Leading the Path to DNA Damage Response Drug Discovery

Integrated Solutions for Targeted DDR Therapies

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 (SPR)
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
Other	WEE1/2, MYT1, POLA, POLG, POLH, MAT2A, PRMT5, TREX1/2, p53Y220C, CHK1/2

Table 1. Target list of different DDR pathways.

Protein Engineering For DDR

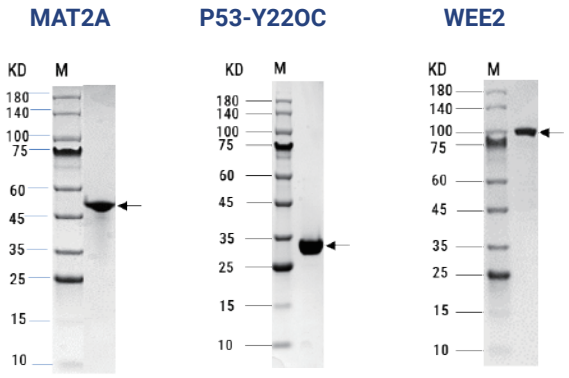


Figure 1. Purified MAT2A, P53-Y220C, and WEE2 proteins at ICE Bioscience (SDS-PAGE data).

- Consistent production, purification, and validation, with custom protein production capabilities.
- Excel in expressing proteins across bacterial, insect, and mammalian systems.
- More than 20 DDR-related proteins purified and validated successfully, demonstrating robust activity in biochemical assays.

No.	Cat. No.	Name	Uniprot#	Host
1	E2204T-H02H	RecQ1	P46063-1; C49-N619	E.coli
2	E2202T-H19H	BLM	P54132; S636-G1298	E.coli
3	S2201T-H03H	WRN	O14191; N500-C946	Sf9
4	S2212T-H56HZ	WRN	Q14191; N517-P1238	Sf9
5	E2204T-H01HU	RecQ4	O94761-1; A427-G1116	E.coli
6	E2204T-H04H	RecQ5	O94762-1; D11-V526	E.coli
7	E2212T-H19H	POLN	Q7Z5Q5; A192-G863	E.coli
8	S2201T-H01H	POLQ	O75417-1; S1819-V2590	Sf9
9	S2201T-H02H	POLQ	O75417-1; M1-M894	Sf9
10	E2202F-H26H	PARG	Q86W56-1; M1-T976; fl	E.coli
11	E2209T-H23H	PARG	Q86W56-1; S448-T976	E.coli
12	S2202T-H27H	PARP1	P09874; A2-W1014; fl	Sf9
13	S2203T-H31G	PARP2	Q9UGN5-1; A2–W583; fl	Sf9
14	S2203F-H32G	PARP3	Q9Y6F1-2; M1-L540; fl	Sf9
15	S2203F-H36G	PARP6	Q2NL67; M1-N630; fl	Sf9
16	S2203F-H40GH	PARP11	Q9NR21-4; M8-H338; fl	Sf9
17	S2203T-H41G	PARP12	Q9H0J9; K500-Q701	Sf9
18	S2203T-H42G	PARP14	Q460N5-6; K1470–K1801	Sf9
19	S2210F-H15G	WEE2	P0C1S8; M1-H567; fl	Sf9
20	E2207F-H12H	MAT2A	P31153-1; N2-Y395; fl	E.coli
21	E2202T-H01H	p53	P04637; S94-T312	E.coli
22	E2207T-H11H	p53[Y220C]	P04637; S94-T312	E.coli
23	E2303T-H08H	PRMT3	O60678-1; C2-Q531; fl	E.coli

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

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 2) 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 2 illustrates examples of biochemical assays tailored for DDR targets.

DDR-related Enzymatic Assays
Unwinding assays
ADP-Glo assays
SYBR green intercalation assays
HTRF assays
Absorbance assays
MTase-Glo assays
Fluorescence polarization assays
Chemiluminescent assays

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

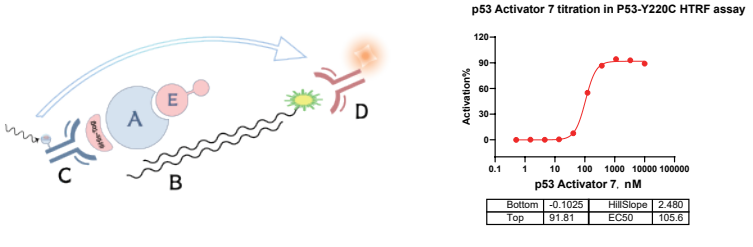


Figure 2. The HTRF-based P53 Binding Assay involves His-tagged P53 protein (A) and biotin-labeled DNA fragments (B) as a specific binding partner. The assay employs a donor-acceptor pair, with His-Tb (C) labeling P53 and Streptavidin-d2 (D) labeling biotin-DNA. (E) Compounds are binding with P53 protein. 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.

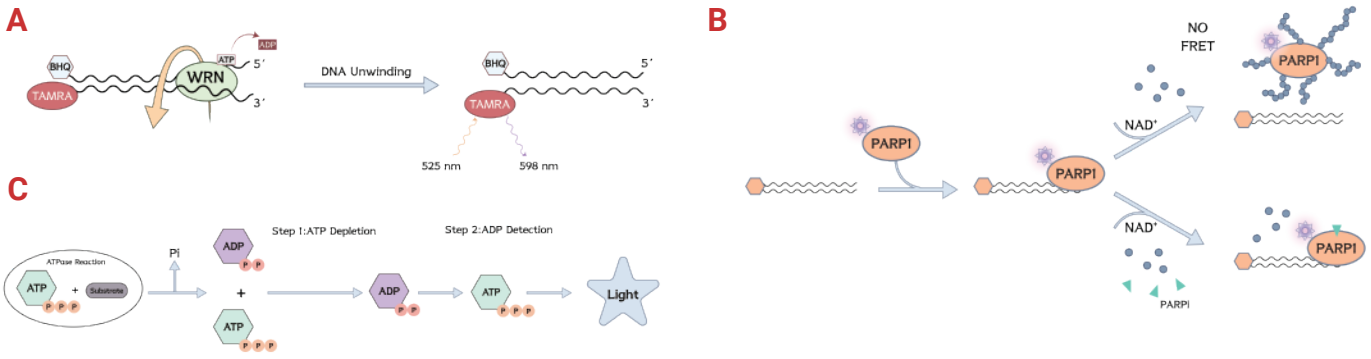


Figure 3. Principles of biochemical assays for DDR.

(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) 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.

(C) By quantifying luminescence, the ADP-Glo assay provides a sensitive and reliable method for monitoring ATP hydrolysis.

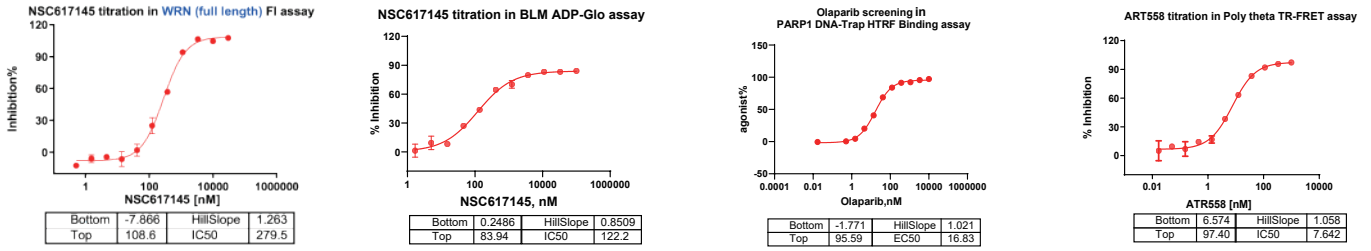


Figure 4. Pharmacology of several compounds in different biochemical assays for DDR.

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 5). 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 6).

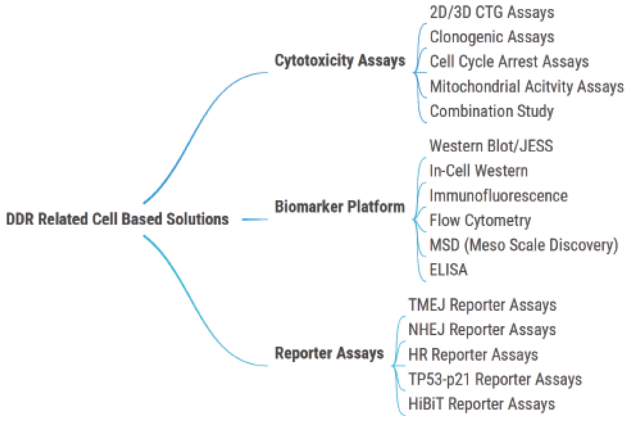


Figure 5. A summarized diagram of DDR related cellular assays.

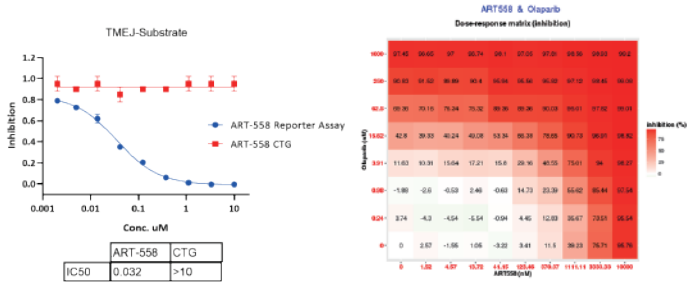


Figure 6. The valuation of ART-558 in TMEJ reporter assay and potency assessment in combination with Olaparib treatment.

PARylation 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 7).

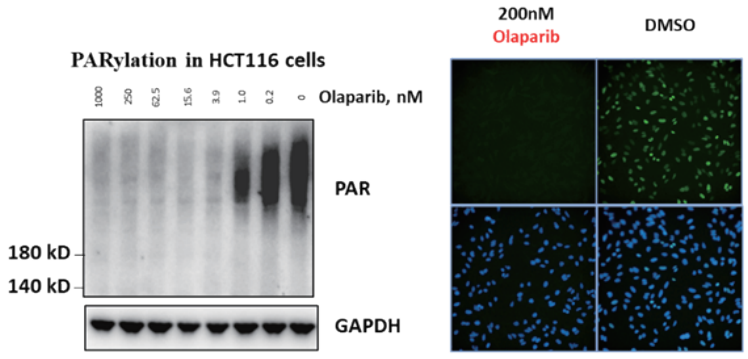
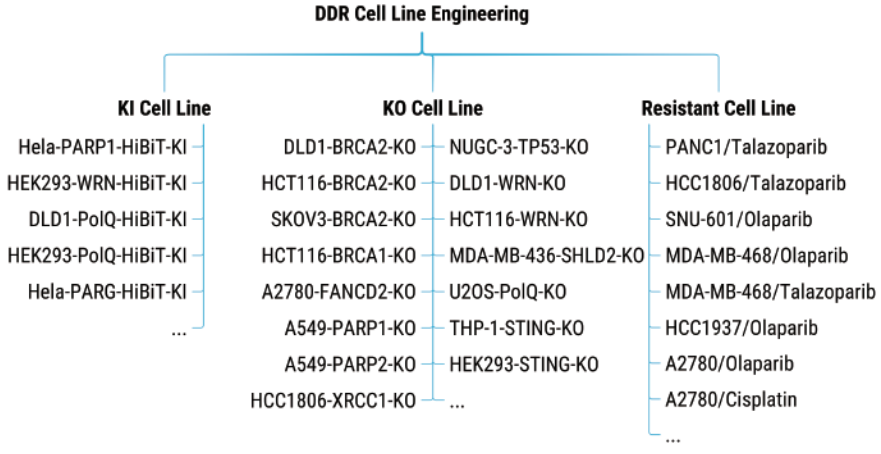
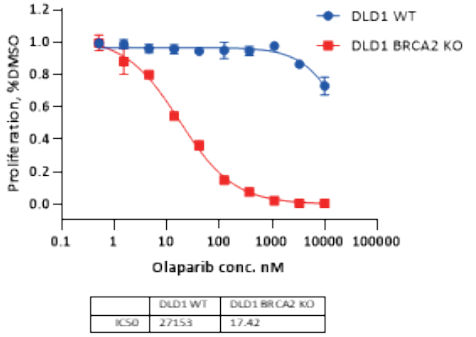


Figure 7. Exemplary data of PARylation and DNA damage evaluation.

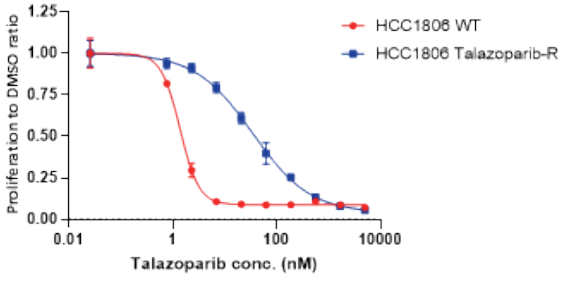
Cell Line Engineering For DDR

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 8).





	DLD1 WT	DLD1 BRCA2 KO
IC50	27153	17.42



	HCC1806 WT	HCC1806 Talazoparib-R
IC50	1.367	34.19

Figure 8. 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 10 cancer types, totaling 80 tumor cell lines, including common tumor cell lines, gene-edited cells such as BRCA-KO, and drug-resistant cell lines (Figure 9). This panel can be used for screening the activity and selectivity of DDR-related inhibitors. Due to the slow-acting nature of DDR-targeting drugs, we are offering assays with a duration of 7-14 days.

- 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.

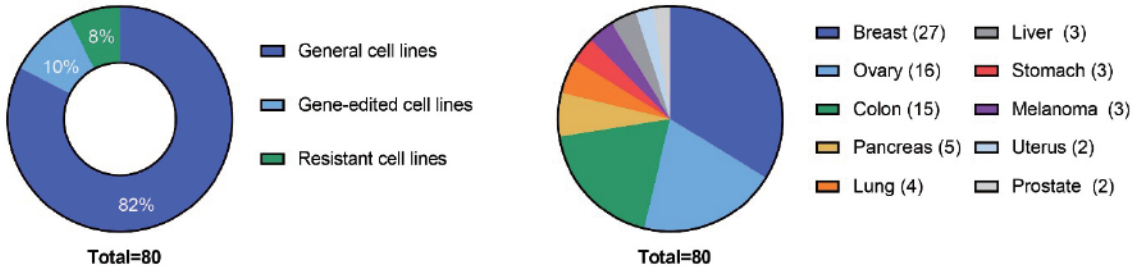


Figure 9. ICECP™ DDR cell panel.

CiPA Cardiac Safety Study

ICE Bioscience, a pioneer in safety pharmacology, introduces advanced solutions for cardiac safety screening. The human ether-a-go-go, hERG assay is essential in drug development to assess potential cardiac safety risks, particularly the risk of Torsades de Pointes (TdP).

- **hERG Patch Clamp Services at Physiological Temperature:** Aligned with the latest FDA updates, these services address historical challenges in S7B studies, offering improved clinical relevance and harmonized voltage protocols.
- **Full cardiac ion channel panel:** Comprehensively assessing the activity and effects of compounds on multiple ion channels which play key roles in governance of the cardiac action potential.

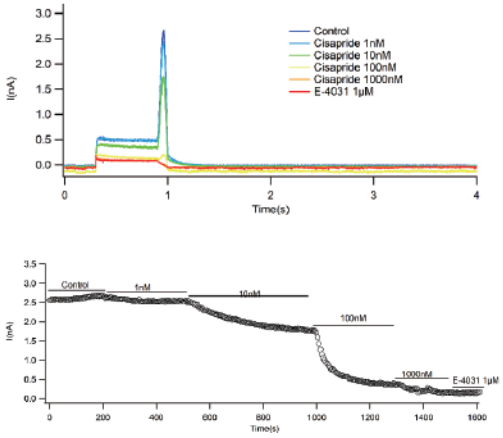


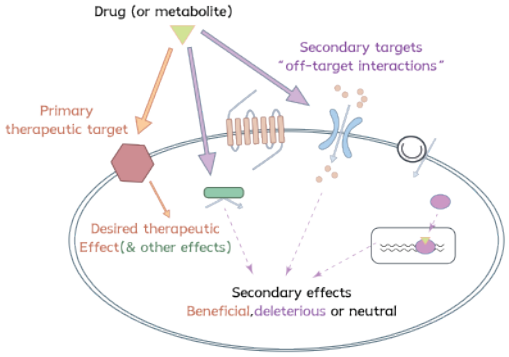
Figure 10. Near physiological temperature (PT), hERG representative current traces and time course plots under different concentrations of Cisapride.

Targets	Function	Reference Compound	Method*
hERG	IKr, rapid repolarization (phase 3)	Cisapride	MPC, APC
Nav1.5	INa, depolarization (phase 0)	TTX, flecainide	MPC, APC
Cav1.2	ICa-L, depolarization (phase 2)	nifedipine	MPC, APC
Kv7.1/minK	IKs/KvLQT, slow repolarization (phase 3)	Chromanol 293B	MPC, APC
Kv1.5	IKur, repolarization (atrial)	4-AP	MPC, APC
Kv4.3	Ito, repolarization (phase 1)	4-AP	MPC, APC
Kir2.1	IK1, repolarization (phase 4)	BaCl2	MPC, APC
Kir3.1/3.4	KAch, repolarization (atrial)	BaCl2	MPC
Kir6.2/SUR2	KATP	Glibenclamide	MPC
Cav3.2	ICa-T, pacemaker current	NiCl2	MPC, APC
HCN2	If, pacemaker current	ivabradine	MPC
HCN4	If, pacemaker current	ivabradine	MP
*MPC= Manual patch clamp, APC= Automated patch clamp (Nanion Patchliner ® /Qpatch HTX48)			

Table 4. ICE Bioscience CiPA full cardiac ion channel panel

In Vitro Safety Pharmacology

Challenges related to safety concerns in drug development persist as a significant hurdle for the pharmaceutical sector. Safety Pharmacology, as outlined by ICH S7A (2001), places a critical emphasis on the evaluation of drugs for off-target effects. In vitro Pharmacological profiling is now more frequently employed at the initial stages of drug discovery to detect unfavorable off-target activity profiles and predict clinical adverse effects to reduce unwarranted attrition.



Targets	SafetyOne44	SafetyIMax90
GPCR	24	37
Ion Channel	8	15
Enzyme	7	32
Nuclear Receptors	2	3
Transporter	3	3
No. of Targets	44	90
No. of Assays	74	138

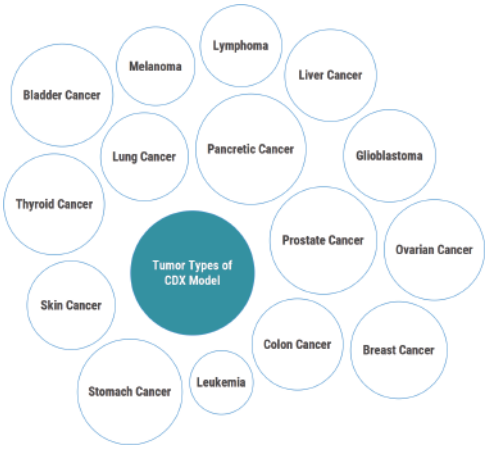
Table 5. In Vitro safety pharmacology functional safety panels.

- **State-of-the-art Functional Safety Panels:** SafetyOne44™ is designed for 44 specific targets, while SafetyMax90™ stands as the most comprehensive functional safety panel available, encompassing up to 90 targets.
- **Specialized Functional Panels:** Tailored to specific research areas. Compared to binding assays, our functional assays offer the advantage of expertise in distinguishing between different modes of action.

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 6). 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 11).



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

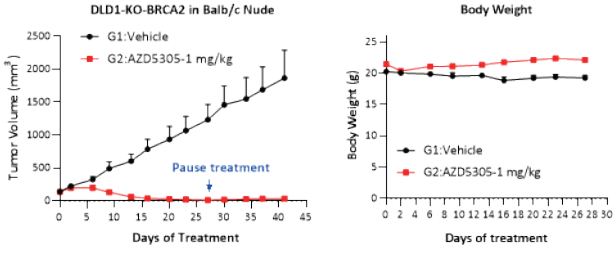


Figure 11. Validated CDX models across various tumor types (top), and an example of efficacy study validation of AZD-5305 against DLD1 BRCA2 KO tumor inoculation (bottom).

Innovative CRO+ Explorer

ICE Bioscience

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.

To learn more, please visit: en.ice-biosci.com