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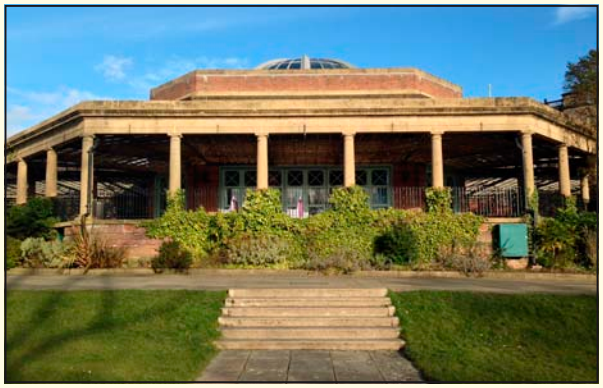

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INDEX TO PRESENTERS *(Name and Abstract Number)*

This index can be found at the end of this document, after the abstract listings.

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Plenary Oral Abstracts

PL1

Digital Pathology for Primary Diagnosis of Screen-Detected Breast Lesions: A Review of the Literature and Experience from Four Centres

Ⓟ BJ Williams[1]; J Besusparis[2]; D Clark[3]; D Snead[4]; A Hanby[1]; R Millican-Slater[1]; E Verghese[1]; A Nijhawan[1]; D Treanor[1]; E Rakha[5]

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Purpose of the study: The rate of deployment of digital pathology(DP) systems for primary diagnosis in the UK is accelerating, with departments seeking to capitalise on efficiency, workflow and quality improvements. The innate flexibility and resilience of digital slides versus standard glass slides could be of great benefit in NHS the breast cancer screening programme (NHSBSP). This study aims to address the safety and benefits of DP to the NHSBSP.
Methods: A literature review was performed to identify studies describing the use of DP for primary diagnosis of breast lesions and pertinent to the NHSBSP. In addition, data from 4 sites, including unpublished experimental and validation data were subjected to detailed concordance:discordance analysis making this the most comprehensive synthesis of digital breast cancer diagnostic data to date.
Summary of results: Detailed concordance analysis of experimental data from 2 histopathology departments reveals complete clinical concordance rates for breast biopsies of 96% (216/225) and 99.6% (249/250). Data from direct comparison validation studies in 2 histopathology departments, utilizing the protocol recommended by Royal College of Pathologists found complete clinical concordance rates for breast histology cases of 99.4% (180/181) and 99.0% (887/896). Discordances encountered in the studies most frequently concerned minor discrepancies in grading attributable to identification of weddelite calcification and differences in mitotic count scoring that is comparable to the published intra-observer concordance figures.
Conclusions: The experience of 4 histopathology laboratories, in addition to our review of pre-existing literature suggests that DP is safe for the primary diagnosis of breast histology specimens, and does not increase the risk of misclassification of breast biopsies.

PL2

Detecting Driver Mutations and Potential Future Drivers in Early Stage Lung Cancer and Investigating the Potential to Expand this to a Sputum Screening Programme

Ⓟ HM Wood[1]; E Verghese[2]; H Slaney[1]; S Dixon[2]; R Milton[2]; M Kefaloyannis[2]; P Tcherveniakov[2]

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Purpose of the study: As personalised medicine becomes more prevalent, it is becoming increasingly important to identify the molecular drivers in cancer. However, personalised treatment can lead to new drivers emerging which are resistant to the original treatment, so knowledge of mutations in sub-populations of cells is also important. In addition, using the detection of mutations in non-tumour samples as a screening tool, so called liquid biopsies, has become a growth area of research.
We sought to use the new high-sensitivity sequencing kits to detect clonal and sub-clonal mutations in a cohort of early stage lung cancer patients. We then attempted to find the same mutations in sputum samples collected before surgery, in a mimic of a potential screening setting.
Methods: Tumour resections and sputum samples were collected from eight early stage lung squamous cell carcinoma patients. DNA was extracted, and libraries prepared with the Agilent SureSelect HS kit, using a lung cancer panel. Samples were sequenced on an Illumina HiSeq, and mutations called using bespoke high-sensitivity pipelines.
Summary of results: All patients showed at least one high cellular frequency deleterious mutation in a known lung cancer driver gene, such as *TP53* and *KRAS*. All of them also showed potential drivers at lower frequencies. None of the driver mutations were detected in the sputum samples.
Conclusions: Sequencing kits designed to have high sensitivity to low cellular frequency mutations can be a useful tool when stratifying patients in a personalised medicine setting, allowing the detection of the likely main driver mutations, and potential drivers that may emerge under the selective influence of a treatment regime. We found no evidence that sputum samples are a useful screening tool using this technology, maybe because few tumour cells or tumour DNA molecules are found in sputum. Other forms of liquid biopsy are likely to be more effective screening techniques.

PL3

Invasive Breadth as a Novel Prognostic Marker for Malignant Melanoma

Ⓟ A Khanna[1]; M Bamford[2]; GS Saldanha[1]

[1]University of Leicester, Leicester, UK; [2]University Hospitals of Leicester, Leicester, UK

Purpose of the study: In the UK Melanoma is the fifth commonest cancer. Histological markers are crucial to staging. The AJCC cancer staging manual 8th edition regards tumour thickness as the strongest predictor for melanoma specific survival (MSS). Histological breadth of invasion has never been investigated. We aim to investigate the impact of histological invasive breadth on survival, using 1004 samples. We hypothesise that breadth is a valid prognostic feature.
Methods: The invasive breadth was defined as the horizontal distance between the lateral most invasive components of a melanoma. Measurements were carried out using hematoxylin and eosin stained slides and standard light microscopy. 1,209 patients with primary invasive melanomas, diagnosed at the University Hospitals of Leicester (UHL) between 2004-2012, were eligible. 1,004 patients were included in the study. Data was acquired through original pathology reports and UHL patient databases. Bivariate analysis of breadth and other prognostic features alongside and univariate / multivariate survival analysis with MSS as primary outcome were carried out using R.
Results: Bivariate analysis showed strong association between breadth and other histological variables such as Breslow thickness (BT) and mitoses. Multivariate analysis showed that breadth was strongly associated with MSS, p value <0.001, adjusted hazard ratio (HR) 1.11(CI 1.07-1.15), however adjusted BT HR was not significant. BT was significant in a multivariate analysis only when breadth was not in the model. Analysis showed that an interaction effect was present between breadth and BT. To explore this further the data set was divided into 2 groups of BT≤ 2mm and BT > 2mm. This showed that breadth was significantly associated with MSS in thicker melanomas, but not thin and vice versa for BT.
Conclusion: This study showed that breadth was strongly associated with MSS even after adjustment and may predict MSS better than BT. Further research is necessary in order to validate our findings.

PL4

Use of T Cell-Specific RNA In Situ Hybridisation as a Novel Test to Distinguish Malignant (Lymphomatous) and Benign (Inflammatory) T Cell Infiltrates

Ⓟ EJ Soilleux[1]; W Day[2]; T Hassanali[3]; M Peccarelli[4]; R Etherington[3]; G Ogg[3]

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Background: Differentiating benign from malignant (lymphoma/leukaemia) lymphocytic infiltrates is an important and common clinicopathological. For T cell infiltrates, there is no equivalent of kappa/lambda immunoglobulin light chain staining, meaning that expensive and time-consuming T cell receptor gene rearrangement PCR studies are needed. We identified two T cell-specific, mutually exclusively expressed, RNA sequences (TRBC1 and TRBC2), corresponding to the two, alternatively employed, T-cell receptor beta constant regions.
Methodology: We analysed the TRBC1/2 gene segments using standard bioinformatic tools, undertook Q-PCR to investigate relative expression levels and developed single and duplex TRBC1/TRC2 segment-specific probes for chromogenic in situ hybridisation (CISH), which we validated on FFPE sections of T-cell lymphoma/ leukaemia lines, T-cell lymphoma (n=100) and corresponding benign tissue (n=100).
Results: The coding regions of TRBC1/2 are very similar at amino acid level, making development of highly specific TRBC1/2 monoclonal antibodies difficult. However, the 3' untranslated regions differ substantially and Q-PCR demonstrated the TRBC1: TRBC2 transcript ratio in peripheral blood mononuclear cells to be very close to 1:1. This was confirmed by single and duplex TRBC1/2 CISH staining of benign T-cell infiltrates. CISH staining of T-cell lymphoma/ leukaemia lines and FFPE sections of T-cell lymphoma demonstrated clear TRBC1/2 restriction (monotypia for TCR), correlating with Q-PCR results. Double (duplex) CISH staining can distinguish between benign (inflammatory) and malignant (lymphomatous) lymphocyte populations in a wide range of tissues.
Conclusion: This is the basis of a novel diagnostic test for T-cell lymphoma, applicable to FFPE sections, that might replace PCR-based clonality studies in the majority of cases, transforming the routine assessment of T cell infiltrates, analogously to kappa/lambda staining for B-cells.

PL5

Prognostic Value of Spatial Interactions of PD-L1$^+$ Cells in Oropharyngeal Squamous Cell Carcinoma: The Pattern Matters

Ⓟ AM Tsakiroglou[1]; D Thomson[2]; K Linton[2]; P Stern[3]; K Oguejiofor[4]; M Fergie[5]; S Astley[5]; C West[1]; R Byers[6]

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Purpose of the study: Immune checkpoint inhibitors, particularly those targeting the PD-1/ PD-L1 pathway of immune-escape, are revolutionising treatment in Oropharyngeal Squamous Cell Carcinoma (OPSCC). There is a need for biomarkers to identify high-risk patients with immuno-incompetent tumours. Observing patterns between cell phenotypes is a promising avenue for biomarker development and to improve our understanding of the tumour micro-environment, the inherent complexity of which is often lost in single-plex immunohistochemical analysis. We aimed to quantify patterns between PD1$^+$ or CD8$^+$ and PD-L1$^+$ cells and test their prognostic significance in OPSCC.
Methods: Diagnostic biopsies from 72 OPSCC patients were stained using multiplex immunofluorescence for CD8, PD1, PD-L1 and CD68. Multispectral scanning and spectral unmixing identified cells positive for each of the markers. The Hypothesised Interaction Distribution (HID) method quantified patterns by the spatial proximity between cells positive for PD1 or CD8 and PD-L1. Patterns were correlated with overall survival.
Results: High frequencies of PD1$^+$ and PD-L1$^+$ (HR 2.64, p=0.042) cells occurring within 30 µm of each other were associated with a poor outcome in patients with HPV negative (n=31) OPSCC. The same effect was observed for co-occurring CD8$^+$ and PD-L1$^+$ cells (HR 2.95, p=0.025).
Conclusions: The HID method can automatically quantify spatial interactions and identify poor-prognosis OPSCC patients. Frequent co-occurrence of PD-1$^+$ or CD8$^+$ and PD-L1$^+$ cells should indicate immune escape through the PD-1/PD-L1 pathway. Future work should validate these finding in larger cohorts and test other interactions between lymphocyte subsets.

PL6

A Quantitative Evolutionary Approach Utilising High Resolution Chromosomal Copy Number Analysis Accurately Stratifies Patients with Ulcerative Colitis and Low Grade Dysplasia by Future Colorectal Cancer Risk

Ⓟ I Al Bakir[1]; K Curtius[1]; N Nasreddin[2]; TSO Clarke[1]; M Moorghen[3]; M Rodriguez-Justo[4]; M Jansen[4]; NA Wright[1]; SJ Leedham[2]; AL Hart[3]; TA Graham[1]

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Introduction: Low grade dysplasia (LGD) in ulcerative colitis (UC) demonstrates a variable risk of progression to colorectal cancer (CRC). Chromosomal copy number alterations (CNAs) are known to occur in UC epithelium. The correlation between LGD CNA burden and future CRC risk is unknown. Shallow whole genome sequencing is a novel, cost-effective technique for high resolution CNA analysis in formalin-fixed, paraffin-embedded tissue.
Methods: We analysed 34 LGD lesions from 22 'progressor' patients who subsequently developed HGD/CRC a median 427 days later (IQR 218-907), and 49 LGD lesions from 45 matched 'non-progressor' patients who remained HGD/CRC-free for >5 years. Histological grading was confirmed by two blinded pathologists.
Results: Both maximal total CNA burden and number of CNA events are greater in LGD of progressor patients than in LGD of non-progressors ($p<0.001$). Specific CNA events occur at much higher frequencies in progressor LGD, including 4q loss, 5p gain, 17p loss and 17q loss (OR>20, $p_{adj}<0.01$). Multivariate analysis combining genetic, clinical and endoscopic data demonstrates CNA burden as the only significant risk factor for future CRC risk ($p<0.001$). Survival analysis of the combined 67 progressor and non-progressor patients demonstrates that those patients bearing LGD with the 25% greatest number CNA events and/or a CNA event on chromosome 17 are much more likely to develop CRC/HGD than the remaining patients (HR 14.8, $p<0.001$). ROC analysis combining clinical and genomic data allows for highly accurate CRC risk prediction, with an AUC of 0.92. Temporospatial phylogenetic analysis in 10 progressor patients with metachronous and/or synchronous neoplasia demonstrates evidence of both clonal expansion (multiple shared CNA events between lesions) and mosaicism (no shared events between lesions).
Conclusion: LGD demonstrates a surprising diversity in CNA burden; some LGD CNA profiles are indistinguishable from the CNA profile of the CRC which subsequently arises in that patient. Shallow whole-genome sequencing output can be used to accurately predict the future CRC risk of LGD.

PL7

Low Levels of Intra-Tumour Heterogeneity in Non-Muscle Invasive Bladder Cancer

Ⓟ JL Griffin[1]; W Wei[2]; P Heath[2]; JWF Catto[3]

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Purpose of the study: Non-muscle invasive bladder cancer (NMIBC) progresses to muscle invasive bladder cancer (MIBC) in up to 20% of cases with an associated increase in mortality and morbidity. Recently, intra-tumour heterogeneity (ITH) has been recognised as an important factor in treatment resistance and aggressive biological behaviour in numerous tumour types. However, ITH has not been investigated in NMIBC. We aimed to assess ITH of gene expression in NMIBC and its relationship to progression to muscle invasive disease.
Methods: We used multi-region sampling of formalin fixed paraffin embedded index NMIBC cases. Total RNA was extracted using the Qiagen Allprep kit. The Nanostring nCounter CancerPathways gene expression panel was used to give an overview of gene expression from networks important in cancer development. Intra-tumour heterogeneity was assessed by mean pairwise 1- Spearman's rank and mean pairwise Euclidean distance of gene expression between pairs of regions within each tumour. Overall tumour heterogeneity was compared between cases that progressed to MIBC and those that didn't.
Results: Ninety-six tumour regions were sampled from 23 patients, 10 of whom progressed to MIBC. Clinical and pathological characteristics were well matched between the two groups. Mean pairwise 1-Spearman's rank was 0.072 for non-progressors and 0.076 for progressors ($p=0.689$). Similarly, mean Euclidean distance was not significantly different between the two groups (20.89 vs. 19.58, $p=0.4777$). On hierarchical clustering, regions from each case clustered together.
Discussion: There are low levels of ITH in NMIBC and no significant difference in ITH is seen between cases that to progress to MIBC vs. those that remain localised. Future work could focus on the change in ITH over time and ITH in other molecular markers such as mutation profiles and miRNA expression.
This project was funded by a Cancer Research UK/Pathological Society Pre-Doctoral Research Bursary.

PL8

Histopathology at Autopsy: Why Bother?

Ⓟ AFI Matkowski

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Background: Frequency of histopathological sampling and attitudes toward it varies nationwide. Inadequate sampling may limit the quality of autopsy reports.
Aim: To assess the value of histopathological sampling and identify barriers to taking it.
Method: Retrospective analysis of 141 autopsies undertaken at Manchester Royal Infirmary, a major teaching hospital, from January to June 2017. The gross and histological findings of the following were considered: brain, heart, kidney, liver, lung, and spleen. The number of pathological diagnoses recorded for each organ were categorised as concordant/refined, discordant, histology needed, and autolysed. Alterations to the recorded cause of death following histopathological sampling were categorised as direct, supportive, irrelevant and inconclusive. Additionally, seven consultant pathologists with post-mortem experience were interviewed. Each was asked six open questions in a semi-structured format. Transcripts were interpreted using thematic coding.
Results: The lung received the highest number (n= 320) and the spleen the lowest number (n= 35) of diagnoses. The organs most frequently requiring histology to reach a diagnosis were the kidney and lung: 52.8% and 28.8%, respectively. Histopathological sampling brought about an alteration to the cause of death in 45% of autopsy reports. In 9.3% of cases histology was not clearly relevant to the documented cause of death. Key barriers to sampling described by pathologists were time constraints and insufficient training.
Conclusion: This study found that histopathology has a major impact on determining an accurate cause of death. Inadequate exposure to post mortem histopathology during training can influence sampling habits as a consultant.

Oral Abstracts

O1

Comparison Between Two Uro-pathologists in Percentage Estimation of Gleason 4 Burden Using Digital Pathology

Ⓟ LM Carmona Echeverria[1]; A Freeman[2]; A Haider[2]; U Stopka-Farooqui[1]; C Cardona Barrena[1]; H Pye[1]; M Emberton[1]; H Whitaker[1]

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Purpose of study: Accurate estimation of Gleason 4 burden has an impact in patient management, using digital pathology we aimed to determine the interobserver reproducibility between two uropathologists (P1 and P2) and the possible factors that influence percentage estimation.
Methods: 30 patients with Gleason 3+4 (n+15) and 4+3 (n=15) from the PROMIS study were included. A total 192 slides were scanned using NanoZoomer-SQ digital slide scanner. Using NDP.View 2 software each core compromised by cancer was manually contoured in yellow, Gleason 4 areas were contoured in black. The most experienced uropathologists (P2) reviewed all scoring and added or removed contours if necessary. For each core a percentage of Gleason 4 was estimated by each uropathologists. The uropathologists were blinded to each other estimations and the original scoring. Wilcoxon matched pairs signed rank test was performed using R.
Summary of results: 426 cores were contoured, with a total of 3619 contours of Gleason 4. The average number of contours was 123 for P1 and 126.1 for P2 (p 0.0024). There was a significant difference in area contoured (p 0.04) with an average of 11.73 and 11.74 for P1 and P2 respectively. Three groups emerged when comparing P1 and P2 Gleason 4 percentage estimation: P1=P2 (n=11), P1>P2 (n=14) and P1<="" p="">

O2

Deep Learning Analysis Identifies Lymphocytic Infiltration as a Prognostic Factor in Patients with Muscle-Invasive Bladder Cancer

Ⓟ GG Christos[1]; N Brieu[2]; NP Ines[1]; JD Harrison[1]; G Schmidt[2]; PD Caie[1]

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Purpose: Muscle-invasive bladder cancer (MIBC) is a highly aggressive disease and regardless of rigorous research the prognosis of MIBC patients has remained immutable during the last three decades. Patients with MIBC have a tendency towards a worse prognosis than patients with non muscle-invasive bladder cancer (NMIBC) and therefore there is a need to significantly ameliorate risk stratification. At present, bladder cancer evaluation depends principally on the clinical gold standard Tumour, Node, Metastasis (TNM) system for staging and patient prognosis. Although TNM staging is very accurate at predicting survival rates at the population level, it is less accurate at personalized prediction and does not adequately reflect the behaviour of the disease. However, features characterizing the immune contexture (IC) in the tumour microenvironment alongside TNM staging could improve the accuracy of patient prognosis. This study reports the identification of Tumour Budding, T-cells (CD3), cytotoxic T-cells (CD8) and programmed death ligand 1 (PD- L1) expression in MIBC patient samples. As a result, the combination of these features yields a superior prognostic signature in MIBC when compared with current TNM staging.
Methods: A computational imaging technology based on machine and deep learning was developed for the evaluation of Pan-cytokeratin, CD3, CD8 and PD-L1 markers across immunofluorescence (IF) labelled whole slide images from 100 MIBC patients, allowing for a comprehensive evaluation of the composition and distribution of distinct populations within the same tissue section.
Results: Our method achieved a more significant cohort stratification (Chisq=56.7, p-value=4.87x10-13) by utilizing the combination of CD8+ T cells infiltration and PD-L1 with TNM staging compared to TNM system alone (Chisq=47.7, p-value=4.45x10-11).
Conclusions: The computation of the IC by image analysis in combination with TNM staging correlates with an improved prognostic outcome.

O3

Improving Adherence to NICE Guidelines for the Follow Up of NMIBC: Are We Over-Investigating?

Ⓟ S Walklett; S Simeen; R Ellis; D Bodiwala

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Bladder cancer is the tenth most common cancer in the UK, non-muscle invasive bladder cancer (NMIBC) accounts for 75% of these diagnoses. NMIBC a spectrum disease that is risk stratified based on histological type, size and foci. The high recurrence rate dictates a costly prolonged regular follow up period. NICE guidelines provide a surveillance scheme post-tumour resection that is based on risk of disease recurrence.
Method: Data from 100 patient's digital records over a 5 month period that had undergone treatment for a histologically confirmed NMIBC was assessed. Each case was risk categorised and the follow up data was then compared with the NICE guideline.
Results: Adherence to the guideline was poor, with a tendency to offer more cystoscopies than suggested by NICE. The low risk group was found to have the worse compliance, with the best compliance being in the high risk patient group. Overall, 77 unnecessary cystoscopies were carried out over the period.
Conclusion: The overall result of these deviations from the NICE guidance was a £17,479 increase in cost over the 5 months. The additional scopes also presented an increased potential for complications and patient distress from the procedure. The audit also incidentally highlighted the inconsistency of recording important details at the time of diagnosis, making risk stratification a challenge. Improved adherence to the guideline and standardized recording of histological type, size and foci would be cost effective, optimize resource allocation and reduce patient discomfort.

O4

Pan-Sarcoma Molecular Fingerprints of Copy Number Change Reveal Distinct Tumour Biology

Ⓟ CD Steele[1]; K Haase[2]; M Tarabichi[2]; S Behjati[3]; MF Amary[4]; R Tirabosco[4]; P Van Loo[2]; AM Flanagan[1]; LB Alexandrov[5]; N Pillay[1]

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Purpose of study: Sarcomas are a heterogenous group of mesenchymal tumours, with differing levels of genomic complexity, from simple-fusion driven tumours such as synovial sarcoma to highly copy number aberrant tumours such as undifferentiated sarcomas. We have previously identified molecular fingerprints in a dataset of 43 undifferentiated sarcomas; here we improve the identification method, and expand the identification to a pan-sarcoma dataset of copy number profiles.
Methods: Molecular fingerprints were extracted from sarcomas from the cancer genome atlas (TCGA) sarcoma dataset, and a series of published and unpublished sarcoma datasets. These included 112 undifferentiated sarcomas and myxofibrosarcomas, 71 uterine and soft-tissue leiomyosarcomas, 118 bone and soft-tissue osteosarcomas, 43 chondrosarcomas, 39 dedifferentiated liposarcomas, 5 malignant peripheral nerve sheath tumours, 6 synovial sarcomas and 10 low-grade sarcomas. Additionally, molecular fingerprints were probabilistically mapped to the human genome in all samples.
Summary of results: Previous molecular fingerprints associated with near-haploidisation and sequential genome doubling were recovered. The prevalence of genome doubling varies substantially between sarcoma subtypes. Chromothripsis amplification was identified on both a diploid and tetraploid background, predominantly observed in dedifferentiated liposarcoma. Fingerprint mapping correctly associated chromothripsis with known hotspots such as chromosome 12 leading to MDM2 amplification in dedifferentiated liposarcoma and parosteal osteosarcoma, however, other hotspots of chromothripsis remain unexplained.
Conclusions: Molecular fingerprints are a powerful tool to investigate the evolutionary history and copy number heterogeneity of sarcomas. The addition of mapping fingerprints allows for strong inference as to the copy number drivers of tumourigenesis in specific sarcoma subtypes, uncovering promising avenues for further research.

O5

Deciphering Tumour Evolution in Neuroendocrine Lung Cancers

Ⓟ L Brownlee[1]; R Veeriah[1]; R Rosenthal[1]; DA Moore[1]; C Swanton (TRACERx Consortium)[2]; M Jamal-Hanjani[1]

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Lung cancer is globally the biggest cause of cancer-related death, resulting in almost 1.6 million deaths per year [1,2]. The neuroendocrine family of tumours comprises 20-25% of all lung cancer diagnoses. Pulmonary neuroendocrine tumours lie on a spectrum of malignant behaviour, which range from the malignant, but relatively indolent, typical carcinoid tumours to small cell lung carcinoma- the most aggressive of the primary neuroendocrine lung tumours. Small cell lung carcinoma usually metastasises to lymph nodes and distant sites faster than other primary lung tumours, and has a bleak prognosis [3]. Although small cell carcinoma and other primary neuroendocrine lung tumours are considered part of the same family, the evolutionary relationship between these tumours is still poorly understood.

The TRACERx study (TRAcking non-small cell lung Cancer Evolution through therapy (Rx)) is a prospective cohort study across multiple UK centres which explores the genomic evolution of non-small cell lung cancers, identifying specific "driver mutations" which impact tumour behavioural phenotypes [4].

We apply the methodology and resources behind TRACERx to a cohort of primary bronchial neuroendocrine tumours, across a range of tumour grades. Multi-region sampling has been performed on each tumour whilst fresh, and whole-exome sequencing has been performed on each region of fresh frozen tumour. Utilising the established TRACERx bioinformatics pipeline, we explore copy number alterations and mutations identified in this group of tumours. We map evolutionary profiles of this tumour family and explore their interrelationships. We differentiate early clonal genetic changes from later sub-clonal changes. We correlate findings with histological subtype and clinical data to give a uniquely rounded perspective on the evolutionary behaviour of these tumours.

This projected has been supported by the Pathological Society and Cancer Research UK Pre-Doctoral Research Bursary.

O6

The Role of Micro-RNAs 21, 200c, 204, 205 and 211 as Diagnostic Biomarkers of Benign, Dysplastic and Malignant Melanocytic Lesions

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Purpose of study: Overlapping histological features between benign and malignant lesions and lack of firm diagnostic criteria for malignancy result in high rates of inter-observer variation in the diagnosis of melanocytic lesions. We aimed to investigate the differential expression of five miRNAs (21, 200c, 204, 205 and 211) in benign naevi (n=42), dysplastic naevi (n=41), melanoma in situ (n=42) and melanoma (n=42), and evaluate their potential as diagnostic biomarkers of benign, dysplastic and malignant melanocytic lesions.

Methods: The expression profile of each miRNA was measured using real time PCR, with machine learning algorithms used to assess the diagnostic potential of differential miRNA expression. The spatial expression of miRNAs was demonstrated with chromogenic in situ hybridisation.

Summary of results: Real time PCR demonstrated differential miRNA expression profiles between benign naevi; dysplastic naevi and melanoma in situ; and invasive melanoma. Random forest accurately classified cases based on miRNA expression profiles with ROC curve analysis of 0.99 for malignant melanoma and greater than 0.9 for all other groups, indicating high accuracy of our panel of miRNAs as a diagnostic test. However, we also examine the significant impact of variable percentage of lesional cells, and of variable spatial expression patterns of miRNAs, on these PCR results. In situ hybridisation confirmed expression of miRNA-21 and 211 in melanocytes, while demonstrating expression of miRNA-205 primarily in keratinocytes, thus calling into question it's value as a biomarker of melanocytic lesions.

Conclusions: We have validated some miRNAs, including miRNA-21 and 211, as potential diagnostic biomarkers of benign, dysplastic and malignant melanocytic lesions. However, we also demonstrate the crucial importance of considering tissue morphology and spatial expression patterns when using molecular techniques for the discovery and validation of new biomarkers.

O7

The Impact of Morbid Obesity and Weight Loss on the Immune Microenvironment of the Endometrium

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Background: Endometrial cancer (EC) has strongest association with obesity of all cancers; a 1.60-fold greater risk is conferred per 5kg/m2 increase in body mass index (BMI). Similarly, surgically induced weight loss reduces risk by up to 81%. It is proposed that this association is related to changes in the microenvironment. Although the immune microenvironment has been previously described in normal and neoplastic endometrium, no study has established if it is altered by weight loss.

Methods: Samples from a previous prospective study of morbidly obese patients undergoing bariatric surgery were utilised. 43 patients, ages 24-60, were included with three successive biopsies: at surgery, two-months and 12-months. Bloods were taken to collect further clinical data. Patients were predominantly pre-menopausal (37/43) with mean baseline BMI of 52.2 (SD=7.2). Multiplex immunofluorescence was used to simultaneously identify cells positive for markers CD8, CD68, CD3, FOXP3, PD1 and CD56. Primary outcomes were quantity of cells at each time point, repeated measures correlation with weight loss and with systemic inflammatory markers.

Results: Mean weight loss over 12-months was 29.2kg (SD=12.6). CD8+ (p=0.015, r=-0.32) cell density increased significantly over the 12-months. There was a significant reduction in inflammatory biomarkers CRP (p=1.38x10-5, r=0.58) and IL-6 (p=0.00082, r=0.46). CD3+ density negatively correlated with IL-6 levels (p=0.0028; r =-0.4896).

Conclusion: CD8+ cell density in the endometrium increased with surgical weight loss. CD3+ cell density rose, inverse to the fall in IL-6. This supports previous literature on EC immune microenvironments, suggesting these cells play a protective role in the endometrium. It may suggest the inflammatory state seen in obesity downregulates the immune system, as do tumours. These findings could have clinical impact in the development of prognostic biomarkers in EC or immunotherapy targets.

O8

Serous Adenocarcinoma of the Endometrium (ESC): A Retrospective Review of Histological Features and Clinicopathological Correlation

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Purpose of the study: Endometrial serous carcinoma (ESC) is an aggressive neoplasm, due to its propensity for metastasis and recurrence. Our aim was to assess various histomorphological features of ESC and to see their clinicopathological correlation with disease-free survival (DFS) and overall survival (OS).

Methods: After Institutional Review Board approval, a total of 205 samples (belonging to 120 patients), diagnosed as ESC from Jan 2009 to Dec 2015, were retrieved and reviewed for various histology parameters. Electronic medical records, files were seen for clinical details and follow up. Receiver operating curves (ROC) were established for the diagnostic performance of depth of invasion (DOI), tumour-free distance (TFD) and myometrial invasion percentage (MI %). Overall survival (OS) and Disease-free survival (DFS) were generated by Kaplan–Meier curves and prognostic significance by Cox regression analysis.

Summary of results: The mean age at diagnosis was 61.8 years, mean tumour size was 4.01 cm. Pure serous carcinoma histology was seen in 67.5%; mixed histology in 32.5% cases. Endometrial intraepithelial carcinoma (EIC) was seen in 40% of cases.Myometrial invasion <1/2 was seen in 37/104 (35%)cases, more than/equal to half in 55/104 (52.8%) cases, while in 5/104 (4.2%) cases, no myometrial invasion was seen; however, 3 of these showed distant metastasis. P53 showed mutated type staining in 91% of cases. Follow-up data was available in 111 (92.5%) patients (median- 24.3 months).The mean DFS was 29.3 and mean OS was 31 months. Percentage myometrial invasion (40% cut off), absolute depth of invasion (6mm cut off) were found to be statistically significant ($p<0.05$) in the univariate and multivariate analysis for DFS. Tumour-free distance to serosa (</=7mm) also showed statistically significant association on univariate analysis with OS.

Conclusion: In ESC, calculating the percentage myometrial invasion and absolute depth of myometrial invasion will be more meaningful.

O9

Expression Patterns of Immunohistochemical Markers p16 and HPV E4 on Biopsy Provide a Reproducible, Potentially Clinically Useful Classification of Cervical and Anal High-Grade Squamous Intraepithelial Lesions (SIL)

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Biopsy pathology usually decides treatment of cervical and anal potentially precancerous lesions, but is poorly reproducible, and does not clearly indicate a progressive lesion. Several studies have shown that grading p16/E4 immunohistochemical (IHC) staining provides a reproducible classification of high-grade (H-) SIL of both cervix and anus, the usual treatment threshold. In 318 women referred for colposcopy, we compared p16/E4 IHC grading of the worst cervical biopsy lesion with methylation of tumour suppressor genes FAM19A4/miR124-2 in cervical cytology (a marker for advanced transforming HSIL). We also examined in 119 women undergoing loop electro-diathermy excision (LEEP) for HSIL, the relation of E4/P16 IHC to the outcome of LEEP. E4 positive staining decreased with increasing SIL grade from 41% in LSIL to 3% in HSIL/CIN3. E4 positivity increased with grade of p16 when p16 expression was limited to the lower 2/3 of the epithelium (r=0.378), but fell with expression at higher levels in the epithelium. Loss of E4 expression was associated with methylation of FAM19A4/miR124-2 and (r=-0.177, p=0.010). 85% of women with ≥lower 2/3 p16 staining/E4-negative HSIL biopsies and 65% with limited p16 staining/E4-positive HSIL biopsies had ≥HSIL in the LEEP specimen (p=0.025). p16 expression in a cervical biopsy is related to viral production and transformation. Combined p16 expression in ≥2/3 of the epithelium and absent E4 relates to methylation, marks advanced HSIL, and indicates likely HSIL on a subsequent LEEP. Grading p16/E4 IHC provides a simple, reproducible, potentially clinically useful biopsy classification of SIL that relates to treatment and merits further clinical study in relation to the natural history and treatment of cervical (and anal) HSIL.

O10

DNA Methylation in Amyotrophic Lateral Sclerosis

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Purpose of the study: ALS can be sporadic (sALS) or familial, with a number genes implicated including C9orf72 (C9ALS) and TARDBP. DNA methylation is an epigenetic mechanism whereby a methyl group is attached to a cytosine, usually resulting in gene expression repression. DNA methylation has been implicated in other neurodegenerative diseases, but little work has been conducted in ALS. We aim to elucidate the role of DNA methylation in motor neurone (MN) decline, without the interactions from other cell types, which may mask MN-specific DNA methylation changes.

Methods: Immunohistochemistry (IHC) was used to determine the pathology of 5-methylcytosine (5mC), TDP43 and 5-hydroxymethylcytosine (5hmC) within cervical spinal cord. From a subset of the same cohort, MNs were extracted from the anterior horn using laser capture microdissection (LCM). DNA was then extracted and analysed using the Illumina Methylation EPIC array.

Results: Immunohistochemistry revealed increased methylation in ALS, with C9ALS displaying the highest global methylation. Interestingly, methylation levels appeared to reduce in the minority of cells that showed loss of nuclear TDP43. Microarray data also showed significantly increased methylation for ALS cases when compared to controls, with PANTHER pathway analysis implicating many known neurodegenerative disease related pathways.

Conclusions: DNA methylation is a contributory factor in ALS, with our data suggesting hypermethylation in particular, is involved in ALS. Further study into the genes and promoters identified could help to elucidate biomarkers for ALS in the future.

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O11

The Use of HOT_ARMS PCR in Liquid Biopsies for the Management of Patients With Colorectal Cancer

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HOT_ARMS PCR is an ultra-sensitive method for mutation detection. It is able to detect and quantify mutant DNA containing single nucleotide variants or indels down to a single copy. HOT_ARMS PCR can generate reliable signals for wild-type even when amplifying 100pg of circulating DNA. We sought to use HOT_ARMS PCR in liquid biopsies to provide data for patient management. 26 colorectal cancer patients were recruited to this study. Pre-/Post-surgery liquid biopsies and corresponding FFPE blocks were collected and analysed for KRAS exon 2 codons 12 and 13 mutations. Mutant allele frequencies of all samples were <3% and the DNA concentration range was 3.78-294ng/ml. 100% sensitivity was achieved by HOT_ARMS PCR (n=12) between FFPE tissue and pre-surgery cell-free-DNA. 7 samples presented surgical clearance and 5 samples had persistent mutant circulating tumour DNA signals in post-operative liquid biopsies. In contrast, nested full-COLD-PCR was deployed on the same cases and achieved 70% sensitivity even with a limit of detection of 0.75% mutant allele frequency. HOT_ARMS is a method of direct mutation detection and is far superior to mutation enrichment methods such as COLD-PCR for both detecting and quantifying mutations in liquid biopsies. Persistent mutant signals in post-operative samples indicate the utility of circulating tumour DNA for patient management. Ultra-sensitive mutation detection systems as characterized by this research are essential for early detection of treatment response. HOT_ARMS is a cheap, closed-tube test which can be universally applied to improve personalised cancer patient management.

O12

An Assessment of EGFR Pathway Mutations and ALK Rearrangements in Lung Cancer by Next Generation Sequencing and Fluorescent In situ Hybridisation in a Regional NHS Genomic Testing Service

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Lung cancer is the most common cause of cancer death in the UK. For metastatic non small cell carcinoma (NSCC), targeted treatments against the epidermal growth factor receptor (EGFR) and rearranged anaplastic lymphoma kinase (ALK) may slow progression. Routine molecular testing of these targets is now recommended to determine eligibility. We aimed to analyse the molecular profiles of all cases tested through a large NHS regional genomic testing service over one year and correlate this with patient and sample factors. Pathology reports were analysed following lung cancer mutation testing for samples submitted between January and December 2018. All cases underwent next generation sequencing (NGS) of PCR products for hotspot mutations in EGFR and for other downstream genes including KRAS, NRAS and BRAF with an Illumina MiSeq. ALK rearrangement was determined by florescent in-situ hybridisation (FISH). Additional clinicopathological data were also collected. Data were collected for 280 patients; 131 male (47%) and 149 female (53%). Smoking history was recorded for 76% of patients (n=212) with a positive history in 92% (195/212).There were 263 tumours diagnosed as NSCC (94%). Where reported (n=277) sample type was 36% primary tumour (n=100) and 64% (n=177) metastasis. Sample type did not appear to influence the molecular result. Where tested, ALK was rearranged in 3% of cases (7/266). The mutation prevalences were 7% for EGFR (19/278), 31% for KRAS (85/275),1% for BRAF (4/275) and no NRAS mutations. The mutation and translocation prevalences were similar to that reported in the literature for EGFR (10-35%), BRAF (1%) and ALK (3-7%). The KRAS mutation rate was higher than expected (15-25%). The study population had a similar mutation frequency for EGFR and BRAF as well as for ALK rearrangement compared to the literature. This shows that routine molecular testing in advanced lung cancer can be successfully undertaken on a variety of sample types to help guide patient treatment.

O13

Enhanced Glutamine Uptake Influence Composition of Immune Cells Infiltrates in Breast Cancer

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Purpose of the study: Cancer cells alter their metabolism in order to satisfy the demands of necessary energy and cellular building blocks. Glutamine availability for growth and progression of Breast Cancer(BC) is important in several BC subtypes. Immune evasion is an additional hallmark of cancer which plays a role in supporting tumour growth and progression.This study aimed to determine whether enhanced glutamine uptake in BC can derive the existence of specific subtypes of immune cells, including the subsequent impact on patient outcome.
Method: Solute Carriers(SLCs) involved in glutamine transport;SLC1A5,SLC7A5,SLC3A2, and immune cell subtypes;T-cell markers(CD3,CD8,FOXP3 andPD1),B-cell marker(CD20),histiocytic marker(CD68) and cancer-related immune marker(PDL1) were assessed using immunohistochemistry on TMA of a large BC cohort(n=803).Patients were stratified into accredited clusters based on SLCs expression, and correlated with the immune cell infiltrates, as well as investigating their associations with patient outcome.The effect of transient siRNA knockdown of SLC1A5 and SLC7A5 on PDL1 was evaluated in MDA-MB-231 breast cancer cell line.
Summary of results: The combined expression of all SLCs(High SLCs cluster) was significantly associated with tumour-related PDL1 and PD1+,CD20+,FOXP3+,and CD68+ immune cells($p<0.001$). In Triple Negative tumours, there were associations between High SLCs and PDL1 together with FOXP3+,CD68+ and PD1+ immune cells $p\leq 0.03$).The expression of SLCs and PDL1,FOXP3+,CD68+ cells was associated with poor survival while the expression with CD20+ cells was associated with better patient outcome ($p<0.001$).Knockdown of SLC7A5, but not SLC1A5, in TN cells significantly reduced the expression of PDL1.
Conclusion: This study provides pre-clinical evidence that altered glutamine pathways in BC, particularly TN tumours, appears to play a role in deriving specific subtypes of inflammatory infiltrates,which either support or counteract its progression.

O14

Normothermic *Ex-Vivo* Perfusion of Human Lymph Nodes: A Feasibility Study

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Purpose of the Study: Precisely how much nodal disease necessitates an axillary lymph node (ALN) dissection in early breast cancer (BC) is contentious. Investigating which ALN metastases will progress, and refining how therapies might halt that process, is clinically important. However, modelling the complex ALN microenvironment is difficult.
Methods: Under appropriate ethical approval, we harvested ALNs from BC patients from whom it was clinically safe to do so. Firstly, ALNs from patients (n = 10) were perfused *ex-vivo* at 37°C for up to 24hrs. After confirming viability, targeted therapies were administered into perfusing ALNs to evaluate perfusion efficacy (n = 3).
Summary of Results: Controlled autologous testing showed that ALNs remain viable after 24 hours of *ex-vivo* perfusion: histology, proliferation and targeted gene expression did not change significantly over time for any perfused ALN compared with a control from time-point zero. During perfusion, although the acid-base balance of the perfused nodes remained stable, the flow rate through metastatic, but not reactive, ALNs increased significantly over time ($p<0.001$). Once viability was confirmed, targeted antibodies (Nivolumab and Trastuzumab) were administered into perfusing ALNs. These permeated the entire node, binding to their cognate receptors; Nivolumab even induced histological cancer cell death. Finally, Adrenalin injection into a perfusing ALN caused intra-nodal lymphovascular dilatation, proving that vasoactive drugs can be used to change the flow rate through ALNs.
Conclusions: We show that normothermic perfusion can keep human ALNs viable *ex-vivo* for hypothesis and intervention testing. This novel model might serve as a translational bridge, in which the efficacy of emerging personalised therapies and the crosstalk between tumour and immune cells could be investigated preclinically. Furthermore, the effects of changes in flow rate on the human ALN microenvironment could be evaluated in real-time.

O15

Prognostic Significance of Isocitrate Dehydrogenase 2 (IDH2): A Biomarker Associated with Lymphovascular Invasion in Breast Cancer

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Background: Lymphovascular invasion (LVI) is associated with metastasis and is a prognostic factor in early-stage invasive breast cancer (BC). Through stringent bioinformatics analysis we identified Isocitrate Dehydrogenase 2 (IDH2) as one of the candidates gene associated with LVI positivity using multiple BC cohorts. This study aimed to evaluate the clinicopathological significance of IDH2 at transcriptomic and proteomic levels using large BC cohorts with long term follow-up.
Methods: IDH2 was probed at transcriptomic [using BC Gene miner, TCGA, and the METABRIC cohort] and proteomic level using immunohistochemistry in a large well-characterised BC cohort (n=859) prepared as tissue microarrays. Association with clinicopathological characteristics, and patient outcome were evaluated.
Results: In METABRIC and TCGA cohorts, overexpression of IDH2 mRNA expression was significantly associated with LVI-positivity (both $p<0.01$), high histological grade and HER2-positivity (all $p<0.05$). IDH2 mRNA expression showed significant positive correlation with IDH2 protein expression ($p=0.002$). At protein level, high expression of IDH2 was associated with LVI-positivity, high histological grade, high Nottingham Prognostic Index, HER2 positivity, large tumour size, and hormonal receptor negativity (all $p<0.01$). Increased IDH2 protein expression was significantly correlated with features of aggressive phenotype including Ki67, EGFR, and E-cadherin loss (all $p<0.05$). High expression of IDH2 mRNA and protein was associated with shorter 10 years of BC specific survival ($p=0.038$). In publicly available datasets using BC gene miner, up-regulation of IIDH2 mRNA was positively associated with poor outcome ($p=0.0002$).
Conclusion: This study confirmed the association of IDH2 expression with LVI status, tumour proliferation and metastasis related biomarkers; results warranting further functional validation and suggesting IDH2 as a potential therapeutic target in BC.

O16

The Cell of Origin of Normal Human Hepatocytes has a Common Ancestor with Biliary Epithelium and Hepatocyte Clonal Expansions Arise from the Portal Tract and Drift to the Central Vein

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In high turnover epithelial tissues such as the intestine, homeostatic cellular replacement is achieved via a pool of stem cells. By contrast, turnover in the normal human liver is slow and the location and necessity of liver stem cells has been hotly debated. Partial hepatectomy has demonstrated that new hepatocytes can be generated from pre-existing hepatocytes, without the requirement of a stem cell population. However, lineage tracing in chronic injury models supports the involvement of liver stem/progenitors. Further complexity has arisen from the demonstration of both periportal and centrilobular neo-hepatocyte generation. This collective knowledge has largely amassed from rodent studies such that far less is known regarding the dynamics of human liver turnover, particularly during normal homeostasis. We have explored hepatocyte dynamics in normal human liver by utilising a combination of methylation of non-expressed genes and mitochondrial DNA (mtDNA) mutations as a molecular clock and clonal expansion respectively. Spatially, we show that clonal hepatocyte expansions are more commonly periportal than centrilobular. Furthermore, by laser-capture microdissection and mtDNA sequencing, we have demonstrated that hepatocytes and ductal epithelial cells have a common cell of origin. Using methylation status, an ancestral relationship was detected from periportal clonal hepatocytes, but not centrilobular. Lastly, using mtDNA next-generation sequencing, we have demonstrated that within clonal hepatocyte expansions, greater genetic diversity exists in centrilobular hepatocytes than those located in the same clonal patch periportally. This is the first study to show the stem cell of origin of normal human liver hepatocytes in the bile duct and stream towards the central vein. The periportal junction of biliary epithelium and hepatocytes has long been suspected as a location of liver stem/progenitors, thus this study demonstrates their involvement in liver homeostasis.

O17

Liver Segment Sampling Using a Tru-Cut Biopsy Needle: Preliminary Data

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Aims: Sampling variation is a known issue in diagnostic liver pathology, limiting the histological interpretation of liver biopsy, and not entirely resolved by sampling protocols of explanted livers. We added to our routine diagnostic samples from explanted livers Tru-Cut biopsies from each liver segment to assess the feasibility of the technique and sampling variation.
Methods: A Tru-Cut needle biopsy was taken from each segment (I-VIII) of livers removed at transplantation. Each biopsy core was placed in a cassette labelled with the segment number. At embedding all segmental biopsies were laid sequentially into a single block, and oriented to have the segment 1 biopsy laid on the labelled side of the glass slides. Sampling variation was assessed on H&E and Sirius red stains using semi quantitative scores (Kleiner for steatohepatitis and Ishak for other aetiologies).
Results: This modified sampling protocol was applied to 17 cirrhotic livers from adult patients six (34%) cases of ALD, two (12%) each of NASH, HCV, PSC, PBC and hemochromatosis and one (6%) of HBV). A precise identification of segments was not possible in some instances (e.g. PSC) due to parenchymal remodelling. There was a variation in sample size and fragmentation. Biopsy surface area range was 9-21 mm^2. In terms of sampling variation, 45/46 (98%) biopsies from the steatohepatitis group showed stage 4 fibrosis, whereas 30 biopsies from chronic biliary disorders (PBC and PSC) showed fibrosis score varying from 3 to 6 in different segments, 6 being the predominant score in 18/32 (56%) biopsies. Eighteen biopsies from the viral hepatitis group showed variation in score ranging from 1-6, 6 being the predominant score in 11/18 (61%) biopsies, all of which belonged to the two cases of HCV cirrhosis.
Conclusion: Bench segmental Tru-Cut explant liver biopsy is feasible but limited by parenchymal remodelling, sample size variation and fragmentation. The underlying aetiology affects sampling variation.

O18

Emerging Evidence of Cancer Stemcellness and Epithelial-Mesenchymal Transition in Hepatocellular Carcinoma Arising on Background of Hemochromatosis

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Purpose of study: Hereditary haemochromatosis (HH) is a risk factor for liver cirrhosis and hepatocellular carcinoma (HCC) with 8 to 33% of affected individuals developing HCC. It is thought that these patients fair worse than non-HH patients, however evidence is limited. HH patients also develop rare mixed subtypes, such as combined hepatocellular cholangiocarcinoma, suggesting that cancer stem cells (CSC) and epithelial-mesenchymal transition (EMT) contribute to the pathogenesis of HCC's in HH. This study aimed to identify whether characteristic features of EMT and CSC were observable in patients with HCC and whether such features were associated with clinical outcomes, clinicopathological features and preferentially concentrated within those patients with HCC on a background of HH (HH-HCC).
Methods: Explants or segmentectomies from 17 HH-HCC and 15 cases of non-HH aetiology HCC (nHH-HCC) were included. Survival rates, clinicopathological and demographic factors were compared. Presence of CSCs and EMT was assessed through immunohistochemical (IHC) staining with 7 antibody panels identifying each process.
Results: Men represent 88% of the HH-HCC and 86% of the nHH-HCC cohorts. HH-HCC had higher rates of combined tumour subtypes, tumour size and lymphovascular invasion, with none reaching statistical significance. HH-HCC patients displayed worse overall survival and decreased mean survival. CSC marker expression was increased in HH-HCC cases, with 56% positive for CSC markers (EpCAM and SALL4) compared to 17% of nHH-HCC. Morphological and IHC characteristics of EMT (loss of E-cadherin, CK18, and gain in vimentin, CD44) occurred with greater frequency in HH-HCC than nHH-HCC (37.5% vs 8.3%).
Conclusion: This study demonstrated that HCC arising on a background of HH has a worse prognosis compared to other aetiologies. CSCs and EMT were more prevalent in HH-HCC cases suggesting a pathogenic role for these processes in tumour progression in HH-HCC.

O19

The Relationship Between DNA Mismatch Repair and Response to FOLFOX-based Pre-operative Chemotherapy in the International Phase III FOxTROT Trial

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FOxTROT is the first international phase III randomised clinical trial to evaluate the effectiveness of preoperative chemotherapy in locally advanced colon cancer. Biomarkers predictive of sensitivity to preoperative chemotherapy have not yet been identified. We investigated the relationship between mismatch repair (MMR) status and chemotherapy response. Patients were randomly assigned in a 2:1 ratio to pre- and post-operative chemotherapy comprising three 2-week cycles of FOLFOX then surgery followed by a further nine 2-week cycles, or to post-operative chemotherapy consisting of surgery followed by twelve 2-week cycles. H&E slides were collected for central pathological review in 904 out of 1052 cases (86%). Immunohistochemistry for MLH1, PMS2, MSH2 and MSH6 was performed in 794 patients (75%). Chemotherapy effectiveness was assessed blinded to trial arm using the AJCC 4 tiered-grading system. Overall, 168 patients (21%) showed deficient MMR (dMMR). When blinded to trial arm, 7 patients showed complete regression, 13 near complete regression, 199 partial regression and 575 poor/no response. dMMR was associated with a significantly higher rate of poor/no response (96% vs. 66%, $p<0.0001$). Both groups had a low rate of complete or near complete regression (dMMR 1.8% vs. proficient MMR 2.7%, $p=0.494$). The trial arms will be unblinded in the near future. Whist the breakdown by trial arm is still awaited, analysis of the first 150 patients has shown that patients in the control arm are classed as poor/no response in 98% of cases. These provisional results therefore strongly indicate that patients with dMMR colon cancer are unlikely to benefit from FOLFOX-based preoperative chemotherapy. dMMR status should therefore be considered mandatory prior to considerations of chemotherapy to offer individualised treatment to patients.

O20

Leukocyte-Associated Immunoglobulin-Like Receptor-1 Confers Poor Prognosis in Invasive Breast Carcinoma: Transcriptomic Driven Study

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Introduction: Leukocyte-associated immunoglobulin-like receptor-1 (LAIR-1/CD305), is a transmembrane glycoprotein carrying immunoreceptor tyrosine-based inhibition motifs (ITIM) and it is reported to be overexpressed in high-grade tumours. Moreover, LAIR-1 plays a key regulatory role in immune cells function and extracellular matrix remodelling reflecting its role in tumour microenvironment homeostasis. The biological role of LAIR-1 in breast cancer (BC) has yet to be elucidated.
Methods: LAIR-1 expression was evaluated at transcriptome [using BC Gene miner, TCGA, and METABRIC cohort] and protein levels using immunohistochemistry in a large well-characterised BC cohort (n=569). In silico differential gene expression was used to evaluate LAIR-1 protein associated signalling pathways. Association with clinicopathological characteristics, immune cell subtype markers and patient outcome were evaluated.
Results: High LAIR-1 expression at the mRNA and protein levels were positively correlated with poor prognostic factors; high tumour grade, high Nottingham Prognostic Index, hormonal receptor negativity, P53, MMP2, MMP14 and MMP15 (all; $p<0.01$). Overexpression of LAIR-1 was also associated with T-cell markers (CD3, CD8, and FOXP3; $p=0.039$) and histiocytic marker (CD68; $p=0.022$). Multivariate analysis revealed that increased LAIR-1 protein is an independent risk factor for shorter BC-specific survival ($p=0.039$). Using BC gene miner, up-regulation of LAIR-1 mRNA was positively associated with shorter patient outcome ($p=0.025$). Inflammation mediated by chemokine and cytokine signalling pathway was the top predicted master regulator of LAIR-1 protein expression ($p=0.002$).
Conclusions: This study provides evidence for the prognostic value of LAIR-1 in invasive BC. Strong positive association with immune cell markers and LAIR-1 warrant further studies to assess them individually and in combination along with the immune checkpoint proteins.
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O21

Immunogenomic Repertoire Profiling of Tumour-Infiltrating B Cells Reveals Sulfated-Glycosaminoglycans to be Major Functional Humoral Antigens in Human Malignancies

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Diffuse-type gastric cancer (DGC), among other gastric cancers (GCs), harbours "genomically stable" genotype with few neo-antigens; therefore, the efficacies of immune-checkpoint blockades alone may not be sufficient against DGCs. In order to understand precise molecular backgrounds of anti-tumour immunity in human DGC micro-environments, I aimed at immunogenetic profiling of tumour-infiltrating T and B cell repertoires for clinical DGCs. It was revealed that mature B cell immunity plays an important role in gastric cancers especially DGCs. With deeper focuses on the B cell repertoires, wide varieties of tumour-specific dominant immunoglobulins were discovered. Biochemical analysis of reconstructed IgGs of such dominant B cell clones showed that some of them exhibited auto-reactivities to abundant cellular proteins; however, it was of note that multiple of the rest of IgGs commonly recognized sulfated-glycosaminoglycans (sGAGs). More than 35% of the tumour-specific dominant IgGs found in human DGC tissues exhibited anti-sGAG nature. Intriguingly, those anti-sGAG human antibodies exhibited growth suppression against not only DGC cells but also various human cancers. Thus, sGAGs were revealed to be major functional B cell antigens in human tumour micro-environments and can be candidate targets of therapeutic antibodies for multiple of devastating human cancers.

O22

Role of Intracellular Interleukin-1 Receptor Antagonist Type 1 in Oral Cancer and the Oral Senescence Program

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Purpose of study: The IL-1 receptor antagonist (IL-1RA) is a potent anti-inflammatory molecule, a major function of which is to inhibit IL-1. In endothelial cells, IL-1RA is also involved in the regulation of senescence and the senescence-associated secretory phenotype (SASP), two potent anti-tumour mechanisms that when de-regulated, can promote cancer development. IL-1RA is frequently downregulated in head and neck cancer (HNSC), but how this is related to the development of HNSC is unknown.
Methods: Using qPCR, western blot, confocal microscopy and immunohistochemistry, we analysed the expression of IL-1RA in a panel of different cell lines and biopsy samples. Transfection of icIL-1RA1 was done using a plasmid and knock down was done using CRISPR/Cas9. Senescence was assessed evaluating p16 and β-Galactosidase activity and SASP factors were analysed using western blot or ELISA.
Summary of results: Intracellular IL-1RA type 1 (icIL-1RA1) is downregulated in oral dysplasia (OD) and oral squamous cell carcinoma (OSCC). Transient re-expression of icIL-1RA1 in OSCC and OD cell lines showed limited or no effects on cell migration, cell proliferation and IL-6 and IL-8 secretion (important cytokines related to epithelial-to-mesenchymal transition ,angiogenesis and tumour growth). IL-1RA expression decreases significantly during normal and dysplastic keratinocyte senescence, which is accompanied by an increase in the expression of IL-1α, IL-1β and IL-6 and IL-8; two main markers of the SASP. Knock-down of icIL-1RA1 in pre-senescent NOK and OD cells caused a significant increase of IL-6 and IL-8.
Conclusions: IL-1RA is downregulated in OD and OSCC, but its phenotypic effects are not clear. IL-1RA downregulation during senescence is correlated with the increase of SASP factors, but this correlation still needs to be confirmed. Current work on CRISPR/Cas9 icIL-1RA1 knocked-out keratinocytes will help to understand icIL-1RA1 functions in senescence.

O23

Optic Nerve Sarcoidosis Presenting as a 'Tumour' at the Optic Disc

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Background: Sarcoidosis is a chronic idiopathic granulomatous inflammatory disease that can affect any major organ system, primarily the lungs, and hence has remarkable heterogeneity in clinical presentation, findings and natural history. Between 25-80% of patients with systemic sarcoidosis will develop inflammatory eye disease and in approximately 20%, it may be the first clinical manifestation of the disease. The uveal tract is most commonly involved, although any segment of the eye and/or orbital structures can be affected. Herein we describe an unusual case of a subretinal 'tumour' at the optic disc.
Case report: A 61-year-old male presented with painful visual loss in the right eye. His previous history included Hodgkins lymphoma and mediastinal sarcoidosis. On examination, his right eye had no light perception, neovascular glaucoma, attenuated retinal vessels and a non-pigmented mass at the optic disc. The left eye was normal. The right was enucleated and sent for histology. Macroscopic examination revealed a whitish mass at the optic disc which histomorphologically showed fine anterior synechiae with focal angle closure and non-necrotising granulomatous inflammation at the optic nerve-head. Special stains for micro-organisms were negative. The appearances were those of optic nerve sarcoidosis. There was no evidence of malignancy.
Conclusion: Neurosarcoidosis is known as the 'great imitator' because it can cause non-specific clinical signs and a variety of symptoms mimicking many other conditions. It remains a challenging aspect of sarcoidosis, requiring prompt treatment to reverse eye damage and prevent permanent visual loss. A multidisciplinary approach is required to optimally manage ocular and systemic manifestations of sarcoidosis.

O24

PDL1 and PDL2 Expression in Plasmablastic and Primary Effusion Lymphomas

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Purpose: Lymphoproliferative disorders have been shown to utilise PD-1/PD-L1 pathway to escape immune recognition. Limited data is available on PD-1 and its ligand expression in immune deficiency lymphomas; very small numbers of cases have been studied. PD-1/PD-L1 blocking agents may be a useful alternative to rituximab in treatment of these CD20 negative lymphomas.
Methods: Tissue microarrays were constructed using archived FFPE tissue for 17 previously diagnosed cases with adequate material (9 plasmablastic (PBL) and 8 primary effusion lymphomas (PEL)). All but one patient was tested to be positive for HIV; one other patient was on immunosuppressive treatment post-renal transplant. Standard immunohistochemistry (IHC) was utilised to determine the percentage of PD-L1 and/or PD-L2 positive tumour cells. RGB images of the IHC stained sections were imported into CellProfiler for analysis. Analysis on PD-1 is currently ongoing. Automated measurement of staining intensity for each cell was carried out by creating a binary mask using haematoxylin counterstain which was overlaid onto the DAB stained raw image.
Results: PD-L1 expression was noted in 8/9 PBLs. In three PBLs 33%, 5% and 3% cells showed strong expression of PD-L1; 1-31% cells showed weak/moderate intensity expression (mean: 13.2%). PD-L2 expression was noted in all 9 PBLs. In four PBLs 9%, 9%, 5% and 3% cells showed strong expression of PD-L2; 12-78% cells showed weak/moderate intensity expression (mean: 38.4%). PD-L1 expression was noted in 6/7 PELs. In one PEL 1% cells showed strong expression of PD-L1; 3-9% cells showed weak/moderate intensity expression (mean: 5.4%). PD-L2 expression was noted in 7/8 PELs. In three PELs 10%, 3% and 1% cells showed strong expression of PD-L2; 5-46% cells showed weak/moderate intensity expression (mean: 21%).
Conclusion: Our study demonstrated the potential benefit of immune checkpoint inhibitors in identification of a subset of patients whose tumours express PD-L1 and PD-L2.

O25

Endocarditis and Sudden Cardiac Death

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Purpose of the study: Endocarditis is growing in incidence due to increased interventions, valve replacements and immunosuppression. It can be difficult to diagnose clinically and if left untreated can present as sudden cardiac death (SCD) with few or subtle preceding symptoms. True incidence of endocarditis related to SCD is unknown.
Methods: Retrospective analysis of our national database of 6000 cases of SCD, 1994-2018, for "endocarditis" as cause of death.
Summary of results: Of 21 cases (0.35% of total), 14(67%) were male and mean age was 32.6 ± 16.0 years. Post-mortem examination showed the aortic valve (AV) was affected in 12(57%), mitral in 5(24%), tricuspid in 3(14%) and pulmonary in 1(4.8%). Two (9.5%) were not valvular, both affecting graft repairs of the greater vessels. Five (24%) had coronary artery septic emboli and infarction. Twelve (57%) had an identifiable valve abnormality, prosthetic valve or previous valve operation, the most common being bicuspid AV (6/50%). Twelve (57%) had prior symptoms but only 7 (33%) had endocarditis diagnosed in life. Vegetations ranged from small, easily-missed irregularities to large and fungating.
Conclusion: This study highlights that although rare, endocarditis is an important cause of SCD in those with valvular disease and/or previous valve surgery. Preceding symptoms can be vague, and most individuals are not diagnosed during life. The absence of a pre-mortem diagnosis in almost 70% of our cohort highlights the need for thorough macroscopic pathological examination of the heart and cardiac valves. The gross appearance of vegetations can vary widely and lesions can be missed at autopsy.

O26

Cancer-Associated Fibroblasts Expressing Proteins Required for Chemotaxis and Cell Migration are Prognostic in Non-Small Cell Lung Cancer

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Lung cancer is the leading-cause of cancer-related mortality with a 5-year survival in NSCLC of approximately 15%. After the success of immune checkpoint inhibitors, other cells in the tumour stroma, in particular fibroblasts, have become the focus of significant interest. In cancer, these cells become activated and are known as cancer-associated fibroblasts (CAFs). However, the prognostic significance and therapeutic targeting of CAFs has so far led to mixed results with studies suggesting CAFs are a heterogenous population. Identifying specific markers and pathways in CAFs which are prognostic in NSCLC might yield novel therapeutic targets as well as providing prognostic information. A systematic review of relevant articles was carried out via Ovid Medline. Data relating to survival were extracted from eligible studies. Pooled hazard ratios (HRs) were calculated for markers where they were examined in more than one study. In addition, identified markers were grouped together based on cellular process. After exclusion of irrelevant titles and abstracts, 273/12120 articles were reviewed in full. This resulted in 49 eligible studies and 24 unique prognostic biomarkers. Meta-analyses showed no single marker had a significant effect on overall survival: CA IX (HR=1.40, p=0.32); α-SMA (HR=3.61, p=0.09); FAP (HR=1.36, p=0.62); and podoplanin (HR=1.72, p=0.30). However, analysis of cellular pathways showed markers involved in cell migration (cMET, α-SMA, podoplanin; HR=2.56, p=0.01) and chemotaxis (HGF, CXCL14; HR=2.63, p<0.01) were significant but not those required for glucose metabolism (IGF-2, GFAT-2; HR=4.71, p=0.25) or the response to hypoxia (MMP2, CA IX; HR=1.56, p=0.29). This study has identified that CAFs expressing proteins required for cell migration and chemotaxis are prognostic in NSCLC. The data also suggests that targeting these pathways in CAFs might yield novel therapeutic options rather than simple blockade of individual proteins.

O27

Pulmonary Botryomycosis: A Mimicker of Malignancy

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We present a case series of pulmonary botryomycosis diagnosed in lung resections, and consider the histological features reported, with the aim of better defining this unusual but not uncommonly seen phenomenon.
Introduction: First described in 1870, botryomycosis is a bacterial pseudomycosis characterised histologically by the presence of mixed Gram positive and Gram negative non-filamentous bacterial colonies, often forming 'granules' within airways, with associated Splendore Hoeppli phenomenon, necrosis, fibrosis, multi-nucleate giant cells and granulation tissue formation. It is considered to be a chronic suppurative infection, arising in patients with impaired immunity.
Method: We carried out a freeword text search, 'botryomycosis', on our pathology system over a 20 year period. Confirmed actinomycosis cases were excluded. Clinical presentation, radiological appearance, microbiology results, histological features and special stains were recorded where available.
Results: 25 cases were identified, of which 16 are included, the remainder had a diagnosis of actinomycosis and therefore excluded. The patients comprise men (7) and women (9) with an average age of 53 years. Ten presentations were operated on for suspected malignancy, 6 with PET positive lung nodules. In two cases, the suspected pre-operative diagnosis was an aspergilloma. Two patients had coexistent low-grade neoplasms causing obstruction. Only 6 cases cultured organisms.
Conclusion: Botryomycosis is a rare infection causing mass-like lesions and should be considered in the differential diagnosis of suspected malignancy, especially when there is cavitation. The diagnosis is often made after surgical resection in this situation.

O28

Multiplex Immunohistochemistry: The Next Generation of Molecular Pathology?

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Purpose of the study: Immunotherapy is a new paradigm in clinical oncology with durable tumour regression and stabilization of disease in advanced cancers, including non-small cell lung cancer (NSCLC). We recently reported on a comprehensive assessment of the PD-L1 diagnostic test in NSCLC. 1) A comparative validation of 22C3 (Dako) and SP263 (Ventana) PD-L1 clones. 2) A description of the PD-L1 reflex test in an accredited laboratory. 3) The role of digital pathology in the scoring of PD-L1. Importantly, we discussed the challenges of PD-L1 interpretation a) calculating the tumour cell denominator; b) peritumoural PD-L1 expression; c) calculation of positive tumour percentages at clinical thresholds; d) relevance of a 100 malignant cell rule. Here, we extend our investigation with the application of multiplexing to improve the accuracy of the PD-L1 diagnostic test.
Methods: Using OpalTM chemistry, on a Lecia Bond Rx automated staining platform, we optimised the multiplex method for sensitive and specific detection of PD-L1-SP263, cytokeratin-AE1 (CK) and CD68-SP1 to comparative routine DAB IHC. Spectral unmixing of each fluorescence channel and FFPE auto-fluorescence removal and was achieved with a Vectra Polaris scanner (40x) with image analysis/interpretation performed with inForm software.
Summary of results: Staining sequence optimisation achieved no discernible difference in antibody sensitivity or specificity following multiple antigen retrieval rounds. Comparison of DAB IHC and multiplex was highly concordant, as was the concordance of digital vs. manual assessment.
Conclusions: Phenotypic diagnostic analysis of PD-L1 is challenging. Making use of a validated multiplex (PD-L1, CK and CD68), we achieved specific visualisation of PD-L1 positive tumour cells. Visualisation of only positive epithelial cells was accomplished by CD68 subtraction. This study may represent a footprint for the validation of a lab-developed test for clinical molecular diagnostics.

O29

FAT Atypical Cadherin 1 (FAT1) is a Novel Marker of Progression from Ductal Carcinoma in situ to Invasive Breast Disease

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Purpose of the study: FAT atypical cadherin 1 (FAT1) is the human homolog of Drosophila FAT, which functions as a tumour suppressor gene and is required for proper morphogenesis. The FAT1 gene product is one of the members of the cadherin superfamily and has been demonstrated to modulate human cell to cell contact and polarity. The altered expression of FAT1 cadherin has been found in a number of solid tumours, however, its role in ductal carcinoma in situ (DCIS) remains to be defined. We aimed to characterise FAT1 protein expression in DCIS and evaluate its prognostic significance.
Methods: Tissue microarrays (TMAs) were constructed from a well characterised cohort of DCIS (n=776 pure DCIS and n=239 DCIS associated with invasive breast cancer). FAT1 was assessed immunohistochemically and its expression was correlated with the clinicopathological parameters and patients' outcome in both pure DCIS and DCIS mixed with invasion.
Summary of results: FAT1 protein showed significant difference in expression between pure and mixed DCIS, with higher expression in pure DCIS (p<0.0001). loss of FAT1 was associated with features of aggressiveness including larger tumour size, high nuclear grade, hormone receptor negativity, HER2 positivity, higher proliferation index and aberrant p53 protein. Loss of FAT1 showed significant association with shorter local recurrence free survival in invasive breast cancer patients (p=0.001) as well as the development of invasive local recurrence in pure DCIS patients (p=0.016).
Conclusions: FAT1 functions as a tumour suppressor and is an independent predictor of development of invasive local recurrence. Low FAT1 expression is associated with poor prognosis in DCIS and might be a potential marker to predict DCIS progression to invasive disease.

O30

Expression and Biological Significance of ADAM28 in Colorectal Adenocarcinoma

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While many colorectal cancers (CRCs) arise from adenomatous polyps through which we call "adenoma-carcinoma sequence", it has been estimated that up to 20% of CRCs likely evolve from an alternative pathway, so-called ''serrated pathway''. However, only 8–10% of CRCs display definitive serrated morphology at diagnosis. We previously examined expression of differentiation and molecular markers that are most likely to be involved in serrated tumour development by using 36 serrated CRCs and found CDX2 loss or BRAF mutations often together. We then generated a mouse model that can be induced concurrent biallelic inactivation of Cdx2 (Cdx2-/-) and expression of mutant BRAFV600E in adult mouse colon epithelium, and the Cdx2-/-/BrafV600E tumours showed a quite similar phenotype to that of CDX2-negative serrated CRCs. Through the validation of global gene expression profile including the mouse models and human CRCs, we focused on ADAM28 as a candidate because ADAM28 was significantly upregulated in Cdx2-/-/BrafV600E tumour and CRCs with low CDX2 expression and BRAFV600E mutation. We examined ADAM28 expression using 12 CRC cases with BRAFV600E mutation and 12 CRC cases with KRAS codon 12/13 mutations and found that ADAM28 was specifically upregulated in BRAF mutant CRC cases. Among 10 CRC cell lines, only HT-29, which has many phenotypic and genetic similarities to serrated CRC, showed a robust expression of membranous type ADAM28, and 9 of 10 CRC cells, except for RKO, showed weak to modest expression of active-form ADAM28. We present a novel transgenic model of human serrated CRC to highlight the suitability of centering on CDX2 loss and BRAFV600E in the pathogenesis. Through the validation of the gene expression profiles, we identified ADAM28 as a promising candidate of new biomarkers and therapeutic targets of CRCs with serrated morphology.

O31

Expression of B7-H3 in Melanoma and its Effects on Patient Prognosis

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Purpose of the study: B7-H3 is an immune checkpoint molecule with a key role in regulating the immune response to cancer through the inhibition of T-cells. B7-H3 is overexpressed in a wide range of human cancers and is generally associated with poor prognosis and negative clinical outcomes. The aim of this project is to explore the relationship between B7-H3 expression in melanoma and patient prognosis with the hopes of identifying B7-H3 as an independent prognostic factor in melanoma. We will also test for association with numerous known clinicopathological factors of melanoma in order to gain a better understanding of the roles of B7-H3 in the progression of melanoma.
Methods: B7-H3 expression was detected by immunohistochemical assay in 328 and 405 cases of primary and metastatic melanoma respectively. Cases were categorised into positive and negative expression of B7-H3, based on the intensity and extent of staining observed. Correlation between B7-H3 expression and overall, recurrence-free and metastasis-free survival was analysed using Log-rank tests and Kaplan-Meier curves. Furthermore, correlation between B7-H3 expression and clinicopathological features was analysed using chi-squared and Fisher's exact tests.
Summary of results: B7-H3 was expressed in 42.6 and 47.4% of primary and metastatic cases, respectively. B7-H3 expression in both cohorts was not significantly associated with patient's survival. However, positive expression of B7-H3 was associated with adverse prognostic parameters in both primary and metastatic melanoma. Within the primary cohort, a significant association was identified with ulceration (p= 0.009) and a trend for positive correlation with microsatellites was found (p=0.061). In the metastatic cohort, B7-H3 expression was significantly associated with lymph node metastasis (p<0.001) and distant metastasis (p<0.001).
Conclusion: Overexpression of B7-H3 in melanoma tissue is significantly associated with adverse prognostic parameters.

O32

The Role of Deep Learning in the Classification of Tumours of Fat

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Purpose of the study: Lipomas are common benign neoplasms of fat with an estimated incidence rate of 2.1 per 1000 people per year. Variation in lipoma histology and the large size of some lipomas require distinction from well differentiated liposarcomas which are malignant and rare and thus prove a histological and clinical challenge. The histological distinction is made by assessing nuclear and architectural features which requires review of multiple sections and the use of ancillary genetic testing. Tumours of fat can therefore present a considerable workload in the general pathology setting and often requires specialist review. Automated whole slide image (WSI) analysis could help address this problem however there are a lack of existing tools and the nature of the tissue presents a computational challenge.
Methods: We developed an iterative framework for quantitative analysis of WSIs using unsupervised clustering and deep convolutional neural networks (CNN) integrated with pathologist feedback. We used 55 liposarcoma and 32 lipoma H&E stained images as the training data with subsets held out for validation and testing. Two CNNs were employed, one using a low magnification (10x) tile based approach to capture architectural features where each WSI was split into an average of 50,000 tiles. We trained another CNN on high resolution spatially tracked nuclei (40x) to capture the nuclear features.
Results: The ability of the "nuclear" CNN to distinguish aberrant from normal yielded an 86.15% overall accuracy (F-score of 0.89) and 81.44% (F-score of 0.839) when using the "architecture" CNN alone. The accuracy for both CNNs was determined at a tile level rather than for the WSI.
Conclusions: Our preliminary results suggest that whole slide image analysis of fatty tumours is feasible but requires refinement. Ongoing work includes incorporating pathologist feedback, integrating both CNNs at the WSI level and then re-training the algorithm on a larger image dataset.

O33

Synovial Chondromatosis, Synovial Chondrosarcoma and Soft Tissue Chondroma: Adding to the Group of Calcifying Tumours with a *FN1* Gene Rearrangement

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Synovial chondromatosis is a rare benign cartilaginous tumour in which a *FN1-ACVR2A* fusion has been described. Malignant transformation to synovial chondrosarcoma occurs in up to 10% of cases. Soft tissue chondromas share similar clinical and histological features with synovial chondromatosis, however, the former rarely recur and have never been reported to become malignant. Here we aim to determine if *FN1* and *ACVR2A* rearrangements represent recurrent alterations in benign and malignant synovial chondromatosis, and to identify recurrent genetic alterations in soft tissue chondromas. ***(Supported by a Path Soc Grant)***

Results: RNA sequencing of 4 synovial chondromatosis (1 malignant) revealed a *FN1-ACVR2A* fusion. RNAseq of 1 soft tissue chondroma revealed a *FN1-FGFR2* fusion, finding confirmed by FISH (*FN1* and *ACVR2A* break-apart probes). Rearrangements were detected in 33/58 synovial chondromatosis and 3/4 synovial chondrosarcomas. Rearrangements of *FGFR1 or FGFR2* were detected in 9/18 soft tissue chondromas. 3 of these 9 tumours revealed a *FN1-FGFR1* fusion, also found in phosphaturic mesenchymal tumours, a tumour which shares histological features with soft tissue chondromas but differ in that the former express FGF23 mRNA. This diagnosis was excluded by the absence of FGF23 expression by RNA in situ hybridisation (RNAscope) and qPCR. *FN1* and/or *AVCRA2* gene rearrangements do not distinguish between benign and malignant synovial chondromatosis. However, copy number alterations in *CDKN2A* were detected in 3/4 synovial chondrosarcomas. In conclusion, recurrent fusions involving *FN1* are observed in synovial chondromatosis, synovial chondrosarcoma and soft tissue chondromas. *ACVR2A* fusion appears to be restricted to synovial chondromatosis, and *FGFR1 or FGFR2* to soft tissue chondromas. Clinical assessment and/or RNAscope help distinguish between soft tissue chondromas and phosphaturic mesenchymal tumours. Genetic alterations in the remaining cases are yet to be identified.

O34

FOxTROT: An International Randomised Controlled Trial Evaluating Neoadjuvant Chemotherapy for Colon Cancer in 1,052 Patients

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Neoadjuvant chemotherapy is established in many solid tumours but has had no previous large-scale evaluation in colon cancer. Patients with operable, non-obstructed colon cancer fit for chemotherapy and surgery with CT-predicted stage T3-4, N0-2 and M0, were randomised 1:2 to control (surgery then 24 weeks of FOLFOX) or novel (6 weeks of FOLFOX, then surgery, then 18 weeks of FOLFOX). RAS wild-type patients allocated to the novel arm had an optional sub-randomisation 1:1 to +/- panitumumab during the neoadjuvant phase. The primary endpoint was freedom from recurrent or persistent disease after two years, by intention to treat. Secondary endpoints included safety; histopathological stage, completeness of resection, DFS and OS. 1,052 pts were randomised between June 2008 and December 2016 at 85 centres in the UK, Denmark and Sweden. In the novel arm, 97% of patients received at least one cycle of neoadjuvant chemotherapy and surgery was attempted in 98% of cases. Neoadjuvant chemotherapy was well tolerated and gave marked histological downstaging, with lower pT ($p<0.0001$) and pN stage ($p<0.0001$) and fewer incomplete resections. (5% vs. 10%, $p=0.09$). Postoperative morbidity was reduced, with significantly fewer anastomotic leaks and complications requiring re-operation. Two year failure was less frequent after NAC (14% vs. 18%, HR=0.77, $p=0.11$), but this difference did not reach statistical significance. This is the first large-scale randomised controlled trial evaluating neoadjuvant chemotherapy for colon cancer. Neoadjuvant chemotherapy is safe and results in reduced postoperative morbidity, improved histological stage and a lower risk of incomplete resection. The observed improvement in two year failure rate fell short of statistical significance. Neoadjuvant chemotherapy for colon cancer improves surgical outcomes and can now be considered a treatment option; longer follow-up and further trials are required to assess its impact on long-term outcomes.

O35

A Novel Artificial Intelligence Based Approach to the Diagnosis of Coeliac Disease, Based on T-Cell Receptor Repertoires

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Current testing strategies in coeliac disease (CD) (serology and histopathological examination of small intestinal endoscopic biopsies) require patients to eat adequate gluten prior to testing, meaning that significant numbers of likely undiagnosed gluten sensitive patients choose not to seek testing. Tests often give equivocal results, meaning that even after an endoscopy patients may remain unsure about whether they are gluten-sensitive. We aimed to develop a more robust and objective test that could identify CD in patients regardless of whether they consumed gluten. DNA was extracted from 60 formalin fixed paraffin embedded (FFPE) biopsy proven cases of CD (histologically Marsh 3B or 3C) and 45 control cases (no histological features of coeliac disease, no history of anaemia or abdominal bloating, biopsy taken for suspected gastro-oesophageal reflux disease). Bulk amplification of the T-cell receptor gamma and delta repertoires was undertaken with Lymphotrack™ and Biomed-2 kits (Invivoscribe) followed by next generation sequencing (Illumina). Novel methods for bioinformatic analysis were constructed in Python and R, using the IMGT database as a reference. We developed a novel algorithm to analyse T-cell receptor repertoires (TCRR), followed by dimensionality reduction and unsupervised nearest neighbour classification (e.g., clustering), grouping together cases with similar TCRR. By modifying the parameters, we could train/ supervise the algorithm to ensure that new cases were correctly clustered. Importantly, biopsies with normal histology from CD patients on a gluten-free diet without raised anti-TTG antibodies were classified as having CD. Our methodology has the potential to revolutionise the diagnosis of CD, so that it no longer relies on either the rather subjective opinion of a histopathologist or on sufficient gluten consumption by the patient. It is also applicable to FFPE biopsies and may, in future, be modified for use as a blood test.

O36

Investigating the Potential of the Faecal Microbiome to Improve Colorectal Cancer Screening

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Colorectal cancer (CRC) patients have a different faecal microbiome to healthy controls. Microbiome data has been shown to improve the sensitivity of CRC screening in small studies collecting whole stool, often transported and stored refrigerated/ frozen. We report the first study to pragmatically translate these findings to a national CRC screening programme, by analysing the microbiome directly from the faeces of processed NHS Bowel Cancer Screening Programme cards, stored and transported at room temperature. DNA was extracted from 1287 cards: 400 for which blood was not detected and 887 for which blood was detected (of which 250 had a normal colonoscopy; 88 a non-neoplastic condition; 291 adenoma; 258 CRC). A technical sub-study was conducted to assess stability by performing extraction replicates 6-23 months post-initial extraction. V4 16SrRNA sequencing was performed. There were no significant differences in bacterial communities of the extraction replicates, with a random forest model being unable to distinguish them, indicating stability. There were statistically significant differences in bacterial communities (weighted and unweighted UniFrac distances) between blood-positive and blood-negative samples and between all of the blood-positive colonoscopy-status groups, apart from the adenoma and non-neoplastic condition groups. The bacteria which differed significantly were in agreement with the existing literature, including an enrichment of *Fusobacterium*, *Parvimonas* and *Porphyromonas* in CRC. Preliminary random forest modelling has shown that samples can be classified with an improvement in accuracy up to 1.5 times baseline. We have demonstrated a method of performing population-level microbiome research. We have verified that the CRC-associated bacteria identified in research studies are present within a bowel cancer screening population. Preliminary work suggests that a microbiome-based model could be used to stratify screened patients.

O37

Unmasking the Tissue Microecology of Ductal Carcinoma In Situ with Deep Learning

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Despite increasing evidence supporting the clinical relevance of tumour infiltrating lymphocytes (TILs) in invasive breast cancer, TIL distribution pattern surrounding ductal carcinoma in situ (DCIS) and its association with prognosis is not well explored. To characterize the tissue microecology of DCIS, we designed and tested a new deep learning pipeline, UNMaSk (UNet-IM-Net-SCCNN), for the automated detection and simultaneous segmentation of DCIS ducts using three patient cohorts. This new method achieved the highest sensitivity and recall over cutting-edge deep learning networks, as well as the highest concordance with DCIS identification based on CK5 staining. Following automated DCIS detection, spatial tessellation centred at each DCIS duct created the boundary in which local ecology can be studied. Another deep learning network was used to identify single cells including TILs. In a small sample set, we found a striking difference between pure DCIS cases and DCIS adjacent to invasive cancer. While pure DCIS cases had significantly more TILs, these TILs tended to co-localize less with DCIS compared to those with adjacent, infiltrating tumour, suggesting a more inflamed tissue ecology local to DCIS in tissue adjacent to invasive breast cancer. Thus, technological developments in artificial intelligence and digital pathology can enable us to quantify the spatial relationship between TILs and individual DCIS ducts, providing a new way to study immune response in DCIS.

O38

A Display Evaluation for Primary Diagnosis using Digital Pathology

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As pathology departments around the world contemplate digitising for primary diagnosis, making an informed choice regarding which displays to purchase in the absence of defined minimum standards, is very challenging. To help inform the procurement of displays within our institution, and to help other departments make similar decisions, we conducted an evaluation of displays with a range of technical specifications. We invited histopathologists within Leeds Teaching Hospitals NHS Trust to take part in a survey evaluation of 8 short-listed displays. The displays were chosen from a range of vendors, and included consumer, professional and medical grade displays. After configuration was optimised, histopathologists blinded to make and model of the displays, were asked to review one H&E slide of a benign nevus on each display and give a score on a Visual Analogue Scale to indicate their preference in terms of image quality and display size. Thirty-eight pathologist participants took part. The preferred display was the most expensive display, which had the highest technical specifications (11.8MP resolution, 2100 cd/m2 maximum luminance, 1200:1 contrast ratio). The least preferred display was the least expensive display, which also had the lowest technical specifications (2.3MP resolution, 300 cd/m2 maximum luminance and 1000:1 contrast ratio). This experiment demonstrates a preference for medical grade displays with the highest technical specifications. As cost becomes implicated in procurement, significantly less expensive medical grade displays with slightly lower technical specifications may be the most cost-effective option.

O39

Unbiased and Objective Artificial Intelligence Identifies Clinically Significant Diagnostic and Prognostic Features

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Purpose of the study: Digital pathology and image analysis can now quantify object-based and spatially resolved data from known histopathological features. However, cancer is a complex disease with multiple cellular interactions occurring within the tumour microenvironment. Undiscovered but clinically significant phenotypic patterns may exist within a patient sample. Artificial intelligence(AI) has the ability to identify such patterns by applying an objective and unbiased approach to the analysis. We utilise AI to predict diagnosis and prognosis in patients by quantifying features captured *a priori*. Furthermore we will describe how the iCAIRD project aims to translate such a deep learning approach into clinical reporting.
Methods: AI was applied to digitised images of patient samples to identify significant features in three clinical examples. 1) Stage II colorectal cancer (CRC) prognosis using immunofluorescence (IF) labelled whole slide images (WSI) (n=173). 2) Stage I & II CRC prognosis using H&E stained WSI (n=75). 3) Diagnosing bladder cancer recurrence from urine cytology samples labelled with IF (n=624).
Results: Automatically inferred phenotypes from H&E labelled stage I and II CRC patients, extracted with no human based annotations, predicted survival with an accuracy of greater than 95% and with an F score of 100%. 125 automatically reported features were extracted from IF labelled urine cytology samples and an AI workflow reported a sensitivity of 95% and a specificity of 70%. Unbiasedly extracted featured from IF labelled stage II CRC were analysed by AI and reported an AUROC of 0.94.
Conclusion: The use of automated image analysis and AI allow novel clinically significant features to be reported without the guidance of human-based training. This works paves the way for AI approaches to be translated into the clinic upon wider validation such as with the iCAIRD digital pathology initiative.

INDEX TO PRESENTERS *(Name and Abstract Number)*