FU to generate resistant variants (continuous growth method with increasing dose). The drug sensitivity of parental and resistant cells was determined by dose-dependent cytotoxicity curve using standard MTT assay. The inhibitory concentration 50% (IC50) values were calculated by non-linear regression test using GraphPad PRISM 5.0 software. The resistance index (RI) was determined as the ratio of the IC50 of the resistant cell line to the IC50 of parental cell line. Relative expression of resistance marker genes to CDDP or 5-FU was determined by qRT-PCR using β-actin method. U-Mann Whitney test was used to compare groups (at statistical significance level of p<0.05). Changes in cell morphology were monitored continuously during the development of resistance using an inverted phase contrast microscope. The stabilisation of drug resistance of cell lines was tested after two months in drug-free medium.

Results and discussions After 10 months of treatment, AGS resistant to CDDP, MKN-28 resistant to CDDP and AGS resistant to 5-FU exhibited an increase of 3.9, 2.6 and 3.4 fold of resistance, respectively. Resistance marker genes for CDDP (ABCC2 and CTR1) and 5-FU (TYMS) were differentially expressed in resistant cells compared to their parental cells. Changes in cell morphology were observed in resistant cells compared to their parental cells. The resistant phenotype was very stable and the values of IC50 and RI had no significant change after 2 months in drug-free medium.

Conclusion The three drug-resistant lines selected by continuous growth method with increasing dose may serve as appropriate models for the study of mechanisms of drug resistance in GC. Further studies are necessary in order to identify the genes involved in the resistant-phenotype which could help to find new targets for GC therapy.

**PO-493 USING FUNCTIONAL GENETIC SCREENS TO UNDERSTAND RESISTANCE TO PARPI IN BRCA-DEFICIENT TUMOURS**

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**Introduction** Error-free repair of double-strand DNA breaks is achieved by homologous recombination (HR), and both BRCA1 and BRCA2 are crucial for this process. Inactivating germline mutations of BRCA1 and BRCA2 genes predispose to breast and ovarian cancers and result in HR deficiency. This defect can be specifically targeted by inhibition of Poly-(ADP-ribose) polymerase (PARP) 1, which leads to the selective killing of HR-deficient tumour cells. These observations have recently resulted in the approval of the first PARP inhibitors (PARPi) for the treatment of patients with germline BRCA-mutated tumours. Although this approach has shown promise, the efficacy of PARPi is limited due to drug resistance, with only a fraction of the BRCA1/2 mutation carriers responding to this therapy. Those who do respond eventually develop resistance and relapse. Although some drug resistance mechanisms have been characterised, many other mechanisms remain to be elucidated. Further investigation is needed to achieve a strategy to overcome drug resistance in order to improve this promising targeted therapy.

**Material and methods** Our aim is to uncover novel mechanisms of resistance and to find promising therapeutic targets able to revert the resistant phenotype. To this end, we will conduct functional genetic screens in PARPi-sensitive and PARPi-resistant 2D and 3D models using shRNA and CRISPR libraries. Furthermore, we will combine multi-omics analysis of BRCA1/2-deficient mammary tumours that acquired PARPi resistance in vivo. Taken together, both approaches will yield...
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a list of candidate genes that we further intend to validate and characterise, both in vitro and in vivo.

Results and discussions Preliminary results yielded a list of candidate genes responsible for resistance or able to re-sensitise to PARPi. Hits are currently being validated in vitro. Furthermore, we intend to characterise the mechanism of action of the validated genes and to further understand its role in DNA repair. Additionally, we intend to validate our findings in vivo and to analyse the relevance of our results in the clinic.

Conclusion With this project we intend to characterise new biological mechanisms that lead to resistance to PARPi and to find new tumour vulnerabilities that could be exploited in order to revert innate and acquired resistance to this therapy. Moreover, it will also allow us to get insights into the biological functions and molecular pathways in which BRCA1 and BRCA2 proteins are involved.

PO-495 PI3K PATHWAY UPREGULATION MEDIATES ACQUIRED RESISTANCE TO PLATINUM AGENTS AND POLYADENORIBOSE POLYMERASE INHIBITORS (PARPi) IN BRCA1-METHYLATED OVARIAN CANCER (OC)

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Introduction BRCA1-methylated OC (BMOC) are specifically sensitive to platinums and PARPi, though acquired resistance to these agents eventually develops. Elucidating underlying druggable resistance mechanisms is needed to enable novel therapeutic options in BMOC.

Material and methods We developed two PARPi resistant (ola-, parb and talozaropar) and one carboplatin resistant OC cell line models (named OVC8RO, OVC8RT and OVC8RC, respectively) derived from the BRCA1-methylated cell line OVC8R5, following continuous (PARPi) or pulsed (carboplatin) drug exposure. Fold resistance (FR) to the parent drug (as determined by the ratio of the resistant cell line IC50 to the parent cell line IC50) suggested clinically relevant resistance models in OVC8RC (FR=4.80±0.43) and OVC8RO (FR=5.71±0.21). OVC8RT displayed higher level resistance (FR=45.61±11.10). We obtained tissue from 5 matched primary and recurrent (post platinum) BMOC patient tumours. Reverse phase protein array (RPPA) was used to examine differential expression and phosphorylation levels of 63 proteins between parent/resistant cell lines, and primary/matched recurrent tumours. 5 day acid phosphatase cytotoxicity assays were used to determine the IC50 of drugs. Synergy in drug combination assays was determined as per the Chou-Talalay method.

Results and discussions Significant increases in PI3K p110a and PI3K p110δ were detected by RT-qPCR in all recurrent cell lines, relative to the parent cell line’s baseline levels (p<0.05), consistent with PI3K pathway upregulation. 3/5 recurrent tumours had increased phosphorylated AKT (T308 or S473), as compared to the corresponding primary BRCA1-methylated tumour. The selective a/8 isoform dominant PI3K inhibitor copanlisib (BAY 80-6946) displayed anti-proliferative effects in both the parent cell line (IC50=76.6±7.4 nM) and all resistant cell lines (IC50=44.0–102.1 nM). In both carboplatin and PARPi-resistant models, combination treatment of copanlisib and the parental drug resulted in synergistic growth inhibition (CI@ ED50=0.27–0.28) and restored sensitivity to the parent drug. When the parental cells were treated with copanlisib in combination with carboplatin or either PARPi, the observed synergism was markedly less (CI@ED50=0.71–0.85) than that observed in the drug resistant models.

Conclusion The addition of copanlisib to carboplatin or PARPi could represent a novel therapeutic strategy in BMOC that has acquired resistance to either carboplatin or PARPi.

PO-496 LOSS OF PARG DRIVES PARP INHIBITOR RESISTANCE IN BRCA2-DEFICIENT MOUSE MAMMARY TUMOURS

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Introduction Inhibitors of poly(ADP-ribose) polymerase (PARP) have recently entered the clinic for the treatment of homologous recombination (HR)-deficient cancers. Despite the success of this approach, resistance to PARP inhibitors (PARPi) is a clinical hurdle, and we poorly understand how cancer cells escape the deadly effects of PARPi without restoring the HR pathway.

Material and methods To tackle this question, we generated matched PARPi-sensitive and -resistant Brca2-mutated mouse mammary tumours. By combining next-generation sequencing with functional genetic screens, we identified loss of poly (ADP-ribose) glycohydrolase (PARG) as a major resistance mechanism.

Results and discussions We demonstrate that PARG depletion restores PAR formation, rescues controlled DNA replication fork progression and promotes the recruitment of downstream DNA repair factors. The potential relevance of PARG in clinical PARPi resistance is underscored by the presence of PARG-negative clones in a subset of human triple-negative breast and serous ovarian cancers. Importantly, acquisition However, the gain of PARPi resistance comes at a cost, as PARP inactivation results in new vulnerabilities that can be exploited therapeutically.

Conclusion We conclude that loss of PARG should be assessed as a potential cause of clinical PARPi resistance. In this case, measurement of PARG activity should further improve clinical decision making for patients with tumours that lack homology-directed DNA repair.

PO-497 MICROTBULE REGULATORY PROTEINS AS PREDICTIVE BIOMARKERS OF TAXANE-BASED CHEMORESISTANCE IN BREAST CANCER?

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Introduction Neo-adjuvant chemotherapy combining taxanes and anthracyclines represents an interesting option for a number