High-Throughput Screening of DDR Molecules Combined with TOPO1 Inhibitors: Identifying Synergistic Pairs for DDR-Based Dual-Payload ADCs in Resistant Cell Lines

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Introduction

The DNA Damage Response (DDR) pathway is the core biological system through which cells detect and repair genomic lesions. Targeting DDR pathways and inhibiting DNA repair mechanisms has emerged as a highly promising strategy in cancer therapy, with significant breakthroughs achieved in the development of DDR inhibitors. Antibody-drug conjugates (ADCs)—which combine the precise targeting of antibodies with the potent cytotoxicity of drugs—represent a promising antineoplastic therapy. However, current ADCs face critical challenges, including non-responsive cancers, drug resistance, and rapid patient relapse, primarily driven by tumor heterogeneity and resistance. To address these issues, dual-payload ADCs have emerged as an innovative strategy: they deliver two cytotoxic agents to enhance efficacy via synergistic effects, mitigate resistance, and enable flexible dosing. Despite their potential, dual-payload ADC development remains complex; cell panel-based studies and drug-resistant cell lines serve as powerful tools to explore effective dual-payload ADCs by evaluating drug combination synergies and elucidating resistance mechanisms. To advance the development of DDR pathway-based dual-payload ADCs, this study leveraged an in-house DDR platform to conduct large-scale synergy screening in ADC-resistant cell lines. The screening panel included over 120 targeted molecules and chemotherapeutics, covering DDR core pathway proteins (e.g., WRN, PARP, ATR, DNAPK), cell cycle regulators, and kinase families such as tyrosine kinases. Screening results revealed that ATR inhibitors combined with Topoisomerase I (TOPO1) inhibitors exhibit strong synergistic activity; their specific molecular mechanisms require further validation. This optimal combination provides a direct candidate for DDR pathway-targeted dual-payload ADC development, with the potential to accelerate the research, development, and translation of next-generation DDR-enabled ADC modalities.

Integrated Drug Evaluation Platform for DDR-Related Targets

DDR Pathways	Targets
HR	ATM, ATR, BLM, RECQ1, RECQ4, RECQ5,HELQ
NHEJ and TMEJ	DNA-PK, WRN, POLQ
SSB and BER	PARP1/2/3/5A/5B/6/7/10/11/12/14/15,PARG, Topo I, APE1,XRCC1
Cell Cycle	WEE1/2, MYT1, p53 Y220C, CHK1/2, CDK family
Others	POLA, POLG, POLH, POLN, MAT2A, PRMT5,TREX1/2, DHX9

- Protein Engineering
- Biophysical Assays
- Biochemical Assays
- Cell Based Assays
- Assay Development

- MOA Studies
- Cancer Cell Panel Screening
- In Vitro ADMET, PK/PD
- In Vivo Pharmacology

- SiRNACRISPR KD/KO
- Cell Line Engineering
- Biomarker Evaluation
- Cell Phenotyping Studies

- Safety Panels
- Kinase Panels
- CiPA Cardiac Safety
- IND Enabling

The DNA damage response (DDR) pathway is frequently dysregulated in a wide spectrum of diseases, with cancer being a primary example. Identifying DDR pathway components as potential drug targets represents a critical pillar of modern drug discovery. DDR encompasses multiple distinct signaling cascades, each specialized in repairing specific types of DNA lesions. Elucidating the key DDR pathways is therefore paramount to drug discovery efforts, as it uncovers actionable targets for therapeutic intervention. To support this process, ICE Bioscience has established a comprehensive panel of assays targeting core proteins across these divergent DDR pathways (Table 1).

DDR as Potential Combination Targets for Dual Payload ADC Development

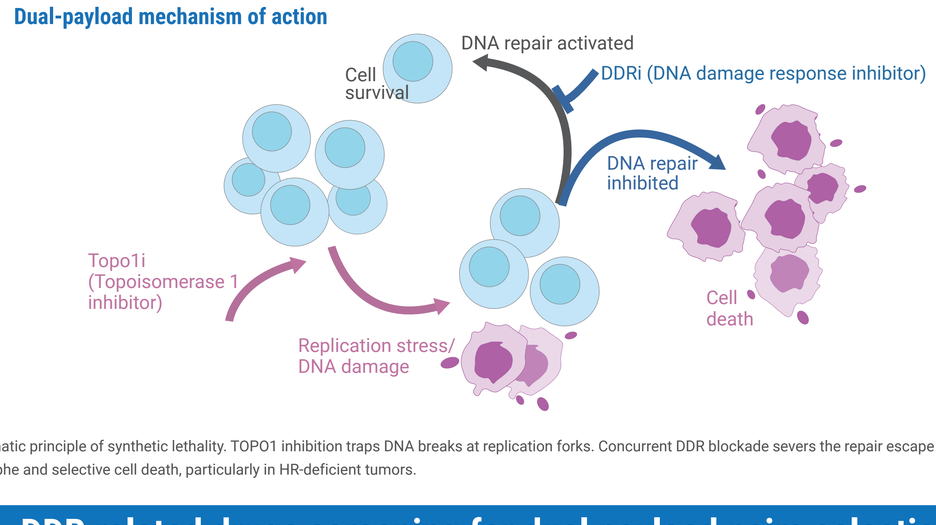


Figure 1. Schematic principle of synthetic lethality. TOPO1 inhibition traps DNA breaks at replication forks. Concurrent DDR blockade severs the repair escape route, leading to replication catastrophe and selective cell death, particularly in HR-deficient tumors.

DDR related drugs screening for dual payload pairs selection

126 Drug Pairs Screening with Fixed Ratio Combination

126 drug pairs (as shown in below table) were screened in ADC/payload-resistant cell lines and corresponding parental cell lins to identify synergistic combinations. 3-day proliferation assays showed DDR-associated targets exhibited significant synergy (IC_{50} shift vs. monotherapy).One DDR target - ATR were further validated in cancer cell models and expanded resistant cell line panel.

Drug A Target			Drug A Name
TOPO1	Dxd	Exatecan	SN-38
Drug B Target			**Drug B Name**
WRN	HRO761		
WEE1	MK-1775		
USP1	KSQ-4279		
PARP1/2	Talazoparib	Olaparib	
DNA-PK	AZD-7648		
CHK1	Prexasertib		
ATR	Ceralasertib	Berzosertib	
PKMYT1	RP-6306		
CDK4/6	Palbociclib	Ribociclib	Abemaciclib
PLK1	GSK461364		
PRMT5	AMG-193	MRTX-1719	
TKR	Selpercatinib	Osimertinib	Crizotinib	Repotrectinib	Alectinib
TKR downstream pathway	BAY-293	Alpelisib	BBO-10203	Everolimus	Dabrafenib
RAS	MRTX1133	Sotorasib	pan-KRAS-IN-5	RMC-6236	
Microtubule	Docetaxel	MMAE			
Chemotherapy	Gemcitabine	Pemetrexed	Cisplatin	Carboplatin	Etoposide
Others	Sorafenib	Triptolide	Tambiciclib	Homoharringtonine	Duocarmycin TM

	Preclinical
	Phase I Clinical Trial
	Phase I/II Clinical Trial
	Phase III Clinical Trial
	Approved (Launched)
	Terminated or withdrawn

DLD-1			Dxd	Exatecan	SN-38
			A_IC50, nM	A_IC50, nM	A_IC50, nM
			19.77	6.02	31.99
Single Group			Combination Group		
Entry	Target	Compound ID	A_IC50, nM	A_IC50, nM	A_IC50, nM	A_IC50, nM
1	ATR	Berzosertib	613.66	32.74	11.93	54.7
2	ATR	Ceralasertib	9098.33	63.24	20.82	67.76

DLD-1-Exatecan R			Dxd	Exatecan	SN-38
			A_IC50, nM	A_IC50, nM	A_IC50, nM
			2849.69	869.74	>15000
Single Group			Combination Group		
Entry	Target	Compound ID	A_IC50, nM	A_IC50, nM	A_IC50, nM	A_IC50, nM
1	ATR	Berzosertib	1928.7	523.25	455.3	1897.35
2	ATR	Ceralasertib	13457.22	2005.78	1155.39	9136.66

NCI-N87			Dxd	Exatecan	SN-38
			A_IC50, nM	A_IC50, nM	A_IC50, nM
			39.03	12.5	38.38
Single Group			Combination Group		
Entry	Target	Compound ID	A_IC50, nM	A_IC50, nM	A_IC50, nM	A_IC50, nM
1	PARP1/2	Talazoparib	>15000	30.77	12.98	32.57
2	WEE1	MK-1775	861.89	68.76	21.48	68.2
3	PKMYT1	RP-6306	8209.87	53.69	16.49	58.66
4	ATR	Berzosertib	2390.5	123.71	76.88	237.71
5	ATR	Ceralasertib	9014.89	126.65	49.40	78.11
6	STAT3	Homoharringtonine	>15000	768.81	785.16	845.31
7	PI3Kα	BBO-10203	486.38	23.19	13.45	18.46

NCI-N87-DS8201 R			Dxd	Exatecan	SN-38
			A_IC50, nM	A_IC50, nM	A_IC50, nM
			3227.5	765.7	10511.43
Single Group			Combination Group		
Entry	Target	Compound ID	A_IC50, nM	A_IC50, nM	A_IC50, nM	A_IC50, nM
1	PARP1/2	Talazoparib	>15000	2174.93	374.3	1899.08
2	WEE1	MK-1775	1808.66	709.64	359.31	489.38
3	PKMYT1	RP-6306	>15000	552.38	249.84	365.12
4	ATR	Berzosertib	4340.8	873.34	372.76	922.68
5	ATR	Ceralasertib	>15000	1841.71	806.98	1138.73
6	STAT3	Homoharringtonine	421.55	445.83	181.98	477.47
7	PI3Kα	BBO-10203	1747.76	1199.27	457.08	998.39

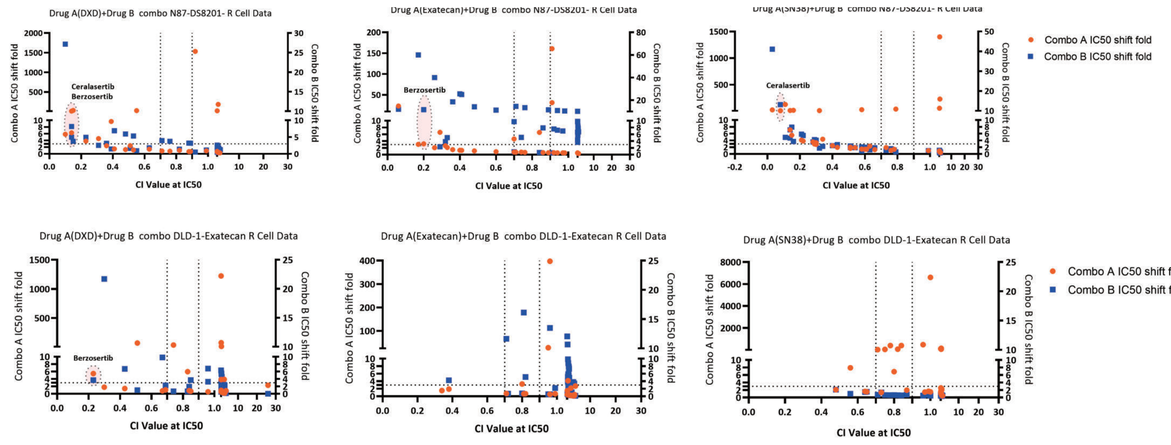


Figure 2. IC50 shift and Chou–Talalay CI at IC50 for fixed-ratio combination.

Combination of DDR inhibitors with TOPO1 inhibitors in Cancer cells

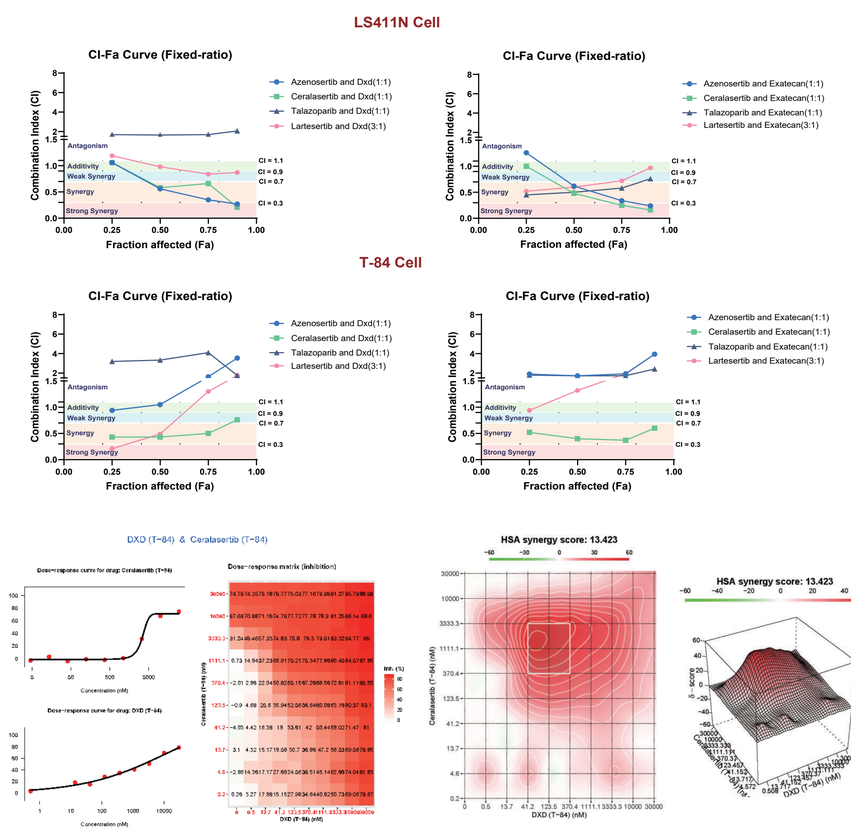


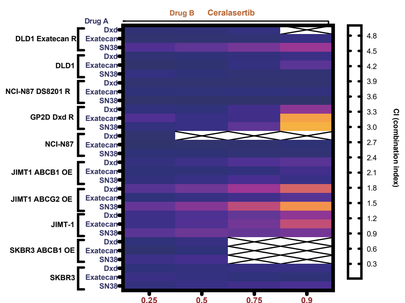
Figure 3. Chou–Talalay CI-Fa curves for fixed-ratio DDRi + TOPO1i combinations in LS411N (upper two panels) and T-84 (lower two panels) cells. dashed grey lines indicate synergy (0.9), additivity (1.1) and strong-synergy (0.7) boundaries. Color code: red ≤ 0.3 (strong synergy), orange 0.3-0.7(synergy), blue 0.7–0.9 (weak synergy), green 0.9-1.1 (additive), no color ≥ 1.1 (antagonism).

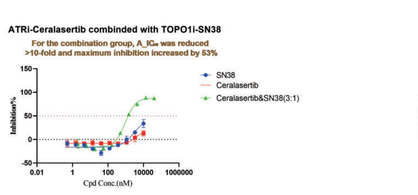
Figure 4. Matrix-combination synergy maps for dual-payload ATRi + TOPO1i pairs. Cells were exposed to 10 × 10 dose matrices (0.1–10 000 nM, 3-fold dilution) for 72 h; viability was assessed by CTG. The left column shows single-agent dose–response curves, while the remaining grids reveal pronounced synergy for Ceralasertib with Dxd (HSA score > 13 across ≥ 2 log concentration windows), confirming strong synergistic activity in T-84 cells.

ATRi and TOPO1 inhibitors pairs combination in ADC and Payload resistant cell panel

No.	Drug Resistant Cells	Status	RI#	STR	RNA-seq	WES	Drug	Drug Type	Target	Cancer Type	Method
1	NCI-N87/DS8201 R	In vitro validated	> 100	√	√	√	DS-8201	ADC	HER2, Topoisomerase I	Gastric	Natural Induction (in vitro)
2	NCI-N87/T-DM1 R	In vitro validated	> 10	√	Ongoing	Ongoing	T-DM1	ADC	HER2, Tubulin	Gastric	Natural Induction (in vitro)
3	HCC1806/IMMU-132 R	In vitro validated	> 10	√	√	√	IMMU-132	ADC	TROP2, Topoisomerase I	Breast	Natural Induction (in vitro)
4	DLD-1/Exatecan R	In vitro validated	> 100	√	√	√	Exatecan	payload	Topoisomerase I	Colorectal	Natural Induction (in vitro)
5	SK-OV-3/Dxd R	In vitro validated	>10	√	√	√	Dxd	payload	Topoisomerase I	Ovarian	Natural Induction (in vitro)
6	GP2d/Dxd R	In vitro validated	> 10	√	√	√	Dxd	payload	Topoisomerase I	Colorectal	Natural Induction (in vitro)
7	HCC1806/Dxd R	In vitro validated	> 50	√	Ongoing	Ongoing	Dxd	payload	Topoisomerase I	Breast	Natural Induction (in vitro)
8	GP2d/SN-38 R	In vitro validated	> 5	√	√	√	SN-38	payload	Topoisomerase I	Colorectal	Natural Induction (in vitro)
9	HCC1806/SN-38 R	In vitro validated	> 20	√	√	Ongoing	SN-38	payload	Topoisomerase I	Breast	Natural Induction (in vitro)
10	NCI-N87/SN-38 R	In vitro validated	> 5	√	Ongoing	Ongoing	SN-38	payload	Topoisomerase I	Gastric	Natural Induction (in vitro)
11	NUGC-4/MMAE R	In vitro validated	> 10	√	√	√	MMAE	payload	Tubulin	Gastric	Natural Induction (in vitro)
12	OVCAR3/MMAE R	In vitro validated	> 5	√	√	√	MMAE	payload	Tubulin	Ovarian	Natural Induction (in vitro)
13	HCC1806/MMAE R	In vitro validated	> 10	√	√	√	MMAE	payload	Tubulin	Breast	Natural Induction (in vitro)
14	SK-OV-3/MMAE R	In vitro validated	> 10	√	√	√	MMAE	payload	Tubulin	Ovarian	Natural Induction (in vitro)
15	HCC4006/MMAE R	In vitro validated	> 50	√	Ongoing	Ongoing	MMAE	payload	Tubulin	Lung	Natural Induction (in vitro)
16	OVCAR3-ABCB1-OE	In vitro validated	/	√	/	/	/	ADC/payload	ABCB1	Ovarian	Genetic Editing
17	OVCAR3-ABCG2-OE	In vitro validated	/	√	/	/	/	ADC/payload	ABCG2	Ovarian	Genetic Editing
18	NCI-N87-ABCB1-OE	In vitro validated	/	√	/	/	/	ADC/payload	ABCB1	Gastric	Genetic Editing
19	NCI-N87-ABCG2-OE	In vitro validated	/	√	/	/	/	ADC/payload	ABCG2	Gastric	Genetic Editing
20	JIMT-1-ABCB1-OE	In vitro validated	/	√	/	/	/	ADC/payload	ABCB1	Breast	Genetic Editing
21	JIMT-1-ABCG2-OE	In vitro validated	/	√	/	/	/	ADC/payload	ABCG2	Breast	Genetic Editing
22	SK-BR-3-ABCB1-OE	In vitro validated	/	√	/	/	/	ADC/payload	ABCB1	Breast	Genetic Editing
23	SK-BR-3-ABCG2-OE	In vitro validated	/	√	/	/	/	ADC/payload	ABCG2	Breast	Genetic Editing
24	HCT116-TOP1-R364H	In vitro validated	/	√	/	/	/	ADC/payload	Topoisomerase I	Colorectal	Genetic Editing

RI#: fold increase in IC_{50} (resistant vs parental)





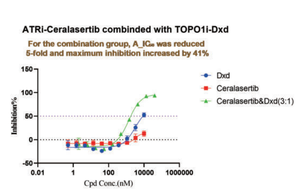
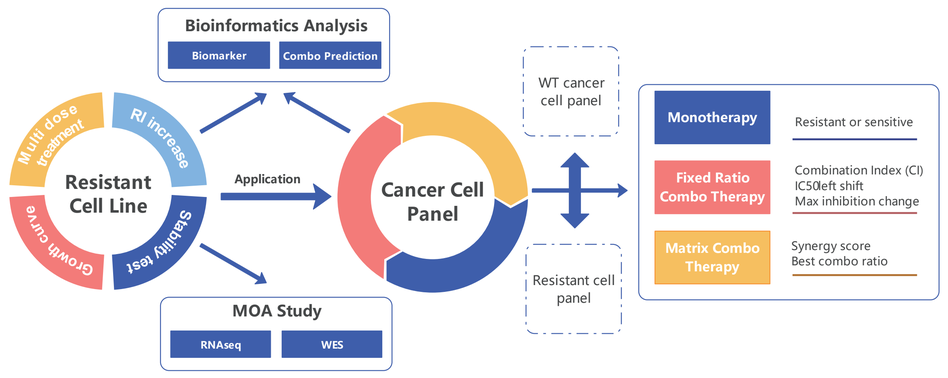


Figure 5. Synergy landscape of TOPO1–DDR combinations across ADC-resistant and wild-type cancer cell lines(A) and IC50 curves(B). Heat map shows the Chou–Talalay combination index (CI) calculated at Fa = 0.25, 0.5, 0.75 and 0.9 (columns) for 10 ADC-related resistant or WT cell lines (rows) and six fixed-ratio drug pairs (matrix columns). Crosses mark conditions where accurate CI could not be determined because of incomplete curves or maximum inhibition < 50 %.

Resistant Cell Panel Workflow



Summary

In this study, Using our ADC/payload-resistant cell line platform, over 120 drug combinations was screened, with one fixed as TOPO1 inhibitors and the other targeting DDR proteins, cell cycle regulators, kinases, or chemicals. Results identified strong synergy between CHK1/ATR inhibitors and TOPO1 inhibitors, offering optimal candidates for DDR-targeted dual-payload ADC development and accelerating next-generation DDR-based ADC research..