A Comprehensive Multi-Omic and High-Throughput Screening Platform of ADC-Resistant Cell Lines: Deciphering Resistance Mechanisms and Accelerating Dual-Payload Strategies

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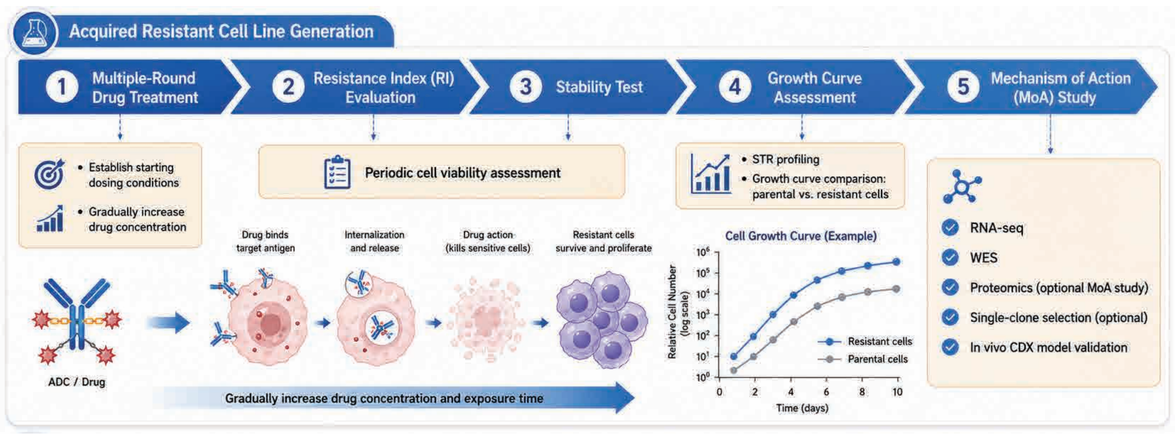
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Introduction

Antibody-drug conjugates (ADCs) are key targeted therapies, yet drug resistance remains a major clinical challenge. To address this, we established a panel of 30 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 Cell Models



Resistant cell panel including:

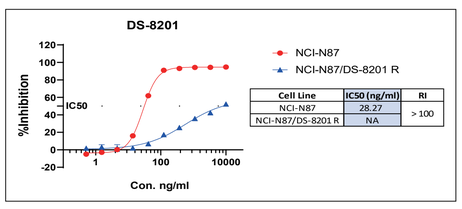
14 cell lines • 6 x **ADC**-induced

16 cell lines • 5 x **Payloads**

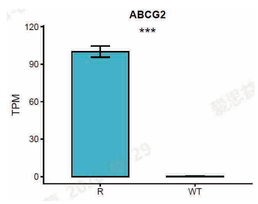
17 cell lines • **RAS** inhibitors

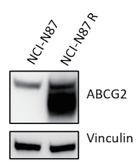
10 cell lines • **Engineered** Isogenics

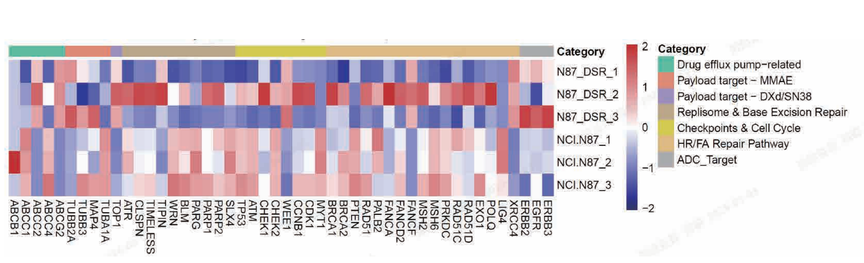
Resistant cell validation - Case 1

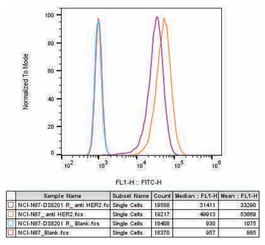


Cell Line	IC50 (ng/ml)	RI
NCI-N87	28.27	
NCI-N87/DS-8201 R	NA	> 100







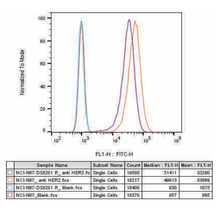


Sample Name	Subset Name	Count	Median : FL1-H	Mean : FL1-H
NCI-N87-DS8201 R_ anti HER2.fcs	Single Cells	19588	31411	33290
NCI-N87_ anti HER2.fcs	Single Cells	19217	48613	53669
NCI-N87-DS8201 R_ Blank.fcs	Single Cells	18408	930	1075
NCI-N87_Blank.fcs	Single Cells	18370	957	995

Resistant cell validation - Case 2

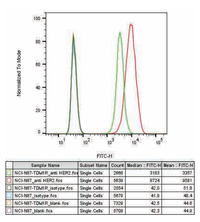
Resistance Index (RI)		ADC/Payload Resistant Cell Lines		
Drug \ Cell Line		ADC(HER2)		
		NCI-N87/DS-8201 R	NCI-N87/T-DM1 R	HCC1954/T-DM1 R
DS-8201	ADC(HER2+Dxd)	> 100	1	> 100

NCI-N87 / DS-8201 R



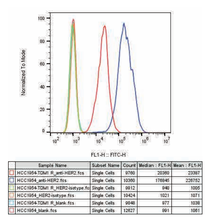
Sample Name	Subset Name	Count	Median : FL1-H	Mean : FL1-H
NCI-N87-TDM1R_anti HER2.fcs	Single Cells	19555	31411	33290
NCI-N87_ anti HER2.fcs	Single Cells	19217	48613	53669
NCI-N87-DS8201 R_ Blank.fcs	Single Cells	19408	930	1075
NCI-N87_Blank.fcs	Single Cells	19370	957	995

NCI-N87 / T-DM1 R



Sample Name	Subset Name	Count	Median : FITC-H	Mean : FITC-H
NCI-N87-TDM1R_anti HER2.fcs	Single Cells	2958	3103	3287
NCI-N87_anti HER2.fcs	Single Cells	5839	8724	9581
NCI-N87-TDM1R_isotype.fcs	Single Cells	2004	42.2	41.8
NCI-N87_isotype.fcs	Single Cells	5870	41.8	46.4
NCI-N87-TDM1R_blank.fcs	Single Cells	7259	42.3	44.8
NCI-N87_blank.fcs	Single Cells	5700	42.2	44.6

HCC1954 / T-DM1 R



Sample Name	Subset Name	Count	Median : FL1-H	Mean : FL1-H
HCC1954-TDM1R_anti-HER2.fcs	Single Cells	9760	35389	37887
HCC1954_anti-HER2.fcs	Single Cells	10390	178946	229192
HCC1954-TDM1R_HER2-isotype.fcs	Single Cells	9812	648	1000
HCC1954_HER2-isotype.fcs	Single Cells	10424	1021	1071
HCC1954-TDM1R_blank.fcs	Single Cells	9948	977	1038
HCC1954_blank.fcs	Single Cells	12457	891	1081

HER2 Expression

The strong DS-8201 cross-resistance in HCC1954/T-DM1-R, but not NCI-N87/T-DM1-R, may be partly explained by the greater reduction in HER2 expression.

Table 1. Multiple ADC/Payload-Resistant Cell Models Determined by Resistance Index (RI).

	Cancer type	Gastric	Gastric	Gastric	Gastric	Gastric	Gastric	Gastric	Breast	Breast	Breast	Breast	Breast	Breast	Breast	Colorectal	Colorectal	Colorectal	Ovarian	Ovarian	Ovarian	NSCLC	NSCLC	
Molecule	Drug Type	NCI-N87/ DS-8201 R	T-DM1 R	NCI-N87/ IMMU-132 R	NCI-N87/ SKB264 R	NCI-N87/ ZD06519 R	NUGC4/ ABBV-399 R	NUGC-4/ MMAE R	HCC1954/ T-DM1 R	HCC1954/ ABBV-399 R	HCC1954/ MRG003 R	HCC1806/ IMMU-132 R	HCC1806/ Dxd R	HCC1806/ SN-38 R	HCC1806/ MMAE R	DLD-1/ Exatecan R	GP2d/ Dxd R	GP2d/ SN-38 R	SK-OV-3/ Dxd R	SK-OV-3/ MMAE R	OVCAR-3/ MMAE R	NCI-H441/ ABBV-399 R	HCC4006/ MMAE R	Molecule
DS-8201	ADC (HER2+Dxd)	> 100	1	> 20	> 100	> 100			> 100	1	3													DS-8201
T-DM1	ADC (HER2+DM1)	1	> 10	> 5	4	> 10			> 20	1	3								3	5				T-DM1
RC-48	ADC (HER2+MMAE)	2		2	1	2			> 100	3	4								> 100					RC-48
IMMU-132	ADC (TROP2+SN38)	> 5	2	> 10	> 20	> 20			1	3	3	> 10	> 20	> 20	2	> 100	> 20		> 10	2	1	2	1	IMMU-132
SKB264	ADC (TROP2+KL610023)	> 5	1	> 10	> 20	> 50			1	2	3	> 5	> 20	> 10	4	> 100	4		> 10	5	1	3	1	SKB264
ABBV-399	ADC (c-Met+MMAE)						> 100	> 100	2	> 100	> 100											> 100	> 100	ABBV-399
MRG003	ADC (EGFR+MMAE)	1	2	4	2	1	1		2	> 5	> 100	4	3	1	> 100		> 100		> 100	> 100	> 100	4	> 100	MRG003
Dxd	Topo1i	> 10	1	> 5	> 10	> 20	1	1	1	1	3	> 50	> 50	> 50	5	> 100	> 10	3	> 100	1	1	2	1	Dxd
Exatecan	Topo1i	3	1	4	5	> 5	1	1	1	1	2	> 10	> 20	> 10		> 100	1	1	> 20	2	1		1	Exatecan
T01-1	Topo1i	4	1	> 5	> 10	> 20	1		1	2	2	> 5	> 20	> 10		> 100	2		> 10	3			1	T01-1
SN-38	Topo1i	> 10	2	> 5	> 20	> 20	2	1	1	3	2	> 20	> 20	> 50	2	> 100	> 10	> 5	> 10	2	1	3	1	SN-38
ZD06519	Topo1i	> 10	1	> 10	> 20	> 20	2	1	2			> 20	> 100	> 20		> 100	> 10	> 5	> 20	2	1		2	ZD06519
MMAE	Tubulin inhibitor	2	2	3	2	3	>5	> 10	2	3	4	1	2	2	> 10	3	> 5		> 10	> 20	> 5	2	> 50	MMAE
DM1	Tubulin inhibitor	2	1	1	1	1	3	2	1	4	4	1	2	3		4	4		> 10	> 20	1	2	> 20	DM1

RI = IC_{50} of resistant / IC_{50} of parental).

Table 2. ABC Transporter-Overexpressing Models

Drugs	SK-BR-3 (Breast cancer)		JIMT-1 (Breast tumor)		NCI-N87 (Gastric cancer)		OVCAR3 (Ovarian cancer)		HCT116(Colorectal cancer)
	Resistance Index (RI)ABCG2 OE	Resistance Index (RI)ABCB1OE	Resistance Index (RI)ABCG2 OE	Resistance Index (RI)ABCB1OE	Resistance Index (RI)ABCG2 OE	Resistance Index (RI)ABCB1OE	Resistance Index (RI)ABCG2 OE	Resistance Index (RI)ABCB1OE	Topoisomerase I R364H
DS-8201	18	4	Insensitive	Insensitive	6	2	Insensitive	Insensitive	Insensitive
Dxd	29	10	15	5	16	6	10	4	56
Exatecan	3	3	5	1	3	2	4	1	44
MMAE	2	> 100	1	> 100	2	23	1	37	
SN-38	24	2	12	2	12	4	17	3	135
ZD06519	24	16	15	9	15	8	10	7	38
T-DM1	2	4	Insensitive	Insensitive	2	2	Insensitive	Insensitive	
DM1	4	73	1	45	2	5	0.2	5	
Irinotecan	8	3	1	2					
IMMU-132					24	5	16	4	58
SKB264					19	14	12	9	35
MRG003							1	> 100	
KL610023									43

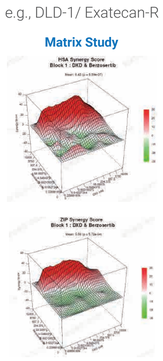
Across multiple cell backgrounds, ABCB1 overexpression preferentially confers resistance to MMAE, whereas ABCG2 has a stronger effect on SN-38, supporting payload-specific efflux mechanisms.

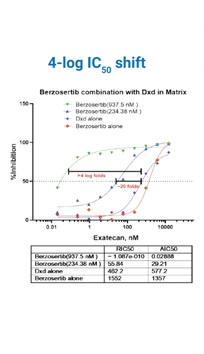
Case study 1:Dual payload pairs Screening in Resistant cell lines

More than 120 drug pairs were screened in ADC/payload-resistant and matched parental cell lines to identify synergistic combinations.



e.g., DLD-1/ Exatecan-R





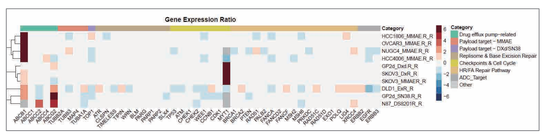
	IN50	AC50
Berzosertib(937.5 nM)	~ 1.067e-010	0.02868
Berzosertib(234.38 nM)	55.84	29.21
Dxd alone	482.2	577.2
Berzosertib alone	1552	1307

Note: 4 oncology inhibitors showed strong synergic effect, the best combination ratio gave 1000~10,000-fold IC50 shift compared to monotreatment

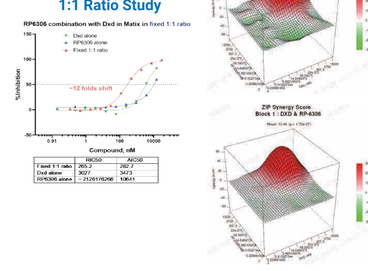
Drug A Name	Drug B Name	Drug B Target	Drug A IC50 Shift in Matrix	Synergy Score (ZIP method)
Dxd	Ceralasertib	ATR	3 log	11.84
SN38			2.7 log	10.05
Dxd	Prexasertib	CHK1/CHK2	3.5 log	13.61
Exatecan			3 log	14.9
SN38			3 log	17.62
SN38	Talazoparib	PARP	20	5.15

* Resistant cell line: NCI-N87 DS8201 R

Case study 2: Overcome resistance based on Omics analysis



RP-6306 + DXd in NCI-N87/DS-8201-R



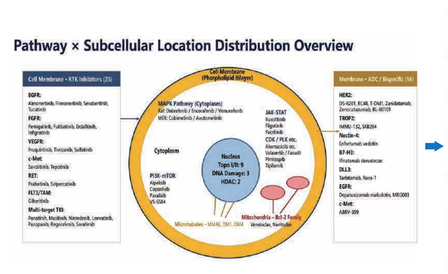
	IC50	AC50
T med 1:1 ratio	265.2	282.7
Dxd alone	3027	3473
RP6306 alone	~ 212817826	10641

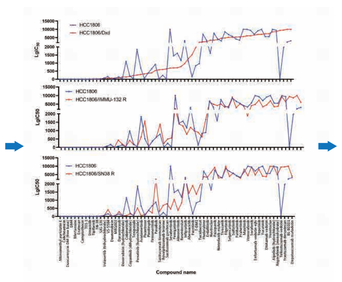
	IC50	AC50
RP6306(2750 nM)	33.38	13.99
RP6306(937.5 nM)	73.58	58.25
RP6306(234.38 nM)	71.51	86.66
RP6306(58.59 nM)	1264	487.9
Dxd alone	3027	3473
RP6306 alone	~ 212817826	10641

HAS/ZIP Analysis

Mini CRC Cell panel test			
Drug B Name	Drug B Target	WT Cell	Drug A IC50 Shift in Matrix
Berzosertib	ATR/ATM	LS411N	2.1-log shift
		NCI-H747	2.0-log shift
		SW480	2.0-log shift
		SW620	1.0-log shift
		T84	2.5-log shift
Ceralasertib	ATR	LS411N	2.3-log shift
		SW480	1.5-log shift
		SW948	2.1-log shift
		T84	2.5-log shift
Prexasertib	CHK1/CHK2	HT-55	2.0-log shift
		LS411N	2.5-log shift
		SW480	2.2-log shift
		SW620	1.6-log shift
		T84	2.4-log shift
RP-6306	PKMYT1	HT-55	2.4-log shift
		LS411N	2.4-log shift
		SW480	2.0-log shift
		SW620	1.4-log shift
		SW948	2.1-log shift
		T84	2.3-log shift

Case study 3: Overcome resistance based on Omics analysis





New sensitive and insensitive inhibitors observed

Compound	HCC1806	HCC1806/IMMU-132 R	RI
Exatecan	0.03	1.2	40
T01-1	0.03	3.0	98
Camptothecin	0.9	7.8	9
SN-38	0.5	10.5	20
Sacituzumab tirumotecan	24	245	10
Cobimetinib	981	420	0.4
MRG003	66	435	7
Sacituzumab govitecan	25	556	23
Ponatinib (hydrochloride)	1,834	617	0.3
Pacritinib	132	897	7
Infigratinib	3,101	1,193	0.4
Pralsetinib	3,365	1,884	0.6
Sevabertinib	10,000	2,411	0.2
Selpercatinib	6,708	2,984	0.4
Enfortumab vedotin-ejfv	7,038	3,468	0.5
Tepotinib	9,137	4,560	0.5
Vemurafenib	10,000	4,879	0.5
Telisotuzumab vedotin	47	9,463	203

Compound	HCC1806	HCC1806/SN38 R	RI
Exatecan	0.03	1.2	40
Tipifarnib	6.7	3.9	0.58
T01-1	0.03	4.3	144
SN-38	0.5	12.7	24
MRG003	65.6	33.1	0.51
Avutometinib	630	52	0.08
Cobimetinib	1,150	60	0.05
Firmonertinib	661	212	0.32
Sacituzumab tirumotecan	24	307	13
Almonertinib	1,626	372	0.23
Sevabertinib	10,000	454	0.05
Ponatinib (hydrochloride)	1,868	521	0.28
Sacituzumab govitecan	25	780	32
Pacritinib	155	1,900	12
Fludarabine	6,960	3,714	0.53
Selpercatinib	6,880	3,869	0.56
Tucatinib	10,000	3,977	0.40
Vemurafenib	10,000	4,273	0.43
Regorafenib (monohydrate)	9,397	4,483	0.52
Telisotuzumab vedotin	47	9,478	203

Compound	HCC1806	HCC1806/Dxd R	RI
Mertansine	0.02	0.12	6
Exatecan	0.03	1.4	44
T01-1	0.03	9	283
SN-38	0.5	21	40
SJG-136	1.1	26	5
VS-5584	160	58	0.4
Daunorubicin	6.34	64	10
Sacituzumab govitecan	141	80	0.6
Cobimetinib	1,109	139	0.1
Teniposide	40	227	6
Ponatinib (hydrochloride)	1,842	261	0.1
Avutometinib	696	297	0.4
Pixantrone	48	333	7
Pixuilib	924	538	0.6
Sacituzumab tirumotecan	24	583	24
Sacituzumab govitecan	25	639	25
Sevabertinib	10,000	689	0.1
Pemigatinib	1,452	703	0.5
Almonertinib	1,612	709	0.4
Infigratinib	3,212	969	0.3
Pacritinib	149	1,509	10
Fludarabine	7,044	2,441	0.3
Pazopanib	7,658	3,487	0.5
Pilgotinib	8,516	4,755	0.6
Mastinib	10,000	5,629	0.6
Telisotuzumab vedotin	47	9,386	203

New sensitive inhibitors will be tested in matrix screening to validate synergic effect.

Summary

We have built a robust ADC-resistant cell model platform, supported by multi-omic characterization. Leveraging this platform, we completed dual-payload and library screening, identifying promising single agents and synergistic combinations. Our platform provides flexible, multi-dimensional solutions to accelerate next-generation ADC discovery.