A Dedicated ADC-Resistant Cell Line Platform for High-Throughput Screening of Synergistic Dual Payload Combinations to Overcome ADC Resistance

Lili Chai, Xue Yang, Wei Liu, Yue Zhai, Yan Zhang, Ying Bi, Ying Meng and Tiejun Bing
ICE Bioscience, INC. Building 16, Yard 18, Kechuang 13th Street, Beijing, 100176
bingtj@ice-biosci.com chaill@ice-biosci.com

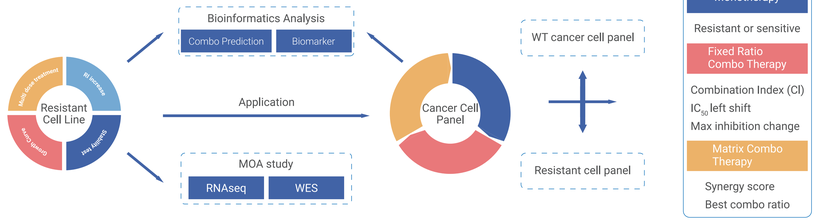
Poster No. 2964

Innovative CRO+Explorer
ICE Bioscience

Introduction

Antibody-drug conjugates (ADCs) represent a promising antineoplastic strategy by combining antibody targeting with potent cytotoxic drugs. However, clinical challenges remain, including tumor non-responsiveness, drug resistance, and disease relapse, largely due to tumor heterogeneity and resistance mechanisms. Dual-payload ADCs have emerged as an innovative approach to improve efficacy through synergistic cytotoxicity, overcome resistance, and support flexible dosing regimens. The development of such ADCs relies on identifying effective payload combinations, which can be efficiently explored using cell panel screening and drug-resistant cell models. In this study, we performed large-scale combination screening in ADC-resistant cell lines to identify synergistic payload pairs. The screening panel included more than 120 targeted agents and chemotherapeutics. Our results identified that ATR inhibitors as well as CHK1/2 inhibitors exhibited favorable synergistic effects in combination with Topoisomerase I (TOPO1) inhibitors, whose detailed mechanisms warrant further validation. This optimized combination serves as a direct candidate for dual-payload ADC development, with the potential to accelerate the discovery and translation of next-generation ADC therapies.

Resistant Cell Panel Workflow



Multi-Drug Resistance Profile in the Resistant Cell Lines

Table 1. Drug Sensitivity Profiles of Established ADC/Payload-Resistant Cell Lines Determined by Resistance Index (RI).

Resistance Index (RI)		ADC/Payload Resistant Cell Lines														
Drugs \ Cells		ADC (HER2)		ADC (TROP2)	Payload (Topoisomerase I inhibitor)							Payload (Tubulin inhibitor)				
		NCI-N87 /DS-8201 R	NCI-N87 /T-DM1 R	HCC1806 /IMMU-132 R	DLD-1 /Exatecan R	GP2d /Dxd R	SK-OV-3 /Dxd R	HCC1806 /Dxd R	GP2d /SN-38 R	HCC1806 /SN-38 R	NCI-N87 /SN-38 R	HCC1806 /MMAE R	SK-OV-3 /MMAE R	NUGC-4 /MMAE R	OVCAR3 /MMAE R	HCC4006 /MMAE R
DS-8201	ADC (HER2)	> 100	1	Insensitive*	Insensitive*	Insensitive*	ND#	Insensitive*	Insensitive*	Insensitive*	> 5	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*
T-DM1	ADC (HER2)	1	> 10	Insensitive*	Insensitive*	> 20	ND#	> 20	Insensitive*	Insensitive*	4	Insensitive*	5	Insensitive*	Insensitive*	Insensitive*
IMMU-132	ADC (TROP2)	> 5	2	> 10	> 100	> 20	ND#	> 20	ND#	> 20	> 10	2	2	Insensitive*	1	1
SKB264	ADC (TROP2)	> 5	1	> 5	> 100	4	ND#	> 20	ND#	> 10	> 5	4	5	Insensitive*	1	1
ABBV-399	ADC (c-Met)	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	ND#	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	> 100	Insensitive*	> 100
MRG003	ADC (EGFR)	1	2	4	Insensitive*	> 100	ND#	3	ND#	1	3	> 100	> 100	ND#	> 100	> 100
HER3-Dxd	ADC (HER3)	Insensitive*	Insensitive*	Insensitive*	ND#	ND#	ND#	Insensitive*	ND#	Insensitive*	Insensitive*	Insensitive*	ND#	Insensitive*	Insensitive*	Insensitive*
Dxd	Payload(Topoisomerase I inhibitor)	> 10	1	> 50	> 100	> 10	> 10	>50	3	>50	> 5	5	1	1	1	1
Exatecan	Payload(Topoisomerase I inhibitor)	3	1	> 10	> 100	1	ND#	>20	1	>10	2	ND#	2	1	1	1
KL610023	Payload(Topoisomerase I inhibitor)	4	1	> 5	> 100	2	ND#	>20	ND#	>10	5	ND#	3	ND#	ND#	1
SN-38	Payload (Topoisomerase I inhibitor)	> 10	2	> 20	> 100	> 10	ND#	>20	> 5	>50	> 5	2	2	1	1	1
ZD06519	Payload(Topoisomerase I inhibitor)	> 10	1	> 20	> 100	> 10	ND#	>100	> 5	>20	> 5	ND#	2	1	1	2
MMAE	Payload (Tubulin inhibitor)	2	2	1	3	> 5	> 10	2	ND#	2	2	> 10	> 20	> 10	> 5	> 50
DM1	Payload (Tubulin inhibitor)	2	1	1	4	4	ND#	2	ND#	3	1	ND#	> 20	2	1	> 20
Irinotecan	Chemotherapeutics	ND#	ND#	ND#	ND#	5	ND#	ND#	5	ND#	ND#	ND#	ND#	ND#	ND#	ND#

Resistance Index (RI) was calculated as the ratio of IC_{50} in resistant cells to IC_{50} in corresponding parental cells (RI = IC_{50}resistant / IC_{50}parental).

Dual payload pairs Screening in Resistant cell lines

1:1 Fixed ratio in Resistant cells

10*10 dose Matrix combination in resistant cells

10*10 dose Matrix combination in Cancer cells

120+ Drug Pairs Screening with Fixed Ratio Combination

Table 2. 126 drug pairs were screened in ADC/payload-resistant cell lines and corresponding parental cell lines to identify synergistic combinations.

Drug A Target	Drug A Name					
TOPO1	Dxd	Exatecan	SN-38			
Drug B Target	**Drug A Name**					
WRN	HRO761					
WEE1	MK-1775					
USP1	KSQ-4279					
PARP1/2	Talazoparib	Olaparib				
DNA-PK	AZD-7648					Preclinical
CHK1	Prexasertib					Phase I Clinical Trial
ATR	Ceralasertib	Berzosertib				Phase I/II Clinical Trial
PKMYT1	RP-6306					Phase III Clinical Trial
CDK4/6	Palbociclib	Ribociclib	Abemaciclib			Approved(Launched)
PLK1	GSK461364					Terminated or withdrawn
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	Duocarmycin TM
Others	Sorafenib	Triptolide	Tambiciclib	Homoharringtonine		

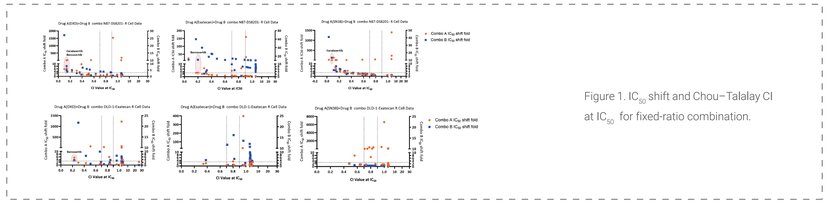


Figure 1. IC_{50} shift and Chou–Talalay CI at IC_{50} for fixed-ratio combination.

ATRi/CHK1/2i and TOPO1 inhibitors pairs combination in ADC and Payload resistant cell panel

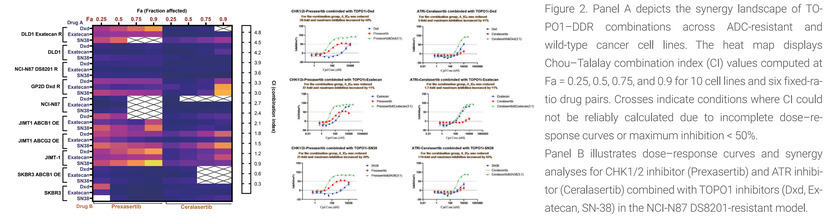


Figure 2. Panel A depicts the synergy landscape of TOPO1–DDR combinations across ADC-resistant and wild-type cancer cell lines. The heat map displays Chou–Talalay combination index (CI) values computed at Fa = 0.25, 0.5, 0.75, and 0.9 for 10 cell lines and six fixed-ratio drug pairs. Crosses indicate conditions where CI could not be reliably calculated due to incomplete dose–response curves or maximum inhibition < 50%.
Panel B illustrates dose–response curves and synergy analyses for CHK1/2 inhibitor (Prexasertib) and ATR inhibitor (Ceralasertib) combined with TOPO1 inhibitors (Dxd, Exatecan, SN-38) in the NCI-N87 DS8201-resistant model.

Dual payload matrix combination testing in Resistant cells

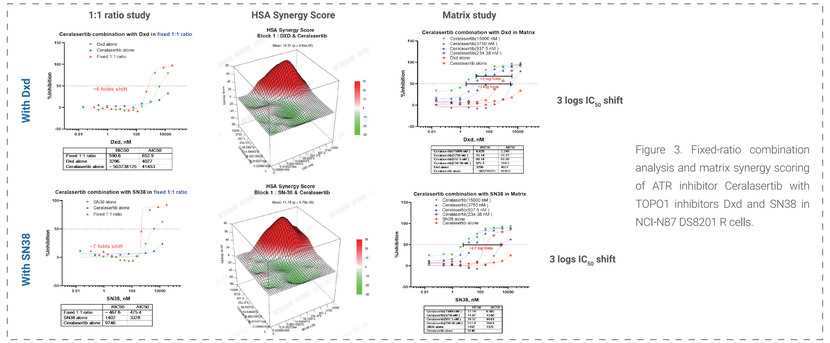


Figure 3. Fixed-ratio combination analysis and matrix synergy scoring of ATR inhibitor Ceralasertib with TOPO1 inhibitors Dxd and SN38 in NCI-N87 DS8201 R cells.

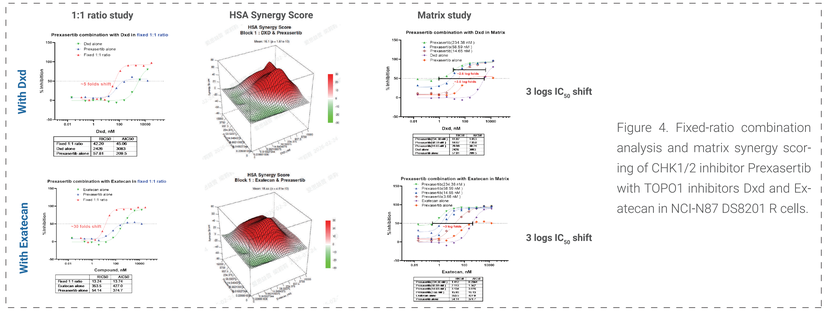


Figure 4. Fixed-ratio combination analysis and matrix synergy scoring of CHK1/2 inhibitor Prexasertib with TOPO1 inhibitors Dxd and Exatecan in NCI-N87 DS8201 R cells.

Table 3. Matrix-combination synergy analysis

Pair #	Drug A Name	Drug A Target	Drug B Name	Drug B Target	Cell Line	IC_{50} Shift Folds in 1:1 Fixed Ratio compared with A/B	Drug A IC_{50} Shift folds in Matrix	Matrix Synergy Score (ZIP method)	Matrix Synergy Score (HSA method)	Indication
1	Dxd	TOPO1	B erzosertib	ATR/ATM	DLD1 Exatecan R	~2 / 4.5	>4 log	5.56	9.43	
2	Exatecan	TOPO1			DLD1 Exatecan R	~3.5/ 7.4	>4 log	10.33	12.61	Strong synergistic effect
3	Dxd	TOPO1			NCI-N87 DS8201 R	~10/ 3.5	~3 log	7.58	6.61	
4	Exatecan	TOPO1			NCI-N87 DS8201 R	~3.8 / 11	~3 log	8.03	8.13	
5	Dxd	TOPO1	Ceralasertib	ATR	NCI-N87 DS8201 R	~6/ >20	~3 log	11.84	10.31	Strong synergistic effect
6	SN38	TOPO1			NCI-N87 DS8201 R	~7/ >20	~2.7 log	10.05	11.19	Strong synergistic effect
7	Dxd	TOPO1	Prexasertib	CHK1/CHK2	NCI-N87 DS8201 R	~68 / 5	~3.5 log	13.61	16.1	Strong synergistic effect
8	Exatecan	TOPO1			NCI-N87 DS8201 R	~30/ 30	~3 log	14.9	18.44	Strong synergistic effect
9	SN38	TOPO1			NCI-N87 DS8201 R	10/ 120	~3 log	17.62	20.55	Strong synergistic effect
13	SN38	TOPO1	Talazoparib	PARP	NCI-N87 DS8201 R	~3/ >20	~20	5.15	8.43	

Dual payload combination testing in cancer cells

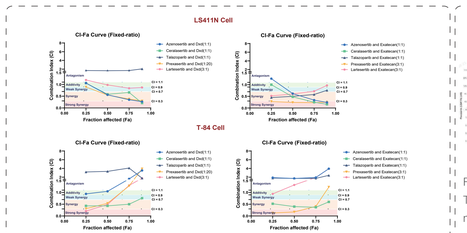


Figure 5. Chou–Talalay CI-Fa curves for fixed-ratio DDRi + TOPO1i combinations in LS411N (upper two panels) and T-84 (lower two panels) cells. dashed grey lines indicate synergy (0.9), additivity (1.1) and strong-synergy (0.7) boundaries. Color code: red ≤ 0.3 (strong synergy), orange 0.3-0.7(synergy), blue 0.7–0.9 (weak synergy), green 0.9-1.1 (additive), no color ≥ 1.1 (antagonism).

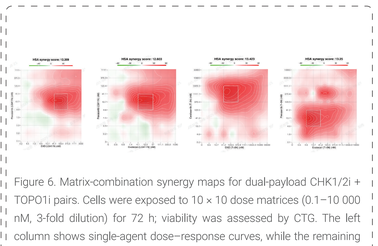


Figure 6. Matrix-combination synergy maps for dual-payload CHK1/2i + TOPO1i pairs. Cells were exposed to 10 × 10 dose matrices (0.1–10 000 nM, 3-fold dilution) for 72 h; viability was assessed by CTG. The left column shows single-agent dose–response curves, while the remaining grids reveal pronounced synergy for Prexasertib with both Dxd and Exatecan (HSA score > 12 across ≥ 2 log concentration windows), confirming strong synergistic activity in LS411N and T-84 cells.

Summary

In this study, using our established ADC/payload-resistant cell line platform, we screened more than 120 drug combinations with a fixed TOPO1 inhibitor component paired with agents targeting DDR proteins, cell cycle regulators, kinases, and other small molecules. Our results demonstrated strong synergistic effects of ATR inhibitors and CHK1/2 inhibitors in combination with TOPO1 inhibitors. Consistent synergistic activity was further validated in tumor cells insensitive to payload monotherapy. Collectively, this study and platform provide optimal candidate combinations for the development of DDR-targeted dual-payload ADCs and advance the research of next-generation DDR pathway–directed ADC therapies.