*Chapter 11 The Topoisomerase I Story- camptothecin from a Happy Chinese Tree 220722br3*

# Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

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## CHAPTER 11

## The Topoisomerase I Story: camptothecin, from a Happy Chinese Tree.

In 1960, the National Cancer Institute (NCI) began a search for anticancer substances in extracts from plants and animals (so called "natural products"). That effort was added to the ongoing testing of large numbers of organic chemicals for anticancer activity. The work was being carried out under the auspices of the NCI's Cancer Chemotherapy National Service Center (CCNSC). Every substance tested in this system received an NSC number to code for it in the database, which had information about chemical structure, origin, and test results in animals and cancer cell lines.

Among the most important discoveries by the natural products effort were camptothecin and taxol, both of which were isolated from plant material by Monroe Wall and Mansukh Wani at the Research Triangle Institute (RTI) in North Carolina (Kohn and Pommier, 2000). Here, I tell the story of camptothecin; the story of taxol is in the next chapter.

According to Wani, when he arrived at RTI in 1962, there was nothing there except 4 walls, and it was only when the 5th 'Wall' joined him at RTI that things started to move. Wall and Wani worked together in a life-long collaboration that yielded some of the most important advances in the history of cancer chemotherapy (Figure 11.1).

Before coming to RTI, Monroe Wall had directed a program at the U. S. Department of Agriculture (USDA) in a search for plant materials that could be used as a starting point for the synthesis of cortisone, which was at the time in short supply. Extracts from those materials, in addition to being tested for substances useful for cortisone synthesis, were also sent to NCI for testing against cancer in tumor-bearing mice.

And so it was that an extract from the Chinese tree *Camptotheca accuminata* (known in China as Xi Shu, meaning “Happy Tree”) (Figure 11.2) was found to have powerful anti-cancer activity. Anticancer search however did not fit in USDA's mandate, and Wall's desire to find the anticancer substance in those extracts had to wait a few years until he moved to RTI.

In 1963, Wall and Mani (Figure 11.1) began their attempt to isolate the anticancer substance from the “happy tree” (Figure 11.2). They began with 20 kg of bark and wood and made extracts using various solvent, which they then tested for anticancer activity in mice. They tested the most active samples at each purification step. The work was slow and painstaking. But by 1966, they had pure camptothecin and had determined its structure by x-ray diffraction (Wall, 1966).

Figure 11.1. Monroe E. Wall (right) and Mansukh C. Wani (left), discoverers of camptothecin and taxol.

Figure 11.2. *Camptotheca accuminata* (Xi Shu, "Happy tree") in the Chengdu Botanical Garden - Chengdu, China. It is native near the warm humid stream banks in Southern China and Tibet. (*Public domain, Wikipedia commons.*)

## *Camptothecin specifically inhibits topoisomerase I.*

The first clue that camptothecin targets a topoisomerase-like enzyme was unknowingly obtained by Susan B. Horwitz in 1973 in an early observation at a time when topoisomerases had not yet been discovered (Horwitz and Horwitz, 1973) (Figure 11.3). That was years before the name "topoisomerase" was invented. She had exposed human cancer cells to camptothecin, a novel anticancer drug, and observed that the cell's DNA strands were broken by the drug.

When the drug was removed, the DNA strand breaks quickly reversed. It seemed that the drug caused repairable or reversible DNA strand breaks. However, there was an additional observation that was so bizarre that it was not mentioned in her paper, perhaps because the paper might then not have been accepted for publication.

About the same time, a similar finding in cultured cancer cells was independently reported by Ann Spataro and David Kessel (Spataro and Kessel, 1972). Also about the same time, Rajalakshmi and Sarma (Rajalakshmi and Sarma, 1973) reported that camptothecin broke DNA strands in the liver of treated rats and that the DNA was repaired surprisingly quickly. According to Dr. Silvio Parodi, who worked with D.S. Sarma under the supervision of Emmanuel Farber at Fels Research Institute in Philadelphia, they were looking at anti-neoplastic agents (especially of natural origin) for their potential carcinogenicity, testing for induction of chromosomal

aberrations and sister chromatid exchanges when they observed the unusual DNA breakage and repair by camptothecin.

A few months before publication of Susan's paper, I visited her laboratory, which was then led by Arthur Grollman at Albert Einstein Medical Center in The Bronx. I had been studying DNA strand breakage and repair by various anticancer drugs, and she therefore told me about her findings with camptothecin. I then asked how long it took for the strands to be repaired. After some hesitation, Arthur Grollman said that the repair was very fast, so fast, even in the cold, that they could not measure it. I asked how that could possibly be. After further hesitation, Arthur said he didn't know, but that maybe there was an enzyme right there by the breaks that resealed them immediately when the drug was removed. That speculation seemed so bizarre that I could not accept it. However, it turned out that Grollman's speculation was right on the mark, and the responsible enzyme was later identified as the then unknown topoisomerase I. Susan Horwitz had observed a new anticancer drug-induced mechanism of DNA breakage and repair that was to have major impact on cancer chemotherapy.

Susan Band Horwitz,

Figure 11.3. Susan Band Horwitz (1937- ), working at Albert Einstein Medical Center, discovered that camptothecin produced rapidly reversible DNA breaks. She also discovered that anticancer drug Taxol blocked microtubules (Chapter 12).

In view of the early evidence that camptothecin caused DNA breaks and that inhibitors of topoisomerase II caused protein-linked DNA stand breaks (see Chapter 10), Leroy Liu and his colleagues tested camptothecin against topoisomerase II. They were surprised to find that there was no effect on topoisomerase II, but found that camptothecin induced topoisomerase I to produce both DNA strand breaks and DNA-protein crosslinks (Hsiang et al., 1985; Hsiang and Liu, 1988). Using the DNA filter elution methods (Chapter 9), Joe Covey, Christine Jaxel, Yves Pommier and I confirmed that indeed camptothecin produced typical protein-linked DNA strand breaks (Covey et al., 1989). As Susan Horwitz and Arthur Grollman had surmised, a DNA-associated enzyme (later identified as topoisomerase I) rapidly reversed the strand breaks; they would have been amazed to know at the time that their postulated reversal enzyme also produced the breaks in the first place.

## *Topoisomerase I solves the problem of over- and under-twisted DNA during transcription and replication.*

Figure 11.4 illustrates one of the cell's major DNA topology problems. As the paired DNA strands separate during transcription or replication, the DNA twists are pushed ahead and would become bunched up to an extent that strand-separation could not continue. In the case of transcription, there is an additional problem behind the bubble of separated strands. When the transcribed RNA emerges (diagram B in Figure 11.4), the complementary DNA strands re-associate, but there are not enough twists to make the stable one twist per 10 base-pairs (Pommier, 2013).

The problem is solved by type I topoisomerases that transiently cleave one DNA strand and allow the strands to swivel and remove the excessive or deficient twists as the DNA or RNA synthesis machinery marches on. After swiveling removes stress on the DNA helix, the topoisomerase rapidly reseals the break (Figure 11.5).

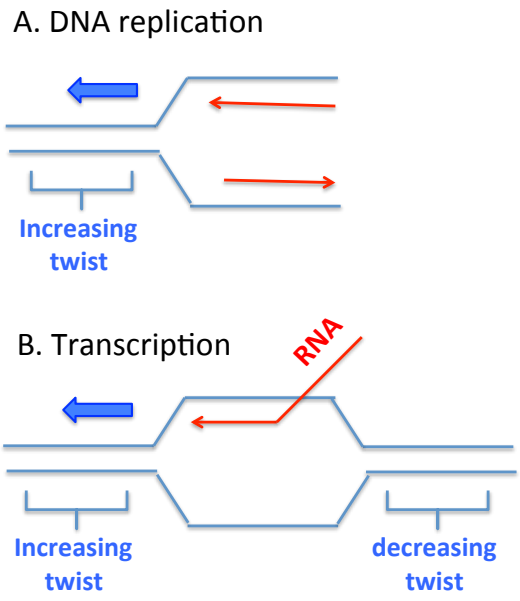



Figure 11.4. The DNA twisting problem in replication (DNA synthesis, A) and transcription (RNA synthesis, B). As the strands separate, the twists are pushed ahead and would impede further strand separation. The excessive or deficient twists are resolved by topoisomerase I, which is bound to and moves along with the replication and transcription machineries. Parallel line pairs represent double-stranded helix. In A, the red lines represent newly synthesized DNA. In B, the red line represents newly synthesized RNA. The strand-separation forks are moving from right to left (fat blue arrows).

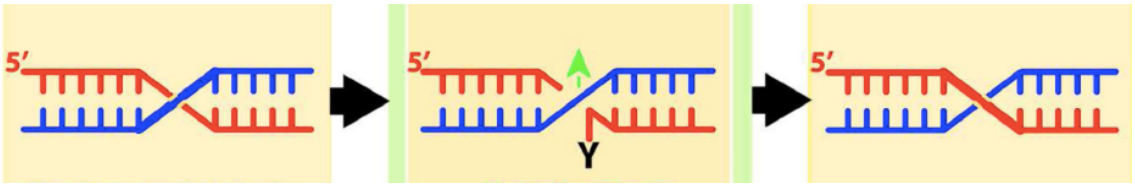



Figure 11.5. The topological problem solved by type 1 topoisomerases. The enzyme breaks one DNA strand, allows the other strand to pass through or swivel around the break, and finally reseals the break. The red strand is broken and the blue strand passes through. As the enzyme breaks the strand, it grabs hold of one end via the enzyme's tyrosine (Y) at the active site (Pommier, 2013) (from *IACS Chemical Biology*).

## *How camptothecin causes DNA damage that kills cancer cells.*

The earliest clue about the actions of camptothecin on DNA came in 1972, when Herbert E. Kann and I obtained evidence that camptothecin reduces the average length of newly synthesized RNA chains, suggesting that camptothecin was prematurely terminating the growth of the RNA chains (Kann and Kohn, 1972). [Herb and I were close friends. He went on to continue both clinical and laboratory cancer research at Emory University in Atlanta but died tragically at an early age of liver failure.]

We had used the then available ultracentrifugation method, but later, in 1990, when electrophoretic methods had been perfected and purified topoisomerase I was available, Ole Westergaard and his colleagues at the University of Aarhus in Denmark showed that camptothecin stops RNA synthesis and that the RNA chains were terminated when they reached 10 base-pairs from the sites where topoisomerase I had become bound to the DNA (Bendixen et al., 1990).

Then, in 1994, we found that camptothecin killed cells when they were in S phase of the cell cycle: the drug selectively killed cells when they were replicating their DNA (Goldwasser et al., 1996). We surmised that cells were selectively blocked and killed in S phase because of collisions between moving replication forks and sites on the DNA where topoisomerase 1 was trapped by camptothecin (Figure 11.6). Our view was based on what was then understood about how topoisomerase I (Top1) operates: it binds DNA in front of moving replication forks and cycles through opening and closing of a DNA strand break, so as to allow the strands to swivel and relieve the accumulating supercoiling of the DNA helix. When topoisomerase I is in the state where it has cleaved the DNA, camptothecin binds and retards the further action of the topoisomerase that would close the DNA break. A double-strand end would form that looks to the cell like a DNA double-strand break (Figure 11.6) (Kohn and Pommier, 2000).

We presumed that a similar, but less lethal, process occurred during the growth of RNA chains which were terminated as was found in the earlier experiments mentioned above (Kann and Kohn, 1972). Collisions due to progress of RNA synthesis, merely generating prematurely terminated RNA chains, apparently produced less toxicity than the DNA double-strand ends produced by collisions during the progress of DNA replication.

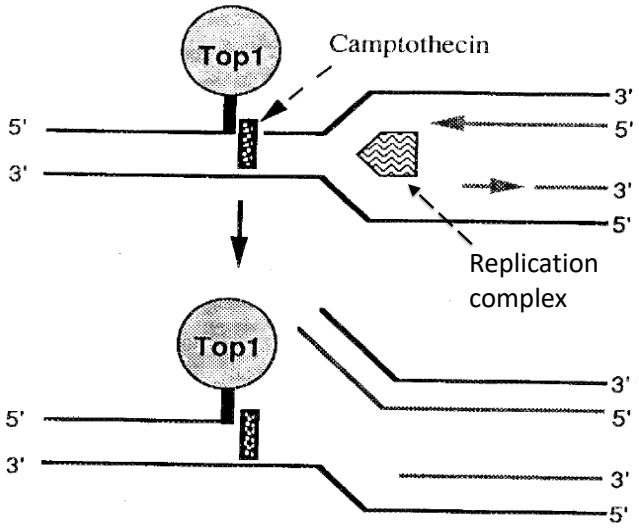



Figure 11.6. How an encounter between a moving replication process and a camptothecin-blocked topoisomerase I (Top1) complex generated a potentially lethal DNA double-strand end, as we envisioned it in 1994 (Pommier et al., 1994). Cells that were not in the process of replicating their DNA, however, were still somewhat sensitive to camptothecin, because of analogous encounters of trapped Top1 by a transcription process (Bendixen et al., 1990).

## *Structure of the camptothecin/topotecan-Top1-DNA cleavage complex.*

Although camptothecin by itself was not noted to insert or intercalate between DNA base-pair, the structure of the Top1-DNA complex trapped in the strand-cleavage state by camptothecin or topotecan nonetheless revealed an intercalation-like structure (Figure 11.6.1) ( Topotecan is a variant of camptothecin; DNA intercalation was explained in Chapter 4.) As Figure 11.6.2 shows, however, the base-pair on one side of the drug is displaced and does not lie flat against the drug as would be required by strict intercalation. Thus, only the base-pair on one side of the drug is flat against it, and that base-pair is preferentially a guanine-cytosine (G:C) pair. That structure was relatively stable, which is why camptothecin induced Top1-DNA cleavage preferentially at sites similar to the structure shown in Figure 11.6.2.

But as just mentioned, the camptothecin or topotecan drug lies flat against only one base-pair: The G:C pair shown in Figure 11.6.2; the drug is as if only *semi*-intercalated. In the absence of Top1, the drug did not show evidence of DNA intercalation. Therefore, the drug must be stabilized by addition interactions with the Top1-DNA cleavage complex. Indeed, the crystallographic structures showed hydrogen bonds from amino acids of the Top1 to the distorted DNA structure, as well as to the drug (Figure 11.7). Especially notable were the three hydrogen bonds between amino acids of Top1 and the oxygen atoms in the E ring of camptothecin (Figure 11.7). That likely accounted for the E ring structure being critical for camptothecin function. Moreover, the hydroxyl group at position 20 of camptothecin is asymmetric and only one of the two possible conformations gave an active drug. It seemed that only one of the conformations points the hydroxyl group at position-20 in the direction required for hydrogen bonding with the Asp533 amino acid of Top1 (Figure 11.7).

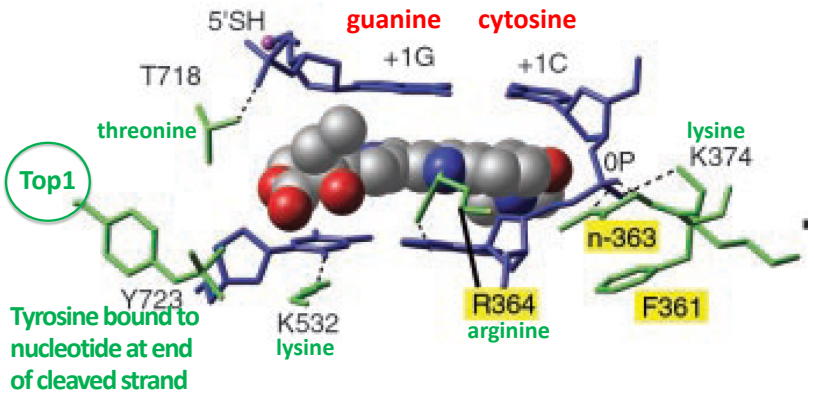



Figure 11.6.1. View of topotecan (a variant of camptothecin) stacked against a G:C base-pair in a complex with DNA and Top1. The cleaved DNA strand is on the left, where tyrosine-723 of Top1 is bound to an end of the cleaved strand. The DNA helix is altered by the inserted topotecan and the Top1-bound end of the cleaved strand. The altered DNA conformation is stabilized by hydrogen bonds to several amino acids of Top1. This structure was based on x-ray crystallography (Staker et al., 2002).

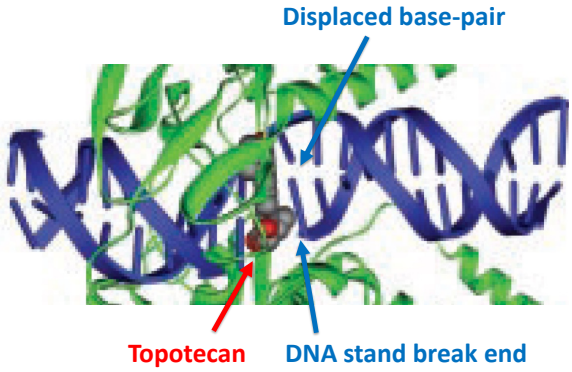


Figure 11.6.2. Topotecan is stacked against the base-pair on topotecan's left. The base-pair to its right is displaced. In this view, the DNA strand near the upper end of the topotecan is intact, whereas the strand near the lower end of the topotecan is cleaved (Staker et al., 2002),

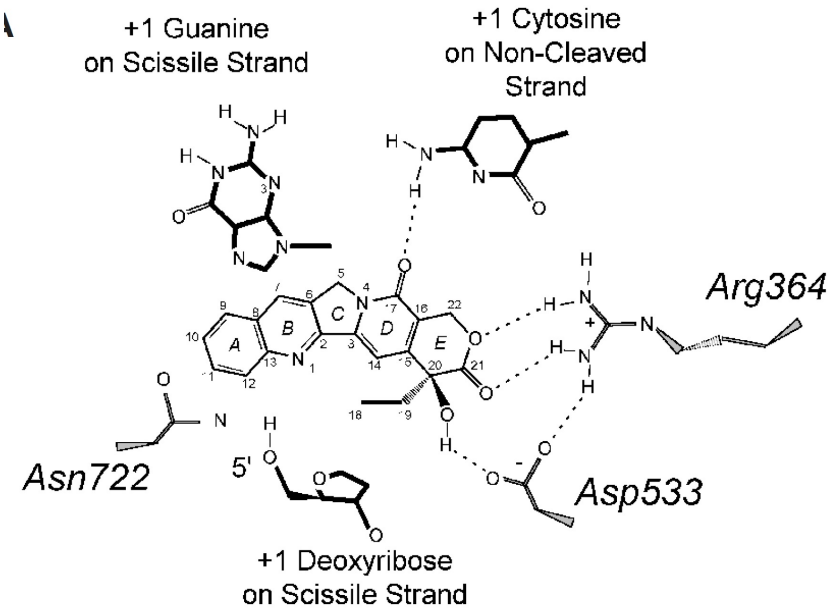



Figure 11.7. In addition to the hydrogen bonds stabilizing the altered DNA conformation shown in the previous Figure, additional hydrogen bonds stabilize the position of camptothecin in the complex. Of particular importance are the three hydrogen bonds involving camptothecin's E ring (Redinbo et al., 1998).

## *How did camptothecin/topotecan kill cancer cells?*

Was the lethal effect of camptothecin due to inhibiting the relaxing of the DNA supercoils that accumulate in front of a replication process? Or was it due to the consequences of the collision itself: the production of DNA double-strand end as shown in Figure 11.4? To address this question, we tested a Top1 mutant that had a change in an amino acid at a critical site for the enzyme's function. In the presence of camptothecin, this particular mutant Top1 functioned as it should in relieving stressful DNA twists but did not produce DNA-Top1 trapped complexes. Camptothecin did not kill cells bearing this Top1 mutation. We inferred, therefore, that the cells were most likely killed as a consequence of a collision of a moving replication fork with a trapped Top1-DNA-camptothecin complex (Pommier et al.,

1999; Urasaki et al., 2001). The potentially lethal effect probably came from the difficulty posed by the DNA double-strand end that is produced (Figure 11.4).

Thus, the very transient camptothecin-induced DNA breakage, originally observed by Susan Horwitz and Arthur Grollman, was found to be due to the drug's effect on Top1 (Hsiang et al., 1985) (Covey et al., 1989). As was the case with topoisomerase II targeted drugs, DNA strand breaks and DNA-protein crosslinks were produced in equal numbers, consistent with one protein bound consistently to one end of each DNA strand break (Mattern et al., 1987). The covalent association of Top1 at each camptothecin-induced DNA break was confirmed by Hsiang and Liu (Hsiang and Liu, 1988). Porter and Champoux then obtained evidence that the trapping of the Top1-DNA breaks was due to reduction by camptothecin of the rate at which the breaks reseal (Porter and Champoux, 1989). These studies clarified the essentials of how camptothecin traps DNA-Top1.

Later studies, however, disclosed that the formation of the disastrous DNA double-strand end shown in Figure 11.4 in cells treated with a topoisomerase inhibitor could be avoided if the drug concentration was not too high. When the growing end of a replicating DNA encountered a drug-induced block, the growing replication fork, instead of proceeding into the blocked region, could temporarily invert, as shown in Figure 11.8 (Ray Chaudhuri et al., 2012). Figure 11.9 shows an electron microscope image of an inverted replication fork.

The new understanding in the 1980's of how camptothecin works greatly revived interest in testing the drug on cancer patients; camptothecin and related topoisomerase I inhibitors have since assumed an important role in cancer chemotherapy. The reversal of the replication fork was mediated in part by poly(ADPR) polymerase (PARP) (Ray Chaudhuri et al., 2012) (see Chapter 30).

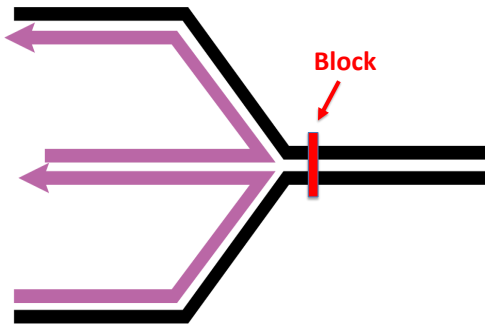



Figure 11.8. Inversion of a replication fork when replicating DNA (purple lines) encountered a block, such as produced by a topoisomerase inhibitor. If the drug concentration was not too high, the replicating strands could invert temporarily until the block spontaneously reversed (Ray Chaudhuri et al., 2012).

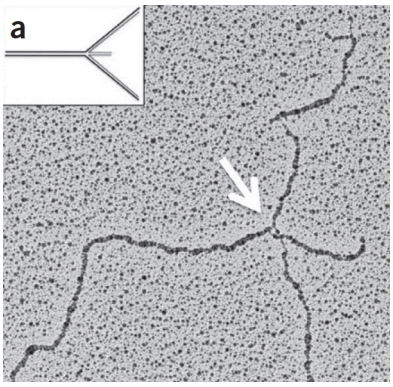



Figure 11.9. Electron microscope image of an inverted replication fork (Ray Chaudhuri et al., 2012). Notice the 4 DNA double-helices emerging from the inversion point (arrow).

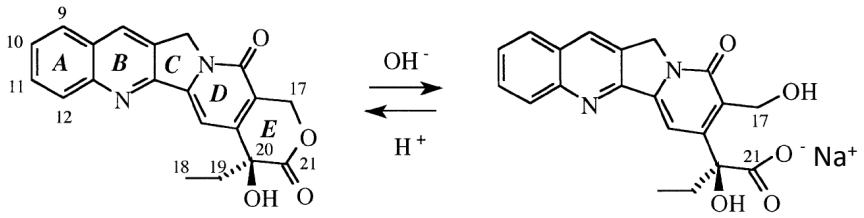
## *Early clinical trials of camptothecin.*

As prelude to trials of camptothecin in cancer patients, testing in animals showed that a dose-limiting toxicity was damage to the lower intestinal tract. The cells of the inner lining of the intestines multiply rapidly in order to renew cells that normally are continually sluffed off. In cancer patients, however, the dose-limiting toxicity was suppression of blood cell production in the bone marrow (Gottlieb et al., 1970). Nevertheless, the rapidly dividing cells, both in the intestines and in the bone marrow, were particularly sensitive to camptothecin.

One of the problems with the early clinical trials of camptothecin was that they used the sodium salt form (Figure 11.10, right), which is inactive and its conversion to the active lactone form (left) in patients was erratic. The camptothecin lactone was the active form, but it was nearly insoluble and therefore difficult to prepare for clinical use. (The solubility problem was later solved by encapsulating the insoluble camptothecin lactone in gelatin capsules for oral administration).

However, the sodium salt was soluble and readily administered. It was therefore used in the early studies when its lack of activity was yet unknown (Muggia et al., 1996). The early clinical experience with camptothecin was discouraging, and the clinical trials were therefore stopped.

Camptothecin studies were resumed 15 years later when its action on topoisomerase I was discovered. Development of camptothecin as an anticancer drug then resumed with renewed intensity, although the laps of 15 years was unfortunate for a drug that was to become very useful for anticancer therapy.




CAMPTOTHECIN (LACTONE) CAMPTOTHECIN (SODIUM SALT)

Figure 11.10. Chemical structure of camptothecin. The active form of camptothecin has a "lactone" structure in the E ring (left). Under alkaline conditions, the lactone ring opens to form the sodium salt (right), which was inactive. Under mild acidic conditions, the sodium salt slowly converted to the active lactone form. Notable also was that the natural active form had its OH group at position 20 pointing up, whereas the isomer whose OH pointed down was inactive. Thus the 3-dimensional structure around position-20 had to be just right for camptothecin to bind to the Top1 protein.

## *Modified camptothecins.*

In 1989, we collaborated with Monroe Wall and Mansukh Wani in testing a large number of modified camptothecins for their activity against topoisomerase I (Jaxel et al., 1989) (Kohn and Pommier, 2000). The results showed where the camptothecin molecule could be modified to increase its potency and indicated where modifications abolished activity. We found out where the camptothecin molecule must remain unobstructed in order to fit into its binding site on the topoisomerase I protein, and where atoms could be added without loss of activity. For example, adding an NH2 group at position 9 on the A ring increased activity, whereas adding an NH2 group at position 12 destroyed activity (Figure 11.10 *left* shows position numbering). Thus, position 12 had to remain unobstructed to allow camptothecin to fit well into its binding site on topoisomerase I. Positions 10 and 11 were free for making small additions. In fact, adding an OH group, especially at position 10, substantially increased camptothecin potency (Jaxel et al., 1989).

Among the modified camptothecins we examined, one of the most potent had a methylenedioxy (-O-$CH_2$-O-) group added to form a 5-membered ring next to the A ring (Figure 11.11) (O'Connor et al., 1990; O'Connor et al., 1991). Although this compound was not pursued for development at that time, it was later rediscovered and called "FL118" (Ling et al., 2012; Ling et al., 2015).

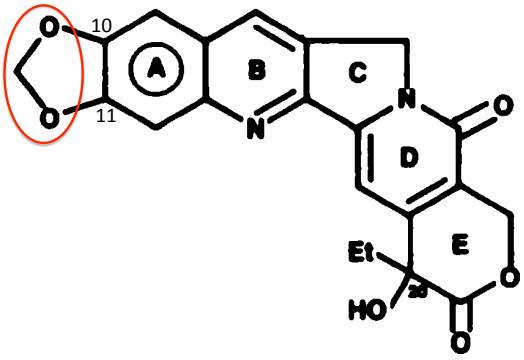



Figure 11.11. 10,11-methylenedioxycamptothecin, a modified camptothecin having increased potency for inhibition of topoisomerase 1 (Jaxel et al., 1989). The addition to the camptothecin molecule is circled red.

## *Topotecan became the most frequently used camptothecin in cancer therapy.*

Our structure-activity findings (Jaxel et al., 1989) helped to design the modified camptothecin, "topotecan", which became commonly used in cancer treatment. Topotecan has a positively charged methylamino group added at position 9 and an OH group added at position 10 (Figure 11.12). The positively charged group solved the solubility problem; its placement at position 9 was in accord with our finding that additions could be made at this position without interfering with the ability of the drug to block topoisomerase I. We had also found that adding an OH group at position 10, which is the case for topotecan, would increase the potency of the drug. Topotecan was relatively easy to make by chemical modification of camptothecin, and it was highly potent against experimental tumors in animals, as well as effective against topoisomerase I in cancer cells (Kingsbury et al., 1991). A potential drawback was that the charged group at position 9 would reduce the ability of the drug to penetrate the blood-brain barrier into the brain. That would be a disadvantage if there were cancer cells in the brain. On the other hand, it might be an advantage if it reduced toxicity induced by the drug action on normal brain cells.

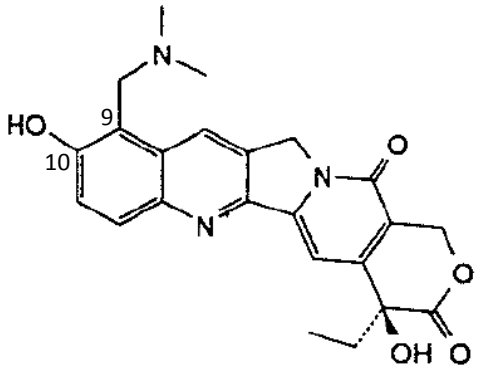



Figure 11.12. Topotecan, a modified camptothecin became used in cancer therapy. The N-containing group added at position 9 became positively charged, and therefore improved the solubility of the drug, so that it could readily be administered to patients. The OH group added at position 10 increased the potency of the drug.

## *Irinotecan*

Another modified camptothecin, irinotecan, also became commonly used in therapy. It was approved by the U. S. Food and Drug Administration in 1996 for the treatment of colon cancer; it was also active against several other types of cancer. Irinotecan is a "pro-drug": it was nearly inactive until a carboxyesterase enzyme, present in liver and other tissues, cut off an inactivating side-chain from the molecule (Figure 11.13A) (Ramesh et al., 2010).

When combined with other drugs, such as 5-fluorouracil and oxaliplatin, it became a key drug for the treatment of metastatic colorectal cancer, and it was also useful against several other types of cancer (Fujita et al., 2015).

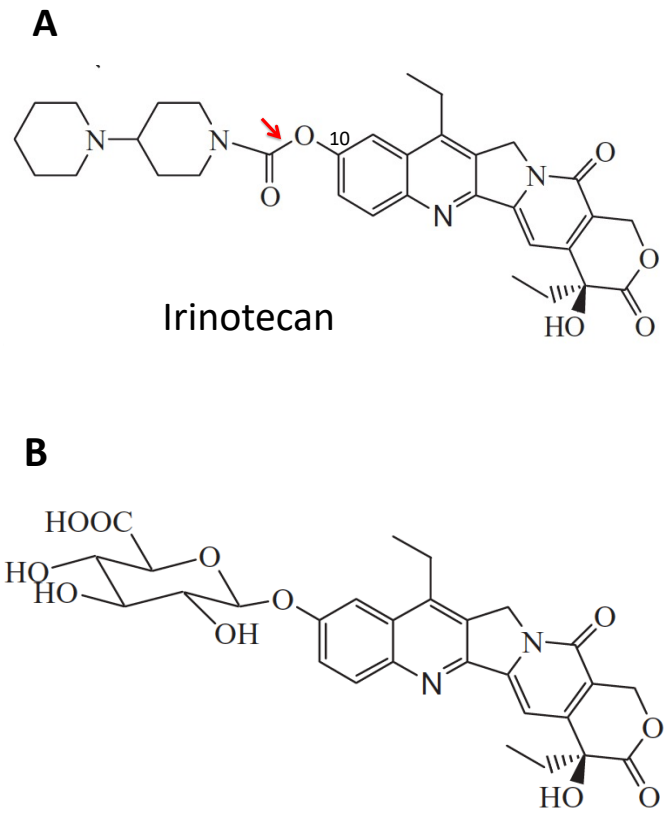



Figure 11.13. **A**. Chemical structure of irinotecan. The side chain on the oxygen at position 10 conferred water-solubility but inactivated the drug. The drug was activated by an enzyme present in tissues that cleaved of the bond indicated by the red arrow (Ramesh et al., 2010). That left an OH group on position10, which increased the potency of the drug (Jaxel et al., 1989). **B**. Chemical structure after replacement of the group at position 10 by a glucose-like (glucuronide) unit through the action of a UGT enzyme. This action by the enzyme inactivated the drug (Ramesh et al., 2010). Absence of this enzyme allowed the amount of available active drug to increase to higher levels and thereby made patients who lack active UGT unusually sensitive to the drug.

Irinotecan produced unusually severe toxicity in some patients. Extensive studies were carried out to find out why that was the case. If the unusually sensitive patients could be identified, their drug dose could be reduced to a safe level. The studies revealed that a frequent cause of the unusual sensitivity was a particular isoform of a gene called UGT1A1 that sensitive patients had in their genome. This gene was found to code for an enzyme called UDP-glucuronosyl-transferase (UGT), whose function will be explained shortly. Among the several genes that code for UGT enzymes, the most troublesome form was UGT1A1*28. People who had only that isoform of the UGT1A1 gene were highly sensitive to irinotecan. The reason for

that was that the UGT enzyme made by that isoform was nearly inactive (Schulz et al., 2009) (Fujiwara and Minami, 2010).

According to Dr. Silvio Parodi, UGT (UDP-glucuronosyltransferase) is a cytosolic glycosyltransferase that catalyzes the transfer of the glucuronic acid component of UDP-glucuronic acid to a small hydrophobic molecule. This was a glucuronidation reaction. The reaction catalyzed by the UGT enzyme involved the addition of a glucuronic acid moiety to a variety of biologically active compounds found in nature.

To understand all that, we have to know what the active UGT enzyme does. After irinotecan has been activated by cutting off the side chain from position-10 (Figure 11.13A), UGT inactivates it again by adding a glucuronide unit (Figure 11.13B). Without active UGT, therefore, the level of active irinotecan was elevated to unusually high levels after the customary dose of the drug (Schulz et al., 2009) (Fujiwara and Minami, 2010). The solution to the irinotecan dosage problem therefore was to determine the UGT status of the patient and adjust the drug dosage accordingly.

A remarkable modification of irinotecan, called etirinotecan pegol, was designed that reduced toxicity and increased anti-tumor potency in mice by slowly releasing the active topoisomerase I inhibitor over long periods of time (Figure 11.14). The structure was designed to link irinotecan to long poly(ethylene glycol) chains in a manner that kept the drug inactive and to slowly and spontaneously release it in its active form (Hoch et al., 2014). Etirinotecan pegol was more effective than the bare irinotecan in suppressing the growth of tumors in mice (Figure 11.15), and clinical trials of this promising designer drug were begun (Alemany, 2014) (Jameson et al., 2013; Lopez-Miranda and Cortes, 2016).

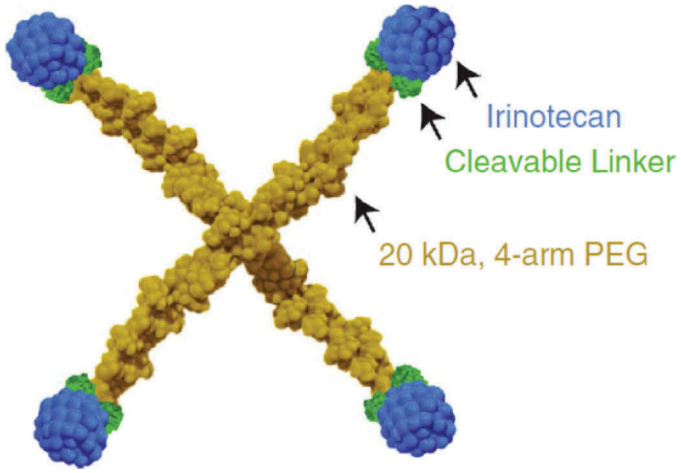



Figure 11.14. Molecular structure of etirinotecan pegol, in which irinotecan molecules were tethered to the ends of poly(ethylene glycol) chains. The linker hydrolysed slowly to release active irinotecan (Hoch et al., 2014).

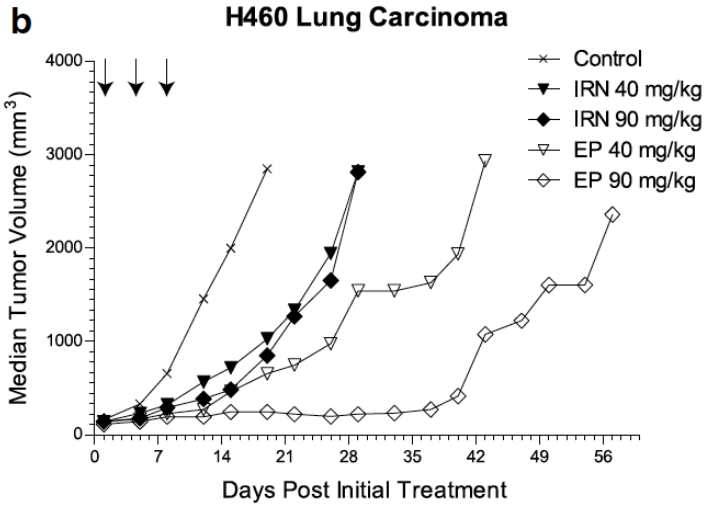



Figure 11.15. Increased effectiveness of etirinotecan pegol (EP) relative to bare irinotecan (IRN) against human lung cancer cells growing as tumors in immune-deficient mice (Hoch et al., 2014). Vertical axis: tumor volume; horizontal axis: time after treatment (arrows show times of EP injection). EP inhibited tumor growth for a much longer time than did IRN. Similar results were reported with several cell lines from other types of human cancer.

Another way to make irinotecan more effective was to incorporate the drug in tiny, microscopic-sized lipid globules called nanoliposomes, from which the drug was

slowly released. In addition, the idea was that the nanoliposomes would be small enough to exit from the tumor's abnormal blood vessels while being large enough to be retained in normal blood vessels. That would selectively deliver the drug to the tumor and reduce toxic effects to normal tissues. Another factor would be that drug within the tumor tissue would only slowly be flushed out, because of the poor lymphatic drainage that was common in tumors. Nanoliposomal irinotecan ("nalirinotecan") has already been approved for clinical use (Ko, 2016).

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