

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

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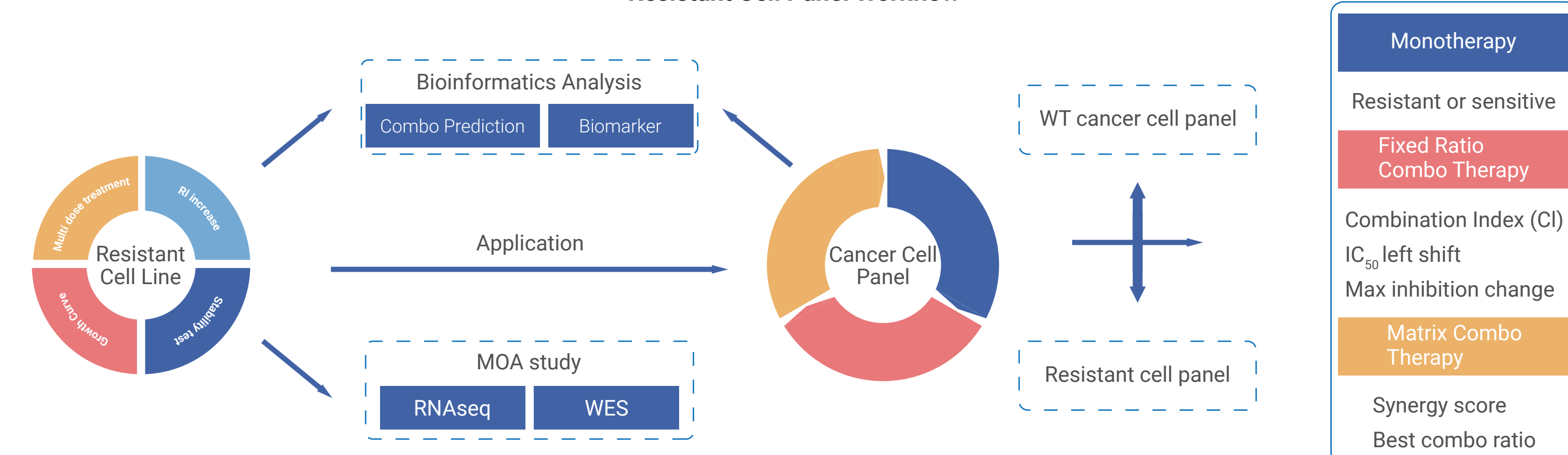


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## Introduction

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.

### 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).

Drugs	Cells	ADC/Payload-Resistant Cell Lines													
		ADC (HER2)				ADC (TROP2)				Payload (Topoisomerase I Inhibitor)					
		NCI-N87 /DS-2201 R	NCI-N87 /T-DM1 R	HCC1806 /IMMU-132 R	DLD-1 /Euxetecan 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	NUCC-4 /MMAE R	QVCAR3 /MMAE R	HCC4006 /MMAE R
DS-201	ADC (HER2)	> 100	1	In sensitive*	In sensitive*	In sensitive*	ND#	In sensitive*	In sensitive*	In sensitive*	In sensitive*	In sensitive*	In sensitive*	In sensitive*	In sensitive*
T-DM1	ADC (HER2)	1	> 10	In sensitive*	In sensitive*	In sensitive*	ND#	In sensitive*	In sensitive*	In sensitive*	In sensitive*	In sensitive*	In sensitive*	In sensitive*	In sensitive*
IMMU-132	ADC (TROP2)	> 5	2	> 10	> 100	> 20	ND#	> 20	ND#	> 20	2	2	In sensitive*	1	1
SKB264	ADC (TROP2)	> 5	1	> 5	> 100	4	ND#	> 20	ND#	> 10	4	5	In sensitive*	1	1
ABBV-399	ADC (c-Met)	In sensitive*	In sensitive*	In sensitive*	In sensitive*	In sensitive*	ND#	In sensitive*	In sensitive*	In sensitive*	In sensitive*	> 100	In sensitive*	> 100	> 100
MRO003	ADC (EGFR)	1	2	4	In sensitive*	> 100	ND#	3	ND#	1	> 100	> 100	ND#	> 100	> 100
HER3-Dxd	ADC (HER3)	In sensitive*	In sensitive*	In sensitive*	ND#	ND#	ND#	In sensitive*	ND#	In sensitive*	In sensitive*	ND#	In sensitive*	In sensitive*	In sensitive*
Dxd	Payload (Topoisomerase I inhibitor)	> 10	1	> 50	> 100	> 10	> 10	> 50	3	> 50	5	1	1	1	1
Euxetecan	Payload (Topoisomerase I inhibitor)	3	1	> 10	> 100	> 10	ND#	> 20	ND#	> 10	ND#	2	1	1	1
KL610023	Payload (Topoisomerase I inhibitor)	4	1	> 5	> 100	2	ND#	> 20	ND#	> 10	ND#	3	ND#	ND#	1
SN-38	Payload (Topoisomerase I inhibitor)	> 10	2	> 20	> 100	> 10	ND#	> 20	> 5	> 50	2	2	1	1	1
ZD6616	Payload (Topoisomerase 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#	5	ND#	ND#	ND#	ND#	ND#	ND#	ND#

Resistance Index (RI) was calculated as the ratio of IC<sub>50</sub> in resistant cells to IC<sub>50</sub> in corresponding parental cells (RI = IC<sub>50</sub> resistant / IC<sub>50</sub> parental). In sensitive\*: 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	Drug B Target	Drug B Name
TOPO1	Dxd	Exatecan	SN-38
WRN	HR0761		
WEE1	MK1775		
USP1	K93-4279		
PARP1/2	Talazoparib	Olaparib	
DNA-PK	AZD-7648		
CHK1	Prexasertib		
ATR	Ceralasertib	Berzosertib	
PKMYT1	RP-6306		
CDK4/6	Palbociclib	Ribociclib	Abermacciclib
PLK1	GS640364		
PRMT5	AMG-193	MRTX-1719	
TKR	Selpercatinib	Osimertinib	Cizotinib
TKR downstream pathway	BAY-293	Alpelisib	BBO-10233
RAS	MRTX1133	Sotorasib	pan KRAS IN-5
Microtubule	Docetaxel	MMAE	
Chemotherapy	Gemtastabine	Pemetrexed	Cisplatin
Others	Sorafenib	Triptolide	Tambociclib

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 <sub>50</sub> Shift folds in 1:1 Fixed Ratio compared with A/B	Drug A IC <sub>50</sub> Shift folds in Matrix	Matrix Synergy Score (ZIP method)	Matrix Synergy Score (HSA method)	Indication
1	Dxd	TOPO1			DLD1 Exatecan R	~2/ 4.5	>4 log	5.56	9.43	
2	Exatecan	TOPO1	Berzosertib	ATR/ATM	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			NCI-N87 DS8201 R	~68/ 5	~3.5 log	13.61	16.1	Strong synergistic effect
8	Exatecan	TOPO1	Prexasertib	CHK1/CHK2	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 <sub>50</sub> shift folds in Matrix	Matrix synergy Score(HSA method)	Indication
1	Dxd	TOPO1			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	Berzosertib	ATR/ATM	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			LS411N	~2.34 log folds	15.07	Strong synergistic effect
10	Dxd	TOPO1	Ceralasertib	ATR	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			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	Prexasertib	CHK1/CHK2	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/IMMU-132 R
- HCC1806/SN-38 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 Resistant cell sensitive	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%

### 10 compounds are sensitive in all 3 resistant cells

Compound	HCC1806/Dxd R	HCC1806/IMMU-132 R	HCC1806/SN-38 R	Type
Risdiplam	RS	RS	RS	RS
Quabalin (Octahydroate)	RS	RS	RS	RS
Osimertinib	RS	RS	RS	RS
Neratinib (maleate)	RS	RS	RS	RS
Neratinib	RS	RS	RS	RS
Digitoxin	RS	RS	RS	RS
Dacomitinib (hydrate)	RS	RS	RS	RS
Dacomitinib	RS	RS	RS	RS
Clofarabine	RS	RS	RS	RS
Afatinib	RS	RS	RS	RS

### 10 compounds are resistant in all 3 resistant cells

Compound	HCC1806/Dxd R	HCC1806/IMMU-132 R	HCC1806/SN-38 R	Δ% inhibition
Trogliptazone	77.7	83.4	55.8	<50
Tiocanazole	98.7	104.3	119.5	>50
Thymol	26.4	6.4	32.8	<50
Pixantrone	74.3	57.9	69.7	<50
Ondansetron (dihydrochloride)	96.4	99.8	75.1	<50
Niraparib (tosylate)	62.5	50.2	41.8	<50
Nicosamide (olamine)	78.1	82.2	85.3	<50
Merimepodib	67.3	57.2	77.8	<50
Clomiphene (citrate)	112.5	87.4	102.9	>50
Berberine (chloride hydrate)	60.4	52.1	45.3	<50

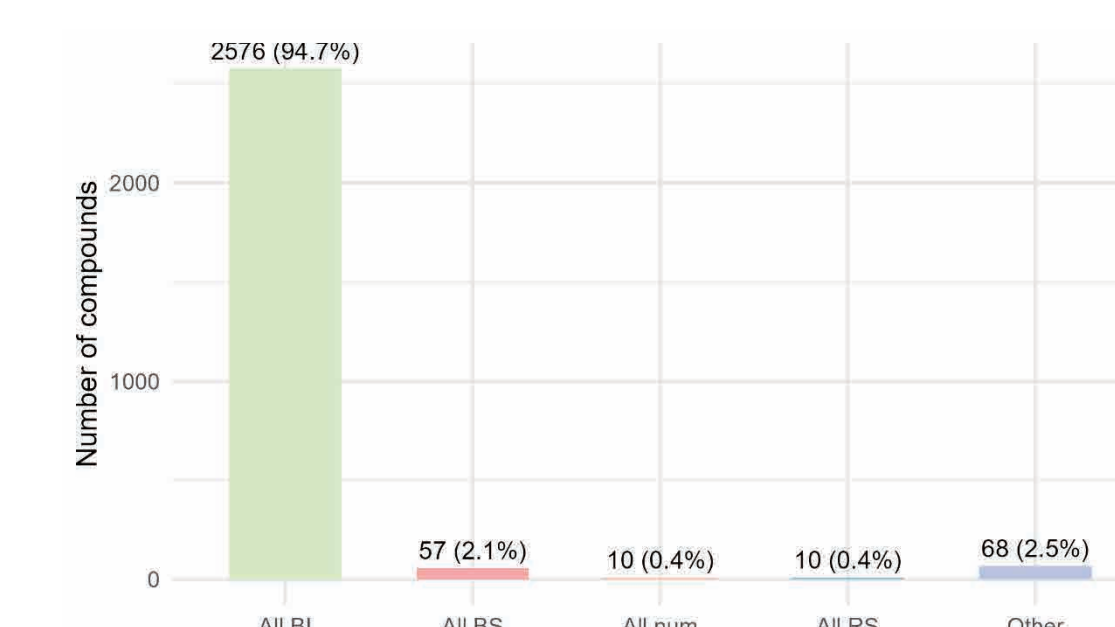


Table 6. Candidates Data Summary

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	