RE promoted both cytostatic and cytotoxic effects in all the colorectal cancer cell lines. We hypothesise that main compounds such as diterpenes (carnosic acid and carnosol) and triterpenes (betulinic and ursolic acid) may be the responsible for the observed antiproliferative activity.

**PO-026**

**72 ROSEMARY (ROSMARINUS OFFICINALIS, L.) EXTRACT INHIBITS CELL PROLIFERATION AND MIGRATION IN COLON CANCER CELL LINES**

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**Introduction** *Rosmarinus officinalis* L., commonly called rosemary, is one of the most widely used and commercialised plant for obtaining botanical extracts which are not only used as a culinary herb for flavouring but also as an antioxidant ingredient in processed foods and cosmetics. This plant has been traditionally used as an herbal medicine for a large variety of disorders. Its beneficial effects have been attributed to the high content in bioactive compounds, such as phenolic diterpenes, flavonoids and other phenolic compounds.

**Material and methods** In this work, a RE obtained using supercritical CO2 extraction has been assayed for the antitumor activity in three colon cancer cell lines (HGUE-C-1, HT-29 and SW480). The effects of ER on different aspects of cell proliferation and migration were determined using RTCA DP instrument (Real Time Cell Analysis Proliferation Assay), wound healing and colony formation assays.

**Results and discussions** Results showed that RE inhibited cell proliferation in a concentration dependent manner in all cell lines. HGUE-C-1 and SW480 cells demonstrated a higher sensitivity against RE on RTCA assay, causing a complete reduction of cell proliferation at 20 and 40 μg/mL. Wound healing experiments showed a significant anti-migrative activity for RE in all the cell lines, without relevant differences between cell lines. Finally, RE treatment also suppressed colony formation in all cell lines.

**Conclusion** Our results support that RE extract enriched in diterpenes and triterpenes shows inhibited cell proliferation, decreased cell migration and colony formation in all the colorectal cancer cell lines tested. We hypothesised that all these effects may contribute to the anticancer and anti-inflammatory properties of rosemary extract.

**PO-027**

**METHYL-β-CYCLODEXTRIN INTENSIFIES THE EFFECT OF MICROTUBE-TARGETING AGENTS BY INCREASING THEIR INTRACELLULAR DRUG ACCUMULATION**

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**Introduction** Microtubule-targeting agents (MTAs) are conventionally used the first-line chemotherapeutic drug for its treatment however, it is accompanied by several limitations like drug resistance and tumour recurrence leading to its extensive failure. The intracellular drug concentration is significantly reduced due to multi-drug transporter proteins triggering the fruitless therapeutic effect. In our work, we suggest a combinatorial strategy involving Methyl-β-Cyclodextrin (MCD) and MTAs which promises to enhance the intracellular drug levels.

**Material and methods** This was a bipartite study that integrates the *in-vitro* effects of MCD in combination with MTAs by analysing inhibition in cell proliferation, cell cycle, and microtube architecture. The second part focuses on the investigation of the underlying mechanism.

**Results and discussions** MCD augments the effect of MTAs on cell inhibition, G2/M cell cycle arrest in HeLa cells. It also exhibits 50% enhanced uptake of two fluorescent drugs crocin and curcumin in the same cell line. Further, investigating the underlying mechanism our data reveals that MCD treatment depolymerizes the actin cytoskeleton, however, the microtubule architecture remains unaffected. Consequently, the actin-dependent functions like traction force, cell stiffness, are also curtailed. It also manifests its inhibition effect on focal adhesion proteins like paxillin and pfaK (phospho focal adhesion kinase) directing towards weaker cell adhesion. This intensifying effect of MCD on MTAs’ action is exerted on breast, liver as well as prostate cancer also. Moreover, it shows promising outcome on a multi-drug resistant cell line.

**Conclusion** Our data suggest a crucial role of MCD in depolymerizing the actin cytoskeleton and thereby increasing cell permeability. Moreover, this amalgamation seems very promising due to its effective outcome in multiple cancer cell types. This sort of combination therapy opens up a new domain of research which can culminate into an effective cancer treatment strategy.

**PO-028**

**EFFECTIVENESS AND MOLECULAR BASIS OF CDK4/6 INHIBITION IN COMBINATION WITH TAXANES IN PANCREATIC CANCER**

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**Introduction** Pancreatic Ductal Adenocarcinoma (PDAC) is among the deadliest human cancers with a 5 year survival rate of less than 5% using the standard of care gemcitabine/nab-paclitaxel. The most frequently disrupted genes in PDACs are first K-RAS and second CDKN2A, which encodes the cyclin-dependent kinase (CDK)4/6 inhibitor p16. Recently, CDK4/6 inhibitors have been approved for breast cancer treatment, and preclinical assays for PDAC are giving promising results.

**Material and methods** PDAC Patient-Derived Xenografts (PDX) models and PDX-derived cell lines were used for *in vivo* and *in vitro* studies, respectively. Cellular studies were performed using proliferation and cell cycle assays in combination with flow cytometry, immunoblotting, fluorescence microscopy and live cell imaging techniques. Drug treatments were performed with the CDK4/6 inhibitor PD-0332991 (Palbociclib), and with Paclitaxel (Taxol) or Nab-Paclitaxel (Abraxane) for *in vitro* and *in vivo* studies, respectively.
Results and discussions Treatment of different PDX-derived cell lines with the combination of taxanes and CDK4/6 inhibitors resulted in a higher anti-proliferative effect than both drugs used as single agents. Cell cycle studies showed that inhibition of CDK4/6 prevented recovery from treatment with taxol. At the molecular level we found that the combined treatment induced a clear interruption in retinoblastoma pathway, even higher than CDK4/6 inhibition in monotherapy. Gene expression profiles comparing single versus combined treatment are currently being performed to further understand the molecular basis underlying the effectiveness of this type of treatment. Moreover, to assess the efficacy of this new combined treatment in vivo, we treated nine PDAC PDX models with PD-0332991 and nab-paclitaxel, following the same schedule. Importantly, eight of them presented an increased tumour growth inhibition in the combination with respect to the monotherapies.

Conclusion Although the molecular mechanism underlying the effectiveness of this treatment is not completely understood yet, our data suggest a good therapeutic value for the combination of CDK4/6 inhibitors and taxanes in PDAC treatment.

PO-029 ANGIOTENSIN-(1–7) PROMOTES MIGRATION OF RENAL CELL CARCINOMACELLS WITH NO EFFECT ON CELL PROLIFERATION

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Introduction Renal Cell Carcinoma (RCC) is the most common kidney with still unclear pathophysiology. The most common types of RCC are clear cell (ccRCC) and papillary (pRCC). Recently, it has been proposed that renin-angiotensin system (RAS), mainly including angiotensin II, is involved in development and progression of RCC. Angiotensin-(1–7) [Ang-(1–7)] is a RAS molecule, acting through Mas receptor (MasR), that has recently gained more attention because its ability to inhibit proliferation and migration of lung, breast and prostate cancer cells. Because high levels of Ang-(1–7) are observed in the kidney, we have decided to investigate role of Ang-(1–7) on RCC cells.

Material and methods The study was performed on Caki-1 and Caki-2 cell lines that represent ccRCC and pRCC phenotype respectively. Cell proliferation was assessed by AlamarBlue assay and wound healing assay was used for quantification of cell migration. Cell were treated with different combinations of 0,001–10 uM of Ang-(1–7) and/or MasR antagonist A-779 for 6–72 hour depending on the experiment.

Results and discussions We found that Ang-(1–7) increase cell migratory abilities of both Caki 1 and Caki 2 cells after 6, 12, 18 and 24 hour (p<0.01). The effect is inhibited by blockade of Mas receptor by A-779, that have no effect on migration when administrated alone. In the proliferation assay, 0,1 uM Ang-(1–7) caused 20% increase of cell proliferation, while 1 uM A-779 inhibited proliferation up to 30%, however, both results were not statistically significant (p>0.05).

Conclusion This study demonstrates potential role of Ang-(1–7) and Mas receptor in pathogenesis of RCC, mostly by promoting cell migration and increasing metastatic potential. Results may help to better understand molecular mechanism underlying progression of this tumour and find new potential targets for therapy. Discrepancy between pro-migratory effect and no impact on cell proliferation, role of Ang-(1–7) and MasR requires further studies and confirmation in animal model of RCC.

PO-030 FUNCTIONAL ANALYSIS OF MASTL MUTATIONS IN CANCER

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Introduction Mastl, also known as Greatwall, is a protein kinase essential for proper chromosome condensation and progression through mitosis and meiosis. It belongs to the AGC kinase family and, particularly, presents a non-conserved insertion of 550 aa at the corresponding T-activation loop site in the C- lobe (usually 20–30 aa). This non-conserved middle region (NCMR) is not considered to have an essential role for Mastl activity but its function in unknown. Mastl is involved in the inhibition of protein phosphatase 2A (PP2A)-B55 complexes to maintain the mitotic state. By using a conditional knockout in mouse generated in our lab, it was shown that mammalian Mastl is essential for mouse embryonic development and cell cycle progression. Mastl was initially found in humans as a gene mutated in thrombocytopenia and preliminary data suggests its overexpression in tumours. However, very little is known about this protein in human disease.

Material and methods Genomic data from repositories of cancer somatic mutations include MASTL NCMR indel mutations, leading to the generation of a truncated protein. Exome sequencing studies in Mastl in gastric cancers show that mutant tumours present microsatellite instability (MSI). We have studied the prevalence of these mutations by sequencing MASTL in a subset of samples from colon and stomach patients.

To evaluate the therapeutic relevance of this kinase, functional assays have been performed, as complementation studies and kinase assays.

To mimic the cancer mutations we have generated a new mouse model using CRISPR/Cas9 technology. We are currently performing several assays such as focus assays, scratch assays, soft agar and colony formation on cells derived from this model. In addition, we are using a chemical-induced colorectal carcinoma model to study the role of these mutant kinases in cancer.

Results and discussions A heterozygous exonic indel mutation has been found in an MSI +CRC from the 21 patient samples sequenced.

Functional assays with the mutant enzyme resulted in a partial rescue in DNA segregation observed in Mastl-null cells. Mastl mutant forms resulted in embryonic lethality in homozygosis. Therefore, our carcinogenesis models are performed in heterozygous mice, thus mimicking cancer mutations.

Conclusion Mastl indel mutations in the NCMR region lead to the expression of truncated shorter forms. These Mastl mutated forms are not able to fully accomplish the role of Mastl in mitosis. Mutant heterozygous mice, mimicking MASTL cancer mutations, are viable and fertile.