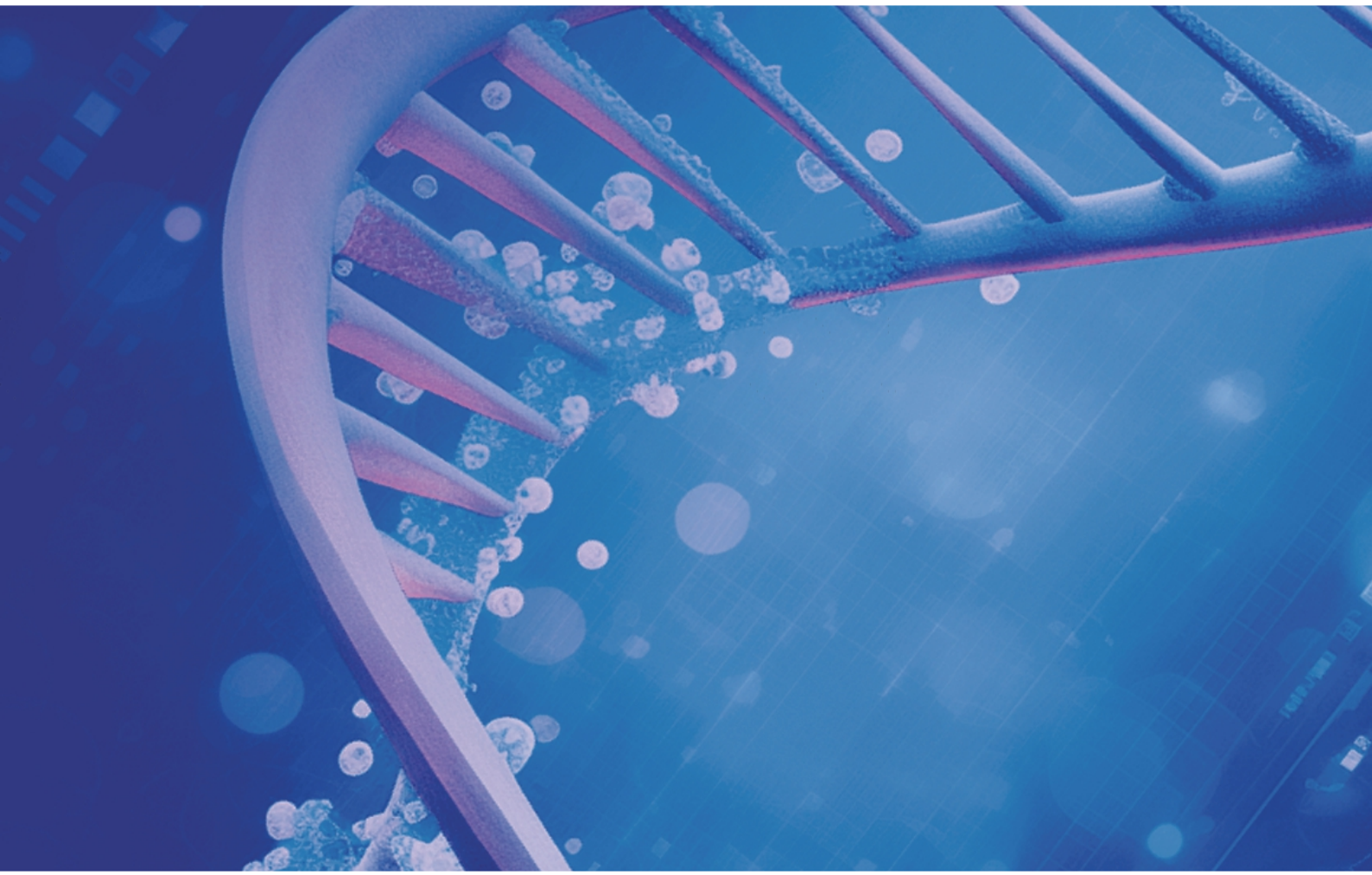
Advance Targeting DNA Damage Response Therapies

DDR Integrated Drug Discovery Services



Innovative CRO+Explorer
ICE Bioscience

DNA Damage Response Solutions For Drug Discovery

DDR (DNA Damage Response) is a collection of processes which cells utilize in order to identify and correct the DNA damage of the genome. DDR plays a pivotal role in drug discovery by serving as a target-rich domain for therapeutic intervention. The failure of DNA repair can eventually lead to malignant tumors or cancers, providing valuable opportunities for precision medicine.

In drug development, DDR research identifies specific molecular targets for therapeutic compounds. This precision allows for the design and development of drugs that selectively modulate aberrant DDR processes, presenting innovative solutions for treatment.

ICE Bioscience provides services for a broad spectrum of targets which cover different DDR pathways, such as single-strand break (SSB) and double-strand break (DSB). Our integrated service platform can support our clients with all-in-one drug discovery solution, which includes protein production, biochemical assays, functional and cell based assays, and in vivo studies, etc. Together, let's unravel the complexities of DDR.

DDR Integrated Drug Discovery Services

- Protein Engineering
- Biophysical Assays
- Biochemical Assays
- Cell Based Assays
- Assay Development

- siRNA/CRISPR KD/KO
- Cell Line Engineering
- Biomarker Evaluation
- Cell Phenotyping Studies

- MOA Studies
- Cancer Cell Panel Screening
- In Vitro ADMET, PK/PD
- In Vivo Pharmacology

- Safety Panels
- Kinase Panels
- CiPA Cardiac Safety
- IND Enabling

DDR Pathways and Targets

DDR pathways are often dysregulated in various diseases, including cancer. Identifying components of DDR pathways as potential drug targets is a critical aspect of drug discovery.

DDR contains multiple distinct pathways which are utilized to repair different types of DNA damage. Understanding the key DDR pathways is paramount in drug discovery, as they unveil potential targets for therapeutic interventions. ICE Bioscience has developed assays against critical proteins of different pathways (Table 1) to support the drug discovery process.

DDR Pathways	Targets
HR	ATM, ATR, BLM, RECQ1, RECQ4, RECQ5
NHEJ and TMEJ	DNA-PK, WRN, POLQ
SSB and BER	PARP1/2/3/6/7/10/11/12/14/15, TNSK1/2, FEN1, Topo I, APE1, XRCC1
Cell Cycle	WEE1/2, MYT1, p53 Y220C, CHK1/2
Others	POLA, POLG, POLH, MAT2A, PRMT5, TREX1/2

Table 1. Target list of different DDR pathways.

Protein Engineering For DDR

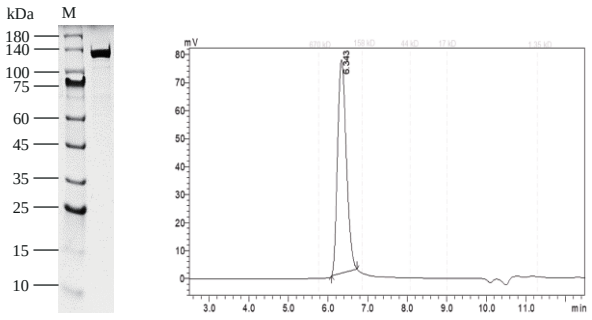


Figure 1. Purified PARP1 protein at ICE Bioscience (SDS-PAGE & SEC-HPLC data).

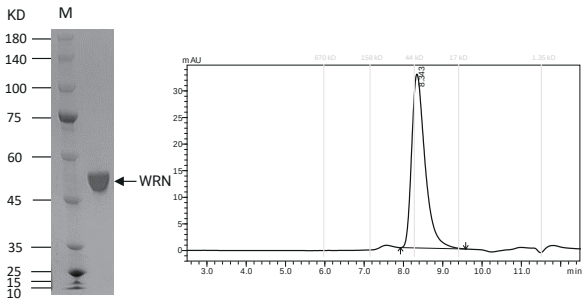


Figure 2. Purified WRN protein at ICE Bioscience (SDS-PAGE & SEC-HPLC data).

No.	Name	No.	Name	No.	Name
1	BLM	22	CDK4/CycD1	42	PARP1
2	CDK1/CycA2	23	CDK4/CycD2	43	PARP10
3	CDK1/CycB1	24	CDK4/CycD3[S259A]	44	PARP11
4	CDK1/CycE1	25	CDK5/p25NCK	45	PARP12
5	CDK1/CycE1	26	CDK5/p35NCK	46	PARP14
6	CDK1/CyclinE2	27	CDK6/CycD1	47	PARP2
7	CDK12/Cyclin K	28	CDK6/CycD3	48	PARP3
8	CDK13/Cyclin K	29	CDK7/CCNH/MNAT1	49	PARP5A
9	CDK14/CyclinY	30	CDK8/CyclinC	50	PARP5B
10	CDK16/CyclinY	31	CDK9/Cyclin K	51	PARP6
11	CDK17/CyclinY	32	CDK9/Cyclin T1	52	POLN
12	CDK17/p35NCK	33	cGAS	53	POLQ
13	CDK18/CyclinY	34	C-Myc	54	RecQ1
14	CDK19/CyclinC	35	DHX9	55	RecQ4
15	CDK2/CycA2	36	HELQ	56	RecQ5
16	CDK2/CycD1	37	MAT2A	57	TREX1
17	CDK2/CycE1	38	MYT1	58	TREX2
18	CDK2/CycE1	39	p53	59	WEE1
19	CDK2/CyclinE2	40	p53[Y220C]	60	WEE2
20	CDK3/CycE1	41	PARG	61	WRN
21	CDK3/CycE1				

Table 2. Recombinant protein products for DDR-related targets.

- Consistent production, purification, and validation, with custom protein production capabilities.
- Excel in expressing proteins across bacterial, insect, and mammalian systems.
- More than 50 DDR-related proteins purified and validated successfully, demonstrating robust activity in biochemical assays.

Biochemical Assays For DDR

ICE Biochemical Platform has pioneered the development of assays for over 1500 targets, encompassing those related to DDR. Our rigorous validation process is applied meticulously to each assay developed. Leveraging the characteristic activity profiles of DDR proteins, we have validated and optimized various biochemical assays (Table 3) to evaluate the potency of drug candidates. We've extended our assay development to include family members of DDR proteins to enhance drug selectivity assessments. Figure 3 illustrates examples of biochemical assays tailored for DDR targets.

DDR-related Enzymatic Assays
ADP-Glo assays
Fluorescence Intensity
HTRF assays
Absorbance assays
MTase-Glo assays
Fluorescence polarization assays
Chemiluminescent assays

Table 3. Different types of biochemical assays available for DDR related targets.

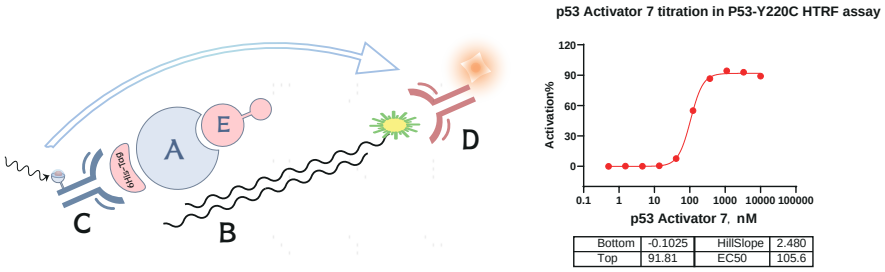
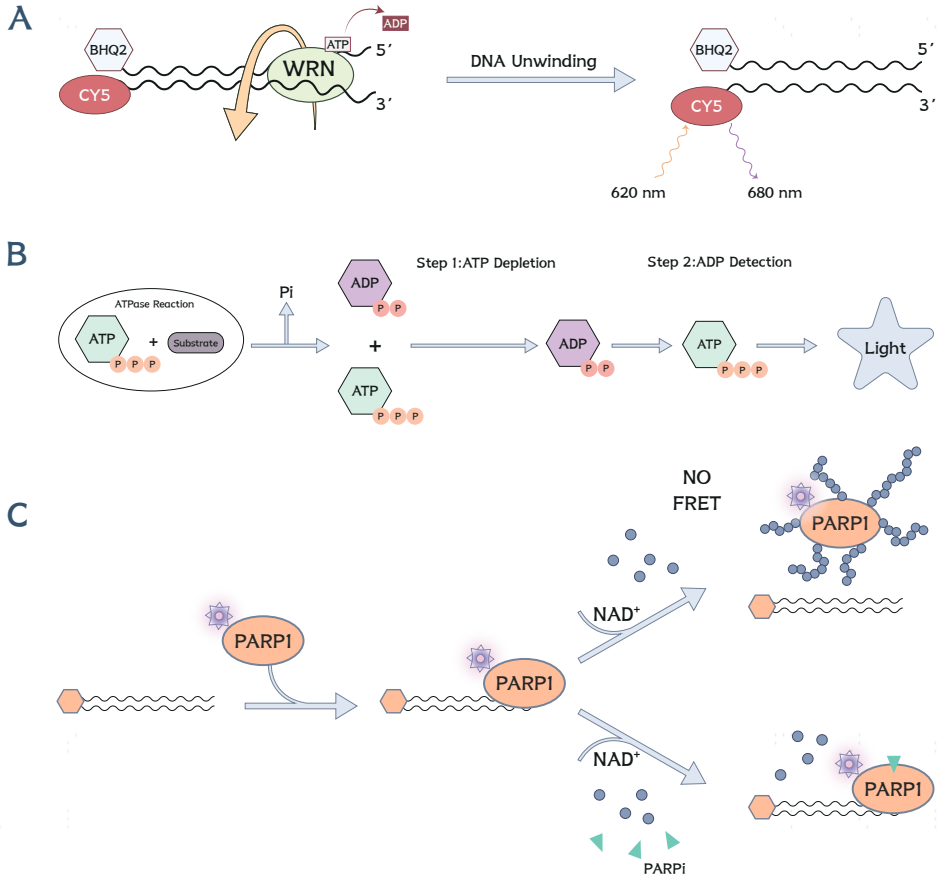
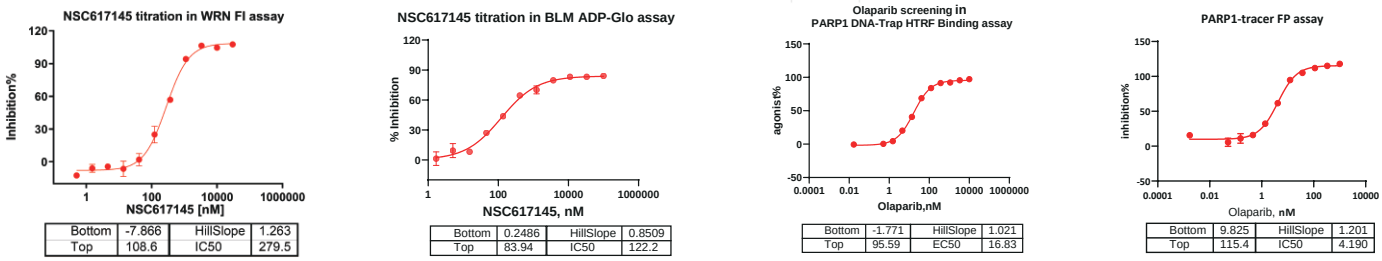


Figure 3. The HTRF-based P53 Binding Assay involves His-tagged P53 protein (A) and biotin-labeled DNA fragments (B) as a specific binding partner. The assay employs a donor-acceptor pair, with His-Tb (C) labeling P53 and Streptavidin-d2 (D) labeling biotin-DNA. (E) Compounds are binding with P53 protein. Proximity-induced fluorescence, resulting from the binding of P53 to biotin-DNA, generates an HTRF signal upon excitation. This luminescent signal is sensitive to changes in the P53-DNA interaction.





Bottom	-7.866	HillSlope	1.263
Top	108.6	IC50	279.5

Bottom	0.2486	HillSlope	0.8509
Top	83.94	IC50	122.2

Bottom	-1.771	HillSlope	1.021
Top	95.59	EC50	16.83

Bottom	9.825	HillSlope	1.201
Top	115.4	IC50	4.190

Figure 4. Principles of biochemical assays for DDR.
(A) The WRN unwinding assay relies on TAMRA fluorescence changes during DNA unwinding. Initially quenched by BHQ, TAMRA fluorescence is activated upon helicase activity initiated by ATP. The ATP-driven unwinding separates DNA strands, allowing TAMRA signals.
(B) By quantifying luminescence, the ADP-Glo assay provides a sensitive and reliable method for monitoring ATP hydrolysis.
(C) Binding to DNA activates PARP1 and in the presence of NAD+, PARP1 ribosylates itself, leading to PARP1 dissociation from the DNA. In the presence of some inhibitors, PARP remains bound to the DNA.

Figure 5. Pharmacology of several compounds in different biochemical assays for DDR.

Cell Based Assays For DDR

In vitro assessment of DDR proteins has been a crucial step during the drug discovery cascade. In order to meet the different requirements in compound screening, our team consistently develops cell based assays for evaluating drug selectivity, cytotoxicity, or their impact on the key biomarkers (Figure 6). ICE Bioscience remains open and enthusiastic about developing novel cell based assays for DDR drug discovery. Polymerase theta (POLQ) has gained increasing attention recently due to its potential contribution to PARP inhibitor (PARPi) resistance. To assess the impact of POLQi, we have successfully developed a TMEJ reporter assay (Figure 7).

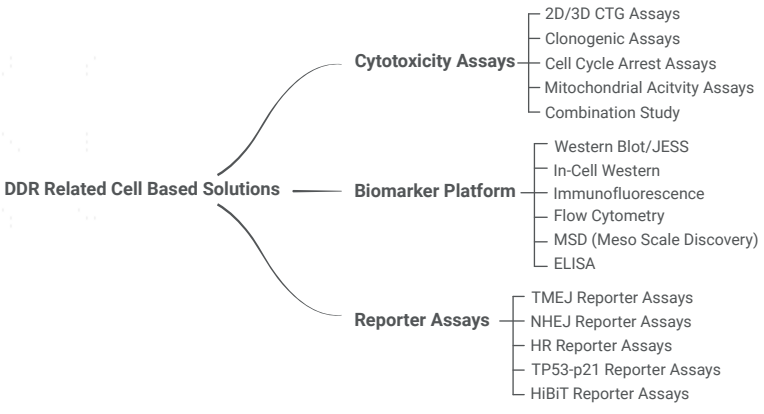
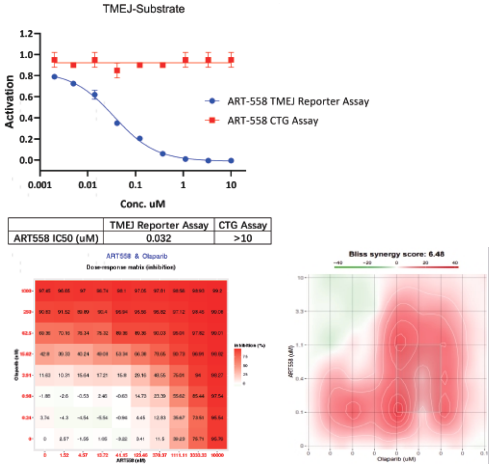


Figure 6. A summarized diagram of DDR related cellular assays.



	TMEJ Reporter Assay	CTG Assay
ART558 IC50 (uM)	0.032	>10

Figure 7. The valuation of ART-558 in TMEJ reporter assay and potency assessment in combination with Olaparib treatment.

PARylation is among our frequently detected DDR biomarker panel (Table 4) and serves as a crucial platform for recruiting proteins involved in the repair of DNA nicks. As poly(ADP-ribose) polymerase (PARP) induces the synthesis of PAR, it emerges as one of the earliest targets for anti-tumor drug development.

To streamline the screening of PARP inhibitors, we have developed various assays which enable us to directly measure the levels of PARylation or assess the accumulation of DNA damage (Figure 8).

DDR Biomarker		
ATM/pATM	KAP1/pKAP1	PLK1/pPLK1
BRCA1/2	MAR	POLQ
CCNE1	MAT2A	Rad51C
CDC2/pCDC2	MDM2	RPA32/pRPA32
CDK1/pCDK1	MYPT1/pMYPT1	SHLD2
CDK2/pCDK2	P21	Ub-PCNA
CDK4/6/7/9	P53	USP1
CHK1/pCHK1	PAR	Wee1
cMyc	PARG	WRN
FANCD2	PARP1/2/7	XRCC1
FANCF	PCNA	γH2A.X
I-SceI	PKMYT1	

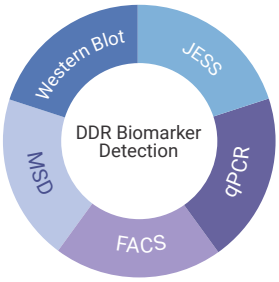


Table 4. DDR biomarker detection methods and target list.

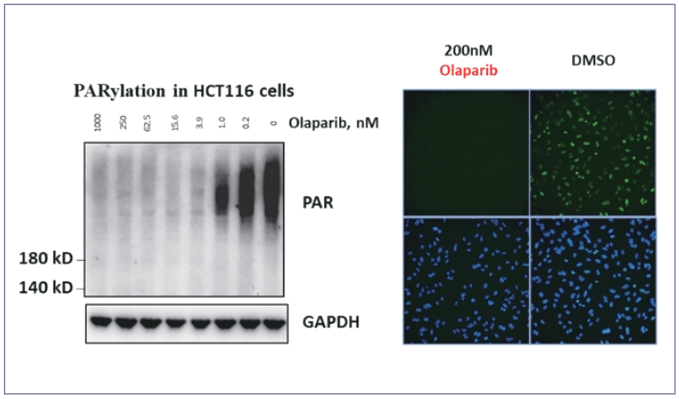


Figure 8. Exemplary data of PARylation and DNA damage evaluation.

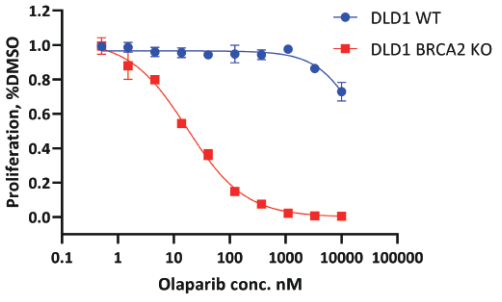
Cell Line Engineering For DDR

Synthetic lethality has been a key mechanism during the development of DDR inhibitors against various diseases, including cancer. ICE Bioscience has devoted considerable efforts to generate knock-out cell lines for different DDR genes. To uncover the mechanisms of drug resistance and thereby facilitate novel drug development, we have established drug-resistant cell lines spanning various tumor types. With the generation of HiBiT knock-in cell lines, which can be utilized for novel degrader screening, our robust cell line engineering platform continues to expand (see Figure 9).

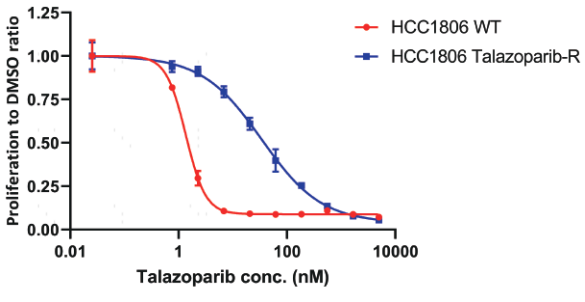
Cell Line Engineering Capabilities	
System	Lentiviral infection
	Electroporation
	Liposomal transfection
	Non-liposomal transfection
Cell type	Reporter cell line
	Over-expression cell line
	Knock-out cell line
	Knock-in cell line
	Knock-down cell line
	Drug resistant cell line

KO Cell Line		
92-1-ASAH1-KO	HCT116-BRCA1-KO	Mel270-ASAH1-KO
A2780-FANCD2-KO	HCT116-CDK2-KO	NUGC-3-TP53-KO
A375-ASAH1-KO	HCT116-MTAP-KO	SK-MEL-28-ASAH1-KO
A549-PARP1-KO	HCT116-PLK2,3-KO	SW1573-SMARCA2-KO
A549-PARP2-KO	HCT116-WRN-KO	THP-1-dual-STING-KO
DLD1-BRCA2-KO	HEK293-CRBN-KO	THP-1-STING-KO
E0771-DDR1-KO	HEK293-NSD3-KO	U2OS-53BP1-KO
EO771-DDR1-KO	HEK293-STING-KO	
FaDu-ATM-KO	HEK293-VHL-KO	
H520-NSD3-KO	HT1080-NSD3-KO	
HCC1806-XRCC1-KO	HT1080-SMARCA2-KO	
HCC95-NSD3-KO	MC38-STK11-KO	

Resistant Cell Line		
22Rv1 Docetaxel R	HCT8 Vincristine R	NCI-H2228 Alectinib R
A2780 Cisplatin R	LNCAP Casodex R	NCI-H2228 Crizotinib R
A375 Dabrafenib R	MCF-7 Tamoxifen R	NCI-H460 Paclitaxel R
A375 Vemurafenib R	MDA-MB-468 5-Fluorouracil R	OVCAR8 Doxorubicin R
BT474 Lapatinib R	MDA-MB-468 Olaparib R	PANC1 Talazoparib R
BT-474 Trastuzumab R	MDA-MB-468 Talazoparib R	PC-9 Afatinib R
H358 Adagriasib R	MIA-paca2 AMG510 R	PC-9 Gefitinib R
HCC1806 Niraparib R	MOLM-13 SHP099 R	PC-9 Osimertinib R
HCC1806 Talazoparib R	MV-4-1 SHP099 R	Ramos Ibrutinib R
HCC4006 Osimertinib R	NALM6 Doxorubicin R	SNU-16 Docetaxel R
HCC827 Gefitinib R	NCI-H1975 Afatinib R	SNU-601 Olaparib R
HCC827 Osimertinib R	NCI-H1975 Osimertinib R	T47D Palbociclib R



	DLD1 WT	DLD1 BRCA2 KO
IC50	27153	17.42



	HCC1806 WT	HCC1806 Talazoparib-R
IC50	1.367	34.19

Figure 9. A partial list of DDR cell line engineering platform (top) and an example of cell line validation for DLD1 BRCA2 KO and HCC1806 Talazoparib-resistant cell lines (below).

ICECP™ DDR Cell Panel

How to utilize the DDR mechanism to inhibit the growth of tumor cells, especially overcoming drug resistance, has been a hot topic in pharmaceutical research over the past decade. Targeting DDR pathways, we have introduced a DDR cell panel that covers 12 cancer types, totaling 75 cell lines, including common tumor cell lines, gene-edited cells such as BRCA-KO, and drug-resistant cell lines (Figure 10). This panel can be used for screening the activity and selectivity of DDR-related inhibitors. To meet different compound assessment requirement, we are offering assays with a duration of various time length.

- Robust, flexible, and tailored profiling of test agents on total 500+ cancer cell lines
- Various assay formats such as 2D proliferation, 3D proliferation, colony formation, and apoptosis, with no assay timeline constraints.
- For generated drug resistant cell lines, we perform RNA-seq-based bioinformatic analysis to investigate the mechanism and provide detailed information about gene expression, enriched pathway and featured gene profiling.

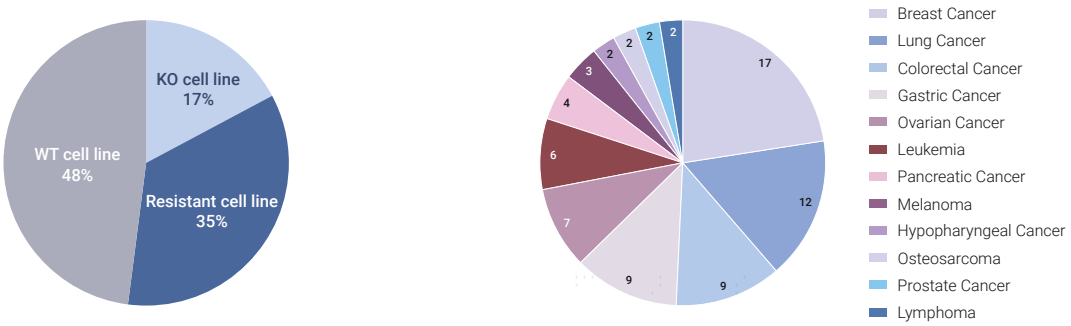


Figure 10. ICECP™ DDR cell panel.

Biophysical Assays For DDR

Biophysical assays contribute significantly to DDR-related drug discovery by providing critical information about the interactions between potential drug candidates and DDR targets. These assays help in understanding the binding mechanisms, assessing the affinity and specificity of interactions, and elucidating the structural and stability changes in target proteins upon ligand binding. ICE Bioscience has extensive experience in key biophysical assays such as Surface Plasmon Resonance (SPR) and nano Differential Scanning Fluorimetry (nanoDSF).

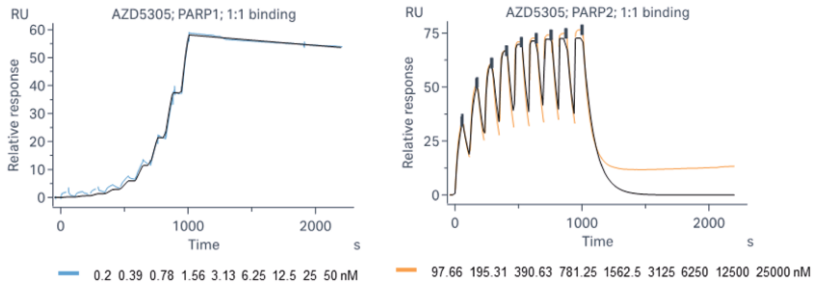


Figure 11. SPR result shows the selectivity of PARP inhibitor AZD5305.

Protein	Compound	Conc.(uM)	Tm D	△ Tm D
P53 Y220C	P53 Activator 7	20uM	43.71	8.11
	P53 Activator 7	20uM	43.43	7.83

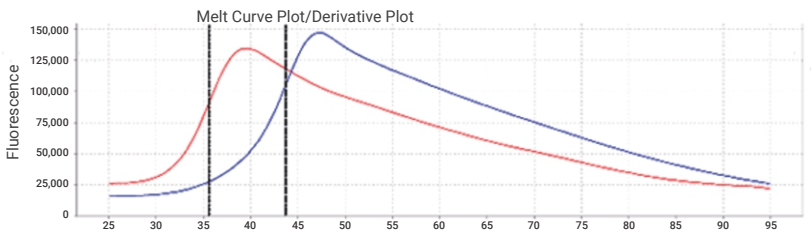


Figure 12. Exemplary TSA data of p53-Y220C.

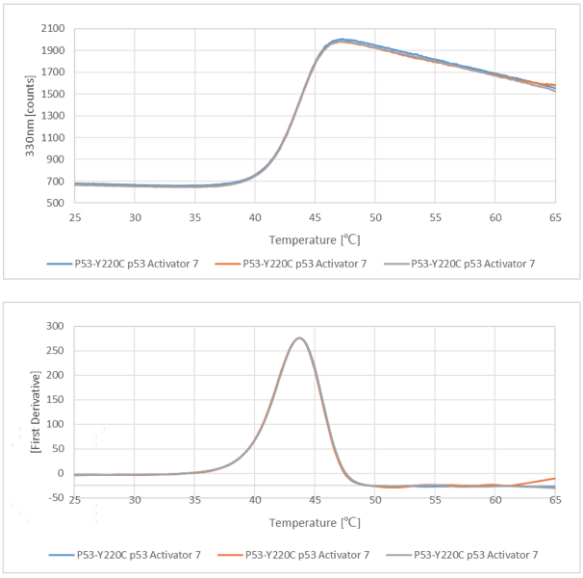


Figure 13. Exemplary nanoDSF data of p53-Y220C.

DMPK and In Vivo Pharmacology

In addition to enzymatic and cell-based assays, in vivo studies are conducted to assess a lead compound in a more complex environment.

Our Drug Metabolism and Pharmacokinetics (DMPK) platform has established comprehensive in vitro ADME assays (Table 5 and exemplary WRNi data in Figure 14.). Furthermore, we have validated various CDX and PDX models in efficacy studies. With these assays, we can provide an integrated solution for DMPK and in vivo pharmacology, tailored to meet the requirements at different stages of DDR-related inhibitor evaluation (Figure 16).

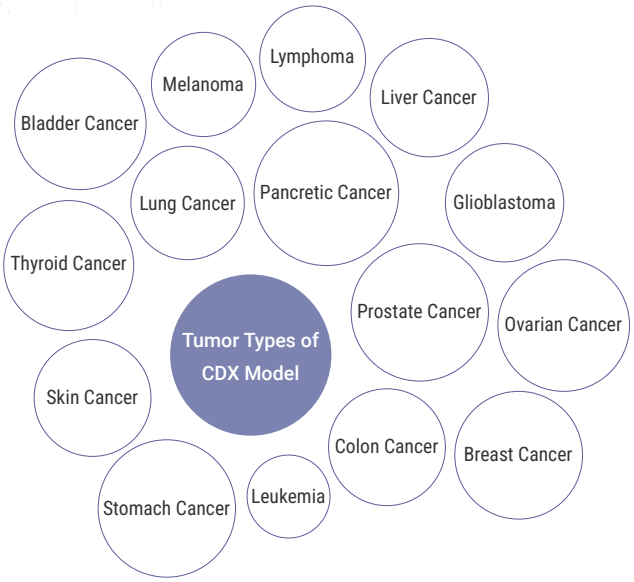
Tier 1 ADME panel

- Solubility
  LC-MS/MS, PBS, SGF, SIF
- Permeability: Caco-2, pH 6.5/7.4
- Protein binding: Plasma, human
- Intrinsic clearance
  Liver microsomes, human
- Lipophilicity
  Log D or Log P

Tier 2 ADME panel

- CYP Inhibition
- CYP time-dependent inhibition
- CYP Phenotyping
- CYP Induction (in progress)
  human hepatocytes
- P-gp substrate assessment (Caco-2)
- BCRP substrate assessment (Caco-2)
- Transporter Inhibition

Table 5. Our Tier 1 and Tier 2 ADME Panels offer in vitro assays for quick and comprehensive analysis.



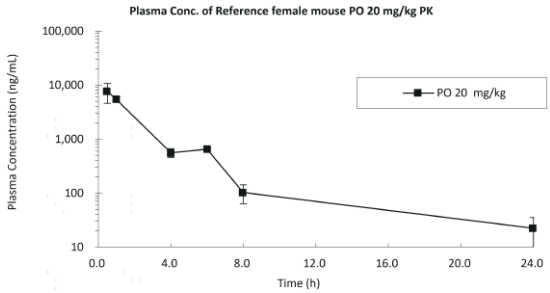


Figure 14. . An example of PK study testing HRO-761 concentration in mouse plasma.

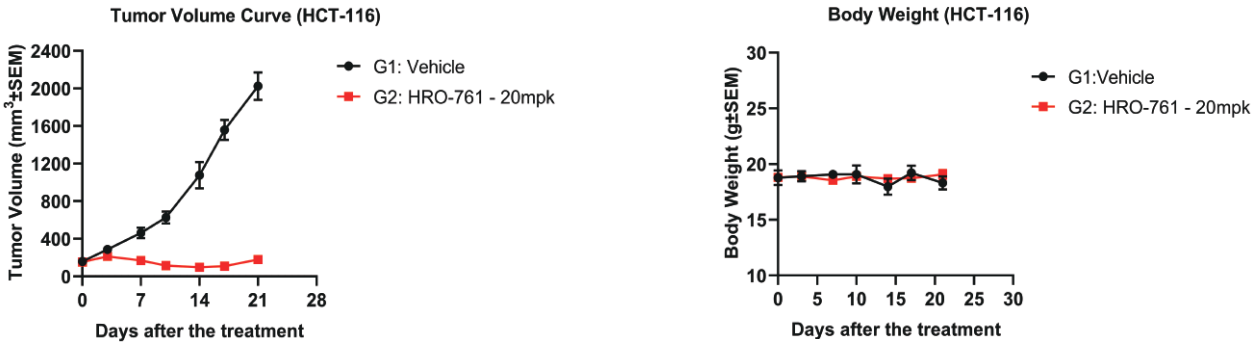


Figure 15. An example of efficacy study validation of WRNi against HCT-116 tumor inoculation.

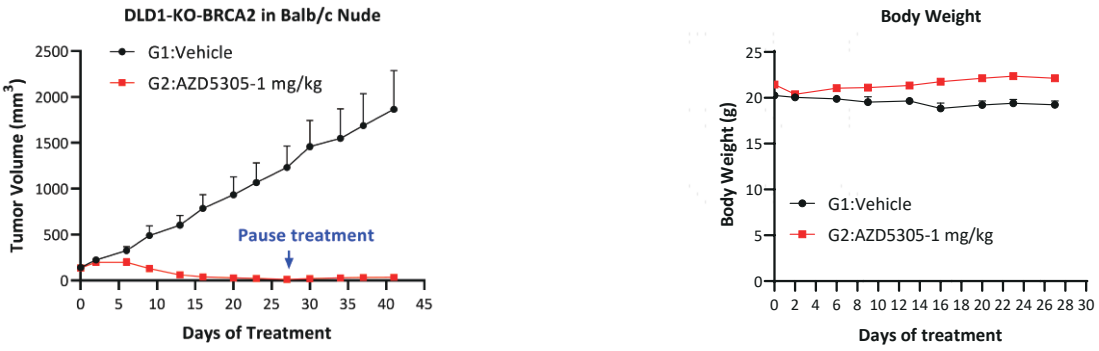


Figure 16. An example of efficacy study validation of AZD-5305 against DLD1 BRCA2 KO tumor inoculation.

ICE Bioscience

Innovative CRO+ Explorer

ICE Bioscience was founded in 2010 as an Innovative CRO+ Explorer company. We specialize in early drug discovery services, spanning from target validation to the identification of pre-clinical candidates. We stand out for our collaborative spirit and expertise in boldly exploring new therapeutic target research. Our commitment to drug discovery services, delivered with enthusiasm and professionalism, empowers clients to overcome challenges, address scientific puzzles, and fulfill our promises to clients, communities, the environment, and global health.

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