# New Life of Topoisomerase I Inhibitors as Antibody–Drug Conjugate Warheads

Yves Pommier and Anish Thomas


## SUMMARY

Antibody–drug conjugates (ADC) allow the delivery of cytotoxic chemotherapeutic agents to tumors. Two ADC delivering topoisomerase I (TOP1) poisons (Enhertu and Trodelvy) have recently been FDA-approved for Her2- and Trop2-expressing solid tumors. In a recent study, a TOP1-anti B7-H4 ADC was described and shown to be synergistic with a novel PARP1-selective inhibitor.

*See related article by Kinneer et al., p. 1086*


In this issue of *Clinical Cancer Research*, Kinneer and colleagues (1) unveil a novel antibody–drug conjugate (ADC) AZD8205 delivering the camptothecin-based payload AZ14170132 to tumor cells expressing cell surface antigen B7-H4 [encoded by V-set domain containing T-cell activation inhibitor 1 (*VTCN1*)]. Taking advantage of the tumor-specific accumulation of this new ADC, they establish the synergistic combination of AZD8205 with a novel PARP inhibitor (AZD5305) specifically targeting PARP1 (2) to avoid bone marrow toxicity.

The clinical topoisomerase I (TOP1) inhibitors, irinotecan and topotecan derived from the natural alkaloid camptothecin, are widely used against a broad spectrum of malignancies including colon, ovarian, pancreatic, and small cell lung cancers (3, 4). They kill cancer cells by poisoning TOP1 cleavage complexes (TOP1ccs; **Fig. 1**, item 5) as TOP1 cleaves the DNA to dissipate the superhelical tensions generated by transcription, replication, and chromatin remodeling (5). The camptothecins are "interfacial inhibitors", a mechanism common to a broad range of natural products, which infers that the higher the drug target, the more poisonous the drug (6). Hence, cancer cells, which generally require elevated TOP1 expression to support their high-level DNA metabolism tend to have enhanced sensitivity to camptothecins.

Cells counter the trapping (poisoning) of the TOP1ccs by degrading TOP1 (7, 8), by removing the TOP1ccs using multiple repair enzymes including but not limited to tyrosyl-DNA phosphodiesterase 1 and PARP1 (9, 10), by transiently halting replication by activating ATR/CHK1 to avoid further damage and enable repair (11, 12), and by repairing the DNA breaks by homology-directed recombination (**Fig. 1**, item 7; refs. 4, 5). This explains the selective cell killing of homologous recombination–deficient (HRD) cancer cells (such as BRCA-deficient cells) by anticancer TOP1 inhibitors (13).

In response to TOP1ccs, cells also engage active cell death pathways (4) as exemplified by the tumor suppressor effects of p53 and the increasing relevance Schlafen 11 (SLFN11), an interferon-inducible innate immune response protein that executes cells with excessive replication stress (**Fig. 1**, item 7; ref. 12). Novel TOP1 inhibitor poisons have been developed, including the one described in the article by Kinneer et al. (1), and deruxtecan, a close derivative of exatecan (14). However, the major limitation of camptothecins is their toxicity to proliferating normal cells of the bone marrow and intestine, which limits the achievable tumor concentrations required for tumor eradication and have precluded therapeutic combinations with TOP1 and PARP inhibitors.

An emerging value of potent TOP1 inhibitors, and a turning point in chemotherapies in general is the use of TOP1 inhibitors as ADC payloads (15, 16). ADCs comprise an antibody targeting a tumor-specific antigen linked to several cytotoxic drug molecules per antibody molecule defining the drug antibody ratio (which is presently limited to 8 drug molecules per antibody; **Fig. 1**, item 1). There are currently 10 ADCs approved by the FDA, and of the three approved in the past 3 years, two are ADC-TOP1 inhibitors (1, 16). Trastuzumab deruxtecan carries an exatecan mesylate (deruxtecan/DX-895f/DXd-8) payload similar to AZD8205, and has shown antitumor activity in Her2-expressing and mutant cancers including breast, lung, and gastric cancers. Trodelvy delivers SN-38, the active metabolite of irinotecan (3), and has shown antitumor activity in breast and urothelial cancers that express Trop-2.

The field of ADC research is highly dynamic across small and large drug companies, biotechnology companies and academic laboratories, with dozens of ADCs in clinical development (15, 16). Achieving the full therapeutic potential of ADCs requires several considerations including: (i) the design of the individual components of ADC—target antigen, antibody, linker and conjugation chemistry, and cytotoxic payload; (ii) patient selection to maximize the likelihood of antitumor response, informed by tumor and host genetic backgrounds; and (iii) establishing rational combinations to increase the depth and duration of responses (summarized in **Fig. 1**).

The target antigen should be expressed preferentially on the surface of a tumor compared with normal cells and must have an extracellular epitope amenable to antibody binding and internalization into target cells where the drug can be released. The target of AZD8205 is the transmembrane glycoprotein B7-H4 (also known as B7S1 or B7x) encoded by the *VTCN1* gene, known to inhibit T cell–mediated immune response and to promote epithelial cell transformation. B7-H4 has also been proposed as a potential target for the treatment of autoimmune diseases (17). Kinneer and colleagues (1) describe a novel IHC protocol using digital pathology to assess B7-H4 expression across a large panel of normal tissues and tumors. Highest expression of B7-H4 was observed in women's malignancies (breast, ovarian, and endometrial carcinomas) and in cholangiocarcinomas, with notable

Laboratory of Molecular Pharmacology and Developmental Therapeutics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

**Corresponding Authors:** Yves Pommier, Developmental Therapeutics Branch, Building 37, Room 5068, NIH, Bethesda, MD 20892. E-mail: pommier@nih.gov; and Anish Thomas, E-mail: anish.thomas@nih.gov

Clin Cancer Res 2023;29:991–3

**doi:** 10.1158/1078-0432.CCR-22-3640

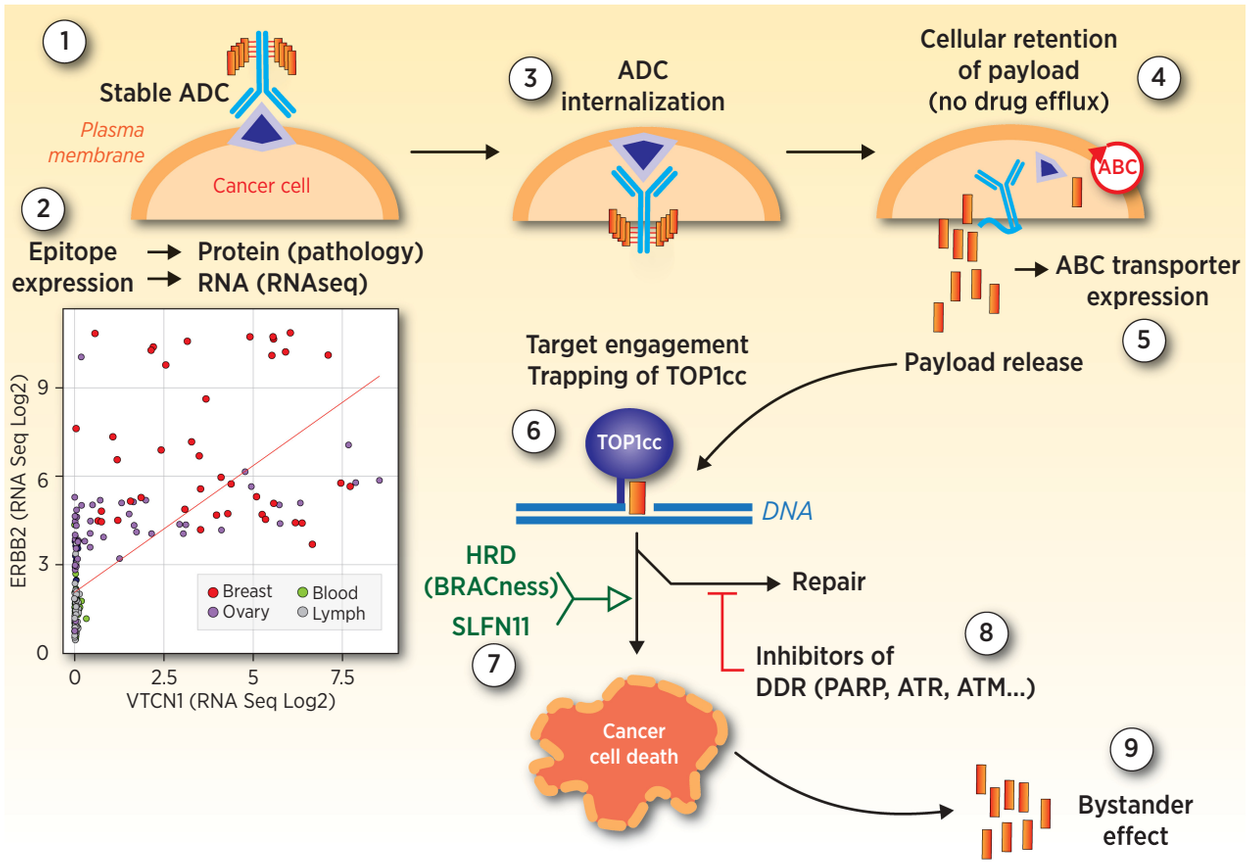


**Figure 1.**

Key parameters to be considered for optimizing TOP1cc-targeted ADCs. 1, The ADC needs to be stable in blood and to not release the payload before it reaches its cancer cell targets. 2, The epitope has to be expressed on cancer but not in normal cells as determined by histology and pathology methods and/or RNA seq. The inset shows the expression of *VTCN1* (which encodes B7-H4) in the 1,019 cancer cell lines of the Broad-MIT (snapshot from https://discover.nci.nih.gov). Note that multiple breast and ovarian cell lines co-express *VTCN1* and *ERBB2* (which encodes Her2). 3, The ADC needs to be internalized into the cancer cells. 4, The payload should be released readily inside the cytosol of the cancer cells. 5, If the payload is known to be substrate for the drug efflux ABC transporter(s), expression of the relevant ABC transporters needs to be determined, as tumor overexpressing such transporter may not respond to the ADC. 6, Target engagement (i.e., trapping of TOP1ccs) can be measured by induction of γH2AX and TOP1 degradation. 7, Determinants of response such as HRD and SLFN11 can be assessed to predict response and potentially select patients. 8, Combination with DNA damage response (DDR) inhibitors such as the PARP1 inhibitor AZD5305 or ATR inhibitors should synergize with the TOP1 inhibitor only in the cancer cells while sparing normal cells. 9, Release of the payload from the dead tumor cells and in the extracellular milieu can attack neighboring cells with low expression of the surface target epitope.

heterogeneity of expression within individual tumors. Many cancer samples co-expressed B7-H4 and HER2 (ERBB2), which is consistent with our analysis of the 1000 cancer cell line data from the NCI, the Sanger and the Broad-MIT Institutes (**Fig. 1**, item 2, inset; https://discover.nci.nih.gov/).

Heterogeneous expression of the target antigen is a challenge for ADCs but may be overcome by the so-called bystander killing effect, which refers to the payload diffusion from antigen-positive tumor cells to adjacent antigen-negative tumor cells (**Fig. 1**, item 9). In the case of sacituzumab-govitecan (Trodelvy) the rapid spontaneous hydrolysis of the linker within and outside the tumor milieu before its penetration into tumor cells has been proposed as mechanism of action (18). The bystander killing capacity of AZD8205 could be attributed to the payload AZ14170132 being highly membrane-permeable allowing controlled linker cleavage at the target site before ADC internalization.

PARP1 and ATR are critical for limiting the TOP1 inhibitor-induced DNA damage and repairing them, and as such, they represent rational targets to enhance the DNA damage from TOP1 inhibitors (10). However, combinations of PARP inhibitors with TOP1 inhibitors have been limited by overlapping hematologic toxicities. Specifically, the currently approved PARP inhibitors have limited selectivity for PARP1 over PARP2, which has been shown to play a role in the survival of hematopoietic/stem progenitor cells. Early data from a phase I/IIa clinical trial suggest that PARP1-selective inhibitor AZD5305 may have improved safety and tolerability compared with first-generation PARP1/2 inhibitors. Kinneer and colleagues (1) combined AZD8205 with AZD5305 (19) in multiple *in vivo* models to find notable antitumor activity in models without known deficiencies in DNA repair and in the PARP inhibitor-resistant setting. The impact of the combination on hematologic parameters needs further study in model systems and ultimately in patients.

AZD8205 is currently being evaluated in a first-in-human, phase I study. Targeted delivery of a TOP1 inhibitor payload by AZD8205 will likely improve upon the toxicity profile of conventional TOP1

inhibitors, and the PARP1-selective inhibitor AZD5305 may further widen the therapeutic window, to enable TOP1–PARP1 inhibitor combinations. Such combinations raise the exciting possibility of extending the benefit of DNA-targeted therapies beyond just tumors with DNA repair deficiencies and could also be effective in PARP inhibitor-resistant tumors and in SLFN11- low/negative tumors.

The therapeutic index of ADC-based combinations may be further widened using the "gapped" schedule (4) wherein the PARP1 inhibitor is administered following a gap or interval after TOP1–ADC that allows for systemic clearance of the TOP1 inhibitor while it is still retained in the tumor. The heterogeneity of B7-H4 expression profile and the potential for bystander effect raise questions about the impact of heterogeneity on drug responses, the optimal biomarker strategy to identify patients who are most likely to benefit (HRD and high SLFN11-expressing tumors; ref. 4), and the best diagnostic assay (RNA vs. protein detection) to identify B7-H4 expression. The time is ripe to unleash the full therapeutic potential of TOP1 inhibitors in its new life as ADC warheads, to hopefully, expand its beneficial effects to a larger population of patients with cancer.

## Authors' Disclosures

Y. Pommier reports grants from EMD Serono Research and Development Institute and Taiho Pharmaceutical Co. Ltd. during the conduct of the study. A. Thomas reports grants from EMD Serono Research and Development Institute, AstraZeneca, Tarveda Therapeutics, Immunomedics, and Prolynx during the conduct of the study. No other disclosures were reported.

Received December 5, 2022; revised December 20, 2022; accepted December 29, 2022; published first January 13, 2023.

## References


1. Kinneer K, Wortmann P, Cooper ZA, Dickinson NJ, Masterson L, Cailleau T, et al. Design and preclinical evaluation of a novel B7-H4–directed antibody–drug conjugate, AZD8205, alone and in combination with the PARP1-selective inhibitor AZD5305. Clin Cancer Res 2023;29:1086–101.
2. Johannes JW, Balazs A, Barratt D, Bista M, Chuba MD, Cosulich S, et al. Discovery of 5-4-[(7-Ethyl-6-oxo-5,6-dihydro-1,5-naphthyridin-3-yl)methyl] piperazin-1-yl-N-methylpyridine-2-carboxamide (AZD5305): a PARP1-DNA trapper with high selectivity for PARP1 over PARP2 and other PARPs. J Med Chem 2021;64:14498–512.
3. Pommier Y. Topoisomerase I inhibitors: camptothecins and beyond. Nat Rev Cancer 2006;6:789–802.
4. Thomas A, Pommier Y. Targeting topoisomerase I in the era of precision medicine. Clin Cancer Res 2019;25:6581–9.
5. Pommier Y, Nussenzweig A, Takeda S, Austin C. Human topoisomerases and their roles in genome stability and organization. Nat Rev Mol Cell Biol 2022;23: 407–27.
6. Pommier Y, Marchand C. Interfacial inhibitors: targeting macromolecular complexes. Nat Rev Drug Discov 2011;11:25–36.
7. Sun Y, Saha LK, Saha S, Jo U, Pommier Y. Debulking of topoisomerase DNA-protein crosslinks (TOP-DPC) by the proteasome, non-proteasomal and non-proteolytic pathways. DNA Repair 2020;94:102926.
8. Sun Y, Saha S, Wang W, Saha LK, Huang SN, Pommier Y. Excision repair of topoisomerase DNA-protein crosslinks (TOP-DPC). DNA Repair 2020;89: 102837.
9. Zhang YW, Regairaz M, Seiler JA, Agama KK, Doroshow JH, Pommier Y. Poly (ADP-ribose) polymerase and XPF-ERCC1 participate in distinct pathways for the repair of topoisomerase I–induced DNA damage in mammalian cells. Nucleic Acids Res 2011;39:3607–20.
10. Murai J, Zhang Y, Morris J, Ji J, Takeda S, Doroshow JH, et al. Rationale for PARP inhibitors in combination therapy with camptothecins or temozolomide based on PARP trapping versus catalytic inhibition. J Pharmacol Exp Ther 2014;349: 408–16.
11. Thomas A, Takahashi N, Rajapakse VN, Zhang X, Sun Y, Ceribelli M, et al. Therapeutic targeting of ATR yields durable regressions in small cell lung cancers with high replication stress. Cancer Cell 2021;39:566–79.
12. Jo U, Murai Y, Takebe N, Thomas A, Pommier Y. Precision oncology with drugs targeting the replication stress, ATR, and Schlafen 11. Cancers 2021;13:4601.
13. Coussy F, El-Botty R, Chateau-Joubert S, Dahmani A, Montaudon E, Leboucher S, et al. BRCAness, SLFN11, and RB1 loss predict response to topoisomerase I inhibitors in triple-negative breast cancers. Sci Transl Med 2020;12:eaax2625.
14. Ogitani Y, Abe Y, Iguchi T, Yamaguchi J, Terauchi T, Kitamura M, et al. Wide application of a novel topoisomerase I inhibitor-based drug conjugation technology. Bioorg Med Chem Lett 2016;26:5069–72.
15. Beck A, Goetsch L, Dumontet C, Corvaia N. Strategies and challenges for the next generation of antibody–drug conjugates. Nat Rev Drug Discov 2017;16:315–37.
16. Jin Y, Schladetsch MA, Huang X, Balunas MJ, Wiemer AJ. Stepping forward in antibody–drug conjugate development. Pharmacol Ther 2022;229:107917.
17. Vaishnav J, Khan F, Yadav M, Parmar N, Buch H, Jadeja SD, et al. V-set domain containing T-cell activation inhibitor 1 (VTCN1): a potential target for the treatment of autoimmune diseases. Immunobiology 2022;227:152274.
18. Santi DV, Cabel L, Bidard FC. Does sacituzumab-govitecan act as a conventional antibody–drug conjugate (ADC), a prodrug of SN-38 or both? Ann Transl Med 2021;9:1113.
19. Illuzzi G, Staniszewska AD, Gill SJ, Pike A, McWilliams L, Critchlow SE, et al. Preclinical characterization of AZD5305, a next-generation, highly selective PARP1 inhibitor and trapper. Clin Cancer Res 2022;28:4724–36.