The University Of Sheffield.

Pathological Society

Understanding Disease

Summer Meeting

3 – 5 July 2012

202nd Scientific Meeting of the Pathological Society of Great Britain & Ireland

Hosted by the Academic Unit of Pathology · University of Sheffield

Venue: The Octagon Centre · University of Sheffield

Western Bank · Sheffield · S10 2TQ

Companion sessions: UK Endocrine Pathology Society · Association of Clinical Electron Microscopists

PROGRAMME ACKNOWLEDGEMENTS

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Note: Competition closes at 15.30 on Wednesday 4 July

STUDENTS' UNION BUILDING · AUDITORIUM	**DAINTON BUILDING · LECTURE THEATRE 1**
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OCTAGON CENTRE · MAIN HALL
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Prof P Shaw, Professor of Neurology, University of Sheffield

UNIVERSITY OF SHEFFIELD · FIRTH HALL
18.30–20.00 **Welcome Reception —** *featuring musical entertainment by "The Djangonauts"*

All details are subject to amendment
Visit our website for further information and updates: **www.pathsoc.org**

Programme Quick Reference Tables

WEDNESDAY 4 JULY 2012

OCTAGON CENTRE · FOYER
07.45 **Registration and Coffee**

STUDENTS' UNION BUILDING · AUDITORIUM
08.00–09.00 **Trainees' Breakfast Session – Meet the Experts:** ***Cardiac Tissue Analysis: From Routine to Complex***
Dr K Suvarna, University of Sheffield

OCTAGON CENTRE · COMPUTER ROOM
09.00–17.30 **Slide Seminar Competition Case Viewing:** ***Gynaecological Pathology***
Note: Competition closes at 15.30 on Wednesday 4 July

STUDENTS' UNION BUILDING · AUDITORIUM
09.00–10.30 **Oral Presentations**
Category: Breast

DAINTON BUILDING · LECTURE THEATRE 1
09.00–10.30 **Oral Presentations**
Categories: Gastrointestinal; Cellular/Molecular

OCTAGON CENTRE · MAIN HALL
10.30–11.00 **Coffee / Trade Exhibition**

STUDENTS' UNION BUILDING · AUDITORIUM
11.00–13.00 **Symposium:** ***Systems Biology – Reverse Engineering The Phenotype***

DAINTON BUILDING · LECTURE THEATRE 1
11.00–13.00 **Symposium:** ***Endocrine Pathology***
— Organised in conjunction with the UK Endocrine Pathology Society

UNIVERSITY HOUSE · ABBEYDALE / FULWOOD ROOMS
13.00–14.00 **Lunch**

OCTAGON CENTRE · MAIN HALL
14.00–15.00 **Poster Viewing and Chairman's Rounds**
Categories: Autopsy/Forensic; Cardiovascular/Pulmonary; Cellular/Molecular; Education and Audit; Endocrine; Experimental Tumour Pathology; Genitourinary/Renal; Gynaecological; Neonatal/Paediatric; Technical Advances

OCTAGON CENTRE · COUNCIL ROOM
14.30–15.30 **UK Endocrine Pathology Society Business Meeting**

STUDENTS' UNION BUILDING · AUDITORIUM
15.00–17.30 **Plenary Oral Presentations**
16.00–16.30 Tea [OCTAGON CENTRE · MAIN HALL]

STUDENTS' UNION BUILDING · AUDITORIUM
17.30–18.30 **The Pathological Society's Doniach Lecture:** ***Only Dead Fish Swim with the Stream — Trying to Understand the Dynamics of the Liver and its Diseases***
Prof MR Alison, Queen Mary University of London

CUTLERS' HALL
19.30–22.30 **Society Dinner**

All details are subject to amendment
Visit our website for further information and updates: **www.pathsoc.org**

Programme Quick Reference Tables

THURSDAY 5 JULY 2012

OCTAGON CENTRE · FOYER
07.45 **Registration and Coffee**

STUDENTS' UNION BUILDING · AUDITORIUM
08.00–09.00 **Trainees' Breakfast Session – Meet the Experts: *My Approach to Soft Tissue Tumours***
Dr K Thway, London

OCTAGON CENTRE · COMPUTER ROOM
09.00–16.00 **Slide Seminar Competition Case Viewing: *Gynaecological Pathology***
Note: Competition closed at 15.30 on Wednesday 4 July

STUDENTS' UNION BUILDING · AUDITORIUM
09.00–10.30 **Oral Presentations**
Categories: Breast; Osteoarticular/Soft Tissue

DAINTON BUILDING · LECTURE THEATRE 1
09.00–10.30 **Oral Presentations**
Categories: Hepatobiliary/Pancreas; Education and Audit; Neonatal/Paediatric

THE OCTAGON CENTRE · COUNCIL ROOM
09.10–10.20 **Association of Clinical Electron Microscopists AGM**
(open to non-members)

OCTAGON CENTRE · MAIN HALL
10.30–11.00 **Coffee / Trade Exhibition**

STUDENTS' UNION BUILDING · AUDITORIUM
11.00–12.30 **Trainees' Symposium: *How to be a Consultant***

DAINTON BUILDING · LECTURE THEATRE 1
11.00–17.00 **Association of Clinical Electron Microscopists (ACEM) (www.acem.org)**
15th Annual Scientific Meeting
(see separate programme)
Sponsored by Leica

STUDENTS' UNION BUILDING · AUDITORIUM
12.30–13.30 **Japanese Pathological Society Lecture: *Pathology of Gastrointestinal Stromal Tumours***
Prof S Hirota, Hyogo, Japan

UNIVERSITY HOUSE · ABBEYDALE / FULWOOD ROOMS
13.30–14.30 **Lunch**

STUDENTS' UNION BUILDING · AUDITORIUM
14.00–14.45 **Pathological Society Annual Business Meeting**

STUDENTS' UNION BUILDING · AUDITORIUM
14.45–15.45 **Slide Seminar Discussion Session: *Gynaecological Pathology***
Prof M Wells, University of Sheffield

All details are subject to amendment
Visit our website for further information and updates: **www.pathsoc.org**

COMPANION MEETINGS

Wednesday 4 July

11.00–15.30 UK Endocrine Pathology Society
11.00–13.00 Symposium jointly organised **[DAINTON BUILDING · LECTURE THEATRE 1]**
14.30–15.30 Business Meeting **[DAINTON BUILDING · LECTURE THEATRE 1]**

Thursday 5 July

09.10–17.00 Association of Clinical Electron Microscopists
09.10–10.30 Annual General Meeting **[OCTAGON CENTRE · COUNCIL ROOM]**
10.30–17.00 15th Annual Scientific Meeting **[DAINTON BUILDING · LECTURE THEATRE 1]**

KEYNOTE AND NAMED LECTURES

Tuesday 3 July

17.30–18.30 Public Lecture **[STUDENTS' UNION BUILDING · AUDITORIUM]**
The Relationships between Patients, the Public and Biomedical Researchers
Prof P Shaw, University of Sheffield

Wednesday 4 July

17.30–18.30 Doniach Lecture **[STUDENTS' UNION BUILDING · AUDITORIUM]**
Only Dead Fish Swim with the Stream – Trying to Understand the Dynamics of the Liver and its Diseases
Prof MR Alison, London

Thursday 5 July

12.00–13.00 Japanese Pathological Society Lecture **[STUDENTS' UNION BUILDING · AUDITORIUM]**
Pathology of Gastrointestinal Tumours
Prof S Hirota, Hyogo

ORAL COMMUNICATIONS **[STUDENTS' UNION BUILDING · AUDITORIUM] –and– [DAINTON BUILDING · LECTURE THEATRE 1]**

Sessions will be held as follows:

Tuesday 3 July	09.30–12.30
Wednesday 4 July	09.00–10.30
Thursday 5 July	09.00–10.30

Note to presenters: Speakers are reminded that no communication may exceed the time allocated on the Programme without the consent of the meeting, obtained through the Chairman.

PLENARY ORAL SESSION **[STUDENTS' UNION BUILDING · AUDITORIUM]**

The eight highest-ranked submitted oral abstracts will be presented on Wednesday 4 July, 15.00–17.30.

PRIZE
A prize for the best presentation, donated by the *Journal of Pathology* will be presented at the Society Dinner.

POSTER VIEWING AND CHAIRMAN'S ROUNDS [OCTAGON CENTRE · MAIN HALL]

Tuesday 3 July 13.00–14.00
Wednesday 4 July 14.00–15.00

PRIZES
Poster round chairs will be circulating during the above times to select the winners of the Pathological Society's Sir Alastair Currie Prize and second and third poster prizes. **Winners** will be announced at the Society Dinner on Wednesday 4 July.

IMPORTANT NOTES FOR PRESENTERS
- Posters should be in place by 09.30 on Tuesday 3 July and **must be removed by** 16.00 on Thursday 5 July.
- Poster boards will be size: 85 cm x 85 cm. **Please do not exceed these dimensions.** Fixings will be provided.
- The presenting author (or another contributor) must attend the meeting and present the poster during the allocated poster rounds in order for the abstract to be published in the *Journal of Pathology On-line Supplement* after the meeting.

SLIDE SEMINAR COMPETITION AND DISCUSSION SESSION
Gynaecological Pathology

VIEWING VIRTUAL SLIDES [OCTAGON CENTRE · COMPUTER ROOM]
Slides images will be available for viewing on:
Tuesday 3 July 09.15–17.30
Wednesday 4 July 09.00–17.30
Thursday 5 July 09.00–16.00

COMPETITION
Note: Competition closes at 15.30 on Wednesday 4 July.
There will be a slide competition using slide images, which will be available during the days and times shown above.

PRIZE
The winner will be announced at the Society Dinner on Wednesday 4 July, the prize being a case of champagne *(which at the discretion of the winner, by tradition, is shared amongst those present at the dinner!).*

COMPETITION CASE DISCUSSION SESSION [STUDENTS' UNION BUILDING · AUDITORIUM]
Thursday 5 July 14.45–15.45

SYMPOSIA

Tuesday 3 July

14.10–17.30 ***Viral Oncogenesis in Head and Neck Tumours*** **[STUDENTS' UNION BUILDING · AUDITORIUM]**

Wednesday 4 July

11.00–13.00 ***Systems Biology – Reverse Engineering the Phenotype*** **[STUDENTS' UNION BUILDING · AUDITORIUM]**
11.00–13.00 ***Endocrine Pathology*** **[DAINTON BUILDING · LECTURE THEATRE 1]**
— Organised in conjunction with the UK Endocrine Pathology Society

TRAINEES PROGRAMME [STUDENTS' UNION BUILDING · AUDITORIUM]

Wednesday 4 July

08.00–09.00 **Meet the Experts:** ***Cardiac Tissue Analysis: from Routine to Complex***

Thursday 5 July

08.00–09.00 **Meet the Experts:** ***My Approach to Soft Tissue Tumours***
11.00–12.30 **Symposium:** ***How to be a Consultant***

CONTINUING PROFESSIONAL DEVELOPMENT (CPD)

This Meeting has been approved by the **Royal College of Pathologists** for the purpose of Continuing Professional Development.

CREDITS

Credits can be accrued as follows:

Tuesday 3 July — (full day only*)	6 credits
Wednesday 4 July — (full day only*)	6 credits
Thursday 5 July — (full day only*)	5 credits

Delegates who are eligible for CPD points should sign in each day and complete the CPD Certificate Request form which will be provided at the meeting.

***PART TIME ATTENDANCE / SELF-ACCREDITATION (SLIDE SEMINAR)**

Delegates who do not attend for the whole day will not be issued with certificates but need to claim credits using the reflective note section of their CPD portfolio. This also applies if you look at the virtual slides, submit answers for the competition and attend the discussion session on Thursday 5 July (14.45–15.45).

SOCIAL EVENTS — WELCOME RECEPTION [UNIVERSITY OF SHEFFIELD · FIRTH HALL]

Tuesday 3 July, 19.00–20.30, *featuring musical entertainment by "The Djangonauts".*
Places are free of charge, please book your ticket(s) when registering on-line.

Direct Link FIRTH HALL

Direct Link DJANGONAUTS

SOCIAL EVENTS — SOCIETY DINNER [CUTLERS' HALL]

Wednesday 4 July, 19.30–22.30.
Tickets are £45, please book your ticket(s) when registering on-line.

Direct Link CUTLERS' HALL

TRADE EXHIBITION [OCTAGON CENTRE · MAIN HALL]

Delegates are encouraged to visit the Trade Exhibition and are requested to support the companies represented there.

PRESENTATION CHECKING AND PREVIEW [OCTAGON CENTRE · COMPUTER ROOM]

INTERNET ACCESS [OCTAGON CENTRE · COMPUTER ROOM]

Wireless access and on-site PCs will be available for delegate use.

MESSAGES

During the Meeting, messages for delegates may be left at the following telephone number: **+44 (0)7964 024118**
There will also be a message board located beside the Registration Desk.

REFRESHMENTS

Tea / Coffee will be served in the Main Hall of the Octagon Centre.
Lunch will be served in the Abbeydale / Fulwood Rooms of University House.

BADGES

Delegates are requested to wear their badges **at all times**.

COATS AND BAGS

Secure facilities will be provided for coats and luggage.

TRAVEL, ACCOMMODATION AND VENUE INFORMATION

Please refer to the meeting website for information: **www.pathsoc.org**

Direct Link
TRAVEL

ENQUIRIES

Enquiries before the Meeting regarding administration should be directed to:

Pathological Society of Great Britain & Ireland
2 Carlton House Terrace, London, SW1Y 5AF
Tel: +44 (0)20 7976 1260
Fax: +44 (0)20 7930 2981
Email: admin@pathsoc.org

Direct Link
ACCOMMODATION

Direct Link
LOCAL PLACES OF INTEREST

DISCLAIMER

The Pathological Society of Great Britain & Ireland cannot be held responsible for any injury or loss sustained during the Meeting.

FUTURE MEETINGS

2013

8–9 January	**Joint Meeting with the Dutch Pathological Society** (NVvP) Utrecht, The Netherlands
18–21 June	**Edinburgh Pathology 2013** *7th Joint Meeting of the British Division of the IAP and the Pathological Society*

2014

31 Aug – 4 Sep	**Joint Meeting with the European Society of Pathology, London**

Fees and Registration

REGISTRATION FEES FEES INCLUDE REFRESHMENTS AND LUNCH				
DELEGATE TYPE	FEE CATEGORIES	DAY or PART DAY		SOCIETY DINNER
		UP TO AND INCLUDING 4 JUN 2012	AFTER 4 JUN 2012	
Pathological Society/UKEPS Members	Ordinary Members, Consultant and/or equivalent position	£ 100	£ 150	£ 45
Pathological Society/UKEPS Concessionary Members	Biomedical Scientists; Honorary or Senior Members; PhD Students; Post-Doctoral Fellows, Technicians and Trainees	£ 50	£ 75	£ 45
Undergraduate Students ❇		£ 50	£ 75	£ 45
Non-Members	Consultant and/or equivalent position	£ 150	£ 225	£ 45
Non-Members Concessionary ❇	Biomedical Scientists; PhD Students; Post-Doctoral Fellows, Technicians and Trainees	£ 60	£ 90	£ 45

ADVANCE REGISTRATION

Direct Link REGISTRATION

Registration is via our on-line facility found on our website. Use the Direct Link to the right.
Advance registration will close at midnight on Friday 15 June 2012.
Thereafter delegates may only register on-site on arrival at the meeting.

❇ CONCESSIONS

Delegates from categories:

Undergraduate Students
Non-Members Concessionary

must provide an identification document as proof of their student or trainee status, including NTNs where applicable. Proof must be by way of a statement from the Head of Department.
A template document is available on our website: **www.pathsoc.org**
Please e-mail documents to: **julie@pathsoc.org** — or fax to: +44 (0)20 7930 2981.

CANCELLATIONS

Please note that a cancellation fee of £20 will be deducted from any refund due for cancellations **received in writing by Monday 4 June 2012**. No refunds will be made after 15 June 2012.

DELEGATE ENROLMENT (AT THE MEETING) [OCTAGON CENTRE · FOYER]

The Registration Desk will be open each day as follows:

Tuesday 3 July — from 08.15
Wednesday 4 July — from 07.45
Thursday 5 July — from 07.45

08.15 **OCTAGON CENTRE · FOYER**
REGISTRATION AND COFFEE

09.15–17.30 **OCTAGON CENTRE · COMPUTER ROOM**
SLIDE SEMINAR COMPETITION CASE VIEWING: *Gynaecological Pathology*
Note: Competition closes at 15.30 on Wednesday 4 July

09.30–12.30 **STUDENTS' UNION BUILDING · AUDITORIUM**
ORAL PRESENTATIONS
Chair: Prof M Pignatelli, University of Glasgow
Dr M Loughrey, Royal Group of Hospitals, Belfast
Category: Gastrointestinal

09.30–09.45 [**O1**] ***PIK3CA Mutation is Strongly Associated with K-ras 12/13 Mutation in Stage II/III Colorectal Cancer***

Ⓟ K Southward[1]; M Taylor[1]; P Chambers[1]; K Handley[2]; L Magill[2]; C Beaumont[1]; S Richman[1]; MT Seymour[3]; DJ Kerr[4]; RG Gray[4]; P Quirke[1]

[1]Leeds Institute of Molecular Medicine, Leeds, United Kingdom; [2]Birmingham Clinical Trials Unit, Birmingham, United Kingdom; [3]CRUK Cancer Centre, Leeds, United Kingdom; [4]University of Oxford, Oxford, United Kingdom

Purpose of the study: Ras and Raf mutations in colorectal cancer are associated with poorer outcome and lack of response to anti-EGFR antibodies. The importance of PIK3CA mutations has not been as extensively investigated. In this study the relationship between PIK3CA, mutations in K-ras, N-ras, B-raf and a number of other clinicopathological features have been examined in Quasar-I, a large phase III adjuvant chemotherapy clinical trial.
Methods: PIK3CA mutation status was investigated in a test set of 900 cases. Tumour DNA was extracted from a formalin-fixed paraffin-embedded block in each case. We have assessed PIK3CA mutations in exon 9 (codons 542/545/546) and exon 20 (codon 1047) by pyrosequencing.
Summary of results: Data was available in 873 cases. The mutation rate was 13.9 %. 83 cases had a mutation in exon 9 and 38 cases had a mutation in exon 20. There were no double PIK3CA mutations. 76 (62.8% of all PIK3CA mutated cases) had a K-ras 12/13 and a PIK3CA mutation ($P \leq 0.0001$). There was no association between PIK3CA mutation and sex, site, stage, tumour content, stromal content, K-ras 61, K-ras 146, N-ras 12/13, N-ras 61 or B-raf V600E mutations.
Conclusion: PIK3CA mutations are associated with K ras mutations and this should be considered when ascribing a poor outcome to PIK3CA mutations.

09.45–10.00 [**O2**] ***Mutation and Expression Profile of Primary Colorectal Cancer and Related Liver Metastasis***

Ⓟ W Fadhil; S Ibrahim; M Ilyas

University of Nottingham, Nottingham, United Kingdom

Colorectal Cancers (CRCs) develop through two major genetic pathways that are characterised by chromosomal instability (CIN) in one and microsatellite instability (MSI) in the other. However, colorectal cancer is considered a genetically heterogeneous and continually evolving disease and genetic changes will accumulate which allow primary tumours to metastasise. If metastasis develops from a dominant clone, most genetic alterations that occur during the development of primary CRC will be maintained in metastases.
In this study we sought to compare the mutation status and expression profile of primary colorectal cancer and matched liver metastasis. Fifty paired primary and liver metastasis CRCs were fingerprinted for mutations in Kras (codons 12/13, 61, and 146), Braf (exons 11 and 15), PIK3CA (exons 1, 9, and 20), and p53 (exon 5-8) by HRM analysis and confirmed by sequencing. Protein expression profiles of p53, BCL2, and P21were analysed by immunohistochemistry (IHC). MSI status was carried out using an in house HRM based methodology and confirmed by IHC staining for the MMR proteins.
The results revealed an overall Kras mutation frequency of 46% both in the primary and the metastases. Braf mutation was found in 3 cases (6%). PIK3CA was in 10% of the primary and 8% of the metastases. P53 mutations frequency was 64% in the primary and 66% in the metastases. Two cases were MSI by PCR and showed deficient MMR expression by IHC. The overall mutation rate concordance between the primary and their paired metastases was 98%. Protein expression profiles for P53, BCL2, and P21 showed highly significant concordance ($p<0.001$) between the primary and related metastatic tumours.
The high concordance between primary and metastases suggests that these alterations are generally acquired prior to metastatic spread. Clinically this means that mutation testing of primary tumour is reasonable when making treatment decisions for metastatic disease.

10.00–10.15 [**O3**]

Recurrence After Radiofrequency Ablation for Barrett's Related High Grade Dysplasia is Due to Persistence of Epithelial Mutations

Ⓟ S Zeki[1]; R Haidry[2]; H Barr[3]; N Shepherd[3]; M Novelli[2]; M Rodriguez Justo[2]; N Wright[1]; L Lovat[2]; SAC McDonald[1]

[1]Barts and the London School of Medicine and Dentistry, London, United Kingdom; [2]University College London Hospitals, London, United Kingdom; [3]Gloucestershire Hospitals NHS Trust, Gloucester, United Kingdom

Radiofrequency ablation (RFA) is an endoscopic method for the ablation of high grade dysplasia (HGD) and intramucosal adenocarcinoma (IMC) in patients with Barrett's oesophagus. A number of patients develop recurrence of dysplasia or cancer (1,2). The reason for this recurrence is unknown. To investigate whether HGD or IMC recurrence is related to the persistence of known cancer driving mutations after radiofrequency ablation. Biopsies and endoscopic mucosal resection (EMR) specimens were obtained before and after RFA for patients with recurrent HGD or IMC. These underwent nested polymerase chain reaction (PCR) sequencing for mutations commonly implicated in cancer progression (TP53, CDKN2A, K-ras). Tissue was obtained for 6 patients before and after RFA, 5 of whom had detectable mutations. All were male (mean age 67 SD +/-2). In 3/5 patients, the same mutation was found in material taken before RFA as after (all TP53 mutations). All 3 patients progressed to IMC having undergone RFA for HGD. In two cases, microdissection was performed on the post-RFA EMR samples. PCR revealed the pre RFA mutation to also be present throughout the IMC specimen. Dysplastic tissue adjacent to the IMC contained a mixture of wild-type or mutated crypts (the same mutation as in the IM) indicating that the IMC was a monoclonal outgrowth and that the persistent mutation was driving the development of the recurrence. In all 3 cases, PCR of a further specimens (performed at a later time point to the first post- RFA EMR), detected the original pre-RFA mutation. The recurrence of dysplasia and cancer after RFA is likely to be due to failure to remove persistent, cancer driving mutations. Further work will need to be done to assess whether this is because of technical errors, or related to specific problems such as 'buried Barrett's'. 1. Shaheen et al., (2009) N Engl J Med, 360:2277-2288 2. Wolfen et al., (2011) Gastroenterology, 141(2):460-468

10.15–10.30 [**O4**]

FGFR2 *Gene Amplification is Related to Prognosis and Lymph Node Status in Two Independent Gastric Cancer Cohorts from the UK and Korea*

Ⓟ HI Grabsch[1]; C Womack[2]; X Su[3]; P Zhan[3]; P Gavine[3]; S Morgan[2]; EJ Jung[4]; YJ Bang[4]; SA Im[4]; WH Kim[4]; E Kilgour[2]

[1]Leeds Institute of Molecular Medicine, University of Leeds, Leeds, United Kingdom; [2]Oncology Innovative Medicines, AstraZeneca, Macclesfield, United Kingdom; [3]Innovation Center China, AstraZeneca, Shanghai, China; [4]College of Medicine, Seoul National University, Seoul, Republic of Korea

Background: Fibroblast growth factor receptor 2 (*FGFR2*, previously called *K-SAM*) was one of the first genes found to be amplified in gastric cancer (GC) more than 20 years ago. The aim of the current study was to compare the frequency of *FGFR2* amplification (*FGFR2*amp) in two independent GC cohorts from the UK and Korea, its relationship with clinicopathological variables, survival and HER2 or cMET status.
Methods: FGFR2, HER2 and cMET status was assessed by fluorescence in situ hybridisation and/or immunohistochemistry in tissue microarrays constructed from 408 UK and 356 Korean GC resection specimens. Gene amplification was defined as a gene/centromeric probe ratio of ≥2.0 after measuring at least 50 tumour cells/case.
Results: 7% UK GC and 4% Korean GC showed *FGFR2*amp. Only 2 of the 26 *FGFR2*amp UK GC showed simultaneous *HER2* amplification. Korean GC with *FGFR2*amp showed neither *HER2* nor *cMET* amplification in *FGFR2*amp GC. *FGFR2*amp was associated with the presence of lymph node metastases in both cohorts ($p<0.0007$) and with diffuse-type histology in the Korean cohort ($p=0.028$). Patients with *FGFR2*amp GC had shorter overall survival in both cohorts. *FGFR2*amp proved to be an independent prognostic marker when age, gender, grade and stage were included in the multivariate analysis model (UK GC: HR 2.37, 95% CI 1.6 - 3.5; $p=0.0001$; Korean GC: HR 2.33, 95% CI 1.28 - 4.25; $p=0.0129$). No relationship was found between FGFR2 status and pT, age or gender.
Conclusions: This study demonstrates a low frequency of *FGFR2*amp and a relationship with prognosis and tumour progression in two large independent GC cohorts from countries with very different GC incidence and ethnic background. In both cohorts, amplification of *FGFR2*, *HER2* or *cMET* appears to be mutually exclusive. Inhibitors to all three genes may be effective in molecularly defined subsets of GC patients with resectable disease.

10.30–10.45 [**O5**]

Hypoxia Response Proteins HIF-1α, CAIX and GLUT1 as Potential Prognostic Biomarkers in Colorectal Cancer

Ⓟ L Sansom; GGA Hutchins; M Shires; G Hemmings; E Tinkler-Hundal; H Grabsch; P Quirke

University of Leeds, Leeds, United Kingdom

Background : There is a need for prognostic and predictive biomarkers in colorectal cancer (CRC). Currently, the use of adjuvant therapy in CRC is directed principally stage and other prognostic histopathological features. Many of these 'high-risk' features are subjective. Biomarkers could thus enhance prognostication and aid therapeutic decisions. Many biomarkers have been suggested, but few validated. We aimed to investigate the potential clinical value of hypoxia response proteins HIF1α, CAIX and GLUT1 as putative prognostic biomarkers in CRC.
Methods : TMA sections containing tissue from 306 CRC patients with were stained by immunohistochemistry using antibodies against hypoxia pathway proteins HIF1α, GLUT1 and CAIX. Slides were manually scored using antibody specific scoring systems. Expression data was compared to clinical outcome data using cancer-specific

(CSS) and overall survival (OS) as primary and secondary endpoints respectively.
Results : High GLUT1 expression was significantly associated with a worse CSS [HR=1.98 (95%CI=1.386-2.853, p<0.0001)] and OS [HR=1.614 (1.190-2.190, p<0.001)] when compared to low GLUT1. Similarly, high nuclear HIF1α expression was associated with lower CSS (HR=1.430, 95%CI=1.027-1.990, p<0.034). No association with survival was seen with CAIX, although non-significant trend was demonstrated whereby increased CAIX was associated with a reduced CSS.
Conclusion : This small study demonstrates HIF1α and GLUT1 as potential prognostic markers in CRC. Tumour hypoxia is associated with worse clinical outcomes.

10.45–11.15 **OCTAGON CENTRE · MAIN HALL**
COFFEE AND TRADE EXHIBITION

11.15–11.30 [**O6**] ***Analysis of Lymph Node Yield in Colorectal Cancer***

Ⓟ D Cilia Vincenti; GI Murray

Pathology Department, Aberdeen Royal Infirmary, Aberdeen, United Kingdom

One of the key prognostic factors in colorectal cancer is the presence or absence of lymph node metastasis. Hence, there is a requirement to identify all lymph nodes as it is postulated that increasing lymph node yield increases the detection of lymph node metastasis. An analysis of lymph node yield from all patients (n=1881) who underwent surgery for colorectal cancer in a single region over a seven year period from 2005 to 2011 has been performed. All the pathology in this study has been performed in a single centre and reported according to the Royal College of Pathologists guidelines. The pathological parameters have been extracted from pathology reports, entered in a database and data analysed using SPSS. The mean lymph node yield for all cases was 16.77 and in 80.3% of cases 12 or more lymph nodes were retrieved. The mean lymph node yield for the cases (n=378) that received neoadjuvant therapy was 16.04. Further analysis focuses on those patients who received primary surgical treatment (n=1503). This set of cases had a mean lymph node yield of 16.96 with 80.6% of cases having at least 12 lymph nodes identified. The mean lymph node yield for colon cancers (n=1341) was 16.71 while the mean lymph node yield for rectal tumours (n=162) was 19.03. Dukes A (n=192) tumours had a mean lymph node yield of 14.63 while Dukes B (n=635) cases had a mean lymph node yield of 17.73 and Dukes C (n=676) cases had a mean lymph node yield of 16.89. The data indicate that at least 12 lymph nodes are identified in most tumours. Although lymph node yield has shown an upward trend, with the mean in 2005 being 15.36 and in 2011 being 19.84, the proportion of Dukes C cases has not shown a similar trend suggesting that increasing the yield of lymph nodes does not necessarily increase the number of lymph node positive tumours.

11.30–11.45 [**O7**] ***The Validity of the Royal College of Pathologists' Stomach Cancer Minimum Dataset in a Population using the Northern & Yorkshire Cancer Registry Data***

Ⓟ O Rotimi; H Grabsch; E Morris

Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom

Purpose of the study: Quality histopathological reporting provides important information for treatment of patients. Sub-standard reporting leads to incorrect staging which impacts on treatment and ultimately survival. Proforma reporting has been introduced as a means of standardisation. This study sought to validate the prognostic significance of the RCPath stomach cancer dataset in a population.
Methods: A retrospective analysis of pathology forms from 1065 resected stomach cancer from 1995 to 2006 was carried out. The variables reported were related to NYCRIS registry survival data using univariate Kaplan-Meier method and Cox proportional regression modelling.
Summary of results: The study validated depth of local invasion, nodal stage and completeness of excision as independent prognostic factors in a population setting. Importantly tumour differentiation is shown to be an independent prognostic factor. Patients recorded as well differentiated tumours had significantly better 3-year survival (61.3%; 95%CI 53.2-68.4%) compared to moderate (38.4%; 95%CI 33.2-43.6%) and poor differentiation (34.1%; 95%CI 30.1-38.1%) especially in the Lauren's intestinal type subgroup. The effect remains significant after adjusting for age and gender with hazard ratio of 1.74 (95%CI 1.11-2.71) for moderately differentiated and 2.1 (95% CI 1.3-3.4) for poorly differentiated tumours compared to well differentiated tumour.
Conclusion: The variables in the RCPath stomach cancer dataset are validated to be of prognostic significance in a population setting. Importantly, the result showed that there is a significant survival differences between the three grades of differentiation. This is in contrast to the current recommendation by the RCPath that well and moderately differentiated tumours be reported together as a single category. We recommend separating this category in the next version of the RCPath stomach cancer dataset guidelines.

11.45–12.00 [**O8**] ***Prognostic Significance of Circumferential Resection Margin Status in Oesophageal Cancer — a Systematic Review and Meta-Analysis***

Ⓟ O Rotimi[1]; DC Greenwood[2]; YK Tu[2]

[1]Department of Histopathology, St James University Hospital, Leeds, United Kingdom; [2]Biostatistics Unit, Centre for Epidemiology & Biostatistics, University of, Leeds, United Kingdom

Purpose of study: The status of the circumferential resection margin (CRM) in oesophageal cancer has been suggested as a prognostic factor but the reports are conflicting. Also, there are two methods of defining positive CRM - within 1mm (Royal College of Pathologists, RCPath, UK) and 0mm (College of American Pathologists, CAP, USA).

Methods: A systematic review was carried out using a protocol and papers that met the inclusion criteria were selected and data extracted from those with required adjusted hazard ratio (HR) for meta-analysis using STATA-11 statistical software. Assessments were made for heterogeneity, publication bias, small study effects and sensitivity analysis for influence.
Summary of results: Fourteen cohort studies were systematically reviewed but nine meta-analysed. The fixed-effects HR for the RCPath criteria is 1.45 (95%CI 1.27 to 1.66; p-value<0.0001)); 2.61 (95%CI 1.90 to 3.59; p-value<0.0001) for the CAP criteria and overall pooled HR of 1.58 (95%CI 1.40 to 1.79; p-value<0.0001). There was significant heterogeneity between the studies (p-value<0.001 and I-squared value of 74.8%). There was evidence of publication bias and small study effects (Egger's test p-value 0.029). None of the studies had undue influence.
Conclusions: This meta-analysis provides evidence that the CRM status in oesophageal carcinoma has prognostic significance. The overall pooled HR of 1.58 (95% CI 1.40 to 1.79) suggests patients with positive CRM have 60% more risk of death compared to patients with a negative margin. This significance is present irrespective of the criteria used for the definition of the margin but the estimate for papers reporting the USA CAP criterion is much higher than those reporting the UK RCPath criterion. The significant heterogeneity and publication bias are limitations to the study.

12.00–12.15 [**O9**] ***Receptor Tyrosine Kinase Over-Expression is a Frequent and Early Event in Oesophageal Carcinogenesis***

Ⓟ AL Paterson[1]; M O'Donovan[1]; E Provenzano[1]; L Murray[2]; H Coleman[2]; B Johnston[3]; D McManus[3]; M Novelli[4]; L Lovat[4]; R Fitzgerald[5]

[1]Addenbrooke's Hospital, Cambridge, United Kingdom; [2]Queen's University Belfast, Belfast, United Kingdom; [3]Belfast Health and Social Care Trust, Belfast, United Kingdom; [4]University College Hospital, London, United Kingdom; [5]MRC Cancer Cell Unit, Cambridge, United Kingdom

Despite being a common event, the timing of receptor tyrosine kinase (RTK) up-regulation during carcinogenesis is poorly understood. We used the metaplasia-dysplasia-carcinoma progression underlying oesophageal adenocarcinoma (OAC) as a model to explore this question. OAC is an aggressive, chemo-resistant malignancy with a rapidly increasing incidence. The key molecular drivers remain unclear and therefore patients are yet to benefit from novel targeted therapies.
We used a cohort of OAC patients (n=367) and two cohorts with pre-invasive disease, one cross-sectional (n=110) and one longitudinal in time (n=91) covering the spectrum from intestinal metaplasia without dysplasia (IM), low grade dysplasia (LGD) and high grade dysplasia (HGD); to determine the frequency and timing of up-regulation of a RTK panel; EGFR, ErbB2, ErbB3, Met and FGFR2.
51% of OACs over-expressed at least one of the RTK panel; with 21% of these over-expressing multiple receptors simultaneously. Up-regulation of RTK expression was an early event, corresponding with LGD development (25% in IM vs. 63% in LGD, p<0.001). The prevalence of RTK co-over-expression increased as IM progressed to LGD, 7% vs. 10% (p=0.06); and as LGD progressed to HGD, 10% vs. 19% (p=0.24). The timing of RTK up-regulation varied depending on the specific receptor; FGFR, ErbB2 and Met over-expression frequently occurred in LGD; whilst EGFR over-expression was predominately seen in invasive disease; and ErbB3 over-expression was uniformly rare.
RTK up-regulation is a frequent and early event in oesophageal carcinogenesis, implying a potentially pivotal role. RTK inhibition offers a novel therapeutic and/or chemopreventative strategy which justifies further investigation.

12.15–12.30 [**O10**] ***Laparoscopic Complete Mesocolic Excision with Central Vascular Ligation for Colon Cancer Produces an Equivalent Specimen to Open Surgery***

Ⓟ NP West[1]; RH Kennedy[2]; JT Jenkins[2]; T Magro[2]; G Luglio[2]; S Sala[2]; P Quirke[1]

[1]Pathology & Tumour Biology, University of Leeds, Leeds, United Kingdom; [2]St. Mark's Hospital, Harrow, United Kingdom

Open complete mesocolic excision with central vascular ligation (CME with CVL) produces an oncologically superior specimen when compared to conventional low tie surgery for colon cancer. We assessed whether laparoscopic CME with CVL in an expert centre produced the same superior specimen to expert open surgery.
One expert laparoscopic centre provided fresh specimen photographs and clinicopathological data on a series of resections performed between February 2010 and July 2011. The plane of surgery was assessed and tissue morphometry performed on the photographs by an independent pathologist using established systems. These were compared to published results from an expert open CME with CVL centre.
In total, 69 laparoscopic colon resections were performed using the CME with CVL technique including 58 for invasive adenocarcinoma. Only 3 cases were converted to open surgery. The median number of lymph nodes resected in the cancer cases was 19 (IQR 14 to 25). The overall mesocolic plane rate was high at 90% and muscularis propria plane surgery was not observed. There was no significant difference in the area of mesentery resected or the distance from the bowel wall to the nearest high vascular tie between the laparoscopic and open CME with CVL specimens.
Open CME with CVL has already been shown to produce a superior specimen when compared to conventional low tie surgery. Laparoscopic colorectal resection is increasing in usage and it is essential that the technique is shown to have equivalence to excellent open surgery. We have shown that laparoscopic CME with CVL produces an equivalent specimen in terms of the amount of tissue resected and respect for the surgical planes. A number of studies have suggested that CME with CVL is associated with improved patient outcomes and this should now be strongly encouraged in both open and laparoscopic colon cancer centres.

09.30–12.30 **DAINTON BUILDING · LECTURE THEATRE 1**

ORAL PRESENTATIONS

Chair: Dr JJ Going, University of Glasgow
Dr E Rakha, University of Nottingham
Category: Breast

09.30–09.45 [**O11**] ***Fatty Acid Binding Protein 7 Expression and its Sub-Cellular Localization in Breast Cancer***

Ⓟ AT Alshareeda; EA Rakha; C Nolan; IO Ellis; AR Green

Nottingham University, Nottingham, United Kingdom

Introduction: FABP7 is a member of the multi gene fatty acid binding protein family. It is expressed in the mammary gland and it has been identified as an inhibitor of proliferation and promoter of differentiation via the JAK/Stat pathway. Although, it is well known that FABP7 has a cytoplasmic pattern of expression, its varying sub-cellular localization between nucleus and cytoplasm has been observed in glioma cell lines and in developing brain. Cytoplasmic FABP7 expression is associated with favourable outcome in basal like breast cancer.
Materials and methods: A well-characterized series of; 1351 unselected cases and 245 ER negative invasive breast cancers with a long term follow up were investigated in this study to assess the sub-cellular localization of FABP7 (Abcam Ltd., Cambridge, UK) using immunohistochemistry.
Results: Nuclear FABP7 expression was associated with high histologic grade (P<0.001), mitotic frequency, pleomorphism and stage, in addition to TN (P<0.001). Interestingly, in multivariate Cox-regression analysis, nuclear FABP7 expression in BLBC was a significant predictor of longer DFI (P=0.025) independent of cytoplasmic expression. Patients with tumours showing only nuclear positive FABP7 expression had a significantly better prognosis than ones with only cytoplasmic expression. Nuclear FABP7 expression showed an association with expression of markers associated with proliferation and cell cycle control including Ki67, p53 and p21 however, cytoplasmic FABP7 was associated only with Ki-67 and P53.
Conclusion: This is the first study elucidating the sub-cellular localization of FABP7 in a large series of breast cancer cases. Our observations demonstrate the considerable heterogeneity in expression patterns of FABP7 within breast cancer and which relates to differences in biological behaviour especially in BLBC. Further investigation of the biology of FABP7 in breast cancer is warranted.

09.45–10.00 [**O12**] ***Topoisomerase II alpha (Top2a) as a Predictor for Response to Anthracycline-based Chemotherapy (ATC-CT): Is it due to Gene Amplification, HER2-Coamplification or Protein Overexpression?***

Ⓟ TMA Abdel-Fatah[1]; MB Lambros[2]; G Ball[3]; R Vatcheva[2]; PD Dickinson[4]; P Moseley[4]; AR Green[5]; IO Ellis[5]; JR Reis-filho[2]; SYT Chan[4]

[1]Clinical Oncology, Nottingham University City Hospital NHS Trust, Nottingham, United Kingdom; [2]The Breakthrough Breast Cancer Research Centre, London, United Kingdom; [3]The Van Geest Cancer Centre, Nottingham Trent University, Nottingham, United Kingdom; [4]Department of Clinical Oncology, Nottingham University Hospitals NHS TRUST, Nottingham, United Kingdom; [5]Division of Pathology, School of Molecular Medical Sciences, University of Nottingham, Nottingham, United Kingdom

Background: In this study we assessed the association between gene copy number (GCN), gene expression (GE) and protein expression of both TOP2A and HER2 and their effect on clinicopathological outcomes and management of breast cancer (BC).
Methods: 1- The associations between response to ATC-CT and both GCN and protein alteration of TOP2A and HER2 were investigated in four independent data bases: a) 250 primary BC treated with neoadjuvant ATC-Neo-CT; pathological complete response (pCR) was used as the primary endpoint (PEP), b) two sets of primary 245 BC and 250 ER- treated with adjuvant ATC-CT and c) 145 primary BC overexpressing HER-2 treated with adjuvant ATC-CT + trastuzumab. 2- The clinicopathological implications of TOP2A GCN and protein expression were evaluated in a series of 1600 consecutive primary BC. 3- The correlations between GCN, GE and protein expression of both TOP2A and HER2 were studied in series of primary BC. Artificial Neural Networks (ANN) and pathway analysis were used to identify genes and biological pathways that related to TOP2A gene alterations.
Results: In ATC-Neo-CT series, the pCR rate was 32/115 (28%) in tumours expressing high levels of Topo2A, compared to 5/74 (7%) in tumours expressing low levels of Topo2A. (p<0.0001). In multivariate analysis, Top2A overexpression was an independent predictor for pCR (p<0.001). TOP2A overexpression was strongly associated with mitotic index (p<0.0001). ANN and pathway analysis reveals that genes associated with TOP2A overexpression are involved in M phase, cell division and metastases.
Conclusions: Alteration in Top2A protein expression is an independent predictor for response to ATC-CT which cloud be explained by: a) Top2A protein is a biomarker for highly proliferating cycling tumour cell and b) Top2a protein is a direct target of anthracycline agents.

10.00–10.15 [**O13**] ***PARP1 Expression and its Relation to BRCA1 Status in the Different Molecular Classes of Breast Cancer***

Ⓟ AA Benhasouna,[1]; A Alshareeda[1]; AR Green[1]; S Madhusudan[2]; IO Ellis[1]; EA Rakha[1]

[1]Department of Histopathology, The University of Nottingham and Nottingham University Hospitals NHS Trust, Nottingham City Hospital, Nottingham, United Kingdom; [2]Laboratory of Molecular Oncology, Academic Unit of Oncology, School of Molecular Medical Sciences, Nottingham University Hospital, Nottingham, United Kingdom

Poly (ADP-ribose) polymerase-1 (PARP1) is a key factor in single strand break repair (SSBR), a subpathway in DNA base excision repair and implicated in tumorigenesis. PARP inhibitors have gained recent attention as targeted therapy for breast cancer (BC) in BRCA1 germline mutation carriers. Recent evidence suggests dysfunctional DNA repair mechanisms may be commonplace in oestrogen receptor (ER)-negative BC and a broader application of PARP inhibitors may be feasible in BC. This study aimed to assess PARP1 (both cleaved (PARP1c) and non-cleaved (PARP1n) forms using immunohistochemistry applied to a large and well-characterised series of early stage (I-III) sporadic BC with long term follow-up including 450 ER- tumours prepared as tissue microarrays. A subset of BRCA1-mutated tumours (no=22) were included as a control group. In addition, tumours with loss of both BRCA1 and RAD51 were used as surrogates for non-functional homologous recombination (HR) DNA repair pathway.

Results: The vast majority of breast carcinomas expressed nuclear PARP1c (95%) and 51% expressed PARP1n protein. All BRCA1-mutated tumours showed expression of PARP1c and only 2 cases lacked expression of PARP1n. Although the difference in PARP1 expression between ER+ and ER- tumours was not significant, there was significant reduction of PARP1n in tumours with defective HR pathway in the whole series (242/1300), in the ER+ subgroup (48/799, p=0.035) and in ER- tumours (174/441, p=0.004). No such association was identified with PARPc. There was no significant association between PARP1 and outcome in the whole series or in the ER- subgroup.

In conclusion, this study indicates there is a subset of sporadic BC which shows dysfunctional DNA repair mechanism including HR pathway and PARP1 expression. Further investigation of this subset of tumours to identify potential candidates for PARP inhibitors is warranted.

10.15–10.30 [**O14**] ***Sumolyation Markers (PIAS gamma and UBC9) are Indicators for Poorer Breast Cancer Specific Survival in Nigerian Women***

Ⓟ AOJ Agboola[1]; AA Musa[2]; BA Ayoade[2]; AA Banjo[3]; EA Rakha[1]; EC Paish[1]; C Nolan[1]; IO Ellis[1]; AR Green[1]

[1]Division of Pathology, School of Molecular Medical Sciences, Nottingham University Hospitals and University of Nottingham, Nottingham, United Kingdom; [2]Olabisi Onabanjo University and Olabisi Onabanjo University Teaching Hospital, Sagamu, Nigeria; [3]Dept of Morbid Anatomy and Histopathology, Olabisi Onabanjo University and Olabisi Onabanjo University Teaching Hospital, Sagamu, Nigeria

Nigerian women have a higher risk of expressing triple negative and BRCA1 deficiency BC with a high mortality rate, prompting speculation that risk factors could be genetic and the molecular portrait of these tumours may be different to those of Western women. BRCA1Sumoylation (PIAS gamma and UBC9) has been implicated in the ER transcription activity, BRCA1 deficiency and triple negative BC.

This study investigated the immunoprofiles of PIASgamma and UBC9 in TMA of 231 and 199 Nigerian women and correlated their protein expression with clinical outcome, pathological responses and the expression of 17 other biomarkers to demonstrate the functional significance in the black women.

The protein expression of PIAS gamma and UBC9 compared with other biomarkers showed an inverse correlation with ER (p<0.001,p=0.002), PgR (p<0.001) and BRCA1(p=0.006, p=0.004) and a positive correlation with triple negative (p<0.001), basal cytokeratin (CK14 expression (p=0.001, p=0.01) and CK5/6 (P<0.001)), EGFR (p=0.01, p<0.001), basal-like breast cancer (p<0.001), p53 (p<0.001), p27 (p<0.001) and MDM4 (p=0.029, p<0.001), KU70/80 (p<0.001), RAD51 (p<0.001, p=0.004), PARP1 (p<0.001), BARD1 (p=0.002, p=0.003) and CHK1 (p<0.001), laminin (p<0.001), MTA1 (p<0.001) and ID4 (p<0.001). Univariate survival analysis showed that those tumours positive for PIAS gamma and UBC9 expression had significantly poorer BCSS compared with those showing negative expression (both p<0.001). In Cox multivariate analysis, PIAS gamma and UBC9 remained significant predictors of outcome for BCSS independent of tumour grade, size and lymph node involvement (p=0.001, p=0.04).

This study demonstrates that PIAS gamma and UBC9 appear to be important in cancer behaviour. Therefore targeting the related functional pathways of tumours arising in Nigerian women could enhance the development of novel targeted therapies.

10.30–10.45 [**O15**] ***Clinicopathological Significance of Ku70/Ku80, a Key Non-Homologous End Joining (NHEJ) DNA Repair Factor in Oestrogen Receptor-Negative Breast Cancer***

Ⓟ AT Alshareeda; AR Green; AA Ben Hansouna; S Madhusudan; IO Ellis; EA Rakha

Nottingham University, Nottingham, United Kingdom

Impaired DNA-damage signalling and repair may play a fundamental role in the pathogenesis of breast cancer. DNA double strand breaks (DSBs) are repaired either by homologous recombination (HR) or non homologous end-joining (NHEJ). It has been confirmed that within basal-like class of breast cancer (BLBC) loss of BRCA1 a key component in HR, is more frequent compared to other BC subtypes. Potential role of impaired NHEJ remains

unclear in BC although this pathway is the predominant DSB repair pathway in mammalian cells outside the S-phase of the cell cycle.
In this study, a well-characterized series of 1208 unselected cases, including; 865 ER receptor negative and 25 known BRCA1 mutation invasive breast cancers with long term follow up were investigated. The aim of the study was to assess the functional status of NHEJ in BC using the immunohistochemical marker Ku70-Ku80 (Abcam Ltd., Cambridge, UK).
Results: Nuclear Ku70-Ku80 expression was significantly associated with high histological grade (P=0.037), tumour stage (P=0.02) and vascular invasion (P<0.001). Positive nuclear Ku70-Ku80 expression was seen in all BRCA1 associated tumours. Nuclear Ku70-Ku80 expression was associated with loss of oestrogen hormone receptors (P=0.025), absence of BRCA1 expression and with p53 expression (P<0.001 and 0.004). Interestingly, in the BLBC Ku70-Ku80 expression was a predictor of outcome independent of chemotherapy (BCSS; P=0.04, DFI; P=0.01). No such association was found in the ER positive cases.
Conclusion, our observations indicate that NHEJ may play a role in BLBC and assessments of proteins associated with DNA repair can potentially be used to sub classify BLBC. DSBs repair mechanisms and their alterations appear to influence prognosis in BLBC and therefore further investigation of the mechanism of this effect is warranted.

10.45–11.15 **OCTAGON CENTRE · MAIN HALL**
COFFEE AND TRADE EXHIBITION

11.15–11.30 [**O16**] ***The Value of Examination of Multiple Levels of Mammary Needle Core Biopsy Specimens Taken for Investigation of Lesions Other Than Calcification.***
Ⓟ AHS Lee; NM Villena Salinas; Z Hodi; EA Rakha; IO Ellis

Nottingham University Hospitals, Nottingham, United Kingdom

It is standard practice to examine multiple levels of needle core biopsies taken for investigation of mammographic calcification, but there is almost no evidence on the value of levels in core biopsies taken for other reasons. This study aimed to assess the value of levels for needle core biopsies taken for investigation of lesions other than calcification. A comparison of the diagnosis on the first level of core biopsies with the diagnosis on levels 2 and 3 was made in 375 breast core biopsies. The diagnosis after examining 3 levels was different from that in the initial level in 4 of 272 (1.5%) of core biopsies taken for reasons other than calcification and in 13 of 103 (13%) biopsies taken for investigation of calcification. This study confirms the value of levels of biopsies taken to investigate mammographic calcification, but suggests that routine levels are of limited value for breast core biopsies taken for other reasons.

11.30–11.45 [**O17**] ***Pleomorphic Lobular Carcinoma of the Breast: is it a Prognostically Significant Pathological Subtype Independent of Histological Grade?***
EA Rakha[1]; CMH van Deurzen[2]; RD Macmillan[1]; IO Ellis[1]; Ⓟ AHS Lee[1]

[1]Nottingham University Hospitals, Nottingham, United Kingdom; [2]Erasmus Medical Center, Rotterdam, Netherlands

Pleomorphic lobular carcinoma is regarded as a biologically aggressive variant of invasive lobular breast carcinoma. However, there is no consensus on the definition and whether this subtype adds useful information to histological grade. 202 grade 2 or grade 3 invasive lobular carcinomas were studied. Tumours were categorised according to the components of histological grade: tubules (T), pleomorphism (P) and mitoses (M). Pleomorphic lobular carcinoma was defined as a carcinoma with a lobular growth pattern and marked nuclear pleomorphism (P3). Breast cancer specific survival was used as the outcome in analysis of prognosis. A non-classical growth pattern was seen more frequently in all subgroups with marked nuclear pleomorphism. Grade 3 pleomorphic lobular carcinomas (T3, P3, M2 and T3, P3, M3) had a worse prognosis than grade 2 (T3, P2, M1) carcinomas. Grade 2 lobular carcinomas with marked nuclear pleomorphism (T3, P3, M1) had a similar prognosis to T3, P2, M1 carcinomas. Non-classical growth pattern was associated with a worse survival than classical growth pattern. Histological grade and nodal status were independent prognostic factors. In conclusion this study shows that histological grade (in particular the mitotic component) when applied to invasive lobular carcinomas is of prognostic importance, but the pleomorphic type, defined according to nuclear morphology, does not provide useful additional prognostic information.

11.45–12.00 [**O18**] ***HAGE (DDX43) Protein Expression is a Powerful Independent Biomarker of Poor Clinical Outcome of Breast Cancer (BC) and Could be a Potential Therapeutic Target for ER Negative BC***
Ⓟ TMA Abdel-Fatah[1]; SE McArdle[2]; C Johnson[2]; P Moseley[1]; A Green[3]; IO Ellis[3]; RC Rees[2]; SYT Chan[1]

[1]Clinical Oncology, Nottingham University City Hospital NHS Trust, Nottingham, United Kingdom; [2]The Van Geest Cancer Research Centre, School of Science and Technology, Nottingham Trent University, Nottingham, United Kingdom; [3]Division of Pathology, School of Molecular Medical Sciences, University of Nottingham, Nottingham, United Kingdom

Purpose: HAGE protein has been shown to be immunogenic and is essential for cancer cell proliferation suggesting it as an ideal candidate for immunotherapy and a useful biomarker in BC. In this study we aimed to analyze the

expression of HAGE in a large well-characterized BC cohorts of patients to determine its prognostic and predictive value .
Patient and methods: HAGE expression was evaluated in breast cell lines, 60 full sections of normal breast tissues, and 1650 early stage BC received CMF or endocrine therapies. Further validation was performed in two independent series of 300 and 396 ER negative (ER-) BC who did not received CT and did received anthracycline CT, respectively.
Results: HAGE was absent in normal breast and its expression was induced by stress in breast cell lines. Overexpression of HAGE (HAGE+) was observed in 8% of BC and was significantly associated with more aggressive clinico-pathological features including: high grade, triple receptors negative (TNT) phenotypes; over-expression of HER2 and p53 mutation. HAGE+ expression tumours showed an adverse outcome with a 2-fold increase in the risk of death, recurrence and metastases compared to tumors with weak/no HAGE expression (HAGE-). Adjuvant CT, either CMF or anthracycline, has a positive impact in high risk ER- BC; in this group of patients HAGE+ expression has no statistically significant difference of increased risk of death, recurrence or distant metastases from those with HAGE- expression. Using a multivariate Cox regression model including other validated prognostic factors, HAGE expression was confirmed as a powerful independent predictor for clinical outcome.
Conclusions: This is the first report which shows HAGE to be a potential predictor for poor prognosis in BC patients, and may be an attractive new target for treatment.

12.00–12.15 [**O19**] ***A Study of Sperm-Associated Antigen 5 (SPAG5) in Predicting Response to Anthracycline (ATC)/ Platinum Chemotherapies (CT) in Breast (BC) and Ovarian Cancers (OVC)***

Ⓟ TMA Abdel-Fatah[1]; G Ball[2]; SE McArdle[2]; C Johnson[2]; P Moseley[3]; A Green[4]; IO Ellis[4]; SYT Chan[5]

[1]Clinical Oncology, Nottingham University City Hospital NHS Trust, Nottingham, United Kingdom; [2]The Van Geest Cancer Research Centre, School of Science and Technology, Nottingham Trent University, Nottingham, United Kingdom; [3]Department of Clinical Oncology, Nottingham University Hospitals, Nottingham, United Kingdom; [4]Division of Pathology, School of Molecular Medical Sciences, University of Nottingham, Nottingham, United Kingdom; [5]Department of Clinical Oncology, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom

Background: Recently TOP2A alteration was found to be a predictor for ATC-CT and our neural network analysis of BC gene expression array (GEA) data has revealed SPAG5 gene as a major hubs in both TOP2A and proliferation pathways. In this study the molecular and clinicopathological functions of SPAG5 was investigated in BC and OVC. Methods: A series of 171 BC was evaluated for SPAG5 gene copy number (using aCGH) and mRNA expression (using GEA) which were validated in independent databases. The expression of SPAG5 protein was evaluated in BC and OVC cell lines and in normal breast tissues and a series of 1650 primary BC. The association between SPAG5 and response to CT was investigated in a) 350 ER negative BC treated with adjuvant ATC-CT, b) 250 BC treated with neoadjuvant (NEO-A)-ATC-CT, and c) 200 primary OVC treated with cisplatinum based adjuvant CT. Results: 5% and 15% of BC showed amplification and gain of SPAG5 locus, respectively, at 17q11.2. SPAG5 mRNA expression displayed a significant correlation with its copy number ($p< 0.0001$). 30% and 20% of ovarian and BC respectively, showed overexpression of SPAG5 protein (+). In BC, SPAG5+ at both mRNA and protein levels showed a significant association with aggressive phenotypes and poor survivals ($p<0.0001$). In ER- BC treated with adjuvant ATC-CT, SPAG5 negative (-) had 7-times higher risk of progression compared with SPRAG+ BC ($p<0.0001$). SPAG5+ BC received NEO-A-ATC based CT achieved 38% pathological complete response (pCR) vs. 6% of SPAG5- ($p<0.0001$). After controlling to other predictors for pCR, SPAG5 was an independent predictor (HR; 2.4; $p=0.001$). Similarly, SPAG5- OVCs were resistant to platinum ($p<0.001$) and independently associated with poor survival ($p<0.001$)
Conclusion: SPAG5 is an important novel gene implicated in the survival of BC and OVC cells and its protein expression is an independent predictor for Anthracycline/ cisplatinum CT.

12.15–12.30 [**O20**] ***Angiogenesis, Vascular Invasion, Hormone Receptor Status, Cell Proliferation and Survival in Breast Cancer.***

ZMA Mohammed[1]; DC McMillan[2]; J Edwards[2]; EA Mallon[3]; JC Doughty[3]; C Orange[3]; Ⓟ JJ Going[1]

[1]Glasgow Royal Infirmary, Glasgow, United Kingdom; [2]University of Glasgow, Glasgow, United Kingdom; [3]Western Infirmary, Glasgow, United Kingdom

Introduction: Established prognostic and predictive factors guiding clinical management of breast cancer include tumour size, grade, nodal stage, and hormone receptor expression. This study sought to compare the prognostic power of vascular invasion and microvessel density in breast cancer with that of more securely established prognostic factors.
Methods: Carcinoma size, grade, nodal metastasis, hormone receptor status (ER, PR, Her2), cell proliferation (Ki-67 index), vascular invasion and microvessel density were derived from full sections or tissue microarrays and their relationships with survival were examined in 384 patients with primary operable invasive ductal breast cancer treated in specialist breast units in Glasgow during 1995-8 (minimum follow up 11.8 years).
Results: Univariate analysis of ER-negative carcinomas showed tumour size ($P=0.018$), involvement of lymph nodes ($P=0.001$), vascular invasion (present/absent; $P=0.036$), microvessel density (by tertiles; $P<0.05$), and vascular invasion with microvessel density in combination ($P=0.02$) all associated with poorer cancer-specific survival. Of these only lymph node involvement was significant on multivariate analysis ($P=0.006$).
Univariate analysis of ER-positive carcinomas showed grade ($P=0.019$), involvement of lymph nodes ($P<0.001$),

Ki-67 labelling index (P<0.001), vascular invasion (P<0.001) and vascular invasion with microvessel density in combination (P<0.001) were associated with poorer cancer specific survival. On multivariate analysis lymph node involvement (P<0.005), Ki-67 index (P<0.001) and vascular invasion (P<0.004) remained independently associated with poorer cancer-specific survival in ER positive carcinomas.
Conclusion: Vascular invasion, but not microvessel density was independently associated with poorer survival in patients with ER positive breast cancer.

12.30–13.00 **UNIVERSITY HOUSE · ABBEYDALE / FULWOOD ROOMS**
LUNCH

13.00–14.00 **OCTAGON CENTRE · MAIN HALL**
POSTER VIEWING AND CHAIRMAN'S ROUNDS

Categories	Poster Numbers	
Breast	P1–P8 [1]	*(Note: P3 is withdrawn)*
Gastrointestinal	P9–P16 [2]	
Head and Neck	P17–P24 [3]	
Hepatobiliary/Pancreas	P25–P27 [2]	*(Note: P25 is withdrawn)*
Lymphoreticular	P28–P31 [4]	
Osteoarticular/Soft Tissue	P32–P34 [4]	*(Note: P30 is withdrawn)*
Skin	P35–P37 [4]	

Chair: [1] Dr JJ Going, Glasgow; Dr RD Liebmann, Kent
[2] Dr P Kitsanta, Sheffield; Prof M Pignatelli, Glasgow
[3] Prof P Speight, Sheffield
[4] Dr R Ali, Sheffield; Prof AM Flanagan, London

14.00–14.10 **STUDENTS' UNION BUILDING · AUDITORIUM**
WELCOME
Prof M Wells, Professor of Gynaecological Pathology, University of Sheffield

14.10–17.30 **STUDENTS' UNION BUILDING · AUDITORIUM**
SYMPOSIUM: *Viral Oncogenesis in Head and Neck Tumours*
Chair: Prof P Speight, School of Clinical Dentistry, University of Sheffield

14.10–14.45 **[S1]** ***Overview of the Biology of HPV and its Role in Carcinogenesis***
Ⓟ Dr NG Powell
Cardiff University, School of Medicine, Cardiff, United Kingdom
Most of our knowledge of the biology of HPV has been gained in the context of cervical disease. Some, but not all, of this knowledge is relevant to HPV infection at other anatomical sites. Some of the paradigms developed for prevention and treatment of HPV associated disease in the cervix are likewise likely to be broadly applicable. The aim of this presentation is to give a brief introduction to the biology of HPV infection and its clinical consequences following anogenital infection. This will include HPV structure, classification, transmission, infection and lifecycle, followed by a short historical perspective on how the link between HPV and cervical cancer was established. The mechanisms by which HPV promotes cellular proliferation will be discussed, with a focus on oncogene function and the mechanisms underlying deregulation of viral gene expression. The presentation will conclude with some thoughts on how the involvement of HPV in pathogenesis can influence disease prevention strategies.

14.45–15.30 **[S2]** ***The Role of HPV in Head and Neck Tumours and Testing for Cooperative Group Clinical Trials***
Ⓟ Prof RCK Jordan
University of California San Francisco, San Francisco, United States
Several etiological agents have been shown to be associated with the development of oral and oropharyngeal carcinoma including the traditional well-established risk factors of tobacco and alcohol abuse. The recently characterized association between HPV16 and the development of oropharyngeal carcinoma has demonstrated that this is another human cancer with an infectious basis. This association has wide clinical impact influencing patient diagnosis, staging, treatment selection and prognosis. In addition, HPV status is increasingly being incorporated into decision-making for large-scale cancer co-operative group clinical trials that assess new therapies for head and neck cancer. This lecture will review the association between HPV16 and oropharyngeal cancer and discuss methods to assess this association in pathology specimens. The role of different methods for HPV testing will be

reviewed and the value of p16 immunohistochemistry as a surrogate assay for HPV16. A new validated H-score will be described for p16 assessment that is being incorporated into co-operative cancer group trials. Finally, how co-operative cancer group trials are using this information and clinical trial decision-making will be discussed.

15.30–16.00 **OCTAGON CENTRE · MAIN HALL**
COFFEE AND TRADE EXHIBITION

16.00–16.45 **[S3]** ***Clinical Trials Investigating Management of HPV Positive Head and Neck Tumours***
Ⓟ Mr R Shaw

University of Liverpool, Liverpool, United Kingdom

Human Papillomavirus (HPV) related head and neck cancer (HNSCC) has differing aetiology, pathogenesis, presentation, demographic, treatment response & prognosis so quite clearly the challenges and endpoints of clinical trials for HPV+ve versus HPV-ve patients are divergent.
The methodological problems associated with de-escalation strategies remain significant, as do the choice of how to de-escalate in terms of maintaining safety. The benefit of EGFR-directed or other targeted approaches remains controversial but much can be learned from the post-hoc analysis of established phase III trials data.
The emergence of HPV as a major sub-group in HNSCC presents great challenges and opportunities for the H&N trialist.

16.45–17.30 **[S4]** ***The Pathology of Virus-Related Carcinomas of the Upper Aerodigestive Tract***
Ⓟ Dr M Robinson

Newcastle University, Newcastle upon Tyne, United Kingdom

The vast majority of head and neck malignancies are squamous cell carcinomas (SCC). In clinical practice the diagnosis of SCC is usually straight-forwards and is based on the recognition of typical morphological features. For poorly differentiated epithelial neoplasms, the detection of specific cytokeratin molecules (CK5/6, CK14) and/or p63 can be used to support a diagnosis of SCC. It is well known that the detection of Epstein-Barr virus (EBV) in non-keratinising nasopharyngeal carcinoma can be used to secure a definitive diagnosis; usually by EBV encoded RNA (EBER) in situ hybridisation (ISH). More recently it has become apparent that the detection of oncogenic human papillomavirus (HPV) in oro-pharyngeal SCC defines a biologically distinct entity that is associated with improved patient survival. Whilst the prognostic information is welcomed by oncologists and patients, HPV testing also has clinical utility for pathologists. The tests can be used to guide the interpretation of sub-optimal biopsy material. In the clinical scenario of a neck lump, testing can be employed to direct the search for an 'unknown primary'. HPV tests can also be used to establish the relationship between oropharyngeal and lung SCCs; metastasis vs. new primary. Testing of oropharyngeal cancers has also identified oncogenic virus in uncommon morphological variants of SCC and rare neuroendocrine (small cell) carcinoma of the oropharynx. This presentation will outline the pathological features of virus-related carcinomas of the upper aerodigestive tract, highlight some of the challenges in diagnosis and provide an overview of the utility of virus testing in clinical practice.

17.30–18.30 **STUDENTS' UNION BUILDING · AUDITORIUM**
PUBLIC LECTURE: *The Relationships between Patients, the Public and Biomedical Researchers*
Chair: Dr SS Cross, University of Sheffield
Speaker: Prof P Shaw, Professor of Neurology, University of Sheffield

18.30–20.00 **UNIVERSITY OF SHEFFIELD · FIRTH HALL**
WELCOME RECEPTION
—with entertainment by "The Djangonauts"

07.45 **OCTAGON CENTRE · FOYER**

REGISTRATION AND COFFEE

08.00–09.00 **STUDENTS' UNION BUILDING · AUDITORIUM**

TRAINEES SESSION – MEET THE EXPERTS

Chair: Dr NP West, St James's University Hospital, Leeds and Chair of Pathological Society Trainees' Sub-Committee

[**S5**] ***Cardiac Tissue Analysis, from Routine to Complex***

Ⓟ Dr SK Suvarna

Sheffield Teaching Hospitals, Sheffield, United Kingdom

Cardiac pathology, from the surgical sample to the autopsy review, covers both routine cases and complicated pathology. This session will be a description of the recommended approach to case analysis, with particular emphasis on autopsy technique. The value and choices of other tests, such as histology, molecular analysis , microbiology and electron microscopy will be debated. The speaker will invite debate from the floor and will discuss the safe and efficient way to analyse cases and when they should be referred for expert review.

09.00–17.30 **OCTAGON CENTRE · COMPUTER ROOM**

SLIDE SEMINAR COMPETITION CASE VIEWING: *Gynaecological Pathology*

Note: Competition closes at 15.30 on Wednesday 4 July

09.00–10.30 **STUDENTS' UNION BUILDING · AUDITORIUM**

ORAL PRESENTATIONS

Chair: Dr R Liebmann, Maidstone and Tunbridge Wells NHS Trust
Dr A Shaaban, University of Leeds

Category: Breast

09.00–09.15 [**O21**] ***External Validation of ImmunoRatio™ Image Analysis Application for Quantification of ERα and Ki-67 in Breast Cancer***

Ⓟ S Sundara Rajan[1]; S Pollock[1]; K Horgan[2]; AM Hanby[1]; V Speirs[1]

[1]University of Leeds, Leeds Institute of Molecular Medicine, Leeds, United Kingdom; [2]Leeds Hospitals NHS Trust, Leeds, United Kingdom

Manual immunohistochemistry (IH) scoring is time consuming. Computer-based image-analysis algorithms have been developed to expedite this process. ImmunoRatio™ (1) is a publically available automated image analysis application for assessing oestrogen receptor (ERα), progesterone receptor (PR) and Ki-67. The study aim was to validate this application by comparing manual and automated scores for ERα and Ki-67 in a pre-stained series of breast carcinomas. IH was performed on tissue microarray sections of breast cancers identified from our archive for ERα (n=100) and Ki67 (n=75), following recommended staining protocols using 1D5 (1: 100; ERα) and MIB-1 (1:100; Ki67), respectively. Stained slides were scanned (Aperio ScanScope™) and scored manually using ImageScope™ viewing software (20x). Corresponding images were saved as JPEG files and uploaded to the ImmunoRatio™ application. ERα was positive in 76 cases scored manually and 81 using ImmunoRatio™. The labelling index for ERα with ImmunoRatio™ correlated well with manual scoring (Spearman, r=0.861; p=0.000). Similarly there was a linear relationship between manual scoring and ImmunoRatio™ for Ki-67 (Pearson, r=0.653; p=0.000). However, the ImmunoRatio™ application lacked specificity in differentiating cancer nuclei from normal, stromal and inflammatory cells as well as some DAB stained nuclear and cytoplasmic areas.
In summary, the correlation between manual scoring and ImmunoRatio™ was superior for ERα compared to Ki-67 in a cohort of breast cancers. There is room for further improvement by increasing the specificity of ImmunoRatio™ in identifying cancer nuclei compared to surrounding normal and stromal elements, particularly if applied to full face sections. Reference: 1. Tuominen et al. 2010. Breast Cancer Res. 12(4):R56.

09.15–09.30 [**O22**] ***Immunonophenotypic Characterization of Oestrogen Receptor Negative Breast Carcinomas Identifies BCL2 as a Prognostic Marker***

Ⓟ AA Benhasouna[1]; AR Green[1]; RD McMillan[2]; IO Ellis[1]; EA Rakha[1]

[1]Department of Histopathology, The University of Nottingham and Nottingham University Hospitals NHS Trust, Nottingham City Hospital, Nottingham, United Kingdom; [2]The Breast Institute, Nottingham University Hospitals NHS Trust, City Hospital Campus, Nottingham, Nottingham, United Kingdom

Introduction: Although prognostic markers have been identified in Oestrogen receptor positive (ER) breast cancer (BC), prognostic markers in ER-negative tumours remain lacking. This study aims to assess the prognostic significance of a panel of proliferation, apoptosis and cell cycle associated proteins in ER-negative BC.
Materials and methods: A consecutive well-characterised and uniformly treated series of 776 ER-negative BC with long term follow-up were assessed for expression of a series of 11 biomarkers; Ki67, BCL2, p53, p21, p27, BRCA1, CK5/6, CK14, CK17, EGFR and HER2 using immunohistochemical staining of tissue microarray tumour tissue

preaprations. The results were correlated with clinicopathological characteristics and patient outcome.
Results: In the ER-negative tumours, HER2 positivity was significantly associated with lymph node positivity but with a lower proliferation (Ki67 and mitotic frequency) status. Independent predictors of outcome are lymph node stage, primary tumour size and BCL2 expression while vascular invasion, proliferation status (Ki67 and mitotic frequency), basal phenotype and HER2 were not. In the ER-negative HER2-negative subgroup, only lymph node stage and BCL2 expression were independent predictors of outcome. Interestingly, in the ER-negative/HER2-negative/lymph node-negative tumours, BCL2 was the only predictor of outcome while established clinicopathological and molecular variables were not significantly associated with outcome. In the HER2-positive subgroup, none of the variables tested apart from lymph node stage were independently associated with outcome.
Conclusion: The results of this study provide further support to the prognostic value of BCL2 in breast cancer and show that BCL2 is an independent prognostic factor in patients with lymph node negative ER-negative HER2-negative breast cancer where other known prognostic factors in breast cancer appear of limited value.

09.30–09.45 [**O23**] ***Further Evidence to Support E-cadherin is not a Tumour Suppressor Gene in Ductal Carcinoma of the Breast: An Immunohistochemical Study***

T Teoh[1]; AR Green[2]; IO Ellis[2]; Ⓟ EA Rakha[1]

[1]The University of Nottingham, Nottingham, United Kingdom; [2]Department of Histopathology, The University of Nottingham and Nottingham University Hospitals NHS Trust, City Hospital Campus, Nottingham, United Kingdom

E-cadherin is recognised as a tumour suppressor gene (TSG) in lobular carcinoma of the breast. Loss of membrane expression of E-Cadherin protein using immunohistochemical (IHC) expression is used diagnostically to differentiate lobular from ductal breast carcinomas. In a previous tissue microarray (TMA) study of 1516 no special type (NST or ductal) carcinoma (Rakha et al Histopathology. 2005; 46(6): 685-693), 7% of cases showed complete absence of membranous IHC expression of E-cadherin. In this study, we investigated E-cadherin IHC expression in these negative NST cases using different antibodies against E-cadherin and other members of the E-cadherin membrane complex; namely β-catenin and p120 on full-face sections. Results: Of the 72 E-cadherin TMA negative cases, 25 tumours were excluded on morphological grounds due to showing features of pure or mixed lobular carcinoma (no=21) or insufficient invasive tumour for assessment (no=4). Of the remaining 47 cases, 30 tumours showed positive membrane expression focally while 17 (36%) were confirmed to have negative IHC expression. On further staining, all these 17 cases showed expression of p120 catenin and β-catenin. Interestingly, 14 cases were also positive for an E-cadherin antibody with specificity for the intracellular domain. In conclusion, our results support the previous observation that E-cadherin is not a TSG in ductal carcinoma of the breast and confirmed that E-cadherin membrane complex is maintained even in the few cases showing absence of E-cadherin protein membrane IHC expression. Using p120 or β-catenin IHC in occasional E-cadherin negative breast carcinoma with ductal morphology may be helpful in diagnostically challenging cases.

09.45–10.00 [**O24**] ***Cadherin Switch in Early Invasive Breast Cancer: a Reverse Phase Protein Microarray Study***

Ⓟ MA Aleskandarany[1]; OH Negm[2]; EA Rakha[3]; MA Hasan[2]; AR Green[2]; P Tighe[2]; IO Ellis[2]

[1]Faculty of Medicine, Menoufyia University, Menoufyia, Egypt; [2]Nottingham University, Nottingham, United Kingdom; [3]Nottingham City Hospital, Mottingham, United Kingdom

Cadherin switching is proposed to occur during epithelial-mesenchymal transition (EMT), where epithelial cadherins are down-regulated, while the mesenchymal cadherins are up-regulated, thereby facilitating motility, invasiveness and proliferation. We aimed at investigating the potential role of this phenomenon in invasive breast cancer (BC) using a quantitative assay of E-cad and N-cad with relevance to distant recurrence.
This study was based on 49 randomly-selected early invasive BC cases with long-term follow-up and prospectively maintained data of broad panel of immunohistochemical markers. Invasive tumourous components were macrodissected from formalin-fixed paraffin-embedded (FFPE) sections. Additionally, in-situ components associated with three of these cases were separately dissected. Using Laemmli buffer, proteins were extracted from FFPE and levels of E-cad and N-cad were assayed using reverse phase protein array (RPPA) with analysis of microarray data by RPP analyser software.
E-cad expression was reciprocally associated with N-cad in luminal and HER2+ BC; where reduced expression of the former in HER2+ was coupled with over-expression of the latter, and vice versa. The association between the expression of both proteins and distant recurrence probability was not statistically significant ($p > 0.05$), however, N-cad, was higher in patients who had developed distant recurrence, in contrast to E-cad expression. Moreover, E-cad was lower while N-cad was higher in invasive components relative to associated in-situ lesions in the three studied cases. Subtle protein changes occurring during EMT could be quantified using RPPA utilising proteins extracted from FFPE. Our data propose that the cadherin switch could be occurring as a continuum of changes from the in-situ to the invasive phase and ultimately into the metastatic phase.

10.00–10.15 [**O25**] ***Quantification of HER2/HER3 Dimerisation Status in Primary Invasive Breast Cancer***

Ⓟ FFT Barros[1]; TMA Abdel-Fatah[2]; EA Rakha[3]; S Chan[2]; AR Green[1]; IO Ellis[2]

[1]University of Nottingham, Nottingham, United Kingdom; [2]Nottingham City Hospital, Nottingham, United Kingdom; [3]University of Nottingham and Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom

Introduction: HER2 plays an important role in breast cancer (BC) progression and provides predictive and prognostic information. Trastuzumab is widely used as targeted therapy in HER2+ BC, but some patients show

innate or develop acquired resistance. HER2 receptor family members function through dimerisation, which can lead to impact on cell function, growth and differentiation; however their value in BC development remains to be defined. This study aims to examine the relationships of HER2/HER3 dimers to BC characteristics and clinical outcome in trastuzumab naive and treated cases.
Methods: HER2 protein (IHC), HER2 gene (chromogenic ISH) and HER2/HER3 dimerisation status (in situ proximity ligation assay (PLA)) was assessed in two BC series prepared in tissue microarray format; a series of consecutive primary operable BC cases (n= 2469) including HER2+ trastuzumab naive cases (TrN, n= 221), the second a cohort of HER2+ trastuzumab adjuvant treated cases (TrT, n= 146).
Results: Our results revealed an inverse association between HER2/HER3 dimerisation status and hormone receptor status (p< 0.001), and a significantly worse outcome amongst cases revealing high levels of HER2/HER3 dimerisation (p< 0.001). Among ER+ cases, HER2/HER3 dimer positive cases were significantly associated with worse prognosis (p< 0.001) overall. However amongst the two HER2+ populations dimerisation status did not show an association with patient outcome.
Conclusion: Tumours exhibiting high levels of HER2/HER3 dimerisation have an adverse prognosis, however in the context of HER2+ BC no association with clinical outcome was observed regardless of use of trastuzumab treatment. Further quantification analysis of dimer/ligand complex using PLA of other HER family members may be useful to identify subset of patients associated with distinct outcome, response to treatment and relationships with HER signalling related biomarkers.

10.15–10.30 [**O26**] ***TOMM34 Expression in Early Invasive Breast Cancer: a Biomarker Associated With Poor Outcome***

Ⓟ MA Aleskandarany[1]; OH Negm[2]; EA Rakha[3]; MAH Ahmed[2]; G Ball[4]; AR Green[2]; P Tighe[2]; IO Ellis[2]

[1]Faculty of Medicine, Menoufyia University, Menoufyia, Egypt; [2]Nottingham University, Nottingham, United Kingdom; [3]Nottingham City Hospital, Nottingham, United Kingdom; [4]Nottingham Trent University, Nottingham, United Kingdom

Mitochondria play fundamental roles in cellular energy metabolism, free radical generation, and apoptosis. Normal cells functioning depend on properly functioning mitochondrial translocation machinery, of which translocase of the outer membrane of mitochondria (Tomm) plays important role. This study aimed at exploring the expression of TOMM34 in breast cancer with emphasis on disease progression.
Gene expression data of 128 invasive breast carcinomas was analysed using artificial Neuronal Network (ANN) with respect to distant metastasis development. TOMM34 expression was studied using immunohistochemistry (IHC) of tissue microarrays in a large well-characterised series of invasive breast cancer (n=1061) with long-term clinical follow-up. Moreover, TOMM34 protein was quantitatively assayed using reverse phase protein microarray (RPPA) using protein extracted from macrodissected formalin-fixed paraffin-embedded tumour tissues representative of 49 invasive breast cancers from the same series. Using ANN analysis, Tomm34 gene transcript was the ninth differentially expressed gene, with up-regulation in cases which developed distant recurrence. IHC expression of TOMM34 was directly associated with tumour grade, nodal stage, tumour size, and definite vascular invasion (p<0.05). Over-expression was associated with shorter breast cancer specific (BCSS) and metastasis free survivals (MFS) (p<0.001), independent of standard prognostic parameters (BCSS; HR=1.396, 95%CI=1.097–1.776, and MFS; HR=1.283, 95%CI=1.019–1.614). In RPPA, cases with distant recurrence showed higher TOMM34 protein expression, although differences were not statistically significant (p>0.05). These findings demonstrate at the translational that TOMM34 protein expression is a marker of poor outcome in breast cancer, underscoring the role played by mitochondrial machinery in breast cancer progression and warrants further prospective validation.

09.00–10.30 DAINTON BUILDING · LECTURE THEATRE 1

ORAL PRESENTATIONS

Chair: Dr P Kitsanta, Sheffield Teaching Hospitals
Dr RFT McMahon, University of Manchester
Categories: Gastrointestinal; Cellular/Molecular

09.00–09.15 [**O27**] ***Immunohistochemical Assessment of Activated RAS and Receptor Tyrosine Kinase Pathways in Gastric Adenocarcinoma***

Ⓟ GNJ Betts[1]; H Valentine[1]; S Pritchard[2]; R Swindell[1]; C Womack[3]; S Morgan[3]; CM West[1]

[1]University of Manchester, Manchester, United Kingdom; [2]University Hospital of South Manchester, Manchester, United Kingdom; [3]AstraZeneca, Alderley Edge, United Kingdom

Surgery is the only curative treatment for gastric cancer with limited options for patients with recurrent or inoperable disease. We measured the expression of biomarkers linked to activation of RAS and receptor tyrosine kinase pathways to explore the prevalence and practicality of measurement in a clinical setting.
FFPE tissue microarrays were constructed from gastrectomy resections of 187 patients from one institution with clinical outcome which was significantly related to T stage, N stage, R margin status and grade. Up to 9 cores were taken from each tumour; invasive edge (IE), luminal surface (LS) and tumour body (TB). Immunohistochemistry (IHC) for pS6, pERK and HER2 were performed. A pan phospho-tyrosine marker (pTyr) was used as a quality control.
High pS6 and pERK (average score >1) were seen in 72.9% and 30% of tumours respectively but showed no

relationship to outcome. A weak association between high HER2 (9.8% of tumours) and better outcome was seen (P=0.041). A strong correlation was seen between pS6 and pERK staining (P<0.0001) across all three tumour regions. Variability between the three regions was pronounced for all three markers, particularly pERK, using Freidman two-way ANOVA and repeated Wilcoxon matched pairs tests. Median scores for LS were significantly greater than TB or IE for all three markers. Low pTyr showed a weak correlation with low pS6 or pERK score. pS6 and pERK are expressed in a subset of gastric adenocarcinomas, implying activation of the RAS pathway but this was not statistically significantly related to outcome. The correlation seen between pS6 and pERK is in common with other studies noting that ERK activation can lead to increased pS6 independent of PI3Kinase activity. Similarly a number of tumours expressed HER2. Heterogeneity of pS6 and pERK presents a challenge in assessment of IHC markers. Expression in LS may be over-representative of the tumour as a whole.

09.15–09.30 [**O28**] ***Beta-catenin Negatively Regulates Kras Expression but Both Proteins Co-operate to Promote Cell Proliferation and Motility in Colorectal Cancer***

S Ibrahem; W Fadhil; B Gelaly; A Nateri; D Jackson; Ⓟ M Ilyas

University of Nottingham, Nottingham, United Kingdom

Wnt signalling and Kras signalling are oncogenic pathways which are commonly activated during the development of colorectal cancer (CRC). However, the interaction between these pathways is still unknown. We investigated whether there was interaction between these pathways and whether overlapping functions led to redundancy in their oncogenic activity. Wnt signalling was inhibited by knockdown of b-catenin and forced expression of dominant-negative TCF4 whilst Kras signalling was inhibited by knockdown of KRAS. Both pathways were inhibited individually and in combination in a variety of CRC cell lines. The effect of these manipulations on the expression of each gene and on a variety of cellular functions was measured. Furthermore, the association between b-catenin expression/subcellular localisation and KRAS mutation was tested in a series of primary CRCs. b-Catenin inhibition caused an increase in Kras mRNA and protein expression whilst knockdown of Kras had no effect on b-catenin expression. Testing of the primary tumour samples showed no association between kras mutation and b-catenin expression/nuclear localisation. Inhibition of b-catenin and Kras individually caused a reduction in cell numbers through both cell cycle arrest and increased apoptosis. This was accompanied by a parallel decrease in cyclin D1 expression and increase Caspase 3 activity. Inhibition of b-catenin and Kras individually also caused a reduction in cell migration and invasion and this was accompanied by an increase in E-cadherin expression. Combined inhibition of b-catenin and Kras had an additive functional effect resulting in marked reduction cell number and marked inhibition of cell motility. We conclude that â-catenin negatively regulates the expression of Kras and this may drive the mutation of KRAS in CRC. When activated together, the Wnt and Kras signalling pathways co-operate to promote cell proliferation and motility.

09.30–09.45 [**O29**] ***Next Generation Sequencing Mutation Detection Compared to Pyrosequencing***

Ⓟ KM Sutton; J Witham; P Chambers; GR Taylor; P Quirke

Leeds Institute of Molecular Medicine, Leeds, United Kingdom

Background: There is a demand for technology that provides cheap, sensitive mutation detection. Pyrosequencing is commonly used in the NHS, however next generation sequencing (NGS) is revolutionising the field. The high throughput allows for whole genome sequencing, but less explored is its potential for targeted sequencing in multiplexed samples.
Methods: Cell-line DNA from SW48 cells containing a heterozygous G12A or G12C KRAS mutation (Horizon Discovery Ltd, Cambridge UK) were titrated with wild-type (WT) SW48 DNA to make serial dilutions of mutant KRAS from 50% to 0.005% and a WT control. DNA was amplified by PCR to produce an 80 base-pair (bp) amplicon for KRAS codon12/13 which was sequenced by pyrosequencing and NGS.
Results: KRAS G12A and G12C mutations could be detected at 5% and 25% respectively with pyrosequencing. The same mutations were detected at 0.5% for G12A and 0.05% for G12C when sequenced with NGS. Reproducibility of NGS was very high; the correlation of two individual runs was 0.9973 and 0.9984 for the G12A and G12C mutations respectively. This was 0.9971 and 0.8768 for pyrosequencing.
Conclusions:NGS has an increase in sensitivity of up to 500-fold over pyrosequencing. One pyrosequencing run allows for 1 mutational site to be tested in 90 samples at 5-25% sensitivity. Modeling the potential of NGS, 90 samples could be theoretically multiplexed to test for up to 1,100 mutational sites and maintain 1% sensitivity or a single NGS run could test one mutational site in 100,000 samples. Although the correlation of pyrosequencing is high, the consistently higher correlation of NGS shows that it is not only highly sensitive but also reproducible. NGS is a powerful technology for large scale sensitive mutation testing.

09.45–10.00 [**O30**] ***Enhanced Stability of MicroRNA Expression Facilitates Classification of FFPE Tumour Samples Exhibiting Near Total mRNA Degradation***

Ⓟ JS Hall[1]; J Taylor[2]; HR Valentine[1]; JJ Irlam[1]; A Eustace[1]; PJ Hoskin[3]; CJ Miller[2]; CML West[1]

[1]The University of Manchester, Manchester, United Kingdom; [2]Paterson Institute for Cancer Research, Manchester, United Kingdom; [3]Mount Vernon Hospital, London, United Kingdom

As degradation of formalin-fixed paraffin-embedded (FFPE) samples limits ability to expression profile, we explored factors predicting success for FFPE profiling and investigated an approach overcoming this limitation. Bladder (n=140, stored 3-8 years) and cervix (n=160, stored 8-23 years) carcinoma FFPE samples were hybridised to Affymetrix Exon1.0ST arrays. Percent detection above background (%DABG) measured technical success. Biological signal was assessed by distinguishing cervix squamous cell carcinoma (SCC) and adenocarcinoma (AC)

using a gene signature. Precursor mir-205 was measured by Exon array and mature miR-205 by qRT-PCR. Eight-old and -young cervix samples were compared using Affymetrix miRNA 2.0 arrays.
RNA quality controls (e.g. RNA integrity number) failed to predict profiling success, but sample age correlated with %DABG in bladder (R^2=-0.30, p<0.01) and cervix (R^2=-0.69, p<0.01). Biological signal was lost in older samples and neither a signature nor precursor mir-205 separated samples by histology. miR-205 qRT-PCR discriminated SCC from AC, validated by miRNA profiling (26-fold higher in SCC; p=1.10x10^{-5}). Median miRNA probeset expression of eight-old and eight-young cervix samples correlated well (R^2=0.95) overcoming the age-related bias of mRNA probesets, suggesting miR-205 stability generalises across microRNA.
Sample age is the best predictor of successful FFPE profiling and microRNA profiling overcomes the limitation of degraded FFPE samples.

10.00–10.15 **[O31]** ***Molecular Testing for Mutations to Improve Pre-operative Management of Patients with Thyroid Nodules***

Ⓟ R Dina[1]; G Tallini[2]; P Economides[3]; L Zakarneh[1]

[1]Hammersmith Hospital, London, United Kingdom; [2]Bologna University School of Medicine, Bologna, Italy; [3]Nicosia Endocrinology Centre, Nicosia, Cyprus

Context: Data accumulated in the last 10-20 years has shown a significant correlation between some molecular alterations and clinicopathological features of thyroid cancer. In particular one of a few mutations along the MAPK pathway (BRAF, RET/PTC, RAS) can be identified on over 70% of all papillary carcinomas. Similarly, although not entirely specific, a few mutations have been associated with Follicular carcinoma (N-RAS, PAX8/PPRg).
Several recent reports have shown solid evidence that molecular diagnosis can effectively improve the pre-operative diagnosis of thyroid nodules indicating the need for large prospective studies.
Aim of the study: to prospectively evaluate the reliability of molecular diagnosis in the pre-operative management of thyroid nodules.
Design: 109 consecutive cases reported at our institution using the RCP Thy1-5 classification were collected. Following the cytology report the residual liquid base cytology samples were stored and sent for molecular analysis to the Diagnostic Pathology Laboratory for PCR- BRAF V600E mutation testing.
Results: 17 cases were classified as Thy4 and Thy 5 (suspicious or diagnostic of papillary carcinoma), 90 as Thy 2 and two as Thy 3 at cytology. Out of the 109 cases 34 did contain evidence of BRAF V600E mutation (1 Thy1, 21 Thy2, 4 Thy3 and 8 Thy5). All Thy 4 and Thy 5 cases were confirmed at histology as papillary carcinomas. Only two cases of Thy 2 had surgery, in both cases benign. All remaining Thy 2 cases with evidence of BRAF V600E mutation in a proportion of cells varying from 1% to 12.5% are being followed up with U/S and FNA cytology without evidence of cancer after 15 months.
Conclusion: Cases which on cytology are classified as Thy2 may harbour a proportion of cells with BRAF V600E mutation. Further follow up and histological correlation is necessary to understand whether molecular analysis in the individual case is clinically relevant.

10.15–10.30 **[O32]** ***Human Colorectal Adenoma Growth is Characterised by Periods of Quiesence and Rapid Clonal Expansion***

Ⓟ A Humphries[1]; B Cereser[2]; M Novelli[3]; M Rodriguez Justo[3]; T Graham[4]; SAC McDonald[2]; NA Wright[2]

[1]Cancer Research UK, London, United Kingdom; [2]Barts and the London School of Medicine and Dentistry, London, United Kingdom; [3]University College London Hospitals, London, United Kingdom; [4]University of California, San Francisco, San Francisco, United States

Little is known about the dynamics and rates of adenoma growth in the human colon. Longitudinal studies demonstrate smaller adenomas to be static over many years, and can even regress. We have recently shown that methylation patterns at CpG loci within non-expressed genes can be used to infer the dynamics of clonal expansion in the normal human colon. By combining clonal mutational analysis with methylation patterns for crypts within adenomas, we aim to learn more about how these pre-malignant lesions grow.
Frozen human adenomas were screened for common tumorigenic mutations known to occur in adenoma progression. Multiple individual crypts across the lesion were then micro-dissected, the mutation(s) detected at screening confirmed and methylation tags at CpG islands of non-expressed genes sampled in order to infer dynamics and patterns of growth.
Crypts within adenomas clonal for APC and KRAS mutations demonstrated significantly less methylation pattern diversity than those from adenomas that were clonal for an APC mutation only, suggesting there had been a recent, rapid clonal expansion in the APC/KRAS mutated lesions. Genetically distinct sub-clones could be identified within adenomas, and analysis of methylation patterns showed them to be expanding rapidly. There was no correlation between the spatial separation of crypts and their respective epigenetic distances within the adenomas studied. In those lesions that had not undergone a recent clonal expansion, there had been a sufficient time period of little or no growth to allow the methylation patterns of neighbouring crypts to diverge.
These data suggest that human adenomas display punctuated growth, with periods of relative quiescence and rapid clonal expansion. More samples should allow the growth rates of genetically distinct lesions and sub-clones within adenomas to be estimated, and may help better inform our management of patients with these lesions.

10.30–11.00 **OCTAGON CENTRE · MAIN HALL**
COFFEE AND TRADE EXHIBITION

11.00–13.00 **STUDENTS' UNION BUILDING · AUDITORIUM**

SYMPOSIUM: ***Systems Biology – Reverse Engineering The Phenotype***

Chair: Dr SS Cross, University of Sheffield

11.00–11.30 ***Bridging the Gap between Computational Biology and Systems Biology***

Prof N Lawrence, The Sheffield Institute for Translational Neurosciences, University of Sheffield

11.30–12.00 [**S6**] ***Barrett's Metaplasia: Exploiting a Tissue-engineered Model to Understand the Response of the Oesophageal Squamous Mucosa to Bile and Acid Exposure.***

NH Green[1]; Z Nicholls[2]; PR Heath[3]; JR Highley[3]; J Cooper-Knock[3]; BM Corfe[2]; JH Wilson[4]; M Rattray[3]; X Liu[5]; S MacNeil[1]; Ⓟ Dr JP Bury[4]

[1]Kroto Research Institute, Sheffield, United Kingdom; [2]University of Sheffield, Sheffield, United Kingdom; [3]Sheffield Institute for Translational Neuroscience, Sheffield, United Kingdom; [4]Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, United Kingdom; [5]Nanjing University of Aeronautics & Astronautics, Nanjing, China

BACKGROUND: The molecular pathogenesis of metaplasia has received relatively little attention even though the phenomenon is often associated with the subsequent development of malignancy. We have focussed on the early pathogenesis of Barrett's metaplasia (BM) of the oesophagus.

METHODS: Since NF-κB activation has been implicated in the early pathogenesis of BM we first assessed the ability of different bile salts to activate NF-κB in squamous cells, identifying taurochenodeoxycholic acid (TCDC) as a specifically effective non-toxic activator of NF-κB. We then subjected a 3-D in-vitro model of the oesophagus to acid (pH4), TCDC and TCDC at pH4 in a pulsatile fashion for two weeks and subsequently performed gene expression analysis and Gene Set Enrichment Analyis (GSEA) to identify other pathways and transcription factors modulated by these regimes.

RESULTS: There were substantial similarities in the gene expression profiles resultant from both acid and TCDC exposure. GSEA confirmed statistically significant modulation of NF-κB targets. Other transcription factors with modulated activity included c-myc and HNF4, the latter a transcription factor with diverse isoforms, involved in liver function and gut development and found in intestinal metaplasia of the stomach. Using immunohistochemistry we confirmed the expression of HNF4α in clinical samples of BM. HNF4α was not detected in our oesophageal tissue cultures, suggesting other isoforms of HNF may operate in this context.

CONCLUSIONS: The ability of TCDC to drive NF-κB at the pathophysiologically relevant concentrations we used is notable given the association of a western diet with both high levels of taurine-conjugated bile salts and oesophageal adenocarcinoma. Secondly, this gene expression data derived from primary human oesophageal squamous cells exposed to controlled simulated reflux suggests a potential role for HNF4 in the very early pathogenesis of BM.

12.00–12.30 [**S7**] ***Why do Patients get Metachronous Colorectal Adenomas?***

JP Bury[1]; S Riley[1]; CA Evans[2]; EA Williams[2]; CA Staton[2]; E Murabito[3]; K Smallbone[3]; Ⓟ Dr BM Corfe[2]

[1]Sheffield Teaching Hospitals, Sheffield, United Kingdom; [2]University of Sheffield, Sheffield, United Kingdom; [3]University of Manchester, Manchester, United Kingdom

Purpose of the study Patients are at greater risk of adenoma if there is a previous history, however to date relatively few studies have addressed the molecular basis for metachronous lesions; the normality of the crypt distant to the adenoma ; and the role of short-chain fatty acids (SCFA) in protection/risk of the index or subsequent adenoma.

Methods Subjects were recruited from GI outpatient lists, biopsies collected for proteomic and immunohistochemical analyses. Biopsies were taken from the mid-sigmoid in all subjects and contralateral to the lesion. Stool samples were analysed for SCFA. Proteomics was undertaken with an iTRAQ workflow. FFPE sections were stained for Ki67, enterchromaffin and neuropilin, and markers identified by the proteomic analysis.

Summary of results Proteomics revealed that the adenoma samples were more different that the macroscopically normal samples, independent of butyrate status. Macroscopically normal samples clustered according to butyrate status. Proteomics revealed that keratins 8 and 19, tubulin and ApoE were altered between the mid-sigmoid and contralateral positions in subjects with adenoma. Multinomial analysis of IHC data showed changes in cellularity, Ki67, Np1, and keratin 8 between the mid-sigmoid samples of normal, adenoma and cancer patients. Multinomial analysis revealed significant differences in the ratios of butyrate and propionate in adenoma and cancer when referenced to the normal group. Metabolic modelling suggests this would alter the acyl-coA:coA-SH ratio in cells. Crypt modelling suggests that slight alterations in cell cycle time or in rate of feedback would cause the levels of observable perturbation in crypt composition.

Conclusions There is evidence of alteration in the macroscopically normal areas of the gut wall and in the function of the microbiota in the presence of an adenoma. The respective cases and models for cause and effect will be presented.

12.30–13.00 **PANEL DISCUSSION:** ***Systems Biology in Pathology Research***

11.00–13.00 **DAINTON BUILDING · LECTURE THEATRE 1**

SYMPOSIUM: *Endocrine Pathology*

Organised in conjunction with the UK Endocrine Pathology Society

Chair: Prof TJ Stephenson, Sheffield Teaching Hospitals

11.00–11.40 [**S8**] ***The Potential of Molecular Biology in Thyroid Cytology and Histology***

Ⓟ Prof M Sobrinho-Simoes

IPATIMUP, Porto, Portugal

Immunohistochemistry, a sort of less sophisticated molecular biology, allows the differential cytological and histological diagnosis of difficult thyroid tumours. Searching for TTF1, thyroglobulin and calcitonin immunoreactivity solves the large majority of cases and the remaining "rare flowers" may be diagnosed using other immunomarkers.

From a diagnostic standpoint, the major problems reside in the separation of follicular adenoma from follicular carcinoma, and in the identification of papillary thyroid carcinomas (PTC) displaying a follicular growth pattern (FVPTC). Molecular biology is not useful at all in the former setting.

The identification of BRAF V600E mutation in FNAB specimens contributes to make the diagnosis of PTC in difficult cases (Unfortunately, the mutation is rare in FVPTC that usually raises more diagnostic problems). The detection of BRAF mutation is also useful in the diagnosis of the oncocytic variant of PTC whereas it is almost useless for distinguishing benign from malignant follicular tumours composed of oncocytes. There is not enough evidence to claim for the diagnostic relevance of other molecular markers such as RAS mutations, PAX8/PPARγ and RET/PTC rearrangements and miRNA clusters. Molecular biology plays also a role in prognosis and therapy selection. While the latter issue is unquestionable (finding specific targets for therapy), there is a lot of controversy on the prognostic relevance of molecular markers in well differentiated thyroid carcinomas. This controversy is particularly obvious in the discussion of the relative importance of the topographical/histopathological features (peripheral location, extrathyroid extension, infiltrative growth pattern, fibrotic stroma) and of the molecular features (BRAF, cMET/HGF, RET/PTC) for the prognostic evaluation of papillary microcarcinomas.

11.40–12.20 [**S9**] ***Follicular Tumours of Uncertain Malignant Potential – Who Needs Them?***

Ⓟ Prof TJ Stephenson

Sheffield Teaching Hospitals, Sheffield, United Kingdom

An intelligent and evidence-based way forward in classifing follicular tumours in a borderline malignant category, or a refuge for the diagnostically-destitute? *That's for the audience to decide.*

The original suggestion of FT-UMP by the Chernobyl Pathologists' Group, for follicular patterned tumours that partially "make it" as a minimally invasive follicular carcinoma (due to partially shown capsular and / or vascular invasion), or as a follicular variant papillary carcinoma (due to the partially developed nuclear features of this) which supposedly didn't need to invade to be malignant, will be traced through to modern day suggestions that the term may have utility.

Contemporary evidence on the biological behaviour and molecular pathogeneses of these categories of tumours will be reviewed. There then follows stakeholder analysis of the purpose of the histopathology report, to look at the alternatives of instituting use of the FT-UMP term compared with simply classifying follicular patterned thyroid tumours as benign or malignant given what we now know about their behaviour provided that they are properly sampled and examined.

The Author believes that a clear position can be taken, but let's see what the audience think, in the light of this talk and the mini-symposium's other presentations.

12.20–13.00 [**S10**] ***Thyroid Microcarcinoma – Definitions and Significance***

Ⓟ Dr MT Moonim

Guy's & St. Thomas' Hospitals, London, United Kingdom

Thyroid microcarinomas are tumours which are less than 1 cm in size and are currently classified as stage pT1a by TNM7. While tumours of all 3 main morphologic cell types have been documented, the vast majority of tumours encountered in clinical practise are papillary microcarcinomas. Most papillary microcarcinomas are sporadic, occur in females, average 1-4 mm in size and are 'incidentalomas' identified either by pathologists sampling goitres or by radiologists while evaluating thyroid nodules. Symptomatic papillary carcinoma accounts for a minority of cases encountered. It is important to determine if disease is unifocal or multifocal as the latter presentation is associated with a higher rate of lymph node metastasis. RET and BRAF mutations have been documented in upto 60% cases. Important prognostic factors while considering treatment include multifocality and patients with a symptomatic presentation. Medullary microcarcinoma can be familial or sporadic with significant differences in presentation, associated pathology, genetics, behaviour and outcome. Distinction from C-cell hyperplasia is important and criteria to make this distinction are discussed.

13.00–14.00 **UNIVERSITY HOUSE · ABBEYDALE / FULWOOD ROOMS**

LUNCH

14.00–15.00 **OCTAGON CENTRE · MAIN HALL**

POSTER VIEWING AND CHAIRMAN'S ROUNDS

Categories	Poster Numbers	
Autopsy/Forensic	P38–P41 [1]	
Cardiovascular/Pulmonary	P42–P44 [1]	*(Note: P44 is withdrawn)*
Cellular/Molecular	P45–P50 [2]	
Education and Audit	P51–P58 [3]	
Endocrine	P59–P65 [4]	*(Note: P60 is withdrawn)*
Experimental Tumour Pathology	P66–P70 [2]	
Genitourinary/Renal	P71–P72 [5]	
Gynaecological	P73–P75 [5]	
Neonatal/Paediatric	P76–P79 [5]	
Technical Advances	P80–P84 [3]	

Chair: [1] Dr M Osborn, London; Dr K Suvarna, Sheffield
[2] Dr RD Byers, Manchester; Prof M Ilyas, Nottingham
[3] Dr JWM Chow, London; Dr SS Cross, Sheffield
[4] Prof TJ Stephenson, Sheffield
[5] Dr P Ramani, Bristol; Prof M Wells, Sheffield

14.30–15.30 **DAINTON BUILDING · LECTURE THEATRE 1**

UK ENDOCRINE PATHOLOGY SOCIETY BUSINESS MEETING

15.00–17.30 **STUDENTS' UNION BUILDING · AUDITORIUM**

PLENARY ORAL PRESENTATIONS

Chair: Prof IO Ellis, Pathological Society Meetings Secretary and University of Nottingham
Prof PA Hall, Editor-in-Chief, *Journal of Pathology*, and King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

15.00–15.15 [**PL1**] ***Epidermal Growth Factor Receptor (EGFR) and Stat3 Signal Through Kras and Have Mutually Opposite Effects on Cten***

Ⓟ S AlGhamdi[1,2]; S Ibrahim[1]; K Balloch[1]; D Jackson[1]; M Ilyas[1]

[1]University of Nottingham, Nottingham, United Kingdom; [2] King Abdullah International Medical Research Center (KAIMRC), King Saud bin Abdulaziz University for Health Sciences (KSAU-HS), Riyadh, Saudi Arabia

Cten is a protein located at focal adhesions and has been reported to be an oncogene in colon, breast, lung and gastric cancer. We have previously shown than Cten is target of K-ras in colorectal and pancreatic cancer. In this study, we investigated whether two other proposed mechanisms i.e. EGFR and Stat3 signalling were involved in regulating Cten expression.

Initially we manipulated EGFR signalling by (i) stimulation with EGF and (ii) inhibition by the PD153035 in the colorectal cancer cell lines SW620 and C32. In C32, EGF stimulation resulted in up-regulation of Kras and Cten whilst exposure to PD153035 resulted in down-regulation of both Kras and Cten. EGFR activation and inhibition was reflected by, respectively, increased and decreased cell motility although the effect of EGFR activation was lost by Cten Knockdown. In SW620, which harbours a KRAS mutation, modulating EGFR activity in this way had no effect on either Kras or Cten.

Stat3 signalling has also been reported to positively regulate Cten. We tested this in SW620 by directly knocking down Stat3 and exposing cells to interleukin-6 (an activator of Stat3). Stat3 knockdown resulted in increased Cten whilst Stat3 activation resulted in downregulation of Cten. Testing for Kras expression showed that Stat3 was negatively regulating Kras and this was reflected in the Cten expression. Functional analysis however showed that inhibition of Stat3 resulted in a reduction of cell motility in a Kras and Cten-independent manner.

We conclude that both EGFR signals through Kras to modulate Cten (and consequently ILK/FAK) and stimulates cell motility. Stat3 however negatively regulates Kras and consequently Cten although its net effect is to stimulate motility through an alternative mechanism.

15.15–15.30 [**PL2**] ***Wild-type K*-ras *Exerts a Tumour Suppressor Effect on Carcinogen-Induced Colorectal Adenomagenesis in the Mouse***

F Luo[1]; G Poulogiannis[1]; H Ye[2]; R Hamoudi[2]; AEK Ibrahim[1]; Ⓟ MJ Arends[1]

[1]University of Cambridge, Cambridge, United Kingdom; [2]University College London, London, United Kingdom

Human colorectal adenomas and carcinomas show K-ras mutations in 40-50% cases, but its contribution to adenoma formation in vivo is incompletely understood. We used a heterozygous K-ras knockout mouse model

to study the effects of K-ras gene dosage on carcinogen induced colorectal adenoma formation. Wild-type and heterozygous K-ras knockout mice were treated with 10 weekly injections of 1,2-dimethylhydrazine (DMH) to induce colorectal tumours. Compared with wild-type K-ras mice, colorectal expression levels of K-ras 4A and 4B transcripts in heterozygous K-ras mice were approximately 50% decreased. Compared with wild-type K-ras mice, heterozygous K-ras mice showed decreased survival from 88% to 82% (1 year after DMH treatment), significantly increased colorectal adenomas from 0.52±0.15 to 0.87±0.14 (mean±SEM per mouse, $p<0.05$), increased total tumour volume by 2.13-fold, increased Ki-67-positive proliferating tumour cells from 7.77±0.64% to 9.15±0.92% (mean±SEM, $p<0.05$), and decreased cleaved caspase-3-positive apoptotic tumour cells from 1.40±0.37% to 0.80±0.22% (mean±SEM, $p<0.05$). The adenomas showed no K-ras or B-raf mutations by sequencing and no significant changes in Erk/MapK or PI3K/Akt pathway activation by immunohistochemistry. However, heterozygous K-ras+/- colorectal adenomas showed reduced RNA expression levels of the p107 and p130 members of the RB family, which may act via E2F family proteins to modulate cell cycle progression. In conclusion, the data collectively show that a 50% reduction of K-ras gene dosage and RNA expression promoted colorectal adenomagenesis, consistent with wild-type K-ras having a tumour suppressor effect on carcinogen-induced murine colorectal adenoma formation.

15.30–15.45 [**PL3**] ***Tumour Necrosis Predicts Benefit From Hypoxia-Modifying Therapy in High Grade and Invasive Bladder Carcinoma***

Ⓟ A Williamson[1]; J Irlam[1]; J Taylor[2]; H Denley[3]; S Agrawal[3]; WD Ryder[4]; A Choudhury[5]; AM Rojas[6]; PJ Hoskin[6]; CML West[1]

[1]University of Manchester, Manchester, United Kingdom; [2]Paterson Institute for Cancer Research, Manchester, United Kingdom; [3]Central Manchester University Hospitals NHS Foundation Trust, Manchester Royal Infirmary, Manchester, United Kingdom; [4]Clinical Trials Coordination Unit, Christie Hospital, Manchester, United Kingdom; [5]Clinical Oncology, Christie Hospital, Manchester, United Kingdom; [6]Cancer Centre, Mount Vernon Hospital, Middlesex, United Kingdom

Purpose: there is no predictive marker of hypoxia-modifying cancer therapy in routine clinical use. Hence, this study was conducted to test the hypothesis that histopathological tumour features might reflect hypoxia and predict benefit from hypoxia-modifying therapy in high grade and invasive bladder carcinoma. Methods: samples were available from 231 patients who participated in the BCON phase III randomised trial comparing radical radiotherapy (RT) with and without carbogen and nicotinamide (CON). Histopathological tumour features examined were: necrosis, growth pattern, appearance of growing margin and tumour stroma ratio (TSR). To explore mechanisms, expression of carbonic anhydrase IX (CA IX), glucose transporter-1 (Glut 1) and Ki 67 were scored on tumour microarrays. Results: tumour necrosis was present in 121 patients (52%) and was the only histopathological tumour feature that had independent prognostic significance in patients receiving RT alone ($p=0.007$). Five-year overall survival (OS) was 48% (RT) versus 39% (RT+CON) (log rank $p=0.32$) in patients without necrosis and 34% (RT) versus 56% (RT+CON) ($p=0.004$) in patients with necrosis. Multivariate analyses showed that the presence of necrosis was a significant predictor of benefit from CON with the risk of death lower compared to RT alone (HR 0.43, 95% CI 0.25-0.73, $p=0.002$). This trend was not observed when there was no evident necrosis (HR 1.64, 95% CI 0.95-2.85, $p=0.08$). Tests for heterogeneity in treatment effect by necrosis strata were significant ($p=0.007$ unadjusted, $p=0.001$ adjusted). CA IX ($p=0.0001$) and Glut 1 ($p=0.013$) were associated with necrosis, but Ki 67 was not. Conclusions: necrosis predicts benefit from hypoxia-modifying therapy in bladder cancer. A prospective trial should be considered using necrosis to select patients for radiotherapy plus hypoxia-modifying therapy versus a novel intervention in those with no tumour necrosis.

15.45–16.00 [**PL4**] ***Integrin-mediated Epithelial-to-Mesenchymal Transition is Suppressed by Hedgehog Signalling in Basal Cell Carcinoma***

Ⓟ KA Moutasim[1]; GW Neill[2]; GJ Thomas[1]

[1]University of Southampton, Southampton, United Kingdom; [2]Queen Mary University of London, London, United Kingdom

Background and Aims: Basal cell carcinoma (BCC) is the most prevalent cancer in the Western world, and its incidence is rising. The pathogenesis of BCC involves deregulated Sonic Hedgehog signalling, leading to activation of the Gli transcription factors. We have previously shown that the epithelial-specific integrin αvβ6 is up-regulated in squamous cell carcinoma, promoting invasion. The aim of this study was to examine the interactions between αvβ6 and Gli1 in nodular and morphoeic BCC.
Methods: Tissue microarrays were generated from paraffin-embedded formalin-fixed BCC cases and immunohistochemistry for a panel of molecular markers was carried out. Gli1 cDNA was introduced into NTert1 and HaCaT skin keratinocytes using retroviral infection, and expression was confirmed by Western blotting. A three-dimensional model of BCC was generated using organotypic gels in an air-liquid interface. EMT was induced by EGF stimulation and EMT marker expression was examined using Western blotting, fluorescence microscopy, flow cytometry and q-RT PCR.
Results and Discussion: Molecular profiling of BCC showed that αvβ6 was significantly upregulated in the more aggressive morphoeic subtype ($p<0.05$). Gli1 significantly down-regulated αvβ6 expression on protein and mRNA levels in vitro ($p<0.01$). αvβ6-dependent TGF-β activation and invasion were also down-regulated by Gli1.
EMT studies showed that Gli1 expression was associated with membraneous E-cadherin localisation. This was recapitulated in clinical speciemens, where E-cadherin was delocalised to the cytoplasm in morphoeic BCCs, which also displayed loss of nuclear Gli1 expression. These results suggest that morphoeic BCC may represent a form of BCC EMT, and that αvβ6 represents a potential target.

16.00–16.30 **OCTAGON CENTRE · MAIN HALL**
COFFEE AND TRADE EXHIBITION

16.30–16.45 [**PL5**] ***Oestrogen Receptor Negative Progesterone Receptor Positive Phenotype in Breast Cancer is a Technical Artefact.***

EA Rakha; Ⓟ J Roberts; Z Ahmed; IO Ellis; AH Lee; A Green
Nottingham City Hospital, Nottingham, United Kingdom

Hormone therapy is currently the mainstay therapy for oestrogen receptor (ER) positive breast cancer. However, several studies have reported identification of a ER-negative (ER-) progesterone receptor positive (PR+) phenotype in up to 10% of cases. The existence and response to therapy of this phenotype remains to be defined. Methods: In this study ER and PR status were assessed in 3000 breast cancer samples including 2000 prepared as tissue microarrays (TMA) from a consecutive series of cases diagnosed between 1988-1998 and 1000 consecutive cases diagnosed on needle core biopsy (NCB) between 2009 and 2011. Surgical excision specimens (full face sections FFS) were examined in relevant cases using different antibodies against ER (6F11, 1D5 and SP1) and PR (SP2 and p636) to confirm staining pattern. Results: Of the 1000 prospective recent NCB tested cases, only 3 tumours were classified as ER-/PR+. However, staining of FFS revealed PR- in all 3 cases (False positive PR on NCB). Of the 2000 TMA samples 60 cases of ER-/PR+ were identified. Staining FFS of those 60 cases using single antibodies (SP1 and SP2) showed that 31 cases were either false negative ER or false positive PR. On repeating staining using different antibodies and more sensitive IHC techniques of the remaining 29 cases revealed ER+/PR+ in 18 cases, ER-/PR- in 9 cases while 2 cases showed ER-/PR+. Examination of those 2 cases revealed ER very weak internal control staining suggesting technical artefacts or suboptimal tissue fixation or processing. In conclusion, our results support the biologically based view that ER-/PR+ phenotype should not exist and when found is likely to represent a technical or methological artefact. Apparent identification of the ER-/PR+ phenotype in a NCB sample should prompt repeating staining on FFS before commencing systemic therapy.

16.45–17.00 [**PL6**] ***Her2 Protein Expression in Advanced Colorectal Cancers from the MRC FOCUS Trial.***

Ⓟ SD Richman; P Chambers; GJ Hemmings; JH Barrett; D Cross; M Taylor; MT Seymour; P Quirke
Leeds Institute of Molecular Medicine, Leeds, United Kingdom

Overexpression and amplification of Her2 has been linked to response to Herceptin in breast and gastric cancers. Current treatments for advanced colorectal cancer (aCRC) now include drugs targeting the epidermal growth factor receptor. KRAS/BRAF mutations confer resistance to anti-EGFr therapy; however, even among patients with KRAS/BRAF wild-type tumours only a minority respond. One hypothetical mechanism of drug resistance is aberrant signalling through the up-regulation of other transmembrane receptors including Her2. We have assessed the frequency of Her2 overexpression in 895 aCRC patients, and related this to KRAS/BRAF status and outcomes. Of 2135 patients enrolled in the MRC FOCUS trial, suitable surplus pathological tumour material was obtained from 895 consenting patients. Tissue microarrays were generated for Her2 immunohistochemistry. Pyrosequencing was used (where DNA was available) to assess mutation status at KRAS codons 12/13, 61 and BRAF codon 600. Of 895 tumours, 18 (2.0%) showed strong membrane staining. A subset of these cases was also tested using FISH and all showed amplification. Her2 membrane positivity was more frequent in KRAS/BRAF-wt tumours, being present in 13/258 (5.0%), compared with 4/284 (1.4%) KRAS/BRAF-mut cases (Fisher's exact test p=0.02). Patients with Her-2 membrane-positive tumours had non-significantly better overall survival (HR 0.81, 95% CI [0.50, 1.32]) and progression-free survival (HR 0.68 [0.42, 1.12]), compared to Her2-negative cases.
Strong membrane staining of Her2 is infrequent in colorectal cancer, but was seen in 5% of patients with KRAS/BRAF-wt tumours. It may be caused by amplification of the Her2 gene, detectable by FISH. Although rare, investigation of anti-Her2 therapy in this group may be warranted.

17.00–17.15 [**PL7**] ***Retinoic Acid-induced Protein 3 : Identification and Characterisation of a Novel Colon Cancer Biomarker***

Ⓟ GGA Hutchins; A Zougman; DA Cairns; E Verghese; S Perry; D Jayne; PJ Selby; RE Banks
University of Leeds, Leeds, United Kingdom

Background: Molecular biomarkers are needed in bowel cancer to precisely define prognosis and predict response to chemotherapy. Using mass spectroscopic and immunohistochemical techniques, we aimed to identify and characterise novel colonic cancer associated proteins which may offer diagnostic and, potentially, therapeutic opportunities.
Methods: Label-free mass spectroscopic proteomic quantitation was employed to profile matched normal colon / colon cancer (CC) tissue pairs for malignancy-associated proteins. The putative diagnostic utility of identified protein(s) was evaluated in 367 CC cases contained within the NCI Progression Colon Cancer tissue microarray (TMA) set using immunohistochemistry (IHC).
Results: Retinoic acid-induced protein 3 (RAI3) was identified as protein over-expressed in CC. Cancer-associated RAI3 over-expression was confirmed by IHC. Despite RAI3 IHC expression within neoplastic epithelium being variable, 76% (n=236) of interpretable CC cases (n=312) displayed uniform cytoplasmic expression. Of particular note, a sub-group of CC cases (n=23, 7.4%) displayed intense cytoplasmic expression, a feature significantly associated with tumour recurrence in Dukes' A-C (stage I-III) patients (HR=3.076, [95%CI=1.738-5.445];p<0.001) when compared to low or negative RAI3 expression. This association retained univariate significance in Dukes' B/

stage II patients only (HR=3.494, [95%CI=1.197-10.20];p<0.022). Significantly, the prognostic capacity of RAI3 was maintained in the stage I-III cohort following multivariate modelling (HR=2.11, [95%CI 1.109 – 4.017], p=0.023). Inter-observer agreement in assessment of dichotomized RAI3 expression was good (Cohen's kappa coefficient κ=0.775 (0.737–0.816), p<0.0001).
Conclusion: RAI3 is a putative prognostic marker that identifies high risk CC patients This study also emphasises the potential value of modern proteomic technology in clinically relevant applications.

17.15–17.30 **[PL8]** ***Molecular Co-expression of YY1 and Bcl-2 is Associated with Unfavourable Outcome in Follicular Lymphoma and Suggests Centriolar Location of YY1 and Role in Control of Proliferation***

Ⓟ HE Sandison[1]; S Usher[1]; A Dunne[2]; JA Radford[1]; KM Linton[1]; RJ Byers[1]

[1]University of Manchester, Manchester, United Kingdom; [2]Paterson Institute for Cancer Research, Manchester, United Kingdom

Ying Yang 1 (YY1) is a transcription factor involved in diverse cellular processes, including both proliferation and apoptosis. It is of prognostic importance in follicular lymphoma (FL), in which increased protein levels are associated with a favorable outcome. Additionally co-localisation of YY1 with the anti-apoptotic protein Bcl-2, which is overexpressed in FL, is associated with favorable outcome in FL. This study aimed to investigate possible molecular co-expression and interaction of YY1 with Bcl-2 in FL.
Duolink in situ proximity ligation assay (PLA), which gives positive signal with very close protein approximation (within 40nm), was used to measure extent of co-expression of YY1 and Bcl-2 in 69 FL samples in a tissue microarray. Three-dimensional (3-D) imaging was used to analyse the data and quantum dot based immuno-electron microscopy (QD-EM) used to visualize YY1 and Bcl-2 protein location.
Positive PLA signals were present in the FL samples at variable frequency (0-196 signals per sample) and Kaplan Meier survival analysis showed association of signal frequency above the 75th centile with unfavourable outcome (p=0.0281). All PLA signals were localised to the nuclear edge, with only one signal per cell, suggesting Bcl-2 and YY1 co-expression at the centriole; QD immunofluorescence (QD-IF) for Plk4, a centriolar protein, also gave one signal per cell at the nuclear edge. QD-EM and 3-D imaging supported YY1 and Bcl-2 localisation at the nuclear edge. There was no correlation between PLA signal frequency and co-localization of YY1 and Bcl-2 (measured by dual QD-IF). The results confirm association of YY1 and Bcl-2 with outcome in FL and suggest co-expression at the centriole. This is intriguing given the reported interaction of YY1 with Plk1 at the centriole and control of cell division at the G2/M checkpoint. This would concord with poor outcome given association of higher Ki-67 with poor outcome in FL.

17.30–18.30 **STUDENTS' UNION BUILDING · AUDITORIUM**

THE PATHOLOGICAL SOCIETY OF GREAT BRITAIN & IRELAND'S 10th DONIACH LECTURE

Chair: Prof AH Wyllie, President, Pathological Society of Great Britain & Ireland

[S11] ***Only Dead Fish Swim with the Stream: Trying to Understand the Dynamics of the Liver and its Diseases***

Ⓟ Prof MR Alison

Barts and The London School of Medicine and Dentistry, London, United Kingdom

The healthy liver exhibits little proliferative activity, but rapid cell cycle entry follows hepatocyte injury. Most regenerative responses involve hepatocytes, but chronic injury induces hepatocyte senescence shifting the burden of regeneration to facultative stem cells in the smallest branches of the biliary tree. Another source of regeneratively-competent cells when hepatocyte regeneration is compromised are 'small hepatic progenitor cells', randomly located within the liver. The role of bone marrow cells (BMCs) in regeneration appears trivial, but we have shown that BMCs functionally contribute to fibrosis in the liver and other organs.
Despite much effort, the kinetic organization of the liver is not resolved. Studies of chimaeric and retrovirally-labelled rodents suggest that daughter hepatocytes remain contiguous, but the orientation of their allocation is random. I was inspired by a pulse-chase analysis of tritiated thymidine-labelled hepatocytes that suggested hepatocytes migrated from the portal areas to the central veins, in effect being a cell lineage system akin to the crypt:villus trajectory seen in the intestine. This STREAMING LIVER hypothesis was derided by purists who worried about thymidine re-utilization, but seemingly verified by tracing the fate of Sox9-expressing portal cholangiocytes in mice, although others failed to find any ingress of these cells into the parenchyma. Of course, the best model of man is man, and we have developed a technique to find clonal populations in human tissue by identifying common mitochondrial DNA mutations. These studies have revealed clonal populations in a portal vein-central vein orientation, supporting the streaming liver hypothesis. We have also examined human female livers heterozygous for X-linked G6PD and the degree of methylation of the promoter regions of non-expressed genes to verify the 'streaming liver' hypothesis.

19.30–22.30 **CUTLERS' HALL**

SOCIETY DINNER

07.45 **OCTAGON CENTRE · FOYER**

REGISTRATION AND COFFEE

08.00–09.00 **STUDENTS' UNION BUILDING · AUDITORIUM**

TRAINEES SESSION – MEET THE EXPERTS: ***My Approach to Soft Tissue Tumours***

Chair: Dr I Proctor, University College London

[**S12**] ***Meet the Experts: My Approach to Soft Tissue Tumours***

Ⓟ Dr K Thway

Royal Marsden Hospital, London, United Kingdom

Soft tissue tumours can seem daunting for histopathology trainees. There are over two hundred types, with many variant patterns of each. As exposure to a significantly large number is limited outside tertiary centres, assessment of malignancy, and subtyping of spindle, epithelioid, pleomorphic or small round cell soft tissue neoplasms can be very challenging. A working knowledge of soft tissue tumours is however important as they will invariably crop up in any general diagnostic surgical pathology workload.

This session will give a practical basis for identifying and diagnosing soft tissue tumours, including in limited biopsy material, breaking them down into morphologic patterns, discussing the salient immunophenotypic and molecular tests and importantly the clinicopathologic aspects.

The aim is to equip attendees with a broad grasp of the important facets of the main soft tissue tumour types, their differential diagnostic considerations, and checklist of essential points to help in the work up for correct identification and appropriate management.

09.00–16.00 **OCTAGON CENTRE · COMPUTER ROOM**

SLIDE SEMINAR COMPETITION CASE VIEWING: ***Gynaecological Pathology***

Note: Competition closed at 15.30 on Wednesday 4 July

09.00–10.30 **STUDENTS' UNION BUILDING · AUDITORIUM**

ORAL PRESENTATIONS

Chair: Prof D Salter, University of Edinburgh
Dr JJ Going, University of Glasgow

Categories: Breast; Osteoarticular/Soft Tissue

09.00–09.15 [**O33**] ***C-Met Expression Correlates with Molecular Sub-type and Outcome in Invasive Breast Cancer***

Ⓟ C Ho-Yen[1]; A Green[2]; I Ellis[2]; S Kermorgant[1]; LJ Jones[1]

[1]Barts Cancer Institute, London, United Kingdom; [2]Nottingham City Hospital, University of Nottingham, Nottingham, United Kingdom

Background: The transmembrane receptor tyrosine kinase c-Met is over-expressed in a sub-set of breast cancers, and is associated with a poor prognosis. Early phase clinical trials of anti-c-Met therapy in breast cancer are ongoing and there is a need to identify the tumour sub-groups most likely to respond.

Aim: The aim of this study was to evaluate c-Met expression in a large cohort of well characterised invasive breast cancers and relate this to molecular sub-type and outcome.

Methods: Tissue microarrays were assembled from 1977 tumours diagnosed at Nottingham University NHS Trust. Sections were cut and immunohistochemistry performed for several markers including ER, PR, Her2, CK5, CK14 and c-Met. Cases were divided into triple negative (TN: ER, PR and Her2 negative) and non-triple negative (NTN). TN tumours were further divided into BL (CK5 and/or CK14 positive) and Unclassified (U: negative for CK5/14).

Results: A score for c-Met reactivity was obtained in 1239 cases. NTN tumours accounted for 82% (n=1020); 18% (n=219) were TN. Of the TN cases, 61% (n=133) were BL and 39% (n=86) were U. C-Met expression showed a positive association with both TN (p=0.008) and BL status (p=0.010). Compared to NTN tumours the BL tumours showed significantly higher c-Met expression (p=0.010). High c-Met expression was associated with lower overall survival (p=0.021) and breast cancer-specific survival (p=0.022).

Conclusion: High c-Met expression is associated with aggressive sub-types of breast cancer and poor outcome. Our findings suggest anti-c-Met therapy may have a role in the treatment of TN cancers, and BL tumours in particular.

09.15–09.30 [**O34**] ***Roles for MicroRNAs in Carcinoma Associated Fibroblasts in Basal-Type Breast Cancer***

Ⓟ LM Gardner; R Drury; AM Hanby; TA Hughes; E Verghese

University of Leeds, Leeds, United Kingdom

MicroRNAs are regulators of gene expression that act at post-transcriptional levels and have key roles in carcinogenesis. However, roles have been examined only in carcinoma epithelial cells and almost nothing is known about potential roles in cancer stroma. A large body of evidence shows that carcinoma associated fibroblasts (CAFs) have strong influences on breast cancer progression. This study aims to examine roles of microRNAs within breast CAFs. We have chosen to focus on basal-type breast cancers because these have a particularly poor

prognosis as compared to other subtypes, partly on account of lack of appropriate targeted therapies. Better understanding of their biology will help in designing improved treatments.
We identified four cases of basal-type breast cancer by immunohistochemistry (ER, PR and Her2 negative; CK5/6 and CK14 positive). Laser micro-dissection was used to prepare samples of normal fibroblasts (NFs, taken from tissue distant from tumour) and CAFs from FFPE resection samples of each case. We examined expression of 671 microRNAs using real-time PCR arrays and relative expressions were determined in the matched pairs of NFs and CAFs. 12 microRNAs were consistently up-regulated and 20 microRNAs were consistently down-regulated in the CAFs. Of these miR-27b and miR-21, which were up-regulated, and miR-573 and miR-30a, which were down-regulated, had an average fold change greater than 4. Of particular interest was miR-21, which is thought to act as an oncogene in epithelial cells, yet we find strong up-regulation in CAFs. Furthermore, in-situ hybridization was used to confirm strong expression in CAFs with relatively low, or undetectable expression in epithelial cancer cells.

09.30–09.45 [**O35**] ***Injectable Hydrogel Delivery System for Mesenchymal Stem Cell Delivery to the Degenerate Intervertebral Disc***

B Barthrop[1]; VL Boyes[2]; S Sabnis[2]; N Chiverton[3]; A Cole[3]; AR Michael[3]; L Breakwell[3]; J Foulkes[4]; C Sammon[2]; Ⓟ CL Le Maitre[1]

[1]Biomedical Research Centre, Sheffield Hallam University, Sheffield, United Kingdom; [2]Materials and Engineering Research Insitute, Sheffield Hallam University, Sheffield, United Kingdom; [3]Sheffield Teaching Hospitals NHS FT, Sheffield, United Kingdom; [4]Smith and Nephew Extruded Films, Sheffield, United Kingdom

Instability of the motion segment as a result of intervertebral disc (IVD) degeneration is well known as a major cause of lower back pain. Here, we investigate the differentiation of mesenchymal stem cells in a novel hydrogel system, which can be maintained as a liquid ex vivo and can potentially be injected into the IVD where it will gel in situ.
Effects on human mesenchymal stem cell viability, migration characteristics, matrix production and differentiation capacity was investigated. Additionally the feasibility of injection through narrow bore needles into degenerate IVDs. In addition effects of the degenerate tissue niche were investigated on MSC behaviour to determine whether inhibitors of degeneration would need to be co-delivered.
Viability of MSCs were unaffected by hydrogel systems, they adhered, migrated and proliferated. Synthesised collagen type II and aggrecan matrix and gene expression following 4 weeks. Liquid hydrogel was successfully injected into degenerated bovine discs. MSC treated with IL-1 induced expression of IL-1, IL-1Ra, MMP3 and MMP13. IL-Ra was successfully incorporated into the hydrogel system.
We have developed a hydrogel system with the potential to deliver MSCs via minimally invasive injection via narrow bore needles, decreasing the risk of damage to the annulus fibrosus. The system is non-toxic, supports MSC growth, differentiation and shows potential to provide mechanical support to the motion segment whilst regeneration takes place. Such a therapy combined with inhibition of degeneration shows immense potential for early and mid stages of spinal degeneration.

09.45–10.00 [**O36**] ***Differential Intracellular Signalling Pathways Induced by IL-1 and CDMP-1 in Human Nucleus Pulposus Cells, and their Potential as Therapeutic Targets.***

Ⓟ J Daniels[1]; KL Phillips[1]; A Cole[2]; A Michael[2]; L Breakwell[2]; N Chiverton[2]; CL Le Maitre[1]

[1]Biomedical Research Centre, Sheffield Hallam University, Sheffield, United Kingdom; [2]Sheffield Teaching Hospitals NHS FT, Sheffield, United Kingdom

Intervertebral disc (IVD) degeneration is a consequence of an imbalance between anabolic and catabolic (particularly IL-1) processes within the IVD. Targeting intracellular signalling may provide a mechanism to inhibit multiple catabolic cytokines simultaneously, as many cytokines share signalling pathways. Differential signalling pathways activated by IL-1 and CDMP-1 (an anabolic factor) were investigated to ensure inhibition of catabolic pathways doesn't affect anabolic pathways.
Human nucleus pulposus cells isolated from surgical samples were extracted via collagenase digestion and expanded in monolayer culture. Following re-differentiation in alginate, cells were treated with IL-1 (10ng/mL) or CDMP-1 (10ng/mL) for 30 minutes. Site-specific phosphorylation of 46 signalling molecules were identified using R&D proteome array. The activation of pERK, pP38 MAPK and pAKT were investigated via BD phosflow techniques and NFκB by immuno-fluorescence. Pre-treatment with SB203580 (20μM, p38 MAPK inhibitor) and Helenalin (10μM, NFκB inhibitor) for 30 minutes, followed by stimulation with IL-1 (10ng/mL) or CDMP-1 (10ng/mL) for 24 hours investigated effects of inhibition of signalling on matrix and matrix degrading enzyme gene expression.
R&D proteome array identified a number of differentially activated pathways, including p38 MAPK. IL-1 induction of p38 MAPK, NFκB, AKT and ERK pathways was confirmed by BD phosflow and immunoflourescence techniques. Inhibition of the NFκB pathway by Helanin reduced the inhibitory effect of IL-1 on the production of aggrecan, but inhibited aggrecan induction by CDMP-1.
IL-1 has been implicated in the pathogenesis of IVD degeneration. The identification of differential intracellular signalling pathways between anabolic and catabolic factors could provide new methods for modulating the catabolic response, and preventing further IVD degeneration.

10.00–10.15 [**O37**] ***Cannabinoids Inhibit IL-1 Signalling Pathways in Human Chondrocytes***

Ⓟ SL Dunn[1]; A Crawford[2]; M Wilkinson[3]; C Le Maitre[1]; RAD Bunning[1]

[1]Biomedical Research Centre, Sheffield Hallam University, Sheffield, United Kingdom; [2]Restorative Dentistry, University of Sheffield, Sheffield, United Kingdom; [3]University of Sheffield Bone Biomedical Research Unit, Northern General Hospital, Sheffield, United Kingdom

A key feature of osteoarthritis and rheumatoid arthritis is the loss of articular cartilage. Matrix metalloproteinases (MMPs) and aggrecanases such as ADAMTS-4 play a key role in the pathogenesis of arthritis leading to the breakdown of extracelllar matrix (ECM). The expression of MMPs is regulated by nuclear factor kappa B (NFκB) which translocates to the nucleus of IL-1 stimulated cells in conditions such as arthritis. Cannabinoids have been shown to reduce joint damage in animal models of arthritis. They have also been shown to prevent IL-1 induced matrix breakdown of collagen and proteoglycan, suggesting a chondroprotective effect of these compounds. Here the effects of cannabinoid WIN-55, 212-2 (WIN-55) on IL-1β induced NFκB translocation to the nucleus in human chondrocytes has been investigated.

Primary human chondrocytes were obtained from patients undergoing total knee replacements. Chondrocytes were cultured in alginate beads and treated with cannabinoid WIN-55 with and without IL-1β stimulation for 30 minutes. Cells were released from alginate beads, fixed and cytospun. Immunofluorescence was used to investigate the translocation of NFκB to the nucleus of chondrocytes.

Chondrocytes stimulated with IL-1β demonstrated NFκB translocation to the nucleus. Chondrocytes treated with IL-1β in combination with WIN-55 showed inhibition of the IL-1β induced translocation of NFκB to the nucleus. The results suggest that WIN-55 can inhibit the translocation of NFκB to the nucleus induced by IL-1β. Thus cannabinoids could act as potential agents to inhibit IL-1 induced cartilage degradation.

10.15–10.30 [**O38**] ***Regulation of Neurotrophic Factor Expression in Nerve Cells and Intervertebral Disc Cells by Inflammatory Cytokines, Implications for Pain Pathways.***

S Thomas[1]; R Colletta[1]; K Phillips[1]; N Chiverton[2]; A Cole[2]; AR Michael[2]; L Breakwell[2]; A Cross[1]; Ⓟ C Le Maitre[1]

[1]Biomedical Research Centre, Sheffield Hallam University, Sheffield, United Kingdom; [2]Sheffield Teaching Hospitals NHS FT, Sheffield, United Kingdom

Intervertebral disc degeneration and prolapse contribute significantly to low back pain and sciatica cases. Here, we investigate the hypothesis that cytokines from degenerate IVDs induce neurotrophic and angiogenic factors in nucleus pulposus (NP) cells and nerve cells and induce neurite outgrowth within nerve cells.

Human NP cells from surgical samples were cultured in 3D culture and stimulation with cytokines: IL-1, IL-6, or IL-8 for 48hrs, RNA extracted and real time PCR performed to investigate expression of NGF, BDNF, NTF3, and VEGF. In addition SHSY-5Y nerve cell line were cultured in the presence of retinoic acid for 7 days prior to stimulation with IL-1, IL-6, IL-8 or TNF to investigate the gene expression of NGF, BDNF and NTF3 together with affects on neurite outgrowth.

Human NP cells showed a biphasic response to IL-1 in terms of NGF expression. A small increase in NTF3 was seen following IL-1 stimulation, whereas IL-6 treatment of NP cells showed a 10 fold increase in NTF 3 expression. SH-SY5Y cells did not express NGF and BDNF but NTF-3 was down regulated by IL-1 and IL-8 treatments but stimulated by IL-6 stimulation. TNF and IL-1 appeared to induce the greatest effects on neurite outgrowth and number of neurites per cell.

Here we demonstrate that a number of cytokines induce a number of neurotrophic factors and angiogeneic factors within human NP cells in 3D cultures and induce neurotrophic factor expression and neurite outgrowth in a nerve cell line suggesting a role for these cytokines both within the nerve ingrowth seen in an intact disc and intact discs and following prolapse.

09.00–10.30 DAINTON BUILDING · LECTURE THEATRE 1

ORAL PRESENTATIONS

Chair: Dr JWM Chow, St George's Hospital Medical School, London
Dr KP West, Leicester Royal Infirmary

Categories: Hepatobiliary/Pancreas; Education and Audit; Neonatal/Paediatric

09.00–09.15 [**O39**] ***Development of a Chimaeric Oncolytic Adenovirus Vector for Pancreatic Cancer Biotherapy***

Ⓟ D Orchard-Webb[1]; N Fox[1]; RM Elghazawy[2]; V Speirs[3]; AM Smith[2]; JPA Lodge[2]; AA Melcher[3]; CS Verbeke[4]; GE Blair[1]

[1]Institute of Molecular and Cellular Biology, University of Leeds, Leeds, United Kingdom; [2]Department of Surgery, St. James' University Hospital, Leeds, United Kingdom; [3]Leeds Institute of Molecular Medicine, St. James' University Hospital, Leeds, United Kingdom; [4]Karolinska Institutet, Stockholm, Sweden

Pancreatic cancer is the fourth most frequent cause of cancer-related mortality in the U.S.A. and around 85% of pancreatic cancers are inoperable at diagnosis. New therapies, including oncolytic virotherapy, are urgently required. Human adenoviruses (Ads) may form the basis of such virotherapies. Most Ad vectors are derived from Ad5 (species C) which utilises the Coxsackie and Adenovirus receptor (CAR) for cellular attachment via the viral fibre. Many tumours, including those of pancreatic origin, display reduced levels of CAR, thus limiting the transduction efficiency of Ad5-based vectors. In contrast, the species B Ads do not interact with CAR but with

CD46 and/or Desmoglein 2, both of which we have shown to be expressed by cultured pancreatic cancer cells and tissues.
To test species B Ads in pancreatic cancer therapy, an organotypic model of pancreatic cancer tissue slices was used. Entry of Ad vectors expressing a fluorescent marker protein (EGFP) was detected 48 hours after infection. Ad-EGFP vectors containing species B fibres (from serotypes 3, 11 and 35) exhibited deeper tissue penetration than Ad5-EGFP. Using a marker for lytic replication of Ads (hexon) in immunohistochemistry, we have shown that wild-type species B Ads replicate in pancreatic tissue slices. We are generating Ad5/serotype B chimaeras in order to improve Ad5 infectivity of pancreatic cancer. In the Ad5/serotype B fibre chimaera that shows the greatest infectivity of pancreatic cancer tissues we are replacing the Ad E1A promoter with one specifically active in cancer to create an infectious, conditionally-replicating oncolytic Ad vector for pancreatic cancer therapy.
We propose that species B Ads infect pancreatic cancer to a greater extent than Ad5 due, at least in part, to the properties of their fibre proteins, and that the introduction of further modifications to Ads may result in useful biotherapies for pancreatic cancer.

09.15–09.30 [**O40**] ***Macrophage Inflammatory Protein-1α (MIP-1 α) in Hepatitis C Virus-Related Hepatocellular Carcinoma: Relation to Clinical Staging and Tumour Angiogenesis***

HA El Aggan[1]; MA Helmy[2]; Ⓟ NMF El Deeb[3]; AE Zeid[1]; MFA Yehia[4]

[1]Department of Internal Medicine, Faculty of Medicine, Alexandria University, Alexandria, Egypt; [2]Department of Clinical Pathology , Faculty of Medicine, Alexandria University, Alexandria, Egypt; [3]Department of Pathology, Faculty of Medicine, Alexandria University, Alexandria, Egypt; [4]Private Physician (Internal Medicine), Alexandria, Egypt

Purpose of the study: Hepatitis C virus (HCV) is a major risk factor for the development of hepatocellular carcinoma (HCC), however, the mechanism of hepatocarcinogenesis in HCV infection is still undefined. MIP-1α is a pro-inflammatory chemokine that is increased in serum of patients with HCV-associated liver disease. This work was designed to study the role of MIP-1α in the development and progression of HCV-related HCC.
Methods: 30 HCV patients with cirrhosis (15 patients with HCC who underwent hepatic resection and 15 patients without HCC) and 15 healthy subjects were included in the study. Serum MIP-1α was measured using ELISA. Immunohistochemical staining of HCC and adjacent non-neoplastic liver was performed using antibodies for MIP-1α, CD68 for assessment of tumour associated macrophage (TAM) count and CD105 for assessment of microvessel density (MVD).
Summary of results: Serum MIP-1α was significantly higher in HCC patients than those without HCC ($P < 0.0001$). MIP-1α was expressed in the tumour cells in 14 HCC patients (93.3%), and was significantly higher in HCC than in adjacent non-neoplastic liver (P=0.0004). The mean TAM count and the mean MVD were significantly higher in HCC than in adjacent non-neoplastic liver (73.47 ± 23.01 vs 31.79 ± 11.55, $P < 0.001$, and 39.55 ± 12.55 vs 13.05 ± 4.62, $P < 0.001$). Serum MIP-1α levels directly correlated with MIP-1α expression in HCC ($P = 0.006$). A positive correlation was found between MIP-1α expression and TAM count in HCC ($P < 0.001$) and both directly correlated with the MVD ($P < 0.001$). The MIP-1α expression, TAM count and MVD in HCC directly correlated with tumour size, grade and stage ($P < 0.05$).
Conclusions: Tumour-derived MIP-1α may promote hepatocarcinogenesis by recruitment of macrophages into tumour tissues and fostering tumour angiogenesis. Therefore, MIP-1α can serve as a potentially useful therapeutic target in HCV-related HCC.

09.30–09.45 [**O41**] ***An Ex-Vivo Model of Human Pancreatic Cancer***

Ⓟ RM Elghazawy[1]; N Fox[2]; D Orchard-Webb[2]; V Speirs[3]; AM Smith[1]; JPA Lodge[1]; CS Verbeke[4]; GE Blair[2]

[1]Dept of HPB & Transplant Surgery, St James's University Hospital, Leeds, United Kingdom; [2]Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom; [3]Leeds Institute of Molecular Medicine, Leeds, United Kingdom; [4]Division of Pathology, Department of Laboratory Medicine, Karolinska Institute, Stockholm, Sweden

Pancreatic cancer is a major leading cause of cancer death in the Western world, with approximately 40,000 deaths per year occurring in Europe and around 26,000 in the USA. The incidence of pancreatic adenocarcinoma has been steadily increasing over the past 20 years and is now the fourth most common cause of cancer deaths. Fatality is due to a lack of effective screening strategies, inconspicuous early symptoms and poor response to existing therapies leading to a median survival of around one year. New forms of treatment for this disease are therefore urgently needed. A critical requirement for pre-clinical studies is a suitable model system in which to analyse the effects of therapeutic agents. Most in-vitro models fail to reflect the complex tissue architecture of an individual tumour. In this study human pancreatic tissue samples, both malignant and normal, were obtained from surgical specimens and processed to precision-cut 250μm x 0.5cm slices. Various elements were introduced to the modelling system (e.g. minimising the time from surgical resection to culture; coated vs uncoated culture plates; using low melting point agarose) in order to maximise the yield from minimal amounts of tissue and optimise the culture conditions. Pancreatic normal and cancer tissue slices could then be cultured for up to four days whilst maintaining good to excellent morphology A comprehensive analysis of tissue morphology, function and viability established that histopathological examination together with a morphological grading was shown to be the best approach for the assessment of the preservation of structural integrity. It was found that cellular death in such cultured slices occurs by necrosis rather than apoptosis. This organotypic culture model presents a representative and reproducible system of the in-vivo situation that will allow the prediction of the clinical efficacy of novel therapies such as gene therapy.

09.45–10.00 [**O42**] ***Undergraduate Histopathology Teaching Using a High-resolution Wall-sized Virtual Microscope***

Ⓟ GGA Hutchins[1]; R Randell[1]; J Sandars[1]; T Ambepitiya[2]; RG Thomas[1]; RA Ruddle[1]; P Quirke[1]; D Treanor[1]

[1]University of Leeds, Leeds, United Kingdom; [2]Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom

Background: High-resolution, wall-sized displays contain many millions of pixels and are physically large. With the advent of glass slide digitalisation, display of virtual slides on wall-sized interactive visualisation systems represents a potentially powerful educational tool. We evaluated this technology in teaching histopathology to medical undergraduates with the aim of enhancing basic knowledge and interest in histopathology.
Methods: Following ethical approval, 2nd year University of Leeds medical students were recruited by tutorial group. 12 students were exposed to virtual slide histopathology teaching on the Leeds "Powerwall" Virtual Microscope immediately following standard tutorials. 10 students from a matching tutorial group served as controls and were not exposed to the intervention. Control/intervention participants completed a questionnaire on enthusiasm for pathology and career intentions. Intervention students also completed a questionnaire on perceived value and usability of the technology. Sessions were video-recorded for analysis of interaction between students, tutor and technology.
Results: Students demonstrated a high level of engagement, pointing to features on slides to answer the tutor's questions, but also pointing to features in order to ask questions. Feedback was positive. Representative student comments included "makes histopathology much more interesting" and "made [histopathology] more engaging and easier to understand". Increased enthusiasm for histopathology was also demonstrated in the questionnaire, where there was a significant difference in change in enthusiasm between the intervention and control group.
Conclusions: The results demonstrate clear potential for wall-sized displays in teaching histopathology to medical students. Expansion of histopathology teaching using this technology is planned.

10.00–10.15 [**O43**] ***3D Virtual Pathology Specimens for Education and Engagement***

Ⓟ J Roulson; PJR Harkin; P Quirke

University of Leeds, Leeds, United Kingdom

Pathology museums provide a valuable educational resource but the traditional museum is not always easy to access, specimens may be unique and there are hazards associated with preservative liquids. Virtual 3D specimens allow interaction with a pathology specimen via a computer interface so it can be viewed easily in many locations. Our project aims to produce 3D virtual specimens which can be used in education and public engagement.
A standard digital camera was used to capture 2D images of pathology specimens which were imported into proprietary software and converted to 3D objects that can be viewed from multiple angles. The 3D specimens can be viewed in a standard web browser and can be rotated and zoomed. Specimens were MRI scanned and the data used to produce a 3D reconstruction which is also viewable over the internet.
The modern practice of pathology almost always precludes the inclusion of new specimens in traditional museums. The use of non-destructive techniques has allowed consented autopsy and surgical material, which would otherwise be unavailable, to be preserved in a virtual museum; and in addition allows better access to current museum specimens. We have produced 3D pathology specimens which can be used for educational and public engagement purposes, allowing anybody to interact with and learn from a realistic pathology specimen.
This project has been funded in part by the Pathsoc Public Engagement Scheme.

10.15–10.30 [**O44**] ***SIDS Implications of Temperature Dependent Toxin Production by Staphylococcus aureus Strains***

Ⓟ TJ McConnell[1]; L Bishop[1]; R Lauder[1]; L Harrison[2]; J Morris[2]

[1]Lancaster University, Lancaster, United Kingdom; [2]Morecambe Bay Hospitals Trust, Lancaster, United Kingdom

Introduction Staphylococcus aureus toxins are implicated in sudden infant death syndrome (SIDS) as there is an inverse relationship between incidence and circulating anti-toxin levels. A recent retrospective review of autopsy reports found that S. aureus was isolated more commonly from cases of SIDS compared to an explained non-infection group. Another study detected the toxins in tissues of over 50% of cases from three different countries. However, these toxins could be due to contamination at sampling. We have hypothesised that toxins not neutralised by IgG will be eliminated to the urine and detected. Here we demonstrate a temperature dependant pattern of secretion which does not support contamination as a source of toxins.
Methods TSST-1 and SEB producing strains of S. aureus were cultured for 24h at 41°C, 37°C, 30°C, 22°C or 4°C in either Brain Heart Infusion broth (BHI) or clean catch mid-stream urine. The OD600 of the suspensions were measured prior to centrifugation at 900g. Toxin levels in the resulting media supernatants were determined by ELISA, gel electrophoresis and western blotting (WB).
Results Maximum bacterial growth was observed at 30°C and 37°C while it was diminished at 22°C and 41°C and undetectable at 4°C. In contrast, maximal TSST-1 release occurred at 41°C (0.10µg/ml) with less at 37°C (0.052µg/ml), while at 4°C, 22°C and 30°C TSST-1 was undetectable. Similar results were obtained for SEB.
Conclusions Contamination of urine samples stored at room or refrigerated temperature does not result in the release of biologically significant levels of toxin. These results support the interpretation of our previous findings that the detection of S. aureus toxins in infant urine results from a transient bacteraemia rather than contamination. ELISA and WB for bacterial toxins in body fluids taken at autopsy can be used to distinguish between genuine infection with S. aureus and contamination.

09.10–10.20 **OCTAGON CENTRE · COUNCIL ROOM**

ASSOCIATION OF CLINICAL ELECTRON MICROSCOPISTS AGM

Open to non-members

10.30–11.00 **OCTAGON CENTRE · MAIN HALL**

COFFEE AND TRADE EXHIBITION

11.00–12.30 **STUDENTS' UNION BUILDING · AUDITORIUM**

TRAINEES' SYMPOSIUM: *How to be a Consultant*

Chair: Prof M Wells, University of Sheffield Medical School

11.00–11.30 **[S13]** ***Your First Year as a Consultant***

Ⓟ Prof TJ Stephenson

Sheffield Teaching Hospitals, Sheffield, United Kingdom

Thought that passing FRCPath, gaining CCT and winning a consultant post at competitive interview were tough and that's a smooth ride thereafter? *Think again!* The presentation starts by considering available evidence that the first year as a consultant is a time of great professional risk, emotional strain and dilemmas. This will be achieved by analysing the difference between being a senior trainee, where goals are clear-cut and the time-table is fixed, to being a new consultant, when there may be many competing priorities and open-ended commitments.
The difference between being "promoted" locally, and moving to an "external" consultant post will be analysed. We will consider the Consultant Contract, Job Planning, Managing Oneself, Relationships with Colleagues, Obtaining Feedback, Mentoring and Sources of Advice, Planning CPD, and Preparation for One's First Appraisal.
The aim is to give constructive and practical advice on avoiding the pitfalls of the first year, so that a sustainable working pattern can be created and that the demands of revalidation can be met, and even first stage management posts can be contemplated.

11.30–12.00 **[S14]** ***Fitness to Practise and the GMC***

Ⓟ Prof M Wells

Department of Oncology, University of Sheffield Medical School, Sheffield, United Kingdom

The main objective of the General Medical Council (GMC) is "to protect, promote and maintain the health and safety of the public". The consultant pathologist in 2012 is part of a multidisciplinary team and participates in: annual appraisal, continuing professional development and external quality assessment schemes. He/she is also preparing for revalidation by the GMC. Professional behaviour includes good clinical care, maintaining good medical practise, teaching and training, appraising and assessing, working relationships with colleagues, probity and health. Unprofessional behaviour can impair fitness to practise, including misconduct resulting in a criminal conviction or caution, drug or alcohol misuse, aggressive, violent or threatening behaviour, persistent inappropriate attitude or behaviour, cheating or plagiarising, dishonesty or fraud and physical or mental health concerns. Deficient professional performance by a pathologist may involve inadequate dissection, sampling or macroscopic description, discrepancies in microscopy or clinical correlation and failure to seek an opinion in an obviously difficult case. The implications for duty of care range from no impact to major harm and the question as to whether a diagnostic error is negligent or not is tested on the Bolam principle, in terms of liability and causation on the balance of probabilities.

12.00–12.30 **[S15]** ***Training Other Pathologists: The Next Step***

Ⓟ Dr DM Bailey

Royal College of Pathologists, London, United Kingdom

The GMC has recently launched a consultation asking whether clinical supervisors should be registered and approved, in the same way that educational supervisors are registered. On the one hand, this gives us an opportunity to weed out poorly performing or discriminatory trainers, but on the other we run the risk of discouraging established and new trainers from from remaining or becoming part of the community of pathologists who pass their skills onto the next generation. Pathology service reconfiguration plans are currently underway which, if pursued to completion will result in microbiology departments with no onsite laboratory, autopsies being handled off-site from hospital pathology laboratories, and merged blood sciences which are run by clinical scientists rather than medical pathologists. Add to this the Modernising Scientific Careers agenda, and the Health and Social Care Bill and its associated white paper, "Liberating the NHS: developing the healthcare workforce", and there has never been a more interesting time to be Director of Training and Educational Standards for the Royal College of Pathologists. Senior trainees who are applying for consultant posts will be looking at very different Job Plans to those we previewed fifteen to twenty years ago, however the curriculum vitae is just as important today as it ever was. In spite of the prevailing culture, many hospital Trusts take training extremely seriously. Having the credentials to start training from day one as a consultant makes a trainee an attractive prospect in many departments with vacant posts. This presentation aims to guide the senior trainee through the maze of factors required to become a consultant trainer in the twenty first century.

11.10–17.00 **DAINTON BUILDING · LECTURE THEATRE 1**

ASSOCIATION OF CLINICAL ELECTRON MICROSCOPISTS (ACEM)
15th ANNUAL SCIENTIFIC MEETING
See separate programme

12.30–13.30 **STUDENTS' UNION BUILDING · AUDITORIUM**

JAPANESE PATHOLOGICAL SOCIETY LECTURE

Chair: Prof CS Herrington, University of Dundee and General Secretary of Pathological Society of Great Britain & Ireland

[**S16**] ***Pathology of Gastrointestinal Stromal Tumour***

Ⓟ Prof SH Hirota

Hyogo College of Medicine, Nishinomiya, Japan

Ws mutant rats are the loss-of-function mutant of the c-kit gene and interstitial cells of Cajal (ICCs) are pacemaker for autonomous gastrointestinal movement. In the 1990's, using Ws mutant rats we found that loss-of-function of the c-kit gene product, KIT receptor tyrosine kinase, resulted in deficiency of ICCs and abnormal movement of gastrointestinal tract in rats.

Based on the results, we hypothesised that gain-of-function mutation of the c-kit gene might induce "ICC tumours". In 1998, we found that most of gastrointestinal stromal tumours (GISTs) express KIT and that most GISTs have gain-of-function mutations of the c-kit gene. In 2003, we and another group found that about half of sporadic GISTs without c-kit gene mutations have gain-of-function mutations of platelet-derived growth factor receptor alpha (PDGFRA) gene that encodes another receptor tyrosine kinase. We also found that familial patients with multiple GISTs have germline gain-of-function mutations of the c-kit gene. In those patients, diffuse hyperplasia of ICCs was observed in myenteric plexus layer of the small intestine as a pre-GIST lesion. We generated gene-targeting mice with gain-of-function mutation of the c-kit gene, and confirmed that the mutation induce ICC hyperplasia and GIST in mice. Thus, GISTs are now considered to be generally KIT- or PDGFRA-driven tumors and originate from ICCs. Imatinib, a selective tyrosine kinase inhibitor, inhibits constitutive activation of mutant KIT and PDGFRA. It is now used for metastatic or unresectable GISTs as a molecular target drug, and shows remarkable effect. Recently, moreover, adjuvant imatinib therapy after resection of GISTs is considered to improve overall survival of GIST patients. Thus, the correct diagnosis of GISTs is very important for GIST practice, and immunohistochemistry of KIT and/or mutational analyses of c-kit and PDGFRA genes are essential for the correct diagnosis of GISTs.

13.30–14.30 **UNIVERSITY HOUSE · ABBEYDALE / FULWOOD ROOMS**

LUNCH

14.00–14.45 **STUDENTS' UNION BUILDING · AUDITORIUM**

PATHOLOGICAL SOCIETY OF GREAT BRITAIN & IRELAND'S ANNUAL BUSINESS MEETING

14.45–15.45 **STUDENTS' UNION BUILDING · AUDITORIUM**

SLIDE SEMINAR DISCUSSION SESSION: *Gynaecological Pathology*

Chair and Speaker: Prof M Wells, University of Sheffield Medical School

15.45 **CLOSE OF MEETING**

Acknowledgments (Trade Exhibition)

as at time of going to press

The Pathological Society of Great Britain & Ireland wishes to acknowledge the support of the following companies participating in the TRADE EXHIBITION

Cirdan Imaging

Cirdan Imaging will be exhibiting their new low-cost PathSuite digital pathology platform at the Summer Meeting.

PathSuite is a modular solution designed to make imaging routine and easy and includes:

- PathStand macro-imaging station
- SPOT Idea microscopy cameras
- Dedicated acquisition and viewing software
- PACS workstation and server options for image storage.

Bringing your laboratory imaging requirements together in one powerful software package.

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Poster Abstracts

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P1

Loss of Dicer Expression is Associated with Breast Cancer Progression and Disease Free Interval

Ⓟ SM Khoshnaw[1]; TMA Abdel-Fatah[2]; CC Nolan[3]; Z Hodi[4]; RD Macmillan[5]; IO Ellis[6]; AR Green[6]

[1]University of Nottingham, Nottingham, United Kingdom; [2]Department of Clinical Oncology, Nottingham University, City Hospital NHS Trust, UK, Nottingham, United Kingdom; [3]University of Nottingham, School of Molecular Medical Sciences, Nottingham, United Kingdom; [4]The Breast Unit, Department of Histopathology, Nottingham City Hospital, Nottingham, United Kingdom; [5]The Breast Unit, Department of Histopathology, Nottingham City Hospital, University of Nottingham, Nottingham, United Kingdom; [6]Department of Histopathology, School of Molecular Medical Sciences, University of Nottingham and Nottingham University Hospitals, Nottingham, United Kingdom

MicroRNA (miRNA) deregulation is a well-known feature of human breast cancer. However, the role of molecules involved in miRNA biogenesis in cancer progression and their correlation with outcome and clinicopathological features in breast cancer is not well understood. Dicer is a protein that plays a pivotal role in the final steps of miRNA processing pathway to produce mature miRNAs from their precursor molecules. The purpose of the current study was to investigate the assumption that Dicer is significant in the development and progression of breast cancer and has clinical relevance as a potential predictive and prognostic target. We have examined Dicer protein in a well-characterised series of unselected invasive breast cancer patients (1174 cases) with long-term follow-up using tissue microarray and immunohistochemistry to investigate its protein expression and correlate it with clinicopathological features and patient outcome. Loss of Dicer expression was associated with loss of BRCA1 cytoplasmic and nuclear expression and with shorter disease free interval in 5 years. Multivariate analysis with adjustment for the other prognostic factors showed that Dicer expression was an independent predictor of disease-free interval in 5 years post diagnosis. Furthermore, a smaller cohort of selected breast cancer cases (24 cases) with distinct stages of tumour progression (Normal, DCIS, invasive and metastases) was investigated for differential Dicer expression in the different tissue components using immunohistochemistry, and a gradual loss of Dicer staining intensity was observed with advancing stage of breast cancer progression, suggesting that Dicer might be central to the process of breast cancer progression.

P2

FOXO3a/FOXO1 and pPRAS40 are Prevalent in Human Breast Cancer and FOXO3a/FOXO1 and pPRAS40 Immunohistochemistry (IHC) Assays Could be Used as Pharmacodynamic Markers of AKT Pathway Activity

Ⓟ A Palmer[1]; S Fenton[1]; C Womack[1]; N Gray[1]; B Reiß[2]; G Bigley[1]; P Elivin[1]; B Davies[1]

[1]AstraZeneca, Macclesfield, United Kingdom; [2]Definiens, Munich, Germany

AKT activity is commonly increased in human tumours and is associated with progression and the development of drug resistance. FOXO3a/FOXO1 and PRAS40 are downstream substrates of AKT and are potential pharmaodynamic markers of AKT pathway activity. AKT mediated phosphorylation of FOXO3a/FOXO1 results in their inactivation and translocation from nucleus to cytoplasm.

We describe the development and validation of IHC assays to quantify FOXO3a/FOXO1 and pPRAS40 staining in tumour xenograft and primary explant models of disease. We also describe the prevalence of FOXO3a/FOXO1 and pPRAS40 in human tissue microarrays and 50 breast cancer whole sections.

Specificity of rabbit monoclonal (mAb) anti-pPRAS40 (Thr246) antibody (CST #2997) and rabbit mAb anti-FOXO3a antibody (CST #2497) were assessed by western blot and IHC. IHC assays were also validated for reproducibility. Comparative evaluation of pathologists by eye score and automated image analysis output generated from IHC was undertaken.

Treatment of a tumour primary explant model with an AKT inhibitor at 100mg/kg p.o. resulted in a translocation of FOXO3a/FOXO1 from the cytoplasm to the nucleus and a 75% reduction ($p<0.005$) in cytoplasmic pPRAS40 staining at 2 hours compared to controls. In human whole breast cancer sections FOXO3a/FOXO1 was present with a combined cytoplasmic/nuclear H score of ≥ 10 in 40/46 (87%) of samples. pPRAS40 was also prevalent in human breast cancer with a cytoplasmic H score of ≥ 10 in 36/46 (78%) samples. There was generally good concordance between pathologist by eye scoring and automated image analysis.

In this study we have demonstrated that both FOXO3a/FOXO1 and pPRAS40 modulation is induced by AKT inhibition and confirm that both these proteins are prevalent in human breast cancer. The data suggests that FOXO3a/FOXO1 and pPRAS40 IHC assays may be useful pharmacodynamic markers of AKT pathway activity in a clinical setting.

P3

This abstract has been withdrawn.

P4

Evaluation of a Tissue Slice System as Pre-clinical Organotypic Breast Cancer Model

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University of Leeds, Leeds, United Kingdom

Breast cancer is a complex and heterogeneous disease with several different molecular alterations involved in its pathogenesis and progression. A model that takes into account the complexity of the disease is required to improve the efficacy of target-based therapy in breast cancer. None of the above can be easily or accurately replicated in routine cell line or animal models. Organotypic tissue slice culture represents an attractive alternative as it preserves tissue in its native state for subsequent analysis. The aim of this study was to evaluate its efficacy in breast cancer.

Following ethical approval, surplus tissue from 7 breast tumours was agarose embedded and 250μ thick sections prepared using a vibrating blade microtome. One tissue section per well was placed (triplicates) in 6-well plates and cultured for 7 days in the presence of either: doxorubin (0.1-100 μM), 4-hydroxytamoxifen (0.1-10nM) or exemestane (0.1-100μM). Fresh drug/vehicle controls were added on days 3 and 5. Tissue slices were then formalin fixed and paraffin embedded before sectioning for morphological evaluation (H&E) and immunohistochemistry (MIB-1, M30).

H&E inspection showed good preservation of tissue morphology with 3D architecture maintained, demonstrating that breast cancer can be recapitulated in vitro in its native state. Following drug treatment changes in proliferation and apoptosis could be evaluated using immunohistochemistry. However the model is delicate and down-stream analysis can be challenging often requiring modification for each sample.

In conclusion we have demonstrated breast cancer tissue slices can be cultured in vitro as an organotypic model which could provide a platform for testing of novel therapeutics.

P5

Audit of 'B3' Breast Core Biopsies Diagnosed as 'Lesions of Uncertain Malignant Potential' and Comparison with Subsequent Diagnoses on Further Biopsy or Excision

Ⓟ KP Lawes[1]; PA Jackson[2]

[1]Leicester Royal Infirmary, Leicester, United Kingdom; [2]Royal Surrey County Hospital, Guildford, United Kingdom

Breast screening core biopsies are divided into 5 categories where B1 = normal tissue or uninterpretable, B2 = benign, B3 = lesion of uncertain malignant potential, B4 = suspicious and B5 malignant. In the Department, previous audits have compared cases where a B3 category has been assigned on biopsy, with the subsequent diagnosis after further biopsy or surgical excision of the lesion. The percentage of lesions which are assigned a B3 category on core biopsy, and are subsequently found to contain carcinoma is 20-25%.

Seventy-five B3 breast core biopsy cases were compared with the subsequent diagnosis after repeat biopsy or surgical excision of the lesion. The aim was to determine the number and percentage of core biopsies which correlated with, or did not match the final diagnosis, and to assess whether the percentage of lesions later found to contain carcinoma was within the range of 20-25%.

Of the 75 cases included in the audit, the core biopsy and subsequent surgical specimen diagnoses correlated in 47 cases (63%) and did not match in 28 cases (37%). In the 16 cases where the final diagnosis was of malignancy (21%), there were 8 cases of invasive carcinoma and 8 of ductal carcinoma *in-situ*. It is possible that the higher grade lesion may not have been sampled by the initial biopsy.

The audit findings demonstrate that 21% of B3 breast core biopsies are taken from a lesion which is subsequently shown to contain carcinoma (*in-situ* or invasive) on further sampling. This is within the previous audit range of 20-25%. The data supports the continued surgical excision or close monitoring of breast lesions classified as 'B3' on core biopsy.

P6

Does the One-Step Nucleic Acid Amplification Intraoperative Technique Predict the Axillary Node Status in Patients with Breast Cancer?

Ⓟ G Pressley; S Ahmad; G Suckling; P Helliwell; J Mathew

Royal Cornwall Hospitals NHS Trust, Truro, Cornwall, United Kingdom

Aim: The One-Step Nucleic Acid Amplification (OSNA) technique is an intra-operative, PCR-based, quantitative technique, for the detection of cytokeratin 19 mRNA within sentinel lymph nodes (SLN) as a predictor of the global axillary lymph node status of a patient. We reviewed the results of the first 100 cases of SLN OSNA done in our institution.

Methods: Patient selection for OSNA was based on the Memorial Sloan-Kettering Cancer Centre SLN metastasis risk stratification algorithm; patients with >25% risk of SLN metastasis were selected for the OSNA procedure. 100 patients (99F:1M; age 37-89 years) who had an intra-operative sentinel node-directed OSNA tests were identified from our database.

Results: OSNA was negative in 64% patients (n=64) and positive in 36% (n=36); of the latter, 56% (n=20) had micrometastasis (MiM) and 44% (n=16) had macrometastasis (MaM). Completion axillary lymph node dissection (CALND) was done in all OSNA-positive (but not OSNA-negative) cases; 25% (n=5) and 31% (n=5) of patients with MiM and MaM respectively, had further CALND positivity, reflecting the positive predictive value of either SLN OSNA-positive state. The sensitivity of this procedure to predict metastasis in CALND is 100% and the specificity was 71%.

Conclusions

*Intra-operative CALND was avoided in two-thirds patients with OSNA-negative SLNs.

*In the remaining third of OSNA-positive SLN patients, intra-operative CALND, avoided the risks and costs of a second operative procedure.

*However, only 27% of the SLN-positive group (10% of all patients) had further (MiM or MaM) CALND positivity.

*The role (and popularity) of CALND as an automatic supplementary procedure to SLN-positivity is being challenged in favour of chemoradiotherapy which is said to give equal or better prognostic results, with less morbidity

*Given current evidence, we have stopped CALND for OSNA-detected MiM and will review our practice for MaM

P7

Invasive Epitheliosis: A Benign Entity Easily Mistaken for Invasive Carcinoma

Ⓟ ME Beauchamp; T Grigor; H Jones

Royal Cornwall Hospital, Truro, United Kingdom

Introduction:

Invasive epitheliosis is an uncommon benign lesion. The histomorphological appearances develop from fibrocystic changes containing florid intraductal hyperplasia that undergo sclerosis, distortion, and entrapment of distorted ducts. The lesions may be mistaken for invasive carcinoma by mammography, gross pathologic examination and light microscopy.

Case report:

A 63 year old female attended the NHS breast screening programme after the identification of microcalcification and a spiculated mass in her breast. The stereotactic core biopsies obtained showed papilloma apparently lacking a myoepithelial cell layer and was consistent with in situ disease. Complete excision was recommended. Light microscopic examination showed scattered dilated ducts some containing focal intraductal hyperplasia without atypia. The residual lesion consisted of a large dilated ducts containing an intraductal epithelial lesion, partly entrapped/infiltrative of surrounding connective tissue and exhibiting papillary features; hyalinised, avascular cores were covered by a fairly thick layer of bland appearing epithelial cells. Immunoperoxidase stains show absence of the myoepithelial cell layer. Although the appearances suggested a benign lesion, lack of myoepithelial layer made the diagnosis difficult. The case was referred for a specialist opinion and the lesion was categorised as infiltrating epitheliosis. Patchy staining for oestrogen receptor supported a benign diagnosis.

Conclusion:

With the advent of breast screening, areas of excessive fibrosis or sclerosis that occur in the breast following stromal involution have become significant because on mammography their appearance mimics cancer.

P8

Factors Affecting Turnaround Time for HER2 Reporting in South East Wales Cancer Network (SEWCN)

Ⓟ J Goom[1]; B Jasani[2]; P Barrett-Lee[3]

[1]Roche UK, Welwyn City, United Kingdom; [2]School of Medicine, Cardiff University, Cardiff, United Kingdom; [3]Velindre Hospital, Cardiff, United Kingdom

HER2 testing in SEWCN is performed at UHW with sections received from 10 hospitals each with varied practice for sample preparation and delivery. Use of HER2 status for planning chemotherapy has raised concern about timeliness of results for MDT decisions. This study aimed to identify factors affecting turnaround times (TATs), which could be improved to meet the new demand.

A project manager, seconded by Roche UK under agreement with the SEWCN, conducted two independent internal audits on HER2 testing timelines a period of 6 months. The first studied HER2 samples sent to UHW from SEWCN for the time from biopsy to HER2 reporting. The second examined in detail 3 hospitals sending requests to UHW, to gather time data from the laboratory request for HER2 testing to reporting. Both data sets validated each other's timelines. In addition interviews were conducted with relevant surgeons, oncologists, histopathologists, biomedical scientists, and medical secretaries at each requesting site and the testing centre.

Of 814 HER2 requests examined, all had IHC and 26% FISH performed. IHC took 15-17 days from the biopsy to MDT reporting, while FISH 19-31 days; including 7 days due to delay caused by batching of 50% samples at one hospital. Results indicate TAT for HER2 reporting could be significantly improved by removal of sample batching. Automation led streamlining of HER2 assay could further reduce TATs down to <1 week. This is the first study to address TAT from the biopsy to HER2 reporting across a population of 1.5 million.

P9

An Audit of Reporting Duodenal Biopsies by a BMS Cut-up Practitioner

Ⓟ M Haynes; R Lindley

Maidstone & Tunbridge Wells NHS Trust, Maidstone, United Kingdom

Introduction: There have been ongoing discussions about expanding the roles of senior BMS to include reporting histopathology specimens in targeted areas. At Maidstone and Tunbridge Wells, we are one of the pioneers in this arena. One of our BMS cut up practitioners has been involved in reporting histological specimens. Histology reports were drafted by the BMS prior to double heading sessions with a consultant histopatholgist. The reports were checked and authorised by the reporting consultant.
Aim: In this study, we assess the level of competency achieved by the BMS in reporting duodenal biopsies. Specific diagnostic criteria were drawn to include a range of histological findings, including normal spectrum, duodenitis, and Coeliac disease.
Method: A retrospective review of the case logbook (Aug. 2010 – Nov. 2011) of duodenal biopsies reported by the BMS (670 cases).
Results: Ten discrepancies were found, including a case of a possible low grade dysplasia and two cases of pyloric metaplasia overlooked by the BMS. It is worth noting that none of the missed cases would have had any impact on patient management.
Nineteen of the cases were deemed 'difficult' by the BMS. Two significant incidental findings of dysplasia and malignancy were identified by the BMS. The sensitivity and specificity for all reports was 98%, positive and negative predictive values of 99% for all reports, 0% false positive and false negative for Coeliac disease.
Conclusion: In this highly targeted area the BMS has achieved high quality reporting comparable to that of a stage B/C histopathology trainee, equivalent to level 3 competency in the RCPath Competency based framework for graded responsibility for specialist registrars and specialty trainees in histopathology and cytopathology (Dec 2009) and has demonstrated the ability to recognise the unusual and unexpected while also demonstrating recognition of personal diagnostic limitations.

P10

A Review of Findings from the NHS Bowel Cancer Screening Programme at a Large Teaching Hospital

Ⓟ TG Palmer[1]; S Brown[2]; P Kitsanta[2]

[1]St James's University Hospital, Leeds, United Kingdom; [2]Northern General Hospital, Sheffield, United Kingdom

We aimed to document the findings and reporting of biopsy material from the NHS bowel cancer screening programme (BCSP) in a large UK teaching hospital centre. Patient details for all BCSP specimens were prospectively collected on a local database. This was used to identify the histopathology reports of all BCSP biopsies taken between 09/05/2008 and 12/11/2010.
A total of 1198 biopsy specimens were identified (mean 1.48 per colonoscopy) with the most common site of origin being the sigmoid colon (35%). Of these, 946 (79%) were shown to contain polyps, which were predominantly low-grade tubular adenomas. Size was documented in 902 polyps (95%), 676 (75%) of which were below 10 mm in maximum dimension. In the 560 endoscopies producing histology, 60 individuals had high risk adenomas (11%), 173 had intermediate risk polyps (31%) and 124 had low risk adenomas (22%). Invasive adenocarcinomas were detected in a further 84 patients (12.2%) from 98 specimens. 35 of these arose in adenomatous polyps. 24 pT1 polyp cancers were detected, all with a Haggitt or Kikuchi stage given (24.4% of all cancers detected compared to 10% nationally). 11 polyp cancers were excised peicemeal and staging was not possible (11% of cancers detected). The rate of adenocarcinoma identified locally was higher than national data (12.2% vs. 10%) as was the rate of adenomatous polyps of all three risk levels. The number of pT1 polyp cancers was high, which may reflect good local endoscopic practice. There were still some cancers that could not be staged with either Kikuchi or Haggitt systems highlighting the difficulties involved in staging biopsy material. Regular audit of BCSP material allows the comparison of local to national data, monitors the quality of histopathological reporting and potentially provides a useful source of feedback to endoscopists regarding polyp size estimation.

P11

The Presence of Macrophages is Related to Tumour Cell Density in Gastric Cancer – A Pilot Study Comparing Automated Image Analysis with Subjective Scoring in Full Sections and Virtual Tissue Cores

Ⓟ S Lovett; A Wright; D Treanor; LC Ward; HI Grabsch

Leeds Institute of Molecular Medicine, University of Leeds, Leeds, United Kingdom

Background: The tumour microenvironment and tumour infiltrating immune cells in particular play an important role in cancer development. Data for gastric cancer (GC) on this subject are still limited. This pilot study aimed to quantify the different types of infiltrating immune cells and relate the findings to clinicopathological parameters and tumour cell density (TCD). At the same time, we performed a feasibility study into subset sampling from full sections and compare results from subjective scoring with fully automated image analysis.
Methods: Full sections from 26 GC were subjected to immunohistochemistry for CD45, CD3, CD20, CD68 and MPO. Whole tumour areas, virtual samples from the tumour centre and edge were analysed by automated image analysis and subjective semiquantitative scoring. The %immunopositive pixels/tumour area was compared to clinicopathological variables and previously measured TCD. Concordance between the measurements of the whole tumour area, centre and edge as well as between image analysis and subjective scoring was assessed.
Results: GC with high TCD were associated with a low number of CD68+ pixels/area but a high number of MPO+ pixels/area (both $p=0.02$). Concordance of results between whole tumour area, centre and edge was good for CD68 ($\kappa=0.6$) and MPO ($\kappa=0.5$) but lower for CD3, CD45 and CD20 (κ: 0.4, 0.3, and 0.2, respectively). Concordance between subjective scores and image analysis was variable, ranging from 56% to 81% ($\kappa=0.2$-0.7).
Discussion: This pilot study identified for the first time a significant relationship between TCD and tumour infiltrating macrophages and MPO+ cells, a finding which needs to be validated in a larger set of samples. Our results suggest that 'random' sampling of cores from full sections for tissue microarray construction may lead to unreliable results when studying lymphocyte subtypes which are not homogenously distributed throughout the tumour.

P12

Lysyl Oxidase (LOX) Expression in Colorectal Cancer – Exploration of Potential Clinical Value

Ⓟ L Sansom; GGA Hutchins; M Shires; G Hemmings; E Tinkler-Hundal; H Grabsch; P Quirke

University of Leeds, Leeds, United Kingdom

Introduction : Lysyl oxidase (LOX) is a copper-containing amine oxidase that is traditionally known for the extracellular catalysis of lysine-derived cross-links in fibrillar collagens and elastin. Recent evidence suggests that secreted LOX may be essential for hypoxia-induced tumour metastasis. To date, LOX expression has been evaluated in many tumour types with high LOX-expressing tumours having poor overall survival. Despite current understanding of LOX, little is known about LOX expression in colorectal cancer (CRC).
Methods : TMA sections containing tissue from 306 CRC patients with were stained by immunohistochemistry using antibodies against LOX. Slides were manually scored using antibody specific scoring systems. Expression data was compared to clinical outcome data using cancer-specific (CSS) and overall survival (OS) as primary and secondary endpoints respectively.
Results : Of 306 cases, 269 were informative for LOX staining. Of these 231 (86%) displayed weak cytoplasmic LOX expression with 38 (14%) showing strong expression. LOX was not associated with any clinicopathological features. No significant association was seen between LOX expression and CSS ($p<0.159$), or OS ($p<0.215$). A trend between increased LOX expression and improved CSS was observed.
Conclusion : LOX expression was not significantly associated with clinical outcome in CRC. In contrast to previous studies, a trend between increased LOX expression and improved clinical outcomes was observed.

P13

Does a Prompt from the Histology Report to Perform Serology to Exclude Coeliac Disease Mean this is Done?

Ⓟ K Lloyd; E Byrne; S Nayagam; H Williams; M Walker

Imperial College NHS Healthcare Trust, London, United Kingdom

Introduction: Lymphocytic duodenosis (LD) is a histological diagnosis comprising normal duodenal architecture, but an intraepithelial lymphocyte (IELs) count >25/ 100 enterocytes. A diagnosis of coeliac disease (CD) can be made in up to 16% of cases where LD is seen (1). CD serology (antibodies to tissue transglutaminase) should be performed in all cases of LD. The aim of this study was to identify the rate of CD serological investigations following a diagnosis of LD.
Methods: All duodenal biopsies taken at St Marys Hospital, London between March and August 2011 were evaluated. The histological reports for all duodenal biopsies coded as abnormal were reviewed. Serology results were checked in all cases where LD was diagnosed.
Results: 280 duodenal biopsies were performed of which 24 met the criteria for a diagnosis of LD (8.6%). The indication for biopsy was anaemia in 12/24, dyspepsia in 4/24, abdominal pain in 3/24, dysphagia in 1/24 and an indication was not indicated in 1/24. Serological testing for CD was carried out prior to or following the diagnosis of LD in 12/24 (50%). In all cases where serology was performed the result was negative.
Conclusion: Opportunities to diagnose CD are being missed in 50% of cases. These figures are similar to an audit carried out in the same department in 2009. Treatment of CD has been shown to reduce morbidity and mortality and reduce the cost of medical care (2-3). Therefore it is important that opportunities to diagnose CD are taken and CD serology is performed in all cases of LD. References

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P14

Analysis of Synchronous Colorectal Cancers

Ⓟ D Cilia Vincenti; GI Murray

Pathology Department, Aberdeen Royal Infirmary, Aberdeen, United Kingdom

Synchronous colorectal cancers are relatively uncommon, and in this study we have identified all resected synchronous colorectal adenocarcinomas in a single region over a seven year period from 2005 to 2011. All the pathology in this study has been performed in a single centre and reported according to the Royal College of Pathologists guidelines. The relevant pathological parameters have been extracted from pathology reports, entered in a database and data analysed using SPSS. Out of 1881 patients, 44 patients (2.34%) were identified that harboured synchronous colorectal cancers in their resection specimens. Thirty-eight patients had one synchronous tumour, five patients had two synchronous tumours, and one patient had three synchronous cancers. The mean age of patients was 75.59 as compared to 68.79 overall. Male patients accounted for 59.1% compared to 53.3% overall. 70.6% of tumours occurred in the same region of the colon, whilst 29.4% occurred in different regions. 51% occurred in the proximal colon as compared to 40.9% overall. 39.2% occurred in the distal colon (31.5% overall). 9.8% occurred in the rectum (27.6% overall). 27.5% were T1 tumours (6.7% overall); 33.3% were T2 tumours (13.1% overall); 35.3% were T3 tumours (60% overall); and 3.9% were T4 tumours (20.2% overall). In our population, synchronous cancers are therefore more likely to affect elderly male patients, more likely to be right-sided and more likely to be of low T-stage. Synchronous tumours are more likely to be in the same segment of the bowel as the index cancer.

P15

Primary Malignant Peritoneal Mesothelioma: Case Report and Review of the Literature

Ⓟ RT Colling[1]; N Banu[2]; N Bhatt[1]; P Cornes[2]; J Pawade[1]

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We present the case of a 58 year old woman with abdominal distension and a history of total body irradiation for acute lymphoblastic leukaemia. Examination revealed significant ascites and cytological analysis of which showed atypical clusters of mesothelial cells. Computed tomographical imaging revealed diffuse thickening of the peritoneum which was then biopsied. Histology from this showed features of primary malignant peritoneal mesothelioma. There was no history of asbestos exposure in this patient however asbestos analysis on the tumour is in progress. Lack of history of asbestos exposure and positive clinical history of radiation exposure suggests that this is a radiation-induced primary malignant peritoneal mesothelioma. Review of the literature, along with this case, supports radiation exposure as an aetiological factor in peritoneal mesothelioma as is already recognised in mesothelioma of the pleura and pericardium.

P16

Signet Ring Cell Change in Ischaemic Enteritis: A Potential Diagnostic Pitfall

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Purpose:
Benign signet ring cell change is an important pseudoneoplastic phenomenon that can mimic signet ring cell carcinoma. Within the gastrointestinal tract, such changes have previously been described in association with ischaemia, pseudomembraneous colitis and polyps. The purpose of this study is to examine the frequency of benign signet ring cell change in small intestinal resections performed for ischaemia at our institution and to emphasise their potential as a diagnostic pitfall.
Methods:
All small intestinal resections performed for ischaemia in our institution over a five year period (2007-2011) were retrieved following a free text and SNOMED based search of the laboratory computer system and the slides were reviewed.
Results:
Forty nine (49) small intestinal resection specimens were identified within the study period. Two (2) of these contained benign signet ring cell change (4.1%). In both cases, the signet ring cells showed mild degenerative atypia. The adjacent mucosa showed ulceration and ischaemic changes. Immunohistochemistry for cytokeratins was positive in the signet ring cells, confirming an epithelial phenotype.
Conclusions:
Benign signet ring cell change is an uncommon finding that can be seen in association with ischaemia in the gastrointestinal tract. The signet ring type cells are epithelial in nature and likely represent a degenerative phenomenon related to ischaemia. The superficial location of the cells, the associated histological findings and the clinical context help to exclude signet ring cell carcinoma in a resection specimen, however this may be more difficult in a biopsy. Knowledge of this phenomenon is important to avoid overdiagnosis of malignancy

P17

Expression of Wilms Tumour Protein (WT1) in Epithelial Salivary Gland Tumours: an Immunohistochemical Investigation

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Despite extensive investigation, the subcellular localisation of WT1 is controversial and little attention has been paid to salivary glands. Being morphologically diverse, epithelial salivary tumours would enable exploring WT1 in various histological subtypes/cell phenotypes. This prompted the present investigation.
Paraffin-embedded, surgical specimens from 79 salivary tumours/adjacent glands were investigated by immunohistochemistry using a monoclonal, anti-WT1 antibody (6F-H2, Dako).
Immunostaining was seen in: normal mucous cells (various; gender independent); 14/14 pleomorphic adenomas (PSAs); 6/6 myoepitheliomas; 4/4 basal cell adenomas; 4/4 canalicular adenomas; 0/7 Warthin tumours; 0/1 oncocytoma; 1/6 acinic cell carcinomas (Cas); 6/11 mucoepidemoid Cas; 1/11 adenoid cystic Cas; 11/12 polymorphous low-grade adenocarcinomas (PLGAs); 1/1 Ca ex PSA; 0/1 salivary duct Ca; and 0/1 clear-cell adenocarcinoma. Stained-cell subpopulations up to 90% were not uncommon in benign non-oncocytic tumours. They were considerably lower in malignant tumours; the exception was PLGA where up to 80% of cells were often stained. Staining was weak to intense, usually cytoplasmic (1 adenoid cystic Ca showed nuclear staining) and variously associated with dyscohesive non-luminal cells in benign biphasic tumours, non-descript cells adjacent to stroma in tumours of simple glandular architecture and mucous tumour cells. Luminal secretions were also stained.
While WT1 is often highly expressed in benign non-oncocytic salivary tumours, patterns supporting a continuum rather than distinct entities, most malignant tumours show decreased expression. Expression is usually cytoplasmic and associated with basal/non-luminal differentiation and, possibly, epithelial-mesenchymal transition. PLGA immunoreactivities may be useful in diagnosis. The significance of immunoreactivities in mucous secretions is speculative.

P18

Expression of FANCD2 in Epithelial Tumours of Salivary Glands in Man: an Immunohistochemical Investigation

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The tumourigenic role of the Fanconi anaemia group D2 protein (FANCD2) has drawn much attention. The intracellular localisation of FANCD2 is, however, controversial and has not been investigated in the full range of human tumours, including those of salivary glands. Being morphologically diverse, epithelial salivary tumours provide opportunity for exploring FANCD2 expression in various histological subtypes/cell phenotypes. This prompted the present investigation.
Archival, paraffin-embedded, surgical specimens from 40 epithelial salivary tumours and adjacent normal/inflamed glands were investigated by immunohistochemistry using a polyclonal, anti-FANCD2 antibody (sc-23584P, Santa Cruz).
Immunostaining was seen in ducts/duct-like structures of normal/inflamed glands respectively. In tumours staining varied from weak to strong, was usually cytoplasmic and occasionally polarised to adluminal rims. In total, 3/9 pleomorphic adenomas (PSAs)/myoepitheliomas; 2/5 basal cell adenomas; 0/2 canalicular adenomas; 3/4 Warthin tumours; 3/5 acinic cell carcinomas (Cas); 3/5 mucoepidemoid Cas; 2/5 adenoid cystic Cas; 1/3 polymorphous low-grade adenocarcinomas; 1/1 Ca ex PSA; and 1/1 clear-cell adenocarcinoma showed FANCD2 immunoreactivity. Stained cell subpopulations ranged between 1-20% in benign tumours and reached 50% in some malignant tumours. Staining was associated with adluminal cells in tumours of biphasic or simple glandular architecture. Serous, mucous or abluminal tumour cells were unstained. 1 acinic cell Ca showed nuclear staining.
FANCD2 was variously expressed in salivary neoplasia, and was mostly cytoplasmic. Increased numbers of immunoreactive cells were seen in malignant tumours; whether this reflects a tumourigenic role of FANCD2 or accumulation of inactivated protein is unknown. Expression was associated with simple luminal/polarised cell phenotypes.

P19

Salivary 'Adenosis': Histology, Histochemistry and Classification

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This investigation aims to increase understanding of phenotypes in salivary lesions variously reported as adenomatous ductal proliferation (ADP), intercalary duct lesions (IDLs) or cystic sclerosing adenosis (ScA), explore possible relationships and suggest a classification.
Histologically detected ADP, IDLs and ScA in surgical specimens were investigated by means of conventional histochemical and immunohistochemical techniques, valuable in characterising secretory events and cytoskeleton.
1) Two circumscribed, non-encapsulated, parotid lesions (1.4-1.7 mm diameter) associated with Warthin tumour (WT) and sialectasis, showed buds/tubules of simple cells with non-distinctive cytoskeleton and no myofilaments, in variable fibro-elastotic stroma. Non-rigid lumens were preferentially associated with tubules wherein occasional cells showed apical secretory granules containing neutral mucosubstances. 2) Two infiltrative, parotid lesions (each 3.0 mm) associated with WT and pleomorphic adenoma + basal cell adenoma, showed packed, usually bilayered, tubules with rigid lumens and variously expressed S-100 protein. Adluminal, columnar cells showed low molecular-weight cytokeratins (CK7, 19) and, often, apical, secretory granules containing neutral mucosubstances. Abluminal cells showed intermediate molecular-weight CKs (5/6, 14) and myofilaments. 3) One infiltrative, palatal lesion (6.3 mm), showed seromucous acini / bilayered tubules, variously surrounded by a layer of myoepithelium, and cords / single files in fibro-elastotic stroma. Parenchymal structures were CK7+, CK19-/+ and S-100 protein +/-, and showed lysosomal activity. Acini showed secretory granules containing mixed, neutral/acidic mucosubstances.
ADP, IDLs and ScA are possibly parts of a continuum, characterised as salivary adenosis, dysplastic in nature. Lesions can be classified according to extent / intricacy of secretion and differentiation of cytoskeleton.

P20

Desmoplasia in Nodal Metastasis of Oral Squamous Cell Carcinoma: Spatiotemporal Considerations

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Desmoplasia is the development of a tumour-associated extracellular matrix containing glycosaminoglycans and myofibroblasts, resulting in a distinct, newly-formed microenvironment. We have shown that desmoplasia in cervical nodal metastases of oral squamous cell carcinoma (OSCC) contains chondroitin-sulphate glycosaminoglycans and myofibroblasts. We now report on relationships between nodal desmoplasia and the natural history of metastasis.
205 paraffin-embedded pN(+) cervical lymph nodes from 89 OSCC cases were investigated by routine histology for desmoplasia in relation to extracapsular-spread (ECS), size and degeneration of the metastasis. Desmoplasia was recorded as intranodal (associated with tumour away from the nodal capsule) or peripheral (associated with tumour facing nodal capsule/ECS).
Desmoplasia was associated with most conventional metastases (142/181, 78%), but with only 2/24 (8%) micrometastases and was preferentially peripheral (115/142, 80%). Intranodal desmoplasia was often absent (60/144, 42%); when present, it was selectively associated with large metastases (>30% of the nodal section surface, 44/72 nodes) showing cystic change (30/44, 68%, $p = 0.0004$). In small metastases desmoplasia was selectively peripheral ($p<0.0001$). ECS presented in 54% of nodes and showed significant association with peripheral desmoplasia ($p<0.0001$).
Early in the natural history of cervical nodal metastasis of OSCC desmoplasia seems selectively associated with a subpopulation of metastatic cells spatially related to ECS; whether this subpopulation is aggressive inherently or after interaction with the desmoplastic/capsular microenvironment is speculative. Later, desmoplasia may be a response to increased microenvironmental pressure related to degeneration of the growing metastatic deposit.

P21

The Effect of XCL1 on Wound Healing in vitro.

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The chemokine receptor XCR1 is present on oral epithelial cells and interaction with its ligand XCL1 stimulates the migration and proliferation of oral epithelial cells in vitro. Expression of XCR1 is also increased in vivo at the edge of oral ulcers and together these results suggest it may play a role in wound healing. The aim of this study is to determine whether XCL1 stimulates oral and skin wound healing in vitro.
Methods: The oral H357 and skin HaCat cell lines were grown in vitro, scratched, and incubated in either keratinocyte growth medium (KGM) (positive control), serum free medium (SFM), or SFM +100ng/ml XCL1 (n=3). Immunocytochemistry was used to confirm XCR1 expression. The scratches were photographed over 24 hours, measured (blinded) using image analysis and results expressed as percentage of wound remaining.
Results: The oral H357 cells healed significantly faster ($p<0.01$) in KGM than SFM (70.2% +/- 1.93; 77.3% +/-1.14 respectively) at 24 hours but no difference was seen between cells in SFM and SFM+XCL1 (77.3% +/-1.14; 77.2% +/-1.07 respectively). Similarly HaCat cells healed faster ($p<0.01$) in KGM than SFM (64.2% +/-2.53; 91.4% +/-0.96 respectively) but cells in XCL1/ SFM did not heal faster than in SFM alone (94.4% +/-0.72; 91.4% +/-0.96 respectively). Immunocytochemistry confirmed XCR1 expression by both H357 and HaCat cells.
Conclusions: These results show under the conditions used XCL1 does not stimulate wound healing even though XCR1 is present. This suggests XCR1/XCL1 may not play a role in wound healing but further work is required to confirm these findings and to establish a role for the expression of XCR1 by epithelial cells. This project was supported by a Pathological Society Intercalated Degree Award.

P22

The Role of HOXD10 in the Development of Head and Neck Squamous Cell Carcinoma

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Background: Head and neck squamous cell cancer (HNSCC) ranks as the sixth most common cancer worldwide. Genetic alterations in these cancers are highly variable and are to some extent correlated with the site and stage of the cancer. Microarray analysis of HNSCC compared with normal mucosa revealed changes in the expression of a number of HOX genes, particularly HOXD10. HOXD10 expression found to be abnormal in some cancers and interacts with different molecules important for cell migration and adhesion.
Materials and methods: Expression of HOXD10 and putative targets was assessed by qPCR. Immunohistochemistry (IHC) and Western Blotting (WB) were used to detect HOXD10 protein in normal and cancer tissues and cell lines. In-silico analysis was used to identify putative targets of HOXD10. The HOXD10 coding region was cloned and transfected into low-HOXD10 expressing cancer cells while siRNA was used to knock it down in high-HOXD10 expressing cells. The resultant phenotype was assessed by MTS proliferation assay, modified transwell migration assay and fibronectin adhesion assay.
Results: Assessment of HOXD10 expression by qPCR showed low expression in normal cells; low to variable in dysplastic cells; high in most primary tumours; but very low in cells derived from lymph node metastases. HOXD10 protein was expressed abundantly in tumour tissues compared to normal tissues ($p<0.05$). Transfection of HOXD10 into naturally low-expressing cells showed an increase in their proliferation and adhesion ($p<0.05$). Silencing of HOXD10 in naturally high-expressing cells found to affect their proliferation and adhesion. The pattern expression of miR-7, miR125b and IGFBP3 showed a correlation with HOXD10 level in a panel of cell lines.
Conclusion: The results suggest that the high expression of HOXD10 might be important in allowing primary tumours to grow and metastasize. The significance of low expression in some cancers is unknown.

P23

Polymorphous Low Grade Adenocarcinoma Arising in a Pleomorphic Adenoma of the Parotid Gland: A Case Report

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We present the case of a 64 year old gentleman presenting with long standing enlargement of the right parotid gland together with recent facial nerve palsy. Clinical examination had identified a firm, partially fixed mass. A subsequent MRI scan showed no significant change when compared to the MRI performed several years previously, and the appearances were felt to be consistent with a pleomorphic adenoma. Core biopsy histology revealed features of a salivary gland neoplasm, but not typical for a pleomorphic adenoma. In view of this and the clinical findings, total parotidectomy was performed. Histological assessment of the mass showed a lesion displaying marked morphological diversity as well as cytological uniformity. Importantly, infiltrative margins were noted at the periphery. In addition, features of a benign mixed tumour were evident in the background.
Overall this lesion had features of a carcinoma arising in a pleomorphic adenoma with a predominant pattern of polymorphous low grade adenocarcinoma (PLGA). PLGA is a well described entity occurring mainly in the minor salivary glands. It may rarely be seen in the major salivary glands where it is often associated with pleomorphic adenoma, as illustrated in this case. It can present a diagnostic challenge due to its architectural variability and close resemblance to other salivary gland tumours. This case also highlights the importance of clinicopathological correlation as well as widespread sampling and careful examination of the entire specimen.

P24

Detection of Oncogenic Human Papillomavirus in Nasopharyngeal Carcinoma

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Nasopharyngeal carcinoma (NPC) accounts for 0.6% of all cancers worldwide with the highest prevalence in South East Asia, Southern China and Northern Africa. The disease is uncommon in Europe with an annual incidence of less than 1 per 100,000, which is due to differences in aetiological and genetic factors. Although the Epstein-Barr virus (EBV) is a well known causative agent in NPC, recent reports have implicated oncogenic Human Papillomavirus (HPV) in a subgroup of these tumours. The recent striking rise of oropharyngeal carcinoma has been attributed to HPV, but the incidence of the virus in NPC is unknown. The aim of this study was to determine the incidence of oncogenic HPV in NPC from tissue archives of two large head and neck cancer centres. Samples were available for fifty-nine patients with clinically validated NPC. The detection of high-risk HPV was carried out by screening all cases for p16 using immunohistochemistry, a sensitive surrogate marker for high-risk HPV oncoprotein expression. All cases with p16 over-expression were then examined by high-risk HPV DNA in-situ hybridisation. Ten cases (10/59, 17%) showed concurrent over-expression of p16 and evidence of high-risk HPV DNA by in-situ hybridisation. Of these 10 cases, 8 occurred in Caucasians and 2 in Blacks. Histologically, there were two conventional keratinising squamous cell carcinoma (SCCs) and eight non-keratinising carcinomas (7 differentiated and 1 undifferentiated). Only one HPV positive case showed co-infection with EBV. The results of this study show that HPV is associated with a subgroup of NPCs. This study also suggests that HPV-related NPC is more likely to occur in the Caucasian population and that the majority are negative for EBV. Larger cohort studies are necessary to validate this data and to determine whether HPV status influences clinical outcome.

P25

This abstract has been withdrawn.

P26

Targeted Inhibition of Tissue Factor Suppresses Hepatic Fibrosis in Carbon Tetrachloride Treated Mice

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Background: Recent evidence suggests a role for the coagulation cascade in promoting liver fibrosis. However, the cellular basis for this relationship is unclear. To explore this relationship we employed two unique transgenic mice strains expressing membrane–tethered tissue factor pathway inhibitor (TFPI) fusion protein driven by a CD31 or an alpha-Smooth Muscle Actin (αSMA) promoter.
Purpose of Study: To evaluate the impact of the targeted inhibition of tissue factor (TF) on effector cells of liver fibrosis in murine hepatic fibrosis induced by CCl4.
Methods: Liver fibrosis was induced in CD31-TFPI, αSMA-TFPI and control mice with 4 weeks carbon tetrachloride administered by intraperitoneal injection. Animals were culled and livers extracted for histological and biochemical analysis. Fibrosis was scored using a four point semi-quantitative system and quantified by digital image analysis to determine percentage area of fibrosis. Immunohistochemistry to determine αSMA expression, a marker of hepatic stellate cell activation was performed and the mean number of activated stellate cells was quantified per high power field.
Summary of Results: The percentage area of fibrosis was significantly less in αSMA-TFPI (2.29+/-1.78) in comparison to control mice (3.66+/-1.52, p=0.02). In CD31-TFPI transgenic mice there was a decrease in fibrosis (2.57+/-3.91) in comparison with control mice (p=0.10). Semiquantitative fibrosis scoring confirmed this.
Both transgenic strains demonstrated a significantly reduced mean number of αSMA stellate cells per high power field in comparison to control mice.
Conclusions: αSMA and CD31 targeted inhibition of TF significantly reduces liver fibrosis and stellate cell activation in a murine carbon tetrachloride model. The data supports the use of this novel murine model as a tool for investigating the cellular biology of the role of coagulation in liver fibrogenesis.

P27

Allografts, Immunosupression and Autophagy: A Conspiracy to Fibrose?

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Classic chronic ductopaenic rejection has all but disappeared from clinical practice in the last 20 years. There has also been a decline in the incidence of acute rejection, but with the emergence of other pathologies, notably chronic rejection in the absence of ductopaenia. While this can be viewed as a triumph there is little information on the optimum protocols for immunosupression in liver allograft recipients. Evidence points to patients with recurrent HCV, classed as tolerant and receiving rapamycin having worse outcomes, with increased graft fibrosis. HCV and rapamycin are both known to effect the cellular process of autophagy; which has been shown to prevent cell death due to acetaminophen toxicity and the prevention of senescence in biliary epithelial cells (BEC).
To establish whether early features of senescence were present in acute rejection, 35 biopsies of orthotopic liver allografts with biopsy proven acute cellular rejection were analysed by immunohistochemistry. There was significant correlation between the senescence marker p21WAF1/Cip and the BANFF grade (p=0.034). Application of oxidative stress to BEC in vitro showed a similar upregulation in p21 ($p<0.01$) with adoption of a senescent morphology. PCR and immunofluorescence analysis of BEC showed static TGF-β1 and increased TGF-β2 expression, confirmed by ELISA ($P=0.001$). Oxidative stress, FK506 and Rapamycin revealed increased levels of autophagy ($p<0.05$). Rapamycin and oxidative stress, but not FK506, increased β6 integrin levels on BEC. Pharmacological inhibition of TGF-βR or autophagy; or peptide blockade of β6 integrin activity prevented in vitro TGF-β activity.
These findings indicate that pleiotropic effects of immunosuppressive agents may play a key role in determining graft outcome. The ability of rapamycin to induce integrin dependent TGF-β signalling may explain its relationship to graft fibrosis.

P28

The Prognostic Value of Skp2 Expression in Egyptian Diffuse Large B-Cell Lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma worldwide. Both morphologically and prognostically, it represents a disease of a diverse spectrum.
S-phase kinase-associated protein 2 (Skp2) is a member of mammalian F-box proteins, which displays S-phase-promoting function through ubiquitin-mediated proteolysis of the cyclindependent kinase inhibitor, p27.
The aim of this study is to evaluate the prognostic value of Skp2 in DLBCL (70 cases) by immunohistochemical staining technique, and its correlation with the clinicopathological features and survival.
Five (25%) control cases (reactive follicular hyperplasia) showed high Skp2 expression compared with 52.9% of DLBCL using 10% as a cutoff point with a significant difference ($P=0.04$). Skp2 was seen staining the large cells in proliferating germinal centres of the control group. High Skp2 expression in DLBCL was associated with several progressive parameters, such as advanced stage ($P=0.036$), involvement of more than one extranodal site ($P=0.05$), and high proliferation ($P=0.0001$). It was also significantly associated with the presence ($P=0.007$) and extent ($P=0.002$) of necrosis and inversely correlated with p27 expression ($P=0.0001$).
From this study, Skp2 expression in DLBCL identified subset of cases characterized by aggressive features such as advanced stage, increased number of extranodal sites, high proliferation, and shorter survival time. The association of Skp2 with necrosis may be a reflection of its ability in promoting proliferative tumour capacity.

P29

Cerebral Involvement in Cutaneous T-Cell Lymphoma – a Case Report

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We present a case of a 49-year old female with a known diagnosis of mycosis fungoides(MF) for 10 years. Skin biopsy in 2003 showed a folliculotropic MF with a classical phenotype of CD4 positive T cells. In January 2012 the patient developed variable right sided hemiparesis, headaches and seizures. Cranial computer tomography scan revealed a left frontal lesion, with additional signal changes on magnetic resonance imaging in the right periinsular, peritrigonal and left temporal regions. Peripheral enhancement with contrast suggested an inflammatory change. A stereotactic biopsy of the left frontal lesion was performed, the histology of which revealed a population of discohesive, pleomorphic cells with accompanying macrophages and mature perivascular lymphocytes. The abnormal cells were CD2, CD3, CD8 positive but unlike the cutaneous disease were now CD4 negative. Additionally some cells also expressed CD30. PCR using Biomed 2 primers from the two sites confirmed an identical monoclonal rearrangement. The tumour was negative for EBV, CMV and HHV-8. This strongly suggested systemic, cerebral involvement of a cutaneous T-cell lymphoma.

DISCUSSION: Mycosis fungoides is the most common of cutaneous T-cell lymphomas with incidence of 4 cases per million population. Cental nervous system involvement is rare and usually follows infiltration of other organs. The above patient's cutaneous disease did not show evidence of progression or transformation at the time of neurological symptoms. A change in tumour cell phenotype from CD4 to CD8, as is the case, is rare but has been documented in literature.

P30

This abstract has been withdrawn.

P31

Audit of Lymphoma Reporting in a Regional Referral Centre

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We retrospectively audited 126 lymphoma cases reported in a regional haematopathology referral centre in one year. The aim was to standardise the criteria for referral request forms, assess turnaround time (TAT) for local and referral cases and reporting against the RCPath national minimum dataset. The request forms and the slides were reviewed by a consultant haematopathologist and a trainee and 6 cases were re-reviewed by a second haematopathologist. The average TAT for all cases was 6 days from receipt but 14 days for referral cases from biopsy date. 36% of cases had the surgeon's details and only 12% had the oncologist/radiologist's details. The other demographic data were recorded in 100% of request forms. 90% of request forms specified the site, but the size of the lesion was only stated in 38%. Almost all the referral cases did not provide this information. All the cases were reported according to the standard dataset following WHO terminology and appropriate immunohistochemical studies. 55 cases used cytogenetics as an aid to final diagnosis. One case was revised from nodular sclerosis to mixed cellularity Hodgkin Lymphoma.

The lack of clinical information resulted in an incomplete report dataset. A new referral form is planned to improve data capture.

P32

Interleukin-1 Regulates Chemokine Expression in the Human Intervertebral Disc

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Within the intervertebral disc (IVD) Interleukin-1 (IL-1) has been shown to regulate cellular processes associated with IVD degeneration; including up-regulation of extra-cellular matrix (ECM) degrading enzymes and down-regulation of synthesis of ECM constituents. Here we investigated the hypothesis that IL-1 also regulates the expression of chemokines in the central Nucleus Pulposus (NP) region of the IVD.

qRT-PCR was used to determine the effects of IL-1 treatment on mRNA expression of the chemokines, CXCL8, CCL2, CCL3, CCL7 and IL-16 in primary human NP cells. Constitutive expression of the chemokines, CXCL8, CCL2 and CCL7 was observed alongside a dose-dependent up-regulation in response to IL-1 treatment ($P=<0.05$). CCL3 was not constitutively expressed by primary human NP cells however expression was induced in response to IL-1 treatment ($P=<0.05$). Constitutive expression of IL-16 and a dose-dependent down-regulation in response to IL-1 treatment ($P=<0.05$) was observed in cells derived from non-degenerate IVDs however, IL-16 expression was not observed in any cells derived from degenerate IVDs.

This study demonstrates the regulatory potential of IL-1 on chemokine expression by native IVD cells. Our findings suggest that IL-1 might be a key in vivo regulator of chemokine expression and that the response of NP cells to IL-1 treatment is altered in cells derived from degenerate IVDs compared to those from non-degenerate IVDs.

P33

Chemokines and their Receptors in the Human Intervertebral Disc

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Chemokines are chemo-attractant cytokines that primarily function to induce the selective directional movement of cells, particularly towards sites of inflammation or infection. Intervertebral disc (IVD) degeneration is a non-inflammatory arthropathy and the IVD is an avascular structure. As such, native IVD cells are the only population found there, except following prolapse where infiltration of leukocytes can occur. Here we investigated whether native Nucleus Pulposus (NP) cells are both the producers and the targets of chemokines within the IVD to explore the hypothesis that chemokines are integral in the maintenance of IVD homeostasis.

Immunohistochemistry was used to confirm and localise production of the chemokines; CXCL8, CCL2, CCL3, CCL4, CCL7 and IL-16, and the chemokine receptors; CXCR1, CXCR2, CCR1 and CD4 to the native cells in the central NP region of 30 human IVDs, from non-degenerate and degenerate study groups. Chemokine expression levels and the grade of IVD degeneration were determined for each tissue sample.

Production of CXCL8, CCL2, CCL3, CCL4, CCL7 and IL-16 was localised to the NP cells. The expression of certain chemokines, including CXCL8 and CCL2 was seen to correlate positively with the grade of degeneration in tissue samples (P=<0.05). Production of CXCR1, CXCR2, CCR1 and CD4 was also localised to the NP cells.

This study demonstrates, for the first time, production of certain chemokines and chemokine receptors in the human IVD. Our findings suggest that an autocrine or paracrine signalling mechanism may exist to regulate cellular processes or to traffic NP cells around the IVD, potentially to sites requiring tissue remodelling or repair.

P34

CXCL1, CXCL2 and CXCL3 in the Pathogenesis of Intervertebral Disc Degeneration

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The roles of chemokines within the avascular structure of the intervertebral disc (IVD) remain to be elucidated. It is known that certain chemokines, and pro-inflammatory cytokines, are produced by native IVD cells. Here we investigated the hypothesis that the chemokines, CXCL1, CXCL2 and CXCL3 are produced within the human IVD and are integral in the pathogenesis of IVD degeneration.

Immunohistochemistry was used to confirm and localise CXCL1, CXCL2 and CXCL3 production to the native cells in the central Nucleus Pulposus (NP) region of the human IVD and to relate expression levels with the characteristic features of IVD degeneration. Further, qRT-PCR was used to determine the effects of pro-inflammatory cytokines, Interleukin-1, Tumour Necrosis Factor and Interleukin-6, on mRNA expression levels of CXCL1, CXCL2 and CXCL3 in cultured primary human NP cells.

Production of CXCL1, CXCL2 and CXCL3 was observed within the human IVD and was seen to correlate, in some instances, with the characteristic features of IVD degeneration. Pro-inflammatory cytokines were seen to regulate mRNA expression of CXCL1, CXCL2 and CXCL3.

This study demonstrates, for the first time, mRNA expression and protein production of the chemokines, CXCL1, CXCL2 and CXCL3 in the human IVD. Our findings suggest that their expression may be regulated by pro-inflammatory cytokines within the IVD. This suggests a role for these chemokines in IVD cell biology and potentially the pathogenesis of IVD degeneration.

P35

An Audit to Determine the Optimum Cutting Distance Between Levels on Skin Punch Biopsies

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Introduction:

In our department skin punch biopsies routinely have three sections cut, and further levels and special stains are requested by the reporting pathologist when appropriate. There was no standard cutting distance between levels until August 2011 when this was standardised to 50 microns. After this change it was felt that more of the punch biopsies were requiring extra levels in addition to those done routinely, leading to an increased workload. The purpose of this audit was to determine if more levels were being requested and, as the Royal College has not issued specific guidelines for the cutting distance between levels, to determine the optimum cutting distance in our department.

Method:

Two audit periods were identified for comparison; the three month period prior to the standardisation of the cutting distance and the three months following the change. A retrospective computer based search for all cases coded "SKX" identified 103 cases in the first group and 118 in the second group. The number of cases which had extra levels requested was compared between the two groups.

Results:

Extra levels were requested on 30/103 (29.1%) of the cases in the baseline group and 48/118 (40.7%) of the cases received after the standardisation of the cutting distance.

Conclusion:

The standardisation of the cutting distance led to an increase in the number of extra levels requested, thereby increasing the workload in the laboratory and adding a delay to the turnaround time. These results were discussed at a departmental meeting and it was decided that the cutting distance should be increased to 100 microns and it was hypothesised that most of the additional levels were being requested on inflammatory skins. A re-audit is ongoing, the results of which will be available for presentation.

P36

When Histology is not Needed. A Case of Cutaneous Dermatobium Hominis Larval Infection.

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In the era of concern regarding case turnarounds, the rapid and brief description of a sample, followed by tissue processing may on occasion be the wrong approach. This case report of a larva, from a cutaneous Myiasis case, did not proceed to histology. Rather, time was taken to fully consider the macroscopy with magnification (hand lens) followed by digital photography. These allowed comparison against text and web resources, allowing the diagnosis of Dermatobium hominis infection. In addition, the images were sent to experts in tropical medicine, who were able to support the interpretation made. The key points from this case were confirming the traditional value of appropriate macroscopy, with enhanced consideration of the features of the case, without recourse to histology. Had the routine processing occurred, then diagnosis might have been more difficult. The approach also has the advantage that the sample is still available as a teaching aid!

P37

The Risk of Malignant Skin Tumours in Renal Transplant Recipients in London and Brighton

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Purpose of the study: To assess the risk of malignant skin tumours in immunosuppressed renal transplant recipients in London and Brighton. Transplant recipients require immunosuppressive therapy to prevent graft rejection which conveys an increased risk of malignancy, especially skin tumours. For kidney recipients prevalence of skin tumours at 10 years followup ranges from 10% - 40% depending on UV light exposure. From 1995-2007 in the UK, mean annual incidence post-transplant has been found to be 11% with prevalences ranging from 14-22%. NICE introduced guidelines to improve outcomes for skin cancer in 2006 and there is a need for recent data and data for the South-East region.
Methods: The pathology records were reviewed for 514 immunosuppressed patients who received a kidney transplant at our hospital between 1995-2008. Details of all skin tumours were recorded and analysed.
Summary of results: 44 (8.6%) of 514 patients developed at least one skin tumour. 33 (10%) males and 11 (6%) females developed a tumour, OR 1.7 (p = 0.11). 195 tumours were found, of which 50.8% were squamous cell carcinomas, 44.6% were basal cell carcinomas and 4.6% were malignant melanomas, BCC:SCC ratio = 1:1.13. The mean time to first tumour was 5.3yrs. The mean no. of tumours per patient was 4.4 with a mean interval of 1yr between occurrences.
Conclusions: Immunosuppressed patients in our cohort had an 8.6% risk of developing a skin tumour. Kidney transplant recipients in South East England today appear to have a slightly lower risk of skin tumours than expected from the evidence prior to 2007. As expected from the literature, the M:F and BCC:SCC ratios were narrowed compared to the normal population. The findings of this study add to the body of evidence which is increasingly important as more transplant surgery is done in this country.
This study would not have been possible without a Pathsoc bursary which was gratefully received.

P38

Sclerosing Encapsulating Peritonitis and Hepatic Cirrhosis

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Sclerosing encapsulating peritonitis is an unusual inflammatory and fibrosing process, which is associated with long term peritoneal dialysis, intraperitoneal chemotherapy and rarely, with hepatic cirrhosis. The main characteristic is of complete or partial encapsulation of the small bowel in a thick fibrotic membrane, an appearance that has been likened to a cocoon. The reported symptoms include episodes of abdominal pain, and nausea and vomiting with associated weight loss. This may progress to acute small bowel obstruction.
A 58 year-old male, presented as an emergency, having previously collapsed at his home address. He was known to have a past history of high alcohol intake causing liver cirrhosis and ascites. He rapidly deteriorated and died.
At post mortem, the deceased was found to have severe micronodular cirrhosis of the liver, with a large volume of purulent ascitic fluid. The small bowel was encased in a thick white-grey fibrotic membrane. Histology demonstrated a variable degree of fibrosis, with moderately dense associated mixed chronic inflammation. In places, the fibrous tissue had a dense lamellar appearance. The macroscopic and microscopic findings indicated a diagnosis of sclerosing encapsulating peritonitis.

P39

Obesity and Sudden Death

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The prevalence of obesity is currently 1.6 billion worldwide and is reported to be rising. Relatively little information is available specifically relating to the causes of sudden death in obese individuals. Evidence suggests that obesity may play a protective role in diseases such as heart failure and pulmonary emboli; this phenomenon is known as the obesity paradox. We made a prospective and retrospective study of 2003 sudden deaths of different BMI groups within the Southampton and surrounding New Forest area of Hampshire, using both post mortem and HM Coroner's reports. In our study, we found that the most common cause of sudden death within the morbidly obese group (BMI ≥40) were of cardiovascular nature, notably cardiac failure (24.1%). Sudden death of an ischaemic cardiac nature was more common within other individuals whose BMI was less than 40. The average life expectancy noted within the different BMI groups observed at autopsy also declined with increasing body mass index with morbid obesity reducing the average age at death by 12 years (BMI ≥ 40 average age at death was 59 years; BMI 20-25, 71 years). In addition, we also found a positive correlation between heart weight and BMI (r^2= 0.225) (p=<0.001), as previously described.
In conclusion, this study suggests that a body mass index (BMI) ≥40 influences the probability of sustaining sudden death from cardiac failure, thus challenging the so-called "obesity paradox". Being a nation of increasing waistlines, more research is required in this area in regards to management and treatment of these "at-risk" patients.

P40

Violent Suicide in South Hampshire

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Purpose of study
Globally, the rate of suicide is estimated at 16 per 100,000 annually, resulting in a suicidal death occurring every 40 seconds. Suicide is a complex entity involving; psychological, biological, environmental, cultural and social factors. Evidence from America has suggested a link between acute alcohol consumption and/or illicit drug use with violent forms of suicide, with a third of these cases having alcohol detected at toxicology. This study aims to quantify the proportion of violent suicides in South Hampshire involving alcohol and to compare this with non-violent methods, such as intentional overdose.
Method
We prospectively examined, over a three year period (2009–12), cases of suicide which were referred to HM Coroner for the jurisdictions of Southampton, New Forest, Portsmouth and East Hampshire. Data was gathered from post mortem examinations and toxicology reports.
Summary of results
The most common form of violent suicide was hanging followed by intentional overdose (24%). The ratio between violent and non-violent suicide was 4:1. In 43% of the violent suicides alcohol was consumed prior to death with 31% of the cases having a blood alcohol level >80mg/100ml (UK driving limit) at the time of death (average level 150mg/100ml). Within the non-violent category the most common method was prescription overdose alone (45%), followed by alcohol/prescription medication combination (17%).
Conclusions
A third of violent suicides in South Hampshire had consumed significant levels of alcohol prior to death. Such levels would have caused marked cognitive and behavioural changes. Worldwide, death by suicide is now one of the three leading causes of death within the 15-44 age group. As the culture of excessive drinking continues in the UK there is a greater need for research into the acute effects of alcohol on suicidality.

P41

Venous Thromboembolic Disease and Obesity: An Audit of Autopsy Reports and Investigation of a Potential Link

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It is important that obesity is included in the medical certificate of cause of death (MCCD) when appropriate, enabling accurate statistics on the impact of obesity on mortality to be compiled. According to RCPath guidelines, recording of height and weight is best practice for all adult autopsies, allowing Body Mass Index (BMI) to be calculated. Obesity is a known independent risk factor for venous thromboembolic disease (VTE) and specifically this study aimed to identify if it was being recorded, where appropriate, in the cause of death in these cases.

All adult autopsies performed during 2011 at a UK teaching hospital were reviewed. BMI was calculated and cause of death recorded. Of 771 full general adult autopsy reports, 690 (89.5%) documented height and weight. The distribution of cases across the different categories of BMI was: 30 (4.3%) underweight (BMI < 18.5), 189 (27.4%) normal weight (BMI 18.6-25), 205 (29.7%) overweight (BMI 25.1-30), 226 (32.8%) obese (BMI 30.1-40) and 40 (5.8%) morbidly obese (BMI 40+).

VTE was the cause of death in 64/690 (9.3%). In each BMI group, VTE was the cause of death in 3 of the underweight cases (10%), 18 of the normal weight (9.5%), 15 of the overweight (7.3%), 23 of the obese (10.2%) and 5 of the morbidly obese cases (12.5%). No underlying factor was given in the cause of death in 25/64 (39%) cases (i.e. cause of death was given as Ia PE/DVT alone). In the obese group, 32% (9/28) had no underlying factor given in the COD. Obesity was included in the cause of death, thus recognising this risk factor, in only 5/28 cases of VTE in the obese and morbidly obese categories.

The great majority of autopsy reports included height and weight, in accordance with RCPath guidelines. There was no significant difference in the incidence of VTE in the different categories of BMI. Obesity was recorded as a contributing factor in the MCCD in only a minority of cases.

P42

The Disappearing Asbestos Fibre

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Following the realisation that lung fibre levels have been trending downwards in cases of mesothelioma, lung carcinoma and asbestosis, a 20 year database review of lung digest fibre assays (n=1094) was undertaken. The yearly mean results clearly demonstrate a falling fibre level with regression coefficient of 0.4393, 0.2319 and 0.1991 for mesothelioma (n=434) carcinoma (n=262) and asbestosis (n=165) cases. In addition, the fibre levels from those exposed, but ultimately determined to have non-asbestos disease (n=233) show a similar falling level and regression coefficient of 0.1519. This suggests that previously cited "significant" fibre levels may currently be misleading. This could reflect steady half-life degradation of asbestos fibres (1 years serpentine; 8-20 years amphiboles). Alternately, it might reflect those in whom the fibre has had longer time at a low level to exert the effect, or a population subgroup with genetic susceptibility of asbestos disease.

P43

Correlation of Microscopic and Clinical Findings in Pulmonary Aspergillosis

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Purpose of the study. *Aspergillus fumigatus* causes a spectrum of infection in humans, including allergic bronchopulmonary aspergillosis (ABPA), aspergilloma, and invasive aspergillosis. Host factors (e.g. cystic fibrosis (CF) and immunocompromise) influence disease presentation. Current diagnostic algorithms include serological, mycological and radiological criteria. We aimed to evaluate if the microscopic morphology of *Aspergillus* correlates with the clinical syndrome.

Methods. During one year at a Scottish teaching hospital, *Aspergillus* was visualised microscopically in sixteen respiratory samples (including one biopsy). Fungal morphology was assessed by an experienced microscopist, blind to the clinical details, who predicted the likely disease form. Images were stored. Clinical details were collected independently. EORTC criteria were used to define invasive aspergillosis. Following a review of the literature, Greenberger's criteria were modified and used to define ABPA, and criteria were established to define aspergilloma.

Summary of results. Five cases had morphology suggestive of ABPA. Three of these met our criteria. One case that did not was a CF patient (a risk factor for ABPA). Of the four cases where morphology suggested aspergilloma, three met our criteria. Only one of the three cases where morphology suggested invasive disease met EORTC criteria. The remaining two occurred in patients with infective COPD exacerbations. Three slides could not be classified and one had morphology suggestive of CF, which did come from a CF patient.

Conclusions. These findings suggest there may be a correlation between the microscopic features of *Aspergillus* and the disease form in ABPA and aspergilloma. A larger study is warranted to confirm these findings.

P44

This abstract has been withdrawn.

P45

An Investigation into the Co-Localisation of CD44 and EGFR in Fibroblasts and Myofibroblasts

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Introduction
Fibroblasts are key mediators in the initiation of wound healing which differentiate into contractile myofibroblasts following treatment with transforming growth factor β1(TGF-β1). One of their main roles includes facilitating wound closure, and the formation of collagen rich scarring. It is suggested that co-localisation of CD44 and epidermal growth factor receptor (EGFR) within myofibroblasts contributes to a signalling pathway thought to facilitate cellular mechanisms promoting the phenotypic characteristics of myofibroblasts.
Aims
The distinction between fibroblast and myofibroblast α-smooth muscle actin (α-SMA) expression will be distinguished. In addition the co-localisation and position of CD44 and EGFR within myofibroblasts will be established.
Methodology
Immunohistochemistry (IHC) and quantitative–polymerase chain reaction (Q-PCR) analysis measured the expression of α-SMA in fibroblasts and myofibroblasts. Westernblotting and IHC detected the presence of CD44 and EGFR within fibroblasts and myofibroblasts. Receptor locations were elucidated via treatment with nystatin and cytochalasin, disrupting lipid rafts and cytoskeleton respectively.
Results
IHC and Q-PCR results showed that α-SMA expression was upregulated in myofibroblasts, providing an adequate marker for cell distinction. IHC and Westernblot analysis revealed increased CD44 and EGFR expression within myofibroblasts. Treatment with nystatin demonstrated receptor co-localisation within myofibroblast lipid rafts.
Conclusion
These results have uncovered an important step in the differentiation of a fibroblast to a myofibroblast, of which a complete understanding may one day lead to the development of scarless wound healing. Further investigation into the signalling pathway(s) propagated by the co-localisation of CD44 and EGFR is required to give an insight into how this differentiation process may be manipulated.

P46

Effectiveness of Immunohistochemical Stains on Cell Block Preparations of Malignant Serous Effusions

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Cytological diagnosis of serous fluids is a cost effective method for diagnosis of metastatic malignancy. Malignant serous effusions can be used to diagnose the possible primary site of a tumour by preparing a cell block and performing immunohistochemistry (IHC). This, in conjunction with radiology, can diagnose the site of primary malignancy and help in providing a tissue diagnosis to form the basis for treatment. Various other ancillary methods including testing of prognostic markers have been used successfully on cell blocks such as Hormone receptors for breast cancer and EGFR mutation analysis in pulmonary adenocarcinoma.
This study was performed to evaluate the effectiveness of immunohistochemistry performed on cell blocks of malignant serous effusions to diagnose the site of possible primary malignancy. We analyzed the reports of 100 cases of malignant serous effusions, 50 cases each from 2010 and 2011. Cell blocks were made in 66 cases (66%). Forty two cases (67%) had sufficient cells to perform IHC. A single primary site was suggested based on IHC and relevant clinical history in 20/42 (48%) cases. In the remaining 22 (52%) cases, several primary sites were suggested based on clinical and radiological assessment. In conclusion, cells blocks and IHC were performed in 42 cases and in these cases a single or several possible sites of primary malignancy, based on immunoprofile, clinical history and imaging were provided, avoiding the use of biopsy for a definite diagnosis. Hence, this approach is a very good diagnostic tool for identifying unknown primary malignancy presenting with serous effusions. We also suggest a panel of the most useful imuunostains to be used in such cases.

P47

Investigation of Human Wound Granulation Tissue to Identify Clinical Surrogate PoP Biomarkers for Antiangiogenic Agents

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Wound granulation tissue as a surrogate model offers several practical and theoretical advantages to clinical trials involving anti-angiogenic agents. As an alternative to tumour sampling wound angiogenesis is a reproducible and physiological process, which can permit continuous observation, repeated assays and therefore, minimize intra/inter subject variability. The aim of this study was to investigate human wound granulation tissue as a surrogate model and to identify clinical surrogate proof of principle (PoP) biomarkers for antiangiogenic agents.
Human wound granulation tissue was obtained from punch biopsies of healthy skin donors, followed by overpunch biopsies at various timepoints (Day 1-14). Formalin fixed paraffin embedded (FFPE) sections were stained by immunohistochemistry (IHC) for the following vascular and angiogenic biomarkers, CD31, CD34, CD105, VEGFR2, VEGFR3, PDGFRβ and αSMA and by immunofluorescence (IF) for αSMA/CD31 co-stain. Subsequent microvessel density analysis (MVD) of IHC stained biomarkers identified vascular biomarkers modulated at key timepoints and indicated an optimal surrogate timepoint range (Day 7-10). Significant up-regulation of all angiogenic biomarkers was also observed when compared to baseline levels in normal human skin. By-eye assessment of αSMA/CD31 IF co-stain indicated the presence of mature pericyte covered vessels as early as Day 0-3, supporting the recently proposed biomechanical (looping) angiogenesis mechanism.
We show that human wound granulation tissue when combined with vascular and angiogenic biomarkers could be employed as a potential surrogate model to measure clinical PoP for neovascular targeting therapies.

P48

Interleukin-17A Stimulates Chemokine, Cytokine and Antimicrobial Peptide Expression by Oral Epithelial Cells

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Purpose of the study:
Periodontal disease is an inflammatory and immune mediated disease. The role of the host defence system in combating pathogenic oral bacteria, such as Porphyromonas gingivalis, is intricate and complex in nature. Two cytokines of interest in periodontal disease are Interleukin-17A (IL-17A) and Interlekin-22 (IL-22), both produced from Th17 cells. These effector cytokines are involved in the immunity of skin and mucosal surfaces via the regulation of antimicrobial peptides (AMPs). This study aimed to determine the role of Porphyromonas gingivalis, antimicrobial peptides (Calgranulin S100A8 and Cathelicidin LL-37) and cytokines (IL-17A and IL-22) in the host cell response to periodontal disease.
Methods:
RNA was extracted from human periodontal tissues (healthy and diseased) and analysed for mRNA expression of IL-17A, S100A8 and LL-37 using qRT-PCR. OKF6 oral mucosal keratinocytes were stimulated with IL-17A, IL-22, planktonic and biofilm oral bacteria for 4 and 24 hours. Supernatants were analysed for IL-8 production using ELISA .Expression of IL-8, S100A8 and LL-37 was analysed using qRT-PCR.
Results:
IL-17A and LL-37 mRNA was elevated in diseased periodontal tissues, compared with healthy tissues. S100A8 gene expression was found in both healthy and diseased tissues. IL-17A stimulation of OKF6 cells resulted in the up-regulation of IL-8 and S100A8, but not LL-37. OKF6 cells stimulated with dead bacteria resulted in an increased IL-8 response, when compared to live bacteria. Biofilm models exhibited an increased IL-8 response compared to planktonic models.
Conclusions:
IL-17A plays an important role in the periodontal immune response to bacterial pathogens. The up regulation of acute inflammatory mediators (such as IL-8) and antimicrobial peptides S100A8 and LL-37 may assist in the removal of any invading microbial threat.
This work was supported by an elective bursary from the Pathological Society

P49

The Anti-cancerous Potential of Polyphenols in the Treatment of Human Myeloid and Lymphoid Leukaemia

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Background: The mortality of leukaemia is still high despite the considerable improvements in chemotherapeutic agents. For this reason, our study aimed to investigate polyphenols as alternative agents for the treatment of leukaemia. The selected polyphenols have been recently shown to be affective in the treatment of solid tumours, although little work has focus on leukaemia. Here, we specifically selected eight compounds from the major classes of polyphenols (quercetin , chrysin, apigenin, emodin, aloe-emodin, rhein, cis-stilbene and trans-stilbene) and have studied their affect on cell proliferation, cell cycle and apoptosis on a panel of human myeloid (KG1a, HL60 THP-1,and K562) and lymphoid (JURKAT, CCRF-CEM, MOLT-3, and U937) leukaemia cell lines.
Methods: The effect of polyphenols on cell proliferation was measured by CellTiter-Glo® luminescent assay; cell cycle was assessed using propidium iodide (PI) staining and flow cytometry and the induction of apoptosis was assessed by caspase 3 activity assay using flow cytometry and Hoescht staining using fluorescence microscopy.
Results: our study showed that quercetin, emodin and cis-stilbene were the most effective polyphenols at inhibiting the cell proliferation , arresting the cell cycle and inducing the apoptosis ($P<0.05$) with IC50 values ranged between 10-50μM following 24hr for all the leukemic types. However, it important to note that the action of the studied polyphenols varied between different leukemic cell lines suggesting that there is a different mechanism of action of each of these molecules. All lymphoid cell lines (JURKAT, CCRF-CEM, MOLT-3, and U937) showed greatest sensitivity to the polyphenolic compounds comparing to the myeloid cell line.
Conclusions: Our findings suggest that polyphenols could be a novel chemotherapeutic drugs candidate for the treatment of lymphoid leukaemia types.

P50

Isolation and Characterisation of Cancer Stem Cells in Solid Tumours

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A growing consensus within the field of cancer research is the existence of rare tumour-initiating cells which exhibit similar characteristics to stem cells.Several markers associated with normal stem cell function have been proposed as suitable markers for isolating CSCs The study outlined here aims to determine the expression of different CSC markers in both cell lines and primary tumour material.
Materials and Methods
3 uveal melanoma (UM) cell lines, 1 cutaneous melanoma (CM) cell line and 1 prostate cancer cell line were used. Fresh UM tumour material was cultured immediately following surgery. Cells were seeded at clonal density to assess their ability to give rise to a stem cell hierarchy. Resulting colonies were graded as holoclones, meroclones or paraclones depending on their ability to give rise to further colonies. Cells were stained for expression of the stem cell markers aldehyde dehydrogenase (ALDH) and Nanog and assessed by flow cytometry.
Results
The cell lines exhibited different capacities to generate a stem cell hierarchy. UM cell lines and the CM cell line were unable to generate a stem cell hierarchy In contrast, all UM short term cultures were able to generate a stem cell hierarchy suggesting that this characteristic is lost in UM cell lines. The prostate cancer cell line PC3 was able to generate a stem cell hierarchy and ALDH expression positively correlated with the formation of holoclones. However, ALDH expression was not exclusive to a holoclone phenotype.
Conclusion
ALDH expression enriches for cells with a higher proliferative capacity and a more primitive colony type in PC3 cells, however is not exclusive to a holoclone phenotype. In contrast, ALDH expression in CM cell line and UM STCs did not correlate with an increased proliferative or clonogenic capacity. Therefore we propose the embryonic stem cell marker, Nanog, may be an alternative marker of CSCs.

P51

Analysis of the Effects of Specialisation on the Quality of Reporting of Stomach Cancer using the Royal College of Pathologists Minimum Dataset in the Yorkshire Region

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Purpose of study: There has been an increasing trend towards specialisation in histopathology. This study examines the effect of specialisation on the quality of reporting of the RCPath stomach cancer dataset.
Methods: An audit of 1065 stomach cancer pathology forms over a 12-year period (1995-2006) was performed. The rate of completeness of the forms, accuracy of the information content and quality of reporting were determined. Accuracy was adjudged by running specific queries to check for discrepancies such as mis-match of the depth of local invasion and the pathological tumour stage. Quality was assessed by the number of lymph nodes retrieved. The impact of specialisation was analysed by comparing median number of lymph nodes retrieved, completion rate of the forms and rate of discrepancies between specialist (Leeds) and non-specialist (11 others) departments. Differences were statistically tested for significance ($p=<0.05$).
Summary of Results: Of the 1065 forms, 31% were submitted from the specialist centre. The median lymph nodes retrieval was statistically significantly different between the two (p-value 0.0001) with a median 22 (IQR 14-30) lymph nodes for specialist centre versus 14(IQR 8-19) for non-specialist centres. Of the 316 forms with discrepancies, 235(74.4%) were from non-specialist centres compared to 81(25.6%) from specialist centre with a p-value of <0.0001. The completion rate of forms was statistically better (p-0.004) in the specialist centre (85.7% complete) compared to the non-specialist centres (78.2% complete).
Conclusions: This regional audit shows that specialisation of histopathologists has significant impact on the completeness of forms, accuracy of information content and quality of reporting. Therefore, further specialisation is recommended to improve the quality of cancer reports and patient management.

P52

A Simple Low Cost Dynamic Electronic Model of Synapse Dysfunction for Neuropathology Education

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Synapse dysfunction is a key part of the pathological process in many neurological diseases including Parkinson's disease and Alzheimer's disease. Although the types of synapse dysfunction can be described to medical students it is difficult to demonstrate the dynamics of the dysfunction. We have constructed a very simple electronic model of synapse dysfunction for undergraduate education. The model consists of a photoresistor connected to a computer through an open source microprocessor board (Arduino). The resistance values are graphically displayed on the computer using a programme written in the open source language Processing. The materials cost about £30. A series of programmes were written in Arduino to be sequentially uploaded to the microprocessor board. The initial programme allows the photoresistor values to pass through unfiltered. Subsequent programmes introduce different types of pathology including threshold values, random noise, hypersensitivity and intermittent signal dropout. The students load the different programmes and apply different levels of light to the photoresistor whilst watching the graphical display. More complexity can be introduced with multiple photoresistors connected in different networks. Qualitative assessment indicated that the model presented a richer understanding of synapse dysfunction than static description alone. We are very grateful to the Pathological Society of Great Britain & Ireland for an educational grant which funded this project.

P53

Using Braitenberg Vehicles to Model Neuropathology in an Educational Context

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In his seminal book 'Vehicles' the Italian neuroscientist Valentino Braitenberg described simple theoretical autonomous robots with a true neuroanatomical basis for their internal wiring that responded to light in different ways. We have facilitated a group of medical undergraduates in the construction of working versions of Braitenberg's vehicles using proprietary robotic construction equipment. Once the students had made working versions they introduced defined errors in the sensor readings or flow of sensor information to simulate neuropathology. If random noise was added to the light sensor readings at levels up to 100% of the sensor value the light seeking robots still reached the target light but their path to the target was longer. At lesser levels of noise there was little effect on performance. Small amounts of intermittent signal dropout also had little effect. The robots thus provided useful dynamic models of the robustness and resilience of simple neural systems that had good educational value. Simulation of neuropathology at a higher level of function was not successful but this was mainly due to the limitations of the proprietary graphical programming language and future developments will include using a C-based language with more complex control structures. We are very grateful to the Pathological Society of Great Britain & Ireland for an educational grant which funded this project.

P54

A Low Cost Open Source Autonomous Robotic Model for the Dynamic Simulation of Movement Disorders

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Neuropathology lesions often produce striking changes in dynamic movements of patients however the pathways behind those changes are often complex and difficult to demonstrate to medical undergraduates. We have developed a low cost open source autonomous robot to provide a dynamic demonstration of some movement disorders. The wheeled robot is based on the open source Arduino microprocessor and programming language. The robot has a light sensor and is programmed to seek brighter light, it also has an ultrasonic distance sensor and is programmed to avoid obstacles. An entire robot costs about £140. Various neuropathologies can be simulated by adding noise to the sensor readings or by altering the movements in the obstacle avoidance routines. Using such alterations we have produced some behaviours that have a similarity to cerebellar ataxia and are produced within the robot by loss of timing synchronisation between sensors and motor output in an analogous fashion to the human dysfunction. Further developments will include simulation of the movement disorders in Parkinson's disease. We are very grateful to the Pathological Society of Great Britain & Ireland for an educational grant which funded this project.

P55

Evaluation of Lymph Node Flow Cytometric Analysis as a Diagnostic Tool for Lymphomas

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Diagnostic haematopathology profoundly depends on multidisciplinary approach from Haematology and Histopathology offering morphological assessment, flow cytometry and immunohistochemistry for immunophenotyping of lympho-proliferative disorders as gold standard analytical tools. The purpose of this study was to compare the utility, sensitivity/specificity and cost-effectiveness of flow cytometry versus immunohistochemistry in diagnosis and subtyping of lymphomas.

This is a retrospective study which included 115 fresh lymph node samples sent for flow cytometry over the period of two years (2010-2011) in whom the final diagnosis was confirmed with histopathology and immunohistochemistry. The overall concordance between the flow cytometry and immunohistochemistry was 76% for all lymph nodes. Amongst the B-cell lymphomas, the concordance rate was as follows: 96% in follicular lymphomas, 100% in chronic lymphocytic lymphomas and mantle cell lymphomas and 45% in diffuse large B cell lymphomas as well as 50% for T-cell lymphomas. Flow cytometric results were suggestive of Hodgkin lymphoma and metastatic lesions in 31% and 61% of cases respectively after correlation with morphological findings. The concordance rate was 97% for the reactive lymph nodes. Light chain restriction was detected in 26 out of 48 cases of B cell lymphomas indicating clonality.

Thus, while morphology and immunohistochemistry are the benchmark diagnostic tests for lymphomas, flow cytometry is also found to be valuable and cost effective tool with short turnaround time. However the rate of false negative result is higher in lesions displaying heterogeneous cell populations or large cells as in Hodgkin lymphoma, diffuse large B cell lymphomas and certain T-cell lymphomas.

P56

The International Collaboration on Cancer Reporting (ICCR): Development of Evidence-Informed Minimum Core Data Sets for Pathology Cancer Reporting

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Cancer pathology data sets (CPDS) are foundational elements for clinical cancer care, surveillance and research. Global standardization of these CPDS is a prerequisite for international benchmarking and epidemiological research but has not been attempted. The Colleges and Associations of Pathology representing the USA, UK, Canada and Australia have formed an international collaboration for cancer reporting (ICCR) which represents a population of approximately 450 million. The ICCR set out to create practical minimum CPDS for translation and use by a broad audience, including countries without advanced medical care. As a pilot, the ICCR established 4 international review panels (RP) to develop CPDS for prostate, lung, endometrial carcinoma and melanoma.

The 4 RPs each comprised 9 physicians: an ICCR pathology lead and 2 representatives from each country. Existing CPDS from each country were collated and the chair facilitated an evidence-based review and harmonisation of the core (required) data elements, permitted responses, non-core (recommended) elements and terminology. Core elements included stage, tumour type and predictive or prognostic data for which there was evidence to support inclusion. While resources to perform a formal evidence-based analysis were not available during this stage of the project, Level III-2 evidence from revised NH&MRC criteria (BMC Med Res Method 2009, 9:34) was set as the threshold and inclusion by consensus alone was discouraged.

All RPs succeeded within the allotted 4 months. Through stringent criteria the RPs were able to remove legacy data elements and to rationalize a total of 118 pre-existing core elements to 66 (endometrium 23/17, prostate 38/18, melanoma 38/20 and lung 19/11). The ICCR, having achieved significant improvements in CPDS, including harmonized terminology, definitions and required responses will extend the process to other languages, jurisdictions and cancer types.

P57

Amended Reports in Surgical Pathology, Edinburgh Pathology Directorate 2009

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Objectives
To estimate the pathological error rate for the surgical amended reports at Edinburgh Pathology Directorate for the year 2009. Furthermore, to classify their risk categories.
Design
67 cases of amended reports were obtained from the surgical pathology file for the year 2009. The total number of cases was 66151. The reasons given for the amended reports were either following Multi Disciplinary Meetings slide review or on request from clinician for case review. The errors were divided into two main categories; the first is authorisation defects and the second is pathologist's interpretation problems. These errors were subdivided into 3 risk categories: major, intermediate and minor (significant, moderate and sever changes respectively)
Results
The surgical pathological error rate was 0.1 %. Authorisation defects accounted for 74.6% (59.7% minor, 10.4 % intermediate and 4.4% major respectively). Pathologist interpretation errors accounted for 25.3 %(2.9% minor, 16.4% intermediate and 5.9% major respectively).
Conclusion
The surgical pathology is entirely dependent on the human interpretation.In the literature, the average pathological error rate ranges between (0.26- 1.2 percent). In this audit, the estimated surgical pathological error rate was 0.1%. The fact that the majorities of the errors were due to authorisation defects, suggested that this process needs to be more robust. The current practice is to authorise reports automatically. This is a quick, speedy and cost effective; however, it may lead to overlooking to some of the details within the pathological reports by overworked pathologist.

P58

An Audit of 62-day Urgent Referral Prostate Biopsies

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Purpose of the study. In 2008 the Scottish Government announced revised targets to reduce waiting times for cancer treatments. 95% of patients referred urgently with suspected cancer are expected to begin treatment within 62 days of receipt of referral. Our centre, which serves a population of approximately 600 000, provides secondary and tertiary care for prostate cancer patients including those referred urgently. We wished to assess the efficacy and sustainability of the Urgent 62-day pathway.
Methods. A retrospective audit of 62-day urgent referral prostate needle biopsies.
Results. From August 2008 to January 2012 only 30% of GP referrals sent as 'urgent 62 day pathway' were progressed after vetting by Consultant Urologists. In 5% a diagnosis of cancer was made clinically in conjunction with very high PSA. 172 prostate needle biopsies (out of a total of 1682 biopsy cases) followed the 62 day route and 90% of these men (median age 69 years) were biopsied within two weeks (mean 10 core biopsy sample). Not all were associated with an elevated PSA (median 11.9ng/mL; range 1.1-596ng/mL). A diagnosis of invasive prostatic carcinoma was made in 58% of cases on first biopsy with the majority reported as high risk disease (48% with Gleason score 8 or above).
Conclusions. A minority of referred cases fulfilled criteria after vetting. Urgent referrals accounted for 10% of needle biopsies during the study period. Referral guidelines seem to cause difficulty and are probably not applied effectively.

P59

An Endocrine Pathology Online Virtual Medical Textbook Generated from HTML Coded Links to Virtual Microscopic Slides

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A simple method for creation of an online endocrine pathology textbook using virtual pathology slides with deep hyperlinking of images from HTML-coded web pages to specific areas of scanned slides using the Aperio virtual slide server system is described. Standard glass haematoxylin and eosin slides are scanned into a virtual slide archive using an Aperio XT scanner and then viewed remotely using proprietary software, Webscope, with a standard computer monitor via the internet. For an example web page for an Endocrine Pathology Slide Atlas see www.ukeps.com and a mirror website at www.slideatlas.com. Descriptive webpage text from the HTML coded web pages to be hyperlinked via HTML hyperlinks to specific area(s) of a virtual pathology slide(s) that is/are of interest. This then allows the addition of multiple HTML webpages to be assembled to create a virtual microscopic slide atlas with text contained on the webpages that then hyperlinks to the relevant area(s) of each scanned virtual slide, so saving time in navigating around the virtual slide, and clearly showing the specific area of the slide that is of interest and of direct relevance to the accompanying text on the associated HTML coded webpage. The hypertext linked web pages can be created directly by the author 'as you write' directly from the virtual slide by cutting and pasting the hyperlinks into the web pages with the hypertext link tool on the Aperio Image Scope. The idea presented appears relatively simple as we are not aware that others have used multiple deep HTML hyperlinks of virtual microscopy slides to HTML coded descriptive text on a webpage for designing an online virtual medical textbook or slide atlas. The same technology could also be used to develop a similar virtual textbook for MRI and CT images using picture archiving systems and DICOM images, or other similar applications in virtual image processing.

P60

This abstract has been withdrawn.

P61

Adult Pancreatic Neuroblastoma, an Unusual Site and Fatal Outcome

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In this report, we describe a classic case of stroma rich neuroblastoma, nodular type in a 22 year old female presented with a pancreatic mass. This rare and unusual presentation elicits several differential diagnostic categories including solid pseudopapillary tumour, pancreatic endocrine tumour, pancreatoblastoma and PNET. In this report, we tried to differentiate between them depending on the histopathological features and using panel of epithelial and neuroendocrine markers. Although of the rarity of pancreatic neuroblastoma as a primary site of origin, however it should be considered in the differential diagnosis of pancreatic masses in children and young adult. Neuropil and ganglionic differentiation are helpful features to recognize neuroblastoma and differentiate them from other small blue cell tumors. The fatal outcome of adult neuroblastoma confirming the independence of age as a prognostic factor in this neoplasm regardless of stage and histology.

P62

Expression of Target Molecules in Japanese Gastroenteropancreatic Neuroendocrine Tumours (GEP-NET) Patients

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Background - The multi-targeted tyrosine kinase inhibitors (TKI), which inhibits vascular endothelial factors (VEGF), its receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), have been reported to have an antitumour activities in neuroendocrine tumours (NET). However the status of these factors in NET of non-pancreatic organs has not been studied. We therefore immunohistochemically evaluated these factors in human gastroenteropancreatic neuroendocrine tumours (GEP-NET).
Design - VEGF, VEGFR and PDGFR immunoreactivity was evaluated in 104 cases of surgically operated Japanese GEP-NETs (stomach: 9, duodenum: 14, pancreas: 62, rectum: 19 cases) and compared to Ki67 labeling index (Ki67 LI), mitotic index (MI) and tumour size of each cases.
Results - VEGF, VEGFR and PDGFR were expressed in 98.1%, 66.3% and 79.8% of all cases, respectively. Ki67 LI was significantly higher in cases with positive VEGF expression than in negative cases (p=0.034, positive: 6.53 ±15.83, negative: 0.065 ±0.050). VEGFR immunoreactivity was significantly correlated with PDGFR immunoreactivity (p=0.002, correlation coefficient= 0.321) but not with VEGF nor proliferative indices or tumour size.
Conclusion - This is the first report to evaluate the immunoreactivity of VEGF, VEGFR and PDGFR in Japanese GEP-NET patients. Results of our present study indicated that VEGF expression in tumour cells contributed to its proliferative activities in GEP-NET. However, the status of both VEGFR and PDGFR were not correlated with tumour proliferative activities. These results also suggest that each targeted molecules in GEP-NET cells were not necessarily correlated each other and therefore, therapeutic strategy of multi-targeted TKI in GEP-NET patients should be considered based on the status of these molecules to be studied in surgical pathology materials of GEP-NET

P63

Accuracy of Assessing Proliferation Indexes in Gastrointestinal Endocrine Tumours

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Purpose of study: The proliferation index (PI) in gastrointestinal endocrine tumours provides prognostic information and is measured using Ki67 immunohistochemistry (IHC). The Royal College of Pathologist (RCPath) recommend calculating PI by counting a sample of 2000 cells. This is time consuming and some pathologists admit to making eyeball estimates of PI rather than formal cell counting. This study aims to compare eyeball estimates of PI with the results of counting 2000 cells.
Methods: Sections from gastrointestinal endocrine tumours were immunostained for Ki67. PI was calculated using three methods: first, manual tally counter of 2000 cells from of the area of highest nuclear labelling using a microscope eyepiece graticule; second, eyeball estimates made by three pathologists within the same area of highest nuclear labelling; third, image analysis of microscope photographs taken from this area using the ImageJ 'cell counter' tool.
Summary of Results: Levels of agreement between methods was evaluated using Bland-Altman plots. Agreement between manual tally and ImageJ assessments was high. Agreement between pathologists' eyeball assessments and ImageJ analysis varied between pathologists but was reasonably high, although as PI increased level of agreement declined.
Conclusions: High levels of agreement between manual tally and ImageJ assessment suggests RCPath methods are an accurate way of assessing PI. Lower levels of agreement between pathologists' eyeball estimates and ImageJ assessment at higher PIs suggests the need for more objective measures when PI is high.

P64

Phaeochromocytomas Associated with PTEN Mutation Demonstarate Tissue Specificity in the Anti-tumour Effect of mTOR Inhibition.

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Transgenic mice harbouring a mutation of PTEN (Pten+/-) develop a number of different tumour types the most frequent being Bcell lymphoma and phaeochromocytoma. Development of the tumoours is further enhanced by the presence of a hypomorphic allele of the LKB1 gene (LKB1+/hypo). PTEN is a phosphatase regulating the activity of the PI3kinase dependent pathway which leads to activation of Akt and further downstream mTOR and S6 riboprotein activation. To test the effectiveness of mTOR inhibition as an anti-tumour agent groups of Pten +/- mice were treated with the mTOR inhibitor AZD8055 or control drug vehicle. While lymphomas responded to the drug treatment by dramatic reduction in bulk and histological presence of apoptosis the phaeochromocytomas were resistant to the anti-tumour effect of the treatment. We analysed the activity of the Akt pathway in phaeochromocytoma using antibodies to phospho-specific epitopes on Akt (Thr 308 PDK1 site, Ser 473 mTOR site), S6riboprotein (Ser235 mTOR site), p-PDK1, GSK3, Bcl2, Cyclin D1 and LKB1 and immunocytochemistry on fixed tissue samples with lymphomas from the same animals for comparison. In both treated and untreated phaeochromocytomas there was strong reactivity for Akt p-308 but not Akt p-473 whereas untreated lymphomas showed reactivity for both but treated lymphomas only for Akt p-308 suggesting that in the phaeochromocytoma mTOR was not activated. This was confirmed by a lack of S6p-235 staining. While cyclin D1 was seen in only a small minority of lymphomas it was uniformly positive in the phaeochromocytomas as was GSK3. These several data suggest that phaeochromocytomas harbouring PTEN mutations arise by a different cellular mechanism from the lymphomas in the same animals and that GSK3 activation of cyclin D1 is involved. These findings reveal tissue specificity in pathway involvement within tumours arising from the same mutation. (Path Soc Summer project.)

P65

Diagnostic Discrepancies in Pathology reports of Neuroendocrine Tumours Referred to a Specialist Centre.

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Aims: Tumour samples from patients with a provisional diagnosis of gastroenteropancreatic neuroendocrine tumour (GEP NET) referred to a specialist tertiary centre undergo histopathological review. We aimed to determine the frequency of discrepancies between local and central histopathology that would potentially influence clinical management.
Methods: Resected tissue or biopsy material from 50 cases of suspected GEP NET referred between 1st January 2011 and 1st February 2012 were reviewed in a regional network centre. Details of the local and tertiary centre pathology reports were compared. The reports were also judged for compliance with the Royal College of Pathologists Minimum Dataset.
Results: There was complete diagnostic agreement in 29 cases (58%). Discrepancies occurred in the remaining 21 cases (42%), including eight morphological discrepancies, six differences in grade or proliferation index score (Ki67), five differences in assessment of perineural or vascular invasion, and two differences in the extent of local invasion. Sixteen of these discrepancies (32% of the total study population) had the potential to meaningfully impact on clinical management. In addition, neither tumour grade nor proliferation index were recorded in thirteen local pathology reports (26%), which would necessitate additional pathological analysis prior to clinical decision making.
Conclusions: This study highlights the importance of tertiary specialist centre histopathology review as recommended by the National Institute for Clinical Excellence (NICE) and standardised reporting using the Royal College of Pathologists Minimum Dataset.

P66

Extracts From Red and White Cabbage Contains Bio-Active Compounds Which Induce Apoptosis in Myeloid and Lymphoid Leukaemia Cell Lines

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Anthocyanins found in many natural fruits and vegetables posses anti-oxidant, anti-angiogenic and anti-inflammatory properties. Red Cabbage contains a plethora of anthocyanins with potential chemo-preventative and pro-apoptotic activities. This study investigated pro-apoptotic effects and anti-proliferative effects of extracts from red and white cabbage on four myeloid (KG-1a, K562, THP-1 and HL-60) and two lymphoid (CCRF-CEM and Jurkat) leukaemia cell lines.
Cellular viability and proliferation was assessed using trypan blue stain and the Cell titer-glow Luminescent Cell Viability Assay. Activation of caspase-3 was measured by flow cytometry and apoptotic nuclear morphology was confirmed using Hoechst stained cells. Cell cycle arrest was determined using flow cytometry of propridium iodide stained cells. Solid phase extraction was used to elucidate the active compounds from white cabbage juice extract (WCJE) and red cabbage juice extract (RCJE), these were then analysed using MS/MS.
Histological analysis confirmed apoptosis of all leukaemia cell lines in a dose- and time- dependant manner following treatment with WCJE and RCJE. Cell viability assays indicated a significant decrease in cell viability after treatment for 24h and 48h. IC50 values were determined for all cell lines. MS/MS analysis of RCJE confirmed the presence several key anthocyanins including Cyanidin as well as non-anthocyanin compound Sulforaphane, which was also found to be present in WCJE.
Extracts from red and white cabbage induced apoptosis and cell cycle arrest in all cell lines, confirming that both WCJE and RCJE hold potential bio -active properties that could be used for the treatment and prevention of leukaemia.

P67

Impact of Anthocyanin Chemical Structure Found in Pomegranate Juice on Leukaemia Treatment.

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Anthocyanins are an abundant group of flavonoids present in pomegranate juice and responsible for its red colour. The six most abundant anthocyanins in pomegranate juice are cyanidin-3-O-glucosie, cyanidin-3, 5-di-O-glucoside, delphinidin-3-O-glucoside, delphinidin-3,5-di-O-glucoside, pelargonidin-3-O-glucoside and pelargonidin-3,5-di-O-glucoside. Studies suggest the anthocyanins show potential for the treatment and prevention of cancers. Anthocyanins have been shown to inhibit cellular proliferation and induce apoptosis in cancer cell lines.
The anti-cancerous effect of eight different anthocyanins was investigated on four leukaemia cell lines (CCRF-CEM, MOLT-3, HL-60 and THP-1). Cells were treated with 0μM to 100μM anthocyanins for 24hours. Cell proliferation was assessed using CellTiter-Glo® Luminescent Cell Viability Assay. The pro-apoptotic actions of anthocyanins were assessed by two assays: Annxien V/Propidium iodide staining and staining for caspase3-activity using flow cytometry.
Delphinidin was found to have the greates inhbitory affect on cell proliferation which was found to be significantly greater than that show by cyanidin and pelargonidin ($P<0.05$). Delphinidin also significantly induced apoptosis in all four cell lines ($P<0.05$). Cyanidin induced apoptosis only in CCRF-CEM and pelargonidin failed to induce apoptosis in any cell lines ($P<0.05$). Anthocyanins containing sugar molecules showed decreased toxicity which correlated with the size of sugar molecule.
These results provide evidence that anthocyanins show anti-cancer effects which are dependent on chemical structure and association with sugar molecules.

P68

Synergistic Action of Falcarinol on Induction of Apoptosis on Human Lymphoid Leukaemia Cell Lines

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Novel therapeutic approaches for leukaemia are urgently needed to improve leukaemia patient's survival. This study investigated the effects of falcarinol- type of polyacetylene isolated from carrots (Daucus carota) alone and in combination with chemotherapy agents and apoptotic induction on three lymphoid leukaemia cell lines to investigate their additive, subadditive or synergistic effects on apoptotic induction.
Flow cytometric analysis was used to detect active caspase 3 following 24 and 48 h treatment and apoptotic morphology was confirmed using DAPI (4', 6-diamidino-2-phenylindole).
Falcarinol treatment has been shown to induce apoptosis in the three leukemic cell lines in a dose and time responsive manner. Treatment with both falcarinol and DR5agonist showed 25% higher levels of caspase-3 activation on Jurkat than cells treated with single agent alone. MOLT-3 (acute lymphoblastic leukaemia patient released following chemotherapy) cell line demonstrated a significant synergistic response indicated by a significant decrease in viability and significant increase in caspase 3 activity following 24 and 48 h incubation with falcarinol combined with 2.5 nM Valcade, 0.5 nM Leptomycin B or 25 μM Sulforaphane. For example 44% of MOLT-3 cells remaining live after 24 h treatment with falcarinol and Valcade vs. 87% and 98% following falcarinol and Valcade alone respectively.
For the first time, this research has shown that falcarinol can act synergistically with a number of agents in leukaemia cell lines, although the effects vary depending on cell type.

P69

The Use of p63 in Distinguishing In Situ and Invasive Tumour in the PyMT Mouse Model of Breast Cancer

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The PyMT mouse is a very useful model of breast cancer in an immunocompetent host. The morphology of the tumours that develop in PyMT mice range from florid hyperplasia through to invasive cancers but it is often difficult to distinguish between in situ and invasive components on standard staining as the morphology does differ from human breast cancer. p63 is a nuclear transcription factor that is present in the nuclei of basal/myoepithelial cells in human epithelium. We have optimised immunohistochemical staining for p63 on a range of tumours from PyMT mice. In normal ducts p63 demonstrates a single continuous myoepithelial layer. In invasive tumours this layer is absent and there are just a few p63 positive cells scattered apparently randomly through the tumours. The most interesting finding was that a discrete p63 layer was absent in many areas which using human morphological diagnostic criteria would have been interpreted as atypical hyperplasia or in situ carcinoma. The absence of a myoepithelial cell layer was confirmed in these areas by an absence of beta integrin staining. Thus the p63 staining demonstrated a much greater area of invasive tumour than would have been anticipated from haematoxylin and eosin staining alone and could be a useful adjunct to tumour assessment in studies using the PyMT mouse.

P70

Variations in *SEPT6* Transcript Expression Reflect Progression of Melanoma.

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While cutaneous melanoma accounts for only 4% of skin cancers, it causes the greatest number of skin cancer related deaths worldwide. The prognosis for advanced melanoma remains poor, with death typically occurring within 1 year for those with metastatic disease. The development of distinct biomarkers may therefore provide a means of clearly identifying melanomas which provide diagnostic difficulty and predicting the likelihood of future metastasis. As Breslow depth to date remains the single most important prognostic factor for melanoma, the need for molecular biomarkers to improve prediction of outcome is urgently required. The septins are an evolutionary conserved family of proteins which have the ability to bind and hydrolyse guanosine-5'-triphosphate, and to form polymers. To date, 13 different septins genes and numerous pseudogenes have been described in humans. We have previously reported that SEPT6 undergoes complex alternative splicing, such that 7 different transcripts of the gene exist, which encode four different polypeptides. Expression profiling of SEPT6 was carried out on 173 samples of FFPE-derived melanocytic lesions, including normal skin, benign naevi, Spitz naevi, in-situ, primary and secondary melanomas. Loss of expression of the individual transcripts SEPT6_v3 and SEPT6_v4 was seen as melanocytic lesions progressed from a benign phenotype to a more malignant phenotype. Conversely, expression levels of SEPT6_v4* were expressed in higher percentages in malignant lesions relative to benign naevi and normal skin. The exact function of this transcript "switching" is not clear, but it may ultimately alter the cell phenotype to one which allows for escape from normal cell regulation and apoptosis, and permits uncontrolled proliferation.

P71

Comparative Analysis of Two- and Three-Marker Immunohistochemical Cocktails in the Diagnosis of Prostate Carcinoma

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Introduction: Immunohistochemistry using antibody cocktails against basal cell specific and cancer-associated markers is important in the diagnosis of prostate carcinoma in needle biopsies. We compared the usefulness for detecting prostate carcinoma of a three marker cocktail of antibodies to α-methylacyl-CoA racemase (AMACR), p63 and cytokeratin 5 with a traditional two marker cocktail of AMACR and p63.
Material and methods: Sixty-six prostate needle biopsies were analysed prospectively. Serial sections were immunostained with the two- and three- antibody cocktails. Blinded slides were assessed individually by two pathologists. Sensitivity and specificity as well as kappa statistics were calculated.
Results: Both antibody cocktails contributed to the detection of prostate carcinoma in needle biopsies. There was fairly good agreement between the pathologists for both the cocktails as indicated by the kappa values. Sensitivity was similar for one pathologist comparing both the cocktails (76.4% and 75.7%), but was slightly lower comparing the three antibody with the two antibody cocktail for the other pathologist (66.6% vs. 77.4%, respectively). Higher specificity values of 90.3% were achieved by both pathologists using the three antibody cocktail as compared with the two antibody cocktail (68.7% and 71.8%).
Discussion and conclusion: Antibody cocktails are important in diagnosing prostate carcinoma in needle biopsies. Specificity was improved when a three antibody cocktail was used. The three antibody cocktail showed similar sensitivity combined with higher specificity in detecting prostate carcinoma in limited needle biopsy material, compared with our traditional two antibody cocktail and should be considered for routine diagnostic use.

P72

Is Human Papilloma Virus has a Role in Bladder Carcinoma of Egyptian Patients?

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Background:Bladder cancer is the second most commonly occuring genitourinary cancer in adults. Egypt has the highest rate of bladder cancer in the world with local factors most probably responsiple for such prevelance. In recent years, viral infections including human papilloma virus(HPV) have been implicated in bladder carcinogenises . HPV is a small circular DNA virus that infects stratified squamous epithelium and has an established aetiological role in tumours of the urogenital tract and anal region. Several previous studies have looked for an association between HPV and bladder cancer development, however, its possible role is still controversal.
Objective: To investigate the possible aetiologic role of HPV in Egyptian bladder carcinoma.
Patients and Methods: 42 Egyptian patients with bladder carcinoma, 17 cases with cyctitis as well as 15 cervical carcinoma cases as a positive control were included in this study. Formalin fixed paraffin embedded tissues were used and stained with; H&E to study histopathologic features, immunohistochemistry for P16 and Ki 67as well as the tissue processed for GP&EIA PCR for HPV expression.
Result: Only one case of bladder carcinoma showed positivity for HPV with complete negativity in the cystitis group. 52% of bladder carcinoma cases showed P16 expression and 21.4% showed overexpression. P16 expression was higher in cases associated with bilharziasis and in transitional carcinoma cases associated with squamous differentiation.
Conclusion: The low prevelance of HPV in this study does not support an aetiologic role of HPV in Egyptian bladder carcinogesis. However, the overexpression of P16 in a subset of bladder carcinoma cases could raise a possiblity for other HPV types that were not detectable by our probe.
Key words: HPV , P16 and bladder carcinoma.

P73

Significance of Complement Split Product C4d Deposition in Placenta of Systemic Lupus Erythematosus (SLE) and Pregnancy Induced Hypertension (PIH)

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Background: SLE and PIH are known to result in premature labour and intrauterine growth retardation, and microscopic features of placenta, as represented by acute atherosis and so on. The exact mechanism of the placental injury of these disorders remains undetermined, although immunologic abnormalities, including a disruption of complement system, might play a role in tissue injury in cases of SLE. Therefore, we investigated the significance of C4d deposition in placentas of SLE and PIH.
Design: Immunostaining for C4d was performed on paraffin-embedded tissue sections of placentas from 21 patients with SLE, 21 with PIH, and 20 control cases. For evaluation of the C4d staining, we employed the H-score. The score was obtained by the formula: 3 x percentage of strongly staining trophoblasts plus 2 x percentage of moderately staining trophoblasts plus percentage of weakly staining trophoblasts, giving a score ranging from 0 to 300. Clinical records were thoroughly reviewed, and a variety of parameters were compared with respect to H-score.
Results: Immunoreactivity of C4d in a linear fashion along with surface of villous trophoblastic membranes was greater in cases of SLE and PIH, compared with control cases. 57% of SLE and 33% of PIH showed intermediate to strong-reactivity of C4d, with an H-score ranging from 14 to 270 and 15 to 105, respectively. All H-scores of control cases were less than 4. Placentas with intermediate to strong reactivity of C4d showed significantly low-placental weights, low-birth weights, and preterm birth in SLE compared to low H-score (0-3) cases for each disorder.
Conclusion: Activation of complement cascade is related to functional abnormality of placenta in PIH and SLE. From the practical point of view, the C4d immunohistochemistry for paraffin-embedded placental tissue may provide information on risk of placental dysfunction in subsequent pregnancies of patients with these two conditions.

P74

A European Society of Pathology Study Testing the Reproducibility of Current Methods for Classification of Complex Endometrial Glandular Lesions

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Purpose of the study: To compare the reproducibility of current classification systems for endometrial hyperplasia and neoplasia. **Methods:** 9 different observers, all international experts in gynaecological pathology (5 from Europe; 4 from the US and Mexico), each independently reviewed all slides once over 1 month using: A) WHO (2003) classification, B) Endometrial Intraepithelial Neoplasia (EIN) terminology and C) classification of the European Working Group (Euro WG) [AJSP 1999;23:1102-8]. Observers 1-3 used method A for the 1st third of the samples, method B for the 2nd third and method C for the remaining 3rd. Corresponding methods for blocks 1, 2 and 3 were B-C-A and C-A-B for observers 4-6 and 7-9 respectively. Thus, each sample was consistently evaluated by three observers per method. The Kappa (κ) values for more than 2 assessments and more than 2 observers were calculated for each method. Confidence intervals were estimated by bootstrap analysis with 5000 replications. **Results:** Both WHO and EIN systems showed poor interobserver agreements (κ 0.3375 and 0.4188 respectively). The Euro WG system showed only moderate agreement (κ 0.5303). Preliminary results for WHO showed partial agreement of 61% and total disagreement of 14%, being poor for complex hyperplasia and moderate for carcinoma. EIN had the worst κ for the diagnosis of endometrial intraepithelial neoplasia (0.2725), full and partial agreement of 36% and 59% respectively were, however, better than WHO and so was the total disagreement of 5%. Confusion of the diagnosis of EIN with "benign" hyperplasia occurred in 13%. Euro WG had a moderate κ of 0.6210 for endometrioid neoplasia and 0.63976 for hyperplasia.
Conclusion: This study confirms the poor reproducibility of the diagnosis of complex endometrial lesions using the current methods and the need for a consensus on simplification and uniformity for the evaluation of these common lesions.

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Mesothelioma Mystery – a Continuing Challenge

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Background: Presented is a young lady who had an open appendicectomy for right iliac fossa pain who was suspected of having endometriosis. She underwent laparascopic examination 8 months latter for persistent pain and was diagnosed with deciduoid mesothelioma. We review the literature emphasising the dilemmas surrounding diagnosis, aetiology and outcome.
Case presentation: At laparoscopic examination multiple ill-defined small nodules were seen on the pelvic peritoneum. Pathology revealed a pleomorphic tumour with morphological and immunohistochemical features of deciduoid mesothelioma. The patient was referred for consideration of peritoneal stripping.
Discussion: Few cases of this variant of epithelioid mesothelioma have been reported following its characterisation in 1994. Initially regarded as a diffuse tumour arising from the peritoneal cavity of young women, it has since been identified in older men and women and arising from non-peritoneal mesothelium. Aetiology remains uncertain with an equivocal association with asbestos exposure.
Initially thought to be aggressive with most patients dying of disease within 2 years, cases with longer survival and even cure have recently been described. This suggests the possibility of a less aggressive variant for whom aggressive treatment could be beneficial. Our case may fall into this group as she had an extended period between initial presentation and diagnosis and a florid associated inflammatory reaction. Morphological features indicative of this subgroup have not been categorised.
Conclusion: Deciduoid mesothelioma needs to be considered in young patients with persisting abdominal pain. Aggressive interventions may improve the prognosis particularly for a possible subset of less aggressively behaving tumours. This group is currently impossible to identify morphologically.

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Clinicopathological Correlation of the Post Mortem Examination in Neonates

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Aim. Post mortem examination often has an important role in the counselling of families after the loss of a baby on the neonatal unit. However, we currently do not know how often the examination gives extra information that may be important for families and/or clinical staff.
Methodology. An eight year retrospective case note review of babies who had post mortem examinations between January 2002 and May 2010. Ante- and post-mortem diagnoses of causes of death were compared and the degree of concordance was assessed and categorised systematically according to a published classification system.
Results. Results from 62 post mortem examinations were available. New information was gained from the post mortem in 18/62 (29%) cases. This ranged from a minor change in diagnosis to a major finding that would have had a major impact on clinical management, including unsuspected fungal and viral infections, necrotising enterocolitis, intestinal atresia, biliary atresia, and new syndromic diagnoses. This new information was considered important for future genetic counselling in 3/62 (5%)cases.
Conclusion. This study shows that extra, potentially important information is frequently found at post mortem examination. These data may be useful in informing discussions with parents who are considering post mortem examination following the death of their baby.

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A Rare Cause of Perinatal Death: Hyperinsulinaemic Hypoglycaemia

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A baby girl was born at 35 weeks gestation following spontaneous labour and delivery. The mother was treated with methadone throughout pregnancy. Over the first 12 hours of life, blood glucose levels fluctuated, and were often ≤ 2.2mmol/L despite IV dextrose infusion. Opiate withdrawal was suspected due to agitation with inadequate suckling, watery diarrhoea and desaturations, and the opiate withdrawal schedule was initiated. The baby suffered an apnoeic episode, followed by bradycardia, and despite full resuscitative measures, could not be revived.

Autopsy revealed normal growth and development. Histological examination of the pancreas revealed diffuse islet cell hypertrophy, with large islet cells containing large and hyperchromatic nuclei, consistent with congenital Hyperinsulinaemic Hypoglycaemia (HH). Toxicological analysis of vitreous humor revealed very low combined glucose and lactate levels (135 mg/L vs 3000-4000 mg/L), consistent with antemortem hypoglycaemia, and free insulin and C-peptide, confirmatory of antemortem hyperinsulinaemia of an endogenous nature.

HH is characterised by inappropriate insulin secretion, leading to recurrent severe hypoglycaemia. HH is a genetically heterogeneous condition with mutations in seven different genes described to date. The most common mutations are in ABCC8 or KCNJ11, which encode the SUR1 and Kir6.2 subunits of the pancreatic β-cell K-ATP channel. Other genes involved are GLUD1, GCK, HADH, HNF4A and SLC16A1. The age at onset and severity of disease is variable. Babies with ABCC8 or KCNJ11 mutations are often macrosomic at birth with severe hypoglycaemic symptoms. Death in the perinatal period is very rare, and diagnosis was complicated in this case by many of the symptoms of hypoglycaemia also being attributable to opiate withdrawal. Premature delivery also renders infants more vulnerable, due to reduced glycogen stores.

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Sudden Unexpected Death in Childhood with Hippocampal Anomalies and a History of Complex Febrile Seizures

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This 23 month old boy had a history of approximately 40 febrile convulsions, the first occurring at the age of 5 months. Some seizures had all of the defining features of *complex* febrile seizures (duration > 15 mins, > 1 seizure during the same febrile illness, focal neurological signs), and some occurred during sleep. An MRI scan, two interictal EEGs and investigations for metabolic disorders were normal. Seven weeks after his last febrile seizure, he was found dead in his cot in the morning, lying face down and prone. He had not been febrile during the previous day.

Autopsy revealed mild brain swelling. On coronal sections, the hippocampi and parahippocampal gyri were asymmetric. Microscopic examination revealed a region of previous necrosis and calcification in the CA1 region of the right hippocampus.

The dentate gyrus of the left hippocampus showed mild hyperconvolution. Increased interstitial neurons were seen in the white matter of both parahippocampal gyri. A collection of subventricular neuroblasts was seen adjacent to the posterior horn of the left lateral ventricle. All other sections from brain showed no significant abnormalities. No significant abnormality was found in other organ systems, and there was no toxicological, virological or microbiological cause of death.

The findings in the brain have previously been described in cases of sudden unexpected death in childhood in the context of febrile seizures, or a family history of febrile seizures. In such cases, death usually occurs during sleep, in a prone position. Death is thought to occur by the same mechanism as sudden death in temporal lobe epilepsy, with an unwitnessed seizure during sleep leading to airway occlusion and death. This child is unusual in the number of febrile seizures he had experienced, and that seizures had previously been witnessed during sleep, lending support to the proposed mechanism of death in such cases.

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Ruptured Aneurysm of the Umbilical Vein

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Aneurysms of the vessels of the umbilical cord and their branches are rare vascular anomalies and can predispose to rupture

We report a case from a 36 year old fit primigravida, non smoker who abstained from alcohol in pregnancy. Her BMI was 20.9. She was low risk and booked for midwifery led care. Her dating and anomaly scans were completed on time with no abnormalities noted. She booked for home delivery.

At term + 14 days she attended the day assessment unit for a review by a doctor as she was declining induction of labour for post maturity. The fetus was assessed and Amniotic Fluid Index (AFI) and cardiotocograph (CTG) were normal.

She continued to decline induction of labour and went into spontaneous labour at term + 17 days. She was transferred from home to the delivery suite because of meconium staining of the liquor after spontaneous rupture of membranes. She progressed to fully dilated and was noted to have a pathological CTG and then a fetal bradycardia. Delivery was expedited with a kiwi ventouse and a 3.6kg live female infant was born with APGARS of 1, 4 and 4.

The placenta was delivered with controlled cord traction and noted to look abnormal at the insertion of the cord.

Baby was transferred to the neonatal intensive care unit (NICU) after being ventilated. She had a cardiac arrest thought to be secondary to hypovolaemia. After 30 minutes, the baby responded to resuscitation and was head cooled for 72 hours on the neonatal intensive care unit. She was discharged on day 12 of life after establishing breastfeeding.

The placenta revealed a large haematoma close to the cord insertion which was shown to contain fetal haemoglobin. Beneath the haematoma there was a 3 cm ruptured aneurysm of a branch of the umbilical vein. In the literature congenital thinning of the vessel wall and necrosis of the vasculature from chronic and severe meconium exposure have been implicated in the aetiology.

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Image Analysis Approach to the Reliable Quantification of Tumour Infiltrating Immune Cells as Immuno-oncology Biomarkers

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Cancer immunotherapy aims to activate immune effector cells to target tumours, or to neutralise suppressive immune mechanisms. However, translation of immunotherapeutic approaches in the clinic will require the development of reliable and robust immune-monitoring strategies for the identification of relevant biomarkers. We have built a platform of immunohistochemistry based assays for the detection of innate and adaptive immune cells in formalin fixed paraffin embedded human tumours, together with a digital imaging approach for the quantification and localisation of infiltrating immune cell populations. Head and neck squamous cell carcinomas have been immunostained for CD8+ cytotoxic T cells, FoxP3+ regulatory T cells and CD45+ haematopoietic cells. Tumour, stromal and necrotic components were separated in digitally acquired images using Genie™ pattern recognition software, and Aperio image analysis algorithms applied to quantify infiltrating immune cells. Due to varied morphology, different GenieTM classifiers were required for each tumour and marker to accurately segment tumours. Despite this, accurate quantification of inter-individual variation in tumour composition was achieved. Immune cell infiltrates predominated in the stroma with a low frequency of immune cells infiltrating tumour cell regions. Analysis of FoxP3:CD8 ratios revealed an immunosuppressive phenotype in the stroma, with elevated FoxP3+ regulatory T cells compared with CD8+ cytotoxic T cells being evident. These data demonstrate that immune cell infiltrates can be localised and quantified accurately in human tumours using a digital imaging approach enabling assessment of inter- and intra- patient variability in baseline immune cell frequencies and the objective assessment of potential changes upon treatment. The conclusion drawn is that this approach could contribute to biomarker strategies for cancer therapies that modulate the immune system.

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Astronomical Algorithms for Automated Analysis of Nuclear, Cytoplasmic and Membranous Immunohistochemical Stains

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Purpose
Personalised cancer medicine has placed increased demands on in situ histopathological assays especially immunohistochemistry (IHC). The major shortcoming of IHC is that it requires manual interpretation by a pathologist returning a semi-quantitative score which can be labour-intensive. Therefore there is an urgent need for methods of automated analysis of IHC stains. Astronomers have developed sophisticated, robust methods for the automated analysis of complex telescopic images of the sky.
Methods
In collaboration with the Institute of Astronomy at the University of Cambridge, we have adapted astronomical algorithms for the analysis of IHC stained digital images of breast cancer using tissue microarrays (TMAs) of 2,258 tumours and compared these results to those generated by manual assessment by pathologists. We developed algorithms for the analysis of IHC stains localising to the nucleus (ER), cytoplasm (BCL2) and membrane (HER2). Spearman's rank correlation and receiver operating characteristic (ROC) analysis were used to assess automated scores against manual scores.
Results
All automated scores showed good-to-excellent correlation with manual scores. For ER, the correlation was 0.82, $p<0.0001$, for BCL2 0.73, $p<0.0001$ and for HER2 0.64, $p<0.0001$. Automated scores showed excellent concordance with manual scores in defining cases as 'positive' or 'negative'. The proportion of cases concordantly classified was 93.2%, 87.3% and 96.0% for ER, BCL2 and HER2 respectively.
Conclusions
Automated analysis of immunostains represents a viable alternative to manual assessment and provides a continuous measure of in situ protein expression in a high-throughput manner. Adaptation of astronomical algorithms to tissue sections constitutes a powerful research tool for the objective investigation of the complexity of microscopic images of tumours.

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Automatic Image Analysis to Calculate the Cancer:Stroma Ratio in Colorectal Cancer

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A key factor in the prognosis of colorectal cancer is the ratio of tumour cells to intra-tumoral stroma (the so called cancer:stroma ratio). Currently cancer:stroma ratio is measured manually, by examining virtual H&E stained slides and point counting all components of the cancer separately e.g. tumour cells, stroma, vessels, inflammation etc. Virtual slides are used to annotate areas of interest on a digital slide image, and previously presented, in-house developed software superimposes points onto the image in a random, systematic fashion. These points are classified one by one by the observer. The analysis of the tumour:stroma ratio requires scoring of at least 300 points per tumour. This is a time consuming (10-60 minutes per case) and laborious task and automating this process would be highly desirable.
These expert-classified measurement points were used as 'ground truth' to develop and validate an automated cancer:stroma detector using image analysis. The virtual slide data is extracted to create pre-classified image patches for image analysis algorithm training and validation. Patches are processed for feature extraction, identifying colour, texture, stain intensity, object size, shape, edges and other visual traits. These features are used as training data for a random forest ensemble classification algorithm, and validated against unseen image patches. Currently, over 50,000 such patches are being used for algorithm training and validation. Initial results show an accuracy of 71% for cancer and 56% for stroma. Further development and refinement of methods is needed to significantly improve the accuracy of the algorithm.

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Testing for Allelic Loss in Colorectal Cancer by High Resolution Melting (HRM) Analysis

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Background:Development of genetic instability is common in human cancers and it is considered as an important event during the progression of colorectal cancer. It usually results in gains or losses of the genetic materials in oncogenes/tumour suppressors e.g. allelic loss following tumour suppressor gene inactivation. Testing for allelic loss is an important tool in cancer research and it is currently done by detection of loss of heterozygosity (LOH) at polymorphic sites using SNPs or microsatellite markers. High Resolution Melting analysis (HRM) allows detection of sequence changes due to formation of DNA heteroduplexes. We hypothesised that allelic loss at a SNP will alter the ratio of heteroduplexes to homoduplexes due to a shift towards a homozygous status and that this would be detectable by HRM.
Materials and Methods: Two SNPs in TP53 and one SNP in Pten were selected from dbSNP data base because they show a high frequency of heterozygosity and 56 cases of CRC were tested. Initially, as proof of principle, microdissected tumour samples from up to six cases were tested by HRM and validated by direct sequencing. Following this, all heterozygous tumours (without microdissection) were tested. Overall, 75% and 50% of cases showed allelic loss in P53 and Pten, respectively, by HRM. The presence of stroma in the non-microdissected cases reduced the signal to noise ratio but still allowed detection of allelic loss. All cases were confirmed by sequencing. We next extended our study by testing 30 non microdissected CRC for LOH of the APC gene using multiple SNPs in the coding sequence of exon 15. Sixteen samples showed heterozygosity at the APC locus and 75% of them showed LOH by HRM.
Conclusion: Our results indicate that HRM would be a suitable and reliable technique for detection of LOH in addition to its use as a rapid and cost effective mutation screening tool.

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Ancillary Staining and Histopathological Services in a Tertiary Hospital in sub-Sahara Africa II: Immunohistochemistry

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Our earlier study had shown that ancillary staining used in a histopathology laboratory in a Nigerian tertiary hospital on 44,813 surgical biopsy specimens from 1991 to 2010 were mostly special histochemical stains (3,104, 6.93%); and that immunohistochemistry was hardly done. In this study, immunohistochemistry was reviewed / analysed in greater detail and found not to have been used at all from 1991 to 2007, sparingly used between 2008 and 2010 (<0.31%) and more frequently used in 2011 (78 out of 3,288 specimens, 2.37%). Of the 11,590 surgical biopsies received between 2008 and 2011, only 90 (0.78%) had immunohistochemistry carried out on them. Multiple immunohistochemistry antibodies were used on some specimens, which translated into 238 antibody usage on 90 specimens. Of the 238 antibody usage, the three most common antibodies and the number of times used, were: oestrogen receptor (ER, 66, 27.73%), HER2/neu (64, 26.89%) and progesterone receptor (PR, 53, 22.27%) while the three least used antibodies were desmin (3, 1.26%), chromogranin (6, 2.52%) and cytokeratin (8, 3.36%). Other immunohistochemistry antibodies used were epithelial membrane antigen (EMA, 17, 7.14%), vimentin (11, 4.62%) and CD45 (10, 4.20%). The underutilization of immunohistochenistry in this facility was due primarily, to high costs; relative frequent use of the triple antibodies for breast cancer - ER, HER2/neu and PR having been made possible through donation of the antibodies by a pharmaceutical company. While immunohistochemistry remains a valuable modern tool in diagnosis, management and prognosis of diseases, its use in this histopathology laboratory and possibly others in sub-Sahara Africa is curtailed by high costs, limited trained personnel and other logistic challenges.

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