PO-008 ANTI-TUMOUR PROPERTIES OF NOVEL MULTIKINASE INHIBITORS IN SARCOMAS: SYNERGISTIC COMBINATION WITH DOXORUBICIN

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Introduction: Cytotoxic drugs like doxorubicin remain as the most utilised agents in sarcoma treatment. However, advanced sarcomas often show resistance to these drugs, mainly through the overexpression of members of the ATP binding cassette (ABC) family which act as efflux pumps for drugs. Therefore, the development of more effective treatments able to prevent drug resistance would improve sarcoma treatments.

Multi-kinase inhibitors provided an efficient way to target several pro-tumorigenic pathways using a single agent and have shown anti-tumour activity in a range of tumours. However, the overactivation of pro-tumoral kinase is common in sarcoma, the effect of multikinase inhibitors in sarcoma has been barely tested. Here, we aimed to study the anti-tumour effect of one of such inhibitors in cell-of-origin sarcoma models and sarcoma patient-derived primary cell lines, as well as to study its ability to prevent drug resistance and synergize with doxorubicin.

Material and methods: Cell survival, apoptotic induction, cell cycle progression and DNA damage were analysed after drug treatments. The existence of synergy between drugs was evaluated using statistic tools. Drug effect on signalling proteins was studied using phospho-antibody arrays and Western blotting analysis. Interaction between drugs and ABC transporters were characterised using substrate and inhibition assays. Finally, in vivo tumour growth and pharmacodynamic response after drug treatments were evaluated in xenografts models.

Results and discussions: Sarcoma cells were sensitive to sub-micromolar concentrations of the multikinase inhibitor, which induced cell cycle arrest, DNA damage and apoptosis. Evaluation of the phosphorylation status of signalling kinases evidenced that PI3K/AKT/mTOR and ERK1/2 were the most highly activated pathways in sarcoma cells and that the drug efficiently inhibited them in vitro and in vivo. By using specific mTOR inhibitors and agonists, we confirmed that the inhibition of this pathway contributed to the cytotoxic effect of the drug. In addition, this drug inhibited the expression and activity of ABC transporters and was not a substrate for them. In line with this ability, we found a synergistic cytotoxic effect when sarcoma cells were treated with combinations of the kinase inhibitor and doxorubicin both in vitro and in vivo.

Conclusion: A novel multikinase inhibitor induced a consistent cytotoxic effect and was able to counteract drug resistance in sarcoma cells, thus highlighting its therapeutic potential when combined with current treatments.

PO-009 A NOVEL INTEGRIN ALPHA 5 BINDING PEPTIDE POTENTIATES EFFECTS OF CHEMOTHERAPY IN PANCREATIC CANCER

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Introduction: Cancer-associated fibroblasts (CAFs) are the key cell type in the pancreatic tumour microenvironment, which induces tumour growth and metastasis. The extracellular matrix produced by CAFs also acts as a barrier to chemotherapy. We have recently identified integrin alpha5 as a novel target overexpressed in CAFs. In this study, we have designed a novel integrin binding peptide (so-called AV3) against ITGA5, comprised of 7 amino acids, and studied its significance in pancreatic cancer.

Material and methods: AV3 and AV3-PEG-5FAM (AV3-FAM) peptides were custom synthesised. Microscale thermophoresis (MST) was performed to determine the binding affinity of the fluorescently labelled peptide (AV3-FAM) against α5β1 and αvβ1 receptor. AV3-FAM binding on primary human pancreatic stellate cells (hPSCs) was studied either with fluorescent microscopy or flow cytometry. Using qPCR and western blot analyses, AV3 therapeutic efficacy was studied on hPSCs. In vivo studies were performed in Panc-1 + hPSCs co-injection tumour model and patient-derived xenograft (PDX) tumour to assess the therapeutic value of AV3.

Results and discussions: AV3-FAM showed a specific and high binding affinity (Kd: 97 nM) to α5β1 but not to a close family integrin αvβ1, indicating its specific binding to α5 (ITGA5). In vitro, activation of hPSCs with recombinant TGFβ1 significantly induced ITGA5 expression. AV3-FAM showed a strong binding to TGF-β-activated hPSCs was confirmed using flow cytometric analysis and fluorescent microscopy. Furthermore, we examined whether AV3 is able to block ITGA5 and thereby inhibit hPSCs activation. Interestingly, treatment with AV3 led to inhibition of TGF-β-induced differentiation of hPSCs, as shown with qPCR and western blot analyses. In addition, AV3 also inhibited TGF-β-induced contractility and p-FAK signalling in hPSCs. In vivo, treatment with AV3 (either
intratumoral 5 mg/kg or intraperitoneal 20 mg/kg injection) inhibited tumour growth in co-injection (Panc-1 + hPSCs) tumour model. Furthermore, we investigated whether AV3 could impair tumour growth in PDX pancreatic tumour model in mice. Intriguingly, co-treatment with AV3 (20 mg/kg, i.p., bis in 7 d.) and gemcitabine (50 mg/kg, i.p., bis in 7 d.) reduced the tumour growth by >80% compared to vehicle group. These effects were found to be due to the reduction of fibrosis in the tumour, as indicated by reduced αSMA and collagen I expression.

**Conclusion** This study shows AV3 is specific against ITGAs which inhibits the activation of PSCs in vivo and inhibits pancreatic tumour growth in vivo.

**PO-010 DUAL CHEMOTHERAPY AND PHOTODYNAMIC THERAPY: A SYNERGISTIC STRATEGY TO IMPROVE CANCER TREATMENT**

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**Introduction** Photodynamic therapy (PDT) is a clinical-approved option in several diseases characterised by uncontrolled cell proliferation, as cancer. PDT is recognised as a minimally invasive and toxic treatment. It is based on the administration of light activatable molecule known as photosensitizer (PS). After irradiation, a photochemical reaction between the PS and molecular oxygen leads to the generation of reactive oxygen species (ROS), which results in tumour regression.

Nowadays different strategies are being introduced in order to enhance PDT effectiveness, such as combination of PDT with chemotherapy or improvement of PS features.

**Material and methods** A new combined PDT-chemotherapy comprising two drugs widespread in clinical research - the hydrophobic Zinc(II)-phthalocyanine (ZnPc) as PS and the common chemotherapeutic agent doxorubicin (DOX) - was tested in tumour cell lines and primary cells from patients affected by metastatic breast cancer. ZnPc was incorporated into nanoliposomes to increase its solubility and uptake into tumoral cells. This dual-therapy was also assayed in vivo (intravenously administrated) in a breast cancer xenograft model following tumour growth by luciferase activity.

**Results and discussions** MTT cytotoxicity assay showed that combination of both therapies remarkably increases the effectiveness of the treatment by inducing a synergistic cell death effect when compared to DOX or ZnPc monotherapy. In addition, annexin-V detection by flow cytometry, analysis of active caspase-3 and cytochrome c by immunofluorescence and time-lapse videomicroscopy corroborated a fine-tunable effect depending on light dose, leading to apoptotic or necrotic mechanism of cell death.

Subcellular location visualised by fluorescence microscopy confirmed internalisation of both drugs. Using DCFH-DA probe, we demonstrate that a significant higher ROS generation into cells was the main cause of the synergistic effect of this combined treatment. Further, mammosphere formation efficiency assay (MFE) showed a reduced breast cancer stem cell activity in established cell line and primary cells obtained from patients, even using DOX at much lower concentration than clinical level. Finally, studies in human breast cancer xenografts indicated a high efficiency also in vivo.

**Conclusion** All these results provide novel and valuable information that contribute to consider chemophototherapy as a promising tool in current antitumoural treatments, potentially overcoming resistance to cancer chemotherapy and targeting cancer stem cells.

**PO-011 DIHYDROARTESMISININ INHIBITS TCTP-DEPENDENT METASTASIS IN GALLBLADDER CANCER**

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**Introduction** Patients with metastatic or relapsed gallbladder cancer generally have a poor prognosis. Therefore, targeting metastasis is one arm of therapeutic strategies to treat gallbladder cancer.

**Material and methods** Levels of translationally controlled tumour protein (TCTP) were measured in samples of gallbladder cancer by immunohistochemical staining. Wound healing, migration and invasion assays were used to investigate the motility of cells. Western blot assay was used to investigate the levels of TCTP and other proteins. Liver metastasis models and lung metastasis models were established to investigate the inhibitory effect of Dihydroartesminisin on gallbladder cancer metastasis.

**Results and discussions** TCTP is aberrantly expressed in gallbladder cancer patients and associated with metastasis and a poor prognosis. Depleting TCTP significantly inhibited gallbladder cancer cell migration and invasion. We found that Dihydroartesminisin as a potent inhibitor of TCTP inhibited TCTP-dependent cell migration and invasion by reducing cell division control protein 42 homolog (Cdc42) activation. In addition, in mice with xenografted tumours, treatment with Dihydroartesminisin decreased gallbladder cancer cell metastases and improved survival.

**Conclusion** These findings provide new insights into the therapeutic activity of Dihydroartesminisin as a treatment for gallbladder cancer metastasis.

**PO-012 PRE-CLINICAL INSIGHT INTO HOW PLATELET COUNT AFFECTS THE ACTIVITY OF HDAC RESMINOSTAT IN COMBINATION WITH THE MULTI-KINASE INHIBITOR SORAFENIB IN HCC**

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**Introduction** In a recent phase I/II clinical trial in hepatocellular carcinoma (HCC), (NCT02400788), the combination of HDAC-inhibitor resminostat (YHI1001) with a multi-kinase inhibitor sorafenib was clinically beneficial compared to sorafenib monotherapy in patients with platelet counts ≥1.5 × 10^7/μl, but not below that threshold. In HCC, platelets have been linked to bad prognosis, tumour growth and metastasis as well as to resistance against the standard treatment with sorafenib. This begs the question how platelets affect HCC cells and modulate their drug response.