Review

Clinical Cancer Research

Targeting Topoisomerase I in the Era of Precision Medicine

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Abstract

Irinotecan and topotecan have been widely used as anticancer drugs for the past 20 years. Because of their selectivity as topoisomerase I (TOP1) inhibitors that trap TOP1 cleavage complexes, camptothecins are also widely used to elucidate the DNA repair pathways associated with DNA–protein cross-links and replication stress. This review summarizes the basic molecular mechanisms of action of TOP1 inhibitors, their current use, and limitations as anticancer agents. We introduce new therapeutic strategies based on novel TOP1 inhibitor chemical scaffolds including the indenoisoquinolines LMP400 (indotecan), LMP776 (indimitecan), and LMP744, and on tumor-targeted delivery TOP1 inhibitors using liposome, PEGylation, and antibody–drug conjugates. We also address how tumor-specific determinants such as homologous recombination defects (HRD and BRCAness) and Schlafen 11 (SLFN11) expression can be used to guide clinical application of TOP1 inhibitors in combination with DNA damage response inhibitors including PARP, ATR, CHEK1, and ATM inhibitors.

Introduction

Humans encodes six topoisomerases, TOP1, TOP1MT, TOP2α, TOP2β, TOP3α, and TOP3β (1) to pack and unpack the approximately 2 meters of DNA that needs to be contained in the nucleus whose diameter (6 μm) is approximately 3 million times smaller. Moreover, the genome is organized in chromosome loops and the separation of the two strands of DNA during transcription and replication generate torsional stress and supercoils that are resolved by topoisomerases.

While TOP1, like all six human topoisomerases removes DNA negative supercoiling (underwinding), only TOP2α and TOP2β resolve DNA knots and intertwined DNA circles (decatenation) as they cleave both DNA strands. While TOP3α resolves hemicatenate and double-Holiday junctions, only TOP3β acts as RNA topoisomerase (1). In all cases, topoisomerases change the topological state of nucleic acids by forming topoisomerase cleavage complexes (TOPCC) that enable an intact DNA or RNA to pass through the topoisomerase-linked breaks made in the DNA (or RNA for TOP3β). The normal activity of topoisomerases relies on the fact that, following topoisomerization, TOPCCs reverse rapidly by the religation of the broken DNA or RNA, which releases the topoisomerases. TOP1 is essential in vertebrates where it is required for genomic stability and for removing both positive and negative DNA supercoils that otherwise lead to the formation of alternate DNA structures such as plectonemes, guanosine quartets, R-loops, and DNA breaks (reviewed in ref. 1).

Anticancer TOP1 Inhibitors Trap TOP1CCs as Interfacial Inhibitors

The plant alkaloid camptothecin and its clinical derivatives, topotecan and irinotecan (Fig. 1A, right) target TOP1CCs by binding at the interface of TOP1CCs (Fig. 1B). They do not bind DNA without TOP1 or TOP1 without DNA, and the binding is stereospecific for the natural camptothecin 20-S isomer (Fig. 1B). Cocrystal studies (ref. 2; Fig. 1B) showed that TOP1CCs are trapped by the reversible binding of a single camptothecin molecule resulting from: (i) stacking of the polycyclic ring scaffold of the drug against the base pairs flanking the DNA nick made by TOP1, and (ii) a network of hydrogen bonds between camptothecin and Asn722, Arg364, and Asp533 of TOP1. Hence camptothecins block the religation of TOP1CCs as archetypal interfacial inhibitors (3). The non-camptothecin indenoisoquinolines in clinical development (Fig. 1A, left; see below) also act by binding at the TOP1–DNA interface (Fig. 1B) and trapping TOP1CCs (4, 5).

Determinants of Response and Pharmacogenomic Signature for TOP1 Inhibitors: TOP1, Replication, HRD, ATR, PARP, and SLFN11

Consistent with the trapping mechanism, TOP1 (Fig. 1B) is required for cell killing by camptothecins with total resistance in Top1-knockout yeast and increased sensitivity by overexpression of TOP1 (6, 7). Moreover, supporting the selective targeting of TOP1 by camptothecins, mutations of TOP1 confer resistance to camptothecins in cancer cells (8). Yet, TOP1CC levels are not sufficient for cellular response (9), and replication fork collisions are a major determinant of cell killing (Fig. 1C; ref. 1). In the absence of replication (and transcription), trapped TOP1CCs

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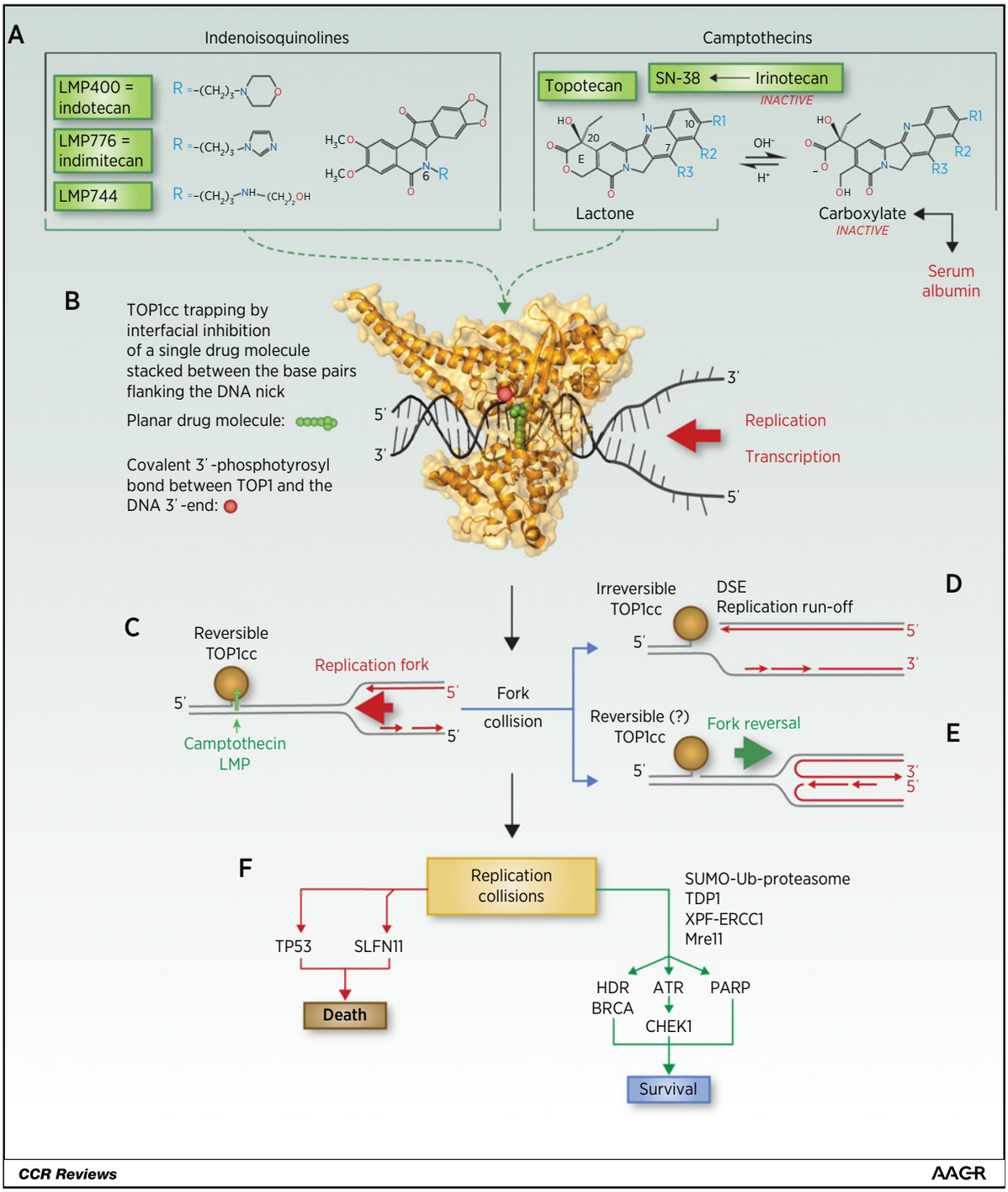

Figure 1.

Outline of the molecular pharmacology and response determinants of clinical TOP1 inhibitors. **A,** Chemical structures of the camptothecin derivatives used in the clinic. R1, R2, and R3 refer to the positions of substitutions that confer water solubility to irinotecan and topotecan. Camptothecins are active in lactone form and are readily inactivated at physiologic pH in the blood and tissues by E-ring hydrolysis to their ring-open carboxylate form (top right), which is sequestered by serum albumin (right). The clinical indenoisoquinoline derivatives, LMP400, LMP776, and LMP744 (left). **B,** Both the camptothecins and indenoisoquinolines trap TOP1CCs by binding at the enzyme–DNA interface. **C,** Replication damage induced by TOP1 inhibitors. **D,** Collision of a replication fork with a TOP1CC on the leading strand for DNA synthesis generates a single-ended DNA double-strand break (DSE: double-stranded end) by replication run-off. **E,** Alternatively, the colliding fork can be remodeled by replication fork reversal (promoted by HLTF, ZRANB3, SMARCL1, RAD51, and PCNA polyubiquitylation) which may convert the TOP1CC to a potentially reversible configuration. Fork restart is promoted by the helicase RecQ1 and the MCM10 replication helicase. PARylation of RecQ1 prevents its activity and thereby keep forks in the reversed configuration. (*Continued on the following page.*)

exhibit limited cytotoxicity as transient TOP1CCs do not damage DNA by themselves. However, upon replication fork collisions, TOP1CCs are converted into irreversible TOP1CCs by replication run-off (Fig. 1D). Fork collisions can also produce fork reversal, which may allow the religation of the replication-trapped TOP1CCs (Fig. 1E). Thus, highly replicative cells are most sensitive to TOP1 inhibitors while quiescent cells are resistant to the drugs.

Studies published in 1988 and predating the FDA approval of camptothecins and the observations of synthetic lethality of PARP inhibitors for homologous recombination deficient (HRD) tumors (10, 11) showed that yeast, which is intrinsically resistant to camptothecins becomes highly sensitive upon inactivation of Rad52, a key component of homologous recombination (HR; refs. 6, 7). Thus, camptothecins were the first drugs identified as synthetic lethal with HRD almost 20 years before the connection was made for PARP inhibitors. The synthetic lethality of TOP1 inhibition in HRD tumors (Fig. 1F, right) remains to be translated clinically.

Trapped TOP1CCs are rapidly SUMOylated ubiquitylated and degraded by the proteasome (12–14). TOP1 degradation is required for the DNA repair enzyme, tyrosyl DNA phosphodiesterase 1 (TDP1) to access and hydrolyze the DNA–protein crosslink between TOP1 and DNA (see Fig. 1B; ref. 1). The importance of TDP1 and existence of endogenous TOP1CCs is exemplified by the severe neurological defects (SCAN1) of patients with TDP1 mutation (15). Alternative TOP1CC repair pathways involve the excision of the DNA segment covalently attached to the TOP1CC by the endonucleases XPF or Mre11 (refs. 1, 16, 17; Fig. 1F, right).

PARP1 and ATR are both critical for drug resistance (Fig. 1F, bottom right). PARP1 limits the toxicity of TOP1CCs by: (i) recruiting TDP1 and enhancing the excition/repair of TOP1CCs (18); (ii) PARylating TOP1, which regulates its nuclear distribution (19) and reverses TOP1CCs (20); and (iii) promoting replication fork reversal (21), which may allow the religation of TOP1CCs (Fig. 1E). ATR and its downstream kinase CHEK1 limit the cytotoxicity of TOP1 inhibitors by transiently arresting replication forks, limiting collisions between replication forks and TOP1CCs (see Fig. 1C) and allowing the repair of broken replication forks (22, 23). This explains the synergy between TOP1 inhibitors and ATR, CHEK1, and PARP inhibitors (22–24).

In addition to repairing DNA damage, cancer cells are programmed for cell death (Fig. 1F, left). This is well-established for TP53 (p53), which drives apoptosis and is genomically inactivated in approximately 50% of cancers. Yet, TP53 is not a reliable determinant of response to TOP1 inhibitors in nonisogenic cancer cells, which is in contrast with the newly identified executioner of cells with replicative damage, Schlafen 11 (SLFN11). SLFN11 was discovered by genome-wide analyses as a dominant response determinant to camptothecins across the NCI-60 panel (refs. 25, 26; http://discover.nci.nih.gov/cellminercdb).

Lack of SLFN11 expression confers high resistance to TOP1, as well as TOP2 inhibitors, PARP inhibitors, platinums, hydroxyurea, and gemcitabine (25, 27). SLFN11 acts independently of ATR and HR by irreversibly blocking replication and HDR (28, 29).

Because response and resistance determinants to TOP1 inhibitors are multifactorial in preclinical models, it is likely that pharmacogenomic signatures will have to be implemented to improve the clinical use of TOP1 inhibitors. Translational signature determinants are beginning to be identified. They include SLFN11, BRCAness/HRD, and ABCG2. Additional factors remain to be identified, and cancer cell line databases and synthetic lethality screens with TOP1 inhibitors are approaches to achieve this goal (26).

Approved TOP1 Inhibitors and Their Limitations

The first camptothecin clinical trial was conducted in the early 1970's (ref. 30; see Fig. 1A, right). In spite of objective responses, clinical trials were not pursued. Fifteen years later, the discovery of TOP1 as the target of camptothecins (31) brought water-soluble camptothecin derivatives back to the clinic, leading to the FDA approval of irinotecan and topotecan in 1996 (Table 1).

Irinotecan is a prodrug. It needs to be converted by carboxylesterases into its active metabolite, SN-38 (Fig. 1A). The pharmacokinetics of irinotecan and SN-38 depend on a pH-dependent equilibrium between the active lactone and inactive carboxylate forms (Fig. 1A; ref. 32). The plasma area under the concentration versus time curve (AUC) of SN-38 is 2%–8% of irinotecan, and SN-38 is 95% bound to plasma proteins (33). SN-38 levels peak at the end of infusion with a mean terminal half-life of approximately 10–20 hours. SN-38 is cleared via glucuronidation (SN-38G) and biliary excretion. A host of transporters are involved in its metabolic transformation, active transport, intestinal absorption, and hepatobiliary secretion (32, 34). Interindividual variability in pharmacogenomics results in marked heterogeneities in efficacy and toxicity of irinotecan.

The dose-limiting toxicities of irinotecan are myelosuppression and diarrhea, with an incidence of about 15%–20%. Early diarrhea within hours of administration is related to a cholinergic surge from inhibition of acetylcholinesterase. Late diarrhea occurring after 24 hours is unpredictable and can be severe or life threatening in 23%–31% patients (33). Direct mucosal cytotoxicity from free intestinal luminal SN-38 or SN-38G deconjugation (by bacterial β-glucuronidase back to SN-38) underlies the late diarrhea. SN-38–induced apoptosis and hypoproliferation in the intestines causes colonic damage with changes in goblet cells and mucin secretion. Individuals who are homozygous for the UGT1A1*28 allele (10% of North Americans; UGT1A1 7/7 genotype) are also at increased risk for neutropenia (35).

Topotecan also undergoes reversible hydrolysis to the open-ring inactive carboxylate form (Fig. 1A), which predominates at physiologic pH (36). Topotecan's terminal half-life is only 2–3 hours, which limits its efficacy because sustained drug exposure is needed to maintain the TOP1CCs until replication/transcription collisions lead to cell death (Fig. 1B and C). The

(*Continued.*) **F,** Collisions of transcription and replication with trapped TOP1CCs induce the degradation of TOP1 by the ubiquitin proteasome pathway and engage the chromatin response by phosphorylation of histone H2AX (γH2AX). TOP1CCs are excised by TDP1 (tyrosyl DNA phosphodiesterase) and the endonuclease XPF-ERCC1. The primary cytotoxic lesions in cancer cells result from collisions between the trapped TOP1CCs and replication forks. These collisions are repaired by HDR (homology directed repair) and activating ATR (Ataxia Telangiectasia related) and CHK1 kinases, as well as PARP. Replication collisions also activate the cell death pathways by engaging p53 (TP53) and Schlafen 11 (SLFN11).

Table 1. Clinical indications, major toxicities, and clinical pharmacology of the FDA-approved camptothecins

Compound	Tumor type	Clinical indication	Major toxicities	Metabolism	Elimination
Irinotecan (Camptosar)	Metastatic colorectal cancer	First-line in combination with 5-fluorouracil and leucovorin Recurrent disease or progression following initial fluorouracil-based therapy	Nausea, vomiting, diarrhea, myelosuppression	Prodrug that requires enzymatic cleavage of the C-10 side chain by an irinotecan carboxylesterase–converting enzyme to generate the active metabolite SN-38. Can also undergo hepatic oxidation of its dipiperidino side chain to form the inactive metabolite 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin.	About 16% (range, 11.1%–20.9%) excreted unchanged in urine. SN-38 is glucuronidated, and both the conjugated and unconjugated forms are excreted in the bile.
Topotecan (Hycamtin)	Metastatic ovarian cancer	Recurrent disease	Myelosuppression	Nonenzymatic hydrolysis of the lactone ring generates the less active open-ring hydroxy carboxylic acid. N-desmethyl is a minor metabolite.	About 26%–41% excreted unchanged in urine over 24 hours. Concentrated in the bile at levels that are 1.5 times higher than the simultaneous plasma levels.
	SCLC	Sensitive disease after failure of first-line chemotherapy			
	Cervical carcinoma	Combination with cisplatin for stage IVB, recurrent, or persistent disease not amenable to curative treatment with surgery and/or radiation			
Irinotecan liposome (Onivyde)	Pancreatic adenocarcinoma	In combination with fluorouracil and leucovorin after disease progression following gemcitabine-based therapy	Diarrhea Myelosuppression	The metabolism of irinotecan liposome has not been evaluated. Irinotecan is metabolized as above.	The elimination of irinotecan liposome has not been evaluated. Irinotecan is eliminated as above.

dose-limiting toxicity of topotecan is myelosuppression with potentially severe neutropenia and thrombocytopenia occurring in approximately 80% and 30% of patients, respectively.

TOP1 Inhibitors in Clinical Development

With the goal of mitigating the shortcomings of camptothecins and their derivatives, several camptothecin analogues derived from modifications to the parent drug are in clinical development. Belotecan hydrochloride is a water-soluble camptothecin analogue and gimatecan is a lipophilic oral camptothecin analogue. However, clinical results do not indicate a substantial benefit of these agents compared with approved camptothecin analogues.

The NCI in collaboration with Purdue University (West Lafayette, IN) developed the indenoisoquinolines to overcome the limitations of camptothecins (ref. 4; Supplementary Table S1) including chemical instability, lability of the TOP1CCs that reverse within minutes upon camptothecin withdrawal (37), active efflux by the ABCG2 (MRP) and ABCB1 (P-glycoprotein) ABC transporters (38, 39), short plasma half-life, and severe diarrhea. Three indenoisoquinolines are in clinical development (Fig. 1A, left): LMP400 (indotecan), LMP776 (indimitecan), and LMP744 (40, 41).

TOP1 Inhibitors as Payloads for Tumor-targeted Delivery

A growing array of tumor-targeted drug delivery strategies are in clinical development including liposomal or nanoparticle formulations and coupling to mAbs (Table 2). Encapsulating camptothecins in a protective environment until they are released in the tumor can overcome the chemical inactivation of camptothecin lactone in the serum, their rapid blood clearance and dose-limiting bone marrow toxicity. Compared with more toxic payloads (such as the highly toxic DNA cross-linking agents or microtubule poisons), the camptothecins allow sufficient tumor delivery while keeping normal tissue toxicity manageable.

Camptothecin derivatives for liposome and nanoparticle delivery

Liposomes and nanoparticles (polymeric micelles, polymeric nanoparticles, and liposomes) provide a physical approach to targeted delivery by preferential accumulation in the tumor owing to pressure created by limited lymphatic drainage and increased permeability of blood vessels–a process termed enhanced permeability and retention (EPR; Table 2). In addition, these formulations protect the drug from degradation, reduce renal clearance, and potentially allow sustained release in the tumor.

Nanoliposomal irinotecan (MM-398, Onivyde) was approved for pancreatic cancers in 2015 (42). The liposome is designed to keep irinotecan in circulation while increasing and prolonging intratumoral drug levels. Compared with free irinotecan, MM-398 exhibits lower C_{max}, longer half-life, higher AUC, smaller volume of distribution, and slower plasma clearance for the released SN-38 (43). In preclinical models, MM-398 administered at doses 5-fold lower than irinotecan achieved similar intratumoral exposure with better antitumor activity (42). Despite the pharmacokinetic benefits and delivery advantage, diarrhea occurs frequently and is severe or life-threatening in 20% of the cases (44). This is

Table 2. Camptothecins as warheads for targeted delivery

Name	Active derivative (payload)	Formulation (target)	Company
Onivyde (MM398)	Irinotecan	Liposome	Ipsen
NLG207 (CRLX101)	Camptothecin	Cyclodextrin-PEG	Newlink
NKTR-102	Etirinotecan	PEG	Nektar
PLX038	SN-38	PEG	ProLynx
Sacituzumab govitecan (IMMU-132)	SN-38	ADC (TROP2)	Immunomedics
Labetuzumab govitecan (IMMU-130)	SN-38	ADC (CEACAM5)	Immunomedics
IMMU-140	SN-38	ADC (HLA-DR)	Immunomedics
Trastuzumab deruxtecan (DS-8201)	DXd	ADC (HER2)	Daiichi Sankyo
Patritumab deruxtecan (U3-1402)	DXd	ADC (HER3)	Daiichi Sankyo
DS-1062	DXd	ADC (TROP2)	Daiichi Sankyo
PEN-866	SN-38	Hsp90-drug conjugate	Tarveda
NK012	SN-38	PEG-polyglutamate	Nippon Kayaku

likely related to the hepatic accumulation of liposomes and biliary release of SN-38-glucuronide.

Although the EPR improves tumoral delivery of nanoparticles, it has been reported to be $\leq$2-fold compared with normal organs (45), and the extent and variability of EPR in tumors is not well-established (46). Nanoparticle delivery efficiency is also influenced by a number of barriers including the mononuclear phagocytic system of the liver, spleen, and other organs, which identify nanoparticles as foreign substances that need to be sequestered, degraded, and eliminated, as well as renal clearance that competes with tumor delivery (47). In animals, the nanoparticle delivery efficiency, that is, the percentage of the injected dose of nanoparticles that reach the tumor is <1% (47). Strategies to improve drug delivery profiles include the use of ligands or targeting moieties to drive nanoparticles to tumors.

Camptothecin derivatives as warhead for antibody–drug conjugates

Antibody–drug conjugates (ADC) use mAbs to target tumor cells expressing specific surface antigens to deliver cytotoxic payloads (48). The four FDA-approved ADCs and most others in development use highly cytotoxic payloads targeting tubulin or cross-linking DNA. Camptothecins being less toxic payloads (see above) are increasingly used to enhance both the therapeutic index and tumor delivery (49). Sacituzumab govitecan (IMMU-132) and trastuzumab deruxtecan (DS-8201) are the two most advanced camptothecin-based ADCs (Table 2) with promising activity.

IMMU-132 consists of SN-38 coupled through a linker to the humanized antitrophoblast cell-surface antigen 2 (TROP2) mAb with a drug to antibody ratio 7.6. TROP2 (encoded by *TACSTD2*) is a transmembrane glycoprotein overexpressed in many epithelial cancers. In the low pH environment of lysosomes and tumors, the linker is cleaved, allowing slow release of SN38. In a phase I trial, the toxicity was manageable. A dose of 10 mg/kg on days 1 and 8 of 21-day treatment cycles was selected for further expansion (50). In a phase I/II trial of third-line or higher line of therapy for metastatic triple-negative breast cancer (TNBC), IMMU-132 produced durable objective responses (51). Among 108 patients, the response rate was 34.3% and the median duration of response was 9.1 months. Neutropenia and diarrhea were less severe and manageable with routine supportive care than with irinotecan. Unlike irinotecan which clears very rapidly from the serum and is poorly converted to SN38, IMMU-132 is cleared with a halflife of approximately 11–14 hours with most SN38 in the serum (>95%) bound to IgG (50). In addition, studies in human tumor xenografts indicate advantageous tumor-targeted drug delivery. IMMU-132 delivered 20- to 136-times more SN-38 to tumors than irinotecan with tumor-to-serum AUC ratio 20- to 40-times higher than with irinotecan (52). Antitumor activity has been observed in patients with platinum-resistant urothelial carcinoma, non–small cell lung cancers, and small-cell lung cancers (SCLC; refs. 53, 54).

DS-8201 consists of camptothecin derivative deruxtecan mesylate (DX-8951f) coupled to a humanized anti-HER2 antibody by an enzymatically cleavable peptide linker with a drug to antibody ratio of 8 (55). In preclinical studies, DS-8201 was effective even in tumors with low HER2 expression and tumors that were resistant to ado-trastuzumab-emtansine (T-DM1), a tubulin inhibitor–based ADC. In a phase I study, no dose-limiting toxicities were observed and the MTD was not reached (56). Consistent with preclinical observations, tumor responses were seen in patients with prior T-DM1 and in low HER2-expressing tumors. DS-8201 is being evaluated in patients with HER2-positive, unresectable, and/or metastatic breast cancer who are resistant or refractory to T-DM1.

Both IMMU-132 and DS-8201 have received FDA breakthrough designations for TNBC and HER2 positive (Table 2). The durable responses suggest that camptothecin payloads have a higher tolerability allowing for higher doses than the more toxic FDA-approved ADCs. The lower frequency of severe adverse events compared with irinotecan in both the ADCs could be attributed to the delivery of the camptothecins in their active, non-glucuronidated form, as IgG-bound SN-38 is protected from glucuronidation (50, 52).

Additional camptothecin-derived ADC are being developed against HER3 (ERBB3), TROP2 (TACSTD2) and carcinoembryonic antigen (CEA) (Table 2).

Combinations of TOP1 Inhibitors with DNA Damage Response Inhibitors and Approaches for Combinations

Combinations with PARP inhibitors are highly effective in cell line and tumor models with and without HRD (24, 57–59). Preclinical data show PARP catalytic inhibition rather than PARP trapping is sufficient for this synergy (24). Despite promising preclinical data, PARP inhibitor combinations have proven challenging in clinic (Table 3). Dose-limiting myelosuppression has severely limited the ability to dose escalate both PARP inhibitor and chemotherapy in several clinical studies (40, 60–65). For example, the PARP inhibitor, veliparib, in combination with topotecan was found highly

Table 3. Dose levels of TOP1 and PARP inhibitors achieved in combination in clinical trials

Combination		MTD	TOP1i % of MTD	PARPi % of MTD	DLT	References
Irinotecan	Olaparib	Irinotecan 200 mg/m^2; q3w olaparib 50 mg qd d1–21	57%	6%	Diarrhea, myelosuppression	63
		Irinotecan 125 mg/m^2 q2w olaparib 50 mg bid d1–5	≈69%	≈12%	Anorexia/fatigue	
Irinotecan	Veliparib	Irinotecan 100 mg/m^2 q1, 8; q3w veliparib 40 bid d1–14	≈80	≈10%	Diarrhea, fatigue, myelosuppression	61
Topotecan	Olaparib	Topotecan 1 mg/m^2/d1–3; q3w olaparib 100 mg bid d1–21	≈40%	25%	Myelosuppression	62
Topotecan	Veliparib	Topotecan 0.6 mg/m^2/d1–5; q3w veliparib 10 bid d1–5	40%	≈3%	Myelosuppression	40
Topotecan	Veliparib	Topotecan 3 mg/m^2 d2, 9, 16; q4w veliparib 300 mg bid d1–3, 8–10, 15–17	≈75%	≈75%	Myelosuppression	60

Abbreviations: DLT, Dose-limiting toxicity; PARPi, PARP inhibitors; TOP1i, TOP1 inhibitors.

myelosuppressive, requiring dose reductions for both agents (40) with the MTD of veliparib and topotecan only 3% and 40% of the respective single-agent MTDs.

With the growing availability of potent and specific DNA damage response (DDR) inhibitors (such as ATM, ATR, WEE1, DNA-PK, and others), pharmacologic inhibition of DDR in patients is an area of intense study. Strategies to enhance antitumor efficacy with DDR inhibitor–TOP1 inhibitor combinations while mitigating the unacceptable normal tissue toxicities are imperative. One approach involves an innovative "gapped-schedule" that incorporates tumor-targeted DNA-damaging chemotherapy delivery and dose scheduling of DDR inhibitors, that is, sequential intermittent dosing as opposed to continuous dosing. In this approach (Fig. 2), the tumor-targeted TOP1 inhibitor is administered first, followed after a 2–3-day gap by the DDR inhibitor. The gapped-schedule ensures that when the DDR inhibitor is introduced, the tumor remains loaded while normal tissue including bone marrow is cleared of the TOP1 inhibitor (Fig. 2). Supporting this concept is preclinical data showing differential effects on DNA damage in tumor versus the bone marrow using targeted DNA-damaging chemotherapy and two ongoing trials to test this concept in clinic (ClinicalTrials.gov Identifier: NCT02769962 and NCT02631733).

Combinations with Immunotherapy

Both innate and adaptive immune responses induced by TOP1 inhibitors are emerging as potential mechanisms to increase the antitumor efficacy of immunotherapies. TOP1 inhibitors augment antigen production in melanoma cells (66) and upregulate the expression of MHC class I and IFNβ in breast cancer cells (67). Overexpression of these antigens enhances recognition of tumor cells by T cells and T cell–mediated cytotoxicity (67, 68). Accordingly, greater tumor control was achieved with MM-398 in combination with anti-PD1/-L1 antibodies in immunocompetent mouse melanoma models (68). In a syngeneic TNBC model, topotecan was shown to activate the stimulator of interferon genes (STING)-controlled innate immune pathway and CD8$^+$ T-cell activation. Notably, the antitumor effects were decreased in mice lacking STING (69).

Tumor-targeted delivery of TOP1 inhibitors may represent an opportunity to capitalize the favorable immunomodulatory effects of TOP1 inhibitors, increased genomic DNA damage, antigen presentation, and inflammatory responses, with less toxicity. A recent study showed that DS-8201 is particularly effective in eliciting antitumor immunity in immunocompetent mouse models with human HER2–expressing cancer cells (70).

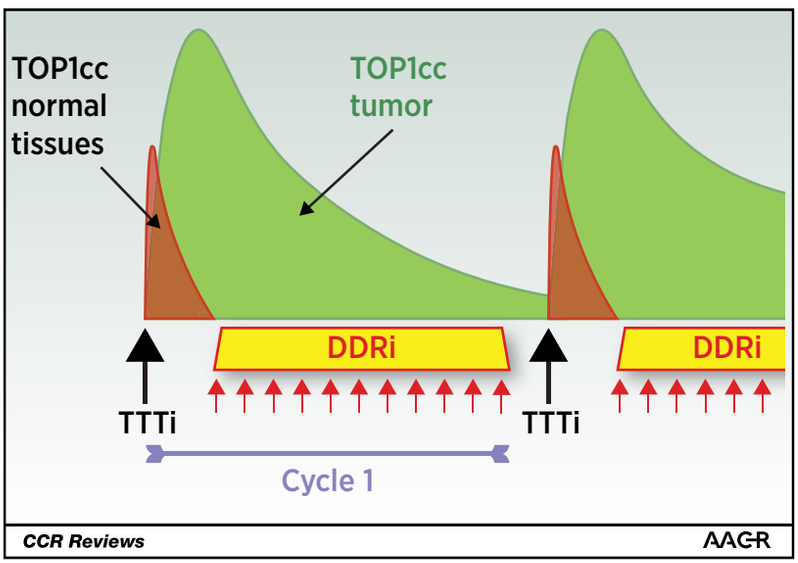

Figure 2.
Rationale for gap-scheduling combination therapies with tumor-targeted TOP1 inhibitors (TTTi) and DNA damage response inhibitors (DDRi; such as PARPi, ATRi or ATMi etc). The TTTi given on day 1 of each cycle initially produces TOP1cc both in normal and tumor tissues (brown area). After a 2–3 day gap, the TTTi is selectively retained in tumor tissues (green area). Treatment with the DDRi is then initiated (red arrows) while TOP1cc are present in the tumor tissues but not in normal tissues. The DDRi is stopped 1–2 days before the next cycle. Such "gap-schedule" avoids overlapping toxicity for normal tissues.

This effect was primarily dependent on the payload, in this case a camptothecin derivative, delivered into HER2-expressing tumors by the ADC. The antitumor effect was accompanied by increased expression of MHC class I in tumor cells, increased expression of dendritic cell activation markers, and increase of tumor infiltrating $CD8^+$ T cells (70).

Conclusions and Future Directions

TOP1 inhibitors are targeted therapies. Like PARP inhibitors, they are synthetic lethal with HRD. Understanding and overcoming the limitations of camptothecins has led to the development of the indenoisoquinolines. Precision therapeutics with TOP1 inhibitors may be achieved by converging approaches: (i) implementing molecular determinants of tumor response, including expression of TOP1 and TDP1, BRCAness and HR, as well as SLFN11; (ii) targeted delivery with tumor-specific antibodies; (iii) improving the warhead such as in the case of the indenoisoquinolines; and (iv) rational and tolerable combination therapies based on mechanistic molecular preclinical models and novel drug delivery schedules.

Disclosure of Potential Conflicts of Interest

Y. Pommier is an inventor of NIH patents LMP400, LMP744, and LMP776. A. Thomas and Y. Pommier report grants from AstraZeneca, Tarveda, Newlink, and Gibson to the National Cancer Institute (CRADA) for conduct of basic and clinical research studies. No other potential conflicts of interest were disclosed.

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