Introduction
- Germline mutations in DNA repair genes BRCA1/2 increase the risk of developing breast and ovarian cancer.
- Germline BRCA1/2 mutations occur in 8-10.7% of unselected epithelial ovarian cancer patients, somatic BRCA1/2 mutations are also frequent (18.2%).
- BRCA1/2 mutated or dysfunctional cells may be sensitive to parp inhibition by synthetic lethality, the inhibition of two major DNA repair pathways.

Aims of Study
- To screen a panel of 43 ovarian cancer cell lines for BRCA1/2 deleterious mutations and BRCA1 gene methylation.
- To determine if BRCA1 deleterious mutated and methylated cell lines are more sensitive to the parp inhibitors Olaparib and Veliparib.

Methods

Results – BRCA1/2 Gene Sequencing
- The BRCA1 and BRCA2 genes were sequenced in a panel of 43 ovarian cancer cell lines.
- Only one cell line had a functionally deleterious mutation in BRCA1 (SNU-251).
- Two cell lines had heterozygous mutations in BRCA1 (IGROV-1) and BRCA2 (OC316), but these have no functional impact on the protein.

Results – BRCA1 Gene Methylation
- BRCA1 gene methylation was examined in the panel of 43 ovarian cancer cell lines. Three cell lines were found to be methylated (A1847, OVCAR9, and UPN-251).
- The methylated cell lines have a corresponding decrease in BRCA1 mRNA expression.
- The SNU-251 cells have similar BRCA1 expression levels to wild type cells. This is due to the location of the qPCR primers. The SNU-251 cell line is a deleterious mutation is at the very tail end of the gene sequence.

Results – Cytotoxicity of Parp Inhibitors
- A smaller panel of 14 cell lines was chosen to investigate the impact of BRCA1/2 dysfunction on sensitivity to parp inhibitors. Eleven wild-type cell lines were compared to two methylated cell lines (A1847 and OVCAR9) and the deleterious mutant (SNU-251).
- Unexpectedly, the SNU-251 cell line with the deleterious mutation in BRCA1 was the most resistant to Olaparib of the panel.
- The BRCA1 methylated cell lines were relatively sensitive to Olaparib. Olaparib may be more useful in treating patients with BRCA1 gene methylation rather than deleterious mutation.

Results – Growth Rate of Cell Lines
- The SNU-251 deleterious mutated cells were the slowest growing cell line in the panel.
- The methylated cell lines were very different in their growth rates. A1847 relatively slow and OVCAR9 the fastest. However, on average the methylated cells were similar in their growth to the wild type cells.

Conclusions
- BRCA1/2 deleterious mutations are much rarer in the cell line panel 1/43 (2.3%) compared to ovarian cancer patients.
- BRCA1 deleterious mutated cells (SNU-251) grow slower in cell culture than wild type cells. BRCA1/2 mutated cells are also capable of reverting to wild type through additional mutations. This suggests that there is a selective pressure against BRCA1/2 mutations in cell culture.
- SNU-251 cells are not more sensitive to Parp inhibition than wild-type cells.
- Patients with BRCA1 gene methylation may benefit from treatment with Parp inhibitors.

Types of BRCA1/2 Mutations
- Full Length Functional Protein
- Full Length Non-Functional Protein
- Truncated Non-Functional Protein
- Each BRCA1/2 mutation is shown to disrupt the functional protein.

Heterozygous or Homozygous?
- Heterozygous mutation means one copy of the gene is mutated.
- Homozygous mutation means both copies of the gene are mutated.

Method – Cytotoxicity of Parp Inhibitors
- Figure 1A: Top SNU-251 showing homogenous deletion. Figure 1B: Top IGROV-1 showing heterozygous deletion; Bottom: BRCA1 Wild type sequence.
- Figure 1C: Top UPH-251 showing homogenous deletions; Bottom: BRCA1 Wild type sequence.
- Figure 1D: Top UPN-251 showing heterozygous deletion; Bottom: BRCA1 Wild type sequence.

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Figure 1A
- Top SNU-251 showing homogenous deletion. Figure 1B: Top IGROV-1 showing heterozygous deletion; Bottom: BRCA1 Wild type sequence.
- Two cell lines had deleterious mutations as well as an additional reversion mutation which has restored the protein back to wild type BRCA1 (UPH-251) and BRCA2 (PEOR1).

Figure 1C
- Top UPH-251 showing homogenous deletions. Bottom: BRCA1 Wild type sequence.

Figure 1D
- Top UPN-251 showing heterozygous deletion. Bottom: BRCA1 Wild type sequence.

Figure 2
- BRCA1/2 DeltaCt
- BRCA1/2 IC50 (uM)
- BRCA1/2 Doubling Time (Days)