of genes associated with stemness such as aldehyde dehydrogenase (ALDH), prominin-1 (CD133) and ATP binding cassette subfamily G member 2 (ABCG2) was detected in all chemoresistant cell line variants. CDDP-resistance could be reverted by ALDH inhibitor disulfiram, hypomethylating agent decitabine and inhibitor of poly (ADP-ribose) polymerase veliparib, when used in combination with CDDP in 3D culture conditions and in vivo.

Conclusion We derived chemoresistant variants of TGCT cell lines as novel clinically relevant model suitable for the evaluation of therapeutic strategies in vitro or in vivo. More importantly, our results suggest novel treatment option for refractory TGCTs with acquired CDDP-resistance.

This work was supported by Slovak Research and Development Agency (APVV-15–0086, APVV-15–0697, APVV-16–0178) and VEGA 2/0124/17. We thank Cancer Research Foundation and League against Cancer for financial support.

Conclusion EveR cells displayed key differences compared to parental cells, including resistance to everolimus and rapamycin but not temsirolimus; suggesting mutations may have occurred in either FKBP12 or mTOR, with current work to sequence these genes on-going. Observed decreases in p.

PO-480 ABSTRACT WITHDRAWN

PO-481 ALTERATION IN EPIGENETIC-RELATED GENES AND HISTONES MODIFICATIONS LEVELS REVEALED AS A POTENTIAL RESISTANCE FACTOR TO OXALIPLATIN IN COLORECTAL CANCER CELLS

Introduction Colorectal cancer (CRC) is the fourth most common cancer worldwide and even though the treatment has evolved, chemotheraphy resistance is still a concern for therapy success and patients’ survival. Cell resistance may be modulated by several mechanisms, including epigenetic modifications; impacting gene expression and cell fate. The aim of this study was to identify epigenetic mechanisms that might control oxaliplatin cell resistance in CRC cells.

Material and methods Gene expression-based risk score was built from the dataset of primary CRC samples of a cohort of 80 (Tsuji - GSE28702) and 36 (Del Rio - GSE72970), using 90 genes with epigenetic roles and defined as the sum of the Cox beta coefficients of each of the 25 genes found with a prognostic value (Episcore). Histones post-translational modifications (PTMs) were evaluated by mass spectrometry and drug interaction analysis was addressed by Sulforhodamine B test and Bliss equation.

Results and discussions Episcore signature revealed 5 genes with a bad prognostic value (ATAD2, HDAC2, HDAC9, MBD1 and MBD2) and 20 with a good one (most of them histone methyltransferases). High-risk patients are characterised by a higher expression of the 5 bad prognostic genes and a lower expression of the 20 good ones. A maximum difference in OS was obtained with an Episcore at $6.098$, splitting patients in Del Rio cohort into a high- and low-risk group of patients (score greater/less than $6.098$, respectively) with 13.3 and 58 months median OS, respectively (p<0.0001).

Episcore was also prognostic in Tsuji cohort (high-risk OS=12 months and low-risk median survival not reached) and when analysing the progression-free survival (patients treated only with FOLFOX regimen). We then evaluated histones PTMs in a 10-fold oxaliplatin resistant clone (HCT116-R1) compared with its parental HCT116 cells, treated or not with oxaliplatin. We found that resistant cells possess lower acetylation levels in histones 3 and 4 and oxaliplatin treatment decreased acetylation levels, for both cell lines. When combining the HDAC inhibitor Entinostar and oxaliplatin, we observed a synergistic effect in oxaliplatin-resistant cells HCT116-R1 and additive/antagonistic effects in the sensitive one.
Conclusion These findings confirm that epigenetic factors have an important prognostic and therapeutic value in patients with CRC. Hence, understanding the functional role of these genes/ modifications in the pathogenesis and drug resistance of CRC is needed (already in progress in our group).

PO-482 EFFICACY OF INHIBITION OF BAD SER99 PHOSPHORYLATION BY A NOVEL SMALL MOLECULE IN CISPLATIN RESISTANT OVARIAN CANCER

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Introduction Human BAD (hBAD) is a pro-apoptotic Bcl-2 family member, whose apoptotic functions can be inactivated through phosphorylation of specific residues including Ser99. Clinically, BAD phosphorylation has been reported to indicate poor survival and cisplatin resistance in ovarian cancer patients. Herein, we investigated the therapeutic potential of inhibiting BAD Ser99 phosphorylation in ovarian cancer.

Material and methods NPB, a novel small molecule which specifically inhibits BAD Ser99 phosphorylation, has been developed in our laboratory. Cell function assays performed include Alamar Blue cell viability assay, caspase3/7 assay, PI-Annexin V apoptosis assay, cell cycle analysis and 3D growth in Matrigel. The CI values are calculated by CompuSyn using Chou-Talalay method. The cancer stem cell-like cell population was examined by ALDEFLOUR and sphere formation assays.

Results and discussions The level of BAD Ser99 phosphorylation is negatively correlated with cisplatin sensitivity in a panel of ovarian cancer cell lines. BAD Ser99 phosphorylation is increased upon acute cisplatin treatment of both sensitive and resistant ovarian cancer cells. The inhibition of BAD Ser99 phosphorylation by NPB alone increased apoptosis, and decreased cell viability, 3D growth, and anchorage-independent growth of both parental and cisplatin resistant ovarian cancer cells. In particular, NPB increased the sensitivity of the parental cells, and partially re-sensitized cisplatin resistant ovarian cancer cells towards cisplatin, with the combination of NPB and cisplatin showing a synergistic effect. An upstream BAD kinase, AKT has also been reported to mediate cisplatin resistance in ovarian cancer cells. Correspondingly, the combination of NPB and AKT inhibitor AZD5363 exhibited strong synergistic effects in both parental and cisplatin resistant ovarian cancer cell lines. We also demonstrated that both the phosphorylation of BAD and its upstream kinase AKT was increased in this CSC-like population. NPB treatment alone was observed to decrease the CSC-like population, while the combination of AZD5363 and NPB produced a synergistic decrease in the CSC-like population.

Conclusion Our preclinical data suggests that NPB, as a novel inhibitor of BAD Ser99 phosphorylation, can potentially be used in combination with cisplatin as a therapy for naïve ovarian cancer patients to increase cisplatin sensitivity. Furthermore, the combination of NPB and AKT inhibitor AZD5363 is a potential therapeutic approach in the treatment of cisplatin resistant ovarian cancer.

PO-483 PHENOTYPE Switching AS AN ESCAPE MECHANISM TO TARGETED THERAPIES IN MELANOMA

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10.1136/esmoopen-2018-EACR25.501

Introduction Melanoma is an aggressive skin cancer with increasing incidence worldwide. The development of BRAF kinase inhibitors as targeted treatments for patients with BRAF-mutant tumours and the introduction of immunotherapies contributed profoundly to an improved overall survival of patients with metastatic melanoma. Despite these promising results, the emergence of rapid resistance to these therapeutic approaches remains a serious clinical issue.

Material and methods To investigate the impact of BRAF inhibitors on miRNomes and transcriptomes, we used in vitro melanoma models consisting of BRAF inhibitor-sensitive and -resistant cell lines generated in our laboratory. Subsequently, miRNA and gene expression analyses were performed in order to identify the underlying mechanisms of resistance.

Results and discussions Regarding miRNome and transcriptome changes, the long-term effects of BRAF inhibition differed in a cell line-specific manner with the two different BRAF inhibitors inducing comparable responses in drug-sensitive melanoma cell lines. Despite this heterogeneity, several miRNAs (e.g. miR-100–5p) and genes (e.g. AXL) were distinctly differentially expressed in drug-resistant versus -sensitive cell lines. Analyses of co-expressed miRNAs, as well as inversely correlated miRNA-mRNA pairs, revealed a switch from a MITF high to an AXL high ratio in a subset of drug-resistant melanoma cell lines that might be regulated by miRNAs. Additionally, the inhibition of AXL reduces growth of BRAF inhibitor-resistant melanoma cells, thus the combined inhibition of BRAF and AXL might be beneficial for patients with metastatic melanoma.

Conclusion In this study, promising miRNAs and genes were identified and associated to BRAF inhibitor-mediated resistance in melanoma, and might be considered as prognostic and/or diagnostic resistance biomarkers in melanoma drug resistance.

PO-484 DEVELOPMENT OF A TWO-STEP SCREENING-AND-CONFIRMATION APPROACH TO EFFICIENTLY IDENTIFY SYNERGISTIC DRUG COMBINATIONS WITH THE PARP INHIBITOR NIRAPARIB

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10.1136/esmoopen-2018-EACR25.502

Introduction Poly (ADP-ribose) polymerases (PARPs) are important players in DNA damage repair. Inhibition of PARP activity is highly effective against cancers deficient in homologous recombination repair due to, a.o., BRCA1/2 mutations. Clinically, PARP inhibitors (PARPi) are used to treat, a.o., ovarian cancers. Although effective, repeated treatment with PARPi may lead to resistance. To identify new synergistic drug combinations, we developed a two-step approach of screening and confirmation using two of our platform technologies SynergyScreen and SynergyFinder. We first screen for synergy in presence of a fixed concentration of compound (e.g., PARPi), followed by