# Arrayed CRISPR assay to evaluate DNA damage response following PARP inhibition

Authors: Gernon G, Pilling J, Zhang B, Quarantotti V, Adam S, Tanriver Ayder E, Kentepozidou E, Leo E, Albertella M, Illuzzi G, Rosenberg L

PARP inhibitors have demonstrated clinical efficacy in cancers with defects in the DNA homologous recombination repair pathway, like BRCAm cancers. Efforts are continuing to identify further patient populations that may receive therapeutic benefit from PARP inhibition. High throughput pooled and arrayed CRISPR screens can be used to identify and mechanistically characterise genes where genetic loss drives resistance or sensitivity to the PARP inhibitor olaparib. In support of this aim we developed a robust and reliable arrayed CRISPR screening assay to evaluate the induction of a DNA damage response following treatment with olaparib.

We used the colorectal cancer cell line DLD1 stably expressing Cas9 and a lipid-based transfection approach to knockout ~200 genes selected from whole genome CRISPR screens, including *BRCA1* and *BARD1* as positive controls. The induction of DNA damage markers, like γH2AX and 53BP1 foci, was measured using high content immunofluorescence imaging following olaparib treatment. We performed extensive optimisation, covering multiple cell seeding densities, lipofectamine conditions, length of CRISRP KO, and length of treatment with olaparib. We applied a statistical workflow to the assay development to ensure deployment of a robust and reliable methodology.

We have successfully developed a screening assay which demonstrates a robust signal window for DNA damage marker induction. Utilising the high content imaging approach, we can now assess additional parameters, such as micronuclei formation and cell cycle analysis. This arrayed screening assay will be used to mechanistically annotate hits from pooled screens helping us to prioritise those to take forwards with the aim of identifying novel patient populations, mechanisms of sensitisation, and develop a greater understanding of the mechanism of action of PARP inhibitors.