anti-ENG monoclonal antibody-drug conjugates, linked to nigrin-b A chain (OMTX503) or cytolysin (OMTX703), appeared as an appealing scenario to test the possible role of ENG as a key target in ES.

Material and methods mRNA levels were evaluated by qRT-PCR. Protein levels were studied by western blot, flow cytometry and immunohistochemistry (IHC). OMTX503 and OMTX703 activity was evaluated by MTT and WST1 assays. OMTX503 and OMTX703 were tested in ES cell line-derived (RM82 and ES8) xenografts and patient-derived xenografts (PDXs). Hematoxylin and eosin staining was performed to assess cell viability. IHC analyses for ENG, MMP14, and Ki67 expression were performed to assess ENG/MMP14 expression and tumour proliferation in samples from ES xenografts and patients.

Results and discussions We evaluated the ENG expression in a set of ES cell lines, related xenografts, PDXs and patient tumours. The consistent heterogeneous ENG expression found among ES sets suggested that the patients presenting high ENG expression in their tumour cells could benefit from anti-ENG treatments. In order to confirm this hypothesis pre-clinically, we assessed the anti-tumoral activity of OMTX503 and OMTX703. Firstly, a significant ENG-dependent anti-proliferating effect was observed in ES cell lines *in vitro*. In two ES cell line-derived xenograft models (RM82 and ES8), we identified a tumour growth impairment in OMTX503-treated animals. Moreover, a significant response to treatment was observed in OMTX703-treated mice, followed by a significantly increased median time of survival ($p<0.019$). Taking into consideration these results, we evaluated OMTX703 in an ES PDX model with high ENG expression. The complete response and increased survival observed in this PDX model treated with 3 weekly doses of 60 mg/kg OMTX703 strengthened the significant anti-tumoral effect of this drug.

Conclusion These results support the role of ENG as an effective target in ES, and suggest the inclusion of novel ADCs —like OMTX703— in the ES therapeutic arsenal for patients with high ENG expression in their tumours.

PO-465 N-GLYCANS OF PSEUDOMYXOMA PERITONEI PRESENT HIGHLY INCREASED FUCOSYLATION AND MULTIFUCOSYLATION CORRELATES WITH HIGH-GRADE MORPHOLOGY

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Introduction Pseudomyxoma peritonei (PMP) is a fatal clinical syndrome, where most often appendix-originating mucinous adenocarcinoma cells grow in the peritoneal cavity filling it with mucinous ascites. *KRAS* mutations are found in nearly all PMPs and *GNAS* mutations in over half of the cases, but other common driver mutations are infrequent. As glycosylation alterations can also contribute to carcinogenesis, our aim was to compare the N-linked glycan profiles of PMP tissue specimens to those of normal appendices.

Material and methods N-glycans were detached from formalin-fixed, paraffin-embedded tissue specimens of 8 normal appendices and 8 low-grade and 8 high-grade PMPs, and analysed by mass spectrometry. The expression levels of glycosylation-related genes were further analysed from PMP microarray data, and the expression of fucosylation-related enzymes and fucosylated glycans was localised by immunohistochemistry and lectin histochemistry. Finally, cell culture experiments were used to study the relationship between fucosylation and mucin expression.

Results and discussions The N-glycan profiles, especially those of neutral glycans, clearly differed between PMPs and normal control samples. The most prominent alteration in PMP was highly increased fucosylation and multifucosylation further showed correlation with the proportion of high-grade morphology in the sample. From microarray data we could demonstrate upregulated mRNA expression of four fucosylation-related enzymes (FUT8, GMDS, GMPPA, and TSTA3) in PMP specimens, and by immunohistochemistry we localised these enzymes into PMP cells. Finally, by using colon carcinoma cell line HCT116 with inherently defective fucosylation, we demonstrated that restoration of fucosylation enhanced *GNAS* mutation-induced upregulation of *MUC2* expression.

Conclusion PMPs show highly increased N-glycan fucosylation as compared to normal appendices, and this glycosylation alteration may be linked to the characteristic mucin overexpression of the disease. Most importantly, multifucosylation correlates with the proportion of high-grade morphology in the specimen and this glycan class may thus provide structures bearing prognostic potential.

PO-466 PI3K-C2A REGULATES MITOTIC SPINDLE ASSEMBLY AND CHEMOTHERAPY RESPONSE IN BREAST CANCER

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Introduction Proper organisation of the mitotic spindle is key to genetic stability but the molecular components of inter-microtubule (MT) bridges that crosslink kinetochore fibres (K-fibres) are still largely unknown. Here, we identify class II phosphoinositide 3-OH kinase a (PI3K-C2α) as a limiting scaffold protein organising the clathrin and TACC3 complex crosslinking K-fibres.

Material and methods Pik3c2a$^{+/-}$ mice were intercrossed with a transgenic strain expressing the activated HER-2/Neu oncogene in the mammary gland. Mice were weekly followed for survival, tumour appearance and growth. Primary Murine Mammary Epithelial Tumour (MMET) cells were derived from early and late stage tumours. Truncating PI3KC2α mutants were generated and interaction with TACC3 was tested. Levels of PI3K-C2α expression were assessed by IHC in breast cancer tissue microarrays (TMA) and correlated with response to chemotherapy.

Results and discussions Loss of PI3K-C2α expression is a frequent occurrence in breast cancer patients (48%) and correlates with local recurrence and metastatic disease. The heterozygous loss of PI3K-C2α initially delays tumour onset

but, on the long run, leads to the convergent evolution of aggressive clones with mitotic checkpoint defects. In line with this, downregulation of PI3K-C2α promotes spindle alterations and aneuploidy, indicating that PI3K-C2α expression is a key determinant of genomic stability. As a consequence of the altered spindle, reduction of PI3K-C2α expression increases the sensitivity to anti-MT drugs, such as paclitaxel, in pre-clinical models and in breast cancer patients.

Conclusion Loss of PI3K-C2α expression is a frequent occurrence in breast cancer patients (48%) and correlates with local recurrence and metastatic disease. The heterozygous loss of PI3K-C2α initially delays tumour onset but, on the long run, leads to the convergent evolution of aggressive clones with mitotic checkpoint defects. In line with this, downregulation of PI3K-C2α promotes spindle alterations and aneuploidy, indicating that PI3K-C2α expression is a key determinant of genomic stability. As a consequence of the altered spindle, reduction of PI3K-C2α expression increases the sensitivity to anti-MT drugs, such as paclitaxel, in pre-clinical models and in breast cancer patients.

PO-467 UNCLARIFIED CASES OF MICROSATELLITE INSTABILITY ANALYSES FOR LYNCH SYNDROME DIAGNOSTICS

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Introduction Lynch syndrome (LS) is an autosomal dominantly inherited form of colorectal cancer (CRC) and is implicated in 2%–4% of CRC cases. LS develops from a mutation in one allele of one of the DNA mismatch repair (MMR) genes, most commonly MLH1 and MSH2, or less frequently MSH6 and PMS2. Loss of functional MMR proteins leads to defects in DNA repair and, subsequently, high DNA microsatellite instability (MSI-High). LS diagnostics currently consists of an analysis of MMR protein expression by means of Immunohistochemistry (IHC) and a molecular analysis to detect MSI. During our 10 year experience in performing LS diagnostics we have encountered few cases where IHC and MSI analyses do not match. This study is to clarify these inconsistent molecular alterations which will contribute to our understanding of LS diagnostics.

Material and methods Of 2335 LS cases 27 (1.1%) showed discrepant IHC and MSI results. To clarify this, IHC and MSI analyses were repeated if possible with different antibodies and different MSI markers (mononucleotide instead of dinucleotide markers). Using MS-MLPA MLH1 hypermethylation, BRAF V600E mutation status and LOH of the MMR genes was analysed. Finally, germline and somatic mutations in MMR genes were analysed by using NGS. Protein modelling was also performed to visualise structural protein changes.

Results and discussions Among these 27 cases, due to the decline of patients for further testing, 4 cases were excluded from further analyses. Of the remaining cases in 2 cases using a different antibody explained the unexpected results and in 2 cases switching from dinucleotide to mononucleotide MSI markers. In respectively 8 and 4 cases somatic and germline mutations explained the IHC and MSI results. Finally, in 2 cases protein modelling of identified mutations explained the presence of protein staining in MSI-high cases. The rest cases cannot be further studied due to the lack of tissues.

Conclusion Based on these results, we have concluded: 1. Mononucleotide markers are more sensitive to detect microsatellite instability. 2. Protein modelling can explain presence of protein staining for mutations that do not cause a major conformational change. 3. Somatic sequencing is a valuable addition to Lynch syndrome diagnostics.

PO-468 LAMININ332 (α3; ß3;γ2) GENES AND PROTEIN EXPRESSION IN CERVICAL CARCINOMAS

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Introduction Laminin332 (α3; ß3; γ2 chains) has been identified as an important macromolecule in cancer invasion and metastasis, influencing cell differentiation, migration, adhesion, cell proliferation and survival.

The α3, β3, and γ2 chains of LAM-332 are encoded by three distinct genes, LAMA3, LAMB3, and LAMC2, respectively. LAMC2 is associated to the invasive and metastatic abilities of several tumour types such as colon and pancreas. However, the molecular role of LAMC2 in cervical carcinomas has not yet been fully elucidated.

We investigated the functional significance of LAMC2 in cervical carcinomas: squamous cell carcinomas (SCC) and adenocarcinoma (ADC) as well as its role in SCC of the cervix.

Material and methods SiHa (SCC) and HeLa (ADC) cell lines were used. The expression of *LAMA3*, *LAMB3*, *LAMC2* and *EGFR* was evaluated by qPCR in the presence/absence of EGF. Lamγ2 levels were evaluated by immunofluorescense. Lamγ2 role in migration and invasion was evaluated by wound healing and trans-well assays in control, EGF stimulated and LAMC2 silenced cells (shRNA). Immunohistochemical study evaluated lamα3, β3, and γ2 chains and EGFR, in formalin-fixed, paraffin-embedded in 122 cases, both *in situ* (n=15) and invasive cervical carcinomas: SCC (n=106) and ADC (n=41). Fisher's exact test was performed for statistical analysis.

Results and discussions EGF stimulates the migration and invasion in SiHa cells. However knocking-down Lamγ2 decreases cell migration and invasion capacities in SiHa cells. Also, the knockdown of *LAMC2* lead to an increased transcription of *LAMA3* and *LAMB3* genes only in SiHa. The immunohistochemical study demonstrated that lamγ2 chain in SCC cases was significantly associated with invasion (p=0.0001) and with that histological type (p=0.0183). In SCC cases, there was also a significant association of lamß3 and γ2 chains overexpression (p=0.008). Lamα3 was associate with ADC type (p<0.0001). EGFR immuno-overexpression was also associated with SCC (p<0.0001).

Conclusion Laminin332 and EGFR are differently expressed in the two most common histological types of cervical carcinomas (SCC and ADC). Lamγ2 expression is associated with SCC in both *in vitro* and clinical context and Lamg2 -