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KEY

Ⓟ = Presenter

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PL1

SOCS2 as a Marker Related to Low Grade and Tumour Morphology in Breast Cancer

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Hypothesis: Suppressor of cytokine signalling (SOCS) family members play a vital role in the activation of the JAK/STAT signalling pathway via a negative feedback loop and have been implicated in the development of cancers. In breast cancer (BC), SOCS2 mRNA has been correlated with oestrogen receptor (ER) positive tumours favouring a good prognosis (BMC Cancer 2007, 7:136). This study aimed to determine whether SOCS2 at the protein level correlates with tumour morphology and low grade in BC.
Methods: Differential expression analysis between tubular and grade matched NSTs were undertaken in the METABRIC cohort. Primary breast cancer tissue microarrays (n=1041) were immuno-stained for SOCS2 and expression patterns correlated with clinico-pathological and molecular variables including outcome.
Results: Differential gene expression analysis on the METABRIC data identified SOCS2 as the top gene with a significant overexpression in the tubular type as compared to low grade NSTs (adjusted p value=0.004). Immunohistochemistry on the Tenovus series showed positive nuclear SOCS2 expression to correlate with tumours of low grade ($p<0.0001$), low proliferation (Ki67 $p<0.0001$), ER/PR positive ($p<0.0001$) phenotype and tubular morphology ($p<0.0001$); as well as negative HER2 status ($p=0.005$) and non-triple negative status ($p<0.0001$). Survival analysis revealed significant associations with long term breast cancer specific survival ($p=0.019$). Positive SOCS2 correlations were also observed with the expression of androgen receptor (AR) ($p<0.0001$) and STAT3 ($p=0.001$), further indicating its role in these two signalling pathways.
Conclusions: Results from this study suggest SOCS2 to be a marker of favourable prognosis: identifying low grade, ER positive breast tumours with particular correlations to the tubular histological tumour type.
Project supported by Career Development Fellowship from PathSoc and NIHR

PL2

Novel Hypoxia-Associated Markers of Chemoresistance in High Grade Serous Ovarian Cancer

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Background: Ovarian cancer is the fifth leading cause of cancer in women and has poor long-term survival, in part, due to chemoresistance. Tumour hypoxia is associated with chemoresistance in ovarian cancer. However, relatively little is known about the genes activated in ovarian cancer which cause chemoresistance due to hypoxia. This study aimed to firstly identify genes whose expression is associated with hypoxia-induced chemoresistance, and secondly select hypoxia-associated biomarkers and evaluate their expression in ovarian tumours.
Methods: Cisplatin-sensitive (A2780) and cisplatin-resistant (A2780cis) ovarian cancer cell lines were exposed to combinations of hypoxia and/or cisplatin as part of a matrix designed to reflect clinically relevant scenarios. RNA was extracted and interrogated on Affymetrix Human Gene arrays. Differential gene expression was analysed for cells exposed to hypoxia and/or treated with cisplatin. Potential markers of chemoresistance were selected for evaluation in a cohort of ovarian tumour samples by RT-PCR.
Results: A wide range of genes associated with chemoresistance were differentially expressed in cells exposed to hypoxia and/or cisplatin. Selected genes [ANGPTL4, HER3 and HIF-1α] were chosen for further validation in a cohort of ovarian tumour samples, n=35. High expression of ANGPTL4 trended towards reduced progression-free and overall survival. High expression of HER3 trended to increased progression-free but reduced overall survival, while high expression of HIF-1α trended towards reduced progression-free and increased overall survival.
Conclusion: This study has further characterized the relationship between hypoxia and chemoresistance in an ovarian cancer model. We have also identified many potential biomarkers of hypoxia and platinum resistance and provide initial validation of a subset of these markers in ovarian cancer tissues.

PL3

Gene Network Analysis Reveals a Novel Pathological Cell Type in Paediatric Focal Cortical Dysplasia

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Introduction: Focal cortical dysplasia (FCD) is a malformation of cortical development that is a frequent cause of multidrug resistant paediatric epilepsy. FCD type IIb is characterised by a population of unique abnormal cells known as balloon cells (BCs). The pathogenesis of FCDIIb is poorly understood and it is unclear if BCs are the key pathological cell or if there are other types of cells that are important in the pathogenesis of the disease.
Methods: Analysis of Affymetrix™ Human Exon 1.0ST microarray data revealed differentially expressed genes (DEGs) between a BC group and a control non-BC group. Ingenuity Pathway Analysis (IPA; bioinformatics software) was used to identify networks of the DEGs. The expression of a micro-network was validated using immunohistochemistry. Double immunofluorescence was undertaken to identify the lineage of cells expressing components of the network.
Results: We identified a network of interacting genes that were upregulated in FCDIIb compared to normally formed cortex or FCD without balloon cells (FCDIIa). Some components of this network were expressed in BCs but others were expressed in novel cell populations. Double immunofluorescence identified a cell with the phenotype of a glial progenitor that was only present in FCDIIb but not in normally formed cortex.
Conclusions: We have identified a novel population of glial progenitors found frequently adjacent to BCs in FCDIIb. Paracrine signaling between BCs and the novel CHI3L1 positive cells is likely to be involved in the pathogenesis in FCDIIb. Further investigations into the role of these cells would give us a better understanding of the molecular abnormalities underlying FCD and possibly provide novel therapeutic targets.

PL4

Post Mortem Microarray and Methylation Studies in Stillbirths with Unexplained IUGR

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Introduction: Intrauterine death (IUD) at ≥24 weeks gestation affects ~1 in 200 pregnancies, with intrauterine growth restriction (IUGR) present in approximately 50%. Although frequently due to placental pathology, genetic abnormalities may also underlie a significant proportion and Silver-Russell syndrome (SRS) may be implicated in some.
Objective: This first comprehensive pathological and genetic study to investigate non-placental IUGR, hypothesises recurring abnormalities common to this group. There is limited experience of these techniques in IUD's. The results of this study will advance the understanding of the genetic influence in the pathogenesis of IUGR and IUD and will inform future routine clinical diagnostic practice to ensure that valuable resources are used in a cost effective manner and may be applicable to prenatal diagnosis in cases of detected IUGR in utero.
Methods: 31 IUD's ≥24 weeks gestation with non-placental IUGR (<3rd centile), with/without congenital anomalies were selected. Standard microarray and MLPA testing for SRS was carried out. Where normal, a higher resolution microarray containing SNP probes was used to test for smaller imbalances, uniparental disomy 7 (UPD7) or loss of heterozygosity (LOH).
Results: One case showed a homozygous deletion of part of the FANCA gene, consistent with Fanconi anaemia and 3 cases remain as uncertain findings. No cases of SRS were identified. The higher resolution microarray did not identify any smaller imbalances and no case of UPD7. 3 cases showing LOH over a gene associated with IUGR are undergoing mutation screening follow-up. **Conclusion:** The comprehensive genetic study of a representative cohort has not shown a frequently recurring underlying genetic abnormality and suggests that causes of IUGR are complex. Further investigations of the cases with findings of uncertain significance will determine whether these are pathogenic.
Acknowledgement: This project is grant funded by Path Soc.

PL5

Identification of a Novel Integrative Prognostic Signature to Stratify High Risk Stage II CRC Patients through Big-Data Image Analysis

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Clinical trials have shown little benefit in treating stage II colorectal cancer (CRC) patients with adjuvant therapy, yet 20-30% of patients will experience disease recurrence. It is imperative to identify this high-risk subpopulation in order to better inform clinical decision making. The invasive front of a CRC tissue section is of particular prognostic value. The morphological invasive growth pattern and lymphatic vasculature are significantly associated with disease-specific death, yet remain in the non-core data items under RCPath guidelines. Reasons cited are observer variability and a lack of standardised quantification methodology. We showcase image analysis as a potential method to standardise the robust quantification of histopathological features. The tumour morphology and lymphatic vasculature at the invasive edge were profiled by implementing a standardised image analysis algorithm to quantify four features co-registered on the same tissue section: tumour budding, poorly differentiated clusters, lymphatic vessel invasion and lymphatic vessel density. Image analysis was subsequently utilised to segment heterogeneous tumour subpopulations across the invasive front and identified an Epithelial to Mesenchymal Transition signature within tumour buds. Furthermore an image analysis based big-data morphometric signature was mined to identify a novel histopathological prognostic feature, which was subsequently validated. Many prognostic biomarkers are reported in the literature, however Tumour, Node, Metastasis (TNM) staging of CRC remains the clinical gold standard. Novel pathological features should aim to augment TNM staging rather than replace it. The significant image based and clinical pathology parameters were therefore integrated to form a highly significant prognostic signature (HR = 7.8; 95% CI, 3.2 - 19.2) which, in our study, improved upon TNM staging alone (HR = 4.26; 95% CI, 1.76 - 10.33).

PL6

Advanced Neoplasia Detection in Colorectal Cancer Screening Using Multiple Stool DNA Markers and Haemoglobin

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Purpose of the study: Molecular tests have the potential to improve current non-invasive faecal immunochemical test (FIT) screening for colorectal cancer (CRC) and advanced precancerous lesions. We examined the performance of a panel of faecal DNA (sDNA) markers and FIT in archival samples from an invitational CRC screening population.

Methods: Whole stool samples were prospectively collected from individuals participating an invitational primary colonoscopy-screening program (COCOS trial). Only participants that provided stool, performed FIT (OC-Sensor) and underwent colonoscopy were selected. The sDNA panel included quantitative molecular assays for KRAS mutations and for aberrant NDRG4 and BMP3 methylation. The performance of the sDNA plus FIT panel was compared to the FIT results alone, by Receiver Operator Characteristic (ROC) analyses.

Results: 1047 individuals (51% male) with a median age of 60 years (range 50-75) were included, of which 7 (0.7%) had colorectal cancer and 104 (9.9%) had advanced precancerous lesions (advanced adenomas or sessile serrated polyps ≥ 1 cm).

The combination of sDNA and FIT was more sensitive than FIT alone for detecting advanced precancerous lesions (49% (50/102) and 25% (26/102), respectively). Specificities among individuals with non-advanced or negative findings (controls) were 89% and 96% for sDNA and FIT testing, respectively.

ROC analysis of CRC and advanced precancerous lesions compared to controls revealed an Area Under the Curve (AUC) of 0.75 for the sDNA plus FIT test, compared to 0.68 for FIT alone. At an equal specificity of 95%, advanced precancerous lesions were detected with higher sensitivity by the sDNA plus FIT test compared to FIT alone (36% vs 28%, p=0.08).

Conclusions: In an invitational colorectal cancer screening cohort, combining stool DNA markers with FIT detected more advanced neoplasia than FIT alone, primarily due to detecting more advanced adenomas.

O1

The Three-Dimensional Anatomy of the Anal Sphincter Complex and its Relevance to Low Rectal and Anal Pathology

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Excellent anatomical knowledge of the anal sphincter complex (ASC) is essential for the treatment and understanding of low rectal and anal pathology. Some of the current descriptions of the ASC are contradictory. In this study, the three-dimensional (3D) anatomy of the ASC is described with relevance to low rectal and anal surgical pathology.

Six human adult cadaveric specimens (three males, three females) were obtained from the Leeds GIFT Research Tissue Programme. Paraffin embedded mega-blocks containing the ASC were serially sectioned at 250 µm intervals. Sections were stained with haematoxylin & eosin, Masson's trichrome and Millers' elastin, from which 3D reconstructions were developed.

The ASC is a complex structure, varying between individuals in the size and distribution of its layers with intermingling of fibres and inconsistency of the longitudinal smooth muscle affecting the creation of the surgical intersphincteric plane. Longitudinal fibres penetrate the internal and external anal sphincter to anchor in the submucosa and ischiorectal fossa. Striated muscle fibres from the external sphincter were identified in the submucosa in four of six specimens.

The ASC is highly complex due to the degree of variation in its structure and intermingling of smooth and striated muscle fibres and their penetration of major structures. This creates potential tissue planes for the spread of infection, fistula extension and tumour spread. The complex anatomy of the ASC also impacts on the staging of low rectal cancers in this region, which requires further investigation.

O2

cTEN Regulates Cell Motility through Snail in Colorectal Cancer

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Cten is upregulated in a number of tumour types and in colorectal cancer expression is associated with advanced Dukes stage, poor prognosis and distant metastasis. Cten is localised at focal adhesions and regulates cell motility but knowledge of underlying signalling mechanisms is sparse. Epithelial to mesenchymal transition (EMT) is a process whereby cells acquire an invasive phenotype to aid cell migration and is found to occur in a number of biological processes including cancer metastasis. We investigated whether Cten increases cell migration through EMT pathways in colorectal cancer.

Cten was forcibly expressed in colorectal cell lines and Snail expression determined by qPCR and western blot. The cycloheximide pulse chase assay was used to assess any changes in Snail protein stability. Further to this, the Transwell migration assay was performed to investigate changes in cell motility.

Forced expression of Cten was shown to increase Snail protein expression in HCT116 and Caco2 cell lines. There was no change in the level of Snail mRNA suggesting that Cten regulates Snail at a post transcriptional level. Inhibition of protein synthesis confirmed this and showed that Cten regulates the stability of Snail protein. Simultaneous forced expression of Cten and knockdown of Snail demonstrated that this relationship was functionally active. Forced expression of Cten increased cell migration ($p<0.05$) which was subsequently lost when Snail was knocked down ($p<0.001$).

We are the first to identify Snail as a downstream target of Cten signalling. This finding advances the understanding of cancer cell motility regulatory networks and further highlights Cten as a potential therapeutic target in colorectal cancer.

Work supported by a Pathological Society grant.

O3

Loss of pTEN Expression is Strongly Associated with the Presence of the BRAF V600E Mutation, and Further Complicates Combination Treatment Strategies for Patients with Advanced Colorectal Cancer

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Treatment for advanced colorectal cancer is moving to combination therapies, targeting multiple signalling pathways. Indeed, MRC FOCUS4 has been designed to assess this. We determined pTEN protein expression, and assessed this in relation to other biomarkers associated with signalling downstream of the epidermal growth factor receptor.

Tissue microarrays were constructed from 2 advanced colorectal cancer (aCRC) clinical trials (FOCUS and PICCOLO) for immunohistochemistry (IHC). Mutation status of KRAS, NRAS, PIK3CA and BRAF was assessed by pyrosequencing. Copy number variation was assessed on Oncoscan® FFPE Assay Kit (Affymetrix Inc.). pTEN protein expression was correlated with mutation status, MMR status, primary tumour location and copy number.

pTEN protein expression for 1288 patients showed complete loss of expression in 85/787 (10.8%) - FOCUS and 64/501 (12.8%) - PICCOLO. BRAF mutation status was significantly different between the pTEN negative and pTEN positive populations ($p<0.0001$), with significantly more pTEN negative tumours having the BRAF V600E mutation. Loss of pTEN expression correlated with genomic deletions involving the pTEN gene. 20/30 (66%) of pTEN negative tumours exhibited loss of the pTEN region (10q), half of which were focal deletions. Only 54/202 (26.7%) pTEN positive tumours showed deletions of this region, and none were focal events. There was no significant difference in either primary tumour site or MMR status ($p=0.1765$) between the pTEN negative and pTEN positive populations.

Signalling pathways do not stand in isolation; they are interlinked in a complex signalling network. Current treatment interventions must target the correct pathway combinations if patients are to benefit from targeted therapy. Our data suggests a subset of patients may require dual AKT and MEK pathway inhibition, in addition to anti-EGFR monoclonal antibody therapy and inhibition of BRAF.

O4

Zonal Differences in PD1 Expression in Centre of Tumour Versus Periphery in Microsatellite Stable and Unstable Colorectal Cancer

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Colorectal cancers (CRC) that show evidence of microsatellite instability (MSI-H) are marked by a high tumour infiltrating lymphocyte (TiL) population which is thought to be prognostic. Programmed cell death 1(PD-1) is a negative regulator of the immune system and targeting the interaction with its ligand PD-L1 offers a potential therapeutic target. We aimed to characterize CD8 and PD-1 expression in both the tumour centre (cT) and tumour periphery (pT) of microsatellite stable (MSS) and unstable CRC.

Methods: Paraffin-embedded tumour blocks were cut at 5um, prepared and stained using specific antibodies for CD8 and PD-1. The pT was defined as the area within a 400x high power field (HPF) from the outline of the tumor. The cT was defined as the area at least one 400x HPF apart from the tumor outline toward centre of the tumor. Images were taken at 40x, 100x, 200x and 400x. Positive cells were averaged across 3 high power fields and classified as high or low positivity.

Results: Forty-two specimens have been analysed to date including 28 MSI-H and 13 MSS tumours. Sixty-eight percent of MSI-H were stage II and 69% of MSS were stage III. In the MSI-H group, a high CD8 count in the cT and pT correlated with and earlier tumour size and stage. PD-1 positivity was seen in 61% of MSI-H cT compared to 0% positivity in the cT of MSS tumours. The periphery of both MSS and MSI-H specimens showed significant PD-1 expression with 71% and 85% of samples showing positivity respectively. There was no association between high or low densities of staining and stage.

Conclusions: Zonal differences exist in the expression of CD8 and PD-1 in microsatellite stable and unstable tumours. A high proportion of MSI-H tumours show PD-1 activity in the centre of the tumour despite an improved prognosis. Further profiling of other T cell populations may help to further understand this expression which may act as a biomarker or provide a therapeutic target

O5

Association of Genomic Aberrations with Disease Recurrence in Stage II and Stage III Colon Cancers

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Biomarkers that are able to distinguish stage II and III colon cancer patients at high risk of developing disease recurrence, who may benefit from adjuvant chemotherapy, are still lacking. Genome-wide profiling of somatic aberrations, including gene point mutations, DNA copy number aberrations (CNA) and structural variants (SV), is expected to provide better insight into the molecular pathology of tumour progression and clinical outcome.

Genome-wide analysis of CNAs was performed using high-resolution comparative genomic hybridization for microsatellite stable (MSS) stage II and III primary colon cancer samples (n=114). In addition, the prevalence of genes suffering from CNA-associated chromosomal breaks, indicative for SVs, was determined. The mutation status of commonly affected *APC, TP53, KRAS, PIK3CA, FBXW7, SMAD4, BRAF* and *NRAS* genes was examined for 60 samples using targeted massive parallel sequencing. Associations of genomic aberrations with disease-free survival (DFS) rates were explored by log-rank tests using 10,000 permutations.

Disease recurrence and DFS rates differed significantly for several CNA-regions ($P<0.05$). A total of 267 genes were recurrently affected by CNA-associated chromosomal breaks (FDR<0.1), among which 168 genes (66%) that were also identified in a previously analysed cohort of 352 metastatic colorectal cancers. Gene point mutation frequencies were in concordance with literature. In a univariate analysis, none of the individual mutated genes appeared to be significantly associated with DFS.

In summary, several associations are found between highly prevalent genomic CNAs and disease recurrence in this cohort of MSS stage II and III colon cancers. Further in-depth analysis is required to unravel underlying biology that contributes to disease recurrence.

O6

Comparison of Histologically Normal Mucosa and Blood as Controls for Targeted Next Generation Sequencing Analysis in Patients with Colon Cancer in the NCRI FOxTROT Trial

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Accurate and reliable methods for assessing the molecular profile of clinical tumour samples are important for the delivery of personalised medicine. When adopting a targeted amplicon sequencing method in combination with next generation sequencing (NGS), it is ideal to call mutations against a control sample to enable artefacts to be removed from the analysis. Blood is considered the gold standard control but may not always be available. We compared the use of histologically normal mucosa to blood as a control in colon cancer.

We examined mutations in 40 colon cancers from the NCRI FOxTROT trial using the Fluidigm Access Array for NGS library preparation. We assessed the use of both blood and normal colonic mucosa as a control for assessing mutations in 11 genes. All samples were tested in duplicate. The work was partly funded by a PathSoc Career Development Fellowship and is presented on behalf of the FOxTROT Collaborative Group.

Mutation calls made using normal mucosa as a control compared to blood were in good agreement; a Mathew's Correlation Coefficient above 0.7 was seen for all of the genes where agreement could be assessed. We found that false positive mutations were due to poorer amplification of the normal mucosa samples and false negatives were due to mutation calls in the normal mucosa.

Overall we found that when assessing mutations in hotspot oncogenes, testing in duplicate and the use of a normal control tissue is not required to make mutation calls. However, where a normal control is required, normal mucosa from the resection margin is a suitable alternative to blood where it is not available.

O7

Sex Cord Tumours Arising in Ovarian and Extraovarian Adenosarcoma: an Unusual Form of Sarcomatous Overgrowth

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We report a series of four unusual ovarian or extraovarian neoplasms composed of an admixture of adenosarcoma and a predominant component comprising a sex cord tumour. The neoplasms occurred in women aged 50 to 69. Three cases arose within the ovary and one was extraovarian (pelvis and abdomen) in location. In all four cases, there were minor areas with morphological features of adenosarcoma with a phyllodes-like architecture and periglandular increased cellularity with mitotic figures. In two cases, the stromal component was morphologically in keeping with a juvenile granulosa cell tumour. In one case, the stromal component had some features of both adult granulosa cell tumour and Sertoli cell tumour within a fibromatous background. The fourth case morphologically could not be categorised as any of the usual types of ovarian sex cord tumour and was categorised as an unclassifiable sex cord tumour. In all four cases, there was immunohistochemical evidence of sex cord differentiation. In each case, we propose that the sex cord tumour arose from a pre-existing adenosarcoma thus representing an unusual form of sarcomatous overgrowth of sex cord elements which can occur within adenosarcomas. This phenomenon is not well described in the literature.

O8

Utility of Serum HE4 in Diagnosis and Prognosis of Endometrial Cancer

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Background: Human epididymis protein 4 (HE4) is a secreted protein that is overexpressed in some cancers. HE4 is emerging as a useful biomarker in diagnosis and follow-up of endometrial cancers. The aim of this study was to evaluate the potential role of serum HE4 in the diagnosis and management of endometrial cancer.
Methods: Patients undergoing surgery for endometrial disease were recruited into this study and had pre-operative serum samples taken, n=157. Demographic, clinical, radiological and laboratory data were reviewed. HE4 and CA125 serum levels were analysed using the Fujirebio Diagnostic ELISA Kits and results correlated with clinicopathological details. Standard cut-off points of 70 pmol/L for HE4 and 35 U/ml for CA125 were used.
Results: HE4 showed a sensitivity of 64% and specificity of 97.50% for detection of endometrial cancer. CA125 had a very low sensitivity of 14% for endometrial cancer diagnosis. HE4 was elevated in all stages of endometrial cancer and demonstrated the ability to distinguish between benign and malignant groups. HE4 also provided information about myometrial space invasion.
Conclusion: HE4 has a role in endometrial cancer diagnosis and prognosis and has the potential to be used in a screening setting or as a triage marker in the primary care setting. For women diagnosed with endometrial cancer, HE4 has the potential to stratify them into treatment regimens where the most appropriate treatment can be delivered resulting in improved quality of life and outcome for endometrial cancer patients.

O9

Platelets Drive Metastatic Changes in Ovarian Cancer Cells

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Background: Ovarian cancer is the 5th leading cause of cancer related deaths in women. Previously we described a dynamic interaction between ovarian cancer cells and platelets in vitro, involving platelet adhesion, activation and induction of pro-survival and pro-angiogenic signals in the cancer cells. This study looked to further investigate this phenomenon in ovarian cancer cells by assessing the molecular changes it induced.
Methods: Cell lines 59M and SKOV3 were used as in vitro models of metastatic ovarian cancer. Platelet cloaking of cells was quantified by flow cytometry. Cells co-cultured with/without platelets for 24hrs were examined by RT-PCR for EMT related changes and by Affymetrix Gene2.0ST arrays for whole transcriptome changes.
Results: Significantly more platelets adhered to SKOV3 cells than 59M cells. While there were different rates of adhesion, the platelets induced similar changes in EMT related genes in both. There was a significant loss in expression of epithelial genes and an increase in mesenchymal genes, indicating the induction of EMT. Whole transcriptome analysis showed that there were a greater number of gene expression changes occurring in SKOV3 cells compared to 59M cells, correlating with the adhesion data. A 32 gene panel of commonly affected genes in both cell lines was identified, many of which form part of an interlinking pathway that is regulated by TGFβ1 and associated with cell adhesion/ECM remodelling. Though only 32 genes overlapped, the biological processes affected in both cell lines were very similar, with 103 of the 148 processes enriched in the 59M data set also seen in the SKOV3 data set.
Conclusion: This study shows that platelets can enhance the metastatic potential of ovarian cancer cells through the induction of EMT and ECM changes. In addition, it has identified a set of 32 genes that hold potential to be in vivo markers of this interaction.

O10

Platelet Cloaked Tumour Cells Suppress NK Cell Immune Surveillance

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Background: During the metastatic cascade, circulating tumour cells rapidly and efficiently adopt a platelet cloak. Platelet cloaking of tumour cells promotes metastatic disease by promoting cellular proliferation, angiogenesis and EMT while inhibiting autophagy and apoptosis. The aim of this study is to examine whether the platelet cloak contributes to tumour cell evasion of NK cell mediated immune surveillance.
Methods: Freshly isolated PBMCs were harvested from healthy donors and stimulated for 18 hours with IL-2 (500U/mL). PBMCs were co-incubated with ovarian (59M and SKOV3), melanoma (Sk-Mel-28) and CML (K562) cell lines that were either uncloaked, or cloaked with washed platelets from healthy donors. The NK-tumour cell receptor ligand systems, NKG2D-MICA/MICB and CD96/CD226-CD155 were examined using NK cell CD107a expression and interferon-gamma production to quantify NK cell mediated recognition and 'killing' of cancer cells.
Results: We first demonstrated that ovarian and melanoma cancer cell lines when cloaked with washed platelets strongly inhibited NK cell antitumor reactivity. Platelet cloaking induced down-regulation of the stress ligands MICA and MICB on the tumour cell coupled with their release into the microenvironment, a known NK cell immune decoy strategy. In addition, platelets significantly down-regulated both CD96 (NK cell) and CD155 (tumour cell), inhibiting NK cell activity. Both mechanisms occur in tandem to comprehensively incapacitate NK cells and promote tumour immune evasion.
Conclusions: Ovarian and melanoma tumour cells are efficiently cloaked by platelets, which facilitates immune evasion by actively suppressing NK cell cytotoxicity and cytokine production.

O11

Role of IGF-IIR/Man-6-P in Glioblastoma Angiogenesis

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Purpose of the study: Glioblastomas (GBM) are the most common and most aggressive primary malignant brain tumours in adults. One of their histopathological hallmarks is the microvascular proliferation; these tumours are among the most angiogenic of malignancies by displaying the highest degree of microvascular proliferation. IGFIIR/Man-6-P is a receptor that belongs to the insulin-like growth factor (IGF) system. The involvement of IGF-IIR/Man-6-P in the process of angiogenesis has been postulated in rare earlier studies. To our knowledge, the role of IGF-IIR/Man-6-P in the neovascularisation of human GBM has never been studied.
Methods: IGF-IIR/Man-6-P expression was evaluated in the vascular compartment from 322 human GBM and from 10 normal adult brain samples by means of quantitative immunohistochemistry on tissue microarray sections. *In vitro* cell line experiments were carried out in order to characterise the IGFIIR/Man-6-P role in angiogenesis.
Summary of results: IGF-IIR/Man-6-P was strongly expressed in the cytoplasm of endothelial cells in hyperplastic vessels and exhibited a dot-staining pattern. We found a higher expression of IGF-IIR/Man-6-P in GBM vessels compared to normal brain vessels (p=0.05). Furthermore, preliminary *in vitro* experiments suggest a role of IGF-IIR/Man-6-P in tube formation but not in growth of the EA.hy926 endothelial cell line.
Conclusions: This work shows a possible role of IGFIIR/Man-6-P in the process of neovascularisation of GBM angiogenesis. Additional investigations are required to confirm the role of this receptor as a direct actor of angiogenesis in GBM.

O12

A Novel View of the Temporal Arteries: Using 3D Histological Reconstruction to Study Microvessel Anatomy

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Purpose of the study: Vasa vasorum (VV) are microvessels which supply vessels that cannot be nourished by diffusion from their own lumina. VV are believed to be a key element in the pathogenesis of vascular diseases. A number of different imaging methods have been used to study the VV but there is still no definitive consensus on their structure. The aim was to describe the normal microvessel anatomy of temporal arteries.
Methods: Human temporal artery, obtained following routine biopsy with ethical approval and patient consent. Samples were embedded into paraffin blocks and serially sectioned at 5 micron intervals. Alternate sections were stained with H&E and scanned to create virtual slides. The slides were aligned, VV were segmented (annotated) and iso-surfaced to generate 3D reconstructions.
Summary of results: The reconstruction shows the structural arrangement of the VV as a complex plexus. No connection to the vascular lumen was visualised. In this segment a hierarchical branching structure was not observed. VV were almost exclusively restricted to the adventitia of the vessel wall. Mean ± SD area of the VV (n = 5283) is 2287.23µm2 (±4956.03). The mean ± SD number of vessels per slide is 60.76 (±15.37). These metrics are based on one arterial specimen.
Conclusion: This method allows us to study the three-dimensional spatial relationships of microvessels within arterial specimens. Furthermore, metric data generated in the process can support the 3D images to study the microvasculature. This method will be applied to diseased arteries in future to generate novel hypotheses about the inflammatory process.
Acknowledgements: This research was supported by a PathSoc intercalated studentship.

O13

Loss of Expression of BAP1 is a Useful Adjunct Which Strongly Supports the Diagnosis of Mesothelioma in Effusion Cytology

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Purpose of the Study: It is controversial whether mesothelioma can be diagnosed with confidence in effusion cytology and therefore an ancillary marker of malignant mesothelial cells would be clinically valuable. BRCA-1 associated protein (BAP1) is a tumour suppressor gene which shows biallelic inactivation in approximately half of all mesotheliomas. BAP1 expression is commonly lost in mesothelioma. We investigated whether loss of BAP1 expression can be used to support a diagnosis of mesothelioma in effusion cytology.
Methods: Immunohistochemistry (IHC) for BAP1 was performed on cell blocks from effusions associated with confirmed mesothelioma cases, effusions containing mesothelial cell atypia, benign effusions, and effusions from patients with lung adenocarcinoma.
Results: IHC for BAP1 was performed on 75 cases of confirmed mesothelioma. 43 (57.3%) showed negative staining in the presence of an internal positive control. In 57 effusions considered to have atypical mesothelial cells in the absence of definitive diagnosis of mesothelioma, 8 cases demonstrated negative staining for BAP1. On follow up, 6 of these patients received a definitive diagnosis of mesothelioma in the subsequent 14 months (2 were lost to follow up immediately). Only 5 of 100 consecutive benign effusions were interpreted as BAP1 negative. 47 patients with confirmed adenocarcinoma demonstrated positive staining for BAP1.
Conclusion: We conclude that loss of BAP1 expression in effusion cytology is quite specific for mesothelioma. Whilst it is not definitive, it can be used to support the diagnosis of mesothelioma in atypical effusions. We caution that interpretation of BAP1 IHC on cell block may be difficult and that convincing positive staining in non-neoplastic cells is required before atypical cells are considered negative. We also note that BAP1 loss is not a sensitive test and cannot be used to exclude mesothelioma.

O14

The South-East of Scotland Experience on the Molecular Detection of EGFR, KRAS and ALK Mutations in Lung Adenocarcinomas

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The approval of novel targeted treatments for EGFR-positive and ALK-positive non-small cell lung cancer (NSCLC) has led to the increased requirement for mutation testing services in South East of Scotland. EGFR mutations are typically found in females, Asians and never smokers whereas KRAS mutations are associated with smoking. ALK rearrangements are commonly found in younger patients and never smokers. This study aimed to determine the prevalence of EGFR, KRAS and ALK mutations in South East of Scotland and to evaluate our experience in testing of ALK with IHC and FISH. Data of all patients tested were collected retrospectively from clinical records. From January 2011 to May 2014, we reported mutation rates of EGFR, KRAS and ALK to be 10.4% (67/643), 35.8% (86/240) and 2.3% (7/304) respectively. In our cohort, an increase in one pack years of smoking resulted in a decrease in the odds ratio of EGFR-positivity (OR 0.94, 95% CI 0.92 - 0.96, p<0.001). KRAS-positivity was associated with a history of smoking, with rates in both former (OR 6.26, 95% CI 2.00-19.56, p=0.002) and current smokers (OR 6.82, 95% CI 2.18-21.35, p=0.001) significantly higher than in non-smokers. The number of smoking pack years had no influence on the rates of KRAS-positivity. ALK-rearrangements were found to be associated with never smokers (p<0.001) and younger patients (≤50 years old) (p<0.001). To date, no false positives were reported for parallel testing of ALK with IHC and FISH. We observed 100% sensitivity (7 IHC+/7 FISH+) and 96.6% specificity (113 IHC-/117 FISH-) when comparing IHC with FISH. In conclusion, the prevalence of EGFR mutation in South East of Scotland has reflected mutation rates reported in West of Scotland. Our findings further support the use of ALK-IHC as a diagnostic screening tool.

O15

HPV and Cell Cycle Protein Expression in Advanced Penile Carcinoma: Results from the TPF Trial

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Purpose of the Study: The molecular mechanisms of metastasis and progression of penile squamous cell carcinoma (PSCC) are unclear. Nobody, to our knowledge, has investigated the expression of cell-cycle proteins in advanced or metastatic PSCC. We aimed to determine the extent of HPV infection in patients with advanced PSCC and its effect on the expression of the key cell-cycle proteins p53, p16INK4A and retinoblastoma (RB).
Methods: Archival paraffin embedded blocks were obtained from 27 primary penile cancers, all patients having developed locally-advanced or metastatic disease. All patients were treated in the Phase II Trial of docetaxel, cisplatin & 5-fluorouracil (TPF) chemotherapy CRUK/09/001 (Nicholson et al. BJC 2013; 109: 2554-9). Samples were analysed immunohistochemically for p16INK4A, p53 and RB protein expression on a tissue microarray. All tumours were HPV typed using PCR.
Summary of Results: HPV DNA was detected in 8/22 (36%) with HPV 16 present in 7/8 (88%). 5 cases were not suitable for analysis. No association was found between HPV and expression of either p16INK4A (p= 0.3426), p53 (p= 0.1365) or RB (p= 1) using Fisher's exact test.
Conclusions: HPV DNA is detected in less than half of progressive PSCC, suggesting either the loss of HPV in advanced disease or that non-HPV related cancers progress more commonly. The lack of correlation between HPV and these cell-cycle proteins suggests that they may undergo somatic mutation that is not driven by HPV, leading to increased growth and invasiveness. Treatment strategies may be hampered by this genetic diversity, which requires further investigation.

O16

Altered Endosome Biogenesis in Prostate Cancer Has Prognostic Potential

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Prostate cancer is the second most common form of cancer in males, and the incidence of this disease is predicted to double globally by 2030. More than 1.1 million new cases of prostate cancer are diagnosed each year and two thirds of these patients are from the Western world. Current diagnostic tests for prostate cancer are limited in both sensitivity and accuracy, and a method for accurate prognosis in these patients is yet to be developed; therefore, there is a need for a sensitive and specific prostate cancer test to implement early and appropriate therapy.
The recent discovery of altered endosomal-lysosomal biogenesis in prostate cancer cells has identified a fundamental change in the cell biology of this cancer that holds great promise for the identification of novel biomarkers that can predict disease outcomes. Investigation of the endosome compartment and endosome biogenesis revealed elevated gene and expression of critical machinery components that are required for endosome biogenesis and endocytosis. Here we demonstrate significantly altered expression of endosomal and lysosomal genes in mRNA microarrays of prostate cancer tissue compared to non-malignant tissue, and that specific endosomal and lysosomal genes are predictive of patient outcomes. Two endosomal tri-gene signatures were identified that had a significant capacity to stratify patient outcomes. Changes in the expression of these genes was further ascertained by qPCR in fresh-frozen prostate tissue specimens, which further implicated altered endosome biology during disease progression, with significant changes in expression observed between aggressive prostate cancer and indolent disease or normal prostate tissue.
These findings support the initiation of a retrospective trial to determine if these new biomarkers can accurately predict clinical progression in prostate cancer patients.

O17

Molecular Pathways Involved in Lymphovascular Invasion: A Biomarker Driven Approach

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Introduction: Lymphovascular invasion (LVI) is an important step in the metastatic cascade. Identification of a molecular signature for the LVI positive phenotype will help identify relevant drivers and pathways. This study aimed to investigate determinants of LVI from a biomarker database.
Methods: Biomarkers (n >200) from a well annotated series (n=1929) were analysed for correlations with LVI [clinical/IHC (D2-40) supplemented]. Proteins with significant associations with LVI were interrogated for pathway enrichment analysis [corrected for false discovery rate (FDR)], using the STRING 9.1 platform incorporating Gene Ontology (GO), KEGG and NCI.
Results: Biomarker analysis related to both clinical/IHC determined LVI identified 35 positively associated markers, 14 in both clinical and IHC categories (e.g. ADA3, CD8, FOXP3, KPNA2). A further 21 markers were negatively associated, 8 in both categories (e.g. Bcl2, BRCA1, MAGE3 and SOX10). Significant pathways ($p<0.001$) unifying the positively associated proteins include metabolism, immune responses (T-cell regulation and differentiation), cell activation and transcription [GO]; T-cell receptor signalling pathways and pathways in cancer and haematopoietic cell lineages [KEGG]. For negatively associated proteins, the following were significant: ubiquitination processes, regulation of the mitosis [GO]; p53 pathways [KEGG] and apoptotic and cell cycle pathways [GO & KEGG]. On cross-validating a subset included in the METABRIC cohort, there were overlapping enrichments for immune response regulation (GO) and haematopoietic cell lineages (KEGG).
Conclusions: These preliminary findings are the first to unify biomarkers for LVI pathway analysis in BC, using protein based data. Within the constraints of selection bias, data mining from immunohistochemistry of multiple biomarkers in relation to biological processes hold promise. *AM supported by the NIHR and the Academy of Medical Sciences

O18

Assessment of HER2 Status on Needle Core Biopsy of Breast Cancer: Impact of Histopathological Concordance

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One of the key recommendations introduced in the ASCO/CAP update guideline recommendation on HER2 testing is the novel concept of "histopathological concordance." It is proposed that certain tumour morphological features such as histologic type and grade should trigger repeating a molecular test in cases of "discordance". In this study we have we have reviewed 3104 breast cancer cases consecutively reported in routine practice in Nottingham in the last 4 years. Data on HER2 status was collected and cases with HER2 assessed on resection specimens (RS) were analysed in details.
Results: of all cases, 98 patients (3%) had HER2 status assessed on core biopsy and the corresponding tumour RS. The main reasons for a repeat were tumour multifocality and morphologically different or heterogeneous tumours. A few cases were repeated because of borderline negative FISH results or neoadjuvant therapy. 18 cases were repeated due to insufficient tumour in the core biopsy. In this study the HER2 status of the index tumour was changed in 2 cases and both were in the borderline result category. HER2 testing of different tumour foci of multifocal or morphological heterogeneous tumours was consistent with that of the index tumour assessed on the core biopsy apart from two cases; one positive and one negative. 17 tumours were upgraded from grade 2 on core to grade 3 on excision and HER2 status did not change. No contribution of hormone receptor or tumour type was identified.
Conclusion: There is excellent agreement between HER2 assessed in core biopsy and RS. Histopathological discordance seems to play a minor role which does not justify test repeat in routine practice.

O19

Molecular Mediators of Mammographic Density

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Purpose of Study: Mammographic density (MD) is a major risk factor for the development of breast cancer though little is known about the biological mechanisms mediating it. Tamoxifen prevents breast cancer in a sub-set of high-risk women in a mechanism that appears to be dependent on reduction of MD. Animal model studies suggest that tamoxifen remodels the mammary stroma to a tumour-inhibitory phenotype. This study aims to analyse the effect of tamoxifen on breast fibroblast function and identify potential pro-tumourigenic pathways contributing to density-associated risk.
Methods: Primary human breast fibroblasts were treated with hydroxytamoxifen (100nm-5μM). Fibroblast function was analysed by measuring: proliferation; expression of stromal proteins fibronectin (FN), LOX and collagen 1; effects on TGF-β signalling via SMAD phosphorylation and upregulation of the myofibroblast marker SMA. Genome wide analysis was performed using RNA-Seq.
Summary of Results: Fibroblasts from 25 patients were treated with tamoxifen. All patients showed reduced proliferation with treatment. In 62% of patients tamoxifen treatment resulted in reduced expression of FN. TGF-β-mediated upregulation of SMA and FN were consistently inhibited by tamoxifen, as was fibroblast contraction of collagen gels. RNA-Seq analysis revealed modulation of a number of metabolic pathways by tamoxifen, including significant upregulation of DHCR7, part of the microsomal antioestrogen binding site (AEBS).
Conclusions: These data indicate that tamoxifen can directly remodel the stromal microenvironment, generating a less 'reactive' stroma. Modulation of AEBS activity has been proposed to be anti-tumourigenic, and also is implicated as a suppressor of Hedgehog signalling. Thus, tamoxifen impacts on multiple pathways to create a tumour inhibitory phenotype.
This work was supported by the Pathsoc Small Grant Scheme.

O20

Mitogen Activated Protein Kinase Signalling Proteins are Associated with Good Prognosis in Breast Cancer and are Mainly Related to Estrogen Receptor

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Purpose of Study: Mitogen Activated Protein Kinases (MAPKs) are three layer signalling transduction molecules that have diverse cellular functions and behaviour in cancer. This study aims to assess the role of a panel of MAPKs biomarkers in breast cancer (BC) and to examine their expression in six BC cell lines.
Methods: Reverse Phase Protein Array (RPPA) was applied to quantify protein expression of MAPKs (15 biomarkers as total and phosphorylated forms) in six BC cell lines with different phenotypes including estrogen receptor (ER)-/+, HER2-/+ and HER2 transfected cells.
Summary of Results: A strong correlations were observed among different proteins involved in MAPKs pathway. MAPKs proteins showed associations with ER status and their differential expression was different between ER-positive and ER-negative cell lines. Importantly, associations between MAPKs proteins and HER2 status (wild and transfected) was mainly seen in the ER negative cell lines.
Conclusions: This study revealed that the high throughput technique of RPPA is useful in testing a panel of biomarkers involved complex biological pathways and networks. MAPKs are mainly related to ER and their association with HER2 was restricted to ER negative status.

O21

Cadherin Switch is More Observed in BRCA1 Mutated than the Basal-Like Breast Cancers

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Purpose of the Study: The Phenotypic features of basal like (BL) breast cancer (BC) resemble those occurring in BRCA1-germline mutation carriers. Several lines of evidence suggesting the overall tendency of basal-like/triple negative BC to spread through vascular rather than lymphatic routes. The latter has recently been attributed to the activation of cadherin switch, an EMT-like phenomenon, in BLBC. This study aims at studying the cadherin switch expression profile TGFB1, a key EMT-trigger, expression in BRCA1 mutated compared to sporadic BC.
Methods: The expression of E-cadherin, N-cadherin and TGFB1 were studied in a subset of germline BRCA1 mutated BC (n= 47) compared to non-selected cohorts of non-lobular sporadic invasive BLBC (n= 422) and non-basal BC (n=1190) using IHC and TMA.
Summary of results: Compared to sporadic BC, BRCA1 mutated cases were of younger age, more grade 3, with more medullary-like tumours, and more LVI positive. E-cad was significantly less expressed in BRCA1 cases than in the sporadic non-basal and in the BLBC. However, N-cad was not significantly expressed in BRCA1, non-basal, and BLBC. TGFB1 was significantly less expressed in sporadic BC, both non-basal BLBC than BRCA1 mutated BC. E-cad/N-cad combinatorial expression phenotypes were significantly different between BRCA1 mutated and non-basal and BLBC. Higher proportions E-cad-/N-cad+ were significantly observed BLBC than non-basal BC. BRCA1 mutated cases displayed the least E-cad+ expression and the highest E-cad-/N-cad+ in the studied series.
Conclusions: Despite the known similarities between BRCA mutated and BLBC, results of this study demonstrate the more occurrence of cadherin switch in BRCA1 mutated breast cancer. E-cad repression appears to contribute more than N-cad gain in BLBC than non-basal BC.

O22

Exploring Molecular Mechanisms Underlying Lymphovascular Invasion in Breast Cancer

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Purpose of the study: Lymphovascular Invasion (LVI) is a crucial step in the metastatic cascade in breast cancer (BC) and is associated with poor prognosis. This study investigated the molecular mechanisms associated with LVI interrogating subsets of the METABRIC series.
Methods: Histological/ immunohistochemistry (D2-40) supplemented LVI were determined in subsets of the METABRIC BC cohort. Cases were stratified into LVI+ and LVI- subgroups. Genes correlating with LVI status were identified in both test (n=179) and validation (n=356) sets from expression profiles using Linear Models for Microarray (LIMMA) data analyses. Biological functions of differentially expressed genes and relevant pathways were explored on multiple platforms.
Summary of results: Initial analysis identified 34362 genes differentially expressed between LVI subgroups. 915 (adjusted $p<0.05$) overlapping transcripts were identified from test and validation sets, some of which have not been previously linked with LVI. Examples of overlapping genes include APPL1, AQR, CD46, CUL4A, MCFD2, PAPOLA, POT1, RANBP2, SNX4, SUMO1, TLK1, ZNF181/644, SNAP23 etc. Biological function/pathway analysis reveals clusters regulating invasion, transcription, immune-regulation, protein binding and catalytic functions. For example, the proteolysis related SUMO pathway was enriched in both subsets.
Conclusions: Global expression profiling combined with robust histopathological characterisation provides a useful platform to decipher molecular pathways relevant to LVI. Further identification of driver genes associated with LVI is underway combining RNA expression with corresponding copy number alterations, followed by functional analysis.
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O23

Role of Insulin like Growth Factor Binding Proteins and Tamoxifen Resistance in Breast Cancer Epithelial Cells

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The development of tamoxifen resistance (TR) in oestrogen-dependent breast cancer (BC) is a therapeutic challenge. Insulin-like growth factor binding proteins (IGFBPs) may play a role in this process. We have investigated the role of IGFBP proteins in TR BC. IGF axis genes were evaluated in MCF-7 (wt) cells and tamoxifen-resistant (TamR) variants using qRT-PCR and confirmed by ELISA, Western, and Ligand blotting. IGFBP-2 & -5 were knocked down by shRNA transfection, and subsequent sensitivity to 4-hydroxytamoxifen (4-HT) was determined via WST-1. Cell migration was investigated by using the Incucyte system. IGFBP-2 expression was evaluated in 424 BC cases by TMA immunohistochemistry. Five out of 10 genes of the IGF axis (IGF-IR, IGF-2R, IGFBP-2, -4 and -5) had the highest expression levels by both parental wt and TamR cells. IGFBP-5 was down-regulated by ~7-fold while IGFBP-2 was up-regulated by ~2-fold in TamR versus wt cells (mRNA and protein levels). Significantly, a knockdown of IGFBP-2 in TamR cells restored sensitivity to (4-HT), reduced ERα expression to 45 ± 11.9% and enhanced cell migration. Expression of IGFBP-2 was significantly (P< 0.001) associated with survival advantage in TR patients. IGFBP-2 and IGFPB-5 are reciprocally regulated in the acquisition of TR by MCF-7 cells. IGFBP-2 may play a role in the development of TR in vitro and its high levels in clinical samples may predict TR.

O24

Exome Sequencing of Invasive Breast Cancer Specimens Identifies Discordant Mutational Evolutionary Changes in Invasive Primary Tumour and Axillary Nodal Metastases

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Purpose of the Study: Several lines of evidence are currently suggesting that the morphologic heterogeneity of breast cancer is mirrored at the genetic level. Understanding the molecular genetic evolution of BC would contribute further insights into the molecular derangements driving disease progression. Moreover, varied clinical outcome and response to similar therapeutic regimen is attributed, at least in-part to intratumoural heterogeneity. NGS can reliably study the genetic events using miniscule amounts of genomic DNA.
Methods: gDNA was extracted from FFPE tissue sections from a case of invasive duct carcinoma (3 primary tumour samples and 3 samples from positive axillary lymph node metastases). Sample preparation and exome enrichment was performed using Nextera Rapid Capture exome kits (illumina, FC-140-1000). Exome sequencing was performed using illumina MiSeq with 15x depth of coverage (following adapter/barcode trimming). Exploratory analyses and data mining were executed regarding variant (s) concordance/discordance between primary tumour samples and their respective metastatic variants.
Summary of Results: Initial findings revealed 37 candidate indels common to all three axillary lymph node samples yet absent from the three primary tumour samples. Several genes have been identified as having frameshift mutations caused by indels. Molecular players previously linked to anti-angiogenesis are amongst the genes affected by indel mutations in their coding sequences that may lead to potential abrogation of protein function.
Conclusions: These initial findings provide the framework for detailed molecular analyses for assessing molecular evolutionary events in primary breast cancer and their corresponding metastases.

O25

c-Myc Function is Associated with Specific Molecular Subtypes of Breast Cancer and Confers Resistance to Endocrine Therapy but not Chemotherapy

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C-MYC is amplified in approximately 15% of breast cancers (BC) and is associated with poor outcome. c-Myc protein is multi-faceted and participates in many aspects of cellular function and is linked with therapeutic response in BC. We hypothesised that the functional role of c-Myc differs between molecular subtypes of BC. We therefore investigated the correlation between c-Myc protein expression and other proteins involved in cell cycle control, proliferation, apoptosis and DNA damage together with clinicopathological parameters, outcome and treatments in early invasive primary BC (n=1,106) using immunuohistochemistry. The METABRIC BC cohort (n=1,980) was evaluated for c-Myc mRNA expression. In whole series, there was significant association between c-Myc protein expression with higher tumour grade, lymph node(LN) positivity and medullary-like tumours. C-myc showed differential association with other proteins in the molecular classes. In luminal A tumours, c-Myc was associated with ATM (p=0.005), Cyclin B1 (p=0.002), PIK3CA (p=0.009) and Ki67 (p<0.001). In contrast, in basal-like tumours, c-Myc showed positive associated with Cyclin E (p=0.003) and p16 (p=0.042) expression. c-Myc was an independent predictor of a shorter distant metastases free survival in luminal A LN+ tumours treated with endocrine therapy (ET; p=0.013). c-Myc expression did not predict patient outcome in the other molecular subtypes with respect to adjuvant treatment. High c-Myc mRNA expression was associated with higher grade and basal phenotype (p<0.001). In luminal tumours treated with ET, c-Myc mRNA expression was associated with BC specific survival (p=0.001). c-Myc function is associated with specific molecular subtypes of BC and confers resistance to ET. The diverse mechanisms of c-Myc function, particularly in luminal A BC, warrants further investigation.

O26

Metasin Axillary Predictive Score (MAPS): A Measure of Axillary Nodal Disease Prediction to Provide an Informed Choice for Breast Cancer Patients and Surgeons

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Purpose of study: Intra-operative sentinel lymph node sampling and molecular analysis empowers the surgeon to carry out axillary clearance as a one-step process. We have recently completed the clinical validation of Metasin, an intraoperative molecular assay for sentinel lymph node analysis in breast cancer patients (1836 cases).
Method: The assay uses 2 positive predictive markers and is quantitative, enabling the prediction of tumour volume using 2 markers CK19 and Mammaglobin. In this study group, 439 patients had positive sentinel nodes and 444 cases underwent axillary clearance. Of the axillary clearance cases, 26% contained positive lymph nodes. 84% were sentinel node (SNB) macrometastases, 5% were SNB micrometastases and 11% were SNB negative or contained isolated tumour cells. Informative data was available for sentinel nodes from 125 positive cases.
Results: Using the qPCR values (from Metasin assays using standardised pre-mixes) and clinical axillary clearance data, the cases have been stratified on the basis of the involvement of other axillary nodes. We have shown a three-tiered predictive grouping exists: Group A includes low tumour volume disease with a nodal positivity of 25% within the axilla (n=16): Group B with a 44% positivity of other nodal involvement (n=80) and Group C with positivity of 73% of axillary clearances (n=29).
Conclusion: The clustering of the Metasin data is dependent on the qPCR results and shows that the cases can be sub-grouped to provide a probability basis for prediction of axillary nodal involvement; dependent on the qPCR cut offs. This gives the patient and surgeon a statistical basis for determining the likelihood of other axillary nodal disease.

O27

External Quality Assessment of BRCA1 and BRCA2 Gene Sequencing: Challenges for Quality in a Changing Diagnostic Environment

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Sequencing of the *BRCA1* and *BRCA2* genes has long been used in genetics laboratories to identify cases of familial breast and ovarian cancer. However, the advent of chemotherapy for ovarian cancer based on PARP inhibitors, which requires the presence of a *BRCA1* or *BRCA2* mutation, is turning this specialist test into a commonly-applied companion diagnostic. At the same time, the introduction of new DNA sequencing technologies is posing challenges even for experienced genetics laboratories. EMQN has been providing EQA of *BRCA1* and *BRCA2* gene sequencing world-wide for 15 years. The rate of serious diagnostic errors has varied from year to year, but the mean has hovered stubbornly around 3%. In EQA, just 3 samples per year are sent out, and the quality and experience of participating laboratories varies greatly. We recently carried out a collaborative study to measure the quality of *BRCA* gene sequencing by traditional and new methods in 20 experienced, expert laboratories from 11 countries. Ten DNA samples (8 with pathogenic mutations, 2 with normal DNA sequence) were sent to each laboratory. Ten labs used next-generation sequencing (NGS) alone, 3 used Sanger sequencing alone, and the others used combinations of Sanger sequencing, NGS, MLPA and other technologies. Seventeen (85%) of labs identified all clinically-significant variants on all 10 samples. Four false negative results were reported by 3 labs. Two were due to deficiencies in the bioinformatics pipeline of the NGS process, while 2 were attributed to a sample swap, and incorrect interpretation of a melting profile. No significant trend was identified with respect to the genotyping accuracy of the different methodologies used. The observed error rate of 2% amongst expert laboratories indicates the complex and challenging nature of this kind of testing. Caution will be required when applying these technologies to sub-optimal FFPE samples in Pathology laboratories.

O28

Good or Bad Sequencing Data? Setting a Benchmark for the Quality of Diagnostic NGS in the Laboratory

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Next Generation Sequencing (NGS) is increasingly being introduced into clinical diagnostic laboratories worldwide. The huge amount of data generated by NGS cannot be duplicated by alternative methods for laboratories to internally validate all results, therefore external assessment of data is required. The UK National External Quality Assessment Scheme (UKNEQAS) for Molecular Genetics and the European Molecular Genetics Quality Network (EMQN) have developed a joint EQA scheme for NGS, with the aims to: (a) assess and improve quality; (b) enable laboratories to benchmark their NGS service against others and against best practice; (c) work towards consistency of reporting clinical results generated by NGS; and (d) contribute towards best practice. EMQN and UKNEQAS offer numerous disease-specific, molecular pathology and technical EQA schemes. The objectives for developing NGS EQA were to make it generic (independent of genes, diseases, platforms, and testing context (e.g,. Somatic, germline etc)) and applicable all users. Two pilot EQAs have been run and 157 labs from 32 countries participated. These labs were sent a genomic DNA sample and asked to sequence either their smallest gene panel or largest single gene which the lab tested, submit technical details, and genotypes at known SNPs. The results were compared against a "consensus EQA genome" established by multiple validations of the DNA. 12187 different genes were tested. Most labs are using small panel of 1-10 genes. 60% of all variants were detected by every lab which tested for them. A detailed summary of the key findings will be presented. Both pilots have proved to be challenging to meet our objectives, however the results have enabled clinical diagnostic labs to start to address the quality of their NGS testing.

O29

CTC-5: A Novel Digital Pathology Approach to Circulating Tumour Cell Characterisation

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Tumours invade the vasculature, which transports circulating tumour cells (CTCs) to distant sites enabling growth of secondary tumours. CTCs hold the potential to monitor: therapeutic response, emergent mutations and act as a screening tool for the early detection of cancer. There are numerous methods to isolate CTCs. Once isolated, EpCAM and/or panCK positivity and CD45 negativity are used to verify CTC status. However, due to the metastasis associated process of Epithelial-Mesenchymal Transition, epithelial markers may be ineffective at identifying all CTCs. To overcome such protein marker based limitations, we have developed a novel staining pipeline (CTC-5) that combines histochemical staining (giemsa) with immunofluorescene (DAPI, EpCAM/panCK, HER3 and CD45) staining and whole slide imaging for robust identification, enumeration and characterisation of CTCs from cancer patients. CTCs are isolated from whole blood using ScreenCell Cyto devices. Cyto devices are then slide mounted, giemsa stained and digitised. Giemsa Staining is washed out and slides are immunofluorescently stained for EpCAM/panCK, CD45, HER3 and counter stained with DAPI. Fluorescently stained slides are digitised. Giemsa stained and four colour immunofluorescent digital slides are processed in silico generating a single z-stacked digital slide for pathological assessment. The CTC-5 staining pipeline has been experimentally validated via CTC characterisation of peripheral blood from Lung, Breast and Ovarian cancer patients, with respect to healthy donor and spiked-in controls. The CTC-5 pipeline overcomes recognised weaknesses in CTC characterisation. Histochemical staining is added to the current gold standard of EpCAM/panCK and CD45 staining, while also preserving a fluorescent channel for assessment of biomarker status (e.g. HER3, apoptosis or platelet cloaking). Such advancements enable robust pathological assessment of CTCs in the clinic.

O30

Histogenic Molecular Mapping (HMM) – A Method for Interrogating Biological Pathways in Tissue Sections

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Thorough interrogation of diseased tissue requires the use of multiple biomarkers in order to investigate biological pathways. Unless fluorescent technology is used, multiple sections are required from each tissue block as each section can only be tested for a limited number of markers. Histogenic Molecular Mapping (HMM) is a technique which used digitized images to evaluate multiple biomarkers. Although each section cut from a block is slightly different from the immediately preceding section, the similarity is sufficient to allow non-linear registration of images of successive sections. If the order is known, multiple sections can be mapped onto each other by registering each with the immediately preceding section. This allows several biomarkers to be mapped into a single "composite" section thereby giving a representation of the pathways activated/expressed in the tissue. We used HMM to investigate the mismatch repair pathway in colorectal cancer. Sequential tissue sections were stained for MLH1, PMS2, MSH2 and MSH6 and then scanned. Bespoke computational algorithms were used for image registration and composite images were binned as either "mismatch repair proficient" or "mismatch repair deficient". Validation of each category could be obtained by quantification of pixels in binarized images or pixel distribution using stereology. Our data show that HMM can be used for interrogating biological pathways in tissue sections and, ultimately, automated diagnosis of disease states.

O31

Personalising Treatment in Locally Advanced Rectal Cancer Using Macrophage Subpopulations to Predict the Degree of Response to Radiotherapy

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Whilst pre-operative radiotherapy is the standard of care in locally advanced rectal cancer (LARC), only half of patients respond. Individualised treatment based on a predictive test could avoid unnecessary radiation exposure in poor responders. Macrophages in the tumour microenvironment with tumoricidal M1 and tumour protective M2 phenotypes could be modulating this response. This study investigated the possible predictive value of M1 and M2 subpopulations in identifying the response to short-course radiotherapy (SCRT).

Pre-treatment biopsies and post-treatment resection samples were taken from 29 patients with LARC given SCRT. Dual-staining immunohistochemistry was performed with CD68, HLA-DR (M1 marker), and CD163 (M2 marker). Samples were scored for hot-and-random spots by Nuance software (version 3.0.2) and compared with tumour response measured by reduction in tumour-cell density. The work was partly funded by a PathSoc Career Development Fellowship.

Samples showing a low score for HLA-DR positive M1 macrophages exhibited a better response to SCRT with a median 80% reduction in tumour cell density (IQR 47 to 85). Those with a high score exhibited a poor response with only a 20% reduction (IQR 0 to 49, p=0·017). No such trends were observed for CD163+ M2 macrophages. The ratio of HLA—DR+ to CD163+ macrophages for biopsy and resection samples was significantly different showing a drop in the HLA-DR positive macrophages in the resection samples (biopsy median 2·53, IQR 1.98 to 3.08; resection median 1·38, IQR 0.96 to 1.80; p=0·024). Assessment of macrophage subpopulations in pre-treatment biopsies appears to predict the degree of response to SCRT in LARC. Further investigation to validate these findings is now required prior to developing a predictive test for use in routine clinical practice. Patients with a poor predicted response could avoid toxic and costly radiotherapy and undergo alternative strategies including chemotherapy.

O32

An Evaluation of Culture Techniques versus 16S Profiling for Investigation of Antibiotic-Mediated Alteration of Microbiota Populations within a Clinically Reflective In Vitro Model of the Human Gut

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Next-generation sequencing technologies (e.g. 16S profiling) are increasingly used to investigate complex bacterial communities. They have advantages over classical methods, as a significant proportion of bacteria are 'non-culturable'. However, they do not distinguish 'viable' and 'non-viable' populations, which may skew results, particularly following antibiotic exposure. Here we report culture and 16S data from a clinically reflective human gut model, describing changes in the gut microbiota following exposure to multiple antibiotics.

A triple-stage chemostat model was inoculated with pooled human faeces from healthy volunteers to establish gut microbiota populations. The model was sequentially exposed to clindamycin (33.9 mg/L, QDS, 7days), vancomycin (125mg/L, QDS, 7days) and fidaxomicin (200 mg/L, BD, 7 days). Specific bacterial populations were enumerated daily on selective agars. Periodically, 16S profiling of gut model samples was performed; DNA was extracted on a QIAXtractor, 16S V4 PCR products were sequenced on an Illumina MiSeq, and resulting data were analysed using QIIME.

Both culture and 16S profiling demonstrated marked alterations in gut microbiota populations following antibiotic exposure. For many populations, notably bifidobacteria and enterobacteria, changes seen by culture correlated with 16S profiling. However, as culture describes numerical changes in populations, and 16S profiling describes proportional changes, results are not always directly comparable. 16S profiling greatly increased microbiome coverage, particularly for clostridia. Population diversity (number of observed species and Shannon index) decreased with sequential antibiotic exposure.

Use of culture and molecular methods in tandem can greatly increase understanding of changes occurring in complex microbial populations.

O33

This abstract is not available before the meeting

O34

Impact of Neoadjuvant Therapy on Cancer-Associated Fibroblasts in Rectal Cancer

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Purpose of the study: Cancer-associated fibroblasts (CAFs) are increasingly recognised as promoters of tumour progression. It is poorly investigated whether cancer management protocols, such as neoadjuvant radio(chemo)therapy, have an impact on CAFs and, by consequence, on tumour progression. This prompted us to study the impact of neoadjuvant radio(chemo)therapy on the α-SMA/epithelial area ratio in rectal cancer, and the impact of this ratio on recurrence-free survival.

Methods: Immunohistochemistry for the CAF marker α-SMA and the proliferation marker Ki67 was performed on sections from 98 rectal cancers of which 62 had undergone neoadjuvant radio(chemo)therapy.

Summary of results: Computer-assisted quantitative analysis showed that the α-SMA/neoplastic epithelial area ratio was higher after neoadjuvant therapy, and that rectal cancers with high α-SMA/epithelial area ratio had low proliferation rates. Interestingly, the α-SMA/epithelial area ratio was an adverse prognostic factor with regard to recurrence-free survival in univariate analysis. In addition, multivariate analysis showed that an α-SMA/epithelial area ratio above 1 provides an independent prognostic value associated with a poor recurrence-free survival.

Conclusions: These results suggest that neoadjuvant treatment has an impact on CAFs in rectal cancer. The correlation of CAFs with decreased recurrence-free survival and abundant experimental data in the literature suggest that under certain circumstances, not yet very well understood, CAFs may favour tumour progression.

O35

Immunohistochemistry Initiates a Complex Screening Cascade in the Detection of Lynch Syndrome

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Background: Lynch Syndrome (LS) accounts for approximately 2-4% of all colorectal cancers (CRC) and is caused by germline mutations in DNA mismatch repair (MMR) genes. Increasing literature supports routine screening for LS using immunohistochemistry (IHC) to detect loss of MMR protein expression on tumour samples. We reviewed different screening approaches at three National Cancer Centres (NCC) and evaluated the impact on genetic referrals and LS diagnoses.
Methods: CRC databases were analysed from January 2005 - December 2013. NCC1 performs IHC upon physician request; NCC2 implemented reflex IHC (rIHC) in November 2008 and NCC3 has been performing rIHC since 2004. Pathology reports were reviewed and the number of genetic referrals in patients exhibiting MMR-d determined. Patients were also evaluated for BRAF testing and those with positive mutations were not considered eligible for referral to genetics unless otherwise indicated. The number of LS patients detected was calculated as a percentage of the total new patient CRCs.
Results: Over a 9-year period 4,049 new CRC in 3,929 patients were diagnosed across the 3 centres. The implementation of universal screening at NCC3 resulted in a MMR-d detection rate of 11%, an increase of 6% and 8% compared to NCC2 and NCC1 respectively. Referrals to genetic counselling on those patients with MMR-d without BRAF mutations or a known result was low across all centres. The number of LS patients diagnosed did increase from 0.7%(NCC1) and 0.6% (NCC2) to 1.1% at NCC3 however the detection rate remained lower than expected. More than 80% of patients referred, elected to undergo germline testing. Of the LS patients identified 79% had mutations in either MLH1 or MSH2.
Conclusions: The implementation of universal screening using reflex immunohistochemistry detects an appropriate number of MMR-d tumours and increases LS detection rates. However adequate resourcing and clinician awareness are needed to ensure that all patients captured

O36

Residual Tumour Cell Density and the Relationship to Survival Following Pre-Operative Chemoradiation in Locally Advanced Rectal Cancer: Results of the NWCOG RICE Trial

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Pre-operative chemoradiotherapy (CRT) is commonly used to downstage locally advanced rectal cancer (LARC). The degree of response is assessed using a number of subjective tumour regression grading systems. Tumour cell density (TCD) has been developed as an objective linear measure of response and may be more sensitive and reproducible.
Patients with MRI-defined LARC received pre-operative CRT using a novel irinotecan-containing regimen, with surgery 9 weeks later. TCD analysis was performed on digitally scanned glass slides. TCD was measured in the pre-treatment biopsy (PTBTCD) and a representative slide from the resection specimen including a 9mm^2 area of greatest TCD (GTCD) and the whole tumour area and/or scar TCD (WTTCD). A systematic sample of 300 random points were inserted into each area using virtual graticule software and manually assessed, TCD was expressed as the percentage of informative points falling on tumour cells. The work is presented on behalf of the NWCOG RICE trial investigators and was part-funded by a PathSoc fellowship.
142 patients commenced CRT and 135 underwent surgery. Median TCD for PTBTCD, GTCD and WTTCD was 38.7%, 7.8% and 1.7% respectively. The number (%) of patients with a TCD of 0% was 0 (0%), 30 (23.6%) and 36 (28.3%) respectively. Distribution of TCD was normal in PTBTCD but highly positively skewed post-resection. Low PTBTCD (split by the median) predicted better 3-year disease free survival (DFS; 76% vs. 60%, p=0.05) although not overall survival (OS; p=0.47). Low WTTCD predicted better DFS and OS (DFS 71% vs. 58%, p=0.05; OS 90% vs. 77%, p=0.02) although no difference was seen for GTCD (p=0.26; p=0.26).
Pre-operative CRT markedly reduces TCD in LARC, and provides a continuous measure to compare different regimens. In the pre-treatment biopsy, lower TCD may predict improved DFS. Following resection, TCD across the whole tumour and scar more accurately predicts DFS and OS than using a selected area of greatest TCD.

O37

This abstract is not available before the meeting

O38

Pathological Response and Specimen Quality Following Long-Course Chemoradiotherapy for Rectal Cancer with a Six vs. Twelve Week Delay: Data From the STARRCAT Randomised Controlled Trial

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Long-course chemoradiotherapy (CRT) is used to down-stage locally-advanced rectal cancer (LARC) prior to resection. An interval period prior to surgery allows for tumour shrinkage to facilitate surgical removal. The optimal time interval remains unclear, with little high-quality evidence to guide clinical decisions about when to operate.
This study explores the pathological outcomes from a pilot randomised controlled trial comparing an interval of 6 weeks versus 12 weeks between CRT and surgery. Thirty one patients were recruited from seven UK centres between June 2012 and May 2014. Photographs were taken of the specimens and assessed by a blinded histopathologist for the quality of the mesorectal dissection. Rates of pathological complete response (pCR), down-staging, and circumferential resection margin (CRM) involvement were determined. Response was also assessed using novel tumour cell density (TCD) assessment where the slides from the resected specimen and baseline biopsy were scanned at 400x magnification, the tumour area selected and 285 to 315 data-points analysed by a blinded expert to describe the percentage of different tissue components. The work was partly funded by a PathSoc Career Development Fellowship and is presented on behalf of the STARRCAT Trial Investigators.
Twenty three patients underwent surgery (10 from the 6-week arm and 13 from the 12-week arm). The mesorectal fascial plane was intact in 7 specimens from the 6-week arm (70%) and 8 from the 12-week arm (62%). Three patients at 6-weeks and two patients at 12-weeks showed a pCR. Only one patient (from the 12-week arm) had an involved CRM. TCD was 0.3% for the 6-week arm and 4.3% for the 12 week arm (p=0.12).
In this small randomised trial, rates of mesorectal quality, CRM status, pCR and TCD were similar following either a 6 or 12 week interval after CRT. Further studies are now needed to clarify whether a longer interval does facilitate on going down-staging.

O39

The Role of Tissue Factor Pathway Inhibitor (TFPI) in Liver Injury

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Introduction: Studies have demonstrated that inhibition of the coagulant cascade is associated with less advanced liver fibrosis and better outcome in acute liver injury. TFPI is a serine protease inhibitor that acts as a homeostatic inhibitor of the coagulation cascade and may be a target to modify outcome in liver disease.
Methods: Transgenic mice carrying a genetic modification that allows cells expressing a-smooth muscle actin (aSMA; e.g. activated hepatic stellate cells) to simultaneously express TFPI were used in models of chronic liver injury (carbon tetrachloride, CCl4) or acute liver injury (paracetamol) and culled at set time points after dosing.
Results: Chronic liver injury: At 24 hours after the last dose of CCl4 the transgenic mice had significantly decreased aSMA expression and tissue inhibitor of metalloproteinase (TIMP) -1 gene expression but no difference in matrix metalloproteinase (MMP) -2 and -9 gene expression compared to wild types. This suggested a microenvironment that would promote fibrosis resolution. However after 24 hours this difference was lost. At all time points there was no significant difference between fibrosis in transgenic and wild type mice as demonstrated by Sirius red staining, hydroxyproline assay and collagen 1a1 gene expression.
Acute liver injury: In paracetamol induced liver injury there was a significant difference in parenchymal necrosis in transgenic mice compared to wild types at 24 and 48 hours after dosing (24 hours: mean necrosis 6% vs. 30% respectively, Mann-Whitney test p=0.008. 48 hours: mean necrosis 2% vs. 20% respectively, Mann-Whitney test p=0.036).
Conclusion: These results suggest that TFPI is an unlikely therapeutic target in chronic liver injury. However in acute paracetamol induced liver injury TFPI appears to rescue the injured liver in a sustained manner from 24 hours after the initial insult and suggests a role for TFPI in managing acute liver injury.
(Research funded by the Pathological Society).

O40

The Liver Biopsy in Alcoholic Hepatitis: Data from the Steroids or Pentoxifylline in Alcoholic Hepatitis (STOPAH) Clinical Trial

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Introduction: Current guidelines recommend the use of liver biopsy to confirm alcoholic hepatitis (AH) in patients who are clinically classified as severe/high risk. This work sought to validate the Alcoholic Hepatitis Histological Score (AHHS) scoring system and further explore the utility of the liver biopsy in AH.
Methods: Two independent histopathologists, blinded to treatment and outcome, centrally reviewed liver biopsies of patients with clinically high risk AH who had been recruited to the STOPAH trial.
Results: 93/208 (47%) biopsies were both adequate in quality and taken between admission and day 5 of trial treatment. 88% (82/93) had histological features diagnostic of AH. Clinically more severe AH was associated a higher rate of AH diagnosis on biopsy (82% of Glasgow Alcoholic Hepatitis Score [GAHS] ≤ 8 vs. 97% of GAHS >8). 65% (53/82) of biopsy proven cases of AH were classified as severe by AHHS. This group had a significantly higher 28 day mortality rate than those classified as mild/moderate (18% vs. 0%, Fisher's exact p=0.02). AHHS severity positively correlated with baseline Maddrey's Discriminant Function and GAHS (r=0.2, p=0.045 and r=0.3, p=0.01). Clinical markers of severe disease positively correlated with biopsy features of severe disease including serum bilirubin with bilirubinostasis (r=0.5, p=<0.0001) and serum white cell or neutrophil count with lobular inflammation (r=0.4, p=<0.001). However elevation of serum alkaline phosphatase and bilirubin were seen to negatively correlate with ductular change (r=-0.2, p=0.04) and Laennec fibrosis grading (r=-0.3, p=0.01) respectively.
Conclusion: This work goes some way towards validating the AHHS classification. The work also highlights the parallels between clinical and histological parameters and documents negative correlations seen in other liver diseases but not previously noted in AH.