Advancing HER2-Targeted ADC Drug Discovery through Resistant Cell Line Generation and Cancer Cell Panel Analysis

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Introduction

Antibody-drug conjugates (ADCs) represent a cutting-edge class of targeted cancer therapeutics, seamlessly integrating the high specificity of monoclonal antibodies with the cytotoxic power of chemotherapy agents. This strategic union facilitates the precise delivery of a potent cytotoxic payload to HER2-expressing cancer cells, where HER2 is a well-established therapeutic target. Despite the promising efficacy of HER2-directed ADCs in treating HER2-positive breast cancer, the emergence of resistance in tumor cells poses a significant obstacle to their therapeutic potential.

HER2 Expressing Database

Top 60 Bottom 60

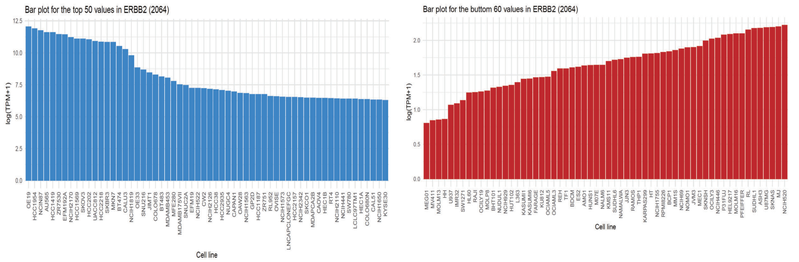


Figure 1. ICE database showed high expression level and Low expression level of HER2 in Cancer cell lines.

Flow Cytometry Assessment of HER2 Surface Expression Ranks Cell Lines

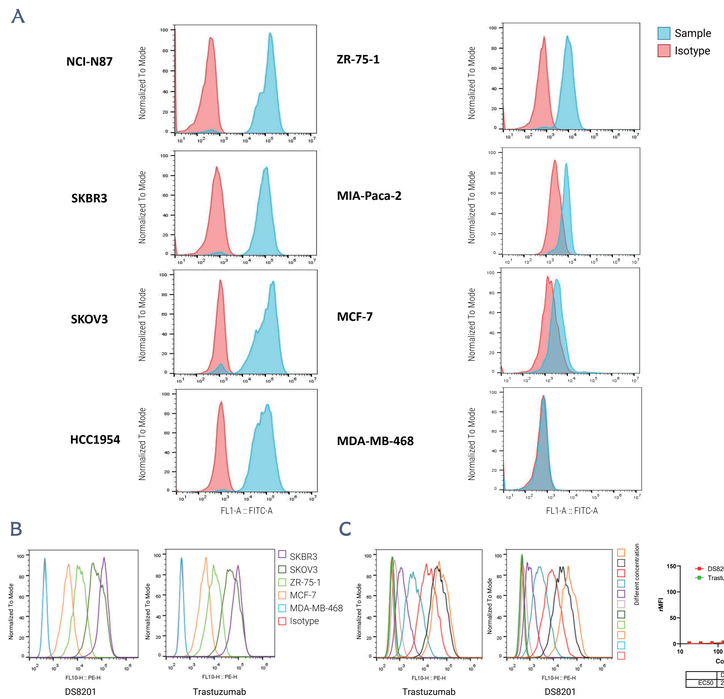


Figure 2. A, HER2 expression level in cancer cell lines detected by Flow. B, Binding assay in tumor cells with different HER2 expression levels. C, Comparison of Antibody and ADC binding EC50 in HER2+ SKOV3 cell. The result showed that NCI-N87, SKBR3, SKOV3 and HCC1954 cells have a high expression level of HER2 which makes them an ideal cell model for studies of breast cancer mono- and combination therapies.

Payload Cytotoxicity and ADC Cell Panel Testing

Payload cytotoxicity test

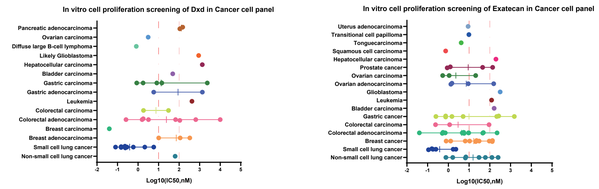
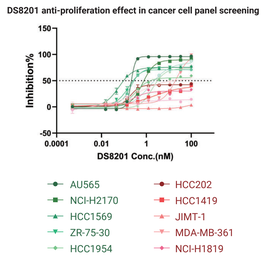


Figure 3. Panels of 40-70 cancer cell lines were used to assess the cytotoxicity of Dxd and Exatecan. The results demonstrated that both compounds exhibited potent cytotoxicity across multiple cancer types.

DS8201 efficacy in Cancer cell panel



Antiproliferative impact of DS8201 highlights its relative selectivity for HER2 overexpressing cell lines

Cell lines	Cancer Type	nTPM value for ERBB2 (Data from The Human Protein Atlas)	HER2 Receptor	Estrogen Receptor	Progesterone Receptor	R_IC50, nM	Max Inhibition%
AU565	Breast adenocarcinoma	3987.3	+++	-	-	0.21	95.98
MDA-MB-361	Breast adenocarcinoma	801.3	+	-	-	21.16	98.80
JIMT-1	Breast carcinoma	403.1	+	-	-	>100	3.20
EFM-192A	Breast carcinoma	3827.8	+++	-	-	0.20	70.34
HCC1954	Breast ductal carcinoma	3431.8	+++	-	-	0.32	55.70
HCC202	Breast ductal carcinoma	1854.2	++	-	-	0.22	42.62
HCC1419	Breast ductal carcinoma	3990.1	+++	-	-	2.25	40.04
UACC-812	Breast ductal carcinoma	2402.3	+++	-	-	1.87	73.39
ZR-75-30	Breast ductal carcinoma	4058.3	+++	-	-	0.15	75.49
HCC1569	Breast metaplastic carcinoma	2321.0	+++	-	-	0.05	75.12
MKN-7	Gastric tubular adenocarcinoma	1288.3	++	-	-	0.45	53.86
NCI-H1819	Non-small cell lung cancer	1050.2	++	-	-	0.22	30.23
NCI-H1781	Non-small cell lung cancer	290.7	+	-	-	1.55	85.08
NCI-H2170	Non-small cell lung cancer	3109.5	+++	-	-	0.69	89.16
SK-OV-3	Ovarian adenocarcinoma	2047.9	+++	-	-	>100	15.50

Figure 4. DS8201 showed antiproliferative activity across various cancer types, including breast, gastric, and non-small cell lung cancers. While effective in many HER2-overexpressing cell lines (e.g., AU565, HCC1954), some HER2-high-expressing lines exhibited weaker responses (e.g., SK-OV-3). This highlights DS8201's therapeutic potential and the variability in efficacy based on cellular context and HER2 expression levels.

Tag-expression Reporter Tumor Cell Panel

In vitro bystander effect assay

A

#	ADC bystander effect assays	Detection methods
1	Ag+/Ag- cell co-cultures with GFP/RFP	Flow cytometry
2	Ag+/Ag- cell co-cultures without GFP/RFP	Flow cytometry
3	Ag+/Ag- cell co-cultures with GFP/RFP	IncuCyte
4	Ag+/Ag- cell co-cultures with GFP/RFP	IncuCyte (Realtime)
5	Co-cultures with Ag+-GFP/RFP cell and Ag-Luc cell	IncuCyte and Microplate Reader
6	Co-cultures with Ag+ cell and Ag-Luc cell	Microplate Reader
7	Medium transfer	Microplate Reader

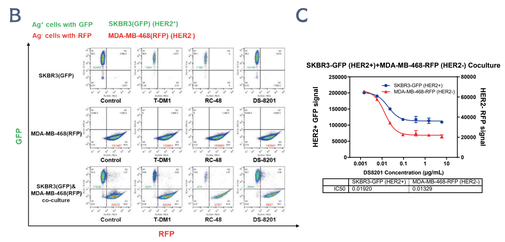


Figure 5. The table sums up the methods to test the bystander effect, like flow cytometry, IncuCyte, and CTG. Figure B shows the bystander effect of DS8201 in co-cultures of HER2-positive SKBR3-GFP and HER2-negative MDA-MB-468-RFP cells detected by flow cytometry. Figure C gives the dose-curve effect of DS8201's bystander effect. Results show DS8201 has a strong bystander effect.

Tag-expression cancer cell panel for bystander assays

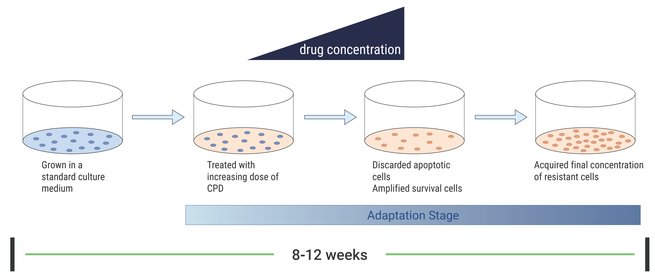
Cancer cell type	GFP cells	High expression targets
Breast cancer	SKBR3-GFP	HER2, TROP2...
Breast cancer	HCC1954-GFP	HER2, TROP2, MUC1, CDH3...
Breast cancer	HCC70-GFP	MUC1, B7-H4, CDH3...
Breast cancer	ZR-75-1-GFP	MUC1, LIV-1, B7-H4...
Ovarian cancer	SKOV-3-GFP	HER2, TROP2, Nectin4, B7-H4...
Ovarian cancer	OVCAR-3-GFP	MSLN, B7-H4, MUC16, CLDN6...
Gastric Cancer	NCI-N87-GFP	HER2, TROP2...
Gastric Cancer	NUGC-4-GFP	CLDN18.2, HER3...
Pancreatic cancer	BXPC-3-EGFP	MET, CEACAM6, CEACAM5, CDCP1, EPHA2...
		...

Cancer cell type	RFP cells	Low expression targets
Breast cancer	MDA-MB-468-RFP	HER2, Nectin4, EGFR, B7-H4, NOTCH3...
Breast cancer	MCF-7-RFP	FOLR1, Nectin4, EGFR, ROR1, PTK7...
Lung cancer	NCI-H1792-RFP	FOLR1, Nectin4, MUC1, ERBB3, CEACAM6...
Lung cancer	NCI_H226-RFP	DLL3, TACSTD2, Nectin-4, CEACAM6, ITGB6...
Colon cancer	SW620-RFP	TACSTD2, CEACAM5...
Pancreatic cancer	MIA-PaCa-2-RFP	CLDN18.2, ITGB6, ST4...
		...

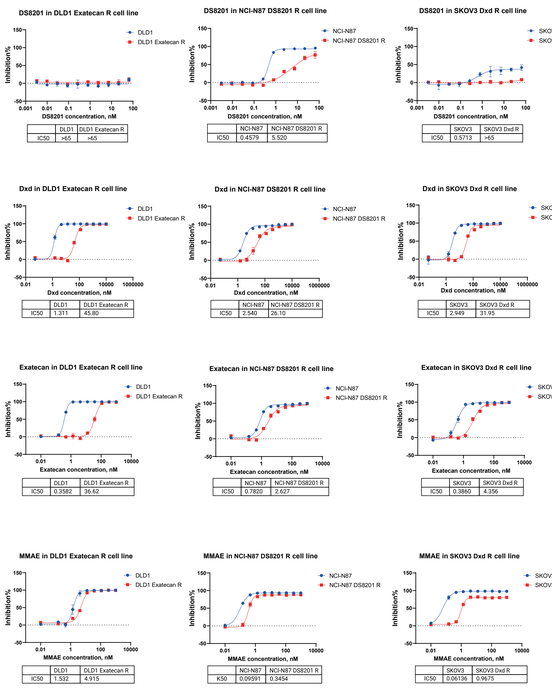
Cancer cell type	Cell lines (ready)
Breast cancer	MDA-MB-231-Luc
Breast cancer	MDA-MB-468-Luc
Breast cancer	MCF-7-Luc-mEGFP
Breast cancer	H1299-Luc-mEGFP
Brain Carcinoma	LN299-Luc-mEGFP
Brain Carcinoma	U251-Luc-mEGFP
Brain Carcinoma	CT2A-Luc-mEGFP
Colorectal Carcinoma	DLD-1-BRCA2-KO-Luc-mEGFP
Colorectal Carcinoma	HCT116-Luc-mEGFP
Lung cancer	NCI-H1975-Luc-mEGFP
Lung cancer	PC-9-Luc
Lung cancer	A549-Luc-mEGFP
Lung cancer	H1299-Luc-mEGFP
Liver Carcinoma	HepG2-Luc
Liver Carcinoma	HepG2-Luc-mEGFP
Hepatoma	HepG2-Luc
Pancreatic cancer	MIA-PaCa-2-Luc
Prostate Adenocarcinoma	PC-3-Luc
Prostate Adenocarcinoma	PANC-1-Luc-mEGFP
Prostate Adenocarcinoma	BXPC-3-Luc-mEGFP
Prostate Adenocarcinoma	MIA PaCa-2-Luc-mEGFP
Leukemia	Jurkat-Luc
Leukemia	K562-Luc
Lymphoma	Raji-Luc

ADC Resistant Cell Line

A



B



DS8201 efficacy summary

Cell lines	Cancer Type	R_IC50, nM	RI
DLD-1	Colorectal adenocarcinoma	>65	/
DLD-1/ Exatecan R		>65	
NCI-N87	Gastric carcinoma	0.458	12.0
NCI-N87 DS8201R		5.520	
SKOV3	Ovarian adenocarcinoma	0.571	/
SKOV3 Dxd R		>65	

Dxd efficacy summary

Cell lines	Cancer Type	R_IC50, nM	RI
DLD-1	Colorectal adenocarcinoma	1.311	35.0
DLD-1/ Exatecan R		45.80	
NCI-N87	Gastric carcinoma	2.540	10.3
NCI-N87 DS8201R		26.10	
SKOV3	Ovarian adenocarcinoma	2.949	10.8
SKOV3 Dxd R		31.95	

Exatecan efficacy summary

Cell lines	Cancer Type	R_IC50, nM	RI
DLD-1	Colorectal adenocarcinoma	0.358	102.3
DLD-1/ Exatecan R		36.62	
NCI-N87	Gastric carcinoma	0.782	3.4
NCI-N87 DS8201R		2.627	
SKOV3	Ovarian adenocarcinoma	0.386	11.3
SKOV3 Dxd R		4.356	

MMAE efficacy summary

Cell lines	Cancer Type	R_IC50, nM	RI
DLD-1	Colorectal adenocarcinoma	1.532	3.2
DLD-1/ Exatecan R		4.915	
NCI-N87	Gastric carcinoma	0.959	<1
NCI-N87 DS8201R		0.345	
SKOV3	Ovarian adenocarcinoma	0.0614	15.8
SKOV3 Dxd R		0.968	

Figure 6. Three ADC-related resistant cell lines for three cancer types (Colon, Gastric, and Ovarian) were generated via procedure A. DS8201 and three other payload molecules were evaluated in the resistant cell proliferation assays (B). Results indicated that DS8201 showed limited efficacy in the DLD-1 and DLD-1 Exatecan R cell lines, as well as in the SKOV3 and SKOV3 Dxd R cell lines. Conversely, MMAE exhibited minimal resistance across all three resistant cell lines compared to Dxd and Exatecan, suggesting distinct resistance mechanisms for these compounds.

Summary

In this study, we detected HER2 expression levels in various tumor cells using ICE's internal database and flow cytometry. We also screened for cancer types and cell lines sensitive to ADC payloads to establish ADC drug models. Additionally, tagged negative and positive cells were constructed to study ADC bystander effects, and resistant cell lines were developed from sensitive ones to investigate resistance and provide models to overcome it. Overall, the ICE platform offers comprehensive evaluation for ADC drug efficacy.