR mechanisms are not associated with increased expression of P-glycoprotein although modifications of the drug transport are involved. As observed by Scheper *et al.*, 1993, several p-glycoprotein-negative MDR cell lines overexpressed a Mr = 110 000 protein (p110) termed lung resistance related protein (LRP) (see the recent review of Izquierdo *et al.*, 1996) which may have a physiological function in several normal tissues, possibly involving a transport mechanism. Many *in vitro* cell lines with increased expression of P-glycoprotein and resistant to drug inhibition were obtained and also many spontaneous human tumors were described to become MDR. For these reasons several '*in vitro*' methods have been developed in order to check the presence of MDR in tumor cells. Some methods are based on the direct detection of P-gp in histological sections of tumoral tissues or cells by using specific monoclonal antibodies labelled with fluorescein (Thiebaut *et al.*, 1987). Other ones check the MDR-1 gene expression by DNA-RNA hybridization techniques (Fojo *et al.*, 1987). Analytic methods based on the cytometry were developed by exploiting the capacity of MDR cells to throw out from the cytoplasm different fluorochromes (Nooter *et al.*, 1991; Ludescher *et al.*, 1991). Also the polymerase chain reaction (PCR) was recently used to analyze the expression of *mdr1* gene in tumor sample from breast cancer patients (Yang *et al.*, 1993). It is important to remember a last method which has the advantage of detecting other MDR mechanisms unrelated to the expression of P-gp. This aim is obtained by cultur-

ing tumor cells in the presence of two different drugs having similar structure but different susceptibility to MDR mediated by P-gp. Thus, the ratios between the IC₅₀ values calculated for the two drugs may be considered as an index of MDR (Baguley *et al.*, 1990).

Drug Resistance to Topoisomerase I Inhibitors

As mentioned above the number of DNA strand breaks induced by topoisomerase inhibitors is dependent on the number of molecules of enzymes in the cells. For this reason, the most simple mechanism of resistance to drugs acting against topoisomerases is consistent with the decrease of cell content or subcellular redistribution of these enzymes (Kanzawa *et al.*, 1990; Danks *et al.*, 1996; Buckwalter *et al.*, 1996). This is the case with the 'cyclic' changes of resistance to topoisomerase-inhibitors which is directly related to the variation of the intracellular content of these enzymes along the cell cycle (Heck *et al.*, 1988; Barronco, 1984). During the Go-G1 phase, many enzymes utilized for DNA duplication process are degraded while their new synthesis starts just before the new DNA replication phase begins (S-phase) (Andrews, 1992). This phenomenon of resistance is called 'cytokinetic resistance' and it seems to play an important role in the protection of normal tissues from toxics ingested with the food or administered during chemotherapy. This type of resistance may be the most probable mechanism which determines the insensitivity of many solid tumors to the chemotherapy. Thus, the quantitative reduction of topo I content seems to be the most frequently occurring event in the development of camptothecin resistance (Sugimoto *et al.*, 1990). A development of resistance to inhibitors of topo I occurring by down-regulation of the target enzyme, thus reducing the production of lethal enzyme-mediated DNA damages has also been suggested (Eng *et al.*, 1990). However, according to some authors, the resistance to topoisomerase inhibitors is not a cycle specific resistance but rather a combination between a decreased quantity of topoisomerases and a diminished direct sensitivity of the enzymes to the drug (Andoh *et al.*, 1987; Kjeldsen *et al.*, 1988). This last mechanism producing resistance was well documented for topo II and was defined as MDR (Altered Topo-MDR) (Beck *et al.*, 1987; Snow *et al.*, 1991).

For topo I the data is not so homogeneous although topo I alterations have been proposed to produce acquired resistance to camptothecin in mammalian cells.

Some cells have been characterized which exhibited altered topo I (Andoh *et al.*, 1987; Gupta *et al.*, 1988) and in some mutants it was even shown that the activity of Topo I extracted from cells resistant to camptothecin was three times as active than topo I from wild type cells (Lefevre *et al.*, 1991). Studies with a mutated camptothecin resistant form of topoisomerase I (TOPO I-K5) indicated a higher cleavage/religation activity of topo I-K5 as a result of improved DNA binding and a concomitant shift in the equilibrium between cleavage and religation towards the religation step (Gromova *et al.*, 1993). Also Tanizawa *et al.* (1992) observed that resistance to camptothecin was associated with a marked reduction of drug-induced DNA strand breaks concluding that camptothecin resistance in DC3f/C10 cells is due to the qualitative alteration of DNA topo I. Ishimi *et al.* (1991) isolated a topo I mutant conferring camptothecin resistance but not able to affect its functions involved in cellular DNA replication. However, while topo II poisons are in general sensitive to MDR1 mediated resistance, the topo I poisons such as camptothecin and its derivatives appear completely insensitive to MDR1 overexpression (Liu, 1990). In fact, alterations of DNA topo I are unrelated to the acquisition of MDR characters (Oguro *et al.*, 1990) but neither does the P-glycoprotein associated with MDR phenotype modify the sensitivity to camptothecin and its derivatives (Giovannella *et al.*, 1991). Moreover, MDR1 overexpression was shown to have little effect on camptothecin cytotoxicity and the capacity of this drug to overcome MDR1 mediated resistance is most likely due to its unimpaired accumulation in MDR-1 cells (Chen *et al.*, 1991). Only very recent experimental evidence points out that MDR cells can be resistant also to camptothecin and its derivatives and that P-gp overexpression diminishes accumulation and toxicity of topo I poisons in hamster and human cells (Hendricks *et al.*, 1992; Yang *et al.*, 1995). Recently also the possible role of p53 has been investigated (Shimizu *et al.*, 1996).

For purposes of this review, drug resistance to camptothecins has been organized around four questions (Pessina, 1993; Prost, 1995). First, does resistance to camptothecins induce cross-resistance to other drug classes? Second, does resistance to commonly used chemotherapeutic agents induce cross-resistance to camptothecins? Third, what are the mechanisms of resistance that can reduce chemosensitivity of tumor cells during therapy with camptothecins? Fourth, are there any known mechanisms of multi-drug resistance that do not affect chemosen-

sitivity to camptothecins?

There is only weak collateral chemosensitivity between topotecan and other agents, and resistance to camptothecins does not usually confer cross-resistance to topoisomerase II inhibitors (e.g., VP-16), microtubule-targeted drugs (e.g., Taxol), or alkylating agents (Ferguson *et al.*, 1988; Gupta *et al.*, 1988; Eng *et al.*, 1990; Pantazis *et al.*, 1994c; Supino *et al.*, 1996; Jensen *et al.*, 1997). Furthermore, a dominant negative p53 genotype that confers cross-resistance to doxorubicin, cisplatin, and Ara-C does not affect sensitivity to camptothecin or paclitaxel (Vasey *et al.*, 1996). Human tumor cell lines resistance to topoisomerase I poisons do not exhibit collateral chemosensitivity between gemcitabine and ara-C (Jensen *et al.*, 1997). Consistent with infrequent cross-resistance patterns, prior myelosuppressive chemotherapy does not alter subsequent camptothecin-induced myelotoxicity (EORTC, 1992).

However, induction of camptothecin resistance under laboratory conditions has been associated with cross-resistance to cisplatin (Fujimori *et al.*, 1996), and cisplatin and topotecan resistance can be related (Niimi *et al.*, 1992; Jensen *et al.*, 1997). These findings contrast with the synergistic activity of co-exposure to cisplatin and SN-38 metabolite of CPT-11 (Fukuda *et al.*, 1996).

There are many mechanisms that could contribute to the resistance of malignancies to camptothecins. Camptothecin resistance can be linked to alterations in topoisomerase I structure (Andoh *et al.*, 1987; Gupta *et al.*, 1988; Jaxel *et al.*, 1989; Lefevre *et al.*, 1991; Ishimi *et al.*, 1991; Beck and Danks, 1991; Kubota *et al.*, 1992; Knab *et al.*, 1993; Tanizawa *et al.*, 1993; Carrigan *et al.*, 1997). This mechanism has been reviewed in detail (Pommier *et al.*, 1996; Nitiss and Beck, 1996; Rothenberg, 1997).

Increased activity of drug efflux transport systems which actively pump natural products out of the cell and minimize intracellular accumulation could be another mechanism of resistance. The best known efflux system in the context of chemotherapy is the *mdr-1* gene product, also known as P170 or P-glycoprotein. Some camptothecins appear to be substrates of P170 whereas others are not, and the presence of ionized functional groups on the camptothecin ring system may be required for interaction with the P170 system (Liu *et al.*, 1990; Giovanella *et al.*, 1991; Chen *et al.*, 1991; Nakatsu *et al.*, 1997; Hoki *et al.*, 1997). Interestingly, topotecan is a P170 substrate whereas 9-amino-camptothecin is not (Hendricks *et al.*, 1992;

Hoki *et al.*, 1997). To our knowledge, the role of the MRP system in camptothecin resistance has not been evaluated experimentally.

An ovarian carcinoma line resistant to cisplatin exhibited cross-resistance to irinotecan in spite of similar levels of intracellular drug accumulation and topoisomerase I activities. However, multiple mechanisms appeared to contribute to the resistance to CPT-11, including glutathione-dependent pathways and diminished intracellular conversion of CPT-11 to its active metabolite, SN-38 (Niimi *et al.*, 1992).

Mutants can be produced that are resistant to camptothecin at the cellular level, even though the isolated topoisomerase I shows no change in sensitivity to camptothecin, if there is increased repair of topoisomerase I-dependent lesions in the DNA (Fujimori *et al.*, 1996). However, these mutants were isolated by selection in camptothecin after treatment with the mutagen MMS, and may not be clinically relevant.

One final observation about cellular resistance to camptothecins should influence the development of new generation camptothecins. There are large inter-species differences in tolerated doses, and rodents can tolerate significantly higher doses of the camptothecins than the human. Differential sensitivity across species can be reproduced *in vitro* using direct drug exposure of hematopoietic progenitors in the bone marrow of the various species (Erickson-Miller *et al.*, 1997). Because of its similar pharmacokinetics in mice and humans (Grochow *et al.*, 1992), topotecan is the best camptothecin to study *in vitro*. Its LD10:MTD ratio of 10 and its ratio of peak plasma concentrations of 22 are replicated *in vitro* by the sensitivity of neutrophil progenitors from mouse and human (Erickson-Miller *et al.*, 1997). Thus, there is increased drug resistance even in normal proliferating rodent cells relative to their human counterparts. The mechanism responsible for this differential tolerance has not been identified, but the findings suggest pharmacodynamic differences that result in relative drug resistance at the cellular level. It will be interesting to discover if differential tolerance is a feature of any drug that inhibits topoisomerase I, or whether it pertains specifically to the camptothecin family of inhibitors or their specific molecular target on the enzyme. It will be important to identify the mechanism because it is responsible in large part for the diminished efficacy of camptothecins against human tumors in patients compared to mouse models (Erickson-Miller *et al.*, 1997). All of these mechanisms of drug resistance reside at the cellular or sub-cellular level. However,

tumors could acquire 'resistance' to camptothecins via physiological mechanisms that lie above the cellular level (reviewed in Pessina, 1993). Since the expression of topoisomerase I is regulated during cell cycle progression, tumors with low growth fractions and high proportions of quiescent (G_0) progenitor cells and stem cells would be refractory to camptothecin therapy, assuming the regulation of the enzyme's expression and function are similar in tumor cells and their normal counterparts. In addition, there could be significant clinical implications to the findings (Beidler and Cheng, 1995; Murren *et al.*, 1996; Taniguchi *et al.*, 1996) that camptothecin treatment down-regulates topoisomerase I levels.

There are some interesting clinical observations that suggest another mechanisms of 'physiological resistance' to camptothecins. CPT-11 is a pro-drug that is metabolized to an active metabolite called SN-38, and this metabolite is further metabolized to a glucuronide conjugate that appears in the plasma of patients treated with CPT-11 (Gupta *et al.*, 1994). Patients exhibiting the lowest extent of metabolism of SN-38 to the glucuronide experience the most severe toxicity (diarrhea), which suggests that drug tolerance in individual patients is determined by how much unconjugated SN-38 gains access to the bile to cause cytotoxicity to the intestinal mucosa (Gupta *et al.*, 1994; Ikuno *et al.*, 1995). In terms of 'drug resistance', these findings suggest that patients with low glucuronyl transferase activity will tolerate lower dose of CPT-11 than other patients. Assuming there is not any difference in tumor sensitivity in these patients, the lower tolerated doses of CPT-11 will result in less efficacious treatment in this patient subpopulation, and the tumors would appear to be 'resistant'. However, the mechanism of tumor resistance is pharmacokinetic in nature, rather than cellular.

Future Perspectives

It seems likely that several factors will continue to drive the development of topoisomerase I inhibitors. In addition to the obvious commercial success of this class of drugs, the relative lack of drug cross-resistance to other classes of antineoplastic agents and its activity in otherwise refractory disease are significant findings. The complex mechanism of topoisomerase I catalysis suggests multiple pharmacological strategies, and new enzyme inhibitors are under development. Furthermore, it may be possible to de-

sign topoisomerase I poisons which are toxic only to cells that have mutations in topoisomerase I that confer resistance to other mechanistic classes. Since some camptothecins are substrates of xenobiotic efflux pumps whereas others are not, it will be important to elucidate the structural features that make the difference between the two. A final point is for the reader to be aware of the fact that a topoisomerase I has been isolated from mammalian mitochondria (Lin and Castora, 1995).

In addition to the obvious cellular and molecular mechanisms (drug efflux pumps, DNA repair, etc.) that contribute to resistance to camptothecins, there are pharmacological mechanisms that lie above the cellular level that are not detectable in *in vitro* assays of antitumor activity. For some camptothecins, these mechanisms determine to a large degree whether a tumor appears 'resistant'. Especially when camptothecins are administered with other medications that can decrease drug tolerance (e.g., inhibit glucuronidation) and therefore decrease delivered dose, these factors need to be considered when drawing conclusions about resistance of tumors to therapy. Apparently, it is not sufficient to analyze only the subcellular mechanisms to understand why a given patient's tumor fails to respond to certain camptothecins. It is also important to incorporate the inter-species differences in percent lactone when designing and extrapolating *in vitro* assays of camptothecins to the *in vivo* setting (Erickson-Miller *et al.*, 1997). *In vitro* evaluations will be most meaningful when they include human plasma (or serum albumin) for human cell assays and murine for murine cell assays to accommodate these species differences in percent lactone at equilibrium. Topotecan is a notable exception and does not show such inter-species differences (Mi and Burke, 1994b).

One serious limitation to the clinical promise of camptothecins is the unfortunate lack of tolerance of the drugs in human patients. The 10-fold or greater sensitivity of human than murine neutrophil progenitors will have to be overcome to achieve the doses in patients that cure mice with human tumor xenografts. Since the mechanism(s) responsible for this large inter-species difference in drug tolerance might be difficult to identify, it may prove difficult to increase human drug tolerance to the level of the mouse via the use of cytoprotectants like amifostine or by transgenic modification of hematopoietic stem cells. It may be more productive to return to preclinical development and identify myelosuppressive camptothecins that are still curative in the human tumor xenograft

models yet show equal potency between mouse and human neutrophil progenitors. Another related issue of importance is to find out whether topoisomerase I plays a more critical role in human than murine hematopoiesis. It will be important to identify the mechanistic classes of topoisomerase I inhibitors that do NOT show inter-species differences in drug tolerance and have a chance at showing the same antitumor efficacy in patients as in xenograft models.

Regardless, topoisomerase I represents a novel and distinct molecular target for cancer chemotherapy, and increased understanding of the cellular and supra-cellular mechanisms that generate refractory disease will be valuable for the optimal development of this drug class. Although this knowledge base is apparently in its infancy, several issues have already been identified that may be unique to camptothecins or generally topoisomerase I poisons.

References

- Abigerges D, Armand JP, Chabot GG, Bruno R, Bissery MC, Bayssas M, Klink-Alakl M, Clavel M and Catimel G (1996) Phase I and pharmacology study of intoplicine (RP 60475; NSC 645008), novel topoisomerase I and II inhibitor, in cancer patients. *Anticancer Drugs* 7: 166–74.
- Andoh T, Ishii K, Suzuki Y, Ikegami Y, Kuzunoki Y, Takemoto Y and Okada K (1987) Characterization of a mammalian mutant with camptothecin resistant DNA topoisomerase I. *Proc Natl Acad Sci USA* 84: 5565–69.
- Andrews BJ (1992) Gene expression-dialogue with the cell cycle. *Nature* 355: 393–4.
- Baguley BC, Holdway KM and Fray LM (1990) Design of DNA intercalators to overcome topoisomerase II-mediated multidrug resistance. *J Natl Cancer Inst* 82: 398–402.
- Baker SD, Wadkins RM, Stewart CF, Beck WT and Danks MK (1995) Cell cycle analysis of amount and distribution of nuclear DNA topoisomerase I as determined by fluorescence digital imaging microscopy. *Cytometry* 19: 134–45.
- Barrett JF, Sutcliffe YA and Gootz TD (1990) *In vitro* assay used to measure the activity of topoisomerases. *Antimicrob. Agents Chemother* 34: 1–7.
- Barranco SC (1984) Cellular and molecular effects of Adriamycin on dividing and non dividing cells. *Pharmac Ther* 24: 303–319.
- Beck WT (1987) The cell biology of multiple drug resistance. *Biochem Pharmacol* 36: 2879–88.
- Beck WT and Danks MK (1991) Mechanisms of resistance to drugs that inhibit DNA topoisomerases. *Semin Cancer Biol* 2: 235–44.
- Beck WY, Cirtain MC, Danks MK, Felsted RL, Safa AR, Wolverton JS, Suttle DP and Trent JM (1987) Pharmacological molecular and cytogenetic analysis of 'atypical' multidrug-resistant human leukemic lines. *Cancer Res* 47: 5455–60.
- Beidler DR and Cheng YC (1995) Camptothecin induction of a time- and concentration-dependent decrease of topoisomerase I and its implication in camptothecin activity. *Mol Pharmacol* 47: 907–14.
- Besterman JM (1996) Topoisomerase I inhibition by the camptothecin analog G1147211C. From the laboratory to the clinic. *Ann N Y Acad Sci* 803: 202–9.
- Boege F, Straub T, Kehr A, Boesenberg C, Christiansen K, Andersen A, Jakob F and Kohrle J (1996) Selected novel flavones inhibit the DNA binding or the DNA religation step of eukaryotic topoisomerase I. *J Biol Chem* 271: 2262–70.
- Bronstein IB, Vorobyev S, Timofeev A, Jolles CJ, Alder SL and Holden JA (1996) Elevations of DNA topoisomerase I catalytic activity and immunoprotein in human malignancies. *Onc Res* 8: 17–25.
- Buckwalter CA, Lin AH and Tanizawa A *et al.* (1996) RNA synthesis inhibitors alter the subnuclear distribution of DNA topoisomerase I. *Cancer Res* 6: 1674–81.
- Burke TG and Mi Z (1993) Preferential binding of carboxylate form of camptothecin by human serum albumin. *Analytical Biochem* 21: 285–87.
- Burke TG and Mi Z (1994) The structural basis of camptothecin interactions with human serum albumin: Impact on drug stability. *J Med Chem* 37: 401–6.
- Carrigan SW, Fox PC, Wall ME, Wani MC and Bowen JP (1997) Comparative molecular field analysis and molecular modeling studies of 20-(S)-camptothecin analogs as inhibitors of DNA topoisomerase I and anticancer/antitumor agents. *J Comput Aided Mol Des* 11: 71–8.
- Champoux JJ (1981) DNA is linked to the rat liver nicking-closing enzyme by a phosphodiester bond to tyrosine. *J Biol Chem* 256: 4805–09.
- Chen AY, Yu C, Potmesil M, Wall ME, Wani MC and Liu LF (1991) Camptothecin overcomes MDR1-mediated resistance in human KB carcinoma cells. *Cancer Res* 51: 6039–44.
- Chen AY, Yu C, Bodley A, Peng LF and Liu LF (1993) A new mammalian DNA topoisomerase I poison Hoechst 33342: cytotoxicity and drug resistance in human cell cultures. *Cancer Res* 53: 1332–37.
- Chin KV, Pastan I and Gottesman MM (1993) Function and regulation of the human multidrug resistance gene. *Adv. Cancer Res* 60: 157–80.
- Chin KV, Ueda K, Pastan I and Gottesman MM (1992) Modulation of activity of the promoter of the human MDR1 gene by ras and p53. *Science* 255: 459–62.
- Clynes M (1993) Cellular models for multiple drug resistance in cancer. *In Vitro Cell. Dev Biol* 29A: 171–79.
- Clynes M, Redmond A and Heenan M (1990) Recent developments in research on multiple drug-resistance in cancer cells. *Cancer J* 3: 34–39.
- Cobb J, Reddy RK, Park C and Handel MA (1997) Analysis of expression and function of topoisomerase I and II during meiosis in male mice. *Mol Reprod Develop* 46: 489–98.
- Creemers GJ, Lund B and Verweij J (1994) Topoisomerase I inhibitors: Topotecan and Irinotecan. *Cancer Treatment Rev* 20: 73–96.
- Cummings J, Macpherson JS, Meikle I and Smyth JF (1996) Development of anthracenyl-amino acid conjugates as topoisomerase I and II inhibitors that circumvent drug resistance. *Biochem Pharmacol* 52: 979–90.
- Dahut W, Brillhart N, Takimoto C, Allegra C, Hamilton JM, Sorensen JM, Arbuck S, Chen A and Grem J (1994) A phase I trial of 9-aminocamptothecin (9-AC) in adult patients with solid tumors. *Proc Am Soc Clin Oncol* 13: A345.
- Danks MK, Garret KE and Marion RC *et al.* (1996) Subcellular redistribution of DNA topoisomerase I in anaplastic astrocytoma cells treated with topotecan. *Cancer Res* 56: 1664–73.
- D'arpa P and Liu FL (1989) Topoisomerase-targeting antitumor-

- drugs. *Biochim Biophys Acta* 989: 163–77.
- Deady LW, Kaye AJ, Finlay GJ, Baguley BC and Denny WA (1997) Synthesis and antitumor properties of N-[2-(dimethylamino)ethyl]carboxamide derivatives of fused tetracyclic quinolines and quinoxalines: a new class of putative topoisomerase inhibitors. *J Med Chem* 40: 2040–6.
- Deffie AM, Alam T, Seneviratne C, Beenken SW, Batra JK, Shea TC, Henner WD and Geldenberg GJ (1988) Multifactorial resistance to Adriamycin: relationship of DNA repair, glutathione transferase activity drug efflux, and P-glycoprotein in cloned cell lines of Adriamycin-sensitive and resistant P388 leukemia. *Cancer Res* 48: 3595–602.
- Endicott JA and Ling V (1989) The biochemistry of P-glycoprotein-mediated multidrug resistance. *Annu Rev Biochem* 58: 351–375.
- Eng WK, Faucette L, Johnson RK and Sternglanz R (1988) Evidence that DNA topoisomerase I is necessary for the cytotoxic effects of camptothecin. *Mol Pharmacol* 34: 755–60.
- Eng WK, McCabe FL, Tan KB, Mattern MR, Hofmann GA, Woessner RD, Hertzberg RP and Johnson RK (1990) Development of a stable camptothecin-resistant subline of P388 leukemia with reduced topoisomerase I content. *Mol Pharmacol* 38: 471–80.
- EORTC (1992) Reports on Topoisomerase I Inhibitors from the 7th NCI-EORTC Symposium on New Drugs in Cancer Therapy. Amsterdam, March 17–20.
- Erickson-Miller CL, May RD, Tomaszewski JE, Osborn B, Murphy MJ, Page JG and Parchment RE (1997) Differential toxicity of camptothecin, topotecan, and 9-aminocamptothecin to human, canine, and murine myeloid progenitors (CFU-GM) *in vitro*. *Can Chem Pharmacol* 39: 467–72.
- Ferguson PJ, Fisher MH, Stephenson J, Li D-H, Zhou B-S and Cheng Y-C (1988) Combined modalities of resistance in etoposide-resistant human KB cell lines. *Cancer Res* 48: 5956.
- Fojo AT, Ueda K, Slamon DJ, Poplack DG, Gottesman MM and Pastan I (1987) Expression of a multidrug-resistance gene in human tumors and tissues. *Proc Natl Acad Sci USA* 84: 265–269.
- Friedman HS, Houghton PJ, Schold SC, Keir S and Bigner DD (1994) Activity of 9-dimethylaminomethyl-10-hydroxycamptothecin against pediatric and adult central nervous system tumor xenografts. *Cancer Chemother Pharmacol* 34: 171–74.
- Fujimori A, Gupta M, Hoki Y and Pommier Y (1996) Acquired camptothecin resistance of human breast cancer MCF-7/C4 cells with normal topoisomerase I and elevated DNA repair. *Mol Pharmacol* 50: 1472–8.
- Fukuda M, Nishio K, Kanzawa F, Ogasawara H, Ishida T, Arioka H, Bojanowski K, Oka M and Saijo N (1996) Synergism between cisplatin and topoisomerase I inhibitors, NB-506 and SN-38, in human small cell lung cancer cells. *Cancer Res* 56: 789–93.
- Funabashi Y, Horiguchi T, Iinuma S, Tanida S and Harada S (1994) TAN-1496 A, C and E, diketopiperazine antibiotics with inhibitory activity against mammalian DNA topoisomerase I. *J Antibiot* 47: 1202–18.
- Funayama Y, Nishio K, Wakabayashi K, Nagao M, Shimoi K, Ohira T, Hasegawa S and Saijo N (1996) Effects of beta- and gamma-carboline derivatives of DNA topoisomerase activities. *Mutat Res* 349: 183–91.
- Gellert M (1981) DNA topoisomerases. *Annu Rev Biochem* 50: 879–910.
- Germann UA (1996) P-glycoprotein—A mediator of multidrug resistance in tumor cells. *Eur J Cancer* 6: 927–44.
- Gerrits CJ, Creemers GJ, Schellens JH, Wissel P, Planting AS, Kunka R, Selinger K, de Boer-Dennert M, Marijnen Y, Hartevel M and Verweij J (1996) Phase I and pharmacological study of the new topoisomerase I inhibitor GI147211, using a daily x 5 intravenous administration. *Br J Cancer* 73: 744–50.
- Giovanella BC, Stehlin JS, Wall ME, Wani MC, Nicholas AW, Liu LF, Silber R and Potmesil M (1989) DNA topoisomerase I-targeted chemotherapy of human colon cancer in xenografts. *Science* 246: 1046–48.
- Giovanella BC, Hinz HR, Kozielski AJ, Stehlin JS, Silber R and Potmesil M (1991) Complete growth inhibition of human cancer xenografts in nude mice by treatment with 20-(S)-camptothecin. *Cancer Res* 51: 3052–55.
- Gobert C, Bracco L, Rossi F, Olivier M, Tazi J, Lavelle, Larsen AK and Riou JF (1996) Modulation of DNA topoisomerase I activity by p53. *Biochem* 35: 5778–86.
- Gottlieb JA, Guarino AM, Call JB, Oliverio VT and Block JB (1970) Preliminary pharmacologic and clinical evaluation of camptothecin sodium (NSC-100880). *Cancer Chemother Reports* 54: 461–70.
- Grochow LB, Rowinsky EK, Johnson R, Ludeman S, Kaufmann SH, McCabe FL, Smith BR, Hurowitz L, DeLisa A, Donehower RC and Noe DA (1992) Pharmacokinetics and pharmacodynamics of topotecan in patients with advanced cancer. *Drug Metab Disp* 20: 706–13.
- Gromova II, Kjeldsen E, Svejstrup JQ, Alsner J, Christiansen K and Westergaard O (1993) Characterization of an altered DNA catalysis of a camptothecin resistant eukaryotic topoisomerase I. *Nucleic Acid Res* 21: 593–600.
- Gupta E, Lestingi TM, Mick R, Ramirez J, Vokes EE and Ratain MJ (1994) Metabolic fate of irinotecan in humans: correlation of glucuronidation with diarrhea. *Cancer Res* 54: 3723–5.
- Gupta M, Abdel-Megeed M, Hoki Y, Kohlhagen G, Paull K, and Pommier Y (1995) Eukaryotic DNA topoisomerases mediated DNA cleavage induced by a new inhibitor: NSC 665517. *Mol Pharmacol* 48: 658–65.
- Gupta RS, Gupta R, Eng B, Lock RB, Ross WE, Hertzberg RP, Caranfa MJ and Johnson RK (1988) Camptothecin resistant mutants of Chinese hamster ovary cells containing a resistant form of topoisomerase I. *Cancer Res* 48: 6404–10.
- Hait WN and Aftab DT (1992) Rational design and preclinical pharmacology of drugs for reversing multidrug resistance. *Biochem Pharmacol* 43: 103–7.
- Heck MMS, Hittelman WN and Earnshaw WC (1988) Differential expression of DNA topoisomerases I and II during the eukaryotic cell cycle. *Proc Natl Acad Sci USA* 85: 1086–90.
- Hendricks CB, Rowinsky EK, Grochow LB, Donehower RC and Kaufmann SH (1992) Effect of p-glycoprotein expression of the accumulation and cytotoxicity of topotecan (SK&f 104864) a new camptothecin analogue. *Cancer Res* 52: 2268–70.
- Hochster H, Liebes L, Speyer J, Sorich J, Taubes B, Oratz R, Wernz J, Chachoua A, Raphael B, Vinci RZ and Blum RH (1994) Phase I trial of low-dose continuous topotecan infusion in patients with cancer: an active and well-tolerated regimen. *J Clin Oncol* 12: 553–59.
- Hoki Y, Fujimori A and Pommier Y (1997) Differential cytotoxicity of clinically important camptothecin derivatives in P-glycoprotein-overexpressing cell lines. *Cancer Chemother Pharmacol* 40: 433–8.
- Horwitz SB (1975) Antibiotics In: Mechanism of action of antimicrobial and antitumor agents. Corcoran W and Hahn FE (eds) Vol III, pp. 48–57, N.Y., Springer Verlag.
- Horwitz SB and Horwitz MS (1973) Effects of camptothecin on the breakage and repair of DNA during the cell cycle. *Cancer Res* 33: 2834–36.
- Houghton PJ, Cheshire PJ, Myers I, Stewart CF, Synold TW and Oughton JA (1992) Evaluation of 9-dimethylaminomethyl-10-hydroxycamptothecin against xenografts derived from adult

- and childhood solid tumors. *Cancer Chemother Pharmacol* 31: 229–59.
- Hsiang H, Liu LF, Wall ME, Wani MC, Nicholas AW, Manikumar G, Kirschenbaum S, Silber R and Potmesil M (1989) DNA topoisomerase I-mediated DNA cleavage and cytotoxicity of camptothecin analogues. *Cancer Res* 49: 4385–89.
- Hsiang T-S (1992) DNA Topoisomerases. *Curr Opin Cell Biol* 4: 396–400.
- Hsiang YH, Herzberg R, Hecht S and Liu LF (1985) Camptothecin induces protein linked DNA breaks via mammalian DNA topoisomerases. *J Biol Chem* 260: 14873–78.
- Hsiang YH, Lihou MG and Liu LF (1989a) Arrest of replication forks by drug-stabilized topoisomerase I-DNA cleavable complexes as a mechanism of cell killing by camptothecin. *Cancer Res* 49: 5077–82.
- Hsiang YH, Wu HY and Liu FL (1988) Proliferation dependent regulation of DNA topoisomerase II in cultured human cells. *Cancer Res* 48: 3230–35.
- Husain I, Mohler JL, Seigler HF and Besterman JM (1994) Elevation topoisomerase I messenger RNA, protein and catalytic activity in human tumors: demonstration of tumor-type specificity and implications for cancer chemotherapy. *Cancer Res* 54: 539–46.
- Hussy P, Maass G, Tümmler B, Grosse F and Schomburg U (1986) Effect of 4-quinolones and novobiocin on calf thymus DNA polymerase α primase complex, topoisomerases I and II, and growth of mammalian lymphoblasts. *Antimicrob Agents Chemother* 29: 1073–78.
- Ikuno N, Soda H, Watanabe M and Oka M (1995) Irinotecan (CPT-11) and characteristic mucosal changes in the mouse ileum and cecum (see comments). *J Natl Cancer Inst* 87: 1876–83.
- Ishii K, Futaki S, Uchiyama H, Nagasawa K and Andoh T (1987) Mechanism of inhibition of mammalian DNA topoisomerase I by heparin. *Biochem J* 241: 111–16.
- Ishimi Y, Nishizawa M and Andoh T (1991) Characterization of a camptothecin-resistant human DNA topoisomerase I in an *in vitro* system for Simian virus 40 DNA replication. *Eur J Biochem* 202: 835–39.
- Izquierdo MA, Scheffer GL, Flens MJ, Schroeijs AB, van der Valk P and Schepers RJ (1996) Major vault protein LRP-related multidrug resistance. *Eur J Cancer* 6: 976–84.
- Jaxel C, Kohn KW, Wani MC, Wall ME and Pommier Y (1989) 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* 49: 1465–69.
- Jaxel C, Capranico G, Wasserman K, Kerrigan D, Kohn KW and Pommier Y (1991) DNA sequence at sites of topoisomerase I cleavage induced by Camptothecin in SV40 DNA. In: Potmesil M and Kohn KW (eds) *DNA topoisomerases in cancer*. Oxford University Press, New York, pp. 182–195.
- Jensen PB, Holm B, Sorensen M, Christensen IJ and Sehested M (1997) *In vitro* cross-resistance and collateral sensitivity in seven resistant small-cell lung cancer cell lines: preclinical identification of suitable drug partners to taxotere, taxol, topotecan and gemcitabine. *Br J Cancer* 75: 869–77.
- Juan CC, Hwang J, Liu AA, Whang-Peng J, Knutsen T, Huebner K, Croce CM, Zhang H, Wang JC and Liu LF (1988) Human DNA topoisomerase I is encoded by a single-copy gene that maps to chromosome region 20q12–13.2. *Proc Natl Acad Sci USA* 85: 8910–17.
- Kafiani CA, Bronstein IB, Timofeen AV, Gromova II and Ter-sikh VV (1986) DNA topoisomerases and regulation of cell proliferation. *Adv Enzyme Regul* 25: 439–57.
- Kantarjian HM, Beran M, Ellis A, Zwelling L, O'Brien S, Cazenave L, Koller C, Rios MB, Plunkett W, Keating MJ and Estey EH (1993) Phase I study of topotecan, a new topoisomerase I inhibitor, in patients with refractory or relapsed acute leukemia. *Blood* 81: 1146–51.
- Kanter PM and Schwartz HS (1982) A fluorescence enhancement assay for cellular DNA damage. *Mol Pharmacol* 22: 145–51.
- Kanzawa F, Sugimoto Y and Minato K, *et al.* (1990) Establishment of a camptothecin analogue (CPT-11)-resistant cell line of human non-small-cell lung cancer: Characterization and mechanism of resistance. *Cancer Res* 50: 5919–24.
- Khon KW, Ewig RAG, Erickson LC and Zwelling LA (1981) Measurement of strand breaks and cross links by alkaline elution. In: Friedberg EC and Hanawalt PC (eds) *DNA repair: a laboratory manual of research procedures*. (pp. 379–401) Marcel Dekker, Inc. New York.
- Kim JS, Gatto B, Yu C, Liu A, Liu LF, and LaVoie EJ (1996) Substituted 2, 5'-Bi-1H-benzimidazoles: topoisomerase I inhibition and cytotoxicity. *J Med Chem* 39: 992–8.
- Kingsbury WD, Boehm JC, Jakas DR, Holden KG, Hecht SM, Gallagher G, Caranfa MJ, McCabe FL, Faucette LF, Johnson RK and Hertzberg RP (1991) Synthesis of water-soluble (aminoalkyl)camptothecin analogues: Inhibition of topoisomerase I and antitumor activity. *J Med Chem* 34: 98–107.
- Kjeldsen E, Bonven BJ, Andoh T, Ishii K, Okada K, Bolund L and Westergaard O (1988) Characterization of a camptothecin resistant human DNA topoisomerase I. *J Biol Chem* 263: 3912–16.
- Knab AM, Fertala J and Bjornsti MA (1993) Mechanisms of camptothecin resistance in yeast DNA topoisomerase I mutants. *J Biol Chem* 268: 22322–30.
- Kubota N, Kanzawa F, Nishio K, Takeda Y, Ohmori T, Fujiwara Y, Terashima Y and Saijo N (1992) Detection of topoisomerase I gene point mutation in CPT-11 resistant lung cancer cell line. *Biochem Biophys Res Commun* 188: 571–7.
- Lefevre D, Riou J-F and Ahomadegbe JC *et al.* (1991) Study of molecular markers of resistance to m-AMSA in a human breast cancer cell line. Decrease of topoisomerase II and increase of both topoisomerase I and the acidic glutathione S transferase. *Biochem Pharmacol* 41: 1967–79.
- Li HL, Fraser TJ, Olin EJ and Bhuyan BK (1972) Action of camptothecin on mammalian cells in culture. *Cancer Res* 32: 2643–50.
- Lin JH and Castora FJ (1995) Response of purified mitochondrial DNA topoisomerase I from bovine liver to camptothecin and m-AMSA. *Arch Biochem Biophys* 324: 293–9.
- Liu LF (1983) DNA topoisomerase-enzymes that catalyse the breaking and rejoining of DNA. *Crit Rev Biochem* 15: 1–24.
- Liu LF (1989) DNA topoisomerase poisons as antitumor drugs. *Annu Rev Biochem* 58: 351–75.
- Liu LF (1990) DNA topoisomerases in drug resistance and cancer chemotherapy. The third Conference on DNA Topoisomerase in therapy. Oct 15–18, New York, p. 25.
- Liu LF, Rowe TC, Yang L, Tewey KM and Chen GL (1983) Cleavage of DNA by mammalian DNA topoisomerase II. *J Biol Chem* 258: 15365–70.
- Loe DW, Deeley RG and Cole SPC (1996) Biology of the multidrug resistance-associated protein, MRP. *Eur J Cancer* 6: 945–57.
- Ludescher C, Gatringer C, Drach J, Hofmann J and Grunicke H (1991) Rapid functional assay for the detection of multidrug-resistant cells using the fluorescent dye rhodamine-123. *Blood* 78: 1385–87.
- Madden KR, Stewart L and Champoux JJ (1995) Preferential binding of human topoisomerase I to superhelical DNA. *EMBO J* 14:

- 5399–409.
- Makhey D, Gatto B, Yu C, Liu A, Liu LF and LaVoie EJ (1996) Coralyne and related compounds as mammalian topoisomerase I and topoisomerase II poisons. *Bioorg Med Chem* 4: 781–91.
- Marshall ES, Finlay GJ, Matthews JHL, Shaw JHF, Nixon J and Baguley BC (1992) Microculture-based chemosensitivity testing: a feasibility study comparing freshly explanted human melanoma cells with human melanoma cell lines. *J Natl Cancer Inst* 84: 341–44.
- Masin JS, Berger SJ, Setrakian S, Stepanik DW and Berger NA (1995) Topoisomerase I activity in squamous cell carcinoma of the head and neck. *Laryng* 105: 1191–6.
- McLeod HL, Douglas F, Oates M, Symonds RP, Prakash D, van der Zee AGJ, Kaye SB, Brown R and Keith WN (1994) Topoisomerase I and II activity in human breast, cervix, lung and colon cancer. *Int J Cancer* 59: 607–611.
- Meikle I, Cummings J, Macpherson JS and Smyth JF (1995) Identification of anthracenyl-dipeptide conjugates as novel topoisomerase I and II inhibitors and their evaluation as potential anticancer drugs. *Anticancer Drug Des* 10: 515–27.
- Mi Z and Burke TG (1994a) Differential interaction of camptothecin lactone and carboxylate forms with human blood components. *Biochemistry* 33: 10325–36.
- Mi Z and Burke TG (1994b) Marked interspecies variations concerning the interaction of camptothecin with serum albumin: a frequency-domain fluorescence spectroscopic study. *Biochemistry* 33: 12540–45.
- Muggia FM, Creaven PJ, Hansen H, Cohen MH and Selawry OS (1972) Phase I clinical trial of weekly and daily treatment with camptothecin (NSC-100880): Correlation with preclinical studies. *Cancer Chemother Reports* 56: 515–21.
- Muller MT, Pfund WP and Mehta VB *et al.* (1985) Eukaryotic type I topoisomerase is enriched in the nucleolus and catalytically active on ribosomal DNA. *EMBO J* 4: 1237–43.
- Murren JR, Beidler DR and Cheng YC (1996) Camptothecin resistance related to drug-induced down-regulation of topoisomerase I and to steps occurring after the formation of protein-linked DNA breaks. *Ann N Y Acad Sci* 803: 74–92.
- Nakatsu S, Kondo S, Kondo Y, Yin D, Peterson JW, Kaakaji R, Morimura T, Kikuchi H, Takeuchi J and Barnett GH (1997) Induction of apoptosis in multi-drug resistant (MDR) human glioblastoma cells by SN-38, a metabolite of the camptothecin derivative CPT-11. *Cancer Chemother Pharmacol* 39: 417–23.
- Niimi S, Nakagawa K, Sugimoto Y, Nishio K, Fujiwara Y, Yokoyama S, Terashima Y and Saijo N (1992) Mechanism of cross-resistance to a camptothecin analogue (CPT-11) in a human ovarian cancer cell line selected by cisplatin. *Cancer Res* 52: 328–33.
- Nitiss JL and Beck WT (1996) Antitopoisomerase drug action and resistance. *Eur J Cancer* 6: 958–66.
- Nooter K and Herweijer H (1991) Multidrug resistance (mdr) genes in human cancer. *Br J Cancer* 63: 663–9.
- Oguro M, Seki Y, Okada K and Andoh T (1990) Collateral drug sensitivity induced in CPT-11 (a novel derivative of camptothecin)-resistant cell lines. *Biomed Pharmacother* 44: 209–216.
- Pantazis P, Early JA, Mendoza JT, DeJesus AR and Giovanella BC (1994a) Cytotoxic efficacy of 9-nitrocarnptothecin in the treatment of human malignant melanoma cells *in vitro*. *Cancer Res* 54: 771–6.
- Pantazis P, Harris N, Mendoza J and Giovanella B (1994b) Conversion of 9-nitro-carnptothecin to 9-amino-carnptothecin by human blood cells *in vitro* (letter). *Eur J Haematol* 53: 246–8.
- Pantazis P, Hinz HR, Mendoza JT, Kozielski AJ, Williams LJ Jr, Stehlin JS Jr and Giovanella BC (1992) 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 camptothecin. *Cancer Res* 52: 3980–87.
- Pantazis P, Mendoza J, DeJesus A, Early J, Shaw M and Giovanella BC (1994c) Development of resistance to 9-nitro-carnptothecin by human leukemia U-937 cells *in vitro* correlates with altered sensitivities to several anticancer drugs. *Anticancer Drugs* 5: 473–9.
- Perez-Stable C, Shen CC and Shen CKJ (1988) Enrichment and depletion of Hela topoisomerase I recognition sites among specific types of DNA elements. *Nucl Acids Res* 16: 7975–93.
- Pessina A (1993) Topoisomerase I in multiple drug resistance. *Cytotechnology* 12: 127–135.
- Pommier Y, Kerrigan D and Hartman KD *et al.* (1990) Phosphorylation of mammalian DNA topoisomerase I and activation by protein kinase C. *J Biol Chem* 265: 9418–22.
- Pommier Y, Zwelling LA, Kao-Shan CS, Whang-Peng J and Bradley MO (1985) Correlation between intercalator induced DNA strand breaks and sister chromatid exchanges mutations and cytotoxicity in Chinese hamster cells. *Cancer Res* 45: 3143–48.
- Pommier Y, Gupta M, Valenti and Nieves-Neira W (1996) Cellular resistance to camptothecins. In Pantazis P, Giovanella BC and Rothenberg ML (eds) *The Camptothecin: From Discovery to the Patient*. New York: Annals NY Acad Sci 52: 2268–78.
- Potmesil M, Hsiang Y-H, Liu LF, Bank B, Grossberg H, Kirschenbaum S, Forlenza TJ, Penziner A, Kanganis D, Knowles D, Traganos F and Silber R (1988) Resistance of human leukemic and normal lymphocytes to drug-induced DNA cleavage and low levels of DNA topoisomerase II. *Cancer Res* 48: 3537–43.
- Pratt CB, Stewart C, Santana VM, Bowman L, Furman W, Ochs J, Marina N, Kuttlesch JF, Heideman R, Sandlund JT, Avery L and Meyer WH (1994) Phase I study of topotecan for pediatric patients with malignant solid tumors. *J Clin Oncol* 12: 539–43.
- Priel E, Showalter SD and Blair DG (1991) Inhibition of human immunodeficiency virus (HIV-1) replication *in vitro* by non cytotoxic doses of camptothecin, a topoisomerase I inhibitor. *AIDS Res Hum Retrovirus* 7: 65–72.
- Prost S (1995) Mechanisms of resistance to topoisomerases poisons. *Gen Pharmacol* 26: 1673–84.
- Qian X and Beck WT (1990) Binding of an optically pure photoaffinity analogue of verapamil LU-49888 to P-glycoprotein from multidrug resistant human leukemic cell lines. *Cancer Res* 50: 1132–1137.
- Ray S, Majumder HK, Chakravarty AK, Mukhopadhyay S, Gil RR, and Cordell GA (1996) Amarogentin, a naturally occurring secoiridoid glycoside and a newly recognized inhibitor of topoisomerase I from *Leishmania donovani*. *J Nat Prod* 59: 27–9.
- Ray S, Sadhukhan PK, Mandal NB, Mahato SB and Majumder HK (1997) Dual inhibition of DNA topoisomerases of *Leishmania donovani* by novel indolyl quinolines. *Biochem Biophys Res Commun* 230: 171–5.
- Riou JF, Grondard L, Naudin A and Bailly C (1995) Effects of two distamycin-ellipticine hybrid molecules on topoisomerase I and II mediated DNA cleavage: relation to cytotoxicity. *Biochem Pharmacol* 50: 424–8.
- Rodriguez-Campos A, Azorin F and Portugal J (1996) Influence of elsamicin A on the activity of mammalian topoisomerase I. *Biochemistry* 35: 11177–82.
- Roninson IB (1992) The role of the MDR1 (P-glycoprotein) gene in multidrug resistance *in vitro* and *in vivo*. *Biochem Pharmacol* 43: 95–102.
- Rose KM, Szopa J, Han FS, Cheng YC, Richter A and Scheer

- U (1988) Association of DNA topoisomerase and RNA polymerase I: a possible role for topoisomerase I in ribosomal gene transcription. *Chromosoma* 96: 411–416.
- Rossi F, Labourier E, Forne T, Divita G, Derancourt J, Riou JF, Antoine E, Cathala G, Brunel C and Tazi J (1996) Specific phosphorylation of SR proteins by mammalian DNA topoisomerase I. *Nature* 381: 80–2.
- Rothenberg ML (1997) Topoisomerase I inhibitors. *Annu Oncol* 8: 837–55.
- Rowinsky EK, Grochow LB, Hendricks CB, Ettinger DS, Forastiere AA, Hurowitz LA, McGuire WP, Sartorius SE, Lubejko BG, Kaufmann SH and Donehower RC (1992) Phase I and pharmacological study of topotecan: A novel topoisomerase inhibitor. *J Clin Oncol* 10: 647–56.
- Rubin E, Pantazis P, Bharti A, Toppmeyer D, Giovannella B and Kufe D (1994) Identification of a mutant human topoisomerase I with intact catalytic activity and resistance to 9-Nitro-camptothecin. *J Biol Chem* 269: 2433–39.
- Saltz L, Sirott M, Young C, Tong W, Niedzwiecki D, Tzy-Jyun Y, Tao Y, Trochanowski B, Wright P, Barbosa K, Toomasi F and Kelsen D (1993) 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. *J Natl Cancer Inst* 85: 1499–507.
- Scheper RJ, Broxterman HJ, Scheffer GL, Kaaijk P, Dalton WS, Heijningen van THM, van Kalken CK, Slovak ML, de Vries EGE, van der Valk P, Meijer CJLM and Pinedo HM (1993) Overexpression of a Mr 110 000 vesicular protein in non-P-glycoprotein-mediated multidrug resistance. *Cancer Res* 53: 1475–79.
- Shen CC and Shen CKJ (1990) Specificity and flexibility of recognition of DNA helical structure by eukaryotic topoisomerase I. *J Mol Biol* 212: 67–78.
- Shimizu T and Pommier Y (1996) DNA fragmentation induced by protease activation in p53-null human leukemia cells undergoing apoptosis following treatment with the topoisomerase I inhibitor camptothecin: Cell-free system studies. *Exp Cell Res* 226: 292–301.
- Slichenmyer WJ, Rowinsky EK, Donehower RC and Kaufmann SH (1993) The current status of camptothecin analogues as antitumor agents. *J Natl Cancer Inst* 85: 271–91.
- Smith PJ (1990) DNA topoisomerase dysfunction: a new goal for antitumor chemotherapy. *Bioassay* 12: 1657–72.
- Snow K and Judd W (1991) Characterization of adriamycin resistant and amsacrine-resistant human leukaemic T-cell lines. *Br J Cancer* 63: 17–28.
- Spicer JA, Gamage SA, Atwell GJ, Finlay GJ, Baguley BC and Denny WA (1997) Structure-activity relationships for acridine-substituted analogues of the mixed topoisomerase I/II inhibitor N-[2-(dimethylamino)ethyl]acridine-4-carboxamide. *J Med Chem* 40: 1919–29.
- Sugarman SM, Zou Y, Wasan K, Poirot K, Kumi R, Reddy S and Perez-Soler R (1996) Lipid-complexed camptothecin: formulation and initial biodistribution and antitumor activity studies. *Cancer Chem Pharmacol* 37: 531–8.
- Sugimoto Y, Tsukahara S, Oh-Hara T, Ise T and Tsuruo T (1990) Decreased expression of DNA topoisomerase I in camptothecin-resistant tumor cell lines as determined by a monoclonal antibody. *Cancer Res* 50: 6925–30.
- Supino R, Caserini C, Orlandi L, Zaffaroni N, Silvestrini R, Vaglini M and Zunino F (1996) Modulation of melphalan cytotoxic activity in human melanoma cell lines. *Anticancer Drugs* 7: 604–12.
- Taniguchi K, Kohno K, Kawanami K, Wada M, Kanematsu T and Kuwano M (1996) Drug-induced down-regulation of topoisomerase I in human epidermoid cancer cells resistant to saintopin and camptothecins. *Cancer Res* 56: 2348–54.
- Tanizawa A and Pommier Y (1992) Topoisomerase I alteration in a camptothecin-resistant cell line derived from Chinese hamster DC3F cells in culture. *Cancer Res* 52: 1848–54.
- Tanizawa A, Beirand R, Kohlhagen G, Tabuchi A, Jenkins J and Pommier Y (1993) Cloning of Chinese hamster DNA topoisomerase I cDNA and identification of a single point mutation responsible for camptothecin resistance. *J Biol Chem* 268: 25463–8.
- Thiebaut T, Tsuruo T, Hamada H, Gottesman MM, Pastan I and Willingham MC (1987) Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci USA* 84: 7735–38.
- Trask DK and Muller MT (1988) Stabilization of type I topoisomerase DNA covalent complexes by actinomycin D. *Proc Natl Acad Sci USA* 85: 1417–25.
- Trask DK, DiDonato JA and Muller MT (1984) Rapid detection and isolation of covalent DNA protein complexes: application to topoisomerase I and II. *EMBO J* 3: 671–676.
- Tsuruo T, Matsuzaki T, Matsushita M, Saito H and Yokokura T (1988) Antitumor effect of CPT-11 a new derivative of camptothecin against pleiotropic drug resistant tumors *in vitro* and *in vivo*. *Cancer Chemother Pharmacol* 21: 71–74.
- Turman MA and Douvas A (1993) A Casein Kinase type II (CKII) like nuclear protein kinase associates with, phosphorylates and activates topoisomerase I. *Biochem Med Metab Biol* 50: 210–225.
- Valenti M, Nieves-Neira W, Kohlhagen G, Kohn KW, Wall ME, Wani MC and Pommier Y (1997) Novel 7-alkyl methylenedioxy-camptothecin derivatives exhibit increased cytotoxicity and induce persistent cleavable complexes both with purified mammalian topoisomerase I and in human colon carcinoma SW620 cells. *Mol Pharmacol* 52: 82–7.
- van der Zee AG, Hollema H, de Jong S, Boonstra H, Gouw A, Willems PH, Zijlstra JG and de Vries EG (1991) 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* 51: 5915–20.
- Vasey PA, Jones NA, Jenkins S, Dive C and Brown R (1996) Cisplatin, camptothecin, and taxol sensitivities of cells with p53-associated multidrug resistance. *Mol Pharmacol* 50: 1536–40.
- Verweij J, Lund B, Beijnen J, Planting A, de Boer-Dennert M, Koier I, Rosing H and Hansen H (1993) Phase I and pharmacokinetic study of topotecan, a new topoisomerase I inhibitor. *Ann Oncol* 4: 673.
- Vinograd J, Lebowitz J, Radloff R, Waron R and Laipis P (1965) The twisted circular form of polyoma viral DNA. *Proc Natl Acad Sci USA* 53: 1104–1111.
- Wall ME, Wani NC, Cook CE, Palmer KH, McPhail AT and Sim GA (1966) Plant antitumor agents. 1. The isolation and structure of camptothecin, a novel alkyloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. *J Am Chem Soc* 94: 3888.
- Wang HK, Liu SY, Hwang KM, Taylor G and Lee KH (1994) Synthesis of novel water-soluble 7-(aminoacylhydrazono)-formyl camptothecins with potent inhibition of DNA topoisomerase I. *Bioorg Med Chem* 2: 1397–402.
- Wang HP and Rogler CE (1991) Topoisomerase I-mediated integration of hepatitis virus DNA *in vitro*. *J Virol* 65: 2381–92.
- Wang JC (1985) DNA topoisomerases. *Annu Rev Biochem* 54: 665–697.

- Wang JC (1996) DNA topoisomerases. *Annu Rev Biochem* 65: 636–92.
- Wang JC (1971) Interaction between DNA and Escherichia coli omega protein. *J Mol Biol* 55: 523–533.
- Wang LK, Johnson RK and Hecht SM (1993) Inhibition of topoisomerase I function by nitidine and fagaronine. *Chem Res Toxicol* 6: 813–8.
- Wang LK, Rogers BD and Hecht SM (1996) Inhibition of topoisomerase I function by coralyne and 5,6-dihydrocoralyne. *Chem Res Toxicol* 9: 75–83.
- Wani MC, Ronman PE, Lindley JT and Wall ME (1980) Plant antitumor agents. 18. Synthesis and biological activity of camptothecin analogues. *J Med Chem* 23: 554–60.
- Xie G, Gupta R, Atchison K and Lown JW (1996) Bisindolylmaleimides linked to DNA minor groove binding lexitropsins. synthesis, inhibitory activity against topoisomerase I, and biological evaluation. *J Med Chem* 39: 1049–55.
- Yang CHJ, Cowan KH and Schneider E (1993) Quantitative PCR for DNA topoisomerase I, II alpha, II beta and mdr1 in cell lines and tumor samples. *Proc Annu Meet Am Assoc Cancer Res* 34: A56.
- Yang CHJ, Horton JK, Cowan KH and Schneider E (1995) Cross-resistance to camptothecin analogues in a mitoxantrone-resistant human breast carcinoma cell line is not due to DNA topoisomerase I alterations. *Cancer Res* 55: 4004–9.
- Zhang H, Wang JC and Liu LF (1988) Involvement of DNA topoisomerase I in transcription of human ribosomal RNA genes. *Proc Natl Acad Sci USA* 85: 1060–1064.
- Zhang H, D'Arpa P and Liu LF (1990) A model tumor cell killing by topoisomerase poisons. *Cancer Cells* 2: 23–27.
- Zhao R, Al-Said NH, Sternbach DL and Lown JW (1997) Camptothecin and minor-groove binder hybrid molecules: synthesis, inhibition of topoisomerase I, and anticancer cytotoxicity *in vitro*. *J Med Chem* 40: 216–25.
- Zhou BS, Bastow KF and Cheng YC (1989) Characterization of 3'-region of the human DNA topoisomerase I gene. *Cancer Res* 49: 3922–3927.
- Zijlstra JG, De Jong S, De Vries EGE and Mulder NH (1990) Topoisomerases, new targets in cancer chemotherapy. *Med Oncol Tumor Pharmacother* 7: 11–18.

Address for correspondence: A. Pessina, Institute of Microbiology, University of Milan, Via Pascal 36 20133 Milan, Italy.