Pergamon

European Journal of Cancer, Vol. 34, No. 10, pp. 1500–1508, 1998

Printed in Great Britain
0959-8049/98/$—see front matter

PII: S0959-8049(98)00229-9

Clinical Oncology Update

Camptothecins: a Review of their Development and Schedules of Administration

J. O'Leary and F.M. Muggia

NYU Medical Center, Division of Oncology, Bellevue C&D Building, 462 First Avenue, Room 556, New York, New York 10016, U.S.A.

Used for centuries in traditional Chinese medicine, camptothecin was rediscovered in the 1950s during a search for compounds that could be used as a source for steroid synthesis. Due to its limited water solubility, a sodium salt was used in the early clinical trials. The severe toxicity and erratic absorption relegated this compound to the research laboratory until the 1980s when the topoisomerase enzyme was identified as the cellular target of camptothecin, the topoisomerase enzyme was found to be overexpressed in cancer cells and a structure–activity relationship was determined for camptothecin. These new developments brought the camptothecins back to the clinical setting for further testing. The various analogues that have been most studied to date include: irinotecan (CPT-11), and its derivative SN-38, topotecan, and 9-aminocamptothecin. Numerous trials have been conducted in an attempt to establish the efficacy in various tumour types, to determine the dose-limiting toxicity and to define the optimal schedule of administration. It seems that large doses of these drugs given on intermittent schedules are not effective. Our hypothesis is that the camptothecins require a prolonged schedule of administration given continuously at low doses or frequent intermittent dosing schedules to be most effective. With these schedules, normal haematopoietic cells and mucosal progenitor cells with low topoisomerase I levels may be spared, while efficacy is preserved.

Key words: camptothecins, 9-aminocamptothecin, topotecan, irinotecan, SN-38, topisomerase
Eur J Cancer, Vol. 34, No. 10, pp. 1500–1508, 1998

HISTORY

In 1958, the extract of the Camptotheca tree was tested by the National Cancer Institute (NCI). Its antitumour activity was established in experimental models. Camptothecin was discovered during the 1950s when thousands of plants were analysed in a search for a source for steroids that would be suitable for the synthesis of cortisone.

During this search, Monroe Wall and Mahsukh Wani discovered camptothecin and its natural analogues [1, 2]. Found in the wood, bark, and fruit of the tree *Camptotheca acuminata*, it has been used in traditional Chinese medicine in the treatment of various illnesses including tumours [3]. The NCI Chemotherapy Programme demonstrated camptothecin's activity against the experimental tumours L1210 murine leukaemia and the rat Walker carcinosarcoma.

Correspondence to J. O'Leary.
Received 23 Mar. 1998; accepted 1 Apr. 1998.

S. Horwitz, D. Kessel and other researchers studied the biochemical actions and showed inhibition of both DNA and RNA synthesis by camptothecins. When the drug was removed, the inhibition of RNA synthesis was totally reversed, whereas DNA synthesis was only partially restored [4–10, 113]. Subsequently, Wall and Horwitz demonstrated that the lactone form of the drug was responsible for its activity and that a pH dependent equilibrium existed between the lactone and carboxylate forms [70].

Camptothecin has limited water solubility, so a water soluble sodium salt was formulated for use in clinical trials. In the late 1960s, Gottlieb and Muggia initiated separate phase I clinical trials at the NCI. The results of these trials and partial results of some phase II studies were published in the early 1970s [11–14]. Antitumour activity was noted in patients with GI tumours. Patients experienced neutropenia, thrombocytopenia, haemorrhagic cystitis, and GI symptoms with significant diarrhoea [15–17]. As a result of these findings, clinical studies with camptothecin sodium came to a halt.

Table 1. *Development of camptothecins (chronology based on published full articles or book chapters)*

1966	Active agent 20(S)-camptothecin isolated and its structure established [2]
1966–1970	Extracts from *Camptotheca acuminata* have antitumour activity [2, 11]
1970–1972	Phase I/II clinical trials of camptothecin sodium salt [12, 14, 15, 18]
1985–1988	20(S)-Camptothecin inhibits DNA topoisomerase I (topo I) [19, 43]
1986–1991	Analogues 9-amino-20(S)-camptothecin (9-AC), CPT-11 and topotecan synthesized and tested [20, 42, 54, 111]
1988–1989	Topo-I is elevated in several types of human malignancies [21, 22]
1989	Topo-I is inhibited by biologically active analogues [23, 39]
1989–1993	Effectiveness of 9-AC, CPT-11 and topotecan against human cancer xenografts [21, 93, 112]
1991–1995	Accelerated clinical development of CPT-11 and topotecan [56, 104, 106–110]
1991–1995	9-AC entered phase I/II clinical trials [56, 97, 104]

During the next 20 years, researchers continued to study the drug in the laboratory (Table 1). In retrospect, it was recognised that the carboxylated form results in insufficient and erratic amounts of the active form being available. Earlier observations confirmed the importance of the lactone, closed ring molecule for drug cytotoxicity.

In the 1980s, there were several developments that renewed interest in clinical research with camptothecins. The topoisomerase I enzyme was identified as the cellular target of camptothecin and its analogues [18, 19]. Topoisomerase I was found to be overexpressed in advanced stages of human colon adenocarcinoma and other malignancies but not in normal tissue [20, 21]. A structure—activity relationship was determined for semi-synthetic and totally synthetic camptothecin derivatives [22]. The totally synthetic analogues, 9AC and 10,11-MDC showed unprecedented effectiveness against human colon adenocarcinoma in immunodeficient mice [23, 24]. At that time, CPT-11 (irinotecan) and topotecan were prepared and tested in experimental and clinical settings. Both of these drugs, joined later also by 9AC (9-aminocamptothecin) underwent broad testing in the early 1990s [25, 26].

Topoisomerase I

Topoisomerase I (Figure 1) is a ubiquitous nuclear enzyme. Its gene has been isolated to chromosome 20q12-13.2 [27–30]. It catalyses relaxation of both positively charged and negatively charged supercoiled DNA (Figure 2). This relaxation function probably removes excessive supercoiling that is generated during replication and transcription [31, 32]. At the target level, camptothecin's activity is very specific. It inhibits the breakage–rejoining reaction of DNA topoisomerase I. This inhibition is most specific for the rejoining step, resulting in the accumulation of reversible intermediate complex known as the cleavable complex which consists of topoisomerase I + camptothecin + the DNA as a ternary complex (Figure 3) [33].

The tyrosine hydroxyl group at site #723 on topoisomerase I cDNA attacks the phosphate group of the phosphodiester linkage resulting in an enzyme-linked single-strand DNA break in which the topoisomerase enzyme is covalently linked to the broken DNA strand [35]. At this stage, the phosphodiester linkage opposite the transient breakage site can swivel. After it swivels, the rejoining reaction starts to take place. This rejoining is initiated by the 5′ hydroxyl group attacking the tyrosyl phosphate bond. The tyrosine residue is released from the phosphate and the single strand break is repaired. Camptothecin interferes with this reaction by blocking the rejoining step [36, 37]. Camptothecin and all of its analogues only bind to the topoisomerase I–DNA complex. It does not bind to the topoisomerase enzyme alone and it does not bind to the DNA alone. The net result is that camptothecin causes fragmentation of chromosomal DNA, kills cells in S-phase and causes extensive chromatid exchanges and chromosomal aberrations [34, 38, 39].

EFFECTS OF SUBSTITUTIONS ON CAMPTOTHECIN AND ITS ANALOGUES

All camptothecins have a basic five ring structure which is essential for activity. A tetracyclic camptothecin analogue has

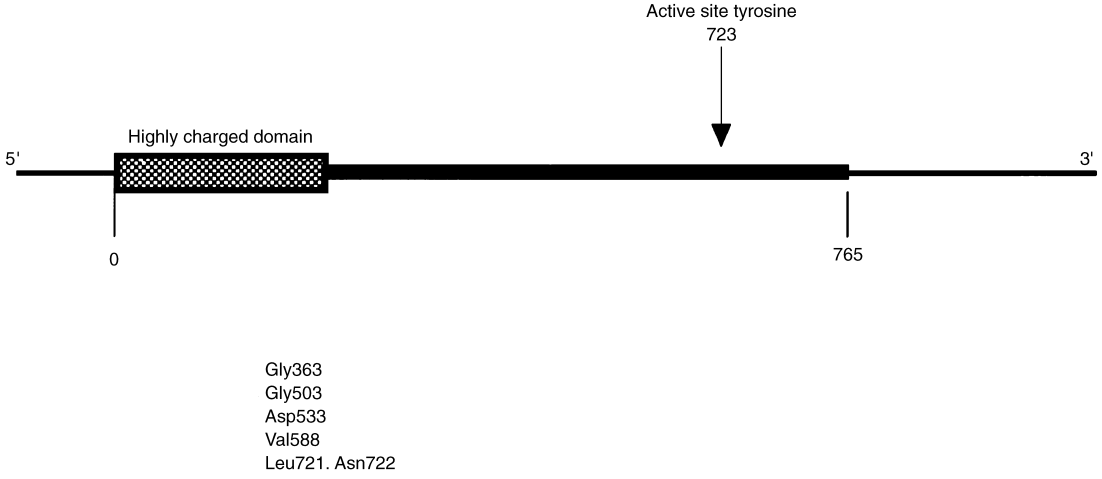

Figure 1. Human DNA topoisomerase I cDNA. The amino acid residues listed represent sites of mutation that can lead to camptothecin resistance. Reproduced by permission of CRC Press Inc. [34].

been prepared and was found to be inactive. The alpha-hydroxylactone at carbon 12 in ring E is an absolute requirement for *in vitro* and *in vivo* activity of camptothecin and its analogues. Specific stereochemistry at carbon 20 (S) isomer is also an absolute necessity for *in vivo* and *in vitro* activity. The (R) isomer is inactive and the racemic mixture has only 50% of the (S) isomer activity.

Substitutions at carbons 9 and 10 by amino groups lead to compounds with greater *in vivo* activity. 9-amino-20 (s)-CPT, 10-amino-20 (s)-CPT, 9-nitro-20 (s)-CPT and 10-nitro-20 (s)-CPT all possess enhanced activity on topoisomersae I [41, 42]. Monosubstitutions at carbons 11 and 12 lower the activity and potency of camptothecin derivatives [41–43]. The addition of an amino group at carbon 12 abolishes activity against topoisomerase I [41, 42, 44]. Substitutions at carbons 10 and 11 with methoxy moieties result in an inactive analogue [45]. However, 10,11-methylene dioxy substitution in ring A of camptothecin results in remarkable enhancement of both *in vivo* and *in vitro* activity [44, 46, 47].

Camptothecin probably binds to the cleavable complex on the face that is proximal to carbons 11 and 12. Hence any moieties that are substituted in these positions may cause unfavourable steric or stereo-electric interactions. Substitutions at carbons 9 or 10 are more distant from this region, thereby causing less steric hindrance. However, bulky substitutions at carbons 9 and 10 may interfere with the active site in the C11 and C12 area resulting in decreased binding to the topoisomerase I–DNA-cleavable complex. 10,11-dimethoxy is an inactive derivative. Steric repulsion between the two methoxy moieties at positions 10 and 11 may cause the methoxy group at carbon 11 to encroach upon the critical region in the vicinity of position 12. In the 10,11-methylene-dioxy compound, the substituted atoms are held together in a coplanar ring form which prevents encroachment on the C12 critical region [40, 49, 50].

IRINOTECAN

Key features

Irinotecan (CPT-11) is a prodrug which possesses limited antitumour activity. It is converted by the enzyme carboxylesterase to a more active compound, SN 38. SN 38 is reported to be anywhere from 200 to 1,000 times more potent than Irinotecan [51]. In a phase I study of CPT-11 performed in San Antonio, serial blood and urine samples were collected from patients and analysed by high performance liquid chromatography [52]. It was found that the peak plasma concentration of CPT-11 occurred immediately after the infusion was completed. The peak plasma concentration of its active metabolite, SN 38 was more variable, occurring anywhere from 30 to 90 min after completion of the infusion. The primary route of clearance for both CPT-11 and SN 38 is biliary excretion. The bile to plasma ratio for CPT-11 is 60:1 and the bile to plasma ratio for SN 38 is 9:1. There is also a minor component of renal excretion for both

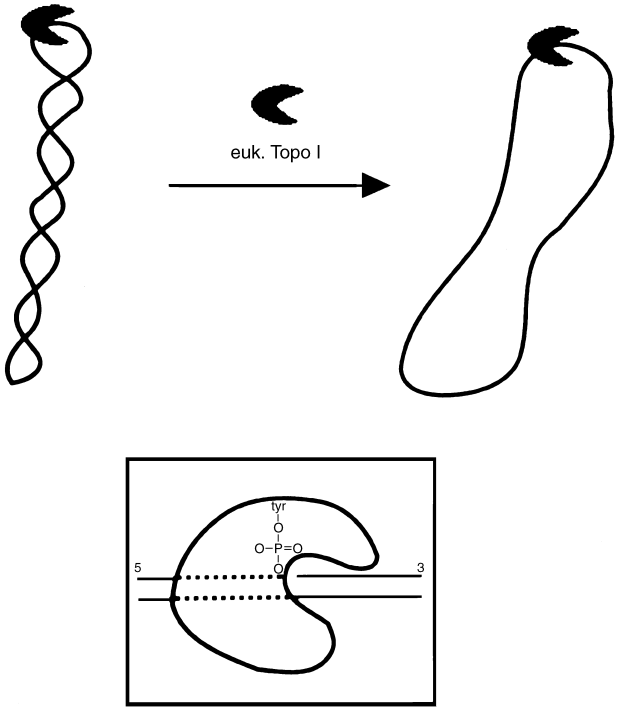

Figure 2. Super-coiled DNA and its relaxation by mammalian DNA topoisomerase I. Reproduced by permission of CRC Press Inc. [34].

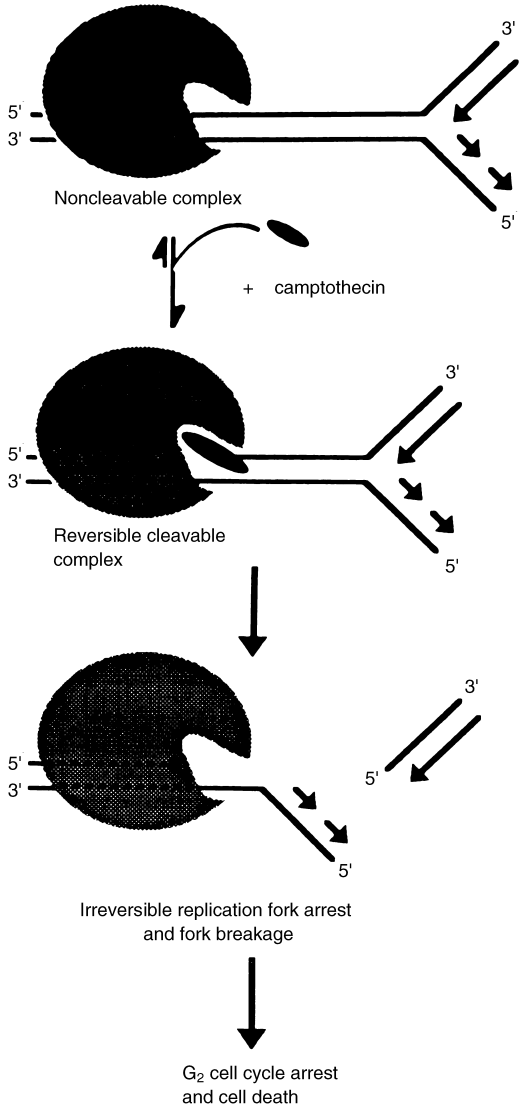

Figure 3. Mechanism of cell killing by camptothecins. Reproduced by permission of CRC Press Inc. [34].

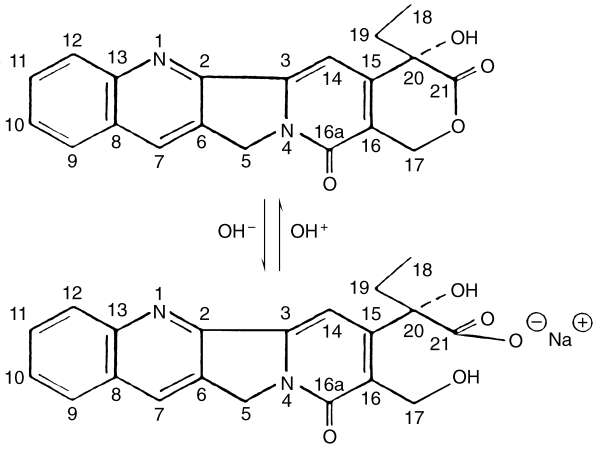

Figure 4. Chemical structure of 20 (S)-camptothecin lactone and its carboxylate salt.

compounds within the first 48 h. The half-life for the lactone form of CPT-11 is approximately 6 h whereas the terminal half-life of SN 38 is about 11–13 h [53].

Review of dose scheduling

Phase I trials were first conducted in Japan, followed by the U.S.A. and Europe. Different routes of administration were studied and the drug was found to be effective if given intravenously (i.v.), intraperitoneally (i.p.) or orally [54, 55].

Different schedules of administration were also studied. The drug was given as an infusion every 3 weeks, as a weekly infusion for 4 weeks, or as a daily infusion for three consecutive days every 3 weeks. The most important dose limiting toxicity (DLT) in the single dose infusions given every 3 weeks was myelosuppression. The most important DLT with the daily $\times 3$ schedule and the weekly $\times 4$ schedule was diarrhoea. With these modes of administration, antitumour activity was noted in colorectal, cervical and mammary tumours [56].

In the initial two phase I studies conducted in the U.S.A., the Johns Hopkins study gave one i.v. infusion over 90 min every 3 weeks and the San Antonio study gave one i.v. infusion over 90 min every week for 4 weeks with a 2 week rest period. A total of 64 patients were entered in these two trials. There were four objective responses. Three of these responses were seen in patients with recurrent colorectal cancer and in 1 patient with cervical cancer. In the Johns Hopkins trial, the maximum tolerated dose (MTD) was established at 240 mg/m^2 and DLT included neutropenia, nausea and vomiting and diarrhoea [57, 58]. The San Antonio trial consisted of 32 patients, most of whom had received prior therapy. 2 patients had a partial response and 11 had stable disease. The MTD was 150 mg/m^2/wk and the DLT was diarrhoea. Myelosuppression was not a major toxicity and clinically significant granulocytopenia was very rare. There were no episodes of neutropenic fever [59].

Clinical trials of CPT 11 were also conducted in France. Three schedules of administration were studied. When the drug was given for 3 consecutive days every 3 weeks, the MTD was 115 mg/m^2 and DLT was neutropenia and diarrhoea. When given weekly for 3 weeks, the MTD was 145 mg/m^2. When given once every 3 weeks, an MTD of 350 mg/m^2 was achieved. Diarrhoea was less severe and myelosuppression became the DLT [60]. In a study conducted in Amsterdam, the drug was given over a prolonged infusion schedule of 14 days every 3–5 weeks. The study consisted of 10 heavily pretreated patients. An MTD of 175 mg/m^2 was achieved, compared with an MTD of 350 mg/m^2 which was achieved with shorter infusion schedules. DLT consisted of both diarrhoea and neutropenia [61].

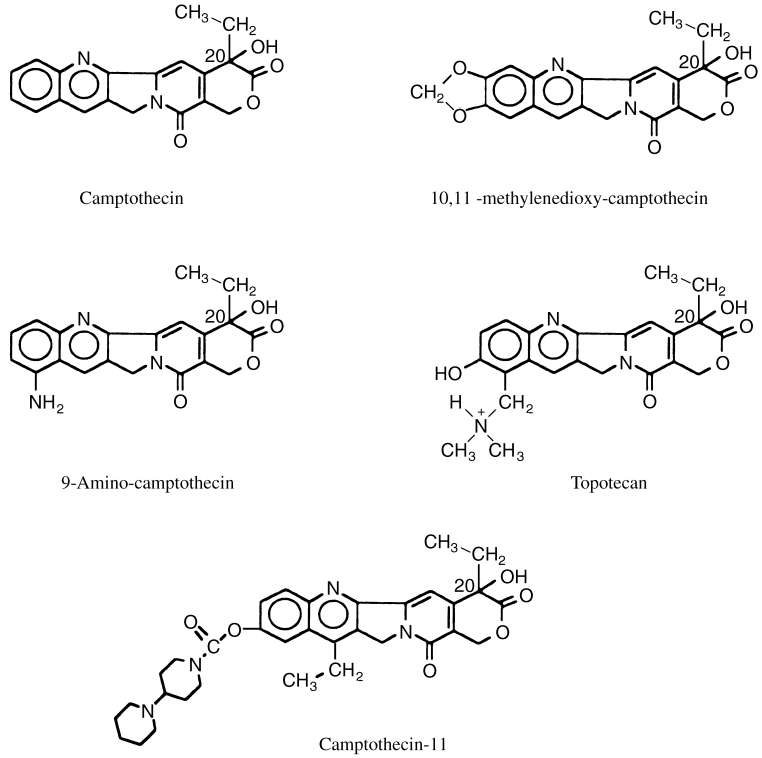

Figure 5. Chemical structures of camptothecin derivatives. Reproduced by permission of CRC Press Inc. [34].

Oral delivery of CPT 11 was addressed in a European study. Among 28 patients, most of whom had advanced colorectal cancer, the drug was given for 5 consecutive days every 3 weeks. There was an overall response rate, including partial response and stable disease, of about 78% [62]. A prolonged schedule of oral administration is currently being tested by several groups in the U.S.A.

In phase II clinical trials of CPT-11 that were conducted by Japanese groups, utilising mainly the weekly or every other week schedules it was found to have a wide range of activities in solid tumours, leukaemia and lymphoma. These studies demonstrated significant clinical activity in patients with small cell lung cancer (RR 37%), non-small cell lung cancer (NSCLC) (RR 34%), previously treated colorectal cancer (RR 32%) [64–66], advanced gastric cancer (RR 23%), previously treated ovarian and cervical cancer (RR 24%), previously treated non-Hodgkin's lymphoma (NHL) (RR 45%), adult T-cell leukaemia/lymphoma (RR 50%). The most noteworthy response rate was that of 32% in previously treated metastatic colon cancer. In this study, the drug was given according to both schedules, once every week or once every 2 weeks and activity was found with either [63].

One of the most troubling side-effects seen in these early clinical trials was diarrhoea. It was seen more frequently when CPT-11 was given on an intermittent schedule such as once a week every week for 4 weeks or when given on a more prolonged schedule such as 3 days every 3 weeks. The diarrhoea can occur early, less than 1 h after the infusion or it can be more delayed, occurring more than 24 h after the infusion. The early onset diarrhoea is associated with abdominal cramping, vomiting, flushing, and diaphoresis. This type of diarrhoea appears to be mediated by increased cholinergic activity. Therefore, it is readily reversible with atropine [67, 68]. The delayed diarrhoea is unresponsive to anticholinergic agents. The most effective intervention against this subacute diarrhoea is early recognition and immediate treatment with antimotility agents. Loperamide should be given at the first sign of abdominal cramping or with the first loose bowel movement. Once grade IV diarrhoea develops, no measures are effective in reversing the process. At this point supportive care with i.v. fluids and electrolyte replacement is all that can be done. The diarrhoea should resolve on its own within 5–7 days. It is impossible to predict which patients will develop diarrhoea. Prior chemotherapy or radiation does not influence the frequency or severity. It has been suggested that patients who have higher carboxylesterase levels convert more CPT-11 to SN 38 and thereby are at increased risk of developing diarrhoea [69]. SN 38 is metabolised by glucoronidation. Patients who have more effective glucoronidation have less gastrointestinal (GI) toxicity as the glucoronidated SN 38 is associated with less diarrhoea [70–72]. Recent attempts at ameliorating toxicity by inhibiting biliary excretion with cyclosporin have been described and may become useful clinically.

TOPOTECAN

Key features

This camptothecin analogue was made water soluble by the presence of a stable, basic side-chain at carbon 9 of the A ring. In preclinical studies, topotecan showed antitumour activity *in vitro* and *in vivo*. The drug was active when given i.v., i.p., subcutaneously (s.c.) and orally against various human cancer cell lines carried as xenografts in nude mice [73, 74]. Greater responses were seen when the drug was given with more frequent and prolonged administration. Continuous infusion was found to be more active compared with intermittent infusions. With the intermittent schedules of administration, DLT was neutropenia, whereas thrombocytopenia was the DLT with the more prolonged infusions and the continuous i.v. infusion. Its non-haematological toxicities such as nausea, vomiting, diarrhoea, fever, rash, fatigue and alopecia are mild and infrequent. Central nervous system (CNS) penetration has been demonstrated. Its cerebrospinal fluid (CSF) level is 32% of its simultaneous plasma levels [75]. Renal elimination is the primary route of excretion of the drug. Forty per cent of the drug can be recovered in the urine during the first 24 h after infusion. The half-life is 3–3.5 h [76].

Review of dose-scheduling

Many schedules of administration have been studied. These include; a 30 min infusion every 3 weeks; a 30 min infusion for 5 consecutive days every 3 weeks; a 24 h infusion every week; a 24 h infusion every 3 weeks; continuous infusion for 72 h every 3 weeks; and as a continuous infusion for 120 h every 3 weeks. More responses were seen in patients who were treated with more frequent dosing or with more prolonged administration. Responses have been seen in patients with small cell NSCLC, platinum refractory ovarian cancer, various GI tumours, breast cancer and leukaemia. Topotecan could be given up to ten cycles without any evidence of cumulative myelosuppression suggesting that it is non-toxic to haematopoietic stem cells. With a 30 min infusion given every 3 weeks, the San Antonio group achieved an MTD of 22.5 mg/m^2 with DLT being neutropenia [77]. At Johns Hopkins, patients were treated with a 30 min infusion on 5 consecutive days every 3 weeks. The same treatment schedule was studied by another institution with similar conclusions. The average MTD was around 1.5–2 mg/m^2/day and DLT was neutropenia [78]. Using the same schedule, a phase I study conducted in Copenhagen showed three partial responses and stable disease in 24 patients among the 48 patients who entered the trial [79]. At Fox Chase, patients were treated with a 24 h infusion every week. The MTD was 2 mg/m^2/wk for up to 3 weeks. This corresponded to a dose intensity of 1.5 mg/m^2/wk. The schedule of a 24 h infusion given every 3 weeks was studied at two separate institutions. With this schedule, a MTD of 5–10 mg/m^2 was achieved, producing a dose intensity of 2–3 mg/m^2/wk. DLT was neutropenia [80, 81]. In a phase I study conducted in Amsterdam, there was no tumour response among 26 patients treated on this schedule [79]. Topotecan as a 120 h continuous infusion was studied by several groups. With this schedule, a very low MTD of 0.68 mg/m^2/day could be delivered. DLTs were thrombocytopenia, neutropenia and severe mucositis. Subsequent studies reduced the continuous infusion schedule to 72 h every 2–3 weeks and were able to achieve an MTD of 1.60 mg/m^2/d. Preclinical studies in mice conducted at NYU showed greater antitumour activity with continuous infusion schedules. Based on these results, a phase I, 21-day continuous infusion protocol was developed. Dosing was started at 0.2 mg/m^2/d. The infusion was first increased by time up to 21 days and was then increased by a dose up to 0.7 mg/m^2/d. All patients had received prior treatment. With this protocol, the major DLT was thrombocytopenia, more so than neutropenia. Most patients who received multiple cycles, required red blood cell transfusions. Non-haematological toxicity was very mild. Responses were seen

in breast, renal cell, ovarian and NSCLC cancers. The 21-day prolonged infusion schedule achieved a dose intensity that exceeded all other schedules. This proves that such a schedule is safe, feasible, well-tolerated and active [82]. More recently, the results of a randomised phase II study were presented at the American Society of Clinical Oncology annual meeting. This Canadian trial randomised previously treated epithelial ovarian cancer patients to topotecan given every day for 5 days every 3 weeks versus a 24-h infusion given once a week for 4 weeks with a 2 week break. The response rate in the weekly administration arm was only 3%, whereas the response rate in the daily×5 schedule was 24% [83].

Based on promising animal studies, there is increased interest in oral formulations using prolonged administration schedules. In murine models, the drug showed good oral bioavailability. In preliminary phase I testing of oral topotecan in humans, the bioavailability was about 32%. These phase I studies are ongoing [84, 85].

AMINO-CAMPTOTHECIN (9-AC)

Key features

When 9-AC was given to mice as an i.v. bolus, s.c. or intramuscular (i.m.) the drug was rapidly absorbed, followed by a high plasma level of the active lactone form. There was rapid drug elimination with a half-life of less than 2 h. This form of administration was toxic and produced only partial responses. At the same doses, when 9-AC suspension was injected s.c., a depot was established with a gradual release into the bloodstream. There was a low peak in the 9-AC plasma lactone level and gradual elimination with a half-life greater than 17 h. There was little to no toxicity and some complete responses were noted in addition to partial responses. These results suggest that a low plasma lactone level, below a toxic threshold and sustained over an extended time, is essential for optimal therapeutic efficacy. The tapered-off plasma level of the 9-AC lactone form with short intervals between treatments may markedly decrease the DLT to normal tissue which have lower topoisomerase I levels, whilst at the same time preserving cytotoxicity against tumour tissue which overexpresses topoisomerase I [56]. 9-AC has effective anticancer activity in mice with small tumours, less than 0.25 cm, as well as in mice with bulky tumours, 2.5–8 cm in size. 9-AC enters cells rapidly and the level of cell-associated drug remains elevated for 6–24 h over drug levels in tissue culture media. The lactone form becomes intercalated in the acyl side-chains in the cell membrane. This region of the cell membrane is hydrophobic and helps to preserve the active lactone form [86–89]. Given via the GI tract, 9-AC is fully active and at higher doses induces complete remissions of human cancer carried as xenografts in nude mice [90, 91].

Preclinical studies in mice demonstrated the effectiveness of 9-AC in colon cancer cell lines that had a high expression of MDR. 9-AC injected s.c. or i.m. at 4 mg/kg twice a week, induced complete remissions in s.c. implanted tumours which were resistant to several clinical anticancer agents [92]. Some of these remissions lasted the entire lifespan of the experimental animal. In mice with liver metastases from a primary colon cancer, compared with treatment with standard 5-FU, 9-AC prolonged the survival of these mice significantly [93–95]. The drug was also found to be effective against melanoma with CNS and adrenal metastases. It was also effective against lung cancer with CNS metastases.

As with all the other camptothecin analogues, poor water solubility produced a major obstacle to the introduction of 9-AC into clinical trials. To increase the solubility of 9-AC, it had to be formulated in dimethylacetamide (DMA) and a special diluent composed of polyethylene glycol and phosphoric acid [96, 97]. This 9-AC/DMA formulation was used in most phase I and II studies. However, this formulation is not compatible with aqueous solutions, requires glass syringes for handling and is toxic. More recently, 9-AC has been formulated as colloidal dispersion (CD) responses were reported in patients with colorectal cancer, ovarian cancer and NSCLC. In both trials, the DLT was neutropenia. Some thrombocytopenia was also seen. In every 2 week schedule given at the NCI, anaemia requiring transfusion was common [99].

Based on the Dana Farber (Boston, Massachusetts, U.S.A.) and NCI results, a dose of 59 $\mu g/m^2/h$ was recommended for phase II evaluation of the 72 h continuous infusion schedule given every 2 weeks with G-CSF support. This dosing was associated with excessive toxicity with a high incidence of grade 4 febrile neutropenia and thrombocytopenia despite G-CSF support. Therefore, the recommended dose for phase II trials was lowered to 35 $ug/m^2/h$ given as a 72 h infusion every 2 weeks without G-CSF support [97].

In another phase I trial conducted at New York University Medical Center (New York, NY, U.S.A.), 9-AC was given as a 21 day continuous infusion. A low-dose infusion was first escalated by time from 7 to 21 days and then escalated by dose. Based on a study that showed an MTD of 59 $ug/m^2/h$ for a 72 h infusion over a 4 week period, this same dose was prorated over the 21 day period to establish a starting dose of 6.2 $\mu g/m^2/h$. This regimen was well-tolerated with a lower toxicity profile whilst at the same time increasing the dose intensity by 50%. DLT was leucopenia and thrombocytopenia. Among 17 patients with ovarian cancer, 1 patient had a complete response, 4 had partial responses and 4 had stable disease for an overall response rate of 52.9%.

4/12 patients with colon adenocarcinoma had stable disease. An overall response rate of 42.8% was seen in 7 patients with breast cancer. The overall response rate among 47 evaluable patients was 38.3% [100–102].

There is an ongoing phase I trial investigating the oral route of delivery. From preclinical studies, it has been determined that 9-AC has good oral bioavailability. Oral delivery achieves 45±10% of the i.v. dose. In this trial, there are two schedules of administration. The drug is given daily for 5 days every 2 weeks or it is given daily for 5 days for 3 or 4 weeks with further continuation determined by the occurrence of DLT. These studies are being conducted in Chicago and in Europe. In a phase I study of 15 patients treated with oral 9-AC at the University of Chicago, no objective responses have been seen thus far. They conclude that the CD formulation of 9-AC has poor oral bioavailability and is not suitable for further clinical development [103].

DISCUSSION

Camptothecins had a very inauspicious beginning. In the early 1970s, they were deemed inappropriate for further clinical investigation due to low antitumour activity and severe toxicity of the sodium salt which had been prepared for patient administration. They were reintroduced into the clinical arena in the early 1990s with the development of new camptothecin analogues with a lower toxicity profile and demonstrated unprecedented effectiveness against human

cancer in xenografts. Dozens of clinical trials are being conducted world-wide with broad application against a wide variety of tumours. Numerous trials have been conducted in an attempt to establish the optimal schedule of administration. It seems that large doses of these drugs given at large intervals are not greatly effective. The camptothecins require a prolonged schedule of administration given continuously at low doses or frequently fractionated dosing schedules to be most effective. With these schedules, normal haematopoietic and mucosal progenitor cells with low topoisomerase I levels may be somewhat spared but more importantly antitumour effects are maintained. These are quite independent of prior treatment, unlike many other drugs. Prolonged drug exposure can also be achieved with the oral route of delivery. There are several ongoing clinical trials studying this mode of administration. Intra-arterial hepatic perfusion and intraperitoneal application or administration will undergo testing, because one may more safely combine these drugs without compromising or even enhancing exposure of the tumour.

Most recently, camptothecins are being combined with other chemotherapeutic agents and other therapeutic modalities. Preclinical experimental studies suggest that when combined with other agents or when combined with radiation, camptothecins may demonstrate synergistic antitumour activity. Topotecan has been studied in combination with cisplatin, carboplatin, cyclophosphamide, paclitaxel, etoposide, doxorubicin and cytosine arabinoside in phase I trials. CPT-11 has been studied in combination with cisplatin, vindesine, cisplatin and vindesine, and etoposide in SCLC and NSCLC in phase I/II trials. Although combination chemotherapy with camptothecins is still novel, some preliminary results appear to be very promising, but at the cost of enhanced and often DLT. Once again the optimal schedules of administration in combination therapy will need to be worked out, with particular attention to the sequence of administration of the different agents. Preclinical studies have demonstrated the radiosensitising effects of CPT-11 and 9-AC. Several phase I studies are currently addressing this issue but it remains a very active area for the development of new clinical trials.

1. Wall ME, Wani MC, Cook CE, Palmer KH, McPhail AT, Sim GA. Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. *J Am Chem Soc* 1996, **88**, 3888.
2. Perdue RE, Smith RL, Wall ME, Hartwell JL, Abbott BJ. *Camptotheca acuminata* decaisne (nyssaceae), source of camptothecin, an antileukemic alkaloid. Technical Bulletin, No. 1415, U.S. Department of Agriculture, Agricultural Research Service, Washington, D. C., 1970.
3. Huang SY, ed. *Seven Hundred Herbal Prescriptions for Cancer Medicine Treatment*. Taipei, Bada Educational and Cultural Publishers, 1986.
4. Bosmann HB. Camptothecin inhibits macromolecular synthesis in mammalian cells but not in isolated mitochondria of *E. coli*. *Biochem Biophys Res Commun* 1970, **41**, 1412.
5. Horwitz SB, Chang CK, Grollman AP. Studies on camptothecin. 1. Effects on nucleic acid and protein synthesis. *Mol Pharmacol* 1971, **7**, 632.
6. Kessel D. Effects of camptothecin on RNA synthesis in leukemia L1210 cells. *Biochem Biophys Acta* 1971, **246**, 225.
7. Wu RS, Kumar A, Warner JR. Ribosomal formation is blocked by camptothecin, a reversible inhibitor of RNA synthesis. *Proc Natl Acad Sci U.S.A.* 1971, **68**, 3009.
8. Abelson HT, Penman S. Selective interruption of high molecular weight RNA synthesis in HeLa cells by camptothecin. *Natl New Biol.*
9. Kessel D, Bosmann HB, Lohr K. Camptothecin effects on DNA synthesis in murine leukemia calls. *Biochem Biophys Acta* 1972, **269**, 210.
10. Horwitz MS, Horwitz SB. Intracellular degradation of HeLa and adenovirus type 2 DNA induced by camptothecin. *Biochem Biophys Res Commun* 1971, **45**, 723.
11. Gottlieb JA, Guarino AM, Call JB, Oliverio VT, Block JB. Preliminary pharmacologic and clinical evaluation of camptothecin sodium (NSC-100880). *Cancer Chemother Rep* 1970, **54**, 461.
12. Muggia FM, Creaven PJ, Hansen HH, Cohen MH, Sealwry OS. Phase I clinical trial of weekly and daily treatment with camptothecin (NSC-100880): correlation with preclinical studies. *Cancer Chemother Rep* 1972, **56**, 515.
13. 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.
14. Gottlieb JA, Luce JK. Treatment of malignant melanoma with camptothecin (NSC-100880). *Cancer Chemother Rep* 1972, **56**, 103.
15. Moertel CG, Schutt AJ, Reitemeier RJ, Hahn RG. Phase II study of camptothecin (NSC-100880) in treatment of advanced gastrointestinal cancer. *Cancer Chemother Rep* 1972, **56**, 96–101.
16. Gottlieb JA, Luce JK. Treatment of malignant melanoma with camptothecin (NSC-100880). *Cancer Chemother Rep* 1972, **56**, 103–105.
17. Muggia FM, Creaven PJ, Hansen HH, Cohen MH, Selawry OS. Phase I trial of weekly and daily treatment with camptothecin (NSC-100880): correlation with preclinical studies. *Cancer Chemother Rep* 1972, **56**, 515–521.
18. Hsiang YH, Hertzberg R, Hecht S, Liu LF. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase. *Int J Biol Chem* 1985, **260**, 14873.
19. Hsiang YH, Liu LF. Identification of mammalian topoisomerase I as an intracellular target of the anticancer drug camptothecin. *Cancer Res* 1988, **48**, 1722.
20. Giovanella BC, Stehlin JS, Wall ME, *et al.* DNA topoisomerase I-targeted chemotherapy of human colon cancer in xenografts. *Science* 1989, **246**, 1046.
21. Potmesil M, Hsiang YH, Liu LF, *et al.* Resistance of human leukemic and normal lymphocytes to drug-induced DNA cleavage and low levels of DNA topoisomerase II. *Cancer Res* 1988, **48**, 3537.
22. Jaxel C, Kohn K, Wani MC, Wall ME, Pommier Y. Structure–activity study of the actions of camptothecin derivatives on mammalian topoisomerase I. Evidence for a specific receptor site and for a relation to antitumor activity. *Cancer Res* 1989, **49**, 1465.
23. Potmesil M, Giovanella BC, Liu LF, *et al.* Preclinical studies of DNA topoisomerase I-targeted 9-amino and 10, 11-methylenedioxy camptothecins. In Potmesil M, Kohn KW, eds. *DNA Topoisomerases in Cancer*. New York, Oxford University Press, 1991, 299.
24. Potmesil M, Giovanella BC, Wall ME, *et al.* Preclinical development of DNA topoisomerase I inhibitors in the United States. In Andoh T, Ikeda, H., Oguro, M., eds. *Molecular Biology of DNA Topoisomerases and its Application to Chemotherapy*. Nagoya, CRC Press, 1993, chap. 29.
25. Negoro S, Fukuoka M, Masuda N, *et al.* Phase I study of weekly intravenous infusions of CPT-11, a new derivative of camptothecin in the treatment of advanced non-small cell lung cancer. *J Natl Cancer Inst* 1991, **83**, 1164.
26. Rowinsky E, Grochow L, Hendricks C, *et al.* Phase I and pharmacologic study of topotecan (SK&F 104864): a novel topoisomerase I inhibitor. *Proc Am Soc Clin Oncol* 1991, **10**, 93.
27. Champoux JJ, Dulbecco R. An activity from mammalian cells that untwists superhelical DNA—a possible swivel for DNA replication. *Proc Natl Acad Sci U.S.A.* 1972, **69**, 143–146.
28. Liu LF, Miller KG. Eukaryotic DNA topoisomerases. Two forms of type I DNA topoisomerases from HeLa cell nuclei. *Proc Natl Acad Sci U.S.A.* 1981, **78**, 3487–3491.

29. D'Arpa P, Machlin PS, Ratrie H, Rothfield NF, Cleveland DW, Earnshaw WC. cDNA cloning of human DNA topoisomerase I: Catalytic activity of a 67.7-kDa carboxyl-terminal fragment. *Proc Natl Acad Sci U.S.A.* 1988, **85**, 2543–2547.
30. Juan CC, Hwang J, Liu AA, *et al.* Human DNA topoisomerase I is encoded by a single-copy gene that maps to chromosome region 20q12-13.2. *Proc Natl Acad Sci. U.S.A.* 1988, **85**, 8910–8913.
31. Yang L, Wold MS, Liu JJ, Kelly TJ, Liu LF. Roles of DNA topoisomerases in SV40 DNA replication *in vitro*. *Proc Natl Acad Sci U.S.A.* 1987, **84**, 950–954.
32. Liu LF, Wang JC. Supercoiling of the DNA template during transcription. *Proc Natl Acad Sci U.S.A.* 1987, **84**, 7024–7027.
33. Liu LF. Biochemistry of camptothecin. In Potmesil M, Pinedo H, eds. *Camptothecins: New Anticancer Agents*. Boca Raton, CRC Press, 1995, 9.
34. Liu LF. Biochemistry of camptothecin. In Potmesil M, Pinedo H, eds. *Camptothecins: New Anticancer Agents*. Boca Raton, CRC Press, 1995, 11–14.
35. Champoux JJ. DNA is linked to the rat liver nicking-closing enzyme by a phosphodiester bond to tyrosine. *J Biol Chem* 1981, **256**, 4805–4809.
36. Hsiang YH, Hertzberg R, Hecht S, Liu LF. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J Biol Chem* 1985, **260**, 14873–14878.
37. Porter SE, Champoux JJ. The basis for camptothecin enhancement of DNA breakage by eukaryotic topoisomerase I. *Nucl Acids Res* 1989, **17**, 8521–8532.
38. Gromova II, Kjeldsen E, Svejstrup JQ, Alsner J, Christiansen K, Westergaard O. Characterization of an altered DNA catalysis of a camptothecin-resistant eukaryotic topoisomerase I. *Nucl Acids Res* 1993, **21**, 593–600.
39. Hsiang YF, Liu LF, Wall ME, *et al.* DNA topoisomerase I-mediated DNA cleavage and cytotoxicity of camptothecin analogue. *Cancer Res* 1989, **49**, 4385–4389.
40. Wall ME, Wani MC. Camptothecin and analogs: from discovery to clinic. In Potmesil M, Pinedo H, eds. *Camptothecins: New Anticancer Agents*. Boca Raton, CRC Press, 1995, 27–35.
41. Wani MC, Nicholas AW, Wall ME. Plant antitumor agents. 23. Synthesis and antileukemic activity of camptothecin analogues. *J Med Chem* 1986, **29**, 2358.
42. Wani MC, Nicholas AW, Manikumar G, Wall ME. Plant antitumor agents. 25. Total synthesis and antileukemic activity of ring A-substituted camptothecin analogs, structure activity. *J Med Chem* 1987, **30**, 1774.
43. Wall ME, Wani MC, Natschke SM, Nicholas AW. Plant antitumor agents. 22. Isolation of 11-hydroxycamptothecin from *Camptotheca acuminata* decne: total synthesis and biological activity. *J Med Chem* 1986, **29**, 1553.
44. Jaxel C, Kohn KW, Wani MC, Wall ME. Structure activity study of the actions of camptothecin derivatives on mammalian topoisomerase I, evidence for a specific receptor site and for a relation to antitumor activity. *Cancer Res* 1989, **49**, 1465.
45. Wani MC, Ronman PE, Lindley JT, Wall ME. Plant antitumor agents. 18. Synthesis and biological activity of camptothecin analogs. *J Med Chem* 1980, **23**, 554.
46. Wall ME, Wani MC, Nicholas AW, *et al.* Plant antitumor agents. 30. Synthesis and structure activity of novel camptothecin analogs. *J Med Chem* 1993.
47. Hsiang YH, Liu LF, Wall ME, *et al.* DNA topoisomerase I-mediated DNA cleavage and cytotoxicity of camptothecin analogs. *Cancer Res* 1989, **49**, 4385.
48. Wall ME, Wani MC. Camptothecin and analogs: from discovery to clinic. In Potmesil M, Pinedo H, eds. *Camptothecins: New Anticancer Agents*. Boca Raton, CRC Press, 1995, 30–33.
49. Jaxel C, Kohn K, Wani MC, Wall ME, Pommier Y. Structure activity study of the actions of camptothecin derivatives on mammalian topoisemerase I, evidence for a specific receptor site and a relation to antitumor activity. *Cancer Res* 1989, **49**, 1465–1469.
50. Wall ME, Wani MC. Camptothecin and analogs: from discovery to clinic. In Potmesil M, Pineddo H, eds. *Camptothecins: New Anticancer Agents*. Boca Raton, CRC Press, 1995, 34.
51. Kawato Y, Aonuma M, Hirota Y, *et al.* Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. *Cancer Res* 1991, **51**, 4187-4191.
52. Kaneda N, . Yokokura T. Nonlinear pharmacokinetics of CPT-11 in rats. *Cancer Res* 1990, **50**, 1721–1725.
53. Rothenberg ML, Rowinsky EK, Kuhn JG, Burris HA, Donehower RC, Von Hoff DD. Clinical trials and pharmacokinetic studies of CPT-11 in the United States. In Potmesil M, Pinedo H, eds. *Camptothecins: New Anticancer Agents*. Boca Raton, CRC Press, 1995, 77–79.
54. Kunimoto T, Nitta AK, Tanaka T, *et al.* Antitumor activity of &-ethyl-10-[4-(1-piperidino)-1-piperidino] carboxylated-camptothecin, a novel water soluble derivative of camptothecin, against murine tumors. *Cancer Res* 1987, **47**, 5944.
55. Tsuruo T, Matsuzaki M, Saito H, Yokokura T. Antitumor effect of CPT-11, a new derivative of camptothecin, against pleiotropic drug-resistant tumors *in vitro* and *in vivo*. *Cancer Chemother Pharmacol* 1988, **21**, 71–74.
56. Potmesil M. Camptothecins: from bench research to hospital wards. *Cancer Res* 1994, **54**, 1433.
57. Yokokura T, Furuta T, Sawada S, *et al.* Antitumor activity of newly synthesized, lactone ring-closed and water-soluble camptothecin derivative in mice. In *Proc Japanese Cancer Assoc, 43rd Ann Meet*. Fukuoka, Japan, 1984.
58. Rowinsky E, Grochow L, Ettinger D, *et al.* Phase I and pharmacologic study of CPT-11, a semisynthetic topoisomerase I-targeting agent, on a single-dose schedule. *Proc Am Soc Clin Oncol* 1992, **11** (abstract), 115.
59. Rothenberg ML, Rowinsky EK, Kuhn JG, Burris HA, Donehower RC, Von Hoff DD. Clinical trials and pharmacokinetic studies of CPT-11 in the United States. In Potmesil M, Pinedo H, eds. *Camptothecins: New Anticancer Agents*. Boca Raton, CRC Press, 1995, 75–77.
60. Chabot GG, de Forni M, Abigerges D, *et al.* Clinical trials and pharmacology studies of CPT-11 and its active metabolite SN-38 in France: preliminary pharmacokinetic–pharmacodynamic relationships. In Potmesil M, Pinedo H, eds. *Camptothecins: New Anticancer Agents*. Boca Raton, CRC Press, 1995, 85.
61. Herben V, Schellens J, Swart M, Gruia G, Benyen J, ten Bokkel Huinink WW. Phase I and pharmacokinetic study of irinotecan with a prolonged (14D) infusion schedule. *Proc Am Soc Clin Oncol* 1997, **16** (abstract 705) 201a.
62. Gerrits CHJ, Verweij J. Oral administration of topoisomerase I inhibitors: results of phase I studies. The *Seventh Conference on DNA Topoisomerases in Therapy, Program and Abstracts* 1996 (abstract) 35.
63. Taguchi T. Clinical studies of CPT-11 in Japan. In Potmesil M, Pinedo H, eds. *Camptothecins: New Anticancer Agents*. Boca Raton, CRC Press, 1995, 67–73.
64. Shimeda Y, Yoshino M, Wakui A, *et al.* Phase II study of CPT-11, a new camptothecin derivative in metastatic colorectal cancer. *J Clin Oncol* 1993, **11**(5), 909.
65. Conti JA, Kemeny N, Saltz L, *et al.* Irinotecan (CPT-11) is an active agent in untreated patients with metastatic colorectal cancer. *Proc Am Soc Clin Oncol* 1994, **13**, 195.
66. Rothenberg ML, Eckardt JR, Burris HA, *et al.* Irinotecan as second line therapy for patients with 5FU-refractory colorectal cancer. *Proc Am Soc Clin Oncol* 1994, **13**, 198.
67. Abigerges D, Chabot GG, Armand JP, *et al.* Phase I and pharmacologic studies of camptothecin analogue irinotecan administered every three weeks in cancer patients. *J Clin Oncol* 1995, **13**, 210.
68. Rowinsky EK, Grochow LB, Ettinger DS, *et al.* Phase I and pharmacologic study of the novel topoisomerase I inhibitor 7-ethyl-1-1 0-[4-(1-piperidino)-1-piperindo]carboxylcamptothecin (CPT-11) adminstered as a ninety minute infusion every three weeks. *Cancer Res* 1994, **54**, 427.
69. Burris HA III, Fields SM, Kuhn JG, Von Hoff DD. Camptothecins: dose-limiting toxicities and their management. In Potmesil M, Pinedo H, eds. *Camptothecins: New Anticancer Agents*. Boca Raton, CRC Press, 1995, 118.
70. Muggia F. Twenty years later: review of clinical trials with camptothecin sodium (NSC-100880). In Potmesil M, Pinedo H, eds. *Camptothecins: New Anticancer Agents*. Boca Raton, CRC Press, 1995, 44.
71. Takimoto CH, Arbuck SG. The camptothecins. In Chabner BA, Longo DL, eds. *Cancer Chemotherapy and Biotherapy: Principles and Practice*. New York, Lippincott-Raven, 1996, 474–476.

72. Gupta E, Lestingi TM, Mick R, *et al.* Metabolic fate of irinotecan in humans: correlation of glucoronidation with diarrhea. *Cancer Res* 1994, **54**, 3723.
73. Chabot GG, de Forni M, Abigerges D, *et al.* Clinical trials and pharmacology studies of CPT-11 and its active metabolite SN-38 in France: preliminary pharmacokinetic–pharmacodynamic relationships. In Potmesil M, Pinedo H., eds. *Camptothecins: New Anticancer Agents*. Boca Raton, CRC Press, 1995, 87.
74. Hochster H. Topotecan clinical trials in the United States. In Potmesil M, Pinedo H, eds. *Camptothecins: New Anticancer Agents*. Boca Raton, CRC Press, 1995, 95.
75. Takimoto CH, Arbuck SG. The camptothecins. In Chabner BA, Longo DL, eds. *Cancer Chemotherapy and Biotherapy: Principles and Practice*. New York, Lippincott-Raven, 1996, 472.
76. Burris HA III, Fields SM, Kuhn JG, Von Hoff DD. Camptothecins: dose-limiting toxicities and their management. In Potmesil M, Pinedo H, eds. *Camptothecins: New Anticancer Agents*. Boca Raton, CRC Press, 1995, 115.
77. Hochster H. Topotecan clinical trials in the United States. In Potmesil M, Pinedo H, eds. *Camptothecins: New Anticancer Agents*. Boca Raton, CRC Press, 1995, 96–100.
78. Rowinsky EK, Grochow LB, Hendricks CB, *et al.* Phase I and pharmacologic study of topotecan: a novel topoisomerase I inhibitor. *J Clin Oncol* 1992, **10**(4), 647–656.
79. Verweij J, ten Bokkel Huinink W, Lund B, *et al.* Clinical trials of topotecan in Europe. In Potmesil M, Pinedo H, eds. *Camptothecins: New Anticancer Agents*. Boca Raton, CRC Press, 1995, 105–109.
80. Haas NB, Ozols RF, O'Dwyer PJ. Phase I trial of topotecan on a weekly 24 hour infusional schedule. *Seventh NCI-EORTC Symp. New Drugs in Cancer Therapy*, (103), Amsterdam, 1992.
81. Haas NB, La Creta FP, Walczak J, *et al.* Phase I pharmacokinetic trial of topotecan on a weekly 24 hour infusional schedule. *Proc Am Assoc Cancer Res* 1992, **33** (abstract 3128) 523.
82. 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**(3), 553–594.
83. Eisenhauer E, Hoskins P, Beare S, *et al.* Randomized phase II study of two schedules of topotecan in previously treated epithelial ovarian cancer. *Proc Am Soc Clin Oncol* 1997, **16** (abstract 1249), 349a.
84. Takimoto CH, Arbuck SG. The camptothecins. In Chabner BA, Longo DL, eds. *Cancer Chemotherapy and Biotherapy: Principles and Practice*. New York, Lippincott-Raven, 1996, 471.
85. Creemers GJ, Schellens JH, Beijnen JH, *et al.* Bioavailability of oral topotecan: a new topoisomerase I inhibitor. *Proc Am Soc Clin Oncol*, 1994, **13**, 132.
86. Potmesil M. Camptothecins: from bench research to hospital wards. *Cancer Res* 1994, **54**, 1431–1439.
87. Potmesil M, Canellakis ZN, Wall ME, *et al.* Pharmacokinetic studies of (-amino-20(S)-camptothecin: cellular partitioning. *Proc Am Assoc Cancer Res* 1992, **33**, 433.
88. Costin D, Silber R, Canellakis ZN, Morse L, Potmesil M. Uptake of 20(S)-camptothecin and analogues by human colon cancer cells or by chronic lymphocytic leukemia. In *Fourth Conference on DNA Topoisomerase in Therapy*. New York, NYU Postgraduate Medical School 1992, 53.
89. Burke TG, Staubus AE, Mishra AK. Liposomal stabilization of camptothecin's lactone ring. *J Am Chem Soc* 1992, **114**, 8318–8319.
90. Giovanella BC, Wall ME, Wani MC, *et al.* Efficacy of camptothecin (NSC 94600, CAM), 9-aminocamptothecin (NSC 60307, 9AC), and 10, 11-methylenedioxycamptothecin (NSC 606174, 10, 11-MDC), in human cancer xenograft models. *Proc. Am. Assoc. Cancer Res*, 1992, **33**, 432.
91. Potmesil M, Giovanella BC. Preclinical development of 20(S)-camptothecin, 9-aminocamptothecin, and other analogues. In Potmesil M, Pinedo H, eds. *Camptothecins: New Anticancer Agents*. Boca Raton, CRC Press, 1995, 52–55.
92. Giovanella BC, Stehlin JS, Wall ME, *et al.* DNA topoisomerase I-targeted chemotherapy of human colon cancer in xenografts. *Science* 1989, **246**, 1046.
93. Potmesil M, Giovanella BC, Liu LF, *et al.* Preclinical studies of DNA topoisomerase I-targeted 9-amino and 10, 11-methylenedioxy camptothecins. In Potmesil M, Kohn KW, eds. *DNA Topoisomerases in Cancer*. New York, Oxford University Press, 1991, 299.
94. Potmesil M, Giovanella BC, Wall ME, *et al.* Preclinical and clinical development of DNA topoisomerase I inhibitors in the United States. In Andoh T, Ikeda H, Oguro M, eds. *Molecular Biology of DNA Topoisomerases and its Application to Chemotherapy*. Nagoya, CRC Press, 1993, 301–311.
95. Potmesil M, Hinz HR, Marcee A, *et al.* Growth inhibition of human cancer metastases: the xenograft model by camptothecin [NCS 94600, CAM], 9-amino-[NSC 603071, 9AC], and 9-nitrocamptothecin. *Proc Am Assoc Cancer Res*, 1992, **33**, 432.
96. Liang MD, Dahut W, Quin MF, *et al.* Preclinical and clinical studies of a new colloidal dispersion formulation of 9-aminocamptothecin. *Proc Am Assoc Cancer Res*, 1996, **37**, 432.
97. Potmesil M, Arbuck SG, Takimoto CH, Liebes L, Hochster H. 9-Aminocamptothecin and beyond: preclinical and clinical studies. *Ann New York Acad Sci* 1996, **803**, 231–247.
98. 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**, 269–276.
99. Dahut W, Takimoto HC, Harold N, *et al.* Phase I and pharmacologic study of 9-aminocamptothecin given by 72-hour infusion in adult cancer patients. *J Clin Oncol* 1996, **14**, 1236–1244.
100. 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**, 553–559.
101. Hochster H, Potmesil M, Liebes L, *et al.* Phase I study of 9-aminocamptothecin by prolonged infusion of 21 days. 9th NCI-EORTC Symposium on New Drugs in Cancer Therapy *Ann Oncol* 1996, **7**(Suppl. 1 abstract 461), 130.
102. Hochster H, Liebes L, Speyer J, *et al.* Phase I and pharmacodynamic study of prolonged infusion 9-aminocamptothecin in two formulations. *Proc Am Assoc Clin Oncol* 1997, **16**, (abstract 704), 201a.
103. Mani S, Iyer L, Janisch L, *et al.* Phase I clinical and pharmacokinetic study of oral 9-aminocamptothecin (9AC)(NSC 603071). *Proc Amer Assoc Clin Oncol* 1997, **16**, (abstract 706), 201a.
104. Costin D, Potmesil M. DNA topoisomerase II as an intracellular target in cancer chemotherapy by anthracyclines. In Liu LF, ed. *DNA Topoisomerases: Topoisomerase-targeting Drugs*. New York, Academic Press, 1994, 51–72.
105. The fifth conference on DNA topoisomerases in therapy. Program and abstracts. New York, NYU Postgraduate Medical School, 1994.
106. Rothenberg M. The current status of Irinotecan (CPT-11) in the United States. In *The Camptothecins: From Discovery to the Patient. Ann New York Acad Sci* 1996, **803**, 272–281.
107. Armand JP, Ternet C, Rixe O. CPT-11: The European experience. In *The Camptothecins: From Discovery to the Patient. Ann New York Acad Sci* 1996, **803**, 282–291.
108. Saito N. Clinical trials of irinotecan hydrochloride (CPT, campto injection) in Japan. In *The Camptothecins: From Discovery to Patient. Ann New York Acad Sci* 1996, **803**, 292–305.
109. ten Bokkel Huinink W, Gore M, Carmichael J, *et al.* Topotecan versus paclitaxel for the treatment of recurrent epithelial ovarian cancer. *J Clin Oncol* 1997, **15**(6), 2183–2193
110. Broom C. Clinical studies of topotecan. In *The Camptothecins: From Discovery to Patient. Ann New York Acad Sci* 1996, **803**, 264–271.
111. Kingsbury WD, Boehm JC, Jakas DR, *et al.* Synthesis of water soluble (aminoalkyl) camptothecin analogues: inhibition of topoisomerase I and antitumor activity. *J Med Chem* 1991, **34**, 98.
112. Chen AY, Chiang Y, Potmesil M, Wall ME, Wani MC, Liu LF. Camptothecin overcomes mdr-mediated resistance in human KB carcinoma cells. *Cancer Res* 1991, **51**, 6039–6044.
113. Gallo RC, Whang-Peng J, Adamson RH. Studies on the antitumor activity, mechanism of action, and cell cycle effects of camptothecin. *J Natl Cancer Inst* 1971, **46**, 789–795.