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PARPi screening cascade to facilitate novel drug discovery

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PURPOSE

The PARP (Poly(ADP-ribose) polymerase) family consists of enzymes vital for cellular functions, notably DNA repair, crucial for genomic stability. Once malfunction, these enzymes can contribute to various diseases, including cancer, neurodegenerative disorders, and inflammatory conditions. PARP inhibitors (PARPi), which target these enzymes, have shown promise as treatments for certain cancers, particularly those with DNA repair pathway defects such as BRCA-mutated cancer. Here, we have developed and validated PARP-related biochemical assays, cell-based assays, drug resistant cell line construction, and in vivo pharmacology models to support novel PARPi discovery.

METHODS

PARP trapping assay: His-tagged PARP1 and PARP2 recombinant proteins were purified and produced in house. A biotin-tagged dsDNA with single strand break was designed in house. The HTRF system consisted with XL665 labeled-streptavidin (SA-XL665) or cryptate-labeled streptavidin (SA-d2), and terbium-labeled anti-his antibody (His-Tb). Upon PARPi treatment, PARP proteins became trapped on DNA, leading to an increase in HTRF signal.

PARylation detection assay: Hela cells were cultured for overnight, then treated with PARPi. The cells were then treated with H_2O_2 to induce DNA damage. The PARylation formation was detected using pADPr antibody (Santa Cruz Biotechnology). Finally, the PARylation signal was normalized to the DAPI staining signal.

Drug resistant cell line construction: The chosen cell line was exposed to gradually increasing concentrations of the drug of interest over multiple passages. The construction was considered successful if the drug sensitivity decreases more than 10 folds when comparing constructed cell line with the parental cell line.

Knock-out cell line constructions: sgRNAs for PARP1 and PARP2 are designed in house. sgRNA and Cas9 protein were transfected in to A549 cells by electroporation. The cutting efficiency was assessed by PCR, followed by monoclonal selection to obtain the KO cell lines, which was further validated by sequencing, western blot, and functional assay.

In vivo efficacy study: Mice were subcutaneously injected with tumor cells. When tumor had reached desired volume, the mice were randomly assigned into vehicle and drug-treated groups. The mice in drug-treated groups were orally administrated with indicated amount of compound daily. The mice in vehicle group were treated with an equivalent volume of DMSO. The tumor volume was monitored on a weekly basis. The tumor weight was weighed at the end of each study.

RESULTS

Biophysical assays contribute significantly to DDR-related drug discovery by providing critical information about the interactions between potential drug candidates and DDR targets. ICE Bioscience has developed assays such as Surface Plasmon Resonance (SPR) to help in understanding the binding mechanisms, assessing the affinity and specificity of interactions, and elucidating the structural and stability changes in PARP proteins upon compound binding (Figure 1).

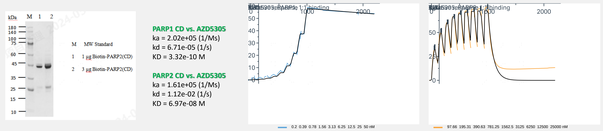


Figure 1. Utilizing the PARP proteins purified in house, ICE Bioscience has validated the binding selectivity of AZD5305 for PARP1 in the SPR assay.

ICE Bioscience has also developed PARP related biochemical assays which includes PARP trapping HTRF assay, fluorescence polarization (FP) assay, and chemiluminescent assay, to assess the potency of PARP inhibitors (Figure 2).

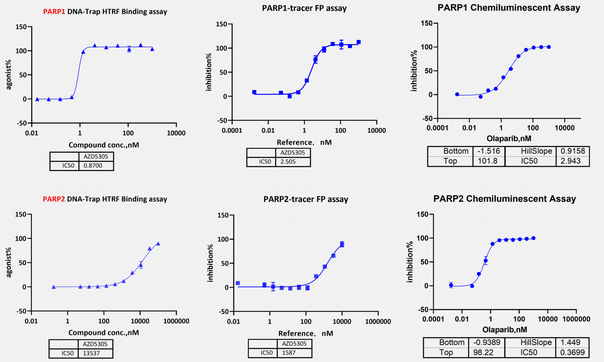


Figure 2. HTRF Binding assay, FP assay and Chemiluminescent assay showing inhibition of the PARP1/2 enzyme complex using a dose-range of AZD5305.

In recent years, the development of PARPi focuses in synthesizing compounds with higher tumor cell sensitivity and better potency against drug resistance. Therefore, we have put great effort in generating PARPi sensitive and resistant cell lines (Table 1). In addition, we provide multiple methods to evaluate the cell viability upon PARPi compound treatment. These methods include Cell-Titer Glo assay, colony formation assay (CFA). We also have extensive experience with the PARP-related combination study. (Figure 3)

HCT116 BRCA1 KO cell	HCC1806/Niraparib R cell line
DLD1 BRCA2 KO cell	HCC1806/Talazoparib R cell line
A549 PARP1/PARP2 KO cell	MDA-MB-468/Olaparib R cell line
A2780 FANCD2 KO cell	MDA-MB-468/Talazoparib-R cell line
HCC1806 XRCC1 KO cell	PANC1/Talazoparib R cell line
	SNU-601/Olaparib R cell line

Table 1. List of PARPi sensitive and resistant cell lines.

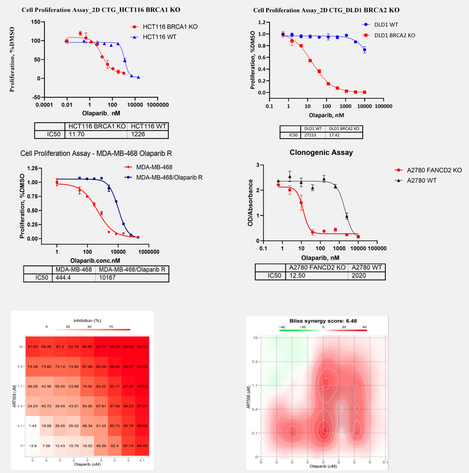


Figure 3. PARPi related cell proliferation assays.

To direct evaluate the function of PARPi on PARylation formation, we have developed immunofluorescence as well Western Blot assays. PARP1 and PARP2 KO cells have been engineered as well to provide insight on PARPi selectivity. (Figure 4)

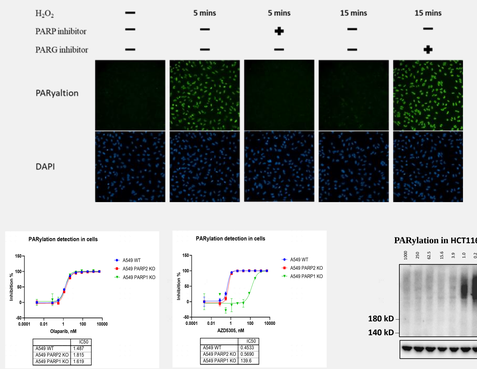
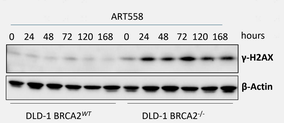


Figure 4. Immunofluorescent assay and Western Blot assay showed dose range inhibition of PARylation in A549 and HCT116 cells.

To further monitor the DNA damage caused by compound treatment, we have validated assays to detect a DNA damage biomarker like γH2AX. (Figure 5)



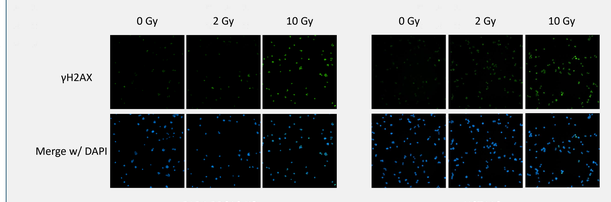


Figure 5. γH2AX detection upon X-ray radiation showed an increase in γH2AX with increasing X-ray irradiation intensity.

To evaluate the in vivo efficacy of PARPi compounds, we have inoculated wild-type, drug-sensitive, and drug-resistant tumor cells into mice and validated the compound efficacy. (Figure 6)

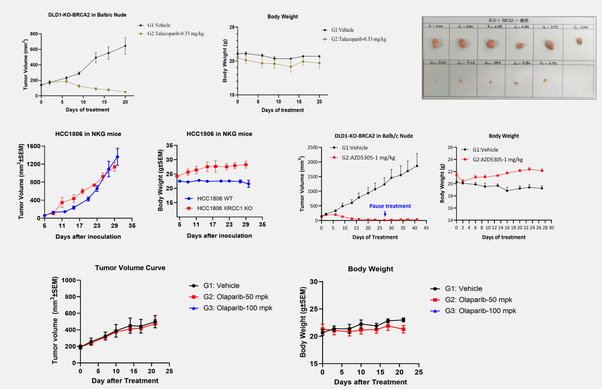


Figure 6. The validated animal models showing the PARPi efficacy against wild-type, drug-sensitive, and drug-resistant tumor cells.

CONCLUSION

We have successfully developed and validated various PARP related assays, which covers biochemical, cell-based, and in vivo models. Together with in-house generated PARP KO and PARPi resistant cell lines, our PARPi screening cascade can provide comprehensive analysis of test compounds thus expedite the drug discovery process.

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