APPLICATION NOTE

Compound screening in BRCA2 (-/-) cell lines: A model for synthetic lethality.

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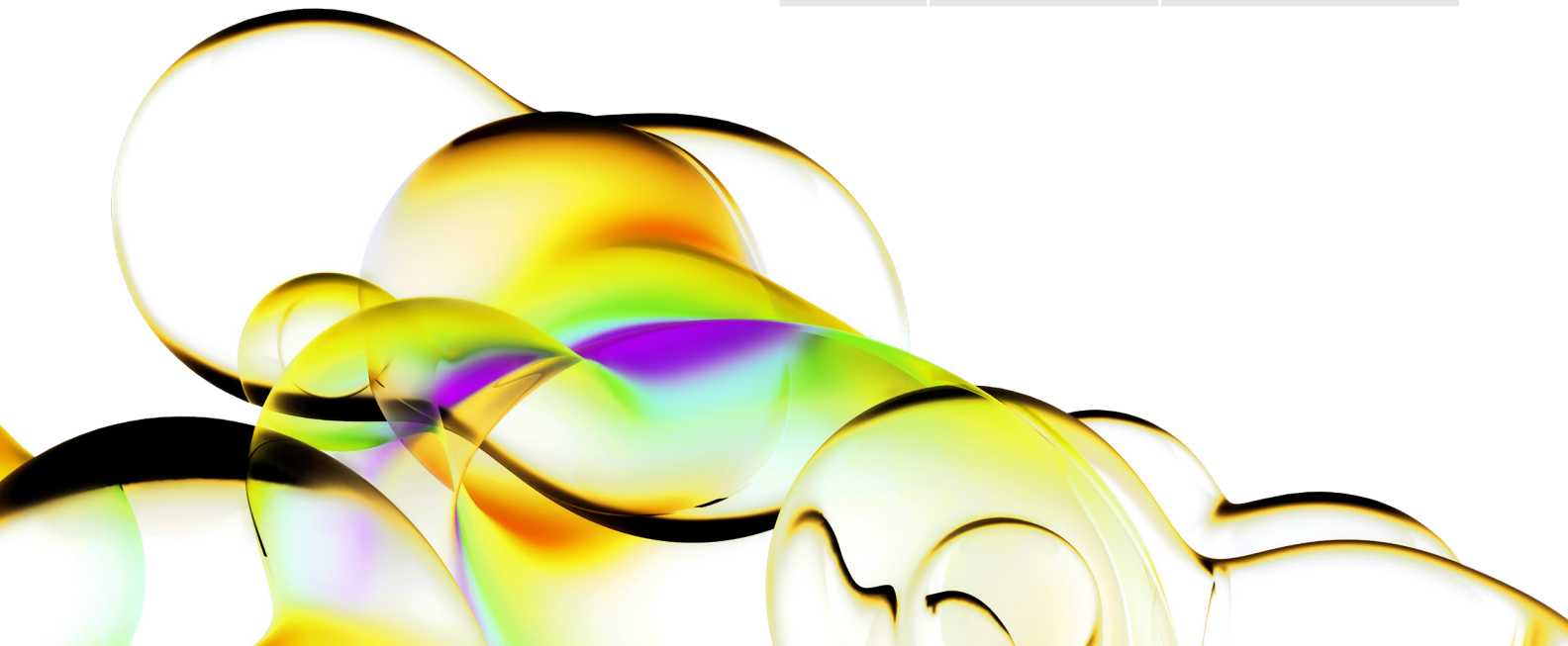
Introduction

Poly (ADP ribose) polymerase (PARP) inhibitors abrogate the repair of DNA single-strand breaks. If unrepaired, single-strand breaks can be converted to double-strand breaks (DSB) and lethal levels of damage may accumulate. Cancer cells deficient in homologous recombination DSB repair e.g. through loss of BRCA2 show increased sensitivity to PARP inhibitors[1,2] exemplifying synthetic lethal approaches for novel cancer therapy.

The Revvity catalogue of cell lines includes a pair of DLD-1 cell lines, which are genetically identical except for their BRCA2 status. Targeted disruption of BRCA2 exon 11 was performed using rAAV gene editing technology to generate DLD-1 BRCA2 (-/-) cells[3]. Historically, cell lines such as Capan-1 have been used to model BRCA2 deficiency[1]. However, these cell lines are often compared to cell lines that differ not only in BRCA2 status but also differ with respect to other gene mutations. In this study we have demonstrated the specific sensitivity of the engineered BRCA2 (-/-) line to a PARP inhibitor.

This Cell Line system allows the role of BRCA2 to be studied without the influence of other factors and offers an attractive method for performing synthetic lethality screens. The permanent and specific nature of the gene targeting technology overcomes limitations of RNA interference strategies such as transient gene disruption and off-target effects. In addition, the isogenic system can be used throughout the drug discovery process, to identify and validate novel targets both *in vitro* and *in vivo*.

Cell line	Genotype	Cat. no.
DLD-1	BRCA2 (-/-)	HD 105-007



Methods

For colony forming assays, cells were seeded into 24-well plates and allowed to adhere overnight. Cells were then treated with compounds and grown for 10 days. To quantify, colonies were fixed and stained with crystal violet solution. Dye was then solubilized and absorbance measured at 590 nM.

For proliferation assays, cells were seeded into 96-well plates and allowed to adhere overnight. Cells were then treated with compounds for 96 hours. Cell viability was quantified using alamar blue.

Results and discussion

Olaparib, a potent PARP inhibitor, was investigated in DLD-1 BRCA2 (-/-) isogenic cells using a colony forming assay. BRCA2 (-/-) cells were highly sensitive to PARP inhibition, showing more than 1000-fold selectivity over parental cells that are BRCA2 (+/+).

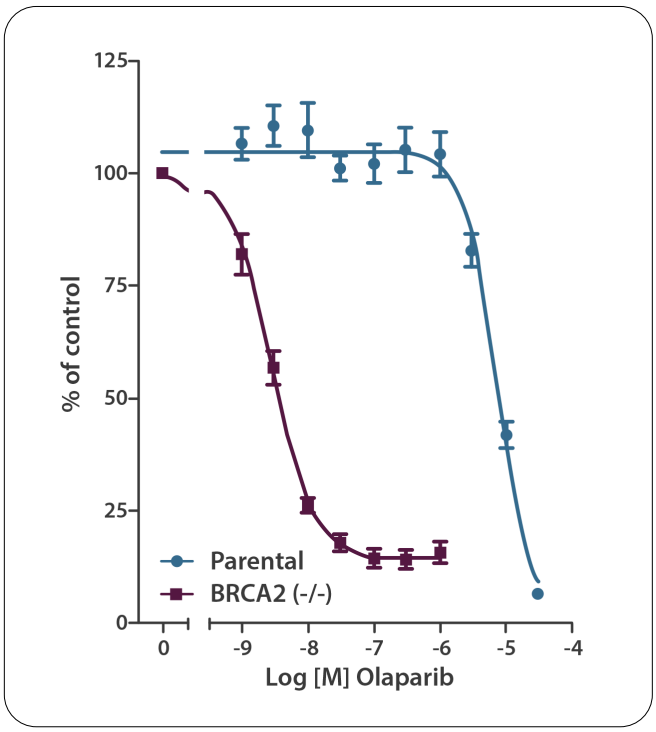


Figure 1: DLD-1 BRCA2 (-/-) cells show selective sensitivity to Olaparib over DLD-1 parental cells in a 10 days colony forming assay.

In addition, a second less potent PARP inhibitor NU1025 and the non- targeted agent gemcitabine were evaluated. NU1025 showed clear selectivity for BRCA2 (-/-) cells, and in keeping with the *in vitro* potency profiles of the compounds[1], was significantly less potent than Olaparib. As expected, gemcitabine was equipotent in both cell lines.

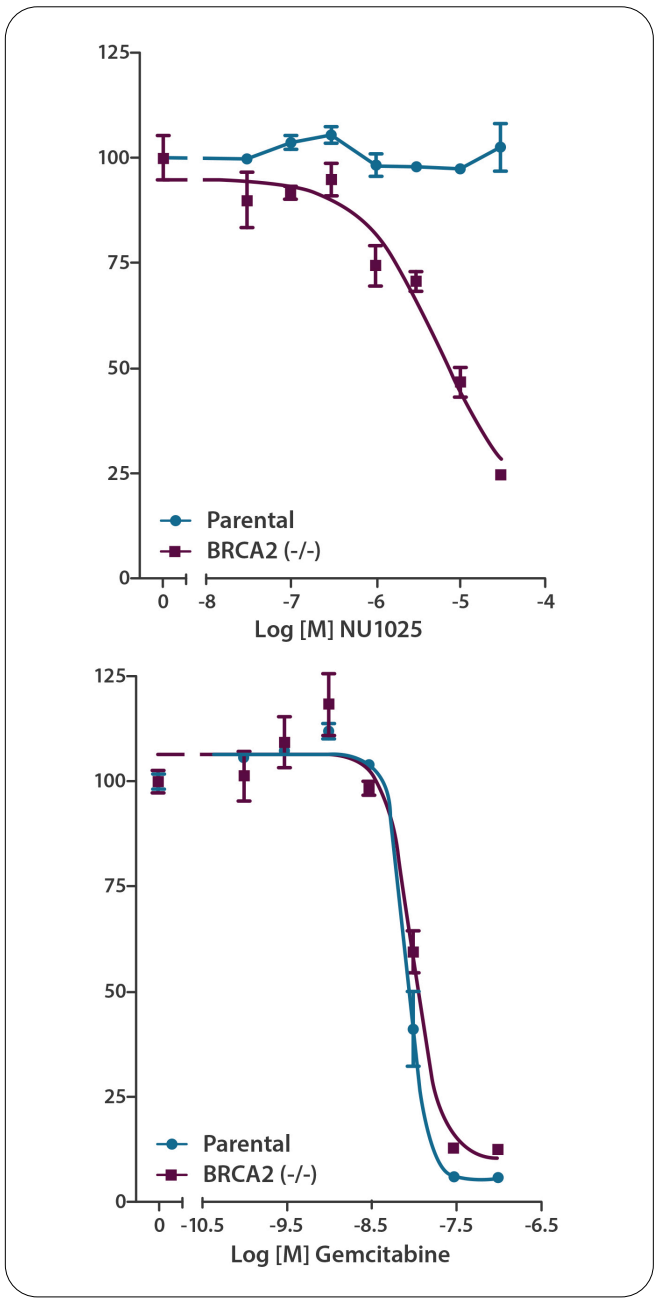


Figure 2: DLD-1 BRCA2 (-/-) cells show selective sensitivity to NU1025 whilst showing no selectivity for the non-targeted agent gemcitabine in a colony forming assay.

The isogenic cell pair was then used to screen the PARP inhibitors in a higher throughput 96-well based proliferation assay.

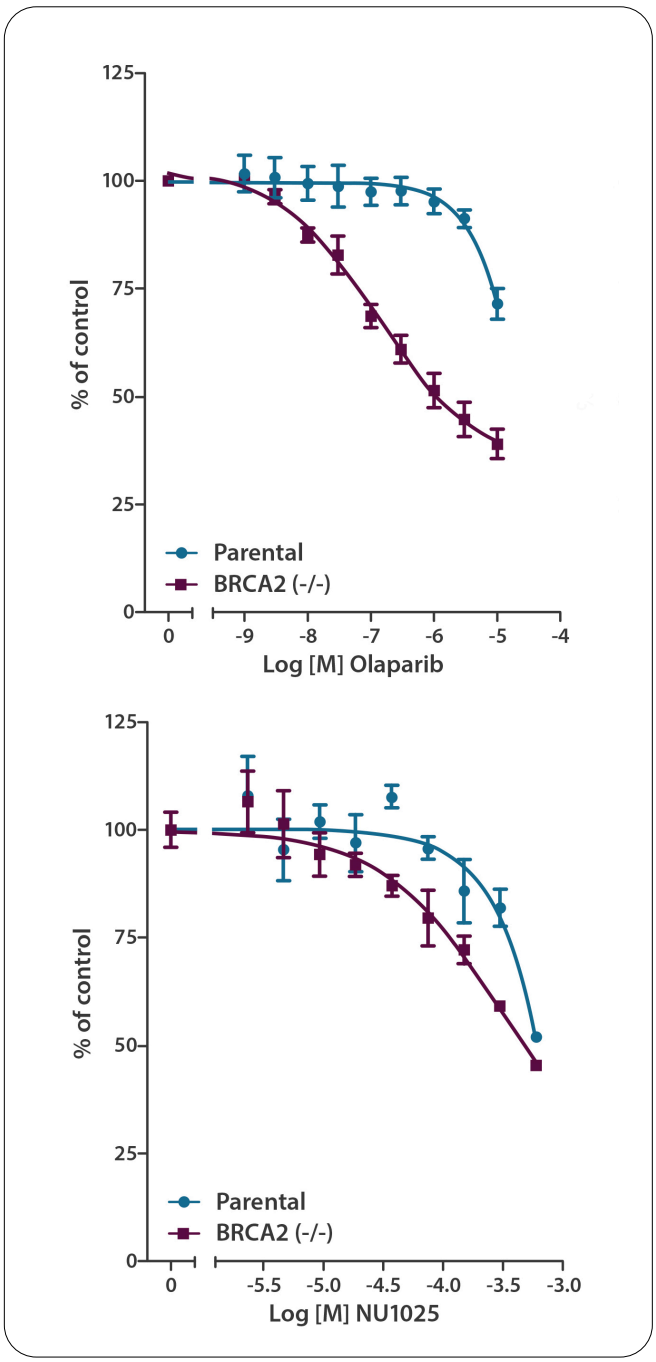


Figure 3: BRCA2 (-/-) cells show sensitivity to potent PARP inhibitors in a 96 hours proliferation assay.

Over the 96 hour proliferation assay, BRCA2 (-/-) cells were clearly sensitive to PARP inhibition, although to a lesser extent than seen with the longer duration colony forming assay. This is consistent with the mechanism of action of the compounds, which require multiple cell division cycles in order for lethal levels of DNA damage to accumulate. Compounds show reduced cellular effects in this format, but the assay remains sensitive enough to rank the potency of PARP inhibitors and therefore would be amenable for high-throughput synthetic lethality screening.

Conclusion

BRCA2 isogenic cells are a powerful and relevant tool for drug discovery and development, enabling a single gene deletion to be studied without the need for large cell panels. The BRCA2 isogenic pair clearly demonstrates the sensitivity of BRCA2 deficient cells to PARP inhibitors, exemplifying the concept of synthetic lethality. Such approaches to drug screening aim to facilitate the discovery of effective and personalized anticancer drugs that exploit vulnerabilities unique to cancer cells by virtue of the mutations they have accrued during tumor progression.

Revvity support

Revvity supplies genetically-defined cell lines, custom cell line generation, reporter gene assay kits, molecular reference standards and assay development and screening services to organizations engaged in academic research; drug discovery and development; clinical diagnostics; and biopharmaceutical process optimization. Please contact us to learn more about how Revvity can support your work.

Cell lines similar to those listed in this Application Note include:

Cell line	Genotype	Cat. no.
HCT116	MLHl (+/-)	HD 104-006
RKO	FANCC (-/-/-)	HD R05-006
RKO	FANCG (-/-)	HD R05-005
HCT116	BLM (-/-)	HD R02-011
HCT116	P53 (-/-)	HD 104-001
RKO		HD 106-002
SW48		HD 103-004
MCFl0A		HD 101-005

References

1. McCabe et al., Cancer Biology & Therapy 2005 (4) p934
2. Farmer et al., Nature 2005 (434) p917
3. Hucl et al., Cancer Res. 2008 (68) p5023

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