A Well-Characterized ADC-Resistant Cell Line Platform for Overcoming Resistance and Dual-Payload Screening

Xue Yang, Wei Liu, Yue Zhai, Yan Zhang, Ying Bi and Tiejun Bing,Fujun Zhang, Ben Wei,Lili Chai

ICE Bioscience, INC. Building 14, Yard 18, Kechuang 13th Street, Beijing, China 100176

Email: bingtj@ice-biosci.com

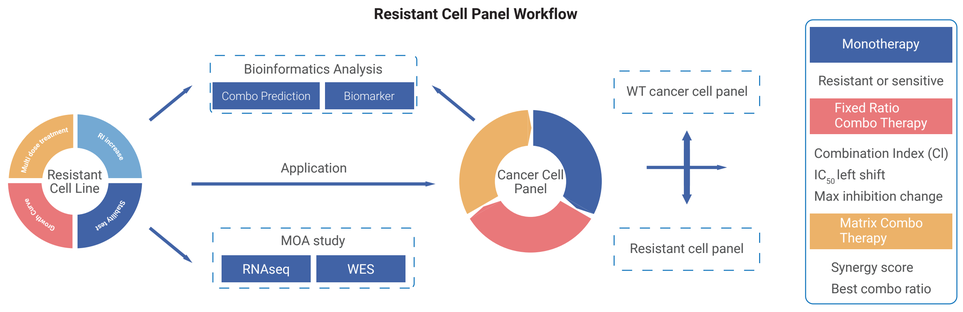
Download FDF

Poster Number : P061

Innovative CRO+Explorer
ICE Bioscience

Introduction

Antibody-drug conjugates (ADCs) are key targeted therapies, yet drug resistance remains a major clinical challenge. To address this, we established a panel of 28 well-characterized ADC-resistant cell lines, validated via resistance profiling, RNA-seq, and WES analysis. This platform enables high-throughput screening of novel payloads and combinations to overcome resistance.



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	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*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*
T-DM1	ADC (HER2)	1	> 10	Insensitive*	Insensitive*	Insensitive*	ND#	Insensitive*	Insensitive*	Insensitive*	Insensitive*	5	Insensitive*	Insensitive*	Insensitive*
IMMU-132	ADC (TROP2)	> 5	2	> 10	> 100	> 20	ND#	> 20	ND#	> 20	2	2	Insensitive*	1	1
SKB264	ADC (TROP2)	> 5	1	> 5	> 100	4	ND#	> 20	ND#	> 10	4	5	Insensitive*	1	1
ABBV-399	ADC (c-Met)	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	ND#	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	> 100	Insensitive*	> 100
MRG003	ADC (EGFR)	1	2	4	Insensitive*	> 100	ND#	3	ND#	1	> 100	> 100	ND#	> 100	> 100
HER3-Dxd	ADC (HER3)	Insensitive*	Insensitive*	Insensitive*	ND#	ND#	ND#	Insensitive*	ND#	Insensitive*	Insensitive*	ND#	Insensitive*	Insensitive*	Insensitive*
Dxd	Payload(Topois omerase I inhibitor)	> 10	1	> 50	> 100	> 10	> 10	>50	3	>50	5	1	1	1	1
Exatecan	Payload(Topois omerase I inhibitor)	3	1	> 10	> 100	1	ND#	>20	1	>10	ND#	2	1	1	1
KL610023	Payload(Topois omerase I inhibitor)	4	1	> 5	> 100	2	ND#	>20	ND#	>10	ND#	3	ND#	ND#	1
SN-38	Payload (Topois omerase I inhibitor)	> 10	2	> 20	> 100	> 10	ND#	>20	> 5	>50	2	2	1	1	1
ZD06519	Payload(Topois omerase I inhibitor)	> 10	1	> 20	> 100	> 10	ND#	>100	> 5	>20	ND#	2	1	1	2
MMAE	Payload (Tubulin inhibitor)	2	2	1	3	> 5	> 10	2	ND#	2	> 10	> 20	> 10	> 5	> 50
DM1	Payload (Tubulin inhibitor)	2	1	1	4	4	ND#	2	ND#	3	ND#	> 20	2	1	> 20
Irinotecan	Chemotherapeutics	ND#	ND#	ND#	ND#	5	ND#	ND#	5	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).

Insensitive*: The parental cell line shows no response to the tested drugs. ND#: Not Done

Case study 1: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

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					
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	Duocarmycin TM
Others	Sorafenib	Triptolide	Tambiciclib	Homoharringtonine		

Table 3. Matrix-combination synergy analysis in ADC and Payload Resistant cell lines

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	

Note: The synergistic parameter > 10 indicates that this drug group shows synergy effect.

Case study 2:Dual Payload Pairs Matrix Screening in Cancer Cell Lines

Table 4. Matrix-combination synergy analysis in WT CRC cell lines

Pair #	Drug A Name	Drug A Target	Drug B Name	Drug B Target	Cell Line	Drug A IC_{50} shift folds in Matrix	Matrix synergy Score(HSA method)	Indication
1	Dxd	TOPO1	Berzosertib	ATR/ATM	COLO 320 DN	~1.56 log folds	10.99	Strong synergistic effect
2	Dxd	TOPO1			HT-55	~2.2 log folds	11 16	Strong synergistic effect
3	Dxd	TOPO1			LS411N	~2.1 log folds	12.58	Strong synergistic effect
4	Dxd	TOPO1			NCI-H747	~2.07 log folds	12.94	Strong synergistic effect
5	Dxd	TOPO1			SW480	~1.98 log folds	16.73	Strong synergistic effect
6	Dxd	TOPO1			SW620	~1 log folds	12.51	Strong synergistic effect
7	Dxd	TOPO1			T84	~2.49 log folds	13.19	Strong synergistic effect
8	Dxd	TOPO1			SW948	~3.43 log folds	19.03	Strong synergistic effect
9	Dxd	TOPO1	Ceralasertib	ATR	LS411N	~2.34 log folds	15.07	Strong synergistic effect
10	Dxd	TOPO1			SW480	~1.5 log folds	10.23	Strong synergistic effect
11	Dxd	TOPO1			SW948	~2.06 log folds	9.98	
12	Dxd	TOPO1			T84	~2.5 log folds	15.36	Strong synergistic effect
13	Dxd	TOPO1	Prexasertib	CHK1/CHK2	HT-55	~2.03 log folds	20.80	Strong synergistic effect
14	Dxd	TOPO1			LS411N	~2.46 log folds	14.39	Strong synergistic effect
15	Dxd	TOPO1			SW480	~2.24 log folds	17.72	Strong synergistic effect
16	Dxd	TOPO1			SW620	~1.63 log folds	20.68	Strong synergistic effect
17	Dxd	TOPO1			T84	~2.36 log folds	11.10	Strong synergistic effect
18	Dxd	TOPO1			SW948	~2.7 log folds	24.30	Strong synergistic effect

Note: The synergistic parameter > 10 indicates that this drug group shows synergy effect.

Case study 3: HTS Compound Library Screening in ADC resistant cell lines

Hit Screening with single dose Sensitive or Resistant → Full doses testing of Candidate cpds RI testing → 10*10 dose Matrix combination Synergy score

Cell lines

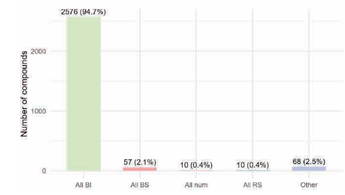
- HCC1806
- HCC1806/SN-38 R
- HCC1806/IMMU-132 R
- HCC1806/Dxd R

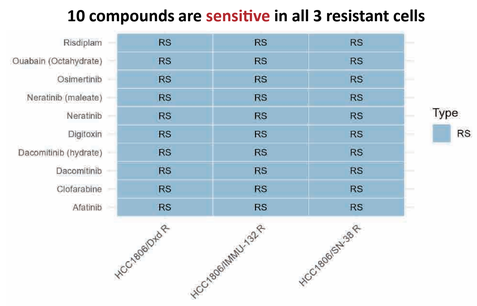
FDA-Approved Drug Library

- Hits screening (2721 compounds, at 100 nM for 7 days)

Table 5. Library Screening in Resistant Cell lines

Item		% Inhibition
△	Difference between 2 cells	Parental cells ≥ 50%,Resistant cells < 50%
RS	Resistantcellssensitive	Parental cells < 50%,Resistant cells ≥50%
BS	Both cells sensitive	Parental cells ≥ 50%,Resistant cells ≥ 50%
BI	Both cells insensitive	Parental cells < 50%,Resistant cells < 50%





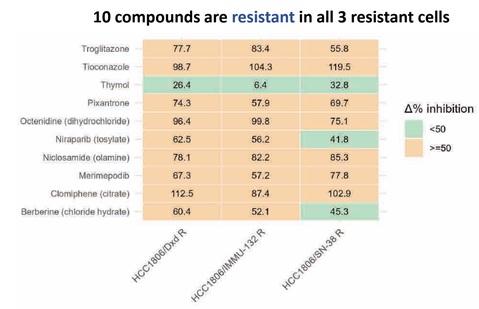


Table 6. Candidates Data Summary

Table 6. Multi-drug Sensitivity (14 compounds)				
Compound_ID	RI			Target
	HCC1806/Dxd R	HCC1806/SN-38 R	HCC1806/IMMU-132 R	
Afatinib	0.40	0.14	0.69	Akt; Apoptosis; Autophagy; c-Met/HGFR; EGFR; p38 MAPK
Almonertinib	0.50	0.17	0.87	EGFR
Cobimetinib (hemifumarate)	0.27	0.05	2.36	Apoptosis; MEK
Dacomitinib	0.10	0.04	0.25	Apoptosis; EGFR
Erlotinib(Hydrochloride)	0.19	0.05	0.64	Autophagy; EGFR
Ibrutinib	0.08	0.02	0.18	Btk; Ligands for Target Protein for PROTAC
Icotinib	0.10	0.03	0.27	EGFR
Lapatinib(ditosylate)	0.22	0.08	0.86	Autophagy; EGFR; Ferroptosis
Lonafarnib	0.03	0.33	0.22	Autophagy; Farnesyl Transferase; Ras
Mobocertinib	0.35	0.12	0.86	EGFR
Neratinib(maleate)	0.12	0.07	0.43	EGFR
Osimertinib	0.20	0.10	0.44	EGFR
Simotinib	0.10	0.02	0.29	EGFR
Trametinib	0.22	0.01	0.38	Apoptosis; Autophagy; MEK

Note: RI < 0.2 indicates the resistant cells are sensitive to the compounds comparing with parental cells.

Table 7. Matrix-combination synergy analysis

Drug A	Drug B	Cell Line	Parameter	Score	Significant
IMMU-132	Erlotinib	HCC1806	Bliss	10.3	***
IMMU-132	Lapatinib	HCC1806/IMMU-132 R	Bliss	10.1	***
IMMU-132	Lapatinib	HCC1806/IMMU-132 R	HSA	10.1	***

Note: The synergistic parameter > 10 indicates that this drug group shows synergy effect.

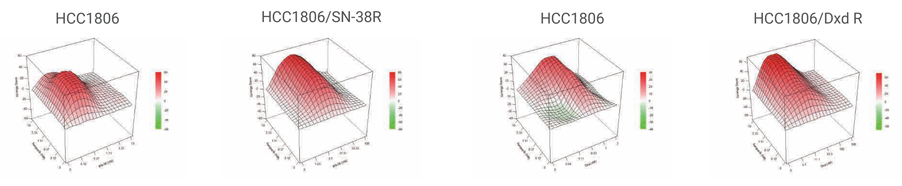
Case study 4: Combination Small Library screening in ADC resistant cell lines

Drug A (single-dose: high + low) Drug B: Customized Library-92 compounds → Combination Candidates IC50 shift ≥ 10 folds → 10*10 dose Matrix combination Synergy score>=10

Table 8. Matrix-combination synergy analysis

Drug A	Drug A Target	Drug B	Drug B Target	Cell Line	ZIP Score
SN-38	TOPO1	Camonsertib	ATR	HCC1806/SN-38 R	12.02
SN-38	TOPO1	Olaparib	PARP1/2	HCC1806	12.46
SN-38	TOPO1	Olaparib	PARP1/2	HCC1806/SN-38 R	10.37
SN-38	TOPO1	Saruparib	PARP1	HCC1806	11.22
SN-38	TOPO1	Saruparib	PARP1	HCC1806/SN-38 R	18.67
Dxd	TOPO1	Olaparib	PARP1/2	HCC1806	10.78
Dxd	TOPO1	Olaparib	PARP1/2	HCC1806/Dxd R	10.93
Dxd	TOPO1	RG7112	MDM2	HCC1806	10.57
Dxd	TOPO1	RG7112	MDM2	HCC1806/Dxd R	16.20
Dxd	TOPO1	Saruparib	PARP1	HCC1806/Dxd R	18.60
IMMU-132	TROP2 ADC	A-485	P300/CBP	HCC1806	15.79
IMMU-132	TROP2 ADC	A-485	P300/CBP	HCC1806/IMMU-132 R	21.48
IMMU-132	TROP2 ADC	Alectinib	ALK	HCC1806/IMMU-132 R	13.49
IMMU-132	TROP2 ADC	Cabozantinib	VEGFR2,MET	HCC1806/IMMU-132 R	12.33
IMMU-132	TROP2 ADC	Infigratinib	FGFR	HCC1806/IMMU-132 R	12.61
IMMU-132	TROP2 ADC	Selpercatinib	RET	HCC1806/IMMU-132 R	13.05

Note: The synergistic parameter > 10 indicates that this drug group shows synergy effect.



Conclusion

We established and fully validated 28 ADC-resistant cell lines covering mainstream ADCs and payload resistance phenotypes. Matrix combination screening identified significant synergistic effects between TOPO1 payloads and ATR/CHK1/PARP inhibitors in resistant cell models. HTS library screening yielded multiple targeted small molecule candidates effective against ADC resistance. This platform enables efficient screening of novel payloads and synergistic combinations to accelerate next-generation ADC and combination therapy development.