HORIZON DISCOVERY

# Application Note

## Compound Screening in DNA Repair X-MAN® Cell Lines

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## Introduction

Errors in DNA repair pathways frequently lead to cancer, however defects in DNA repair proteins often render cancer cells more sensitive to DNA targeted agents such as Etoposide. Etoposide forms a complex with DNA and topoisomerase II and prevents ligation of cleaved DNA resulting in accumulation of single or double-strand DNA breaks and leads to apoptosis and cell death. By exploiting the increased sensitivity of tumors harbouring DNA repair protein deletions to DNA damaging agents, more efficient and targeted treatments could be identified.

Horizon Discovery has used its propriety rAAV gene engineering technology to generate X-MAN® Cell Lines deficient in key DNA repair genes that can be used to investigate drug resistance and sensitivities.

The response of LIG4 (LIGIV) and PRKDC (DNA-PKc) (-/-) cell lines to treatment with Etoposide was studied. These proteins play a role in Non-Homologous End Joining (NHEJ) and they are both associated with disease; LIG4 is associated with T cell leukemia whilst loss of PRKDC has been linked to gastric tumors[1,2].

The use of cell lines allowed the effects of these deletions to be viewed in isolation, without the interference of other genetic alterations.

## Cell Lines Used

| Cell Line | Genotype | Cat. No. |
|---|---|---|
| HCT116 | LIG4 (-/-) | HD R02-063 |
| HCT116 | PRKDC (-/-) | HD R02-049 |

## Methods

For Western blotting, cell lysates were separated by SDS-PAGE and Western blotting performed.

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 8 days. To quantify, colonies were fixed and stained with crystal violet solution. Dye was then solubilized and absorbance measured at 590nm.

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.

## Cell Line Characterization

Western blots confirmed that both the LIG4 and PRKDC proteins were not expressed by the relevant knockout HCT116 cells.

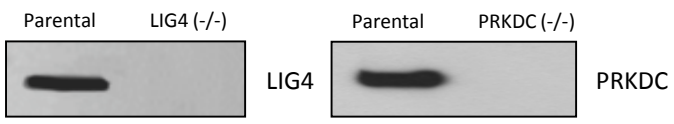


**Figure 1**. Both cell lines were negative for their respective proteins by Western blot.

## Results and Discussion

The HCT116 LIG4 and PRKDC (-/-) cells were found to be highly sensitive to topoisomerase II inhibition by Etoposide in a medium throughput colony forming assay (CFA) and a higher throughput 96-well based proliferation assay.

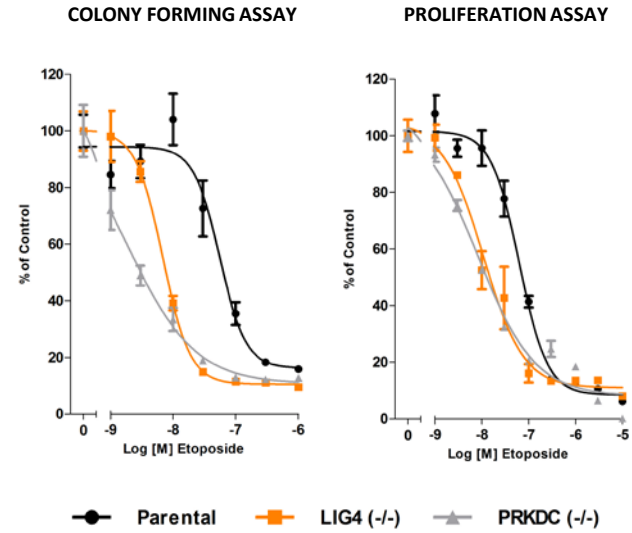


**Figure 2**. LIG4 and PRKDC (-/-) cells show selective sensitivity to Etoposide over HCT116 Parental cells in an 8 day colony forming assay and a 96 hour proliferation assay.

| | $IC_{50}$ nM Etoposide | |
|---|---|---|
| | CFA | Proliferation |
| HCT116 Parental | 70 | 63 |
| HCT116 LIG4 (-/-) | 7 | 11 |
| HCT116 PRKDC (-/-) | 2 | 8 |

**Table 1**. Comparison of Etoposide IC50s obtained in colony forming (CFA) and proliferation assays.

## Conclusions

Horizon Discovery's X-MAN® Cell Lines allow single genetic modifications to be studied in isolation. Use of the LIG4 and PRKDC (-/-) cell lines showed that deletion of a single component of the NHEJ pathway is sufficient to render cells sensitive to Etoposide.

## Horizon Support

Horizon supplies genetically-defined cell lines, custom cell line generation, *in vivo* models, 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 Horizon can support your work.

Additional cell lines relevant to this Application Note include:

| Cell Line | Genotype | Cat. No. |
|---|---|---|
| DLD-1, MCF10A | BRCA1, BRCA2 | HR |
| RKO | FANCC, FANCG | HR |
| DLD-1, HCT116 | CHEK1, CHEK2 | DNA Damage Effector |
| DLD-1 | ATR | DNA Damage Sensing |
| DLD-1, HCT116, MCF10A, RKO, SW48, NALM-6 | TP53 | Various |
| HCT116, NALM-6, RPE, BJ | PRKDC | NHEJ |
| HCT116, NALM-6 | XRCC6, XRCC5 | NHEJ |
| HCT116, RPE | XRCC4 | NHEJ |
| HCT116, NALM-6 | LIG4 | NHEJ |
| RPE | DCLRE1C | NHEJ |
| HCT116 | LIG3 | BER |
| HCT116 | MLH1 | MMR |

**Table 2**. Repair Pathway Definitions: HR - Homologous Recombination; NHEJ – Non-Homologous End Joining; BER – Base Excision Repair; MMR – Mismatch Repair.

## References


- Nijnik A, *et al*. J Clin Invest. 2009 June 1; 119(6): 1696–1705.
- Lee HS, Yang HK, Kim WH, *et al*. Cancer Res Treat 2005;37:98-102.





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