The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Pathological Society
Understanding Disease

Nottingham Pathology 2016

Plenary Oral and Oral Abstracts

9th Joint Meeting of the British Division of the International Academy of Pathology and the Pathological Society of Great Britain & Ireland

28 June – 1 July 2016

Hosted by

Academic Unit of Molecular Pathology
Division of Cancer and Stem Cells
School of Medicine, University of Nottingham

Venue

East Midlands Conference Centre and Orchard Hotel
The University of Nottingham, University Park, NG7 2RJ

Companion Sessions

AIDPATH · British Lymphoma Pathology Group
Association of Clinical Electron Microscopists
UK Cardiac Pathology Network · Renal EQA

KEY

Ⓟ = Presenter

PRESENTER'S INDEX

To be found at the end of this document, after the abstract listings.

ABSTRACT REVIEWERS

Dr MF Amary, London
Prof MJ Arends, Edinburgh
Dr C Bacon, Newcastle-upon-Tyne
Dr EW Benbow, Manchester
Prof DM Berney, London
Dr T Brenn, Skin
Dr L Browning, Oxford
Dr L Burke, Cork
Prof SS Cross, Sheffield
Dr J Dormer, Leicester
Prof M-Q Du, Cambridge
Prof RM Feakins, London
Prof S Fleming, Dundee
Prof AJ Freemont, Manchester
Dr TR Helliwell, Liverpool
Prof CS Herrington, Edinburgh
Prof S Hubscher, Birmingham
Prof M Ilyas, Nottingham
Dr M Khan, Nottingham
Dr S Manek, Oxford
Dr S Martin, Nottingham
Prof RFT McMahon, Manchester
Prof GI Murray, Aberdeen
Prof AG Nicholson, London
Dr M Osborn, London
Dr S Paine, Nottingham
Dr N Pillay, London
Dr D Poller, Portsmouth
Dr CM Quinn, Dublin
Prof E Rakha, Nottingham
Dr G Saldanha, Leicester
Prof G Thomas, Southampton
Dr D Treanor, Leeds
Prof P van der Valk, Amsterdam
Dr A Warren, Cambridge
Dr KP West, Leicester

PROGRAMME ACKNOWLEDGEMENTS

This Programme is jointly published by:
The British Division of the International Academy of Pathology and The Pathological Society of Great Britain & Ireland

Cover picture credits:
The publishers wish to thank the following for granting permission to use their photographs:
Top/Main picture……Photographer – Gerry Molumby
Lower two pictures……Experience Nottinghamshire

This publication was designed and printed in England by Byte & Type Limited, Birmingham (Tel: 0333 666 4321)

PL1

Molecular Mediators of Mammographic Density

Ⓟ A Ironside; J Gomm; L Haywood; I Goulding; J Wang; A Guerra; C Chelala; JL Jones

Barts Cancer Institute, London, UK

Background: Mammographic density (MD), created predominantly by increased stromal tissue, is a major risk factor for breast cancer, though little is known about the biological mechanisms mediating it. Tamoxifen prevents breast cancer in high-risk women via mechanisms that appear dependent on reduction of MD. Animal models suggest tamoxifen remodels the mammary stroma to a tumour-inhibitory phenotype.
Aims: to analyse the effect of tamoxifen on breast fibroblast function and identify pathways contributing to the density-associated risk.
Methods: Primary human breast fibroblasts from normal, high risk or breast cancer patients were treated with hydroxytamoxifen (4-OHTam, 100nM-5μM). Fibroblast function was analysed by measuring: proliferation, expression of stromal proteins fibronectin (FN), LOX and collagen 1; effects on TGF-β signalling and expression of the myofibroblast marker SMA. Genome wide analysis was performed using RNA-Seq.
Results: Fibroblasts from 25 patients were treated with 4-OHTam. All patients showed reduced proliferation with treatment. 62% of patients showed reduced FN expression. TGF-β-mediated upregulation of SMA and FN were consistently inhibited. RNA-Seq analysis revealed downregulation of Wnt signaling, a pro-fibrogenic and pro-tumourigenic pathway, and modulation of many metabolic pathways, including components of the microsomal anti-oestrogen binding site (AEBS). Binding of tamoxifen to the AEBS inhibits ChEH activity promoting an anti-tumourigenic phenotype. The effects of tamoxifen on fibroblasts could be replicated using tesmilifene, a commercially available inhibitor of ChEH.
Conclusion: These data indicate that tamoxifen can directly remodel the stromal microenvironment, generating a less 'reactive' stroma. Thus, tamoxifen impacts on multiple pathways, many independent of the oestrogen receptor, to create a tumour-inhibitory microenvironment. This offers exciting potential for patient monitoring and alternative cancer prevention strategies.

PL2

Exome Sequencing Identifies MSS51 Mitochondrial Translational Activator as a Putative Metastasis Associated Gene in Invasive Breast Carcinoma

MA Aleskandarany[1];Ⓟ M Diez-Rodriguez[1]; E Nuglozeh[2]; A Elmouna[2]; MF Fazaludeen[2]; I Ashankyty[2]; S mian[2]; CC Nolan[1]; IO Ellis[1]; AR Green[1]; EA Rakha[1]

[1]Nottingham City Hospital, Nottingham, UK; [2]Molecular Diagnostics and Personalised Therapeutics Unit, University of Ha'il, Ha'il, Saudi Arabia

Purpose: Understanding the molecular genetic evolution of BC would contribute further insights into the molecular derangements driving its progression. Metastatic progression in BC occurs through the integration of multiple molecular factors/pathways. However, there are as yet unknown biomarkers associated with adverse clinical outcome in breast cancer, in particular development of metastatic disease.
Methods: Exome sequencing was performed using illumina MiSeq to sequence gDNA extracted from FFPE tissue sections from different tumour quadrants and corresponding axillary lymph node metastases. Exploratory analyses and data revealed an indels in MSS51 gene common to all axillary lymph node samples yet absent from primary tumour samples. MSS51 was assessed in a large series of invasive BC (n=810), and MSS51 was explored in the TCGA data and the prognostic significance was assessed in the publicly available gene expression datasets (bc-GenExMiner v3.2).
Summary of Results: MSS51 indel showed a high impact on protein function as determined by in-silico prediction modelling algorithm (SnpEff version 4.1). Increased MSS51 protein expression was associated with lower nuclear grade, more tubule formation, histologic types of excellent/good prognosis, triple negative BC, ER+, PR+, E-cadherin+, and N-cadherin-. Using the BC Gene Expression Miner, only one dataset of the seven publicly available datasets (n=1250) showed border-line significance with distant recurrence. Within the TCGA data, cell 2015, 20/971 (2%) cases showed genetic alteration with non-significant impact on patients' survival. Gene co-expression showed the highest correlation with genes encoding ribosomal proteins.
Conclusions: MSS51 gene has been identified as having frameshift mutations caused by indels. Reduced protein expression showed association with features of less differentiated BC with cadherin switch-like alteration. Therefore, MSS51 is a potential metastasis-associated gene warranting further studies.

PL3

Histological and Genomic Markers of the Switch from In Situ to Invasive Behaviour in Early Lung Adenocarcinoma

Ⓟ DA Moore[1]; A Crate[1]; G Wilson[2]; JPC Le Quesne[1]

[1]University of Leicester, Leicester, UK; [2]Crick Institute, London, UK

Lung adenocarcinoma generally arises from benign precursors in a stepwise manner. This is well characterised at the tissue level but, compared to other common malignancies, is poorly understood genomically. In particular it is not known which mutations drive the switch from benign in situ lesions to lethal invasive disease. This deficit is due to both the scarcity of human tissue samples exemplifying the switch, and to difficulties in identifying which early tumours best exemplify this behaviour. This study firstly identifies a subgroup of small tumours from our large diagnostic archive which exemplify the switch from in situ growth to adenocarcinoma, and then uses these cases to identify genomic events which lead to the development of invasive lung adenocarcinoma from precursors.
We reviewed 250 sub-20mm lung adenocarcinomas resected over 10 years and developed a scoring system to identify cases which show a genuine shift from in situ to invasive disease. Example cases were selected and in situ and invasive tumour regions separated using laser capture microdissection. DNA extracted from the tissue was subject to both targeted and whole exome next generation sequencing.
Cases which appear to demonstrate true in situ growth rather than a 'lepidic cancerisation' phenomenon showed a lower rate of nodal involvement at resection (p=0.015). Laser capture microdissectin followed by panel and whole exome next-generation sequencing of the selected cases exemplifying the step change demonstrated mutations which were acquired in the shift to the invasive phase of the disease, and subclonal mutations within the in situ component which were highly enriched within the invasive focus.
The adenoma carcinoma sequence in the lung is only beginning to be understood. Histological assessment of early lesions is key to selection of appropriate cases. Early sequence data has identified a range of genomic alterations associated with the acquisition of invasive behaviour in early tumours.

PL4

UCA1 Overexpression is a New Independent Prognostic Marker in Bladder Cancer Associated with Increased Patient Survival

Ⓟ L Lebrun[1]; D Milowich[1]; M Le Mercier[1]; J Allard[2]; YR Van Eycke[2]; T Roumeguere[3]; C Decaestecker[4]; I Salmon[1]; S Rorive[1]

[1]Department of Pathology, Erasme University Hospital, Université Libre de Bruxelles (ULB), Brussels, Belgium; [2]DIAPath, Center for Microscopy and Molecular Imaging (CMMI), Académie Universitaire Wallonie-Bruxelles, Gosselies, Belgium; [3]Department of Urology, Erasme University Hospital, Université libre de Bruxelles (ULB), Brussels, Belgium; [4]Laboratories of Image, Signal processing and Acoustics (LISA), Brussels School of Engineering/Ecole Polytechnique de Bruxelles, Brussels, Belgium

Background: Non-coding *RNAs* have been shown to play important roles in carcinogenesis via complex mechanisms, including transcriptional and post-transcriptional regulation, as well as chromatin interactions. *Urothelial-carcinoma-associated-1* (*UCA1*, a long non-coding RNA) was recently shown to have tumourigenic properties in urothelial bladder cancer (UBC), demonstrated by enhanced proliferation, migration, invasion and therapy resistance of UBC cell lines *in vitro*. These findings, mainly provided by in vitro cellular assays, suggest that *UCA1* is associated with aggressive tumour behaviour and could have prognostic implications in UBC.
Methods: Chromogenic in situ hybridization and immunohistochemistry were carried out on tissue-microarray to characterise *UCA1mRNA*, p53 and KI-67 expression in 208 UBC, including 145 non-muscle-invasive and 63 muscle-invasive cases. We investigated statistical relations between *UCA1* expression and UBC pathological features, patient prognosis as well as expression of p53 and KI-67.
Results: *UCA1* was observed in the tumour cells of 166/208 (80%) UBC tested. No expression was noted in normal stromal and endothelium cells. Patients with UBC that overexpressed *UCA1* had a significantly higher survival (p=0.006) compared to patients whose UBC didn't overexpress *UCA1*. This prognostic factor was independent of tumour morphology, grading, staging and concomitant CIS. In addition, the absence of *UCA1* expression was associated with a high Ki67 proliferative index and two different patterns of p53 expression (strong nuclear expression or complete absence of staining).
Conclusion: Our work identified *UCA1* as a possible new independent prognostic marker in UBC that could play a pivotal role in bladder cancer carcinogenesis.

PL5

Algorithm for Assessment and Prognostic Significance of MYC and BCL2 Expression and Rearrangements in Diffuse Large B-cell Lymphoma (DLBCL) — All Wales Lymphoma Panel Study

Ⓟ MR Pugh; A Joshi; RL Attanoos; SD Dojcinov

All Wales Lymphoma Panel, University Hospital of Wales, Cardiff, UK

Background: "Double expresser phenotype (DEP) as well as co-rearrangements of MYC and BCL2 ("double hit genotype (DHG)) are adverse prognostic indicators in DLBCL. There is no consensus regarding immunohistochemistry (IHC) and FISH for MYC/BCL2 in routine diagnosis of DLBCL.
Aims: To determine the prognostic impact of MYC and BCL2 expression and rearrangements and establish a rational testing algorithm.
Methods: All DLBCLs from August 2012 – October 2015 were prospectively assessed. Cases were assigned 'germinal centre' (GC) or 'activated B-cell' (ABC) type according to the Hans criteria. IHC was performed for BCL2 and MYC with FISH for MYC, BCL2 and BCL6 rearrangements. Clinical follow up and survival information was collated.
Results: 468 cases were assessed (mean age 69; 53% M; 47% F). 50.8% did not show evidence of co-expression or co-rearrangement of MYC and BCL2 ("NOS type)(60% GC, 40% ABC). Of the rest, DHG was seen in 3.7% (100% GC). DEP represented 34.8% (32% GC, 68% ABC) of which 10% showed single MYC or BCL2 rearrangements. All DHGs showed double expression of MYC and BCL-2. 16% of cases showed other genetic abnormalities (not translating into DEP or DHG). Triple rearrangements were seen in 1%. Significant difference ($p<0.05$) was noted in the overall survival between the NOS, DEP and DHG (80%, 60%, 25%, respectively; 35 month follow up). When DEP cases were excluded from the ABC group, there was no survival difference between GC/ABC types.
Conclusion: IHC+FISH for MYC+BCL2 confirm prognostic significance in DLBCL. DHG confers poorest prognosis of GC DLBCL. DEP in the ABC type confers the prognostic difference between the GC/ABC types. The proposed algorithm is: all DLBCLs tested for MYC+BCL2 by IHC; GC type co-expressing MYC+BCL2 tested for MYC by FISH; If rearrangement present, FISH for BCL2 is applied. There is no rationale to test DLBCLs of ABC type by FISH for MYC+BCL2.

PL6

Investigating the Mechanisms Underlying Oligodendrocyte Dysfunction in C9ORF72 ALS

Ⓟ A Lorente Pons; PJ Shaw; PG Ince; J Cooper-Knock; A Ramachandran; J Kirby; JR Highley; JD Wood

Sheffield Institute for Translational Neuroscience (SITraN), Sheffield, UK

Purpose of the Study: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterised by motor neuron degeneration. Oligodendrocyte dysfunction has also been shown to be a feature of ALS and pathological ubiquitylated cytoplasmic inclusions are well documented in oligodendroglia (gUCIs). Myelin basic protein (MBP) messenger RNA (mRNA) must be transported to oligodendrocyte processes for local translation. The C9ORF72 gene harbours the expansion of a GGGGCC repeat in many ALS cases (C9ALS). The GGGGCC motif binds heterogeneous nuclear ribonucleoprotein A2 (hnRNPA2), which is essential for the assembly of MBP mRNA transport granules. This may lead to sequestration of hnRNPA2, reducing its availability to bind MBP mRNA, thereby causing myelin dysfunction in C9ALS.
Aims: To characterize oligodendrocyte pathology in post mortem tissue from C9ALS, sporadic ALS cases (sALS) and controls (Ct).
Methods: We used immunohistochemistry to quantify gUCIs in motor (MCx) and frontal cortices and spinal cord, and to quantify hnRNPA2, MBP, dipeptide repeat-inclusions (DPRs) and oligodendrocyte precursor cells in the white matter underlying the MCx (MCx WM).
Summary of Results: C9ALS cases showed more gUCIs in the cortex compared to sporadic ALS (sALS) and Ct cases. Both C9ALS and sALS cases show increased gUCIs in the SC in comparison with Ct cases. These C9ALS cases also show that the number of gUCIs in the white matter underlying the motor cortex correlates negatively with the amount of MBP, the proportion of cells expressing hnRNPA2, and the number of OPCs in the same area. These C9ALS cases rarely showed DPRs and did not show a specific pTDP43 pathology in the MCx WM.
Conclusions: MCx gUCIs, but not SC gUCIs, are specific of C9ALS. The lack of some myelination markers in the MCx WM of C9ALS could be related to proteins included in the gUCIs which are still unknown, but not to DPRs.
Funded by the Pathological Society and "la Caixa Foundation.

PL7

Cten Knockout Using CRISPR/Cas9 Abrogates Cell Migration and Invasion in Colorectal Cancer Cell Lines

Ⓟ H Thorpe; A Asiri; H Otifi; M Ilyas

University of Nottingham, Nottingham, UK

Cten has oncogenic activity in colorectal cancer (CRC) and is associated with advanced Dukes' stage, poor prognosis and distant metastasis. Cten regulates cell migration but knowledge of downstream activity is sparse. We sought to use CRISPR/Cas9 technology, to knockout Cten in CRC cell lines to investigate Cten signalling. SW620 is a CRC cell line which endogenously expresses high levels of Cten. Cells were transfected with a episomal Cas9-gRNA-GFP construct targeted to exon 3 of the Cten gene. Cells were sorted according to GFP expression and clonally expanded to form homogenous populations. Clones were screened for mutation using High Resolution Melting and these were confirmed by sequencing. Western blotting was used to confirm Cten knockout and investigate Cten downstream signalling. Transwell cell migration and invasion assays were performed to assess the functional activity of Cten knockout cells in comparison to the control SW620 cell line.
One clone was identified which contained a 19 and 20 nucleotide deletion at the Cten alleles. These mutations were predicted to result in a frameshift with consequent protein truncation and loss of protein expression was confirmed by Western blotting. The knockout of Cten resulted in a reduction in Snail, ILK ad FAK expression. Functional assays revealed that knockout of Cten expression was associated with a profound reduction in cell migration and invasion when compared to wild-type cell lines ($p<0.001$ for each).
In summary, we have successfully created a Cten knockout cell line which allows the interrogation of Cten signalling. This is an advancement on recent investigations and allows for the creation of Cten rescue models to better determine Cten activity. A more valid in vitro model will help better understand signalling mechanisms regulating cancer metastasis. This work was supported by a grant from the Pathological Society.

PL8

An In-Depth Examination of Genomic Heterogeneity in Disseminated Colorectal Cancer

Ⓟ TG Palmer; HM Wood; M Taylor; W Fateen; IM Carr; P Chambers; P Quirke

University of Leeds, Leeds, UK

An understanding of tumour heterogeneity in advanced cancer is central the effective use of targeted therapies in solid malignancies; up to this point only a superficial understanding of this process has been gained in colorectal cancer (CRC).
Aims: We investigated mutation profiles and copy number variation in over 500 primary and metastatic tumour samples from 8 CRC patients to perform an in-depth analysis of the genomic heterogeneity in CRC tumours.
Methods: Multiple samples from primary and secondary tumours were retrieved at autopsy and from previous surgical resections from 8 consented adults. Next generation sequencing (NGS) for copy number variation (CNV) and pyrosequencing for KRAS, NRAS, BRAF and PIKC3A activating mutations were performed on each deposit to determine the degree of genetic heterogeneity in each individual. Mismatch repair (MMR) status was also established by immunohistochemistry.
Results: Analysis of up to 75 samples per patient revealed a range of therapeutically important mutations in KRAS, NRAS and BRAF that demonstrated minimal inter-tumour heterogeneity. However, CNV analyses showed a range of aneuploidy ranging from minimal CN change to gross chromosomal instability producing complex evolutionary patterns. The severity and complexity of these changes correlated with MMR status and distribution of disease.
Conclusions: This is largest examination of tumoural heterogeneity performed in solid malignancy and demonstrates important genomic features of MMR deficient and proficient colorectal tumours.

O1

Expression of MET and SOX-2 in Non Small Cell Lung Cancer with Correlation of Histological Features

Ⓟ AM Quinn[1]; D Ganguli[2]; L Franklin[3]; C Keeling[4]; H Bradley[3]; J Harris[2]; R Byers[4]; D Nonaka[5]; W Newman[2]; F Blackhall[5]

[1]University Hospital South Manchester, Manchester, UK; [2]Genomic Medicine, St. Mary's Hospital, Manchester, UK; [3]Cancer Research UK, Manchester, UK; [4]Manchester Royal Infirmary, Manchester, UK; [5]Christie Hospital, Manchester, UK

Background: Targeted molecular therapies which exploit genetic alterations in non small cell lung cancer have the potential to dramatically influence patient outcomes. Emerging targets of interest include the receptor tyrosine kinases MET and FGFR1 and transcription factor SOX2. This study aimed to evaluate the expression of MET and FGFR1 in 66 lung adenocarcinomas (ACAs) and SOX-2 expression in 41 pulmonary squamous carcinomas (SCCs) by immunohistochemistry.
Methods: Histology of each case was reviewed. Ten of the MET IHC slides were scanned by the image software analysis programme Defininens Developer XD 2.3 using the Tissue Studio portal (v4.0). Attempts at copy number analysis of MET and FGFR1 were attempted by fluorescent in situ hybridisation, however data were not analysable due to poor quality DNA.
Results: ACAs were subclassified according to their predominant pattern of growth (13 lepidic, 11 acinar, 16 solid, 26 papillary). FGFR1 staining was largely negative with just a few cases showing patchy cytoplasmic staining. SOX2 nuclear staining tended to be intense and diffuse; the median H-score estimated was 180. For MET staining, the M-score defines a positive sample as one with ≥ 50% tumour staining of 2+ or 3+ intensity (≥ 50% 1+ or 0 is reported as negative). 48 ACAs were MET negative and 18 MET positive. 7 of the MET-positive ACAs had a solid pattern of growth. A H-score of 150 or more was likely to yield a positive M-score ($p < 0.0001$). The H-score calculated from the automated scan was correlated with the histopathologist manual H-scores (Pearson $r = 0.85$). There was an improved overall survival with early versus late stage cancers ($p = 0.001$). Using a H-score of 150 as a cut-off MET expression did not seem to alter with survival.
Conclusion: MET expression may be increased in solid ACA, and could potentially predict copy number changes and exon 14 alterations.
This study was supported by a Pathological Society Small Grant.

O2

Histopathological Assessment of EBUS Specimens: The Nottingham Experience

Ⓟ A Loona[1]; R Rummery[2]; D Baldwin[1]; IN Soomro[1]

[1]Nottingham University Hospitals NHS Trust, Nottingham, UK; [2]Royal Derby Hospital NHS Foundation Trust, Derby, UK

Introduction: EBUS-TBNA was introduced at our hospital in 2009 and is used to investigate mediastinal masses and mediastinal lymphadenopathy, predominantly in the staging of lung cancer, but also other malignancies and benign conditions such as sarcoidosis and infections. Most commonly sampled lymph node stations are, 4R, 4L and 7 with 2R, 10, 11 and 12 also frequently sampled.
Aim: To measure the specificity, sensitivity and accuracy of EBUS histopathological assessment in relation to the final clinical diagnosis in benign and malignant conditions and to measure the specificity, sensitivity and accuracy of the initial clinical impression in relation to the EBUS diagnosis.
Materials and Methods: In Nottingham the EBUS-TBNA procedure was introduced in 2009 and we have collected data on 819 cases at the time of this analysis. For the present study we analysed data on a sample of 200 cases, 100 cases from 2009 and 100 cases from 2014. These cases were followed up for clinical outcome after the final diagnosis. We collected histopathology data from the hospital database,clinical data from the clinicians and patient's electronic notes.
Results: In malignant conditions, the histopathological diagnosis in comparison to final clinical diagnosis in malignant conditions had 94.7% accuracy, 94.7%sensitivity and 98.9%specificity. In benign conditions, the sensitivity was 98.9% and specificity is 94.9%. The accuracy was the same as for malignant disease. The initial clinical impression had a sensitivity of 99%, specificity 66.7% and accuracy 85% in malignant conditions whilst in benign conditions, the values were sensitivity 66.7%, specificity 99% and accuracy 85%. In summary, in this experienced centre, EBUS-TBNA has very good sensitivity, specificity and accuracy for benign and malignant conditions and compares with the previous studies.

O3

Impact Evaluation of BDIAP / IBMS Sponsored Training Workshops in Basic Histology and Immunohistochemistry in East Africa

Ⓟ O Rotimi[1]; P Jackson[1]; E Vuhahula[2]; A Kalebi[3]; AJ Howat[4]

[1]St James's University Teaching Hospital, Leeds, UK; [2]Muhibili National Hospital, Dar-es-Salaam, Tanzania; [3]Lancet Pathology, Nairobi, Kenya; [4]Department of Histopathology, Royal Blackburn Hospital, Blackburn, UK

Introduction: A situational assessment of histopathology practice in East Africa revealed poor quality of H&E sections and a serious need to improve the technical quality. A consultant pathologist and biomedical scientist (BMS) from the UK in collaboration with local organisers delivered workshops to train BMS and pathologists in the region.
Methods: 160 participants from 6 countries (Tanzania, Kenya, Uganda, Rwanda, Malawi and Seychelles) attended 4 workshops (each one-week) over 2 years. This impact evaluation using a mail questionnaire survey was performed one year after the workshops to assess the workshops effectiveness in improving the technical capacity. 25 participants (20 BMS and 5 pathologists) from 5 countries responded to the survey.
Findings: All trainees claim to currently use the taught basic histology and special staining techniques. Similarly, trainees have started performing quality control and assurance procedures. 18 respondents reported that the interaction between pathologists and BMS occurred more since the workshops on the subject of recognition and correction of artefacts. Respondents rated the organization of the workshops high and appreciated their usefulness in terms of their improved skills and performance, provision of international exposure and inter-lab networking and increased awareness of current histopathology practices. They face challenges in applying all they learnt in their labs with problems of lack of supply of reagents. They recommended that sponsors should continue the workshops, visit labs to assess the post-training practice and support labs to enable post-training application of taught skills.
Conclusions: The workshops have indeed improved histopathology technical capacity of participants and they have been sensitized to improve quality of diagnosis through recognition and correction of artefacts, performance of quality control and improved interaction between pathologists and lab scientists.

O4

Remote Teaching of Histopathology Using Scanned Slides via Skype® Between the UK and Nigeria

O Rotimi[1];Ⓟ NO Orah[2]; A Shabaan[3]; AO Daramola[4]; FB Abdulkareem[4]

[1]St James's University Teaching Hospital, Leeds, UK; [2]Lagos University Teaching Hospital (L.U.T.H.), Idi Araba, Nigeria; [3]Queen Elizabeth Hospital, Birmingham, UK; [4]Lagos University Teaching Hospital, Idi Araba, Nigeria

Purpose of Study: Web based learning is a major component of distance education. We explore its applications for pathology teaching in resource limited sub-Saharan Africa.
Methods: The participants were consultant pathologists and trainees drawn from tertiary institutions in Nigeria. They viewed the digital slides via the Leeds virtual pathology website following which interactive lectures were given via Skype®. Questionnaires were administered via SurveyMonkey® to all participants of twelve sessions between 2014 and 2015.
Summary of Results: Nine consultant pathologists and thirty two trainees participated in this survey. Twenty-nine (69%) of the respondents thought it was fairly easy to navigate the system, 11 (26.2%) thought it was easy while 2 (4.8%) felt it was difficult. Twenty-six(61.9%) respondents found it fairly easy to make a make a diagnosis, 13 (31%) thought it was easy, while three (7.1%) noted that it was difficult. Twenty-four (57.1%) respondents had a fairly smooth user experience, 12 (28.6%) experienced occasional crashes while six (14.3%) reported a smooth experience. Almost all (97.6%) of the respondents felt the pathology teaching was beneficial to their local pathology practice and all indicated their desire for more of such sessions.
Conclusions: The beneficial applications of internet based lectures make it a viable cheaper, faster and cost effective alternative to face-to-face lectures in delivering education to resource-limited countries.
This project was for the purpose of continuing medical education and was funded by an Educational Grant (EGS 2013 10 05) from the Pathological Society of Great Britain & Ireland.

O5

Development of a Pathology Training Programme in Zambia

Ⓟ RJ Byers[1]; AJ Merritt[2]; C Marimo[3]; V Mudenda[3]; K Fleming[4]

[1]Manchester Royal Infirmary, Manchester, UK; [2]Blackburn Royal Hospital, Blackburn, UK; [3]University Teaching Hospital, Lusaka, Zambia; [4]Centre for Global Health, Washington, USA

Pathology in Zambia shares problems with many other low income countries, especially low levels of staffing, equipment and reagents, variable service standards, long TAT, limited IHC tests and limited quality assurance. Until recently it had just 7 pathologists, in 5 departments, for a population of 13M. There was no local training programme prior to 2011, with trainees sent to UK/US/ and South Africa, most of whom did not return to work in Zambia. To address this, a Zambian training programme was supported with funding by DfID through the Tropical Health Education Trust (THET).

The 4 year training programme started in September 2011 with 4 students, followed by 3 in 2012 and 4 in 2014. Initially students rotate for 5 months through microbiology, chemical pathology and haematology, followed by cellular pathology for the remainder of the 4 years. All aspects of cellular pathology are covered and in the 3rd year students undertake 2 x 4 month international placements (Aga Khan Hospital and Kenyata National Hospital, Nairobi, Kenya, and Toronto), together with a 4 month research project. Graduation in the 4th year takes place after passing final practical and theoretical examinations and submission of research dissertation. External faculty visit from the UK to provide specialized input in liver, forensic, GI, urology and haematopathology, and cytology is supported locally and by external placements. There have been logistical hurdles but 2 of the first cohort of 4 students graduated in 2015 and will support training of subsequent cohorts, demonstrating success of the programme and providing a foundation for future growth.

O6

The Merits of Reverse Phase Protein Array for Molecular Classification of Breast Cancer

Ⓟ OH Negm; AA Muftah; MA Aleskandarany; MR Hamed; DAJ Ahmad; CC Nolan; MD Rodriguez; PJ Tighe; IO Ellis; EA Rakha; AR Green

University of Nottingham, Nottingham, UK

Purpose of the Study: This study aimed to confirm the ability of Reverse Phase Protein Array (RPPA) as alternative to IHC for molecular classification of breast cancer using the Nottingham Prognostic Index Plus (NPI+).

Methods: Proteins were extracted from archival formalin-fixed paraffin-embedded (FFPE) breast cancer tissues using different extraction protocols. Three preparation methods, full-face sections, Laser capture microdissection and macrodissection, were used to assess the yield and quality of protein extracts. Ten biomarkers used for the NPI+ (ER, PgR, HER2, Cytokeratins 5/6 and 7/8, EGFR, HER3, HER4, p53 and Mucin 1) were quantified using RPPA and compared to results determined by IHC.

Summary of Results: The commercial available Q-proteome FFPE Tissue Kit produced significantly higher protein concentration and signal intensities. The intra- and inter-array reproducibility assessment indicated that RPPA using FFPE lysates was highly reproducible and robust technique. The protein yield from the microdissected cases was insufficient to check the targets of interest using RPPA. Expression of the biomarkers individually and in combination using RPPA was highly consistent with IHC results. Macrodissection of the invasive tumour component gave more reliable results with the majority of biomarkers determined by IHC, (80% concordance) compared with full-face sections (60% concordance).

Conclusions: RPPA could be reliably used in molecular classification of BC, such as the NPI+, through fast and reliable proteomic quantification of multiple proteins in BC samples allowing patient stratification according to BC progression, risk of recurrence, and response to therapy.

O7

Vesicular Transporter Proteins in Invasive Breast Cancer: Clinicopathological Relevance of Synaptosomal-Associated Protein-23 (SNAP23)

Ⓟ SN Sonbul[1]; A Mukherjee[1]; MA Aleskandarany[1]; R Russell[2]; O Rueda[2]; AR Green[1]; CC Nolan[1]; M Diez-Rodriguez[1]; E Provenzano[3]; C Caldas[3]; IO Ellis[1]; EA Rakha[1]

[1]Department of Pathology, School of Medicine, University of Nottingham, Nottingham, UK; [2]CRUK Cambridge Research Institute, University of Cambridge, Cambridge, UK; [3]Addenbrooke's Hospital, Cambridge Breast Unit, Cambridge University Hospital NHS Foundation Trust, Cambridge, UK

Purpose of the Study: Vesicular transportation and efficient focal exocytosis of proteins are essential for progression and metastasis of invasive breast cancer (BC). In our search for genes driving lymphovascular invasion (LVI) in BC utilising the well-characterised METABRIC cohort, Synaptosomal-associated protein-23 (SNAP23), a member of the SNARE protein family, was revealed as an important differentially expressed gene correlated with LVI status.

Methods: To further validate the role of SNAP23 in BC, the cytoplasmic (CP) and membranous (MB) expression levels of SNAP23 was assessed immunohistochemically on a large annotated series of BC (n= 1240).

Summary of Results: Protein expression of SNAP23 was observed in the membrane (57.3%) and cytoplasm (33.7%) of invasive tumour cells. SNAP23-MB expression was associated with site-specific distant metastasis, namely to the pleura (75.9%;p<0.041). Also, SNAP23-MB was positively correlated with the BRCA1-associated RING domain protein BARD1 (58.8%;p<0.027) while the expression of SNAP23-CP was negatively associated with the Metastasis-associated protein MTA1(62.5%;p<0.022). No significant association between SNAP23 and LVI was identified at the protein level.

Conclusions: SNAP23 may mediate pathways of the invasion-metastasis cascade and could potentially govern the tropism of metastatic BC cells to specific distal organs. Though not directly correlated with LVI at the protein level in spite of correlations at the transcriptome level, it may be an important passenger in the complex molecular cascade controlling metastatic mechanisms.

**AM supported by NIHR and the Academy of Medical Sciences.*

O8

The Prognostic Significance of KRAS-Induced Actin-Interacting Protein (KRAP) Expression in Invasive Breast Cancer

Ⓟ C Joseph[1]; R Russell[2]; M Craze[1]; C Nolan[1]; E Provenzano[3]; C Caldas[4]; A Green[1]; E Rakha[5]; IO Ellis[5]; A Mukherjee[5]

[1]Department of Histopathology, School of Medicine, University of Nottingham, Nottingham, UK; [2]CRUK Cambridge Research Institute, Cambridge, UK; [3]Addenbrooke's Hospital, Cambridge University Hospital NHS Foundation Trust, Cambridge, UK; [4]CRUK Cambridge Research Institute, Addenbrooke's Hospital, Cambridge, UK; [5]Department of Histopathology,Nottingham University Hospitals NHS Trust, Nottingham, UK

Introduction: KRAP, a gene involved in cell proliferation, differentiation, and apoptosis, is upregulated by the activated K-Ras pathway [Xiao et al, PLoS One. 2014:9(10)]. However, its role in breast cancer (BC) is unclear. Differential expression analysis from the METABRIC series identified KRAP as a gene correlated with survival in low grade ER positive BCs (adjusted p=0.04). This study examined KRAP expression at the protein level in BC and its correlations with clinicopathological variables and survival.

Methods: Immunohistochemical (IHC) expression of KRAP was studied in a large series of invasive BCs (n=1047) and correlated with clinico-pathological and molecular variables. Patient outcome was assessed through Kaplan-Meier survival curves.

Results: On IHC, high KRAP expression was observed in the cytoplasm (82.1%) of invasive BC cells. Significant correlations were observed between high KRAP protein expression and low grade (p=0.001), low stage (p=0.023), ER+PR+ (p<0.001) BCs with good Nottingham Prognostic Index (p=0.003). Positive correlations with GATA3 (p=0.005), AURKA (p=0.0001), STAT3 (p= 0.005) and p21 (p= 0.014) and negative correlations with p53 (p=0.016) protein expression indicate possible roles in allied signalling pathways. Loss of KRAP was associated with poorer breast cancer specific survival on both univariate (p=0.014) and multivariate analysis (p=0.037).

Conclusions: Results highlight KRAP as a novel marker for low grade, ER positive BCs of favourable prognosis, with loss of KRAP resulting in poorer outcome. Investigating interactions with related signalling pathways will further unravel the role of KRAP in breast tumourigenesis.

**Project supported by NIHR and CDF from PathSoc.*

O9

PALB2 is Downregulated in Sporadic Triple Negative Invasive Breast Cancer and is Associated with Poor Prognosis

Ⓟ S Gurhan[1]; MA Aleskandarany[1]; CC Nolan[1]; M Diez-Rodriguez[1]; D Caracappa[2]; RD Macmillan[1]; S Madhusudan[1]; IO Ellis[1]; EA Rakha[1]; AR Green[1]

[1]Nottingham City Hospital, Nottingham, UK; [2]S. Maria della Misericordia Hospital, University of Perugia, Perugia, Italy

Purpose of the Study: The Partner And Localiser of BRCA2 (PALB2) plays a critical role to achieve successful DNA Double Strand Break (DSB) homologous recombination repair (HRR). PALB2 is important in the localisation and stability of BRCA2 at DNA damage sites. Moreover, an interrupted BRCA1-PALB2 interaction results in defective HRR. This study aimed to investigate the expression of PALB2 in sporadic and BRCA mutated breast cancer (BC).
Methods: The expression of PALB2 was assessed in two independent clinically annotated cohorts of BRCA mutated cases (n: BRCA1=44, BRCA2=24, total=68) and sporadic cases (n=874) using immunohistochemistry (IHC). Expression was correlated to clinicopathological parameters, molecular biomarkers and patients' outcome.
Summary of Results: Within the sporadic BC cohort low PALB2 expression was significantly associated with higher tumour grade, ductal NST and medullary-like histological type, larger tumour size (p=0.015) poor NPI (p<0.001), ER- (p=0.004), PR- (p=0.026), CK14- (p=0.011), high Ki67 (p<0.001), BCL2- (p<0.001), E-cadherin- (p<0.001), ATM- (p<0.001), BRCA1- (p<0.001), BRCA2- (p=0.008), RAD51+ (p=0.009) ATR+ (p=0.002), and in triple negative (TN) and HER2+ BC (p=0.001). Within BRCA mutated cases, none of the clinico-pathological or the molecular characteristics demonstrated significant relationship with PALB2 expression. No significant difference was found between BRCA1 mutant, BRCA2 mutant, and sporadic BC regarding PALB2 expression (p>0.05). Reduced PALB2 and expression was significantly associated with shorter survival in the sporadic but not in the BRCA mutant cohorts.
Conclusions: Low PALB2 expression demonstrated significant associations with features of poor prognosis especially in the TN and HER2+ BC molecular subtypes. These findings reinforce the importance of the PALB2 relationship with HRR in invasive BC, and underscore the BRCA-ness features within TN BC.

O10

Radiological, Histological Features and Outcome of Classical Lobular Carcinoma In Situ with Comedo-Necrosis; A Multi Institutional Series

Ⓟ AM Shaaban[1]; N Elgeredly[2]; N Sharma[3]; S Sundararajan[4]; Y Maurice[5]; J Loane[6]; A Manoharan[7]

[1]Queen Elizabeth Hospital Birmingham, Birmingham, UK; [2]Stockport Foundation Trust, Stockport, UK; [3]St James's University Hospital, Leeds, UK; [4]Royal Stoke University Hospital, Stoke-on-Trent, UK; [5]Wirral University Hospital NHS Foundation Trust, Wirral, UK; [6]Western General Hospital, Edinburgh, UK; [7]The Royal Oldham Hospital, Oldham, UK

Introduction: Classical lobular carcinoma in situ (LCIS) with comedo necrosis is a recently described entity that is distinguished from pleomorphic LCIS (PLCIS) by lack of high-grade nuclei. Little is known about its natural history and optimal management.
Methods: Cases from 6 large breast cancer screening units were reviewed by specialist breast pathologists. Comprehensive imaging, histological and follow up data were collected.
Results: 17 cases fulfilled the diagnostic criteria (all were e-cadherin –ve). The mean age was 60.7yrs (range 42 – 86). Except for one FH screening patient, all were identified during routine mammography. 11 cases presented with calcification and six with stromal deformity. Imaging size ranged from 3 – 50mm. The lesion was the sole core biopsy diagnosis in 4 cases. Three lesions (17.6%) were multifocal. LCIS was associated with PLCIS in 4 cases (23.5%), DCIS in 5 (29.4%) and invasive carcinoma in 6 cases (35.3%). All three invasive carcinomas identified were of lobular type (classic and pleomorphic). Where lymph node sampling was done, 3/4 cases showed nodal metastasis, of which two had extensive disease (20 positive nodes). For pure LCIS with necrosis on core biopsy, excision biopsy showed similar changes with no associated DCIS, PLCIS or invasive disease. No recurrences and or deaths were reported in the study patients after a follow up of up to 30 months.
Conclusion: LCIS with necrosis is a rare entity that presents at an older age compared with classical LCIS. Mammographic calcification is the main presentation and the lesion can be multifocal. The lesion was associated with either in situ or invasive carcinoma in 53% of cases. In isolation , no cases were upgraded on further sampling. The data indicate that this lesion is a more aggressive form compared with classical LCIS and support categorising lesions on core biopsy as B4 for excision. Further prospective multicentre data collection is warranted to inform management of this rare disease.

O11

How Close are we to Standardised Extended *RAS* Gene Mutation Testing? The UK NEQAS Experience.

Ⓟ SD Richman[1]; J Fairley[2]; R Butler[3]; Z Deans[2]

[1]Leeds Institute of Cancer and Pathology, Leeds, UK; [2]UK NEQAS for Molecular Genetics, Edinburgh, UK; [3]Cardiff and Vale UHB - Medical Genetics, Cardiff, UK

Since 2008, *KRAS* mutation status in exon 2, has been used to predict response to anti-EGFR therapies. Over recent years, a growing body of evidence has demonstrated that *NRAS* status, in addition to *KRAS* status is predictive of response. Many retrospective 'extended *RAS*' analyses have now been performed on clinical trial material. Despite this, are we really moving towards such an extended screening practice in reality?
Data was analysed from four consecutive UK NEQAS for Molecular Genetics Colorectal cancer EQA schemes (during the period 2014 to 2016), with up to 110 laboratories (worldwide) participating in each scheme. Testing of 4-5 colorectal tumour samples is required per scheme. Laboratories were asked to provide information on which codons were routinely screened, and provide genotyping and interpretation results for each sample.
At least 85% of laboratories routinely tested *KRAS* codons 12, 13 and 61. Over the four schemes, there was an increase in the number of laboratories routinely testing *KRAS* codons 59, 117 and 146. There was an increase in the number of laboratories during this period introducing next generation sequencing technologies (NGS). The pattern of 'extended testing' was reassuringly similar for *NRAS*, although overall, fewer laboratories currently test for mutations in this gene. Alarmingly, still only 36.1% and 24.1% of participating laboratories met the ACP Molecular Pathology and Diagnostics Group and ASCO guidelines respectively, for extended *RAS* testing in the latest scheme Run.
Despite recommendations both in the UK and USA on extended *RAS* testing, there has clearly been, based upon these results, a delay in implementing 'extended testing'. Inadequate testing, results in patients being subjected to harmful treatment regimens, which would not be the case, were routine practice altered, in line with evidence-based guidelines.

O12

Characterisation of Arachidonic Acid Metabolising Enzymes in Colorectal Cancer

Ⓟ A Alnabulsi[1]; R Swan[1]; B Cash[2]; A Alnabulsi[2]; GI Murray[1]

[1]University of Aberdeen, Aberdeen, UK; [2]Vertebrate Antibodies, Aberdeen, UK

Colorectal cancer is one of the most common type of cancer in the developed world. Arachidonic acid is converted by individual cytochrome P450 enzymes to epoxyeicosatrienoic acids which have been implicated in various cancer-associated biological processes. Thus, the aim of this study was to profile the expression of arachidonic acid metabolising enzymes in primary colorectal cancer and assess the association between their expression and prognosis.
Monoclonal antibodies to CYP4A11, CYP4F11, CYP4V2 and CYP4Z1 were developed and successfully characterised by immunoblotting and immunohistochemistry.
The antibodies were then used to profile the expression of those enzymes by immunohistochemistry on a large and well characterised colorectal cancer cohort (n=650) arrayed on a tissue microarray. The immunohistochemistry results were interpreted by light microscopy using a semi-quantitative scoring system.
For each antibody, immunoblotting using lysate overexpressing the relevant protein resulted in a band migrating at the expected molecular weight. The expression of CYP4A11 (p<0.001), CYP4F11 (p<0.001) and CYP4V2 (p<0.001) were significantly higher in primary tumour when compared to normal mucosa. The presence of strong CYPA11 immunoreactivity was associated with significantly reduced survival in the whole patient cohort (χ^2=7.234, p=0.007) and in mismatch repair (MMR) proficient tumours (χ^2=9.404, p=0.002). CYP4F11 immunoreactivity was significantly associated with poor survival in MMR deficient tumours (χ^2=4.684, p=0.03).
In conclusion, arachidonic acid metabolising enzymes CYP4A11, CYP4V2 and CYP4F11 are significantly overexpressed in patients with colorectal cancer and high expression of CYP4A11 is significantly associated with poor survival.
RS was supported by the Jean Shanks Foundation.

O13

Comparative Analysis of Primary Tumour and Matched Metastasis in Colorectal Cancer Patients; Investigate Genomic, Transcription and Translational Profiles

Ⓟ OH Negm[1]; HH Karim[1]; K Manger[2]; J Hassall[1]; W Fadhil[1]; PJ Tighe[3]; M Ilyas[1]

[1]University of Nottingham, Nottingham, UK; [2]Nottingham University Hospitals NHS Trust, Nottingham, UK; [3]School of Life Sciences, University of Nottingham, Nottingham, UK

Purpose of the Study: Approximately 50% of patients with primary colorectal carcinoma develop liver metastases. This study proposes to investigate genetic discrepancies between primary colorectal cancer (CRC) and their respective metastasis.
Methods: A total of 22 pairs of primary CRC and metastasis were tested. Mutation profiling across 26 cancer-associated genes was undertaken. Expression of a panel of six miRNAs was tested and protein expression of 20 genes was measured using Reverse Phase Protein Array (RPPA).
Summary of Results: Among the primary tumours, the mutation frequencies were: TP53 (86%), APC (64%), KRAS (41%), PIK3CA (9%), SMAD4 (9%), NRAS (9%), BRAF (4%), GNAS (4%), FBXW7 (4%), and CDH1 (4%). In primary vs metastasis, four mutations were detected in primary which were not detected in metastasis and two mutations were detected in metastasis which were not present in the primary. However, no significant differences were seen in mutation profiles between primary CRC and metastasis. Of the six miRNA, three had significantly higher expression levels in the metastases than the corresponding primary tumour (miR-20a ($p<0.02$), miR-21 ($p<0.02$), miR-31 ($p<0.03$) and miR-92a ($p<0.02$)). Regarding RPPA data, only the protein expression of CD34 has significantly increased in the metastases than primary cases ($p<0.04$).
Conclusions: Our data show that the mutation profiles of primary tumours and metastases are similar and, for mutation-based stratification, analysis of the primary tumour is sufficient. There are some differences in miRNA and protein expression although the mechanism of change and functional significance of these remains uncertain.

O14

ADAM-17/FHL2 Colocalisation Suggests Interaction and Role of These Proteins in Colorectal Cancer

L Verset[1]; J Tommelein[2]; C Decaestecker[3]; I Salmon[1]; O De Wever[2]; Ⓟ P Demetter[1]

[1]Department of Pathology, Erasme University Hospital, Brussels, Belgium; [2]Department of Radiation Oncology and Experimental Cancer Research, UGent, Ghent, Belgium; [3]Digital Image Analysis in Pathology (DIAPATH), CMMI, Gosselies, Belgium

Purpose of the Study: We showed that high FHL2 expression in colorectal cancer is associated with poor prognosis. In order to elucidate FHL2 implication in colorectal carcinogenesis, we wanted to identify a potential partner of FHL2 in this process. ADAM-17 is a metalloprotease belonging to the ADAM family; it is the main sheddase of the EGF pathway. FHL2 deficiency leads to decreased activity of ADAM-17 in mouse macrophages.
Methods: We studied FHL2 and ADAM-17 expression in HCT8E11, HT29, SW480 and SW620 colon cancer cell lines by immunocytochemistry. To highlight possible interaction between FHL2 and ADAM-17, we used the Duolink® kit for proximity ligation assay (PLA) on SW480 cells. We also performed PLA on 10 biopsies and 9 surgical specimens (validation cohort) of colorectal adenocarcinoma and on matched normal colonic mucosa from the same patients. Furthermore, 10 biopsies of colorectal adenoma with matched normal colonic mucosa were selected. For quantification, 5 (biopsies) and 20 (validation cohort) pictures (magnification x400) of the malignant, adenomatous and normal tissues were taken. PLA signals were counted and mean number of PLA signals and of PLA signals/nucleus were calculated for each sample.
Summary of Results: All cell lines showed FHL2 immunoreactivity; strongest positivity was observed in SW480 cells. ADAM-17 was expressed in all cell lines. PLA signals were present in SW480 cells. Quantitative analysis revealed that the interaction between FHL2 and ADAM-17 is more frequent in malignant than in normal tissue ($p=0.005$). Mean number of ADAM-17/FHL2 PLA signals was higher in colorectal adenocarcinoma than in adenoma with low-grade dysplasia ($p=0.0004$).
Conclusions: FHL2 interacts with ADAM-17 in normal, dysplastic and malignant colon epithelial cells. Colocalisation of these proteins is more frequent in malignant than in normal and dysplastic cells, suggesting a role for ADAM-17/FHL2 interaction in the development of colorectal cancer.

O15

Short Term Pathology Results from the First World Wide Randomised Trial of Robotic Versus Laparoscopic Resection for Rectal Cancer (ROLARR)

Ⓟ NP West[1]; DG Jayne[1]; A Pigazzi[2]; H Marshall[1]; J Croft[1]; N Corrigan[1]; J Copeland[1]; T Rautio[3]; N Thomassen[4]; H Tilney[5]; M Gudgeon[5]; P Bianchi[6]; JM Brown[1]; P Quirke[1]

[1]University of Leeds, Leeds, UK; [2]University of California, Irvine, USA; [3]Oulu University Hospital, Oulu, Finland; [4]Aarhus Hospital, Aarhus, Denmark; [5]Frimley Park Hospital, Frimley, UK; [6]Ospedale La Misericordia, Grosseto, Italy

Purpose of the Study: The clinical benefit of robotic surgery in rectal cancer, and many other cancers, is unknown.
Methods: We undertook the first worldwide randomised trial of robotic versus laparoscopic resection for rectal cancer (ROLARR) between 2011 and 2014. 1276 patients were assessed for eligibility by 40 surgeons from 26 sites across 10 countries. 471 (36.9%) of these patients were randomised; 234 to laparoscopic (LAP) and 237 to robotic (ROB) surgery. 466 patients underwent operation with 456 (97.9%) undergoing the allocated treatment.
Summary of Results: The primary end point was overall rate of conversion to open surgery, which was LAP 28/230 (12.2%) vs. ROB 19/236 (8.1%) (adjusted OR 0.614, 95% CI 0.311 to 1.211, $p=0.158$). No differences were seen in bladder or sexual function at 6 months. 76.4% of tumours were stage pT2 or pT3. Mean lymph node yields were high in both arms (23.6, SD 12.43) and 35.9% of cancers were node positive. The overall circumferential resection margin involvement (CRM+) rate was 26/459 (5.7%) with similar odds between the arms (adjusted OR 0.785, 95% CI 0.350 to 1.762, $p=0.557$). No distal margin and one laparoscopic proximal margin were involved by tumour. Local pathological assessment of the quality of surgery following anterior resection was of the highest standard in 75.3% of cases, with no difference between the laparoscopic or robotic groups. Central review of the slides and photographs is currently on-going.
Conclusions: ROLARR has shown that both laparoscopic and robotic rectal cancer surgery, when performed by experienced surgeons, can achieve excellent short-term outcomes with low CRM+ rates, low conversion rates to open surgery, high lymph node yields, respectable pathological specimens, and low rates of postoperative bladder and sexual dysfunction. It sets a new standard for the quality of pathology examination in bowel cancer trials.
Acknowledgement: This work is supported by a PathSoc Career Development Fellowship.

O16

Parietal Cell, Chief Cell Densities and Intragastric Acidity are Reduced in Healthy Volunteers with H.pylori Infection Relative to Ininfected Volunteers

Ⓟ MH Derakhshan[1]; DR Mitchell[1]; AA Wirz[1]; SA Ballantyne[2]; A Weir[2]; KEL McColl[1]; JJ Going[1]

[1]University of Glasgow, Glasgow, UK; [2]NHS Greater Glasgow & Clyed, Glasgow, UK

Introduction: H.pylori is negatively associated with gastroesophageal reflux disease (GORD) and oesophageal adenocarcinoma. We studied healthy volunteers to determine if H.pylori is associated with reduced gastric secretory cell densities and peptic secretions.
Methods: 31 H.pylori positive (Hp+) and 29 H.pylori negative (Hp-) subjects were matched for age, gender and BMI. Orientated biopsies were taken at 11 standard locations: gastro-oesophageal junction (GOJ; x4), mid-body lesser curve, incisura angularis, distal body greater curve, mid body greater curve, fundus and antrum. Parietal and chief cells (PC, CC) were counted in sections immunostained for H+/K+ATPase and pepsinogen I. High resolution 12-sensor pHmetry and 36-sensor manometry were performed fasting and after a standard meal. The squamocolumnar junction (SCJ), marked with two endoscopically placed clips, was located radiologically relative to probes.
Results: Both PC and CC densities were lower in Hp+ than Hp- subjects in 10 / 11 gastric locations (all $p<0.01$). Median PC/CC densities (greater curve, mid-body) were 235/285 mm-2 (Hp+) versus 361/401 mm-2 (Hp-); $p<0.001$. Cardiac mucosa was longer (1.5mm Hp+ vs 0.7mm Hp-, $p<0.01$). Fasting median pH at all sensors > 1.1cm distal to peak LES pressure was greater in Hp+ subjects. pH transition was further from the SCJ (2.6cm Hp+ v. 1.2cm Hp-; $p=0.034$). Postprandial intragastric acidity was less in Hp+ than Hp- subjects at sensors 2.2-4.4 cm distal to peak LES pressure (all $p<0.05$), i.e. the postprandial acid pocket was less evident in Hp+ than Hp- subjects. LES length (4.2cm v 4.2cm; $p=1.0$) and distance from peak LES pressure to SCJ (1.3cm v 1.8cm; $p=0.19$) were the same.
Conclusions: Most H.pylori infected subjects have reduced parietal and chief cell densities and intragastric acidity compared to uninfected subjects. Acidity is most reduced close to the GOJ. Reduced peptic stress may explain the negative association of H.pylori infection with GORD and oesophageal adenocarcinoma.

O17

Longitudinal Single Cell Clonal Analyis Reveals Evolutionary Stasis and Predetermined Malignant Potential in Non-Dysplastic Barrett's Esophagus

Ⓟ P Martinez[1]; MR Timmer[2]; CT Lau[2]; CC Maley[3]; TA Graham[1]; KK Krishnadath[2]

[1]Barts Cancer Institute, London, UK; [2]Amsterdam Medical Centrum, Amsterdam, Netherlands; [3]Arizona State University, Phoenix, USA

Surveillance of Barrett's esophagus allows us to study the evolutionary dynamics of a human neoplasm over time. We used multicolour fluorescence in situ hybridization on brush cytology specimens, from two time points with a median interval of 37 months in 195 non-dysplastic patients, and a third time point in a subset of 90 patients at a median interval of 36 months, to study clonal evolution at single cell resolution. Baseline genetic diversity significantly predicted progression and remained in a stable dynamic equilibrium over time. Clonal expansions were rare, being detected once every 36.8 patient years, and growing at an average rate of 1.58cm2 (95% CI: 0.09 – 4.06) per year, often involving the p16 locus. This suggests a lack of strong clonal selection in Barrett's and that the malignant potential of 'benign' Barrett's lesions is predetermined, with important implications for surveillance programs.

O18

The MERCURY II Study: Prospective Validation of a Low Rectal Cancer Assessment System Using Magnetic Resonance Imaging, and Development of a Local Recurrence Risk Stratification Model

NJ Battersby[1]; P How[1]; BJ Moran[2]; S Stelzner[3];Ⓟ NP West[4]; G Branagan[5]; J Strassburg[6]; P Quirke[4]; P Tekkis[7]; BG Pedersen[8]; A Gudgeon[9]; RJ Heald[10]; G Brown[11]

[1]Pelican Cancer Foundation, Basingstoke, UK; [2]North Hampshire Hospital, Basingstoke, UK; [3]Dresden-Friedrichstadt General Hospital, Dresden, Germany; [4]University of Leeds, Leeds, UK; [5]Salisbury District Hospital, Salisbury, UK; [6]Vivantes im Friedrichshain, Berlin, Germany; [7]The Royal Marsden NHS Foundation Trust, London, UK; [8]Aarhus University Hospital, Aarhus, Denmark; [9]Frimley Park Hospital, Frimley, UK; [10]Pelican Cancer Foundation, Basingstoke, UK; [11]The Royal Marsden Hospital, Sutton, UK

Purpose of the Study: Surgery for low rectal cancer is associated with poorer oncological outcomes. There is a high rate of pathological circumferential resection margin (pCRM) involvement and unacceptable variations in permanent colostomies. This study aimed to validate a pre-operative magnetic resonance imaging (MRI) assessment system that determined the relationship between the tumour and the low rectal cancer surgical resection plane (mrLRP).
Methods: MERCURY II was a prospective, observational, multicentre study that recruited 279 patients with adenocarcinoma 6 cm or less from the anal verge between 2008 and 2012. Patients underwent routine pre-operative MRI, and were assessed according to the following criteria: mrLRP "safe or "unsafe, extramural venous invasion (mrEMVI), depth of spread, lymph node status, tumour height, and tumour quadrant. MRI-based treatment recommendations were compared against final management and pCRM outcomes.
Summary of Results: Overall pCRM involvement was 9.0% (95% CI 5.9 to 12.3). Patients with no adverse MRI features and a "safe mrLRP underwent sphincter-preserving surgery without pre-operative radiotherapy with a pCRM rate of 1.6%. The pCRM rate increased for an "unsafe compared with "safe pre-operative mrLRP (OR 5.5; 95% CI 2.3 to 13.3). Post-treatment MRI reassessment indicated a "safe ymrLRP in 33 of 113 (29.2%), none of whom had ypCRM involvement. In contrast, persistent "unsafe ymrLRP resulted in 17.5% ypCRM involvement. Other independent MRI assessed risk factors were EMVI (OR 3.8; 1.5 to 9.6), tumours less than 4 cm from the anal verge (OR 3.4; 1.3 to 8.8), and anterior tumours (OR 2.8; 1.1 to 6.8).
Conclusions: The study validated MRI low rectal plane assessment, significantly reduced pCRM involvement (previously 30%) and avoided overtreatment through selective preoperative therapy and rationalised use of permanent colostomy.
Acknowledgements: The work is supported by a Pathsoc Career Development Fellowship.

O19

Exploring the Suitability and Stability of Guaiac Faecal Occult Blood Test Cards to Isolate Microbial DNA for 16srRNA Sequencing

Ⓟ M Taylor[1]; HM Wood[1]; S Halloran[2]; P Quirke[1]

[1]Leeds Institute of Cancer and Pathology, Leeds, UK; [2]University of Surrey, Guildford, UK

Purpose of the Study: The human gut microbiome has been linked to many human diseases, with a growing interest in the influence it might have on the development of colorectal cancer. The aim of this study was to investigate the feasibility of using the National Health Service Bowel Screening Programme (NHSBCSP) guaiac faecal occult blood test (gFOBt) cards for microbial analysis by profiling matched gFOBt and fresh stool samples. We also explored whether the long term storage of gFOBt cards had a detrimental effect on microbiome analysis.
Methods: Three healthy volunteers each provided three separate fresh stool samples. These samples were applied to gFOBt cards, and the microbial DNA was isolated from the paired fresh stool and gFOBt card samples. We also examined the stability of microbiome extract in fresh stool samples on gFOBt cards stored at room temperature over a period of 2 weeks to 2 years. The V4 region of the 16srRNA gene was amplified, and libraries sequenced on an Illumina MiSeq. Data was analysed using the QIIME software.
Results: Between 16971 and 85790 reads per sample were obtained (median 44685). Samples were grouped both by volunteer, and by sample type (fresh or gFOBt). Groups were compared in a variety of ways: visual inspection of taxa, within sample beta diversity, principle component analysis and t-tests of weighted and unweighted UniFrac distances. By all methods, samples grouped by volunteer and not by sample type. Analysis of the different time points showed no appreciable differences with increased storage time.
Conclusions: This study identified good concordance between microbial DNA isolated from gFOBt and fresh stool samples. Samples stored over 2 years showed no detrimental changes in the bacteria identified. This study has important implications for the ease of performing large scale microbiome studies, and the potential for future population or patient screening for colorectal cancer and other pathologies.

O20

Digital Microscopy is Non-inferior to Optical Microscopy in the Diagnosis of Dysplasia in Barrett's Oesophagus: A Validation Study

Ⓟ C Marchessoux[1]; PV Kaye[2]; A Mukherjee[2]; S Paine[2]; A Haider[2]; M Ilyas[3]

[1]Barco, Kortrijk, Belgium; [2]Nottingham University Hospitals, Nottingham, UK; [3]University of Nottingham, Nottingham, UK

Background and Aims: Digital Microscopy (DM) is considered by many as an alternative to traditional Optical Microscopy (OM). However, it is uncertain whether DM is equivalent to OM in difficult cases where discrimination of diagnostic categories may rely on subtle morphological variations. We compared DM with OM in the notoriously difficult area of dysplasia in Barrett's oesophagus (BO).
Materials and Methods: Digitized images of H&E stained sections and immunostaining for p53 were examined in 69 cases of BO. Images were viewed by 3 expert GI pathologists and 2 non-expert pathologists. Inter-observer variation for (i) the scoring of p53 and (ii) for diagnosis of dysplasia was evaluated using several different metrics. Data obtained using the DM were compared with data previously obtained using the OM.
Results: Data analysis in all metrics showed equivalence of DM and OM. For example, for p53, weighted kappa values for the 3 experts were OM – 0.688, DM – 0.814. For all 5 pathologists, the value was DM – 0.793. For diagnosis of dysplasia, weighted kappa values were OM – 0.45, DM – 0.457. Comparison of the consensus DM diagnosis with the consensus OM diagnosis showed very good concordance for p53 scoring and good for dysplasia diagnosis (kappa value 0.96 and 0.76 respectively).
Conclusions: The performance of Pathologists in the diagnosis of dysplasia in Barrett's oesophagus is nearly identical when using either DM or OM. Our study supports DM as a non-inferior means of delivering diagnostic services to OM.

O21

Ankyrin 3 Protein may Influence DNA-Damage Response Pathways and the Immune Response in Invasive Breast Carcinoma

SN Sonbul;Ⓟ M Pigera; A Mukherjee; MA Aleskandarany; AR Green; M Diez-Rodriguez; C Joseph; CC Nolan; IO Ellis; EA Rakha

Department of Pathology, School of Medicine, University of Nottingham, Nottingham, UK

Purpose of the Study: Ankyrin3 (ANK3), extensively studied in neuronal signalling, is a member of the adapter protein family maintaining the plasma membrane scaffold in epithelial cells through interactions with E-Cadherin. It has been implicated in colorectal carcinogenesis [BMC Genomics. 2011 Oct 14; 12:505] and bladder tumour recurrence [Oncotarget, 2016 Jan 19; 7(3): 2629]. However, its role in breast cancer (BC) remains unknown. This study investigated the protein expression of ANK3 in BC and correlations with clinicopathological variables.
Methods: Immunohistochemistry procedure was applied on tissue microarray (TMA) sections of well-characterised series of BC (n=1234). Statistical analysis was performed for clinicopathological correlations and associations with other biomarkers.
Summary of Results: The expression of ANK3 protein was cytoplasmic; with the majority of cases (67.6%) showing high expression levels. Significant positive associations were identified with DNA-damage response markers including p53 (p=0.029), CHK1 (p=0.01), PARP1 (p=0.001), BARD1 (p=0.001), Ku70/Ku80 (p= 0.034). In terms of immune-related markers, high ANK3 expression was positively associated with B lymphocyte marker, CD20 (p=0.036) and T lineage regulator FOXP3 (p=0.005). No direct associations were observed between ANK3 and clinico-pathological parameters.
Conclusions: ANK3 protein in BC interacts with DNA damage response pathways including p53 suggesting a role for membrane scaffold proteins during DNA damage repair. The positive association of CD20/FOXP3 and ANK3 expression potentially mirrors the complex partnership on immune system regulation by novel signalling proteins like ANK3. Further functional characterisation will unravel the pathobiology of ANK3 in BC.
**MP and AM supported by grants from the PathSoc.*

O22

Further Evidence to Support that Oestrogen Receptor Expression in Breast Cancer is Bimodal

Ⓟ AA Muftah[1]; MA Aleskandarany[1]; SN Sonbul[1]; CC Nolan[1]; M Diez Rodriguez[1]; C Caldas[2]; IO Ellis[1]; AR Green[1]; EA Rakha[1]

[1]University of Nottingham, School of Medicine, City Hospital Campus, Division of Cancer and Stem Cells, Nottingham, UK; [2]Addenbrooke's Hospital,Cambridge University Hospital NHS Foundation Trust, Cambridge, UK

Although oestrogen receptor (ER)-negative breast cancers (BC) do not respond to hormone therapy, the response of ER-positive BC is reported to be variable which may suggest a dose dependent effect. This study aimed to assess the pattern of ER-expression at the protein (IHC) and transcriptome (microarray-based gene expression) levels.
Methods: ER IHC expression was assessed on a large series of BC including 3649 core biopsies and 1887cases prepared as TMA stained using different but recent more sensitive detection systems. ESR1 mRNA expression was assessed on the METABRIC study (1980 cases). The gene expression data was analysed by Linear Models for Microarray Data (LIMMA) and results were compared with protein levels.
Results: Bimodality of ER IHC expression was evident on our series with 92.2% and 89.2% of the cases showed completely negative (0%) or highly positive (≥70%) expression on the cores and TMA respectively. While weak positive cases (1-10%) and intermediate-expression (11%–69%) cases were infrequent (2.7% & 5.1% and 1.6% & 9.2% respectively). Interestingly, re-staining full-face sections of the low and intermediate groups revealed the large majority often related to the negative or strongly positive classes. The METABRIC data not only showed significant correlation between ESR1 mRNA and protein expression but also confirmed the bimodality of ER at the mRNA levels.
Conclusion: Our study provides further evidence that ER expression is essentially bimodal and that this bimodality is observed at both mRNA and protein levels. The reported poor survival of BC patients with low ER-expression in the early clinical trials may be related to the inclusion of ER-negative cases.

O23

Exploring Molecular Portraits Defining Low Grade in Breast Cancer

Ⓟ A Mukherjee[1]; R Russell[2]; OM Rueda[2]; S Hamarsheh[3]; C Caldas[4]; E Provenzano[5]; IO Ellis[3]

[1]Department of Histopathology, Nottingham University Hospitals NHS Trust, Nottingham, UK; [2]CRUK Cambridge Research Institute, Cambridge, UK; [3]Department of Histopathology, School of Medicine, University of Nottingham, Nottingham, UK; [4]CRUK Cambridge Research Institute, Addenbrooke's Hospital, Cambridge, UK; [5]Addenbrooke's Hospital, Cambridge University Hospital NHS Foundation Trust, Cambridge, UK

Introduction: The low grade (LG) oestrogen receptor (ER) positive breast cancers (BCs) may be considered as the least aberrant on the BC evolution scale. It is essential to identify the drivers of neoplasia within the LG group in contrast to high grade BCs. This study investigated the molecular portraits associated with low grade ER positive BCs interrogating subsets of the METABRIC (Molecular Taxonomy of Breast Cancer International Consortium) series.
Methodology: Histological Grade 1 ER positive ductal NSTs (n=96) were compared with higher grade ER positive ductal NSTs (n=818) on the METABRIC BC cohort. Genes correlating with grade were identified from expression profiles using Linear Models for Microarray (LIMMA) analyses. Differentially enriched pathways were explored on multiple platforms (GSEA, Metacore).
Results: Initial analysis identified 3275 genes significantly differentially expressed between grade 1 versus higher grade ER positive NSTs (adjusted p value <0.05). Top ranked genes positively correlated with low grade include the laminin related protein, Netrin4; an endothelial nitric oxide synthase regulator, Nostrin; the circardian gene CRY2; extra-cellular matrix regulator, COL16A1; a mammary restricted cytochrome, CYP4Z1 etc. Top ranked genes correlated with high grade include pituitary transforming genes PTTG1, PTTG3P; ubiquitination genes UBE2S, UBE2C and mitosis regulators CDK1, AURKA, MAD2L1, NUSAP1 etc. Pathway analysis on GSEA and Metacore analysis reveal significant overlapping enrichments in proteinaceous extracellular matrix pathways for Grade1 while mitotic pathways dominate in higher grade ER+ BCs.
Conclusion: Expression profiling alongside histopathological characterisation provides useful molecular portraits relevant to grade. Further analysis of copy number alterations followed by functional analysis is deemed useful.
**This work is supported by NIHR and the Pathological Society of Great Britain & Ireland.*

O24

Nostrin as a Marker Related to Low Grade and Better Prognosis in Breast Cancer

S Hamarsheh[1]; M Craze[1]; C Joseph[1]; E Provenzano[2]; R Russell[3]; C Caldas[4]; AR Green[1]; E Rakha[5]; IO Ellis[5];Ⓟ A Mukherjee[5]

[1]Department of Histopathology, School of Medicine, University of Nottingham, Nottingham, UK; [2]Addenbrooke's Hospital, Cambridge University Hospital NHS Foundation Trust, Cambridge, UK; [3]CRUK Cambridge Research Institute, Cambridge, UK; [4]CRUK Cambridge Research Institute, Addenbrooke's Hospital, Cambridge, UK; [5]Department of Histopathology,Nottingham University Hospitals NHS Trust, Nottingham, UK

Purpose of the Study: Through interrogation of the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) cohort, Nostrin, a member of protein adaptors that regulates nitric oxide synthase, was identified as one of the top genes with significant upregulation in Grade 1 as compared to higher grade ER positive BCs of no special type (adjusted p value <0.0000001). This study aimed to determine whether Nostrin at the protein level correlates with grade and other clinico-pathological variables in BCs.
Methods: Breast cancer tissue microarrays (n=708) were immuno-stained for Nostrin and expression patterns correlated with clinico-pathological and molecular variables as well as patient outcome. Results were validated in other BC cohorts [Breast Cancer Gene Expression Miner].
Results: : Immunohistochemistry showed high Nostrin expression to correlate with tumours of low grade (p<0.0001), ER/PR positive (p<0.05) phenotype and lobular morphology (p<0.001); as well as negative HER2 status (p=0.003). Loss of Nostrin expression was associated with increased lymphovascular invasion, metastases and poorer NPI (p<0.002) and poorer long term breast cancer specific survival (p=0.019). Positive correlations were also observed with the expression of STAT3 and CDC42 (p<0.001) proteins, indicating possible interacting pathways. Validation on multiple datasets on the breast cancer gene miner show that loss of Nostrin expression is related to poorer relapse free survival in ER positive BCs even when adjusted for NPI and proliferation (p<0.0001).
Conclusions: Results from this study suggest Nostrin to be a marker of favourable prognosis in BC identifying low grade, ER positive breast tumours with loss resulting in poorer prognosis.
**Project supported by NIHR and Career Development Fellowship from PathSoc.*

O25

Phenotypic and Immunophenotypic Characterisation of Recurrent Breast Cancer

Ⓟ DY Al-Bazz; MA Aleskandarany; CC Nolan; M Diez-Rodriguez; IO Ellis; EA Rakha; AR Green

University of Nottingham, Nottingham, UK

Purpose of the Study: Approximately 10–30% of patients with primary breast cancer (BC) subsequently develop a recurrence. Associated with the progression of distant metastasis and cancer specific death, local recurrence (LR) is an adverse prognostic event. However, limited clinical data is available concerning the biological parameters of recurrent BC. This study aimed to characterise LR and assess the impact and contribution on the outcome of BC.
Methods: 1,353 patients presenting with primary BC at Nottingham City Hospital between 1987 and 1998 with biological and outcome data were selected. Primary and Recurrent tumour FFPE sections were assessed for ER, PgR, HER2, Ki67, CK5/6, and EGFR using immunohistochemistry. Statistical analysis was performed using Pearson Chi-squared analysis.
Summary of Results: A total of 274/1353 (20%) primary BC cases resulted in LR; 108 (39%) of these patients did not progress to either a regional or distant recurrence during follow-up (median 219 months). There was a significant association between younger age, premenopausal status, smaller tumour size, PgR+, and CK5/6- and development LR (all $p<0.05$). There were positive associations between LR and altered expression of DNA repair proteins (BARD1, PARP1, PIAS and SMC6L1), inflammatory marker (CD8), stem cell markers (CD24 and STAT3) and the cell lineage and differentiation protein TWIST2 (all $p<0.05$). Comparing the LR and corresponding primary tumour there were differences in grade, HER2, PR, ER, CK5/6, Ki67, and Triple Negative status (all $p<0.05$).
Conclusions: As receptor status may alter in LR, it prudent not to treat patients with a LR on the basis of their primary tumour biology. Further investigation of the biological factors which trigger the development of LR is warranted.

O26

Research Studies and Level of Diagnostic Concordance in Breast Pathology: Lessons from the UK EQA Scheme

EA Rakha[1];Ⓟ MAH Ahmed[1]; AH Lee[2]; Z Hodi[2]; M Aleskandarany[1]; D Coleman[3]; RA Bennett[4]; C Hawkes[1]; IO Ellis[1]

[1]Division of Cancer and Stem Cells, School of Medicine, The University of Nottingham, Department of Histopathology, Nottingham, UK; [2]Department of Histopathology, Nottingham University Hospitals, City Hospital Campus, Hucknall Road, Nottingham, UK; [3]Cancer Screening Evaluation Unit, Institute of Cancer Research, Sutton, Surrey, UK; [4]Nottingham University Hospitals NHS Trust, Nottingham, UK

Purpose of the Study: Some recent studies have reported an unexpectedly high level of diagnostic discordance among breast pathologists which have raised concerns (JAMA.2015;313(11):1122-32).
Aims: To assess the diagnostic agreement among breast pathologists in the UK.
Methods: 240 cases of breast lesions included in the UK NHSBSP breast pathology interpretive external quality assurance (EQA) scheme in the last ten years were reviewed. In this scheme one representative H&E stained slide from each case is circulated to an average of 600 participants, which resulted in 144,000 readings in total. Data on diagnostic categories (benign, and malignant; in-situ and invasive) were collected. Slides from cases with diagnostic discordance ≥5% were reviewed by 3 pathologists.
Results: Out of the 240 cases, 35 cases (14.6%) were identified. The reasons for diagnostic discordance included one or more of the following: 1– Scheme methodology limitations including i) lack of guidelines on coding of certain lesions namely phyllodes tumours and lobular neoplasia (n=7 cases), and ii) use of glass slides with variable representation of the same lesion on the different slides, an average of 75 slides per paraffin block are circulated per case (n=12 cases). iii) inclusion of certain morphologically challenging lesions that can't be interpreted without the aid of ancillary techniques or consultation. These included papillary lesions; benign with sclerosis, epithelial proliferation, and/or atypia (n=6) and malignant papillary lesions (n=6), complex sclerosing lesions (n=3) and intraductal epithelial proliferative lesions (n=5). 2– Pathologist-related factors: reviewing expert pathologists identified over- and under-diagnosis in 8 cases (3.3% of all cases) including 5 papillary lesions.
Conclusions: The performance of breast pathologists is extremely high. Limitations of the EQA system should be addressed to assess the true performance. More complex papillary, proliferative and sclerosing lesions often require additional diagnostic work-up and difficult cases should trigger consensus opinion or expert referral.

O27

Expression of CUL4A in invasive Breast Cancer: a Biomarker of Good Prognosis Related to Tumour Differentiation

Ⓟ MA Aleskandarany[1]; R Surridge[1]; S Sonbul[1]; A Elmouna[2]; I Ashankyty[2]; E Nuglozeh[2]; MF Fazaludeen[2]; CC Nolan[1]; M Diez-Rodriguez[1]; IO Ellis[1]; AR Green[1]; EA Rakha[1]

[1]Nottingham City Hospital, Nottingham, UK; [2]Molecular Diagnostics and Personalised Therapeutics Unit, University of Ha'il, Ha'il, Saudi Arabia

Purpose of the Study: The contribution of cellular proteins that modulate biological processes leading to disease progression remain as challenging task. Cullin-4a (CUL4A) is a ligase playing a critical role in the ubiquitination pathway. The covalent binding of ubiquitin to target proteins is a major post-translational process that alters their functions and thus regulates a diverse array of cellular processes including the degradation of DNA damage-response proteins, chromatin regulation, and cell cycle regulation.
Methods: CUL4A expression was assessed in a large clinically annotated cohort (n=988) of invasive BC with long-term follow-up using IHC and TMA. The Cancer Genome Atlas (TCGA) and bc-GenExMiner v3.2 were used for validation.
Summary of Results: CUL4A showed cytoplasmic and nuclear expression where over-expression showed significant association with lower grade ($p<0.001$), less mitotic figures ($p<0.001$) more tubule formation ($p<0.001$), histological types of excellent/good prognosis ($p<0.001$) good NPI prognostic subgroup ($p=0.01$), negative LVI ($p=0.023$), ER+ ($p<0.001$), PR+ ($p<0.001$), HER2- ($p=0.003$) and luminal-A subtype ($p<0.001$), and DNA damage response proteins [BRCA1/2, PARP-1, BARD1, Rad51, ATM, ART, and CHEK1. High expression of CUL4A was significantly associated with an improved survival ($p<0.001$) and time to distant metastasis ($p=0.04$), independent of tumour size, stage and grade. CUL4A showed 3.41% CNA and/or mutations in the TCGA dataset with non-significant impact of these derangements on outcome. Although pooled CUL4A mRNA data (n=3925) showed non-significant association with outcome, over-expression was significantly associated with better outcome in 4 datasets, and with worse outcome in 3 datasets.
Conclusions: Our results identified the scaffold protein CUL4A as a biomarker of good prognostic impact in BC. Its expression pattern alludes to a role in tumour differentiation; findings worthy of further investigation.

O28

Multiple Coagulation Factor Deficiency Protein-2 (MCFD2) as a Potential Prognostic Germ Cell Marker in Invasive Breast Carcinoma: a Transcriptome-Driven Study

Ⓟ SN Sonbul[1]; A Mukherjee[1]; R Russell[2]; OM Rueda[2]; MA Aleskandarany[1]; AR Green[1]; CC Nolan[1]; M Diez-Rodriguez[1]; E Provenzano[3]; C Caldas[3]; IO Ellis[1]; EA Rakha[1]

[1]Department of Pathology, School of Medicine, University of Nottingham, Nottingham, UK; [2]CRUK Cambridge Research Institute, University of Cambridge, Cambridge, UK; [3]Addenbrooke's Hospital, Cambridge Breast Unit, Cambridge University Hospital NHS Foundation Trust, Cambridge, UK

Purpose of the Study: Genes driving the development of lymphovascular invasion (LVI) in breast cancer (BC) remain unknown. In this study we attempted to validate at the protein level differentially expressed genes correlated with LVI in BC and examined associations with clinicopathological and molecular features.
Methods: Subgroups of positive and negative LVI status were identified in subsets of the METABRIC BC cohort using morphology and immunohistochemistry (IHC D2-40). A differential transcriptomic profile correlating with LVI status in both subgroups was identified from microarray data analysis, (LVI+, n= 91) and (LVI–, n= 83). The copy number aberration (CNA) gain ratio was calculated for each transcript in both subgroups. IHC protocols were implemented in a large series of invasive BCs (n= 1282). Clinicopathological and molecular significance of MCFD2 expression was determined statistically.
Summary of Results: Multiple coagulation factor deficiency-2 (MCFD2) ranked first in CNA gain ratio, (score = 4.56). IHC demonstrated that MFCD2 is expressed in the cytoplasm of invasive tumour cells. Low cytoplasmic expression of MFCD2 was found to be significantly associated with increased risk of development of regional recurrence, ($p = 0.021$). High cytoplasmic expression of MFCD2 showed a significant positive correlation with the p53 coactivator TADA3 ($p=0.039$) but negative association with the growth factor TFF1 ($p=0.007$). No significant association between protein expression and LVI could be identified in our cohort.
Conclusions: The results obtained endorse that the higher cytoplasmic expression levels of MCFD2 is correlated with good prognosis and better outcomes in invasive BC but does not support its role in driving the development of LVI.
**AM supported by NIHR and the Academy of Medical Sciences.*

O29

Diagnosing Breast Fibroepithelial Lesions: How Accurate are Core Biopsy Categories in Assessing for Phyllodes Tumour and How Well do Fibroepithelial Diagnoses in Excisions Predict Their Behaviour?

Ⓟ WJ Dalleywater[1]; MM Al-kaabi[2]; J White[3]; N Khirwadkar[3]; Y Mir[3]; E Rakha[4]

[1]Leicester Royal Infirmary, Leicester, UK; [2]Nottingham University Hospitals, Nottingham, UK; [3]Royal Liverpool and Broadgreen Hospitals, Liverpool, UK; [4]University of Nottingham, Nottingham, UK

Phyllodes tumours (PT) are rare breast fibroepithelial lesions, which may mimic fibroadenoma (FA), a more common entity, clinically and histologically. Histological diagnosis may be difficult when there is limited tissue available for assessment, in particular differentiating benign PT from FA. The aim of this study was to evaluate the range of diagnoses of fibroepithelial lesions in breast core biopsies and excisions, and their follow-up outcomes.

Data were extracted from the laboratory records of 3 histopathology departments for all breast excisions where the pathological diagnosis was PT. The data included demographic details, specimen type, and linked records of core biopsies and previous specimens showing fibroepithelial lesions - all cases without any linked records were excluded from analysis. We stratified the data on grade of tumour (benign, borderline, malignant) and evaluated how core biopsy or previous specimen results related to diagnosis of PT, and how grade affected follow-up outcome.

We identified 441 cases of PT over 25 years, of which 194 were referred cases. 70% had a core biopsy result; a further 3.5% had a previous specimen result. Of these, 116 were benign, 39 borderline and 18 malignant. In those with core biopsy results, 79% were at least suspicious of PT (B3-B5). All except 1 of the remainder were diagnosed as FA (B2) – in these 30, 26 went on to have a benign PT and 4 were borderline. In those with a previous history, 9/11 had had a FA. After follow-up, 3% of benign, 18% of borderline and 17% of malignant lesions had local recurrence and there was 1 distant metastasis in the malignant group.

Core biopsy is a useful initial investigation in evaluating women with fibroepithelial lesions, but lacks sensitivity in diagnosing patients with benign PT. Further work should focus on how this can be improved to assist with guiding appropriate treatment. Borderline and malignant PT have similar local recurrence behaviour, distinct from benign PT.

O30

Intra-Operative Assessment of Sentinel Lymph Nodes by Selective-Scanning Raman Spectroscopy

Ⓟ DW Shipp[1]; EA Rakha[2]; I Notingher[1]

[1]University of Nottingham, Nottingham, UK; [2]Nottingham University Hospitals NHS Trust, Nottingham, UK

Intra-operative assessment of sentinel lymph nodes (SLN) in breast cancer is important for surgical management of the axilla. Current techniques for this are either not cost effective, lack sufficient accuracy, or utilize the tissue precluding subsequent histological confirmation. Raman spectroscopy (RS) offers a promising alternative due to its high accuracy and non-invasive nature. RS is a non-destructive, optical technique that enables the identification of tumours with high sensitivity and specificity. Current applications of RS require very long times for scanning whole tissue sections. In this pilot study, we use tissue auto-fluorescence to quickly identify suspicious regions on the surface of sliced fresh lymph. Suspicious regions are then scanned by RS. This method, called Selective-scanning Raman Spectroscopy (SSRS), reduces the measurement time for a SLN surface from hours to less than 20 minutes with maintenance of the highest degree of accuracy. SSRS is entirely non-destructive and requires no exogenous labels, so the entire nodal tissue can be processed for histological examination. In this pilot study of 9 cases (4 benign nodes and 5 cancers), we demonstrate the ability of SSRS to discriminate between tumour and lymphoid tissues with high sensitivity and specificity. Further, the speed and accuracy of SSRS provide evidence of its potential clinical utility for intraoperative assessment of tumours in the SLN as well as other tissue. This has the potential to reduce second axillary surgery and be cost efficient.

O31

The Significance of Reporting Perineural Invasion in 988 Conservatively Treated Prostate Cancer Patients with Long Term Outcome

Ⓟ V Parameshwaran[1]; BV North[1]; H Møller[2]; P Scardino[3]; J Cuzick[1]; L Beltran[1]; DM Berney[1]

[1]Queen Mary University of London, London, UK; [2]Kings College University of London, London, UK; [3]Memorial Sloane Kettering Cancer Centre, New York City, USA

Purpose of the Study: The reporting of perineural invasion (PNI) is a required item in the latest Royal College of Pathology dataset. However its significance as an independent prognostic factor is debated. We evaluated the presence of PNI and its effect on death from prostate cancer (PC) in conservatively managed patients.

Methods: Cases of PC were identified from three cancer registries in the UK from men with clinically localised PC diagnosed by needle biopsy from 1990–2003. The endpoint was death from PC. Clinical variables included PSA, clinical stage, and disease extent. Patients treated radically within 6 months, those with objective evidence of metastases or who had prior hormone therapy were excluded. Follow up was through cancer registries up until 2012. Deaths were divided into those from PC and those from other causes, according to WHO criteria. All cases were reviewed by 2 expert uropathologists.

Summary of Results: 988 cases were identified and there were 169 (17.1%) PC deaths. PNI was present in 284 (28.7%). On univariate analysis PNI was significantly associated with death (HR 2.27, 95% CI 1.67-3.08 p=1.45x10-7). However when using multivariate Cox regression including Gleason score, PSA, clinical T stage and the percentage of disease, PNI was not a significant factor in predicting death from disease (HR 1.37, 95% CI 0.93-2.03, p=0.116) and all helpful information was contained within Gleason score, PSA and T stage.

Conclusions: The reporting of PNI is difficult and may be time consuming. We suggest that reporting in biopsies should be optional as it does not appear to contribute useful information beyond standard clinico-pathological parameters.

O32

Vascular Insulin-Like Growth Factor Receptor Type 2 (IGF2R) Expression is Upregulated in Malignant Tumours

Ⓟ AL Trépant[1]; N D'Haene[1]; J Allard[2]; YR Van Eycke[2]; C Decaestecker[2]; I Salmon[1]; P Demetter[1]

[1]Department of Pathology, Erasme University Hospital, Brussels, Belgium; [2]Digital Image Analysis in Pathology (DIAPATH), CMMI, Gosselies, Belgium

Purpose of the Study: Insulin-like growth factor receptor type 2 (IGF2R) is a receptor belonging to the insulin-like growth factor (IGF) system. Involvement of IGF2R in the process of angiogenesis has been postulated in rare earlier studies. In previous work we demonstrated IGF2R expression in brain vessels and in particular in hyperplastic vessels of glioblastoma which is known as one of the most angiogenic malignancies. Since accumulating evidence suggests that tumoural angiogenesis is heterogeneous, we aimed to investigate whether this expression is restricted to glioblastoma vessels.

Methods: Vascular IGF2R expression was evaluated by means of computer-assisted quantitative immunohistochemistry in tissue microarray sections from 17 colonic adenocarcinomas (ADC), 10 gastric ADC, 10 gastro-intestinal stromal tumours (GIST) , 20 pulmonary ADC , 15 pulmonary squamous cell cancers, 7 prostatic ADC, 4 renal tubulopapillary carcinomas, 14 renal clear cell carcinomas and 10 urothelial carcinomas. From these tumours, we also studied matched normal tissue except for GIST and urothelial carcinoma.

Summary of Results: Our quantitative analysis revealed vascular expression of IGF2R in all tumours without significant difference between different tumour types. We also observed higher expression of IGF2R in tumoural vessels compared to normal vessels from the same patient (global p-value <0.001).

Conclusions: This work reveals that IGF2R is expressed in blood vessels of different tumour types. Furthermore, our results suggest an upregulation of vascular IGF2R expression in malignant tumours.

O33

Digital PCR Analysis of Circulating Tumour DNA in a Broad Cohort of Sarcoma Patients

Ⓟ A Gutteridge[1]; VM Rathbone[1]; R Gibbons[2]; F Amary[2]; N Archard[1]; K Davies[1]; J Brown[1]; M Jorgensen[1]; M Gupta[1]; AM Flanagan[1]; T Forshew[1]

[1]University College London – Cancer Institute, London, UK; [2]Royal National Orthopaedic Hospital NHS Trust (Histopathology), Stanmore, UK

It is now well established that many solid tumours release DNA, cell-free, into the blood of cancer patients. It is believed that this circulating tumour DNA (ctDNA) is released through either apoptosis or necrosis of cells. By screening for cancer-specific mutations it is possible to detect and quantify the tumour fraction potentially enabling a broad range of cancer biomarker applications from non-invasive diagnosis to early relapse detection. To date very little has been published describing ctDNA levels in sarcoma patients.

Digital PCR is currently the most sensitive and specific method for detecting and quantifying mutant DNA molecules. The BioRad QX200 digital PCR platform is also both cost-effective and scalable. Using the QX200 platform, we have developed assays for 15 different point mutations including 12 tumour hotspots within the genes *IDH1*, *IDH2* (central chondrosarcoma , n=33), *H3F3A* (giant cell tumour of bone, n=26), *CTNNB1* (desmoid fibromatosis, n=5), *GNAS1* (fibrous dysplasia, n=4 and intramuscular myxoma, n=6), and *MYOD1* (spindle cell rhabdomyosarcoma, n=3) and 3 patient specific mutations (chordoma).

It was possible to detect mutant DNA in select individuals with chondrosarcoma, giant cell tumour of bone (GCT), chordoma, and spindle cell rhabdomyosarcoma. Pre-treatment levels were variable and did not correlate with tumour size. In the 4 individuals analysed post-surgery a clear drop in ctDNA levels was visible. The highest levels of ctDNA detected were from patients with high grade disease (chondrosarcoma), and a patient with a GCT who suffered a femoral fracture.

This study demonstrates for the first time that sarcomas with a broad range of tumour types release ctDNA at low levels. Further large studies are needed to define where there is clinical utility for detecting this DNA.

This research was supported by Sarcoma UK and a grant from the Pathological Society.

O34

Breaking the Ice: Digital Pathology for Remote Frozen Section Diagnosis — A Two-Year, Single-Centre Experience of 267 Cases

Ⓟ J Griffin; P Kitsanta; SK Suvarna; B Perunovic; J Bury

Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK

Purpose of the Study: Following a reorganisation of diagnostic services at our institution, the histopathology department moved to a different site from surgical specialities that required intraoperative frozen section diagnosis. We designed a digital pathology solution where the frozen section sample was dissected by an advanced Biomedical Scientist under video conference consultant supervision and the resulting slides then scanned and viewed remotely. We assessed the concordance of digital and glass frozen section diagnoses.

Methods: Details of cases that required frozen section between February 2014 and January 2016 were retrieved from a prospectively compiled database. Specimen reports and request forms were reviewed and data analysed using an Excel spreadsheet.

Summary of Results: Digital frozen section examination was performed in 267 cases. The majority of cases were thoracic (80%) and upper gastrointestinal (15%). Intra operative consultation was requested to determine presence or absence and type of tumour in 223 (83.5%) cases, to assess margins in 26 (9.7%) cases and to determine if a tumour was resectable in 18 (6.7%) cases. Two cases were excluded due to incomplete data. The digital slide frozen section diagnosis was concordant with the final glass slide diagnosis in 247 (92.5%) of cases. Eight lesions were regarded as likely primary lung neoplasia but necrosis, poor differentiation or unusual morphology prevented confident further classification, five tumours were only found on processing of the entire frozen section sample, three tumours diagnosed as neoplasia of uncertain type were metastases and two tumours were obscured by inflammation or fibrosis. None of these results led to incorrect clinical management.

Conclusions: Digital pathology can be safely used for frozen section diagnosis. We have implemented a multidisciplinary intraoperative diagnostic service encompassing remote macroscopic dissection supervision and whole slide imaging.

O35

Demonstration of mRNA Light Chain Restriction in Follicular Lymphomas Using Ultrasensitive In Situ Hybridisation

Ⓟ A Warford; M Rahman; D Ribeiro

University of Westminster, London, UK

Purpose of the Study: The demonstration of immunoglobulin light chain restriction by immunocytochemistry is one of the diagnostic tests used to confirm B cell malignancy in formalin fixed paraffin embedded (FFPE) tissues. An alternative approach involves the demonstration of mRNA, but sensitivity concerns have hindered its adoption for use in the instance of low copy expression, such as in follicular lymphoma. The Recently developed RNAscope (Advanced Cell Diagnostics Inc) technology offers a step change in both sensitivity and specificity for the demonstration of low copy mRNA. To assess this a RNAscope manual kit was applied to FFPE cases of follicular lymphoma and control tonsil tissues.

Methods and Results: Demonstration of peptidylprolyl isomerase B (PPIB), a ubiquitously and moderately expressed mRNA target, was undertaken first to assess mRNA conservation and to establish optimal pre-treatment conditions. 10 of 14 cases were determined as suitable for subsequent hybridisation with the kappa and lambda probes. Light chain mRNA restriction was demonstrated in 5 of 7 follicular lymphomas and the expected distribution of kappa and lambda in the B lymphocyte compartments was shown in the 2 of 3 tonsil samples.

Conclusions: The results confirm that low copy number mRNA expression can be demonstrated in FFPE tissue using the RNAscope technology. Furthermore, in the context of follicular lymphoma, the technology could be considered for the diagnostic demonstration of light chain restriction.

O36

Evaluation of Relative Sensitivities and Specificities of BRAF Mutation Detection Methodologies, Using the Paradigm of Hairy Cell Leukaemia

Ⓟ B Cardus; RT Colling; EJ Soilleux; A Hamblin

Oxford University, Oxford, UK

BRAF mutations (eg V600E) are found in a wide range of neoplasms and targeted BRAF inhibitors (vemurafenib, dabrafenib) can provide effective treatment for BRAF-mutated malignancies. Accurate, sensitive, cost-effective methods for mutation detection are needed to guide use of potentially effective, but expensive, treatments. In order to evaluate relative sensitivities and specificities of BRAF mutation detection methodologies, we used hairy cell leukaemia (HCL) as a paradigm, as around 95% of HCL cases are BRAF-mutated.

We used 10 control, 6 non-HCL lymphoma/ leukaemia and 11 HCL-containing bone marrow trephine (BMT) specimens to compare sensitivity of 3 sequencing-based BRAF detection methodologies (Sanger sequencing, pyrosequencing and targeted Next Generation Sequencing (NGS)) and an automated polymerase chain reaction (PCR) assay (Idylla, Biocartis). We also immunostained BMTs with a BRAF V600E-mutation specific antibody (VE1, Roche Ventana).

Idylla and immunostaining detected a BRAF mutation in 11/11 cases of HCL, while pyrosequencing detected the mutation in 10/11 cases (mutation-negative case had low level HCL infiltration). Targeted NGS detected the mutation in 6/10 cases and failed on 1 case, while Sanger sequencing detected the mutation in 7/8 cases and failed on 3 cases. No method identified a BRAF mutation in any trephine that did not contain HCL histologically.

We conclude that the novel VE1 BRAF-V600E-mutation-specific antibody and the Idylla platform provide the most sensitive and cost-effective techniques to detect the BRAF-V600E-mutation mutation in HCL BMTs. The disadvantage of these technologies is their limited ability to multiplex BRAF mutation analysis with analysis for a range of other mutations, which is possible with targeted NGS and may be increasingly relevant as more personalised treatment strategies become available with the advent of novel targeted therapies.

O37

Loss of Bone Marrow Mesenchymal Stem Cells in Myeloproliferative Neoplasms as an Indicator of Niche Dysregulation

Ⓟ E Bishop; A Mead; EJ Soilleux

Oxford University, Oxford, UK

In myeloproliferative neoplasms (MPNs), proliferation of at least one marrow lineage occurs and mutant haematopoietic stem cells (HSCs) clonally expand and disrupt the bone marrow environment. HSC quiescence is usually maintained via regulation by a niche, which also contains sympathetic nerve fibres and mesenchymal stem cells (MSCs). Mouse studies show that neoplastic HSCs dysregulate sympathetic innervation, causing MSC loss. We investigated whether there is a similar loss of MSCs in human MPN patients.

We identified bone marrows from individuals with chronic myeloid leukaemia (CML, n=13), essential thrombocythaemia (ET, n=14), polycythaemia vera (PV, n=10), primary myelofibrosis (PMF, n=15) and no myeloproliferative neoplasm (n=20). We undertook double immunostaining for the mesenchymal stem cell marker, nestin, and the blast cell and vascular endothelial marker (CD34). We then counted the number of MSCs and blood vessels in three different x20 fields for each trephine.

This approach demonstrated that the number of MSCs was significantly reduced in the bone marrow of MPN patients ($p<0.0001$). The average number of MSCs per x20 field in the normal group was 3.35 +/-0.22 compared with 0.55 +/-0.06 in 52 MPN trephines ($p<0.0001$). A novel finding was that marrows from CML patients (Philadelphia chromosome positive (Ph+ve) by definition) showed significantly lower numbers of MSCs compared with the other Ph-ve subtypes, 1.55 +/-0.19 to 0.33 +/-0.10. There was a negative correlation between numbers of MSCs and blood vessel density, presumably because blood vessel density increases in myeloproliferative neoplasms, but no significant correlation between MSC numbers and degree of fibrosis.

This study supports the hypothesis that loss of MSCs is a fundamental part of HSC niche dysregulation in patients with MPNs.

O38

Ipilimumab Induced Colitis: Is the Epstein Barr Virus (EBV) Implicated?

Ⓟ MR Pugh[1]; M Morgan[2]; SD Dojcinov[1]

[1]All Wales Lymphoma Panel - University Hospital of Wales, Cardiff, UK; [2]University Hospital of Wales, Cardiff, UK

Purpose of Study: Ipilimumab (IPL) is a CTLA-4 inhibitor used for metastatic melanoma, and has been associated with immune related adverse events, including colitis. Exclusion of infective causes is advocated in the assessment of such cases, however, there is no published literature examining the role Epstein Barr virus (EBV). The aim of this study was to establish whether EBV is implicated in IPL induced colitis (IIC).

Methods: : Biopsy and resection specimens of IIC diagnosed at the University Hospital of Wales were identified from 2010 to 2015. *In situ* hybridisation for EBER was performed on all cases with immunohistochemistry for CD20, CD3, CD4, CD8, CD30, CD15, PAX5, PD1, PDL1 and CMV.

Results: 7 specimens from 4 patients were identified. 2 of the patients had undergone serial biopsies, 1 of which underwent a colectomy. 1 patient showed increasing positivity for EBER in sequential biopsies within lymphoid tissue, with florid positivity in the resection specimen primarily in the base of the ulcers. The associated lymphoid infiltrate in these EBER+ specimens included CD20+, CD30+, CD15+ and PAX5+ positive blasts, some with Hodgkin-like features. In this case the features were consistent with EBV+ mucocutaneous ulcer (EBVMCU). All other biopsies were EBER-. PDL1 was expressed in the histiocytes at the base of the ulcers from all cases but no significant PD1 expression was seen.

Conclusion: EBV+ lymphoproliferation manifesting as EBVMCU may complicate at least a subset of established IICs, in particular, those that progress to colectomy.

The findings also suggest that inflammation and subsequent tissue damage may be the initial pathogenetic step in the development of EBVMCU. PDL1 has previously been implicated in the pathogenesis of EBV+ lymphomas and may play a role in the development of EBVMCU.

O39

Evaluation of Pro-Survival Signalling Pathways as Novel Therapeutic Targets in Chronic Lymphocytic Leukaemia

Ⓟ D Kluczna; S Jayne; M Vogler; MJS Dyer; S Macip

University of Leicester, Leicester, UK

Purpose of the Study: Chronic Lymphocytic Leukaemia (CLL) is one of the most common adult leukaemias and is currently incurable. Notch 1 mutations are one of the independent prognostic factors for a poor prognosis and Richter syndrome, a life threatening complication of CLL. We performed a pre-clinical evaluation of effectiveness on primary CLL cells of commercially available small molecule inhibitors, which target different pro-survival signalling pathways. The project aims to propose novel treatment regimens through stratification of sensitivity results by patient's mutation status. This would improve therapy outcomes and reduce the reliance of cytotoxic therapies.

Methods: Genotyping of 160 patients (Set 1: 105 randomly selected patients; Set 2: 45 patients with tumour clones harbouring Trisomy 12) was completed using PCR to amplify the exon 34 of Notch1 gene, followed by Sanger Sequencing. Primary CLL cells were stimulated either with CD40 and IL-1 supplemented media (RPMI, 10%FBS) or with RPMI medium with 10% Human serum prior to treatment. The effect of inhibitors were evaluated using MTT viability assay and FACs analysis, and Western Blot was performed to investigate their mechanism of action.

Summary of Results: Notch 1 mutations were found in 12.4% and 31.1% of patients in set 1 and set 2 respectively. We used different target inhibitors in stimulated primary cells (RO4929097, IMD 0354 and ABT 199, among others) and we observed that patients with Notch-1 mutations showed higher sensitivity to some and increased resistance to others. Using this information, we designed combination therapies that maximized the effects on the mutant cells.

Conclusions: Notch 1 mutation status can be used as a stratification tool and it is an important factor determining cell's sensitivity to a number of targeted inhibitors. The project lays solid foundations towards personalised medicine in leukaemia treatment in the clinic.

O40

Extending the Concept of Monotypia to T-cells: 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; M Salimi; T Hassanali; G Ogg

Oxford University, Oxford, UK

The differentiation between benign and malignant (eg lymphoma or leukaemia) lymphocytic infiltrates is an important and common clinico-pathological question in routine healthcare practice. While this is possible for B cell infiltrates using kappa/lambda immunoglobulin light chain staining, there is not an equivalent for T cell infiltrates, which currently require expensive and time consuming T cell receptor gene rearrangement PCR studies to be undertaken. We have identified two T cell-specific RNA sequences (X and Y), which show mutually exclusive expression. The corresponding protein sequences are not significantly different, but the sequences differ significantly enough at the RNA level, to mean that chromogenic in situ hybridisation (CISH), rather than immunohistochemistry, is the most appropriate detection method.

We used RT-PCR to determine the relative frequencies of sequences X and Y in peripheral blood T-cells. We designed oligonucleotide probe sets for CISH-based detection of sequences X and Y and used them with various manual and automated detection methodologies.

RT-PCR indicates that 50% of T cells in peripheral blood express sequence X and 50% express sequence Y, with each individual T cell only expressing one type of these sequences (X or Y). Sequences X and Y can be demonstrated in tissue sections using CISH detection methodologies with significant amplification and demonstrate mutually exclusive expression on T-cell leukaemia/ lymphoma lines. Our CISH results are in agreement with RT-PCR results on the same material.

This is the basis of a novel diagnostic pathology test, which will have the potential to transform the routine assessment of T cell infiltrates in a manner analogous to the way kappa/lambda staining has done for B cells. It is therefore likely to impact upon international diagnostic guidelines with global health economic implications.

O41

Clinical Value of Subclassification of Focal Segmental Glomerulosclerosis in IgA Nephropathy

SS Bellur[1]; F Lepeytre[2]; O Vorobyeva[1]; S Troyanov[2]; HT Cook[3];Ⓟ ISD Roberts[1]

[1]Oxford University Hospitals, Oxford, UK;
[2]Hôpital du Sacré-Coeur de Montréal, Montréal, Canada; [3]Imperial College, London, UK

Purpose of the Study: Focal segmental glomerulosclerosis (FSGS) is a common finding in IgA nephropathy (IgAN). We assessed segmental sclerosing lesions in the Oxford Classification cohort and correlated histology with clinical features and outcomes.
Methods: There was segmental sclerosis in 76% biopsies, 137 with slides available for review. We subclassified FSGS lesions and evaluated the associations between histology and the clinical features and outcomes (proteinuria, rate of renal function decline, survival from a 50% reduction in GFR or ESRD). The reproducibility of assessment of FSGS lesions was determined.
Results: Biopsy review and subclassification of FSGS lesions identified 38% of patients with podocyte hypertrophy, 10% hyalinosis, 9% resorption droplets within podocytes, 7% tip lesions, 3% perihilar sclerosis and 2% endocapillary foam cells. Reproducibility was good/excellent for tip lesions, hyalinosis and perihilar sclerosis, moderate for podocyte hypertrophy and poor for podocyte resorption droplets, adhesion only and endocapillary foam cells. Podocytopathic features (podocyte hypertrophy and tip lesions) correlated with initial proteinuria. During follow-up of patients without immunosuppression (IS), those with podocytopathic features had greater proteinuria, a faster rate of renal function decline and worse renal survival compared to patients with FSGS without podocytopathic features and those without FSGS. In individuals with podocytopathic features, immunosuppressive treatment was associated with a better survival compared to patients without IS.
Conclusions: In IgAN, the presence of podocyte hypertrophy or tip lesions, markers of podocyte injury, are reproducible. These podocytopathic features are strongly associated with the initial proteinuria and, in untreated patients, carry a worse prognosis. Our findings support reporting these features alongside the Oxford Classification S score.