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 IC_{50} of the resistant cell line to the IC_{50} of parental cell line. Relative expression of resistance marker genes to CDDP or 5-FU was determined by qRT-PCR using $2^{-(ddCt)}$ 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 clones 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 IC_{50} 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 TARGETING THE DRUG RESISTANCE EPIGENETIC DRIVER SMYD3 AS A NEW STRATEGY TO POTENTIATE CHEMOTHERAPEUTIC EFFECTS

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Introduction Human cancers arise from a combination of genetic and epigenetic changes. Epigenetic factors regulate chromatin structure, affecting biological processes and promoting cancer. Drugs that target epigenetic modifiers are a new therapeutic challenge, due to the reversibility of epi-modifications. Indeed, epigenetic drugs might sensitise cancer resistant cells to chemotherapy. The SMYD3 histone methyltransferase has an oncogenic role in several cancer types. It is overexpressed in various cancers and promotes cell proliferation, making it a potential target for drug discovery.

Material and methods We performed a virtual screening to identify new compounds able to inhibit SMYD3 and then evaluated phenotypic and molecular changes in cells treated with the selected molecule 4- (aminocarbonyl)-N-(4-bromophenyl)−1-piperidineacetamide (BCI-121). Its inhibitory action was assessed by *in vitro* methylation and surface plasmon resonance assays. To characterise SMYD3 role in cancer response to therapy, we tested potential changes in the sensitivity of cancer cells treated with a combination of BCI-121 and S-phase-specific drugs. Finally, we investigated SMYD3 contribution in DNA repair by evaluating 53 BP1 nuclear foci formation.

Results and discussions We observed that SMYD3 is overexpressed in several cancer cell lines, with cells expressing high levels of SMYD3 being highly sensitive to its genetic depletion or pharmacological inhibition by BCI-121. BCI-121 reduces proliferation by arresting cancer cell cycle at the S/G2 boundary. Of note, cell cycle plays a key role in chemosensitivity, particularly for drugs displaying targeted cell cycle effects. Our results showed that pre-treatment with BCI-121 significantly increased cytotoxicity of S-phase agents. Breast cancer cells exposed to DNA damaging agents showed increased levels of nuclear SMYD3 following activation of the repair signals, and an accumulation of unrepaired DNA lesions after SMYD3 genetic ablation. We also evaluated the potential of combined treatment with BCI-121 and S-phase drugs in Triple Negative Breast Cancer (TNBC), which does not usually respond to common therapies. TNBC cells overexpressing SMYD3 confirmed the efficacy of the combined treatment.

Conclusion New therapeutic strategies focused on SMYD3 targeting might overcome cancer resistance to existing drugs, thus allowing not only to reduce dose and side effects, but also to treat cancers not usually responding to common therapies.

PO-494 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