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REVIEW ARTICLE

DNA topoisomerase-targeting chemotherapeutics: what's new?

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Abstract To resolve the topological problems that threaten the function and structural integrity of nuclear and mitochondrial genomes and RNA molecules, human cells encode six different DNA topoisomerases including type IB enzymes (TOP1 and TOP1mt), type IIA enzymes (TOP2α and TOP2β) and type IA enzymes (TOP3α and TOP3β). DNA entanglements and the supercoiling of DNA molecules are regulated by topoisomerases through the introduction of transient enzyme-linked DNA breaks. The covalent topoisomerase–DNA complexes are the cellular targets of a diverse group of cancer chemotherapeutics, which reversibly stabilize these reaction intermediates. Here we review the structure–function and catalytic mechanisms of each family of eukaryotic DNA topoisomerases and the topoisomerase-targeting agents currently approved for patient therapy or in clinical trials, and highlight novel developments and challenges in the clinical development of these agents.

History

In 1971, James C. Wang discovered a new class of enzyme from *Escherichia coli* that catalyzes alterations in the topology of duplex DNA molecules, which are now referred to as DNA topoisomerases. The enzyme he described (protein ϖ or prokaryotic DNA topoisomerase I) had the ability to resolve negative superhelical turns (or underwinding of DNA strands) within closed circular, duplex DNA without the necessity of an energy cofactor [1]. He hypothesized a reaction mechanism of concerted DNA strand nicking and religation mediated by the formation of an enzyme–DNA covalent intermediate. In 1972, Champoux and Dulbecco reported a similar enzymatic activity from mouse embryo cells that was also able to relax DNA superhelical turns and later demonstrated the covalent linkage of the enzyme to a single nicked DNA strand by a phosphodiester bond to tyrosine [2, 3]. As with prokaryotic DNA topoisomerase I, the formation of a covalent enzyme–DNA intermediate obviated the need for an energy cofactor in the DNA nicking/closing reaction cycle of the DNA. It took almost 30 years before Pommier and co-workers identified a mitochondrial-targeted isoform of Champoux and Dulbecco's eukaryotic TOP1, TOP1mt, which is only found in vertebrates [4].

In 1976, Gellert and colleagues isolated DNA gyrase, a distinct class of DNA topoisomerase from *E. coli*. This enzyme was able to introduce negative supercoils into DNA; however, the reaction required the cofactors ATP and Mg^{2+} [5]. Subsequent studies defined the formation of transient, protein-linked double-strand DNA breaks [6]. In 1980, Liu and Alberts [7] coined the term type II DNA topoisomerase to describe enzymes such as DNA gyrase or bacteriophage T4 topoisomerase that alter DNA topology by reversibly breaking both strands of duplex DNA. A eukaryotic type II DNA topoisomerase (TOP2α) was subsequently isolated from human HeLa cells, followed a few years later by the discovery of a second type II isoform, TOP2β, respectively [8–12].

Yeast TOP3 was the first type IA enzyme identified in eukaryotic organisms as a result of its role in homologous

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recombination [13, 14]. As with type II enzymes, vertebrates have two isoforms of TOP3: TOP3α and TOP3β [15, 16].

Since the original descriptions of topoisomerases, the function, structures, and mechanism of type I and type II classes of enzymes have been extensively studied [17–21]. DNA topoisomerase-targeted compounds, many of which are derived from natural products, have been shown to specifically bind to and stabilize the covalent topoisomerase–DNA reaction intermediates. Analogs of these compounds are FDA approved and/or are in development as antimicrobial and cancer chemotherapeutics [18, 22–27]. These chemotherapeutics enhance the stability of normally transient enzyme–DNA covalent complexes, which are then converted into lethal DNA lesions/breaks through interactions with replication and transcription machinery, or during chromosome segregation [19, 21, 23, 28–33]. For the purpose of this review, we will focus on chemotherapeutics that target eukaryotic topoisomerases. Human cells express six DNA topoisomerases representing three subfamilies: Type IA (TOP3α and TOP3β), type IB (TOP1 and TOP1mt), and type II (TOP2α and TOP2β) [17–19, 21].

Physiological function

DNA topoisomerases function to resolve topological problems that are intrinsic to the helical, duplex structure of DNA and are generated during DNA metabolism and gene expression. These enzymes catalyze the relaxation of DNA supercoils, and the decatenation and unknotting of DNA helices and strands. As such, DNA topoisomerases play essential roles in DNA replication, transcription, and repair, and in the condensation and segregation of chromosomes during mitosis [17–19].

Eukaryotic topoisomerases are classified as type I or type II enzymes, based on the number of strands in duplex DNA that are transiently cleaved during the reaction cycle (one strand for type I and staggered double-strand breaks for type II). Categorization into IA, IB, and IIA subfamilies is based on differences in structure and mechanism (Table 1) [17–19, 34]. Type IA and type IB topoisomerases are largely monomeric enzymes that cleave one strand of duplex DNA. However, they do not share sequence or structural similarities, they form distinct phospho-tyrosyl linkages, and they exhibit different mechanisms of DNA supercoil relaxation (Fig. 1a, b). Type II topoisomerases induce a double-strand break, function as a dimer, and require ATP hydrolysis for the alterations in enzyme structure that accompany DNA strand passage (Fig. 1c). Recently, structural homologs of the type II archaeal topo VIB enzyme subunit have been identified in plants and animals and have been implicated in the formation of meiotic double-strand DNA breaks, after interaction with the SPO11 subunit [35]. While exhibiting structural and mechanistic similarities to type IIA enzymes, these topoisomerases are currently not therapeutic targets.

To catalyze alterations in DNA topology, all DNA topoisomerases utilize successive transesterifications, where the active site tyrosine acts in the nucleophilic attack on the DNA phosphodiester backbone to form a transient phospho-tyrosyl linkage with the nicked DNA. This enzyme–DNA covalent complex is also referred to as a cleavable complex, as it may be irreversibly trapped with denaturing agents. Topological strain in the DNA is then released via topoisomerase-mediated strand passage for type IA and type II enzymes, or by a mechanism of enzyme-controlled strand rotation for type IB enzymes [19] (Fig. 1). Subsequent religation of the DNA strand is accomplished by a second transesterification reaction involving the

Table 1 Overview of eukaryotic DNA topoisomerases

	Type IA		Type IB		Type II	
Gene	*TOP3A*	*TOP3B*	*TOP 1*	*TOP1mt*	*TOP2A*	*TOP2B*
Protein	TOP3α	TOP3β	TOP1	TOP1mt	TOP2α	TOP2β
Cellular localization	Nuc[a] Mito[b]	Nuc[a]	Nuc[a]	Mito[b]	Nuc[a] Mito[b]	Nuc[a] Mito[b]
Genomic location	17p11.2	22q11.22	20q12	8q24.3	17q21.2	3p24.2
Protein size (kDa)	109.3[c] 112.3[d]	96.7	90.7	69.8	170	180
# Subunits	1		1		2	
DNA Strand Cleaved	Single strand		Single strand		Double strand	
Enzyme–DNA linkage	5′-phospho-tyrosyl		3′-phospho-tyrosyl		5′-phospho-tyrosyl	
Mechanism of relaxation	Single strand passage		Rotation		Double-strand passage	
Cofactor	Mg^{2+}		None		ATP & Mg^{2+}	

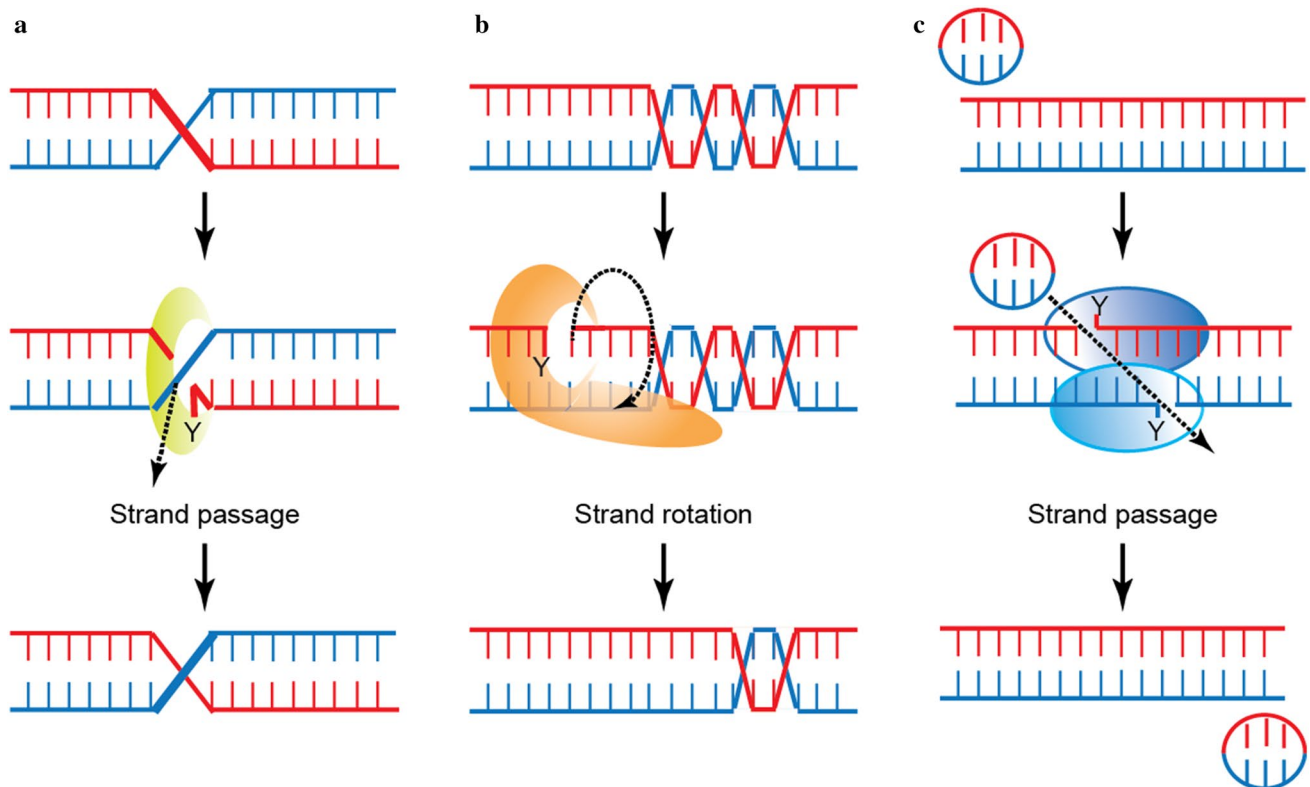[a] Nuclear; [b] Mitochondria; [c] size TOP3α; [d] size TOP3β

Fig. 1 Three distinct catalytic mechanisms of topoisomerases. Each of the three types of DNA topoisomerases exhibits unique catalytic mechanism to alter DNA topology. **a** Type IA acts as a monomer and cleaves one strand forming a 5′-phospho-tyrosyl linkage within the protein–DNA complex. The opening of the cleaved strand allows the non-cleaved strand to pass through for DNA relaxation or (de)catenation. The ends of the cleaved strand religate to restore the phospho-diester backbone of the DNA and allowing the enzyme to dissociate from the DNA 5′-end. **b** Type IB also acts as a monomer and cleaves one strand of duplex DNA, but forms a transient 3′-phospho-tyrosyl bond. DNA relaxation occurs by the controlled rotation of the free 5′-end of the DNA around the non-cleaved strand. **c** Type IIA topoisomerases act as homodimers, with each subunit cleaving one strand and forms a 5′-phospho-tyrosyl linkage, resulting in a staggered double-strand break within the protein–DNA complex. DNA relaxation or (de)catenation occurs by passage of a double-stranded DNA molecule through the enzyme-linked double-strand break. Religation of the cleaved strands restores DNA integrity. See text for more details

nucleophilic attack of the free −OH group of the DNA end to resolve the phosphoryl-tyrosine bond. In these reactions, a water molecule has been posited to act as a general acid–base catalyst [34, 36].

Structure and function of DNA topoisomerase subfamilies

Type I topoisomerases

Eukaryotic type I topoisomerases can be divided into two subclasses; type IA includes topoisomerase III (TOP3α and TOP3β), and type IB consists of nuclear topoisomerase I (TOP1) and the mitochondrial isoform (TOP1mt) found only in vertebrates (Table 1). With the exception of the dimeric TOP1B from *Trypanosoma* and *Leishmania* parasites [37, 38], these enzymes function as monomers and consist of conserved structural elements. Sequence and mechanistic similarities of TOP1 and TOP1mt suggest that they share a common ancestral gene [39]. However, TOP1mt contains a much shorter N-terminal domain (51 residues versus 215 residues for nuclear TOP1), which contains a mitochondrial localization signal (MLS) and lacks the nuclear localization signal (NLS) found in TOP1. The core, linker, and C-terminal domains of these isoforms are sufficient for catalytic activity and show high degrees of identity (73, 57, and 75%, respectively). Both type IB isoforms are also the cellular target of the camptothecin (CPT) class of chemotherapeutics [4, 28, 40, 41]. In cells, TOP1mt is not affected by the parental compound CPT, but can be targeted by the topotecan analog, which is able to enter the mitochondrial matrix [42, 43]. However, it remains unclear what role targeting TOP1mt plays in the therapeutic response of cancer patients to toptotecan, or the prodrug CPT-11.

Mammalian cells express two isoforms of TOP3 (TOP3α and TOP3β). TOP3α can be found in the nucleus

and mitochondria [44], and functions with the BLM helicase in a complex with RMI1 in the resolution of Holliday junctions and in replication fork recovery [45, 46]. TOP3β has also been shown to possess RNA topoisomerase activity on circular RNA (circRNA) substrates and its function in a ribonucleoprotein complex is required for normal neurodevelopment [47]. The TOP3 isoforms act to maintain genome stability and neurodevelopment and have not been the target of drug discovery efforts. Conversely, the prokaryotic type IA enzymes exhibit structural and mechanistic similarities to TOP3 and are potential targets for antibiotics to treat tuberculosis [26]. Thus, there may be complications associated with the chemotherapeutic targeting of tuberculosis in human populations where TOP3α and TOP3β are ubiquitously expressed.

The structures of type IA topoisomerases are generally described as a *padlock*, where domains I, III, and IV form the bottom of the structure (aka *the lock*), and domain II forms a *loop* that facilitates opening and closing of the nicked single strand of DNA [19, 48]. Mechanistically, type IA topoisomerases generate a transient 5′-phospho-tyrosyl bond that covalently links the enzyme with the 5′-end of the nicked single strand of duplex DNA (Fig. 1a), in a reaction that is Mg^{2+} dependent, but ATP independent. Type IA enzymes selectively catalyze the relaxation of negative supercoils in duplex DNA, where the formation of the covalent enzyme–DNA complex is facilitated by the propensity for negatively supercoiled duplex DNA to locally unwind. Consequently, type IA enzymes are unable to relax overwound, or positively supercoiled DNA. These enzymes also act as decatenases to disentangle precatenanes formed during DNA replication, and can catalyze the knotting/ unknotting of single-stranded DNA. In all cases, these enzymes act by a strand transfer mechanism whereby the nonscissile strand is passed through the protein-linked nick in the opposite strand. Subsequent nucleophilic attack by the free 3′-hydroxyl end on the 5′-phospho-tyrosyl linkage results in religation of the DNA strand, allowing for dissociation of the enzyme from the DNA (Fig. 1a).

The type IB subfamily of topoisomerases does not share sequence, mechanistic, or structural homology with type IA or type II topoisomerases. Type IB enzymes are unique in the formation of covalent intermediates with a 3′-phospho-tyrosyl linkage, as opposed to the 5′-linkage observed with all other classes of topoisomerases (Fig. 1b). Unlike type IA enzymes, type IB topoisomerases are able to relax positive and negative supercoils, through a mechanism of DNA strand rotation that does not require Mg^{2+} [34]. In this case, topological stresses in the duplex DNA substrate, rather than the enzyme itself, dictates DNA strand unwinding or rewinding. However, as with other topoisomerases, the cleavage and religation of DNA strands does not require ATP.

The most widely studied type IB enzyme is human DNA topoisomerase I, for which several X-ray crystal structures have been resolved, all of which contain DNA substrates and, in many instances, drugs such as CPTs that target TOP1B [49, 50]. However, the N-terminally truncated TOPO70 enzyme used for these structural determinations lacks residues 1–174 of human TOP1B and, in these TOPO70 structures, only residues 203–765 have been resolved (Fig. 2a). Nevertheless, TOPO70 is able to bind DNA, is catalytically active, and is comparable to TOP1mt in size, reaction mechanism, and presumably tertiary structure [34]. TOPO70 consists of a core domain (core-subdomain I, II, & III), an extended pair of alpha helices
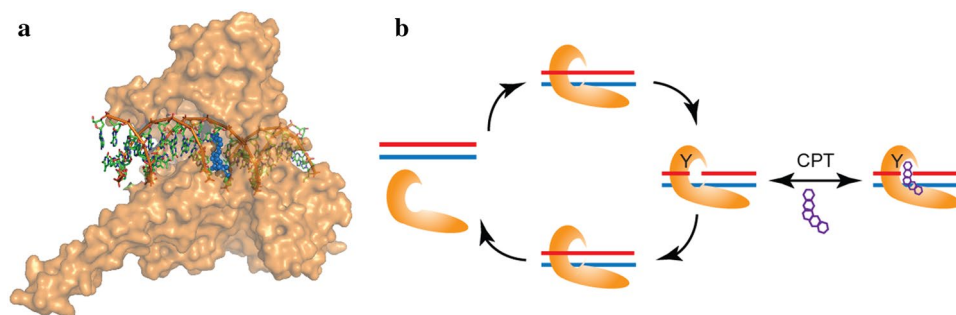

Fig. 2 Type IB DNA topoisomerase. **a** Human TOP1B (TOPO70) crystal structure with DNA and topotecan (TPT). Surface representation of the N-terminally truncated TOPO70 (lacking the first 174 residues) in orange with DNA (*sticks*) and TPT (*blue balls*), PDB# 1K4T [100]. All structure figures were generated using MacPyMol 0.99rc6 (DeLano Scientific, San Carlos, CA). **b** Schematic representation of eukaryotic DNA topoisomerase I (TOP1B) catalytic cycle where the covalent enzyme–DNA intermediate constitutes the sole cellular target of camptothecin (CPT). Topotecan and irinotecan as FDA-approved CPT analogs. TOP1B non-covalently circumscribes duplex DNA. The active site tyrosine cleaves one strand forming a 3′-phospho-tyrosyl bond, which covalently links the enzyme to the 3′-end of DNA. After DNA relaxation by a controlled rotation mechanism, the DNA ends religate and TOP1B may dissociate from the DNA. CPT intercalates into the cleavage site preventing religation of the DNA ends. The reversible drug-stabilized enzyme–DNA complex can be converted into lethal DNA lesions in collisions with processive replication or transcription machinery. See text for more details

that form the linker domain, and a C-terminal domain that contains the active site tyrosine [49, 50]. The core domain forms a "C"-type protein clamp, where domains I and II form the "cap," while domain III forms the base of the "C." The coiled-coil structure of the linker domain runs like a guided ruler away from the "C"-clamp and positions the active site tyrosine of the C-terminal domain within the enzyme active site formed by the core domain of the protein clamp. The "C"-clamp cradles the DNA, while in co-crystal structures that contain CPT or other TOP1 poison, the linker domain is positioned at an oblique angle to the double-stranded helical DNA that extends from the clamp, $3'$ to the site of cleavage in the nicked DNA strand (Fig. 2) [49, 50]. In contrast, the linker domain is not resolved in covalent TOPO70–DNA complexes that lack drug [49]. In conjunction with reconstitution studies reported by Champoux and colleagues, these data suggest that the physical linkage and the flexibility of the linker domain impact the intrinsic sensitivity of TOP1B to CPT [51].

In terms of reaction mechanism, type IB enzymes first bind non-covalently to double-stranded DNA, where the "C"-clamp completely circumscribes the DNA, and the active site tyrosine is poised for nucleophilic attack of the phosphodiester backbone bond of the scissile DNA strand. Upon DNA strand cleavage and concomitant linkage of the enzyme to the $3'$- phosphoryl DNA end, the free $5'$-hydroxyl DNA end rotates within the protein clamp, around the non-cleaved strand, to alleviate DNA strand over- or underwinding (positive or negative supercoils, respectively). The covalent complex is resolved by a second transesterification reaction, involving the nucleophilic attack by the $5'$-hydroxyl DNA end to reform the phosphodiester bond in the DNA. The enzyme subsequently dissociates from the non-covalent enzyme DNA complex or initiates a new DNA cleavage–religation cycle at the same site (Figs. 1b, 2b).

Type II topoisomerases

Eukaryotic type II topoisomerases function as homodimers and transiently cut both strands of a DNA duplex. Then, through a strand passage mechanism (Fig. 1c), these enzymes catalyze the relaxation of positively and negatively supercoiled DNA, or the decatenation/unknotting of entangled double-stranded DNA molecules. Mammalian cells express two type IIA isoforms (TOP2α and TOP2β). TOP2α expression is cycle dependent, increasing in S phase and peaking during G_2/M phase, to assist in the untangling of DNA duplexes during replication. This enzyme also catalyzes the relaxation of positive supercoiled DNA more efficiently than negative supercoils and decatenates mitotic chromosomes during cell division. In contrast, TOP2β is constitutively expressed

in replicating and quiescent cells. TOP2β plays a critical role during neuronal cell development and has been implicated in the regulation of gene transcription and DNA recombination, yet acts equally efficiently in the relaxation of positively and negatively supercoiled DNA [52–55].

Human TOP2α and TOP2β share considerable mechanistic, structural, and sequence identity with each other and the related type II enzyme expressed in the yeast *Saccharomyces cerevisiae*, being 68% identical in amino acid sequence to each other and 40% identical to yeast TOP2. Numerous structures of the C-terminal catalytic core and N-terminal ATPase domains of eukaryotic DNA type II enzymes have been separately resolved, as well as a near-complete structure of yeast TOP2 (Fig. 3) [56–59]. Given the overall conservation of the eukaryotic and bacterial type IIA family of enzymes, the compilation of these structures provides detailed mechanistic insights into type II enzyme catalysis and drug targeting [21, 57, 59, 60].

A series of dimer interfaces form three gates in the type IIA homodimer through which a DNA duplex is passed (Fig. 4). A series of coordinated, ATP-dependent conformational changes in enzyme promoter structure allow for gate opening and closing, to pass one DNA duplex (the T-segment) through a staggered double-stranded break in a second segment of duplex DNA (G-segment). The N-gate is formed by the N-terminal ATPase domain of each promoter, which consists of the GHKL (Gyrase, Heat-shock protein 90, histidine Kinase,
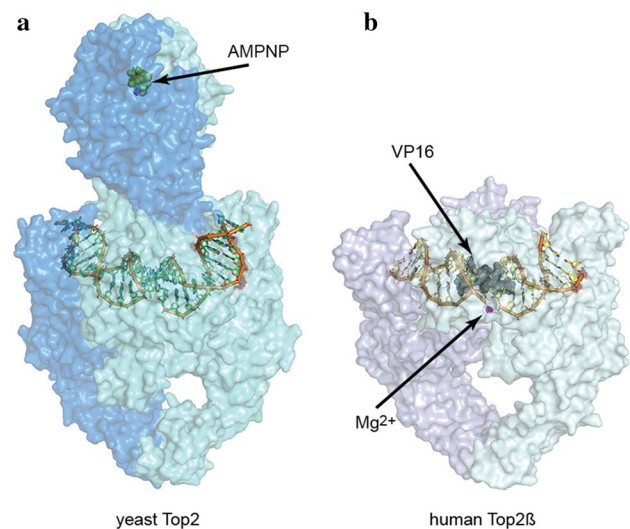

Fig. 3 Crystal structures of eukaryotic type IIA topoisomerases. **a** Near-complete structure of yeast TOP2 PBD# 4GFH [59]. TOP2 protein surface is shown in *blue/cyan* with DNA (*sticks*) and the non-hydrolysable ATP analog AMPNP (*green balls*). **b** C-terminal catalytic core surface structure of human TOP2ß shown in *pale blue/pale cyan*, DNA (*sticks*), and etoposide -VP16- (*gray balls*) from PDB# 3QX3 [57]
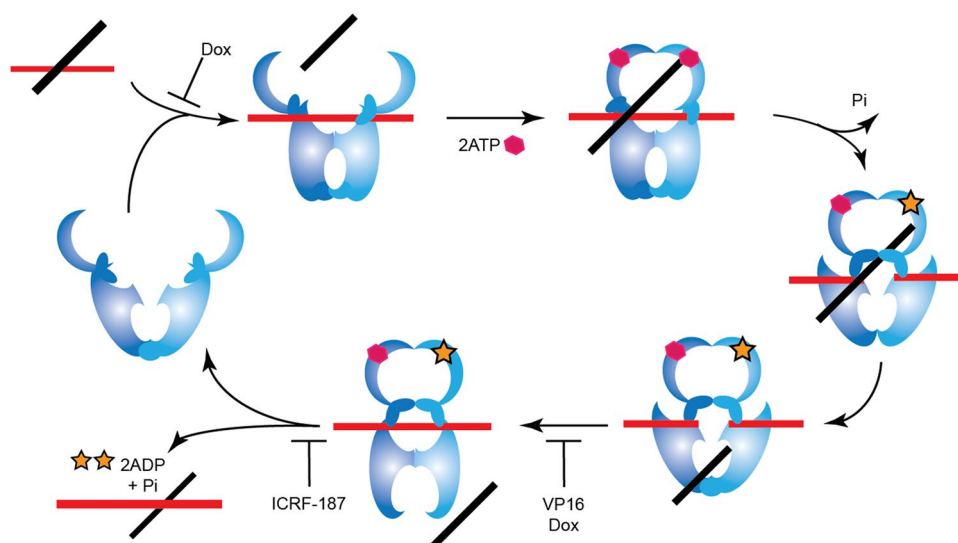
Fig. 4 DNA Topoisomerase II catalytic cycle. Topoisomerase II binds two duplex DNAs. First, the Gated or G-segment DNA stands enters the open N-gate formed by the dimeric ATPase domains. The Transported or T-segment DNA (*black*) then enters via the N-gate. The T-segment DNA may be from the same molecule as the G-segment DNA (during DNA relaxation or (un)knotting), or from another DNA molecule [(de)catenation]. Subsequently, two ATP molecules bind to the ATPase domains, stimulating domain dimerization to close the N-gate. Upon hydrolysis of one ATP molecule, G-segment DNA strands are cleaved and covalently linked to one subunit via a 5′-phospho-tyrosyl bond. The T-segment DNA is then transported through the cleaved G-segment (G-gate). The strands of the G-seg-

ment are religated and the remaining ATP is hydrolyzed. Upon dissociation of the two ADP molecules, the T-segment is released through the C-terminal "C-gate." Closing of the C-gate is followed by separation of the ATPase domains to allow the G-segment to leave the enzyme complex. Doxorubicin (Dox) or adriamycin can inhibit the catalytic cycle at two different steps. Dox blocks the religation of the cleaved G-segment, which is also the mechanism of action for etoposide (VP16). However, Dox can also interfere with DNA binding at the start of the catalytic cycle, preventing TOP2-mediated DNA relaxation or (de)catenation. ICRF-187 prevents the hydrolysis of the second ATP molecule, preventing separation of the ATPase domains or opening of the N-gate. See text for more details

and mutL) domain and a structure-relaying transducer domain [54, 59, 61]. These domains are linked to the catalytic core of the enzyme, which forms the DNA-gate and comprises a metal-chelating TOPRIM (topoisomerase/primase) domain, a winged-helix domain (WHD) containing the active site tyrosine, and a tower domain. The third dimerization interface, the C-gate, consists of a coiled-coil domain that links the structural elements of the DNA-gate to the largely disordered C-terminal domain (Fig. 4) [21, 56, 57, 60–62].

As shown in Fig. 4 and Table 1, type IIA topoisomerase catalysis is dependent on ATP and Mg^{2+} and involves the preferential binding of duplex DNA crossovers (the G- and T-segments) [63]. ATP binding induces dimerization of the N-gate, to trap the G- and T-segments within the closed protein clamp. Subsequently, the active site tyrosines in each promoter cleave opposite DNA strands to form staggered 5′-phospho-tyrosyl bonds. The broken G-segment is then pulled apart to allow the T-segment to be transported into the bottom C-gate. Hydrolysis of one ATP molecule promotes T-segment passage through the DNA-gate. The G-segment is then religated and the T-segment is released through the C-gate. Hydrolysis of the second ATP resets the open N-gate and the G-segment is released. Recent

structural studies suggest that limited conformational changes in the WHDs may couple DNA cleavage/religation with the allosteric control of C-gate opening [56].

Topoisomerases as drug targets

The intertwining of DNA strands and helices, produced during essential cellular processes of replication, recombination, transcription, and chromosome segregation, must be resolved in order to maintain cell viability and genome stability. DNA topoisomerases provide an important solution for resolving such topological problems. However, they do so through the formation of a covalent enzyme–DNA reaction intermediate, which is in itself a potentially toxic lesion. Indeed, targeting of topoisomerase–DNA complexes has been widely exploited in the identification and development of antibacterial and anticancer chemotherapeutics [17, 18, 23, 26]. The majority of these agents are termed "poisons" to indicate a mechanism of covalent enzyme–DNA complex stabilization (Figs. 2a, 3b), versus "inhibitor," which would signify the lack of DNA binding or cleavage by the enzyme. A critical aspect of these differences in the mechanism of drug action is that in an isogenic cell system, increased expression of the enzyme

would increase the cytotoxic activity of a "poison" through increased production of drug-stabilized topoisomerase–DNA adducts. In contrast, elevated levels of enzyme would confer resistance to "inhibitors." FDA-approved topoisomerase IB (Fig. 5) and type IIA (Fig. 6) poisons, such as topotecan and etoposide, respectively, are analogs of naturally occurring poisons, camptothecin (Fig. 5a, b) and podophyllotoxin (Fig. 6a), respectively. (For drug information, see: https://www.cancer.gov/publications/dictionaries/cancer-drug).

In addition, many of the topoisomerase-targeting agents act as interfacial inhibitors (poisons) [64]. Interfacial inhibitors are different from competitive (orthosteric) inhibitors and non-competitive (allosteric) inhibitors since they interact at the interface between two or more molecules. TOP1B-targeting CPTs are a great example of interfacial inhibitors; these compounds interact with the protein–DNA interface, effectively acting as additional nucleotides to prevent DNA religation (reviewed in [65]). Moreover, elegant work in yeast showed that the TOP1B inhibitors act as poisons rather than catalytic inhibitors [66, 67].

TOP1B poisons

Camptothecin was isolated from the chinese tree *Camptotheca acuminata* and identified in a natural products screen

for anti-leukemic activity [41]. Although active in cancer clinical trials, low solubility and adverse drug reactions limited clinical development [68, 69]. Subsequent development efforts yielded several water-soluble CPT analogs, with improved toxicity profiles. Two of these—topotecan and the prodrug irinotecan—are FDA approved for the treatment of ovarian and lung cancer, and colorectal carcinoma, respectively [22, 23, 28]. All CPT analogs reversibly stabilize the TOP1B–DNA complex. In this ternary drug–enzyme–DNA complex, CPT-induced misalignment of the 5'-hydroxyl group and the scissile tyrosine–DNA bond prevents DNA religation (Fig. 2). However, these protein-linked DNA lesions are not toxic in themselves. Rather, the collision of DNA replication forks with the ternary complexes, or the positively supercoiled DNA domains induced by these complexes, produces the irreversible DNA lesions and double-strand breaks that ultimately lead to cell death [33, 70, 71]. Thus, the cytotoxicity of these drugs is S phase dependent.

A common aspect of all CPT analogs is a planar pentacyclic structure, where the conjugated rings are labeled A–E. The lactone ring is highly susceptible to hydrolysis, and at physiological pH, ring E is in equilibrium between the closed lactone and open carboxylate forms (Fig. 5a). However, the lactone form is more lipophilic, which facilitates cellular uptake, and is required for productive
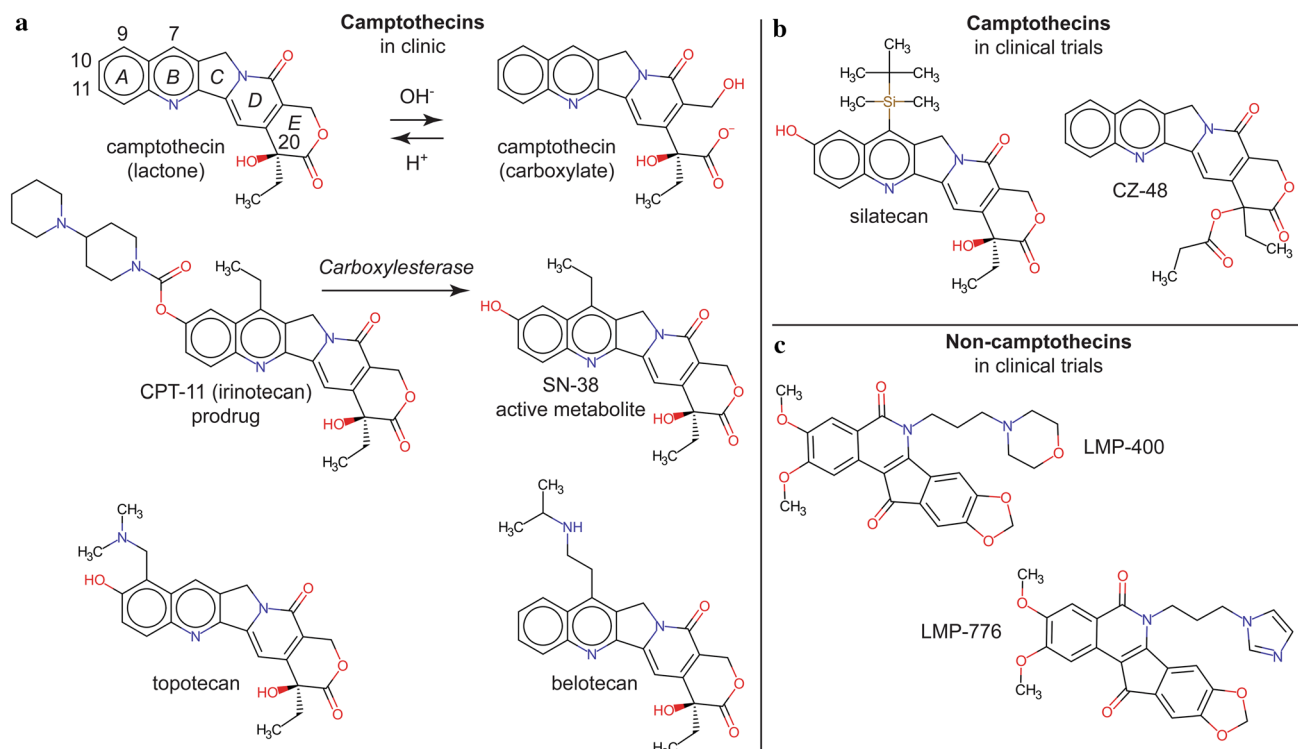

Fig. 5 Structures of DNA topoisomerase IB inhibitors. Shown are the structures of camptothecin or analogs that are currently FDA approved (**a**), or in clinical trials (**b**). **c** Non-camptothecin agents currently in clinical trials
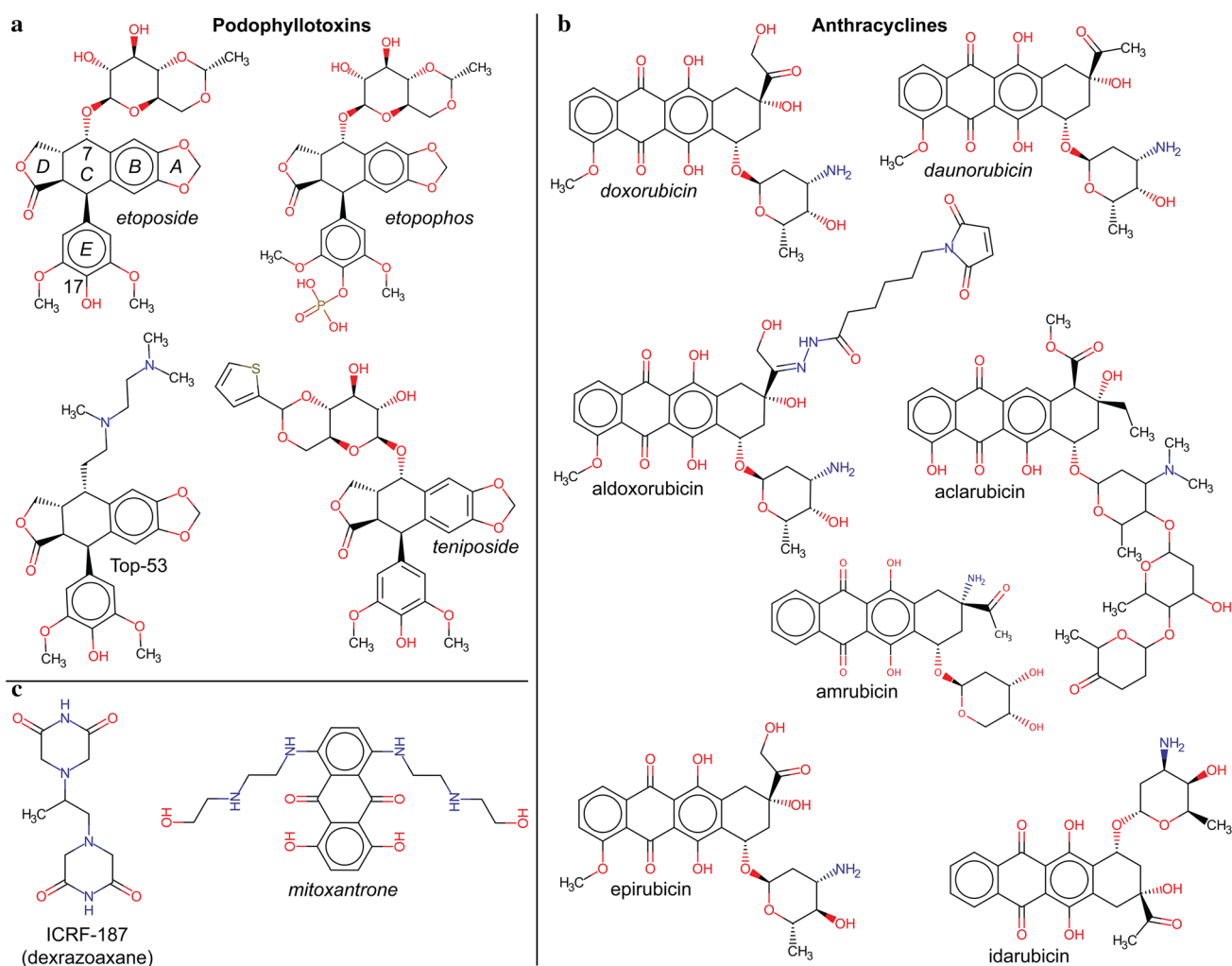
Fig. 6 Structures of DNA topoisomerase II inhibitors. Shown are agents that are currently FDA approved (*italic*), or in clinical trials (normal font): **a** podophyllotoxin analogs, **b** anthracyclines analogs, and **c** other agents

interactions with the TOP1B–DNA complex. The addition of a methyl group to the E ring, to form a 7-member β-hydroxyacetone in homocamptothecin, slows ring opening and enhances potency [72]. Several homocamptothecins have been evaluated in clinical trials; however, irreversible ring opening and increased protein binding have limited further clinical application. Another E ring modification in the prodrug camptothecin-20(S)-O-propionate hydrate (CZ48) is the addition of propionate ester, which stabilizes the lactone ring (Fig. 5b). Subsequent cleavage by cellular esterases yields active CPT. CZ48 is currently in phase I clinical trials.

Extensive analog development has determined that modification of the C, D, and E rings reduces the cytotoxic activity of CPT [23, 70, 73, 74]. On the other hand, modifications to the A ring at positions 9, 10, and 11 and position 7 of the B ring have been shown to be well tolerated and provide improvements in pharmacologic properties and

potency [23, 70, 73, 74]. The semisynthetic CPT derivatives irinotecan and topotecan received FDA approval in 1996 (Fig. 5a). Topotecan is a water-soluble derivative, with position 8 and 9 substituents, and is also approved for oral use. Bone marrow suppression, primarily neutropenia, is the dose-limiting toxicity. Irinotecan (CPT-11) is a prodrug with substituents at positions 7 and 10, which, when cleaved by serum or cellular carboxylesterases, yields the active metabolite SN-38 (Fig. 5a). Dose-limiting toxicities for irinotecan include neutropenia and diarrhea [75]. The latter results from the elimination of SN-38 and its glucuronidated form in the bile and feces. The activity of bacterially expressed β-glucuronidase in the gut generates active SN-38, which in turn induces epithelial cell damage associated with diarrhea. Prophylactic treatment with antibiotics can mitigate this dose-limiting toxicity [76].

Numerous A- and B-ring substituents have been evaluated in preclinical and clinical trials, including 9-amino

CPT and 9-nitro CPT analogs, and hexacyclic derivatives with rings at positions 7/8 or 9/10. The addition of alkyl groups at position 7 was found to increase the cytotoxic activity of CPT. Among these analogs, the improved water solubility of belotecan (CKD-602) results from a basic nitrogen incorporated into an alkyl chain at position 7 (Fig. 5a). This analog is currently in clinical trials and is approved in South Korea for the treatment of small cell lung cancer and ovarian cancer. Among the most potent CPT analogs is gimatecan (ST1481), an oxyiminomethyl derivative (position 7) that is not a substrate for ATP-binding cassette transporters and, as a consequence, is orally bioavailable [77, 78]. Limited phase I–II trials were completed in 2015, while the results from a Chinese phase II clinical trial (2016) have yet to be reported. Silatecan contains an alkylsilyl group at position 7 and an −OH group at position 10, which reduces protein binding and allows the drug to cross the blood–brain barrier (Fig. 5b) [79]. This CPT analog, also known as DB-67 or AR-67, is currently in phase II clinical development. Another 7-silyl CPT, karenitecin (BNP1350), has completed clinical trial evaluations and is not currently in active trials [73, 80, 81].

A novel class of non-camptothecin TOP1B "poisons" is indenoisoquinolines (Fig. 5c). These synthetic molecules exhibit improved chemical stability and longer stabilization of the covalent TOP1B–DNA intermediate than that observed with CPTs [82]. Of a series of over 400 analogs, two derivatives, indotecan (LMP400) and indimitecan (LMP776), are currently being evaluated in phase I clinical trials. LMP400 shows no significant gastrointestinal problems, as are observed with CPT-11, but induce some myelosuppression [83]. Moreover, indenoisoquinolines can overcome multidrug resistance, as they are not substrates for the drug efflux transporters, which limits the oral bioavailability of CPTs [84].

Type IIA topoisomerase poisons

In the mid-1960s, two podophyllotoxin analogs, the epipodophyllotoxin etoposide and the closely related teniposide (Fig. 6a), were identified from a series of synthetically derived compounds exhibiting potent antineoplastic activity [85]. Podophyllotoxin is derived from podophyllin, an alcohol extract of dried roots and rhizomes of the plant *Podophyllum peltatum*, also known as mandrake [86]. In 1983, etoposide received FDA approval as a cancer chemotherapeutic, although its mechanism of action was not fully understood [85]. The following year, Leroy Liu and colleagues reported etoposide and teniposide "poisoning" of DNA topoisomerase II [87]. Similar to CPT poisoning of TOP1B, etoposide stabilizes the topoisomerase II–DNA reaction intermediate (Fig. 3b), preventing religation of the

DNA strands, which causes cell death (Fig. 4). Teniposide was approved by the FDA in 1992.

Etoposide comprises three chemical moieties: a polycyclic core (A–D rings), a glycosidic group (attached to position 4 position of the C ring), and a pendant ring (E ring) [25, 85]. The pendant ring is crucial for the activity of etoposide. Modification of the C-4 glycosidic group can also affect drug activity; however, it does not aid etoposide interactions with topoisomerase II [85]. Teniposide differs from etoposide only by a thienyl glycoside group in place of a methyl (Fig. 6a) [24].

Based on the activity of these epipodophyllotoxins, considerable efforts were made to develop more effective analogs. Structure–activity relationship (SAR) studies of A and B rings resulted in decreased potency relative to etoposide, as did an extensive SAR campaign of E-ring substituents. However, the addition of an E-ring phosphate ester in the prodrug etopophos improved the water solubility of etoposide and allowed for oral bioavailability. Etopophos is readily converted to the parent compound and is currently in the clinic. Another E-ring analog, CAP7.1, a prodrug that is converted to etoposide by carboxylesterases, is in clinical trials in Germany. C-ring substituents, particularly at position C7, have yielded improved activity profiles, with several analogs [such as TOP-53 (Fig. 6a)] in preclinical development [88].

Other classes of FDA-approved topoisomerase II "poisons" include the anthracycline antibiotics, daunorubicin and its hydroxylated congener doxorubicin (Adriamycin), which are derived from *Streptomyces*, and their analogs idarubicin and epirubicin, respectively (Fig. 6b). These drugs intercalate into double-stranded DNA and interfere with the cleavage–ligation cycle of DNA topoisomerase II. Doxorubicin is perhaps one of the most active cancer therapeutics developed (Fig. 4). However, a serious side effect is cardiomyopathy, which can lead to congestive heart failure. Initially attributed to doxorubicin generation of oxygen free radicals, recent studies suggest that cardiac toxicity results from drug poisoning of the TOP2β isoform of topoisomerase II [89]. Other factors have also been implicated in doxorubicin-induced cardiotoxicity. TOP1mt deficiency was recently linked with enhanced doxorubicin-induced cardiomyocyte toxicity [90]. TOP1mt is a critical factor in normal mtDNA homeostasis and may assist in repair of damaged mtDNA [91]. Indeed, Khiati and co-workers observed numerous genomic variants for TOP1mt, within the general population, which have the potential to impair TOP1mt [90].

As with other topoisomerase-targeted drugs, analog development has been pursued to improve the safety profile and efficacy of anthracyclines. One such derivative of doxorubicin, amrubicin, has received approval in Japan; however, it has only been awarded orphan drug

status by the FDA (Fig. 6b). Another analog, esorubicin, exhibited reduced cardiotoxicity, but was also associated with increased myelosuppression. Several approaches have been shown to be more effective in reducing the risk of cardiotoxicity, without affecting tumor response to doxorubicin, including the use of the bisdioxopiperazine topoisomerase II catalytic inhibitor dexrazoxane [ICRF-187] (Figs. 4, 6c). Another approach is the development of nanoparticle formulations that limit drug delivery to the heart, such as liposomal PEGylated doxorubicin (DOXIL). Other formulations in clinical development include glutathione PEGylated liposomal doxorubicin (2B3-101) for selective delivery to the brain, HER2 antibody liposomal doxorubicin (MM-302) for targeting of HER2-expressing tumors, and a co-polymer of N-(2-Hydroxypropyl) methacrylamide (HPMA) and doxorubicin (FCE2806) with no cardiotoxicity. Aldoxorubicin is a 6-maleimidocaproyl hydrazone derivative prodrug undergoing clinical evaluation, where doxorubicin is released in the tumor microenvironment following cleavage of the acid-labile hydrazine linker.

A third class of FDA-approved topoisomerase II poisons is mitoxantrone (Fig. 6c). It is a DNA-reactive agent that intercalates into DNA through hydrogen bonding, and causes crosslinks and strand breaks [92, 93]. It binds to topoisomerase II forming a cleavable complex that will induce DNA strand breaks and successively apoptosis [94].

In contrast to poisons, topoisomerase II catalytic inhibitors do not stabilize the covalent enzyme–DNA intermediate at pharmacologically relevant concentrations. In addition to the bisdioxopiperazine dexrazoxane (ICRF-187) mentioned above in the discussion of doxorubicin, other examples of structurally unrelated classes of inhibitors include the bisdioxopiperazine ICRF-193, the anthracycline aclarubicin, merbarone, and the coumarin drug novobiocin [95]. Of these, dexrazoxane and aclarubicin are currently in clinical development (Fig. 6).

Clinical challenges

As discussed above, numerous TOP1B- and topoisomerase II-targeted agents and analogs have been developed and assessed in preclinical and clinical studies. Clinicaltrials.gov currently lists over 900 active clinical trials of topoisomerase-targeted agents, ~800 of which are cancer trials (for an example of open clinical trials, see Table 2). Of these, ~430 are in the US, 57 in Canada, 200 in the EU, and 160 in China. However, only a handful of these trials involve novel drugs as single agents. Topoisomerases have proven critical cancer targets, with a significant number

Table 2 Drug combination with DNA topoisomerase inhibitors in clinical trials

Drug category	Drug class	Drug combination[a]	
		TOP agent[b]	Other drug(s)
Analgesics	Analgesics, Non-Narcotic	Etoposide	Sirolimus; Celecoxib; Cyclophosphamide
Anti-infective agents	Anti-HIV agents	Etoposide, Doxorubicin	Vincristine; Aldesleukin; Carboplatin; ch14.18/CHO; Cisplatin; Cyclophosphamide; G-CSF; Busulfan; Melphalan
Anti-inflammatory agents	Methylprednisolone acetate	Doxorubicin	Cyclophosphamide; Obinutuzumab; Polatuzumab Vedotin; Prednisolone; Prednisone; Rituximab
Anticoagulants/Fibrinolytic agents	Calcium Heparin	Idarubicin	CX-01; Cytarabine
Antineoplastic agents	Camptothecin	Olaparib	CRLX101
Antirheumatic agents	Cyclooxygenase Inhibitors	Topotecan	DFMO; Celecoxib; Cyclophosphamide
Central nervous system depressants	Valproic Acid	Doxorubicin	
Dermatologic agents	Podophyllotoxin	Etoposide	Cisplatin; Proton beam radiation therapy
Gastrointestinal agents	Sorbitol	Irinotecan, Dasatinib	Rapamycin; Temozolomide
Lipid-regulating agents	Pravastatin	Idarubicin	Cytarabine; pravastatin sodium
Micronutrients	Niacin	Irinotecan	Sorafenib
Psychotropic drugs	Antidepressive agents	Idarubicin	Cytarabine; Indoximod
Renal agents	Ofloxacin	Levofloxacin	Aspirin
Reproductive control agents	Methotrexate	Topotecan, Etoposide	Alisertib; methotrexate; cisplatin; carboplatin; cyclophosphamide; vincristine

This table represents the 435 open cancer trials listed at clinicaltrials.gov, which contain topoisomerase-targeting agents in the treatment schedule

[a] Shown is one example of treatment drug combination; for more information, please visit www.clinicaltrials.gov

[b] DNA topoisomerase-targeting chemotherapeutic

of FDA-approved agents as effective cytotoxic therapies for the treatment of solid and liquid malignancies in adult and pediatric cancer patient populations. Nevertheless, current clinical challenges are focused primarily on two critical issues: (1) combining these effective agents with other cytotoxic and molecularly targeted agents, and (2) new formulations to improve the pharmacologic properties of these drugs and overcome the limitations of scheduling.

Etoposide, doxorubicin, and irinotecan have been incorporated in multiple chemotherapeutic regimens for the treatment of a wide range of solid malignancies. In addition to clinical investigations of new combinations of cytotoxic drug regimens and multi-modality regimens with radiation, current clinical trials include combining these topoisomerase poisons with molecularly targeted therapeutics, such as poly(ADP-ribose) polymerase (PARP) inhibitors, signaling inhibitors (rapamycin analogs), immune checkpoint modulators, and DNA damage checkpoint inhibitors (Table 2). Obviously, the challenge here is to develop robust preclinical models, such as patient-derived xenografts (PDX), and gene expression/editing technology-based screening (RNAi and CRISPR/Cas9) to define the synthetic lethal interactions predictive of pathway interactions that may be exploited with novel drug combinations. An interesting example is preclinical studies demonstrating the increased bioavailability of irinotecan and topotecan, induced in mice by HER tyrosine kinase inhibitor targeting of the ABCG2 drug transporter [96, 97].

In the context of topoisomerase catalysis, the existence of specific tyrosyl-DNA phosphodiesterases, TDP1 and TDP2, allows the development of targeted synthetic lethal drug combinations. TDP1 and TDP2 are able to hydrolyze the covalent phospho-tyrosyl linkage between the topoisomerase active site tyrosine and the phosphoryl end of the DNA. TDP1 preferentially resolves a 3′-phospho-tyrosyl link to the 3′-DNA end, while TDP2 prefers a 5′-phospho-tyrosyl bond which attaches TOP2 to the 5′-DNA end (review in [98]). Consequently, synthetic lethality would be predicted to result from the inhibition of TDP1 or TDP2 activity in combination with a TOP1B or a TOP2 poison, respectively. However, this concept will have to wait for future validation, as no TDP (1 or 2) inhibitors are currently available for clinical evaluation.

As described above for doxorubicin, the clinical development of novel nanoparticle delivery systems is also being exploited to avoid non-malignant tissue toxicity, improve tumor targeting, and address issues of drug stability, oral dosing, and scheduling. In the case of CPT targeting of TOP1B, the S-phase dependent cytotoxic activity of these agents presents challenges in the optimal daily dosing of drugs to maximize therapeutic efficacy (Table 2). Efforts to increase the half-life of CPT analogs could provide for prolonged exposure of S-phase populations of tumor cells,

without repeated dosing. Along these lines, the FDA has approved a long-lived, PEGylated nanoliposomal formulation of irinotecan (Onivyde) for use in combination with fluorouracil and leucovorin, for the treatment of metastatic adenocarcinoma of the pancreas after disease progression. Santi and colleagues at Prolynx recently reported the development of PEG conjugates of SN-38, where β-elimination of phenol ether linkages releases SN-38 with a predictable half-life [99]. Irinotecan or its active metabolite SN-38 has also either been PEGylated (e.g., NKTR102) or attached to nanoparticles (e.g., NK012) to enhance pharmacodynamics and tumor delivery. Moreover, a nano-formulation of CPT in CRLX101, which is water soluble, is currently in clinical trials for the treatment of small cell lung cancer in combination with the PARP inhibitor Olaparib. For current updates of clinical trial of these modified TOP1 and TOP2 inhibitors, see https://clinicaltrials.gov/. The application of these and other novel technologies promises to overcome some of the obstacles in drug dosing and delivery that have hampered the clinical application of what are otherwise extremely active cancer therapeutics.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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