DRUG-RESISTANT CANCER CELL PANEL FOR SCREENING THERAPEUTIC STRATEGIES

Lili Chai, Tingting Yang, Zhu Meng, Zhengtai Li, Tiejun Bing, Xue Yang, Wei Liu and Cong Huang
ICE Bioscience, INC. Building 14, Yard 18, Kechuang 13th Street, Beijing, China 100176
Email: bingtj@ice-biosci.com

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Poster Number: P036

Introduction

Drug resistance is a major challenge in cancer therapy, often causing relapse after initial successful treatment. Exploring resistance mechanisms and developing new treatments are key goals in oncology. Several promising targets for anticancer drug development have been identified or are under active investigation. For instance, KRAS mutations are prevalent in pancreatic, colorectal, and lung cancers, driving uncontrolled cell proliferation. DNA damage repair (DDR) mechanisms normally maintain genomic stability by repairing DNA damage, but defects in DDR pathways can make cancer cells more susceptible to targeted therapies like PARP inhibitors. EGFR resistance is frequently observed in clinical settings, and the emerging field of antibody-drug conjugate (ADC) therapies is also encountering drug resistance challenges. Drug-resistant cell lines are valuable tools for studying these resistance mechanisms and discovering novel therapies.

Drug Resistant Cell Line Generation

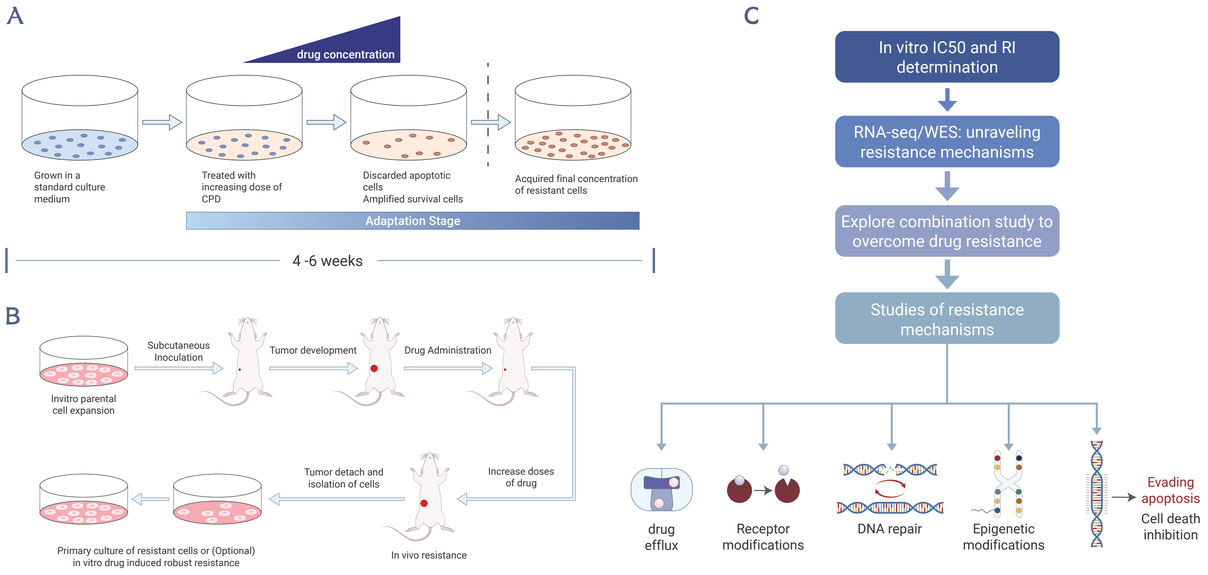


Figure 1. Schematic of Resistant Cell Line Generation. Panel A illustrates the workflow for generating drug - resistant cell lines using in vitro methods. Panel B outlines the process of creating drug - resistant cell lines employing in vivo approaches. Panel C presents the workflow for verifying resistant cell lines and exploring their resistance mechanisms.

Cancer Type-Dependent Efficacy Evaluation of SOC and Research-Stage Drugs in Drug-Resistant Cell Lines

Table 1 SOCs and Research-Stage Compounds in This Study

Drug Name	Target	Characteristic	Highest Phase	Indications	Test cancer type in this study
Osimertinib	EGFR	Small Molecule	2015 Launched	Non-Small Cell Lung Cancer,others	NSCLC
Afatinib	dual EGFR/HER2	Small Molecule	2013 Launched	Non-Small Cell Lung Cancer,Breast Cancer,others	NSCLC
Poziotinib	Pan-HER/EGFR	Small Molecule	Discontinued	Non-Small Cell Lung Cancer,Breast Cancer	NSCLC
Crizotinib	ALK	Small Molecule	2011 Launched	Non-Small Cell Lung Cancer,Breast Cancer	NSCLC
Adagrasib	KRAS G12C	Small Molecule	2022 Launched	Non-Small Cell Lung Cancer,others	NSCLC
RMC6236	Pan RAS	Molecule Glue	Phase III	Non-Small Cell Lung Cancer,others	NSCLC
Capmatinib	MET	Small Molecule	2020 Launched	Non-Small Cell Lung Cancer,others	NSCLC
Alpelisib	PI3Kα	Small Molecule	2019 Launched	Non-Small Cell Lung Cancer discontinued,Breast Cancer	NSCLC
Selpercatinib	RET	Small Molecule	2020 Launched	Non-Small Cell Lung Cancer,others	NSCLC
RMC9805	KRAS G12D	Molecule Glue	Phase II	Non-Small Cell Lung Cancer,others	NSCLC
Brigatinib	ALK/EGFR	Small Molecule	2017 Launched	Non-small-cell lung cancer	NSCLC
Osimertinib	EGFR	Small Molecule	2015 Launched	Non-Small Cell Lung Cancer,others	BC
Afatinib	dual EGFR/HER2	Small Molecule	2013 Launched	Non-Small Cell Lung Cancer,Breast Cancer,others	BC
Poziotinib	Pan-HER/EGFR	Small Molecule	Discontinued	Non-Small Cell Lung Cancer,Breast Cancer	BC
Tamoxifen	SERM	Small Molecule	1975 Launched	Breast cancer	BC
Palbociclib	CDK4/6	Small Molecule	2015 Launched	Breast cancer	BC
Lapatinib	HER2/EGFR	Small Molecule	2007 Launched	Breast cancer, others	BC
5-Fluorouracil	Thymidylate Synthase Inhibitor	Small Molecule	\	\	BC
Gartisertib	ATR	Small Molecule	Discontinued	Breast cancer	BC
Exatecan	Topoisomerase I	Small Molecule	Discontinued	\	BC
PARG-IN-4	PARG	Small Molecule	\	\	BC
Paclitaxel	EGFR	Small Molecule	Launched	Breast cancer, others	BC

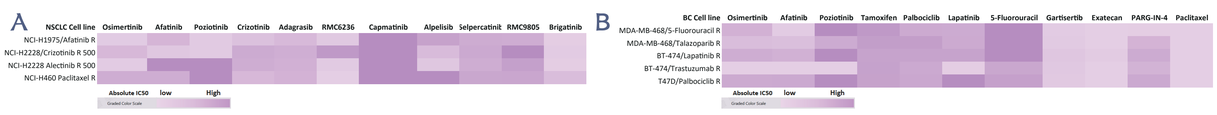


Figure 2. Cancer Type-Dependent Efficacy Evaluation of SOC and Research-Stage Drugs in Drug-Resistant Cell lines.
A, The drug activity assessment of standard-of-care (SOC) and investigational agents across various drug-resistant non-small cell lung cancer (NSCLC) cell lines and breast cancer (BC) cell lines. B, The evaluation of drug activity for SOC and investigational compounds in different drug-resistant breast cancer cell lines.

Generation and Mechanistic Investigation of WRN-Resistant Cell Lines

Resistant cell line generation

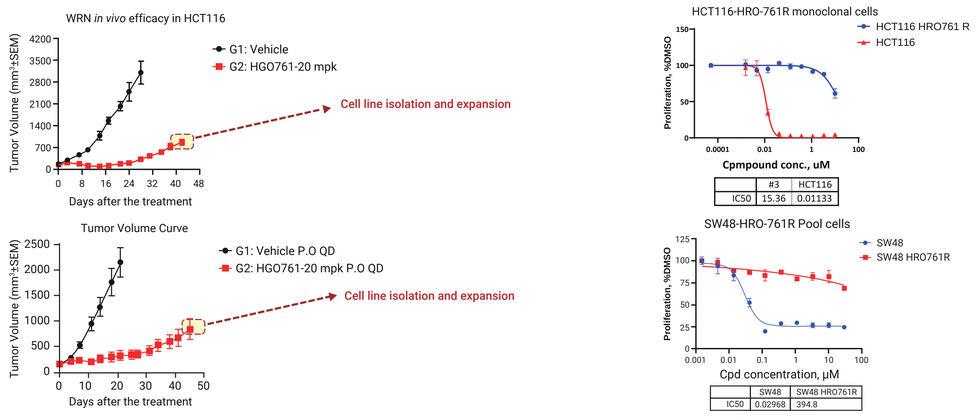


Figure 3. Establishment of WRNi resistant cell lines. During in vivo drug efficacy assessment, tumors initially regressed but resumed growth around day 40 of treatment. Tumor cells were subsequently isolated and subjected to in vitro culture with sustained drug exposure to propagate resistance, ultimately yielding HRO761 resistant cell lines.

WRNi and DHX9i Efficacy in WRNi Resistant Cell Line

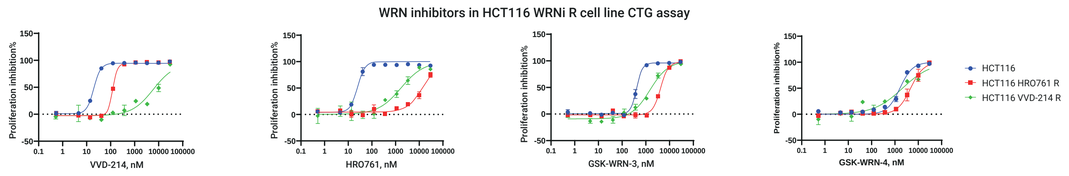


Table 2 IC50 Summary

Drug Name	Target	HCT116	HCT116 HRO761 R		HCT116 VVD214 R	
		IC50, nM	IC50, nM	Resistant Index(RI)	IC50, nM	Resistant Index(RI)
VVD-214	WRN	19.69	122.20	6.21	5980.00	303.71
HRO-761	WRN	25.25	148852.00	5895.13	1692.00	67.01
GSK-WRN-3	WRN	424.20	4271.00	10.07	1495.00	3.52
GSK-WRN-4	WRN	1634.00	4692.00	2.87	2104.00	1.29
ATX-968	DHX9	278.10	423.80	1.52	3428.00	12.33

Figure 4. WRN inhibitors (HRO761, VVD214) and DHX9 inhibitor (ATX-968) were tested in HRO761 resistant and VVD214 resistant cell lines. Results showed that in HRO761 resistant cell lines, only HRO761 exhibited strong resistance, while other WRN inhibitors and DHX9 inhibitors showed weak resistance. Similarly, in VVD214 resistant cell lines, only VVD214 had strong resistance, and HRO-761 showed some resistance, and other tested inhibitors displayed weak resistance. This indicates that different inhibitors have distinct resistance mechanisms. IC50 values are summarized in Table 2.

Resistance Mechanism Exploration

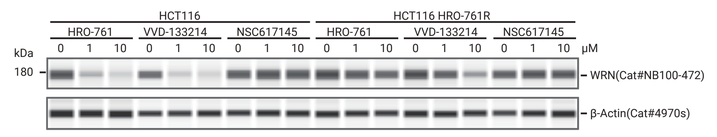


Figure 5. HCT116 and HCT116 HRO 761 resistant cells were exposed to HRO761, VVD - 214, or NSC617145. WRN protein levels were assessed via JESS. Results demonstrated that, unlike in wild type cells, these compounds failed to degrade WRN in resistant cells, indicative of a key resistance mechanism.

- Small molecule library containing 1000 compounds
- Compound duration-7 days
- Readout: Proliferation inhibition%

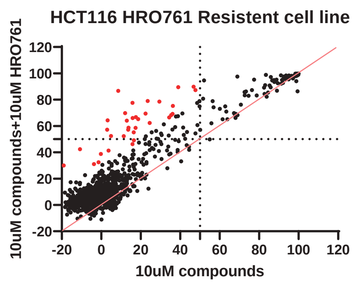
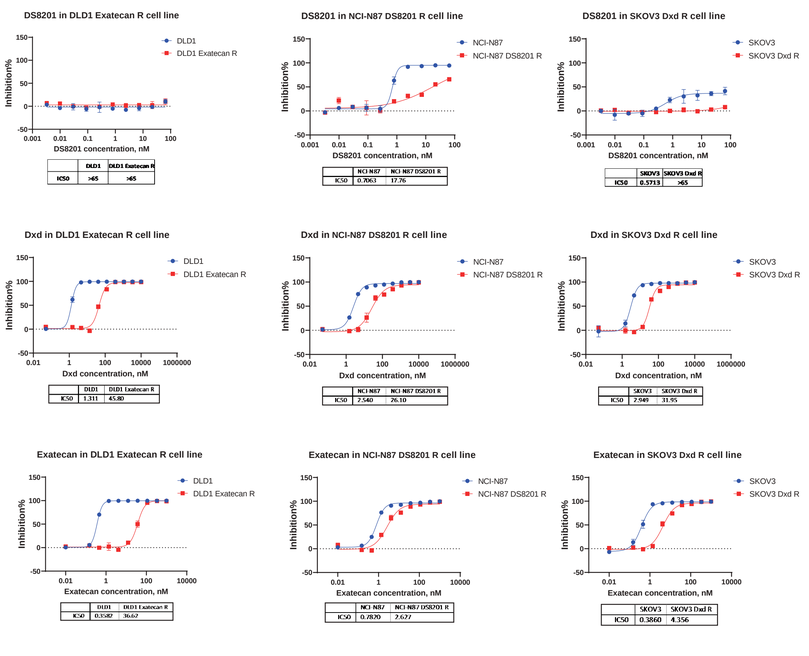


Table 3 Compounds Summary with Synergistic Effect

Compound Name	10uM compounds Only（inhibition%）	Combined with 10uM HRO761 (inhibition%)	Target	Pathway
Masoprocol	8.6	86.7	Lipoxygenase	Metabolic Enzyme/Protease
Propyl gallate	12.1	69.8	Others	Others
Monobenzone	23.5	79	Others	Others
Alfuzosin (hydrochloride)	3	57.2	Adrenergic Receptor	GPCR/G Protein; Neuronal Signaling
Nitrofurantoin	-11	42	Antibiotic; Bacterial	Anti-infection
Cilnidipine	12.9	64.1	Calcium Channel	Membrane Transporter/Ion Channel; Neuronal Signaling
Luteolin	39	89.5	Apoptosis; Autophagy; Endogenous Metabolite; Keap1-Nrf2	Apoptosis; Autophagy; Metabolic Enzyme/Protease; NF-κB
Bacampicillin (hydrochloride)	16	66	Antibiotic; Bacterial	Anti-infection
Temsirolimus	29.4	78.5	Apoptosis; Autophagy; Bacterial; mTOR	Anti-infection; Apoptosis; Autophagy; PI3K/Akt/mTOR
Tenofovir amibufenamide	18	67	HBV	Anti-infection
Rezafungin (acetate)	22.4	69.4	Fungal	Anti-infection
Sumatriptan	5	52	5-HT Receptor	GPCR/G Protein; Neuronal Signaling
Pinaverium bromide	19	65	Calcium Channel	Membrane Transporter/Ion Channel; Neuronal Signaling
Danthron	13.8	58.7	AMPK; Autophagy; Bacterial; Virus Protease	Anti-infection; Autophagy; Epigenetics; PI3K/Akt/mTOR
Capivasertib	14	57	Akt; Autophagy	Autophagy; PI3K/Akt/mTOR
Menadione	52	95	Endogenous Metabolite	Metabolic Enzyme/Protease
Cortisone	46.8	89.8	Endogenous Metabolite; Glucocorticoid Receptor	Immunology/Inflammation; Metabolic Enzyme/Protease; Vitamin D Related/Nuclear Receptor
Furazolidone	17	59	Antibiotic; Apoptosis; Bacterial; Parasite	Anti-infection; Apoptosis
Levonordefrin	11	52	Adrenergic Receptor	GPCR/G Protein; Neuronal Signaling
Fidaxomicin	48	87	Antibiotic; Apoptosis; Bacterial; DNA/RNA Synthesis	Anti-infection; Apoptosis; Cell Cycle/DNA Damage

Figure 6. A library of approximately 1000 small molecules was assessed in the HRO761 Resistant cell line at a concentration of 10 μM, both as single agents and in combination with 10 μM HRO761. The results revealed several compounds that demonstrated potential synergistic effects. These compounds were implicated in pathways such as metabolism, autophagy, and cell cycle regulation, details showed in table 3.

Elucidating the Resistance Mechanisms of ADC-Related Resistant Cell Lines



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	0.7063	25.4
NCI-N87 DS8201R		17.76	
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	0.0959	3.6
NCI-N87 DS8201R		0.345	
SKOV3	Ovarian adenocarcinoma	0.0614	15.8
SKOV3 Dxd R		0.968	

Figure 7. 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.

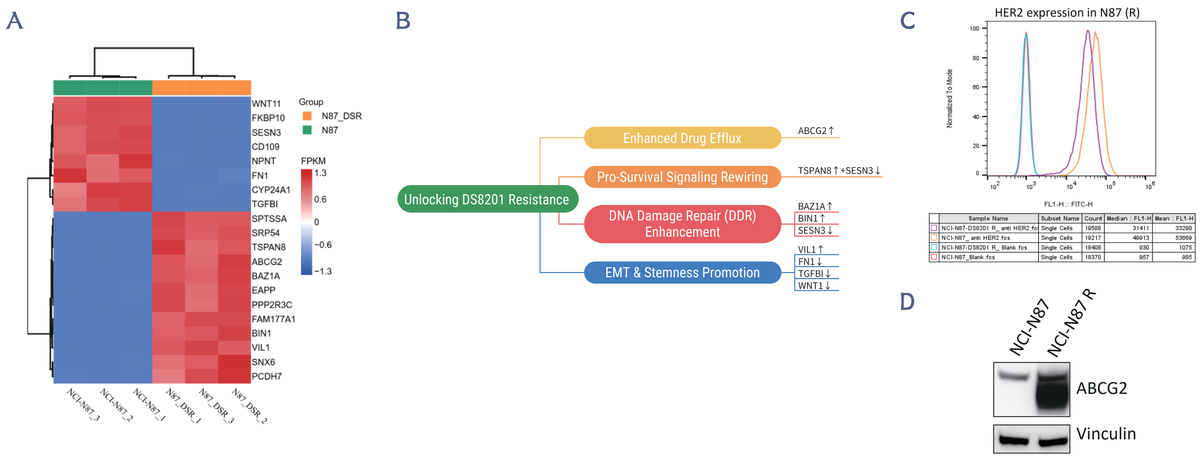


Figure 8. Bioinformatics analyses were conducted using RNA-seq data from NCI-N87 WT and DS8201-resistant cell lines. Panel A displays the differential gene expression profiles between parental NCI-N87 cells and DS8201-resistant derivatives. Panel B outlines the pathway enrichment analysis of differentially expressed gene clusters. Panel C confirms reduced HER2 expression in resistant cells through flow cytometry. Panel D provides Western blot validation of markedly upregulated ABCG2 in DS8201-resistant cells, implying a drug-resistance mechanism potentially linked to heightened ABC transporter activity and diminished HER2 expression.

Summary

In this study, we successfully constructed over 30 drug-resistant cancer cell lines, spanning key oncology targets such as KRAS, EGFR, PARP, DDR-related targets, and ADC-related targets, as well as major cancer types like non-small cell lung cancer, breast cancer, colorectal cancer, gastric cancer,etc. We assembled a cell panel from these lines to evaluate the efficacy of standard-of-care therapies in NSCLC and breast cancer via in vitro proliferation inhibition assays. This work aims to uncover combination therapies or new drug development opportunities. We also delved into WRN-related resistance mechanisms by examining how drugs alter WRN expression levels and employing bioinformatics to identify potential resistance genes. Furthermore, we screened approximately 1,000 FDA-approved small molecules in combination with resistant-strain drugs to find synergistic agents capable of overcoming resistance. Regarding ADC drugs, we identified DS8201 resistance as resulting from decreased HER2 expression and increased ABC transporter expression. These findings provide a foundation for clinical practice and drug development. Overall, our ICE drug resistance cell platform and cell panel platform continuously develop resistance models, bridge clinical practices with resistance mechanism research, and serve as a cornerstone for advancing personalized medicine and improving therapeutic outcomes in oncology, thereby driving progress toward precision medicine and enhancing therapeutic efficacy in oncology.