Material and methods Immunohistochemical scoring for total-STAT3, pY705 and pS727 was done in 75 paired core-biopsy and post-NACT TNBC samples. Bioluminescence resonance energy transfer based sensor (PhosphoBRET) was developed by N-terminal fusion of STAT3 with Nanoluc (donor) and TurboFP635 (acceptor) and validated on multiple cancer cell lines (MCF7, A549, HT1080, PC3) or drug inhibitors (Niclosamide, Stattic). We also engineered 3'UTR STAT3shRNA cells overexpressing Nanoluc-STAT3 variants i.e. Wild type [wt], Y705F, S727A, K685R and performed biochemical as well as phenotypic assays to study biological role of STAT3 pathway.
Results and discussions Over 90% TNBC cases showed positive staining for pS727 as compared to pY705 STAT3 indicating hyperactivation of non-canonical pathway. 3'UTR STAT3 shRNA cells over-expressing S727A PTM mutant, showed decreased pY705 expression along with abrogation of K685 acetylation. The level of downstream STAT3 targets like Myc, cyclin D1 etc. remained intact while some of the novel target genes (Her2, E-Cadherin and ERα) showed elevated expression in S727A and K685R mutants. Additionally, S727A or K685R mutant also exhibited 2-fold decrease in overall cell proliferation and survival potential. The designed STAT3-PhosphoBRET sensor demonstrated upto 3-fold gain in BRET ratio ($p<0.01$) using IL6 or EGF ligand. Further, PhosphoBRET sensor expressing either Y705F or S727A or K685R showed increased BRET ratio ($p<0.001$) implicating higher STAT3 dimerization and activation. Additionally, the PhosphoBRET platform was also extended to test various STAT3 inhibitors, of which Niclosamide was identified as a potent dual blocker (pS727 and pY705) of STAT3 activation for the first time.
Conclusion Predominant expression of pS727 STAT3 in TNBC cases along with *in vitro* data implicate crucial role of non canonical (pS727 and acK685) pathway in controlling STAT3 dimerization and downstream function independent of pY705 activation. Hence, future drug design strategy should aim both arms of STAT3 pathway to completely abrogate its oncogenic function.

PO-186 GENERATION OF THE GLI1, GLI2 AND GLI3 KNOCK-OUT OVARIAN CANCER CELL LINES USING THE CRISPR/CAS9

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Introduction Hedgehog signalling pathway is a developmental signalling pathway which is dormant in most adult differentiated tissues, but aberrantly activated in various tumours. In ovarian tumours it can be activated in a canonical way, by the SHH ligand, or the non-canonical way, by upregulation of the GLI transcription factors. Expression of GLI1 is usually associated with tumour progression in a clinical setting, but GLI2 and GLI3 also play a role by modifying the activity of GLI1 and transcription of their common transcriptional targets. In ovarian cancer, GLI3 protein is expressed in the full-length activator form, and not the shortened repressor form which is the predominant form for GLI3 protein.
Material and methods To study the roles of the three GLI proteins in detail, we used the CRISPR/Cas9 guided gene editing to generate knock-out lines for each of the three GLI proteins. The sgRNA were designed using the online tool at crispr.mit.edu web site, and cloned into the pX330 vector expressing the sgRNA and the Cas9 protein. The sgRNA were designed to target the region close to the ATG site of each of the three GLI proteins, and after Cas9 nuclease activity the double stranded DNA break should be repaired by non-homologous end joining, which generates indels and leads to frameshift. Frameshift close to the ATG often leads to early termination of the protein, generating a knock-out. The SKOV3 ovarian cancer cell line was transfected with each of the vectors, the cells were split to the density of a single cell/well in a 96-well plate, and cell lines were expanded from single cells to obtain homogenous lines.
Results and discussions SKOV3 ovarian cancer cell lines with knock outs in each of the three GLI proteins, GLI1, GLI2 and GLI3 were generated. The knock-out was confirmed by qRT-PCR and Western blot, and DNA was sequenced to show the exact genetic modification of the lines leading to the knock-out. The modified cell lines showed differences in cell morphology in relation to the original SKOV3 cell line immediately after expansion from the single cell.
Conclusion The Hedgehog signalling pathway plays an important role in ovarian cancer. Cells harbouring knock out for the effector proteins of the Hedgehog signalling pathway, GLI1, GLI2 and GLI3 show distinct changes in cell morphology and viability. The Hedgehog signalling pathway, and more specifically GLI proteins, may be a good potential therapeutic target in management of ovarian cancer.

PO-187 DEFECTIVE LIVER REGENERATION ABILITY OF SOS1-KO AND SOS1/2-DKO, BUT NOT SOS2-KO MICE

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Introduction To ascertain specific functional role(s) of the Sos1 and Sos2 Ras-GEF activators we investigated phenotypic effects of single or combined disruption of Sos1 and/or Sos2 in adult mice by using a tamoxifen-inducible Sos1-KO system. Upon TAM induction, the resulting Sos1/2-DKO animals die precipitously (in about 2 weeks) whereas single Sos1-KO or Sos2-KO adult mice are perfectly viable. Histological examination of DKO mice showed that the internal organs most severely affected by concomitant Sos1 and Sos2 loss include the liver and gallbladder in the gastrointestinal (GI) tract.
Material and methods To determine cause(s) of the quick death of Sos1/2-DKO mice, we analysed blood and GI tissues from 6–8 week-old mice of 4 relevant Sos genotypes (WT, Sos1-KO, Sos2-KO and Sos1/2-DKO) similarly treated with TAM for 13 days. Different biochemical parameters were quantitated in blood serum and liver samples, and various IHC assays were also performed on different organs of the GI tract. Liver regeneration was characterised by means of partial hepatectomy studies of WT, Sos1-KO and Sos2-KO animals previously treated with TAM for 10 days.
Results and discussions Combined loss of Sos1 and Sos2 in DKO mice resulted in markedly reduced levels of total serum protein and increased serum levels of lactate dehydrogenase, creatinine kinase and other liver enzymes, suggesting the occurrence of substantial liver failure in these animals. Histological analysis of the DKO animals showed a quick overall

structural degeneration of the liver accompanied by increased levels of oxidative stress in the hepatic lobules. We also observed marked distension of the gallbladder, perhaps linked to decreased CCK levels in the gut mucosa cells. The dilatation of the gallbladder together with the observed decrease of serum triglycerides and fat in all the organs analysed point to significant impairment of the lipidic metabolism in Sos1/2-DKO mice. Finally, our partial hepatectomy studies in single Sos KO mice showed that hepatic regeneration critically depends on the presence of Sos1, whereas Sos2 appears to be dispensable for this process of organ recovery after liver damage.

Conclusion Our data suggest functional redundancy of Sos1 and Sos2 for overall homeostasis of GI organs, especially liver and gallbladder. Our studies also show that Sos1, but not Sos2, is the main, critical RasGEF required for liver regeneration and tissue repair upon organ injury.

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PO-188 LOSS OF UPAR FUNCTION AND SUPPRESSION OF MALIGNANT CELL BEHAVIOUR BY A GPI-SPECIFIC PHOSPHOLIPASE C

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Introduction The urokinase receptor (uPAR) is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein that promotes tissue remodelling and tumour progression. uPAR is highly expressed in many cancers and correlates with poor prognosis. uPAR mediates matrix degradation through protease recruitment and enhances tumour cell migration and signalling through vitronectin binding and interaction with integrins. Full-length uPAR is released from the cell surface, resulting in a soluble form (suPAR), but the mechanism and functional significance of uPAR shedding have been elusive.

Material and methods Cell biological and biochemical assays; super-resolution microscopy; homology modelling; knockdown/knockout studies; xenograft model; patient survival analysis.

Results and discussions We find that uPAR is released from the cell surface through GPI-anchor cleavage by a multi-pass membrane glycerophosphodiesterase, termed GDE3, acting as a GPI-specific phospholipase C (PLC), leading to loss of uPAR function. By contrast, GDE3's closest relative GDE2 fails to cleave uPAR. By shedding uPAR from the cell surface, GDE3 abrogates uPAR-driven cell adhesion, spreading and lamellipodia formation on vitronectin. In breast cancer cells, high GDE3 expression depletes uPAR form distinct basolateral membrane microdomains resulting in a less transformed phenotype, as revealed by reduced matrix degradation, cell motility and colony formation. Furthermore, elevated GDE3 expression reduces tumour progression in a xenograft model and correlates with higher survival probability in breast cancer patients. Our results establish GDE3 as a cell-intrinsic GPI-specific PLC that sheds uPAR to attenuate malignant cell behaviour.

Conclusion GDE3 is the first mammalian GPI-specific PLC that negatively regulates the uPAR signalling network, thereby suppressing the malignant phenotype of uPAR-positive cancer cells. Future studies should address how GDE3 activity and its substrate specificity are regulated.

PO-189 ABSTRACT WITHDRAWN

PO-190 *IN SILICO* AND *IN VITRO* SCREENING OF POLYPHENOLIC COMPOUNDS FOR THEIR MTOR MODULATORY CAPACITY

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Introduction The phosphoinositide 3-kinase (PI3Ks)/protein kinase B (PKB or AKT)/mammalian target of rapamycin (mTOR) signalling pathway is essential for tumorigenesis and metastasis in many types of human tumours and some tumour cells owe their hyperproliferative capacity to the overactivation of this pathway, in response to mutations that abolish some repressing genes. Therefore, the inhibition of mTOR and the subsequent activation of autophagy has been postulated as a therapeutic target in certain types of tumours. Therefore, the control of the route governed by mTOR can be a therapeutic target, both positively and negatively, depending on the type of tumour.

It is economically unfeasible to analyse libraries of millions of bioactive compounds *in vitro*. For this reason, in recent years *in silico* methodologies are being used since reduce the size of these libraries to an affordable number of compounds that can be tested *in vitro*.

In this work we studied the modulatory effect on mTOR in the highly invasive HCT-116 colon cancer model of 11 compounds of plant origin, that were selected an *in silico* screening.

Material and methods

Citotoxicity assay Cell viability was measured through the MTT assay and the dose-response curves were used to calculate the optimal concentrations of each compound to study its activity upon mTOR.

In silico screening A library of compounds, previously selected with ADMET criteria, were tested *in silico* with molecular docking experiments, using AutoDock/vina.

Protein expression The expression of mTOR and activation (phosphorylated form) were measured by western blot. β-actin was used as a loading control and PARP as a viability control.

Results and discussions The results of IBMC-C, IBMC-F, IBMC-H and IBMC-J compounds show a decrease similar to that caused by rapamycin, that is a mTOR inhibitor, immunosuppressant and kinase inhibitor.

Conclusion Compounds IBMC-C, IBMC-F, IBMC-H and IBMC-J may have potential to inhibit the proliferation of