APPLICATION NOTE

Compound screening in *BRCA2* (–/–) GeneArt Engineered Cell Model

A model for synthetic lethality

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 Invitrogen™ GeneArt™ Engineered Cell Models collection includes a pair of isogenic DLD-1 cell lines, which are genetically identical except for their *BRCA2* status. Targeted disruption of *BRCA2* exon 11 was performed using recombinant adeno-associated virus (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 with respect to other gene mutations. In this study, we have demonstrated the specific sensitivity of an engineered *BRCA2* (–/–) cell line to PARP inhibitors.

The GeneArt Engineered Cell Model 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*.

ThermoFisher SCIENTIFIC

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 Invitrogen™ AlamarBlue™ Cell Viability Reagent.

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 1,000-fold selectivity over parental cells that are *BRCA2* (+/+) (Figure 1).

In addition, a second, less-potent PARP inhibitor NU1025 and the nontargeted 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).

The isogenic cell pair was then used to screen the PARP inhibitors in a high-throughput 96-well proliferation assay (Figure 3). 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.

Conclusions

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.

References

1. McCabe N, Lord CJ, Tutt AN et al. (2005) *BRCA2*-deficient CAPAN-1 cells are extremely sensitive to the inhibition of Poly (ADP-Ribose) polymerase: an issue of potency. *Cancer Biol Ther* 4(9):934–936.
2. Farmer H, McCabe N, Lord CJ et al. (2005) Targeting the DNA repair defect in *BRCA* mutant cells as a therapeutic strategy. *Nature* 434(7035):917–921.
3. Hucl T, Rago C, Gallmeier E et al. (2008) A syngeneic variance library for functional annotation of human variation: application to *BRCA2*. *Cancer Res* 68(13):5023–5030.

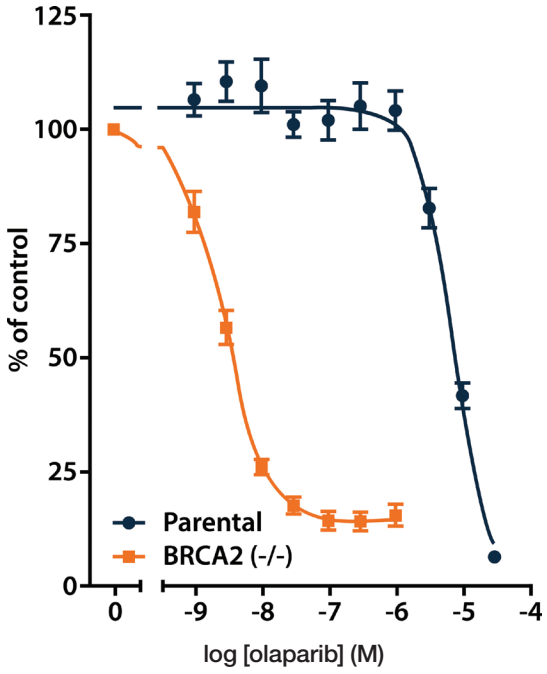

Figure 1. DLD-1 *BRCA2* (–/–) cells show selective sensitivity to olaparib over DLD-1 parental cells in a 10-day colony-forming assay.

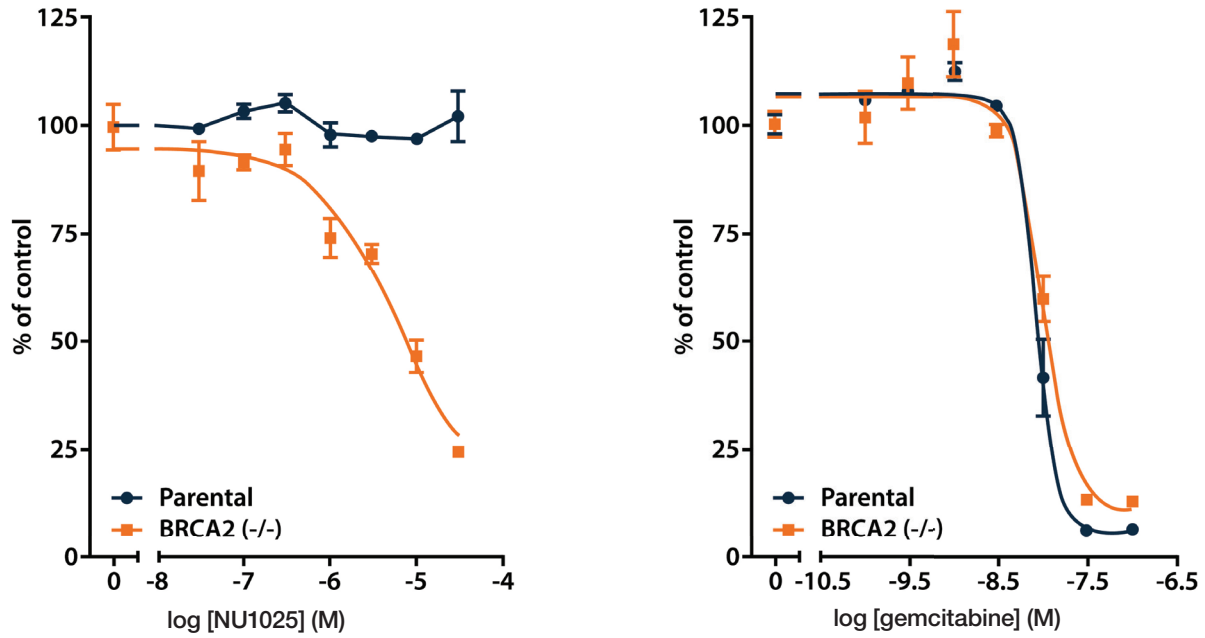

Figure 2. DLD-1 *BRCA2* (–/–) cells show selective sensitivity to NU1025 while showing no selectivity for the non-targeted agent gemcitabine in a colony-forming assay.

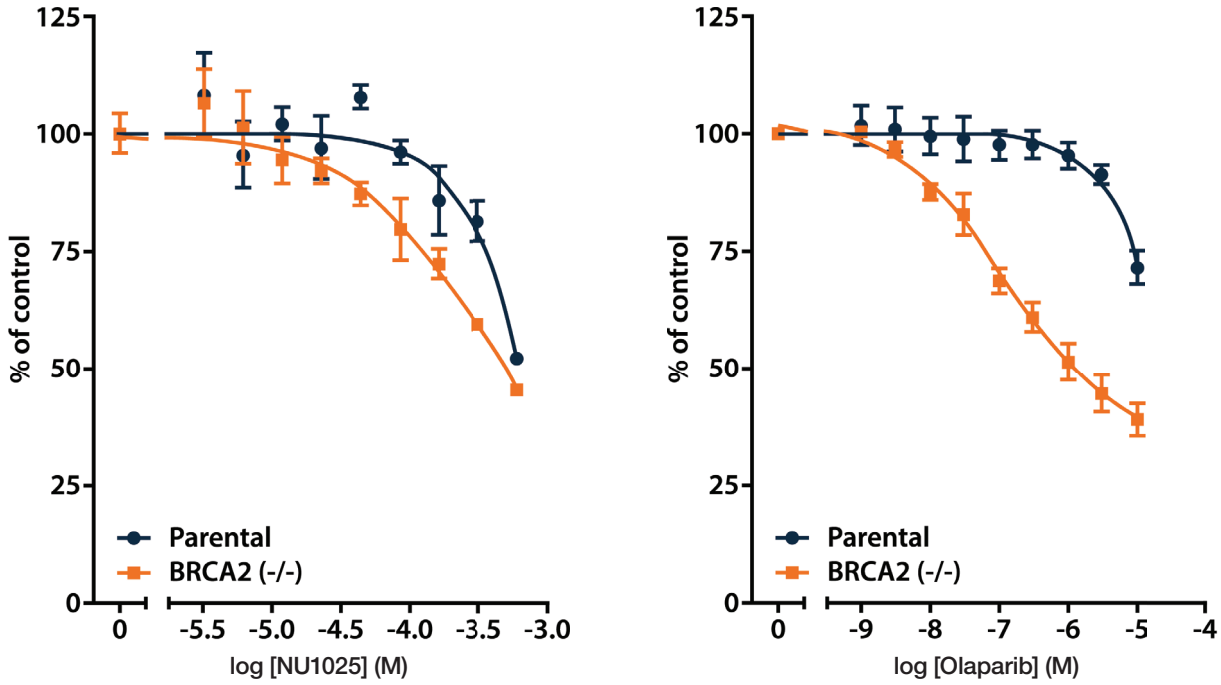

Figure 3. *BRCA2* (–/–) cells show sensitivity to potent PARP inhibitors in a 96-hour proliferation assay.

invitrogen

Ordering information

Cell line	Gene	Genotype	Cell ID No.
DLD-1	*BRCA2*	*BRCA2* (–/–)	HD 105-007

To consistently culture the world's largest collection of ready-to-go CRISPR engineered cell lines, pair the cells with Gibco™ media and reagents. Please refer to the cell line data sheets for specific products.

Related products ordering information

Product	Quantity	Cat. No.
RPMI 1640 Medium	500 mL	11875-093
Fetal Bovine Serum, charcoal stripped, USDA-approved regions	50 mL	12676011
DMEM/F-12 Medium	500 mL	11330-032
Horse Serum, heat inactivated	500 mL	26050088
AlamarBlue Cell Viability Reagent	100 mL	DAL1100

Accelerate your cancer research with GeneArt Engineered Cell Models. Find out more at **thermofisher.com/cancercelllines**

ThermoFisher SCIENTIFIC