The camptothecin derivatives topoisomerase I (TOP1) inhibitors, irinotecan and topotecan, are FDA approved for the treatment of colorectal, ovarian, lung and breast cancers. Because of the chemical instability of camptothecins, short plasma half-life, drug efflux by the multidrug-resistance ABC transporters, and the severe diarrhea produced by irinotecan, indenoisoquinoline TOP1 inhibitors (LMP400, LMP776, and LMP744), which overcome these limitations, have been developed and are in clinical development. Further modifications of the indenoisoquinolines led to the fluoroidenoisoquinolines, one of which, LMP517, is the focus of this study. LMP517 showed better antitumor activity than its parent compound LMP744 against H82 (small cell lung cancer) xenografts. Genetic analyses in DT40 cells showed a dual TOP1 and TOP2 signature with selectivity of LMP517 for DNA repair-deficient tyrosyl DNA phosphodiesterase 2 (TDP2)- and Ku70-knockout cells. RADAR assays revealed that LMP517, and to a lesser extent LMP744, induce TOP2 cleavage complexes (TOP2cc) in addition to TOP1ccs. Histone γH2AX detection showed that, unlike classical TOP1 inhibitors, LMP517 targets cells independently of their position in the cell cycle. Our study establishes LMP517 as a dual TOP1 and TOP2 inhibitor with therapeutic potential.

**Introduction**

Topoisomerases play critical roles in genome organization and stability. They remove supercoils and DNA intertwining induced by essential DNA processes including replication, transcription, and chromatin remodeling (1–4). Topoisomerase I (TOP1) induces single-strand breaks, allowing the DNA to rotate on itself, while topoisomerases II (TOP2α and TOP2β) induce DNA double-strand breaks (DSB), allowing a DNA helix to pass through another. Both classes of enzyme act by forming transient catalytic intermediates called topoisomerase cleavage complexes (TOPcc). Rejoining of the DNA strand(s) restores DNA integrity. Unresolved TOPccs lead to protein-linked DNA single- or double-strand breaks that are lethal as cells undergo replication or transcription (2). For this reason, TOP1 and TOP2 inhibitors are widely used for cancer treatment. TOP1ccs are targeted by the clinical camptothecin (CPT) derivatives topotecan and irinotecan, and CPT derivatives are the only chemical class of TOP1 inhibitor approved by the FDA (5). TOP2ccs are targeted by etoposide, doxorubicin (as well as other anthracycline derivatives), and mitoxantrone (4, 6). Although TOP1 and TOP2 inhibitors are highly specific for their respective enzyme targets (TOP1 and TOP2, respectively), they share the same mechanism of action (7); they poison TOPccs by impeding DNA resealing as the drugs stack with the base pairs flanking the break made by the topoisomerases while making amino acid hydrogen bonds with the topoisomerases. This mode of inhibition, termed interfacial inhibition, was first discovered for the topoisomerase inhibitors and has been generalized to other natural products targeting macromolecular interfaces (7).

Following the trapping of TOPccs by topoisomerase inhibitors, cells must repair the irreversible topoisomerase-linked DNA breaks induced when replication and/or transcription machineries collide with the TOPccs. Tyrosyl DNA phosphodiesterase 1 and 2 (TDP1 and TDP2) remove the topoisomerase polypeptides covalently linked to the ends of the breaks (8–10); and the associated DSBs are repaired by two main pathways: homologous recombination (HR) and nonhomologous end joining (NHEJ). HR primarily repairs TOP1ccs during S-phase when replicated DNA is available as repair template (11–14). In contrast, NHEJ relies on Ku70/80 and DNA-dependent protein kinase complexes, which directly joins the broken ends in nonreplicating cells. NHEJ is the primary repair pathway for TOP2ccs following their processing by TDP2 (8, 14, 15). Notably, inactivation of NHEJ by knocking-out Ku70 in chicken lymphoblastoid DT40 cells increases resistance to CPTs (14). Indicating that toxic replication intermediates are generated by NHEJ in response to TOP1ccs while NHEJ effectively repairs TOP2ccs (14, 15).

The indenoisoquinolines are new TOP1 inhibitors that have been developed to overcome the limitations of CPTs (5, 16). Three indenoisoquinolines derivatives are in phase I/phase II clinical trials, LMP400 (indotecan), LMP776 (indimitecan) and LMP744 (5, 17, 18). Unlike the CPTs, the indenoisoquinolines are chemically stable, induce persistent TOP1ccs, are not substrates of drug efflux by ABC transporters and have an extended plasma half-life (17, 18). Moreover, unlike irinotecan, they do not cause severe diarrhea (5, 17, 18). We recently reported the anticancer activities of second-generation indenoisoquinolines, the fluoroidenoisoquinolines, in which the addition of a fluorenine at position 3 replaces the methoxy groups at position 2, 3 of the clinical indenoisoquinolines (Fig. 1A; refs. 19–21). We showed that among these fluoroidenoisoquinolines, LMP517 generated the most DNA damage, which correlated with its potency for trapping TOP1ccs (19). The aim of this study was to identify the molecular
mechanisms explaining the enhanced LMP517 potency and to compare it with its parent clinical derivative LMP744.

**Material and Methods**

**Cells and reagents**

The DT40 chicken lymphoma and the TK6 human lymphoma cell lines were obtained from Dr. Shunichi Takeda, Laboratory of Radiation Genetics, Graduate School of Medicine in Kyoto University (Kyoto, Japan). All the mutant cell lines were previously authenticated by Southern blotting and/or RT-PCR and/or Western blotting. DT40 cells were cultured at 37°C with 5% CO₂ in RPMI1640 medium (11875-093, Invitrogen) supplemented with 1% chicken serum (16110-082, Invitrogen), 10 nmol/L β-mercaptoethanol (M-3148, Sigma-Aldrich), penicillin-streptomycin (15140-122, Invitrogen), and 10% FBS (100-106, Gemini Bio-Products). All cell lines were kept for maximum 45 days after thawing and tested for *Mycoplasma* with MycoAlert Mycoplasma Detection Kit (Lonza). H82 cells were obtained from the NCI repository. For the HCT116 Fluorescent Ubiquitination-based Cell Cycle Indicator (FUCCI) cells, we obtained mKO2-hCdt1(30/120)/pCSII-EF-MCS and mAG-hGeminin(1/110)/pCSII-EF-MCS from Dr. Hiroyuki Miyoshi and Dr. Atsushi Miyawaki.
(RIKEN Institute, Japan; ref. 22). HEK293T cells were transfected with either construct using Lentiv-X packaging system (Takara). The harvested lentiviral particles from both constructs were used to infect HCT116 cells, which were then FACs sorted for dual-positive cells (FUCCI). The brightest single clone was selected and used for this study.

Topotecan, CPT, etoposide, and LMP744 were provided by the NCI Drug Developmental Therapeutics Program (DTP). LMP517 was synthesized in the Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University (West Lafayette, IN; ref. 21).

Survival assays

DT40 cells were seeded at 5,000 cells per well in 96-well white plates (catalog no. 6005680, PerkinElmer Life Sciences) and exposed to the indicated concentrations of LMP744, LMP517, etoposide, or topotecan for 72 hours, performed at least three times with in-experiment triplicates. Cellular viability was determined using ATPlite 1-step kit (catalog no. 6005680, PerkinElmer) and exposed to the treatment for 2 hours with drugs. After treatment, slides were fixed in paraformaldehyde 4% for 10 minutes at room temperature then permeabilized with 0.02% Triton X-100 for 5 minutes at room temperature. After rinsing with PBS, the slides were blocked for 1 hour in PBS-BSA 8% at room temperature and incubated for 2 hours with primary antibody at room temperature (Abcam ab22551 mouse anti-γH2AX). After washing with PBS, cells were incubated with secondary antibody for 1 hour at room temperature, washed with PBS and mounted with Vectorshield with DAPI (Vector Laboratories). The primary antibody for histone γH2AX was mouse monoclonal anti-γH2AX Ser140 antibody, clone JBW301 (Abcam: ab22551) and the secondary antibody was a chicken anti-mouse Alexa 647 (A21463 Invitrogen).

Mouse antitumor experiments

Athymic nude mice (nu/nu, female, 20–25 g, 8–12-week-old) from Charles River, were transplanted with 5 million H82 human small-cell lung cancer cells (SCLC). When the tumor volume reached between 100 and 125 mm3, the animals were randomized into treatment groups based on tumor volume and body weights using the StudyLog software. Ten mice for LMP744, vehicle arm and for LMP517 were used. The animals were treated with either LMP744 (10 mg/kg) administered intravenous (i.v.) or with LMP517 (10 mg/kg) administered i.v. push via tail vein once a day for 5 consecutive days once (1 cycle) or twice (2 cycles). LMP744 and LMP517 were dissolved in 10 mmol/L citric acid, 5% dextrose. The three axes (millimeters) of tumors were measured with a caliper to calculate tumor volume. Measurements were made every 3 or 4 days. Maximum allowable weight loss tolerated was defined as: [(ATP signal in treated cells)/(ATP signal in untreated cells)] × 100.

Recombinant proteins

Human TOP1 was purified from baculovirus as described previously (23). Human TOP2α was purified from yeast strains JEL1 top1A transformed with 12-URA-B 6 × His-hTOP2α. Induction of TOP2 by galactose as described previously (24). Yeast cells were lysed in equilibration buffer [300 mmol/L KCl, 10 mmol/L imidazole, 20 mmol/L Tris HCl pH 7.7, 10% glycerol, and protease inhibitor cocktail (Sigma-Aldrich, catalog no. P8215)] by glass bead homogenization. Lysates were incubated with Ni-NTA resin and washed using wash buffer #1 (300 mmol/L KCl, 30 mmol/L imidazole, 20 mmol/L Tris HCl pH 7.7, 10% glycerol, and protease inhibitors) then wash buffer #2 (150 mmol/L KCl, 30 mmol/L imidazole, 20 mmol/L Tris HCl pH 7.7, 10% glycerol, and protease inhibitor cocktail). hTOP2α and β were eluted on a Poly-Prep chromatography column (Bio-Rad, catalog no. 7311550) with elution buffer (150 mmol/L KCl, 300 mmol/L imidazole, 20 mmol/L Tris HCl pH 7.7, 10% glycerol, and protease inhibitors). The peak protein fractions were dialyzed in dialysis buffer (750 mmol/L KCl, 50 mmol/L Tris HCl pH 7.7, 20% glycerol, 0.1 mmol/L EDTA, and 0.5 mmol/L DTT) and His tag was removed using Tobacco Etch Virus (TEV) protease.

TOPI and TOP2 cleavage assay

Topoisomerase plasmid cleavage assay was carried out as described previously (25). In brief, 250 ng of pBR322 supercoiled plasmid DNA and 1 μg of recombinant TOP1 or TOP2α were incubated in 20 μL TOP1 reaction buffer containing 50 mmol/L Tris-HCl, pH 7.5, 100 mmol/L KCl, 1 mmol/L DTT, 10 mmol/L EDTA and 5 μg/mL acetylated BSA or in 20 μL TOP2 reaction buffer containing 20 mmol/L Tris-HCl, pH 7.5, 10 mmol/L MgCl2, 150 mmol/L KCl, 1 mmol/L ATP, 1 mmol/L EDTA, 1 mmol/L DTT, and 30 μg/mL acetylated BSA (TOP2) in the presence of various concentrations of drugs at 37°C for 30 minutes. The reactions were terminated by adding 2 μL 10% SDS, 0.75 μL of 500 mmol/L EDTA, pH 8.0, and 2 μL 0.8 mg/mL proteinase K and further incubated for 2 hours at 30°C. DNA samples were electrophoresed in 0.8% agarose gels containing 0.5 μg/mL ethidium bromide.

Histone γH2AX detection

HCT116 and HCT116 FUCCI cells were plated at 50,000 cells per well in 4-well chamber slides, incubated for 72 hours and then treated with drugs. After treatment, slides were fixed in paraformaldehyde 4% for 10 minutes at room temperature then permeabilized with 0.02% Triton X-100 for 5 minutes at room temperature. After rinsing with PBS, the slides were blocked for 1 hour in PBS-BSA 8% at room temperature and incubated for 2 hours with primary antibody at room temperature (Abcam ab22551 mouse anti-γH2AX). After washing with PBS, cells were incubated with secondary antibody for 1 hour at room temperature, washed with PBS and mounted with Vectashield with DAPI (Vector Laboratories). The primary antibody for histone γH2AX was mouse monoclonal anti-γH2AX Ser140 antibody, clone JBW301 (Abcam: ab22551) and the secondary antibody was a chicken anti-mouse Alexa 647 (A21463 Invitrogen).

Results

The fluoroindenoisoquinoline LMP517 has an improved antitumoral activity over LMP744

To test the antitumoral activity of LMP517, we xenografted nude mice with the SCLC cell line H82 (19) and compared LMP517 with its parent indenoisoquinoline LMP744 in one or two treatment cycles (5 days of treatment per cycle). LMP744 and LMP517 (chemical structures in Fig. 1A) both had an maximum tolerated dose (MTD) of 10 mg/kg. At this concentration, only LMP517 induced a reduction in tumor growth (Fig. 1B) with marginal body weight lost (Supplementary Fig. S1). LMP517 treatment resulted in an average survival of 30 days for 1 cycle and 36 days for the 2 cycles protocol versus 19 days for both one cycle and two cycles of LMP744 (Fig. 1C). This result demonstrates the increased efficacy of the fluoroindenoisoquinoline LMP517 over LMP744 in this model system. The following experiments were performed to elucidate the molecular pharmacology of LMP517 and understand why it is more potent than its parent counterpart LMP744.

LMP517 displays a comparable phenotype with etoposide in isogenic TOP- and ku70-knockout DT40 cells

Cells have redundant pathways to excise unresolved covalent complexes between topoisomerases and DNA (2). The most specific
pathways involve TDP1 and TDP2, which hydrolyze the covalent bond between the tyrosine of the topoisomerase polypeptides (TOP1 and TOP2, respectively) and the DNA backbone. TDP1 inactivation inhibits the repair of TOP1cc and increases sensitivity to CPT, and to a lesser extent to etoposide (26, 27). Conversely, TDP2 inactivation increases TOP2cc accumulation and sensitizes cells to etoposide (9, 28). Here we used DT40 TDP1- or TDP2-deficient cells (referred as DT40 tdp1 and DT40 tdp2, respectively; refs. 27, 28) to determine the impact of TDP1 and TDP2 on the activity of LMP517.

As expected, DT40 tdp1 cells were hypersensitive to CPT with an IC_{50} of 2 nmol/L versus 15 nmol/L for DT40 WT (Fig. 2A). DT40 tdp2 cells were also sensitive to CPT, consistent with the role of TDP2 as secondary repair pathway for TOP1ccs (9, 28) (IC_{50}: 5 nmol/L for DT40 Tdp2; Fig. 2A). LMP744 showed a similar sensitivity pattern as CPT with an IC_{50} of 6 nmol/L versus 25 nmol/L for DT40 WT cells and 15 nmol/L for DT40 tdp2 cells.

Conversely, DT40 tdp2 cells were most sensitive to etoposide (IC_{50}: 28 nmol/L vs. WT >125 nmol/L) and tdp1 cells were also hypersensitive to etoposide, consistent with a role of TDP1 as alternative pathway for excising 5'-phosphotyrosyl adducts (27, 29). Notably, among the three cells lines, tdp2 cells showed the highest sensitivity to LMP517 (IC_{50}: 11 nmol/L, 18 nmol/L, and 32 nmol/L in tdp2, tdp1, and WT cells, respectively; Fig. 2A). This unexpected result suggested that LMP517 might act like etoposide (by TOP2 poisoning) in addition to being a TOP1 poison.

Repair of TOP2cc by TDP2 generates direct substrate for NHEJ (2, 8, 9, 15) whereas NHEJ exerts the opposite effect in response to TOP1cc by initiating toxic NHEJ (14). We used this difference to further determine whether LMP517 could be acting as a TOP2 poison.
DT40 Ku70-deficient cells showed the expected resistance to topotecan while being hypersensitive to etoposide, LMP517, and LMP744 (Fig. 2B). The phenotypes observed in TDP1-, TDP2-, and Ku-deficient DT40 cells suggest that LMP517 could act as a TOP2 poison.

**LMP517 is a dual TOP1 and TOP2 inhibitor in biochemical assays and in HCT116 and TK6 cells**

To directly determine the effects of LMP517 on TOP1 and TOP2, we performed biochemical assays with negatively supercoiled plasmid pBR322 in the presence of recombinant TOP1 or TOP2, using CPT and etoposide as positive controls. As expected, both LMP517 and LMP744 trapped TOP1ccs (Fig. 3A). Indeed, when TOP1 was applied to supercoiled DNA in the presence of LMP744 or LMP517, we observed extensive accumulation of nicked DNA, as in the case of CPT (Fig. 3A, lane C, ref. 30). Notably, in the presence of TOP2, we observed both linearized and nicked DNA with LMP517 or LMP744, which are characteristic results obtained with trapping of TOP2 by etoposide (Fig. 3A, lane E at right; ref. 31). The induction of nicked and linear DNA species is consistent with TOP2 trapping by LMP517 and LMP744.

To test whether LMP517 also acts as a dual TOP1 and TOP2 inhibitor in cells, we treated human lymphoblast TK6 and colon carcinoma HCT116 cells for 1 hour with LMP517, CPT, etoposide, or LMP744 and detected TOP1cc and TOP2cc by Radar assay (32). Figure 3B shows that LMP517 induces both TOP2α and TOP2βccs in addition to TOP1ccs in both cell lines tested (Fig. 3B and C). In HCT116, we also tested the indenoisoquinoline LMP744 and found that it also induced TOP2ccs, although to a lesser extent than LMP517 (Fig. 3C).

**Figure 3.** LMP517 induces TOP1cc and TOP2cc both with purified enzymes and in human cancer cells. **A**, Recombinant TOP1 and TOP2 applied to the plasmid pBR322 in the presence of CPT (C), etoposide (E), LMP744, or LMP517. **B**, TOP1, TOP2α, and TOP2βccs detected in lymphoblast TK6 cells after treatment with CPT, etoposide (ETP), LMP517, or LMP744. **C**, TOP1, TOP2α, and TOP2βccs detected in colon cancer HCT116 cells after treatment with CPT, ETP, LMP517, or LMP744.
Together, these results demonstrate that LMP517 is a dual inhibitor of TOP1 and TOP2 (both TOP2α and TOP2β) in biochemical and cellular assays.

LMP517 induces histone γH2AX in both G1- and S-G2, cell-cycle phase cells, consistent with its dual activity against TOP1 and TOP2.

It is well established that TOP1ccs trapped by CPTs induce DNA damage primarily in replicative cells (S-phase; refs. 2, 33) while etoposide targets cells in all phases of the cell cycle by trapping both TOP2α and TOP2β (2, 34). To test the induction of DNA damage by LMP517 and whether such damage is dependent on replication, we used HCT116 Fucci cells, which express tagged cell-cycle reporter peptides, mAzami-Green–tagged N-terminus of geminin (green channel) or mKusabira-Orange2–tagged N-terminus of CDT1 (red channel). This dual labeling allows the detection of cells in the S-G2- or G1-phase, respectively (22).

We treated HCT116 Fucci cells with CPT (1 μmol/L), etoposide (50 μmol/L), LMP517 (1 μmol/L), or LMP744 (1 μmol/L) for 1 hour and detected DNA damage with γH2AX antibodies (Fig. 4A). LMP517 induced DNA damage starting from 50 μmol/L with extensive γH2AX at 1 μmol/L (Fig. 4A; Supplementary Fig. S2). When treated with CPT, HCT116 cells were divided in two groups: γH2AX-positive and γH2AX-negative (Fig. 4A). γH2AX-positive-negative cells were mainly mK02-positive cells (G1-phase cells; Supplementary Fig. S3; ref. 35). In contrast, etoposide treatment induced γH2AX signal in nearly all cells (Fig. 4; Supplementary Fig. S3), demonstrating that etoposide targets cells regardless of their cell-cycle phase. LMP744 induced γH2AX-positive cells, similar to CPT, mainly in S-G2-phase cells (GFP-positive; Fig. 4B; Supplementary Fig. S3). This result is consistent with the conclusion that LMP744 acts primarily as a TOP1 inhibitor in HCT116 cells (36).

Notably, cells treated with LMP517 did not show a bimodal γH2AX signal distribution (Fig. 4A). Analyses of the GFP and RFP signals and cell-cycle distribution showed that, like etoposide, LMP517 induced γH2AX signal in G1-phase cells (88% and 89% of total G1-phase cells, respectively; Fig. 4B, left). Only a small percentage of G1-phase cells showed γH2AX signal when treated with CPT or LMP744 (29% and 23% of total G1-phase cells, respectively; Fig. 4B, left). These results demonstrate that LMP517, like etoposide and contrary to CPT and LMP744, induces DNA damage in cells in G1-phase of the cell cycle, consistent with cellular damage induced by TOP2 trapping.

**Schlafen 11, NHEJ, and BRCAness are determinants of response to LMP517**

Schlafen 11 (SLFN11) is an established dominant determinant of response to both TOP1 and TOP2 inhibitors (37–39). We recently reported, in isogenic human leukemia CCRF-CEM cells, an increased resistance to LMP517 in the absence of SLFN11 expression (19). To determine the predictive value of SLFN11 expression in nonisogenic cells, we analyzed the activity of LMP517 across the NCI-60 cell line panel using CellMinerCDB (Fig. 5A; refs. 40, 41). A significant correlation was observed between the activity of LMP517 and SLFN11 expression. However, some cells seem to respond in the absence of SLFN11 and other, SLFN11-positive, were not hypersensitive to LMP517. LMP517 induced DNA damage in all phases of the cell cycle like etoposide. A, Total γH2AX signal after 1-hour treatments with CPT (1 μmol/L), ETP (50 μmol/L), LMP517 (1 μmol/L), or LMP744 (1 μmol/L). Dots correspond to individual cells (n = 888 for each of the 5 sets). The shaded area corresponds to γH2AX-positive cells defined by a γH2AX signal above a threshold set at 24,000 AU (arbitrary unit; 5% of the nontreated (NT) cells). Dose-response for LMP517 at lower concentrations is included in Supplementary Fig. S2. B, Left: Quantification of the ratio of γH2AX-positive G0-cells over the total G0-cells after 1-hour treatments with CPT, etoposide, LMP744, or LMP517. Right: Quantification of the ratio of γH2AX-positive S/G2-cells over the total S/G2-cells after 1-hour treatments with CPT, etoposide, LMP744, or LMP517. Bars: SD between three independent experiments. Significance between CPT and other treatment is displayed (***, P < 0.0005, ****, P < 0.00005).
Figure 5. SLFN11 and HRD are determinants of response to LMP517. A, Correlation between SLFN11 expression and the antiproliferative activity of LMP517 across the NCI-60. Each dot corresponds to a cell line (see key to the right and http://discover.nci.nih.gov/cellminercdb). B, Predicted response of LMP517 across the NCI-60 by including SLFN11 expression, XRCC6 (KU70) copy number, and APLF expression [CellMinerCDB “Multivariate Analyses” (41)]. C, Relationship between SLFN11 expression, Ku70 (XRCC6) copy number, and APLF expression and the antiproliferative activity of LMP517 across the NCI-60 cell lines. Cell lines (individual columns) are ranked by drug sensitivity. Color scale: Red represents high drug sensitivity and high gene expression. Blue is the opposite. D, Cell viability of DT40 WT, BRCA1-, BRCA2-, and PALB2-knockout cells after 72 hours’ treatments with increasing concentration of LMP517. Bars: SD between three independent experiments. Statistically significant differences (P values < 0.05) between WT and KO cells are annotated with black stars. E, Cell viability of DT40 WT and BRCA1-knockout cells after 72 hours’ treatments with increasing concentration of olaparib without or with 3 nmol/L of LMP517. Bars: SD between three independent experiments.

Discussion

Our results demonstrate that the fluoroindenoisoquinoline LMP517 (19, 20) displays improved antitumoral efficacy in murine H82 (SCLC) xenograft compared with its parent compound LMP744 (see Fig. 1), which was recently introduced in phase I clinical trials based on its antitumor activity in dog lymphomas (18). Hence, LMP517 has the potential to be developed as second-generation indenoisoquinoline inhibitor. Further studies are warranted to determine LMP517 potency against other models and determine its clinical potential.

We show that LMP517 acts by dual targeting of TOP1 and TOP2 at nanomolar concentrations. This conclusion is based on our current results. First, LMP517 exhibits selective antiproliferative activity toward TDP2-defective cells compared with TDP1-deficient cells and also toward Ku70-defective cells (see Fig. 2) like the TOP2 inhibitor etoposide and unlike the TOP1 inhibitors: CPT, topotecan, LMP400 (indotecan), and LMP776 (indimecan: refs. 5, 14). Second, LMP517 traps both TOP1 and TOP2ccs in biochemical assays and in human
colon carcinoma HCT116 and lymphoma TK6 cells (see Fig. 3). Third, LMP517 induces DNA damage at nanomolar concentrations (19) in nonreplicating G1-phase cells, similar to etoposide and distinct from CPT (see Fig. 4; refs. 34, 43, 44). And finally, similar to the other established TOP1 and TOP2 inhibitors, SLFN11 (37, 39) and BRCA- ness (HRD; refs. 11, 14) are dominant determinants of response to LMP517 (see Fig. 5).

Comparing LMP517 with its parent indenoisoquinoline LMP744 reveals that the addition of a fluoride and removal of the methoxy groups in the A-ring (see Fig. 1) increased the ability of LMP517 to target TOP2. In our recent study of LMP517, we also noticed the ability of LMP517 to intercalate into DNA at high concentration as it has been previously established for LMP744 (36). This intercalating effect of LMP517 and LMP744 may explain the inhibitory effect on TOP2 as several chemical classes of DNA intercalating agents effectively trap TOP2ccs (45–47) by stacking with the base pairs flanking the TOP2cc, including the clinically used anthracyclines (doxorubicin, daunorubicin, epirubicin, idarubicin), mitoxantrone, and ansamycin, as well as ellipticine derivatives (6, 43). Indeed, DNA intercalation of drugs and ligands such as benzo[a]pyrene carcinogens at a TOP2 cleavage site can directly block the DNA religation by TOP2 (7, 48, 49).

Our results provide groundwork for the potential clinical development of LMP517 as a dual TOP1 and TOP2 inhibitor and potentially as a tumor-targeted delivery payload (5, 50).

Disclosure of Potential Conflicts of Interest

Y. Pommier has ownership interest in a patent. No potential conflicts of interest were disclosed by the other authors.

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