Results and discussions Bioinformatics analysis showed that a higher expression of ENG correlated with worse disease-free survival (DFS) (p=0.00023), whereas MMP14 showed a similar trend without reaching statistical significance (p=0.077). These results differ from skin melanoma DFS (p=0.74 for ENG, p=0.22 for MMP14). Pathway enrichment analysis showed that higher ENG expression is associated with a higher abundance of CD8+ T-cells (p<0.002), endothelial cells (p<0.003) and fibroblasts (p<0.001). In the 2 patient cohorts, differential levels of ENG/MMP14 were detected. In vitro, cell line MUM2B expressed higher levels of ENG/MMP14. UM cell lines were highly sensitive to treatment with ADM OMTX503, with IC50 in the sub-nM range. Sensitivity was dependent on ENG expression. Knockout of MMP14 resulted in lack of protein expression in the single cell derived clones. The effect on ENG shedding and malignant features upon lack of MMP14 is currently being analysed.

Conclusion ENG/MMP14 are expressed in UM cell lines and in patient tumour samples. Results suggest that higher levels of ENG are associated with a more aggressive phenotype. ENG is an attractive target, and ENG-targeting biologics such as OMTX503 show promising in vitro activity. In-depth evaluation of the role of ENG/MMP14 in UM is ongoing.

PO-460 GENE EXPRESSION-BASED PROBABILISTIC GRAPHICAL MODELS IDENTIFY THREE INDEPENDENT BIOLOGICAL LAYERS IN COLORRECTAL CANCER

Introduction Colorectal cancer (CRC) is the fourth cause of cancer death in the world. The Colorectal Cancer Subtyping Consortium (CRCSC) defined four consensus molecular subtypes (CMS): immune, canonical, metabolic and mesenchymal. This study bases on the hypothesis that there are two independent biological layers, as our group has previously seen in other malignancies, such as triple negative breast cancer, melanoma or bladder cancer. So, the aim of this study is to put the colorectal cancer subtypes in context with that hypothesis.

Material and methods Colorectal cancer tumour datasets were obtained from Control Expression Omnibus (GEO). A merged database was constructed, and limma R package was used to remove batch effect. A probabilistic graphical model, followed by successive sparse k-means and consensus cluster analyses were used to classify CRC tumour samples from the merged dataset.

Results and discussions Through this approach, three independent classifications, immune, molecular and adhesion-related, were established. Molecular classification showed different groups of tumours that present differential expression of diverse colorectal biomarkers. The immune classification further divided patients into high immune activity and low immune activity subgroups. And the adhesion-related assignment divides patients in two subgroups with prognostic value. We showed that immune and adhesion-related layers, when studied together, add its prognostic value defining low risk and high risk subgroups. We finally compare our classifications with the consensus molecular classification.

Conclusion Molecular information obtained using a novel analytical approach to classify colorectal cancer tumour samples allowed us to establish new classifications based on different biological layers. We have demonstrated that our classifications based on the immune and the adhesion-related layers have prognostic value. Ultimately, to deepen the knowledge of CRC makes it possible to propose new therapeutic strategies and could also be used for diagnostic and prognostic purposes.

PO-461 A TRANSCRIPTOMIC AND MOLECULAR APPROACH TO UNCOVERING ACHAETE-SCUTE COMPLEX HOMOLOG 2 (ASCL2) AS A POTENTIAL NOVEL DRIVER GENE IN BREAST CANCER

Introduction Breast cancer is highly heterogeneous and despite ongoing advancements in diagnostics and targeted therapeutics, incidence and mortality continues to rise. Thus, there is a need for greater characterisation of driver genes to understand their mechanistic role in breast carcinogenesis and their respective signalling pathways.

With the advent of high-throughput technologies in transcriptomics, it is now possible to examine thousands of genes in parallel, generating an unprecedented amount of information. However, the task of synthesising the vast amount of OMICS’ data produced and extracting the clinically significant results is a prominent challenge. The aim of this study was to use in silico and in vitro methods to identify and functionally investigate a novel genetic driver gene that plays a key role in breast carcinogenesis.

Material and methods Gene expression profiles were obtained from public databases (Array Express and Gene Expression Omnibus) and subjected to an extreme variation analysis. Pathway analysis tools were used to identify a novel potential candidate gene for further investigation. Gene expression was assessed in vitro by quantitative real-time RT-PCR (qRT-PCR) to ensure concordance with transcriptomic data. siRNA knockdown was carried out in breast cancer cells, and sufficient knockdown was validated using qRT-PCR. Subsequently, to assess the effect of gene knockdown, assays were used to measure proliferation, wound-healing and apoptosis.

Results and discussions We have established a basic, novel and user-friendly pipeline for in silico pathway analysis of transcriptomic data. This has allowed for selection and in vitro validation of a candidate driver gene, Achaete-scute complex homolog 2 (ASCL2), seen to be elevated in breast cancer.

This gene is a transcription factor and regulator of stem cell identity, as well as a known Wnt-target gene. Functional work has suggested that ASCL2 may be involved in the migration of breast cancer cells, as upon siRNA knockdown of ASCL2, cellular migration was inhibited compared to negative controls. Additional analysis has revealed that ASCL2 knockdown alters...
the expression of various Wnt-associated genes, such as Survivin (BIRC5) and CD44.

Conclusion This work suggests that ASCL2, functioning within the Wnt signalling pathway, may be a potential novel driver of breast tumourigenesis. Investigation using both in vitro methods and data mining, will continue to further elucidate the functional role of ASCL2 in breast cancer tumourigenesis.

FUNCTIONAL EX VIVO ASSAY REVEALS HOMOLOGOUS RECOMBINATION DEFICIENCY IN BREAST CANCER BEYOND BRCA GENE DEFECTS

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Introduction Tumours of germline BRCA1/2 mutated carriers show homologous recombination (HR) deficiency (HRD), resulting in impaired DNA double strand break (DSB) repair and high sensitivity to Poly-(ADP-Ribose)-Polymerase (PARP) inhibitors. Although this therapy is expected to be effective beyond germline BRCA1/2 mutated carriers, a robust validated test to detect HRD tumours is lacking. In the present study we therefore evaluated a functional HR assay exploiting the formation of RAD51 foci in proliferating cells after ex vivo irradiation of fresh breast cancers (BrC) tissue: the RECAP test.

Material and methods Fresh samples of 170 primary BrC were analysed using the RECAP test. The molecular explanation for the HRD phenotype was investigated by exploring BRCA deficiencies, mutational signatures, amount of tumour infiltrating lymphocytes (TILs) and microsatellite instability (MSI).

Results and discussions RECAP was completed successfully in 148 out of 170 samples (87%). 24 tumours showed HRD (16%), while 6 tumours were HR intermediate (HRi) (4%). HRD was explained by BRCA deficiencies (mutations, promoter hypermethylation, deletions) in 16 cases. Several non-BRCA deficient HRD tumours showed BRCA1 related mutational signatures, suggesting that they are likely also bona fide HRD cases. HRD tumour showed an increased incidence of high TIL counts (p=0.023) compared to HR proficient (HRP) tumours and MSI was more frequently observed in the HRD group (2/20, 10%) than expected in unselected BrC (1%) (p=0.017).

Conclusion The RECAP test is a robust assay detecting both BRCA1/2 deficient and BRCA1/2 proficient HRD tumours. This test identifies approximately 50% more patients that may benefit from PARPi treatment than BRCA gene testing only.

MULTIPARAMETRIC ANALYSIS OF LUNG CANCER TISSUE SECTIONS USING IMAGING MASS CYTOMETRY

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Introduction A novel tool for multiparametric molecular pathology analysis of tissue sections is described. Imaging Mass Cytometry (IMC), a new mass spectrometer-based technology, provides visualisation and spatial distribution of up to 40 markers in a single tissue section with 1 μm resolution. IMC uses metal-tagged antibodies (mAbs), a pulsed UV laser for ablating regions of interest, and a stream of inert gas to transfer material generated by the laser to the mass cytometer.1

Material and methods Tissue microarray (TMA) of human lung cancer was stained according to IMC protocol2 using a mix of 23 mAbs.

The samples were inserted into the ablation chamber of the Hyperion Imaging System. CyTOF® Software v6.7 and MCD Viewer 1.0 were used for data acquisition and rendering of pseudo three-colour images. Quantification of selected markers was done with Definiens Tissue Studio® (DTS) software. Standard HPA protocol was used for immunohistochemical (IHC) staining.

Results and discussions IMC analysis of human lung cancer TMA stained with a panel of 23 mAbs revealed that core morphology was consistent with anatopathological tumour classification. Immune cell phenotype and spatial distribution assessed by IHC and IMC were equivalent.

IMC generated data was used to quantitate PD-L1 marker expression. Percentages of cells expressing different levels of PD-L1 and average intensity of PD-L1 related signal were calculated for tumour and stroma regions of each core. In addition, PD-L1 corresponding signal intensity was calculated in cytoplasm and membrane compartments of marker-positive cells.

Conclusion IMC technology is a powerful tool for multiparametric histopathological analysis. It allows researchers to simultaneously detect up to 40 markers with single-cell detection resolution equivalent to conventional IHC. Using DTS software we demonstrate quantitation of multiple markers in lung tumour sections probed with a panel of 23 mAbs.

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ENDOGLIN-TARGETED THERAPY DEMONSTRATES STRONG PRECLINICAL ANTI-TUMOUR ACTIVITY IN EWING SARCOMA USING ANTIBODY-DRUG CONJUGATES

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Introduction Ewing sarcoma (ES) is a bone/soft tissue neoplasia putatively originated from mesenchymal stem cells (MSC). Among the proteins that define the MSC signature, endoglin (ENG, CD105) is considered one of the highly expressed molecules. This protein is a transmembrane co-receptor of the TGF-β family. The poor outcome experienced by ES patients with disseminated disease highlights the necessity of developing new therapeutic strategies. The generation of two novel