estrogens. We hypothesise that cancer cells adapt to utilise 4AD to promote resistance and survival in AI resistance.

**Material and methods** Molecular changes in a breast cancer cell model which have transitioned from AI sensitive (MCF7 cells) to AI resistant (LetR cells) were assessed using RNA sequencing. Alterations in the steroid microenvironment occur as a result of AI therapy and responses to these steroids was confirmed using MTS, mammosphere and invasion assays. Mass spectrometry (LC-MS/MS) analysis identified androgen-mediated, AR protein interactions. The clinical relevance of AR in AI resistance was also evaluated in a cohort of primary breast cancer patients (n=375).

**Results and discussions** LetR cell growth is AR dependent as the anti-AR drug Enzalutamide inhibits cell growth. Furthermore, Enzalutamide abrogates mammosphere formation and self-renewal of LetR cells. We have found LetR cells to be responsive to 4AD, and treatment with this steroid drives a more aggressive phenotype in vitro. RNA sequencing data highlighted a non-canonical cytoplasmic AR signalling mechanism. Our research suggests that 4AD initiates rapid second messenger signalling in the AI resistant setting. LC-MS/MS analysis identified androgen-mediated, AR protein interactors unique to our resistant model particularly associated with cell-cell adhesion and invasion. Evaluation of AR protein in a cohort of primary breast cancer patients found AR expression in 90% of patients, with 25% expressing high intensity AR staining. Analysis is ongoing into this subset and its association with clinical pathological parameters. 

**Conclusion** AR and its ligand 4AD may be a significant driving factor in AI resistance and may play a role in facilitating a more invasive phenotype. Further investigations into AR interactors identified will help elucidate mechanisms of resistance to AI therapy, and these novel AR protein partners may aid the identification of patients who would benefit from anti-AR therapy.

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**PO-179**

**IDENTIFICATION OF C-MET AND INSULIN RECEPTOR HETERODIMER AS A MEDIATOR FOR HEPATOCELLULAR CARCINOMA**

**Introduction** Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related deaths and obesity is predicted to be the leading cause for HCC in the following decades. Hyperglycemia and hyperinsulinemia are the two obesity-related factors associated with poor prognosis and survival. Hepatocyte growth factor (HGF) receptor, c-Met overactivation is common and results in higher proliferation and metastasis of HCC cells and drug resistance. In HCC patients, c-Met activation is often observed in the absence of HGF.

**Material and methods** Cells were starved for 24 hours before hyperglycemic (HG, 25 mM) and/or hyperinsulinemic (HI, 100 nM) induction. Lysates were analysed by western blotting using the antibodies detecting activatory phosphorylations of c-Met, IR, Erk 1/2 and Akt. Receptor dimerization was studied by coimmunoprecipitation and colocalization using live-cell imaging. c-Met(SU11274) and IR(HMPA-(AM)3) inhibitors were used to reverse the effects of activations. Tumour cell metabolism was analysed using Seahorse Analyzer with cells expressing predominantly either IR isoform A or B. Proliferation and adhesion capacities were investigated using real time cell analysis system xCELLigence for 96 hours. Tumour cell survival in HG/HI blood circulation was simulated by circulation under shear stress for 4 hours in a fluidic system. Migration and invasion are studied using Boyden chamber.

Results and discussions c-Met expression and activation was elevated when induced by high glucose/insulin. Also c-Met and IR were found to form a heterodimer upon HG/HI induction which explains a mechanism of c-Met activation even when HGF is absent in the tumour microenvironment. Both IR isoforms A/B were found to colocalize with c-Met and mediate the effect. In the presence of inhibitors, receptor activations and colocalizations were disrupted. c-Met inhibition resulted in a shift in tumour cell metabolism; diminished glycolysis and oxidative phosphorylation, which offers a role for rendering the cells more aggressive. Elevated adhesion, proliferation, migration, invasion and survival were observed upon induction which are controlled by Erk 1/2 and Akt activations, reversed by c-Met inhibition.

**Conclusion** Here we identified that obesity-related prognostic factors, hyperglycemia and hyperinsulinemia, increase invasive and metastatic capacity of HCC cells through transactivation of c-Met by IR, which might also be a mechanism for drug resistance and offer an approach for targeted therapy.

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**PO-180**

**DISECTING THE EFFECTS OF EGF STARVATION ON EGFR SIGNALLING IN THE MOUSE SMALL INTESTINE USING 3D ORGANOID CULTURE SYSTEMS**

**Introduction** The epidermal growth factor receptor (EGFR) signalling pathway has been established as a key driver of cell proliferation, growth and migration. Activating mutations as well as amplifications of the receptor gene have been found in a number of solid tumours such as glioblastoma, non-small cell lung cancer and colorectal cancer. For this reason, this pathway has been exploited therapeutically in various malignancies through the use of anti-EGFR monoclonal antibodies and small molecule tyrosine kinase inhibitors. Understanding how the pathway components are wired in both normal and cancerous tissues is crucial to develop effective therapies and overcome drug resistance. However, one understudied aspect of EGFR signalling is how it operates in settings where the endogenous ligand is deficient. Thus, the aim of the current study is to investigate how such network is wired in the mouse intestinal tissue in settings where growth factors are supplemented versus depleted.

**Material and methods** Intestinal organoid cultures were prepared from the mouse small intestine according to previously published protocols. We starved the organoids for various time periods of endogenous EGF and perturbed the pathway with inhibitors and growth factors. The levels of phospho-ERK (pERK) were determined using a capillary Western blot assay, while levels of other phosphoproteins besides ERK were measured by a magnetic bead-based immunoassay.
**Results and discussions**

EGF starvation of mouse intestinal organoids for 24 hours resulted in an unexpected upregulation of pERK levels compared to EGF supplied controls. Blocking EGFR tyrosine kinase activity in starved organoids resulted in a significant reduction of pERK levels. Similarly, MEK inhibition caused a profound drop of pERK in starved settings. In contrast, inhibition of phosphoinositide 3-kinase (PI3K) did not seem to affect pERK. These results show that increased pERK levels upon EGF starvation of organoids are mediated via an activation of EGFR tyrosine kinase domain and the downstream RAS-RAF-MEK axis. Furthermore, it was found that EGF addition to starved organoids resulted in a decrease in pERK levels in a time-dependent manner. This reduction in pERK was observed specifically following EGF addition and not addition of other ligands.

**Conclusion**

EGF depletion of mouse intestinal organoids paradoxically activates the EGFR signalling and MAPK axis resulting in an increase in pERK levels. The mechanism by which this paradoxical activation occurs is still under current investigation.

**PO-181 ANAPLASTIC LYMPHOMA KINASE ADDICTIVE NEOBLASTOMA CELL LINES ARE ASSOCIATED WITH GROWTH UPON TREATMENT WITH MEK INHIBITOR TRAMETINIB**

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**Introduction**

The RAS-MAPK pathway is a major player in initiation and progression of multiple cancers where it can be hyper-activated by upstream regulatory proteins, such as receptor tyrosine kinases, phosphatases and GTPase-activating proteins, which is accompanied by a decrease in tumour aggressiveness. Manipulation of PDGFRB levels in a decrease in the signal transducer and activator of transcription (STAT) and NPM-ALK signalling pathways dampen tumour development and dissemination in a STAT-independent manner. Recent studies from our lab identified AP-1 transcription factors JUNB and cJUN as downstream effectors of PDGFRB, which directly up-regulate platelet derived growth factor receptor beta (PDGFRB) expression in lymphoma cells. We demonstrated that besides increased receptor expression its ligand, PDGFB levels are also elevated in both mouse ALCL tumours and human ALCL patient plasma. Furthermore, therapeutic inhibition of PDGFRB with the kinase inhibitor imatinib resulted in rapid and sustained remission in late-stage therapy-resistant ALCL patients.

**Material and methods**

Despite these discoveries, the underlying mechanisms and the nature of PDGFRB signalling in lymphoma formation and progression still remain to be resolved. To elucidate the mechanisms of PDGFRB signalling in ALCL, we have crossed a murine ALCL model, which expresses NPM-ALK under the CD4 promoter, to PDGFRB floxed mice and a CD4 promoter driven Cre recombinase, to yield a specific deletion of PDGFRB in T cells (CD4-NPM-ALK-CD4-PDGFRB). Cells from these mice have significantly prolonged survival rates compared to NPM-ALK only mice. In vitro deletion of PDGFRB results in a decrease in lymphatic vessels, which is accompanied by a decrease in tumour dissemination. Moreover, in vivo deletion of PDGFRB results in a decrease in the signal transducer and activator of transcription (STAT) and NPM-ALK signalling pathways dampening tumour aggressiveness. Manipulation of PDGFRB levels in vitro, via CRISPR/Cas9 mediated knockout or overexpression, in primary tumour cell lines isolated from CD4-NPM-ALK-CD4-PDGFRB mice have significantly prolonged survival rates and reduced tumour growth, due to increased apoptosis. CD4-NPM-ALK-CD4-PDGFRB tumours exhibit a decrease in lymphatic vessels, which is accompanied by a decrease in tumour dissemination. Moreover, in vivo deletion of PDGFRB results in a decrease in the signal transducer and activator of transcription (STAT) and NPM-ALK signalling pathways dampening tumour aggressiveness. Manipulation of PDGFRB levels in vitro, via CRISPR/Cas9 mediated knockout or overexpression, in primary tumour cell lines isolated from CD4-NPM-ALK-CD4-PDGFRB mice have significantly prolonged survival rates and reduced tumour growth, due to increased apoptosis.

**Conclusion**

Our results contraindicate the use of MEK inhibitors as an effective therapeutic strategy in ALK-positive neuroblastoma.

**PO-182 EXAMINING THE FUNCTION OF PDGFRB IN ANAPLASTIC LARGE CELL LYMPHOMA**

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**Introduction**

Anaplastic large-cell lymphoma (ALCL) is an aggressive non-Hodgkin T-cell lymphoma (NHL) most commonly diagnosed in children and young adults. A majority of ALCL tumours harbour the translocation t(2;5)(p23;q35), resulting in the fusion of Nucleophosmin (NPM) to the Anaplastic lymphoma kinase (ALK) gene. The oncogenic fusion protein (NPM-ALK) is constitutively expressed and consequently contributes to the pathogenesis and progression of about 70% of ALCL cases. Recent studies from our lab identified AP-1 transcription factors JUNB and cJUN as downstream effectors of NPM-ALK, which directly up-regulate platelet derived growth factor receptor beta (PDGFRB) expression in lymphoma cells. We demonstrated that besides increased receptor expression its ligand, PDGFB levels are also elevated in both mouse ALCL tumours and human ALCL patient plasma. Furthermore, therapeutic inhibition of PDGFRB with the kinase inhibitor imatinib resulted in rapid and sustained remission in late-stage therapy-resistant ALCL patients.

**Material and methods**

Despite these discoveries, the underlying mechanisms and the nature of PDGFRB signalling in lymphoma formation and progression still remain to be resolved. To elucidate the mechanisms of PDGFRB signalling in ALCL, we have crossed a murine ALCL model, which expresses NPM-ALK under the CD4 promoter, to PDGFRB floxed mice and a CD4 promoter driven Cre recombinase, to yield a specific deletion of PDGFRB in T cells (CD4-NPM-ALK-CD4-PDGFRB).