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Drugging Topoisomerases: Lessons and Challenges

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Abstract

Topoisomerases are ubiquitous enzymes that control DNA supercoiling and entanglements. They are essential during transcription and replication and topoisomerase inhibitors are among the most effective and most commonly used anticancer and antibacterial drugs. This review consists in two parts. In the first part ("Lessons"), it gives background information on the catalytic mechanisms of the different enzyme families (6 different genes in humans and 4 in most bacteria), describes the "interfacial inhibition" by which topoisomerase-targeted drugs act as topoisomerase poisons and describes clinically relevant topoisomerase inhibitors. It generalizes the interfacial inhibition principle, which was discovered from the mechanism of action of topoisomerase inhibitors, and discusses how topoisomerase inhibitors kill cells by trapping topoisomerases on DNA rather than by classical enzymatic inhibition. Trapping protein-DNA complexes extends to a novel mechanism of action of PARP inhibitors and could be applied to the targeting of transcription factors. The second part of the review focuses on the challenges for discovery and precise use of topoisomerase inhibitors, including targeting topoisomerase inhibitors using chemical coupling and encapsulation for selective tumor delivery, use of pharmacodynamic biomarkers to follow drug activity, complexity of the response determinants for anticancer activity and patient selection, prospects of rational combinations with DNA repair inhibitors targeting tyrosyl-DNA-phosphodiesterases 1 and 2 (TDP1 and TDP2) and PARP, and the unmeet need to develop inhibitors for type IA enzymes.

1. Foreword

The first part of this assay summarizes the known mechanisms by which drugs target topoisomerases, complementing and updating more detailed reviews.[1–12] The relatively unknown mechanism of action of topoisomerase inhibitors can be traced to the complexity of the topic with 6 different genes in humans cells and bacteria, drugs acting as interfacial inhibitors that trap ternary complexes, and drug cytotoxic mechanisms mediated by the trapping of topoisomerases on DNA rather than by classical enzymatic inhibition. The second part of the review addresses the remaining challenges for the development and precise use of topoisomerase inhibitors for the treatment of cancers and infections.

2. DNA topoisomerases

Topoisomerases are universal and present in eukaryotes, archaebacteria and eubacteria.[13–19] Human cells encode six topoisomerases whereas bacteria generally contain only 4 topoisomerases and lack the type IB enzymes (Table 1 and Fig. 1).[5] The ubiquity of topoisomerases stems from DNA's double-helical (duplex) structure and length, which promote DNA entanglement in the compacted nucleus of eukaryotic cells or the nucleoid of bacteria. The opening of duplex DNA and separation of its two strands during transcription

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and replication generate supercoiling (torsional tension) on both sides of the open DNA segment. Excessive positive supercoiling tightens the DNA and prevents further strand separation thereby stalling the polymerases. Negative supercoiling behind the polymerases, on the other hand, tends to extend DNA strand separation and facilitates the formation of abnormal nucleic acid structures such as R-loops, which can stall RNA polymerase when the transcripts remain bound to the unwound DNA template. Negative supercoiling also promotes the formation of non-canonical DNA structures such as z-DNA, intramolecular hairpins and guanosine quartets (G4's). Topoisomerases prevent the formation of such potentially deleterious structures by removing free supercoiling.

Topoisomerase remove supercoiling by different mechanisms. Type IB enzymes work by letting the broken strand rotate around the intact strand (Fig. 1B),[20–23] whereas type IA and type IIA enzymes work by passing one strand or one duplex, respectively, from the same DNA molecule through the single- or double-strand break generated by the topoisomerase in another duplex (Fig. 1 and Table 1).[15,24,25]

Replication of circular DNA molecules and chromatin loops produces interlinked DNA products (catenanes)[5] that need to be removed by the strand passing activities of topoisomerases. While type IIA enzymes (Fig. 1C and Table 1) act as full decatenases, passing one duplex through another, the strand passing activity of type IA enzymes is restricted to single-stranded DNA segments adjacent to duplex regions (Fig. 1A), which enables Top3 to pass a DNA single-strand and resolve hemicatenanes and double-holiday junctions following replication of supercoiled DNA.[26]

Topoisomerases always break DNA by transesterification reactions using an active site tyrosine as the nucleophile that attacks the DNA phosphodiester backbone. Type IA and IIA enzymes break the DNA by attacking and bonding to the 5'-phosphate whereas type IB enzymes break DNA by covalent attachment to the 3'-phosphate (Table 1 and Figs. 1 and 2). The resulting 3'-hydroxyl ends in the case of type IA and IIA enzymes and 5'-hydroxyl ends in the case of the type IB enzymes reverse the phosphotyrosyl bonds, thereby enabling the release of the topoisomerase and religation of the DNA (Figs. 1 & 2). The nicking-closing activities of topoisomerases are remarkably fast (up to 6000 cycles per minute for Top1 and 250 for Top2);[6,23] yet the enzymes are susceptible to the drugs selectively when the DNA is in the cleaved state (Fig. 2).[23]

3. Topoisomerase inhibitors and the interfacial inhibition principle

The molecular mechanism of action of topoisomerase inhibitors, i.e. their specific binding at the interface of topoisomerase-DNA complexes, led to the interfacial inhibition concept.[6,27,28] We proposed this hypothesis initially for Top2 inhibitors to explain the sequence selective trapping of Top2cc by different drugs; namely the preference for an adenine at position −1 in the case of doxorubicin and other anthracyclines (Fig. 3C),[29] and for cytosine −1 and adenine +1 for etoposide and m-AMSA, respectively.[30,31] Similarly for Top1, camptothecin preferentially traps a subset of Top1cc; those with a guanine +1.[32] A unifying model emerged by which one drug molecule stacks against the base pairs flanking the topoisomerase-induced cleavage site (Fig. 2C & G).[29,30]

To account for the stereospecificity of camptothecins (i.e. only the natural 20-*S*-isomer is active against Top1; see Fig. 3A) [33] and for the high drug resistance of specific Top1 mutants,[34] we also proposed that the enzyme forms specific amino acid contacts with the drug as it stacks against the bases flanking the cleaved DNA. This led to the ternary complex hypothesis with the drug simultaneously interacting with the DNA and the enzyme (Fig. 2C & G).

It took over 10 years to confirm by x-ray crystallography the Top1-DNA-camptothecin model (Fig. 2D) with camptothecin stacking against the +1 guanine and forming a hydrogen bond network with the enzyme.[35–38] The confirmation of the Top2cc trapping interfacial model (Fig. 2C) for antibiotics and anticancer drugs was obtained more recently (Fig. 2G).[39–42] The interfacial inhibition principle extends beyond topoisomerase inhibitors. A large number of natural products act as interfacial inhibitors not only by binding at the interface of nucleic acids and proteins (α-amanitin, aminoglycoside antibiotics), but also at the interface of polypeptides that form multi-proteins complexes and move around each other to perform their biological function (vinblastine, colchicine, rapamycin, brefeldine A, benzodiazepines, anesthetics).[6]

4. Mechanism of action of topoisomerase inhibitors: trapping of topoisomerase-DNA complexes versus catalytic inhibition

Topoisomerase inhibitors are exquisitely selective and without ambiguity eligible as "targeted therapies". Clinically relevant Top1 inhibitors (Fig. 3) do not affect Top2, and, conversely, Top2 inhibitors do not trap Top1 enzymes. Furthermore, the inhibitors of bacterial topoisomerases (gyrase and Topo IV) are inactive against host cell topoisomerases (Top2 and Top1), which accounts for their antibacterial potency without impact on the host genome.

The therapeutic mechanism of action of topoisomerase inhibitors revealed another new paradigm for drug action (in addition to interfacial inhibition detailed above): enzyme poisoning rather than catalytic inhibition drives drug activity. This concept first emerged for the antibacterial topoisomerase inhibitors and was demonstrated for the anticancer topoisomerase inhibitors soon after Top1 was discovered as the target of camptothecin.[43] Indeed, yeast cells lacking Top1 are immune to camptothecin.[44,45] Similarly, human cancer cells depleted for Top1 become resistant to camptothecin,[46] implying that Top1 is required for the cytotoxicity of camptothecin, whereas lack of Top1 catalytic activity (as in cells lacking Top1) is tolerated. Biochemical evidence for the requirement of Top1 for the cytotoxicity of camptothecins and non-camptothecin Top1 inhibitors is supported by the formation of Top1cc in cells treated with Top1 inhibitors.[46–49] Induction of Top1cc in biochemical system is actually routinely used to discover and evaluate Top1 inhibitors.[50,51]

Genetic evidence for Top2 requirement for the anticancer activity of Top2 inhibitors (Table 1) or for the requirement of gyrase or/and topo IV for the antibiotics (Table 1) has been more difficult to obtain than for Top1 inhibitors because cells lacking type IIA topoisomerases are not viable. Nevertheless, several independent studies established that reducing Top2 (and Top1) levels in tumors minimizes drug activity.[34,46,52–54] Conversely, the therapeutic activity of doxorubicin is correlated with Top2 overexpression in the case of amplification of the TOP2A locus together with the HER2 locus on chromosome 17 in a subset of breast cancers.[55] Biochemical evidence for the trapping of Top2-DNA complexes (Top2cc) by anticancer drugs is relatively straightforward and multiple assays can be used to detect the Top2cc not only with recombinant Top2[56] but also in cells.[57–59]

Because the cytotoxic effect of topoisomerase inhibitors requires and is positively correlated with the levels and activity of topoisomerases, assays are being developed to measure the enzymes in patient samples to monitor drug anticancer activity (see Table 2 and section 8).[60,61]

The enzyme poisoning mechanism of action first identified for topoisomerase inhibitors has recently been extended to poly(adenosine diphosphoribose) polymerase (PARP) inhibitors. Indeed, as in the case of Top1 inhibitors, knocking out PARP renders cells immune to PARP inhibitors, and treating cells with PARP inhibitors such as olaparib produces PARP1 and PARP2 DNA complexes.[62] These findings imply that the remarkable activity of PARP inhibitors in breast and ovarian cancers and in Ewing sarcoma can be related, at least in part to the trapping of PARP-DNA complexes when the cancer cells are deficient in homologous recombination repair (BRCA and Fanconi anemia genetic deficiencies).[62] The fact that three very important classes of anticancer drugs, the Top1, Top2 and PARP inhibitors act by poisoning protein-DNA complexes suggests the possibility that other DNA processing protein complexes, such as transcription factors including the Myc-Max heterodimer could be targeted by interfacial inhibitors. Interfacial inhibitors would lock them on the DNA and thereby initiate a cytotoxic cascade to kill cancer cells.

5. Anticancer Top1-targeted drugs

Camptothecin derivatives are the only FDA-approved Top1-targeted anticancer drugs (Fig. 3A). They are water-soluble semi-synthetic derivatives of the plant alkaloid, camptothecin. The potent anticancer activity of camptothecin was known for ≈ 20 years[33] before the identification of Top1 as its molecular target.[43,47]

Topotecan (Hycamtin®) is routinely prescribed for ovarian cancer, and recurrent small cell lung cancer (SCL).[63] Irinotecan (Camptosar®, Campto®) is widely used in gastrointestinal (colorectal and gastro esophageal) malignancies.[63] Topotecan and irinotecan are also used in primary brain malignancies (glioblastomas), sarcomas, and cancers of the cervix. Irinotecan is a prodrug, which is readily hydrolyzed to its active metabolite, SN-38 by carboxyl esterase (Fig. 3A).

Dose-limiting toxicities are myelosuppression for both topotecan and irinotecan and diarrhea for irinotecan. Bone marrow toxicity is common to all other classical cytotoxics and is probably related to the high proliferative index of bone marrow cells and to cell death priming.[64] Severe diarrheas are only observed with irinotecan and their mechanism is not fully understood. It has been related to the hepatic elimination of SN-38 and its glucuronated metabolite that produce high intestinal concentrations of SN-38.[63]

In spite of their potent anticancer activity, all the camptothecins (Fig. 3A) suffer from well-defined limitations.[3] In addition to their dose-limiting toxicity, which prevents the use of curative doses,[65] camptothecins are rapidly inactivated by E-ring opening (Fig. 3A). Indeed, the E-ring α-hydroxylactone spontaneously hydrolyzes within minutes at physiological pH to camptothecin carboxylate, which is sequestered by its high affinity binding to serum albumin. Irinotecan and topotecan are also substrates for the drug efflux transporters, especially ABCG2.[66] Finally, both irinotecan and topotecan are formulated for IV administration and oral formulations are not been pursued because of the intestinal toxicity of irinotecan.

To avoid the dose-limiting toxicity of camptothecins and their short half lives and to reduce normal tissue toxicity while increasing drug delivery to tumors, camptothecins have been conjugated to a macromolecular core as in etirinotecan pegols (NKTR-102). NK-102 is in advanced clinical development (Phase III) for ovarian, breast and colon cancers. The macromolecular conjugation allows slow drug release with lower peak concentration, extended half-life (up to 15 days), and enhanced tumor penetration through leaky tumor vasculature. Another comparable approach is liposomal formulation (see section 8.3).

The chemical instability of camptothecins has been impossible to overcome by simple semi-synthetic derivations of any of the camptothecins including topotecan and irinotecan derivatives.[3,33] The intact α-hydroxylactone E-ring is indeed critical for the binding of camptothecins to the Top1-DNA cleavage complex.[35–38]

Two families of non-camptothecin Top1 inhibitors that overcome the E-ring instability of camptothecins are in clinical development (Fig. 3B).[4,67] The indenoisoquinolines were discovered by screening the NCI Developmental Program drug database for compounds producing cytotoxicity profiles highly correlated with camptothecin across the 60 diverse cancer cell lines of the NCI drug screen [the NCI-60[68]].[3,69,70] Two derivatives are currently in clinical trials indotecan (LMP400) and indimitecan (LMP776). They are developed by the NCI and Purdue University, and licensed to Linus Oncology. In addition to their chemical stability, the indenoisoquinolines offer several advantages over the camptothecins. They target additional genomic sites, their cleavage complexes are markedly more persistent than for camptothecins,[51,71] they overcome multidrug resistance drug efflux pumps[51] and they produce less bone marrow suppression at equal antitumor activity [72].

The other non-camptothecin in clinical trials is 8,9-dimethoxy-5-(2-N-methylaminoethyl)-2,3-methylenedioxy-5H-dibenzo[c,h][1,6]naphthyridin-6-one (Genz-644282; SAR402674) (Fig. 3B), was developed from a structure-activity relationship conducted around the dibenzo[c,h][1,6]naphthyridin-6-one compound family.[80] Genz-644282 has equivalent or superior activity in xenograft models compared with standard drugs and has a favorable cytotoxic profile *in vitro* in bone marrow and tumor cell colony forming assays.[81,82] The compound was licensed to Genzyme by Rutgers University and is now in the drug development portfolio of Sanofi (SAN).[67] Genz-644282 has comparable Top1-targeting activity as the indenoisoquinolines.[73] Both the indenoisoquinolines (LMP776 and LMP400, indimitecan and indotecan) and Genz-644282 appear active and relatively well tolerated in Phase I clinical trials. [83]

6. Anticancer Top2-targeted drugs

All the drugs shown in Figure 3 (panel C–F) inhibit Top2 by targeting Top2cc and inhibiting their religation,[1,5,7,11,74] most likely through interfacial inhibition (see section 3 and Fig. 2). They offer a broad spectrum of chemical diversity,[11] potency,[75,76] sequence selectivity,[31] and ability to trap concerted Top2cc.[5,29,75–77] We refer to "concerted Top2cc" as those where both strands of the DNA are cleaved simultaneously with a canonical 4 base pair overhang stagger (Figs. 1 & 2).

The chemical diversity of Top2-targeted drugs was recently reviewed, and we invite the reader to consult the excellent overview of C. Bailly.[11] We will focus on the clinical use and chemical biology of prototypical Top2-targeted drugs and the drugs in clinical trials.

The anthracycline daunorubicin (daunomycin) was discovered in the 1950's from Streptomyces soil bacteria as an extremely potent anticancer drug. It remains used today primarily for the treatment of acute leukemia.[78] Doxorubicin (adriamycin), another bacterial toxin, was discovered soon after daunorubicin and is more widely used.[78] It is active in first line therapy for breast cancers, bone and soft tissue sarcomas, bladder cancers, anaplastic thyroid cancer, Hodgkin's and non-Hodgkin's lymphomas and multiple myeloma.[79] Epirubicin (4'-epi-doxorubicin), an active isomer of doxorubicin (Fig. 3C) was developed later (FDA approval in 1999) to limit the side effects of doxorubicin, possibly due to its faster elimination. Epirubicin is used in breast, esophageal and gastric cancers.[79] The molecular pharmacology and mechanism of action of anthracyclines are complex. In addition to their anti-Top2 activity, anthracyclines are potent DNA intercalators and generate reactive oxygen intermediates. Their effects on Top2[80] first proposed by Kohn and coworkers[81] was demonstrated well after their approval by the FDA as anticancer agents.

The Top2 inhibitory effect of anthracyclines exhibits notable peculiarities. First, because of the very effective DNA intercalation of anthracyclines, the trapping of Top2 cleavage complexes, which is achieved at submicromolar drug concentration decreases as drug concentration increases, resulting in a bell-shape concentration response with lack of trapping of Top2cc at or above 10 μM concentration.[56,82] Second, compared to etoposide, anthracyclines trap Top2cc with high selectivity at limited genomic sites with an adenine at the −1 position (see Fig. 2).[29] Third, most of the Top2cc are concerted and correspond to DNA double-strand breaks.[75] Finally, the reversal to Top2cc is slow upon drug removal, explaining the potent effects of the anthracyclines on Top2.

Besides bone marrow suppression, which is common to all topoisomerase-targeted anticancer drugs, anthracyclines are cardiotoxic. This dose-limiting and cumulative cardiotoxicity was until recently primarily attributed to the generation of reactive oxygen species.[78] However, a recent study has linked cardiotoxicity to top2β targeting in the nucleus and subsequent mitochondrial damage.[83] Although interesting, this possibility will probably require further studies to elucidate whether doxorubicin could damage mitochondria more directly by targeting mitochondrial Top2β.[84]

Etoposide (VP-16; Vepesid®) (Fig. 3D) is widely used in oncology for a broad range of solid tumors including small cell lung cancers, testicular and germ cell tumors, endocrine tumors, osteosarcomas and Ewing's sarcomas, neuroblastomas and Kaposi sarcoma. Like doxorubicin, etoposide was developed clinically and approved by the FDA (in 1983) without knowing that Top2 was its molecular target. It is a semi-synthetic demethylepipodophyllotoxin derivative without activity on tubulin (by contrast to the podophyllotoxins). Etoposide stands apart from other Top2cc-targeted drugs for the following reasons. First, it is the most selective Top2cc-targeted drug currently in the clinic. As it does not act as a DNA intercalating agent, the Top2cc produced by etoposide form in a monotonic manner without decrease at high drug concentration.[85–87] Second, the Top2cc trapped by etoposide are frequently uncoupled ("non-concerted") with the majority being single-strand breaks instead of the expected double-strand breaks during concerted inhibition[86,87] (see beginning of this section), suggesting that etoposide traps Top2 homodimers asymmetrically with a single drug molecule bound into one of the two breaks (see Fig. 2G but with a single drug molecule and only one of the homodimers trapped in the cleavage complex). Third, as mentioned in section 3, the base sequence preference of etoposide is determined by the presence of cytosine at position −1.[30] Fourth, etoposide produces a very high frequency of Top2cc compared to the intercalating Top2 inhibitors.[88,89] Fifth, the Top2cc trapped by etoposide are readily reversible upon drug wash out, which is different from the anthracyclines. Finally, etoposide traps both Top2α and β very effectively,[59,90] whereas doxorubicin tends to target more selectively cellular Top2α over Top2β.[91] The trapping of Top2β has been related to the induction of secondary leukemia in patient previously treated with etoposide,[92] and linked to Top2β-mediated DNA translocations (see section 8.2).[93,94]

The anthracenedione mitoxantrone (Novantrone®) (Fig. 3E) was developed as a synthetic analog of anthracyclines at the American Cyanamid Laboratories in the late 1970s[78] and approved by the FDA in 1996 for prostate cancer. Like anthracyclines, mitoxantrone is both a potent DNA intercalator and Top2cc poison. Its reduced potential to undergo redox reactions compared to doxorubicin may explain its reduced cardiotoxicity.[78] It is used in first line therapy for pediatric and adult acute leukemia[79] and second line therapy for breast, prostate cancers and hematological malignancies.[79] Mitoxantrone is also approved for worsening forms of multiple sclerosis since 2000. Mitoxantrone has to be used with caution because of its risks of cardiotoxicity and secondary leukemia in relationship with Top2β poisoning.[94]

Four Top2-targeted drugs are presently in clinical development: F14512, vesaroxin, C-1311 and XK469.[11] F14512 is a demethylepipodophyllotoxin derivative with a spermine side chain (Fig. 3D) targeting cells overexpressing the polyamine transport system (PTS).[11] F14512 also binds Top2cc more persistently than etoposide probably because of the DNA binding of its spermine moiety. Voreloxin is an intercalative quinolone derivative in Phase II–III clinical development in combination with cytarabine for relapsing and refractory acute myeloblastic leukemia.[95] DNA intercalation is important for its activity. C-1311 (Symadex; Fig. 3F) is an iminodazoacridinone derivative with tight DNA interactions both by DNA intercalation and possibly alkylation (reviewed in [11]). The fourth Top2-targeted drug in clinical trials is the quinoxaline R(+)-XK469 (Fig. 3F), which has been reported to target Top2β specifically.[96] However, its mechanism of action is complex with reported inhibition of protein kinases such as MEK, ERK and cdk1 (see [11]).

ICRF-187 (Dexrazoxane) (Fig. 3G) differs from the other Top2-targeted drugs because it acts as a catalytic inhibitor rather than by trapping Top2cc.[1,5,12,97] It is not used as a cytotoxic anticancer agent but as a modulator of anthracyclines' cardiotoxicity[78] and to treat extravasations resulting from intravenous anthracycline injections. Merbarone (Fig. 3G) is another catalytic inhibitor of Top2 useful for cellular and mechanistic studies.[12] Finally, recent studies suggested that sodium salicylate, aspirine's active component can act as catalytic Top2 inhibitor.[98]

7. Top2-targeted antibiotics

Bacterial type IIA topoisomerases (Fig. 1 and Table 1) [for details see [5,9,99,100]] have been the target of antibiotics since the discovery of the antibacterial activity of novobiocin, coumermycin and nalidixic acid in the 1960s. Quinolones target the GyrA subunit of gyrase and the ParC subunit of Topo IV[5] by interfacial inhibition[39–41] (see section 3[6]). Since coumermycin was never developed for clinical use and novobiocin has been withdrawn from the market, there are presently no clinical antibiotic targeting the GyrB subunit of gyrase (Table 1).[5].

Prokaryotic Top2s are excellent targets because: 1) they are essential in all bacteria; 2) cleavage complexes are bactericidal (not just bacteriostatic); 3) their targeting does not affect host human enzymes (their selectivity is at least three order of magnitude higher for prokaryotic over eukaryotic enzymes); 4) the high degree of homology between gyrase and Topo IV[5] enables the targeting of both enzymes and therefore the killing of a broad spectrum of bacteria with a single drug.

Quinolones (Fig. 3H) are totally synthetic and are among the most successful antimicrobials both clinically and economically.[9] The first quinolone was discovered 50 years ago as an impurity in a batch of chloroquine,[101] 14 years before the identification gyrase as its molecular target.[102] Several generations of fluoroquinolones have evolved since the 1960s to extend their activity from Gram-negative urinary infections to Gram-positive bacteria and to a broad range of infections including anaerobic infections and multidrug-resistant tuberculosis (Fig. 3H).[9]

8. Challenges and speculations

This last section is a selected list of questions, challenges and possible answers regarding topoisomerase biology and drug targeting. Topics are treated independently from each other and can be read one at a time. They are also outlined in Table 2.

8.1. Lack of drugs targeting type IA topoisomerases (Table 2 # 1)

All bacteria contain type IA (Topo I and Topo III) along with type IIA topoisomerases (see Table 1). It is generally accepted that bacterial Topo I primary removes hypernegative supercoiling, while Topo III decatenates newly replicated intertwined daughter DNA molecules.[15] A similar division of labor may apply to the two human topoisomerase III. As Top3α as a prevalent "post-replicative DNA hemicatenane resolvase" in association with BLM helicase (see section 2), one might speculate that Top3β is the prevalent "hypernegative DNA supercoiling relaxase". The rationale for targeting bacterial type IA topoisomerases stems from the fact that Topo I trapping by genetic alterations,[103] similar to the trapping of bacterial type IIA by interfacial inhibitor antibiotics, produces rapid bacterial cell death.[104] However, there is presently no clinical drug available to target bacterial type IA topoisomerases and efforts have been limited, primarily spearheaded by Yuk-Chin Tse-Dinh.[105] The query for such a novel class of antibiotics can benefit from the recent crystal structures of a covalent Topo I-DNA intermediate[103] and Topo I and III complexes with single-stranded DNA.[106,107]

It would also be useful to have small molecule inhibitors for eukaryotic type IA enzymes to explore the biology of Top3α and Top3β in human cells (see Table 1) and potentially develop Top3 inhibitors as anticancer agents (Table 2, sections 1–2).

8.2. Selective targeting type IIA topoisomerases: Top2α versus Top2β (Table 2 #2)

The currently used Top2-targeted anticancer drugs are dual inhibitors of Top2α and β.[1,59,90,91] Yet, Top2β rather than Top2α is implicated in the adverse effects of Top2-targeted drugs, namely the therapy-related acute myeloid leukemia (t-AML) resulting from balanced chromosome translocations involving the mixed-lineage leukemia locus (*MLL*) at chromosome 11q23 in one third of t-AML patients following etoposide and mitoxantrone treatments.[93,94,108] A recent study also showed that Top2β poisoning is implicated in the cardiotoxicity of anthracyclines.[83]

In addition to these toxic side effects related to Top2β, the fact that Top2α (*TOP2A*) is highly expressed and sometimes amplified in tumors such as breast and colon cancers along with *ERB2*,[109,110] and that Top2β is acting as a housekeeping gene with broad roles at promoter sites,[108,111] legitimates the discovery and design of drugs selective for Top2α versus Top2β. This conclusion questions the validity of further developing the Top2β-specific inhibitor R(+)XK469 (see Fig. 3F and section 6).[96] The challenge of finding Top2α-specific inhibitors should be relatively easily achievable as both Top2 enzymes are readily available for biochemical and screening assays. Second, rationale drug development could take into consideration the prior knowledge that anthracyclines (Fig. 3C) tend to be Top2α-specific whereas demethylepipodophyllotoxin derivatives (Fig. 3D) are very effective against Top2β. Third, recent drug-enzyme-DNA co-crystal structures could be used to rationalize the chemical design of Top2α-specific drugs.[24,42]

8.3. Targeted drug delivery and therapeutic index enhancement (Table 2 #2)

Topoisomerase inhibitors are effective but suffer from limited tumor selectivity. Their side effects and dose-limiting toxicities are due to the poisoning of normal cells, which, like cancer cells, require topoisomerases for survival and growth, and are often primed for apoptosis.[64] This is especially the case of bone marrow progenitor and rapidly dividing intestinal cells. In fact, only doubling the maximal-tolerated drug dose might be sufficient to markedly improve the therapeutic index of topoisomerase inhibitors. For instance, mice, whose bone marrow tolerate camptothecin better than humans can be cured with camptothecins because they tolerate higher drug exposures. [65] A similarly enhanced therapeutic index might also account for the activity of camptothecins in pediatric tumors because children tend to tolerate relatively higher drug exposures than adults.

Several approaches are being implemented to target topoisomerase inhibitors to tumors while sparing normal tissues. Etirinotean pegol (NKTR-102) couples the active metabolite of irinotecan (SN-38) to polyethylene glycol, limiting the release of SN-38 to normal tissues with tight vasculature, whereas the drug is released into tumors by their intrinsically leaky blood vessels. A comparable approach is to encapsulate topoisomerase inhibitors into nanoparticles, which are preferentially sequestered in tumors with uneven blood flow and taken up by tumor cells. The ultimate approach would be to take advantage of tumor-specific components selectively expressed at the surface of tumor cells. In which case, the nanoparticles could be designed to dock with such cell surface receptors to deliver their drug payload to tumor cells. An original approach is being pursued for the Top2-targeted demethylepipodophyllotxin derivative, F14512 (see section 6). Attaching a polyamine (spermine) via a glycine linked (Fig. 3D) drives the update of the drug to cells overexpressing the polyamine transport system (PTS). This “Trojan horse” approach[11,112] is giving promising results in AML clinical trials where the patients with leukemic cells with enhanced PTS are being identified with a (99m)Tc-HYNIC-spermine scintigraphic probe. [113] Finally, to our knowledge, no attempt is being made to use topoisomerase inhibitors as cytotoxins in antibody-drug conjugates (ADC).[114]. This is probably justified by the fact that topoisomerase-targeted drugs are active at micromolar rather than picomolar concentrations and therefore would require a heavy payload for the ADC approach to work.

8.4. Elucidating and targeting the repair pathways for topoisomerase-induced DNA damage: tyrosyl-DNA-phosphodiesterases (Table 2 #3–5)

Because of the covalent attachment of topoisomerases to one of the cleaved DNA ends (see Figs. 1 & 2), cells need to remove Top1 from the 3’-ends and Top2 from the 5’-ends. Two main mechanisms are used (Fig. 4): precise cleavage of the tyrosyl-DNA bond by phosphodiesterases or endonuclease cleavage and elimination of the DNA strand attached to the topoisomerase.

Tyrosyl-DNA-phosphodiesterase I (TDP1)[115,116] was discovered by Nash and coworkers.[117,118] Although TDP1 is conserved from yeast to humans, it is dispensable for the repair of Top1-mediated DNA damage because parallel pathways mostly represented by endonucleases (Fig. 4) are used to excise the Top1-DNA adducts. This explains why cells are selectively sensitive to TDP1 inactivation when they are also deficient in endonuclease pathways such as XPF/ERCC1 (Rad1/Rad10 in yeast) [119,120] or Mus81/Eme1 (Mus81/Mms4 in yeast) [121] or when they are deficient in cell cycle checkpoints.[122] TDP1 functions in coordination with other repair complexes. Before TDP1 can process the tyrosyl-DNA bond, Top1 needs to be denatured or/and felled by the proteasome.[123–126] TDP1 acts in a complex with XRCC1, PNK, ligase III and PARP.[127] PNK is required to remove the 3’-phosphate left by TDP1 before DNA ligase(s) and polymerase(s) can process the 3’ terminus. Recent studies suggest that the coordinated functions of TDP1 and PARP can account for the potentiating effect of PARP inhibitors in combination with Top1 inhibitors.[120] The discovery of TDP1 inhibitors is ongoing.[116] They would be particularly suited in combination with Top1 inhibitors for patients whose tumors are deficient in the endonuclease pathways (ERCC1-XPF, Mre11, Mus81-Eme1, CtIP).[120] The existence of robust biochemical assays and crystal structures of TDP1 bound to its tyrosyl-DNA substrate[128–130] should facilitate the optimization and discovery of TDP1 inhibitors.

Tyrosyl-DNA-phosphodiesterase 2 (TDP2) was discovered more recently by Caldecott and coworkers after finding that the polypeptide encoded by TTRAP (TRAF and TNF receptor-associated protein), and previously associated with cellular stress responses and inhibition of NFkB activation, was the prevalent cellular 5’-tyrosyl phosphodiesterase responsible for resistance to Top2cc-targeted drugs.[131] TDP2 has also been linked to viral replication.[132–134] Because TDP2 generates 5’-phosphate termini, it is conceivable that

TDP2 functions in coordination with the NHEJ repair pathways including Ku and DNA-PK. The screening of TDP2 inhibitors has just begun, and, as in the case of TDP1 should be facilitated by the recent elucidation of TDP2 crystal structures.[135,136]

It is important to stress that TDP1 and TDP2 are mechanistically and structurally very different despite they both function as monomers, prefer single-stranded DNA substrates,[137,138] and can serve as a backup for each other.[131,137,139,140] TDP1 functions without divalent metal in a two-step reaction involving a transient covalent intermediate between the DNA 3'-end and its catalytic histidine (H263 in humans). The release of TDP1 from the 3'-DNA end requires a second histidine (H493 in humans) whose mutation is the cause of the neurodegenerative disease SCAN1.[141,142] (for scheme see [116]) TDP1 structure shows a pseudo-dimeric fold with a catalytic site formed by the juxtaposition of 2 HKD motifs.[116,129,143] On the other hand, TDP2 belongs to the Ape1, DNase I superfamily and uses Mg^{2+}/Mn^{2+} coordination to hydrolyze 5'-phospho-tyrosyl bonds in one step (without covalent intermediate).[138]

In spite of the detailed knowledge of TDP1 and TDP2 molecular biology, less is known regarding the integration of TDP's in the cellular repair pathways; i.e. which repair components are upstream and downstream of and parallel to their activities. More is presently known for TDP1, which is part of the XRCC1 repair complex with ligase III, PNK and PARP (see above), than for TDP2. Yet, both enzymes appear to function downstream from the proteasome. The relationship between the TDP's and the non-homologous end-joining (NHEJ) and homologous recombination (HR) pathways remains to be clarified. The impact of proteasome inhibitors on the repair of topoisomerase cleavage complexes is also a potentially interesting avenue.[120,126,144–147] Finally, further investigations are needed to elucidate the cellular cofactors and regulators of TDP2.

8.5. Role of poly(ADP-ribose) polymerases (PARP) in the repair of topoisomerase-induced DNA damage and rational for combination therapy (Table 2 #5)

PARP inhibitors are highly synergistic with Top1 inhibitors but not with Top2 inhibitors, which fits with PARP activation by Top1 but not by Top2 inhibitors.[120,148,149] PARP activation by Top1cc is both transcription- and replication-dependent[120] and tightly coupled with TDP1 activity (our unpublished data). One possible model is that conversion of Top1cc into DNA damage by transcription and replication collisions recruits the proteasome, which prepares the DNA ends for processing by the TDP1-PARP complex, in which PARP acts as a cofactor of TDP1 to facilitate its stability and recruitment to the DNA damage sites along with XRCC1, PNK and ligase III. Combination of both PARP and TDP1 inhibitors together with Top1 inhibitors is unlikely to be synergistic because of the overlapping (epistatic) roles of TDP1 and PARP in the repair of Top1cc. On the other hand, either TDP1 or PARP inhibitors are likely to be beneficial in tumors with endonuclease (ERCC1) defects (see Fig. 4 and section 8.4 above).

8.6. Clinical determinants of response to anticancer Top1 and Top2 inhibitors and precision medicine (Table 2 #3–5)

Topoisomerase inhibitors are effective chemotherapies that should only be prescribed to patients who should benefit from the drugs. Otherwise, ineffective regimens delay access to the correct treatment, select for drug resistance and produce costly side effects. Because of the redundant repair pathways involved in the survival of cancer cells targeted by topoisomerase inhibitors, it has been difficult to pinpoint single determinants of response to anticancer topoisomerase inhibitors. Topoisomerases are required,[34,44–46,52–54] yet there is no simple linear correlation between topoisomerase levels and drug response.[60,61] Two main approaches are should define cancer-related defects predicting drug response (or lack of).

First, step-by-step molecular biology analyses of the DNA repair (TDP's, endonucleases, double-strand break repair; see above) and stress response (cell cycle checkpoints, survival and death) pathways should build the cellular response network and identify the most critical parameters that determine the cellular response to topoisomerase inhibitors. The second approach is to use the genomic analyses of tumors as in the TCGA program[a] and cell lines [68,150,151]. For instance, siRNA screening recently identified the protein kinase TAK1[152] and gene expression correlations identified the potential helicase and cell cycle regulator SLFN11 as critical determinants of response to topoisomerase inhibitors.[150,153]

In the future, precision medicine[b] with topoisomerase inhibitors will require the establishment of a genomic (or molecular biology) signature of the tumor that matches the activity of the drugs (Table 2 #4). In parallel, it is critical to set up pharmacodynamic biomarkers to monitor the response of the tumor within a few days after initiating the treatment. Such biomarkers could be related to topoisomerase and DNA damage response.[60,61,72,154] Pursuit of therapy would then be based on quantitative tumor response.

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[a]http://cancergenome.nih.gov/
[b]http://dels.nas.edu/Report/Toward-Precision-Medicine-Building-Knowledge/13284

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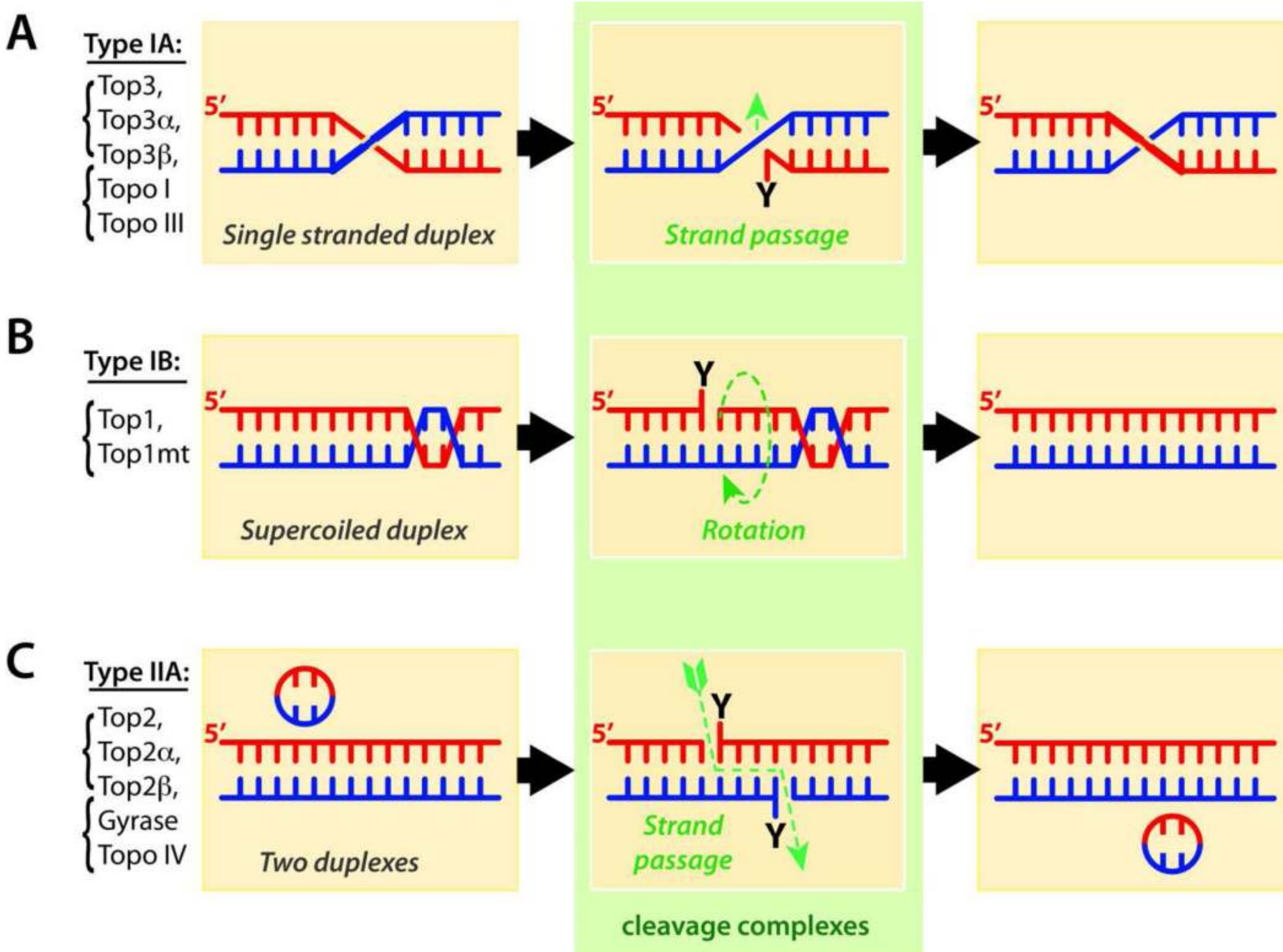

Figure 1.

Differential catalytic mechanisms of topoisomerases. Reactions are represented from left to right. Type I enzymes cleave one strand to process DNA entanglements whereas type II cleave both strands by concerted action of each Top2 monomer (see Table 1). Type IA and IIA enzymes (panels A and C) cleave DNA by covalently attaching their catalytic tyrosine to the DNA 5'-end. Type IA enzymes cleave a single-stranded segment and let another single-strand pass through the break, whereas type IIA let a duplex pass through the concerted breakage of both strands. For both type IA and IIA enzymes, the 3'-ends are tightly bound during strand passage, which keeps the passing DNA in an enzyme cavity before resealing of the ends (not shown; for details see [15,24,25]). By contrast to type IA and IIA enzymes, type IB topoisomerases (panel B) form 3'-phosphotyrosine bonds and relax DNA supercoiling by controlled rotation of the broken 5'-end around the intact strand.[23,155]

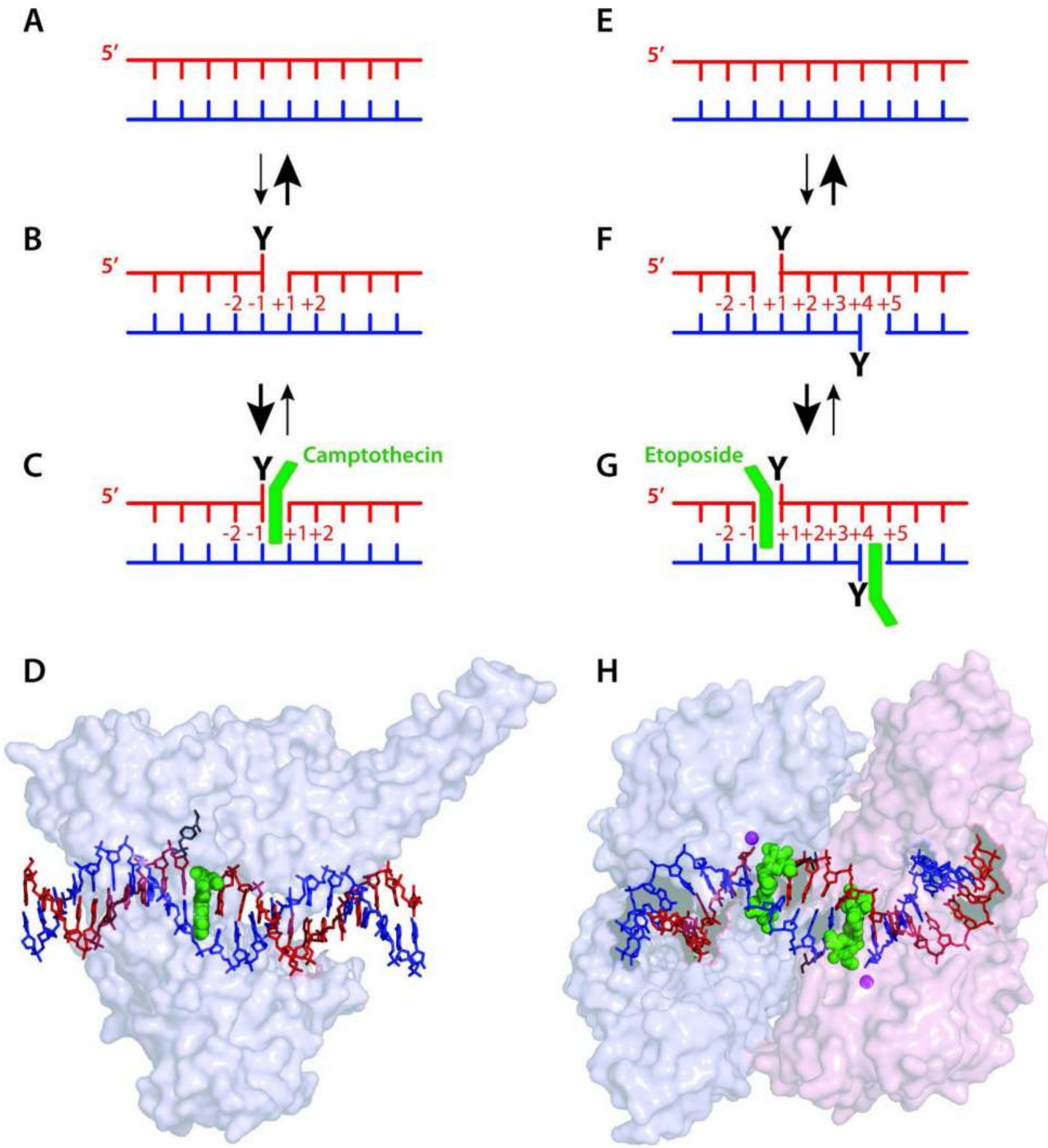

Figure 2.

Interfacial inhibition for Top1 (left) and Top2 inhibitors (right). Under normal conditions, Top1 and Top2 cleave and religate DNA very rapidly (A–B and E–F). Religation is faster than cleavage and cleavage complexes are transient. Drugs (green) (C–D and G–H) bind reversibly (C and G) at the interface of the cleaved DNA and the enzyme by forming a ternary complex (see text for details). The pdb coordinates for D and H are 1T8I and 3QX3, respectively.[6]

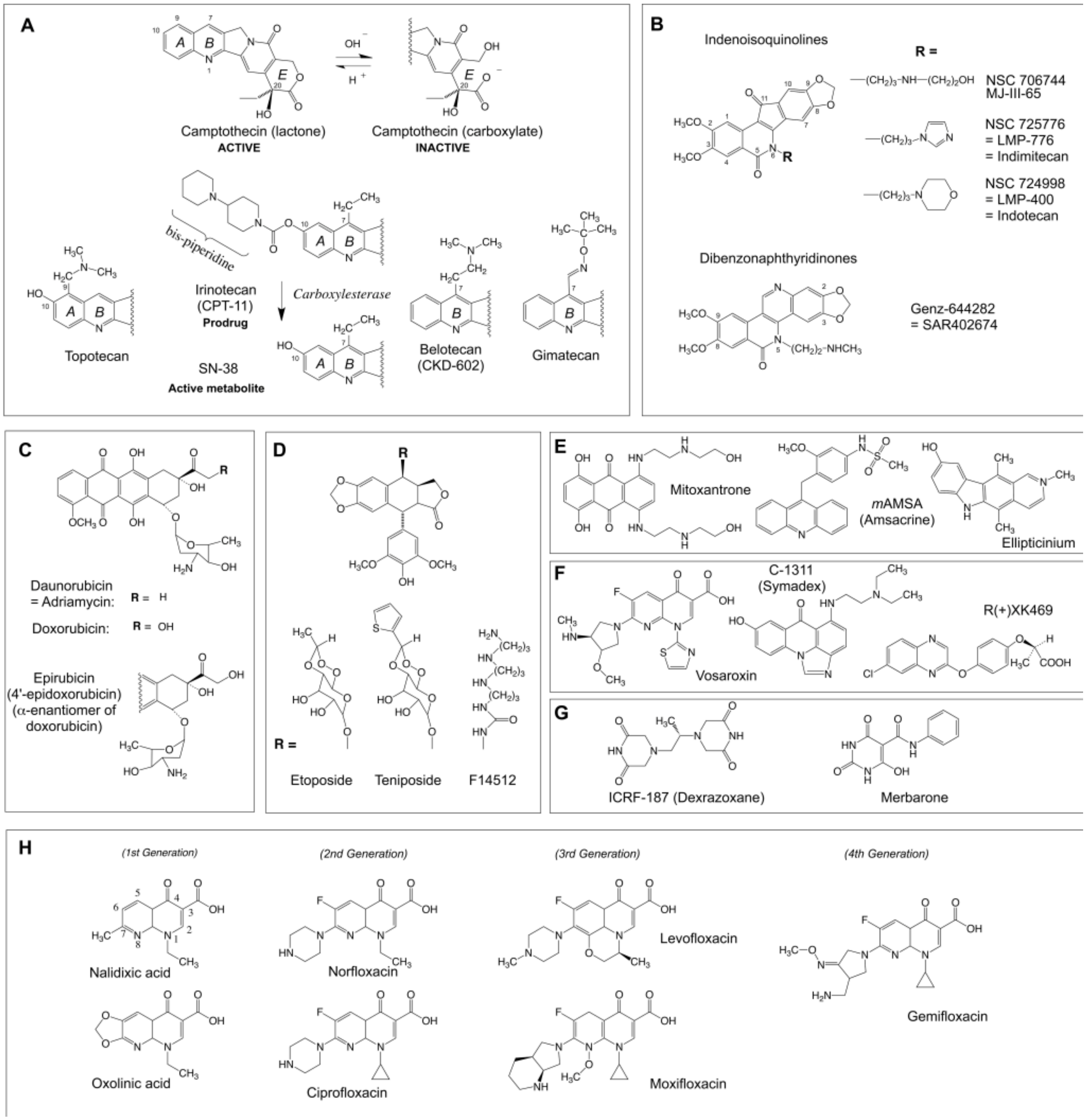

Figure 3.

Structure of anticancer and antibacterial topoisomerase inhibitors. **A**: Camptothecins. **B**: Non-camptothecin Top1 inhibitors in clinical trials. **C**: Anthracyclines. **D**: Demethylepipodophyllotoxin derivatives, including the clinical trial drug F14512 with its spermine side chain. **E**: Other Top2cc-targeted intercalative drugs. **F**: Three Top2cc-targeted drugs in clinical trials in addition to F14512 shown in panel D. **G**: Top2 catalytic inhibitors. **G**: Quinolone antibacterials.

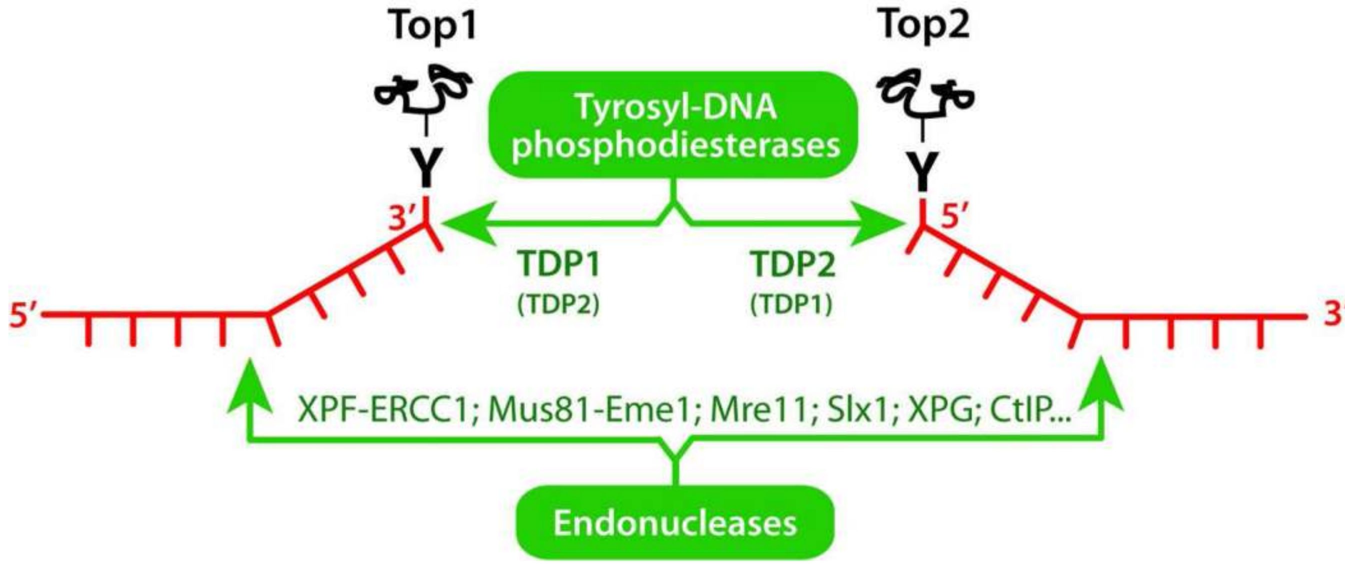

Figure 4.
Schematic representation of the two main repair pathways removing topoisomerase-DNA complexes.

Table 1

Classification of topoisomerases

Type	Polarity	Mechanism	Humans			Bacteria		
			Genes	Proteins	Drugs	Genes	Proteins	Drugs
IA	5'-PY	Strand passage	*TOP3A*	Top3α	none	*TOPA*	Topo I	none
			TOP3B	Top3β	none	*TOPB*	Topo III	none
IB	3'-PY	Rotation	*TOP1*	Top1	anticancer	usually		
			TOP1MT	Top1mt	none (?)	none		
IIA	5'-PY	Strand passage	*TOP2A*	Top2α	anticancer	*GYRA*	Gyrase	Antibiotics
						GYRB		
		ATPase	*TOP2B*	Top2β		*PARC*	Topo IV	
						PARE		

Type I enzymes are monomeric and cleave one strand of DNA for catalysis. Type II enzymes are homodimeric (humans) or heterotetrameric (bacteria), and cleave both strands of duplex DNA with a 5'-four-base overhang (see Fig. 2).

Table 2

Challenges for the discovery and use of topoisomerase inhibitors

Challenges	Possible answers (new approaches)
1. New topoisomerase targets	• Type IA (TopA and Top3) inhibitors • Top2β-specific inhibitors
2. New topoisomerase inhibitors (in addition to #1 above)	• Chemically stable camptothecins • Non-camptothecin Top1 inhibitors • Top1 catalytic inhibitors • New Top2 inhibitors with novel structures • Orally bioavailable inhibitors • Targeted delivery (nanoparticles for time-staggered and tumor-specific delivery)
3. Pharmacodynamic (PD) biomarkers to rapidly evaluate tumor drug response	• Top1 and Top2 cleavage complexes induction • Top1 and Top2 down-regulation • DNA damage (γ-H2AX) • Apoptotic response (caspase activation) • Additional PD biomarkers based on further elucidation of the molecular DNA repair pathways and DNA damage responses (DDR) downstream from topoisomerase poisoning in model systems
4. Cancer patient selection	• Identification and implementation of predictive biomarkers and “drug response signatures” (based on OMIC tools: tumor gene expression and somatic mutations, proteomic and metabolomic) for patient stratification • New predictive biomarkers based on molecular biology and pharmacology studies in model systems • High tumor Top1 and Top2 levels • Pharmacogenomics tests (germline mutations affecting drug pharmacokinetics and metabolism)
5. Optimize drug combinations	• Based on the further elucidation of the molecular DNA repair pathways and DNA damage responses (DDR) downstream from topoisomerase poisoning in model systems • Based on synthetic lethality related to tumor-specific defects (ERCC1-deficiency for combining Top1 and PARP inhibitors) • Based on system pharmacology in model systems to reveal the pathways (molecular networks) and novel genetic and molecular determinants that drive tumor response • Based on experimental data obtained in model systems • Based on non-overlapping drug toxicities and side effects (current approach) • Based on clinical trials (current empirical approach)