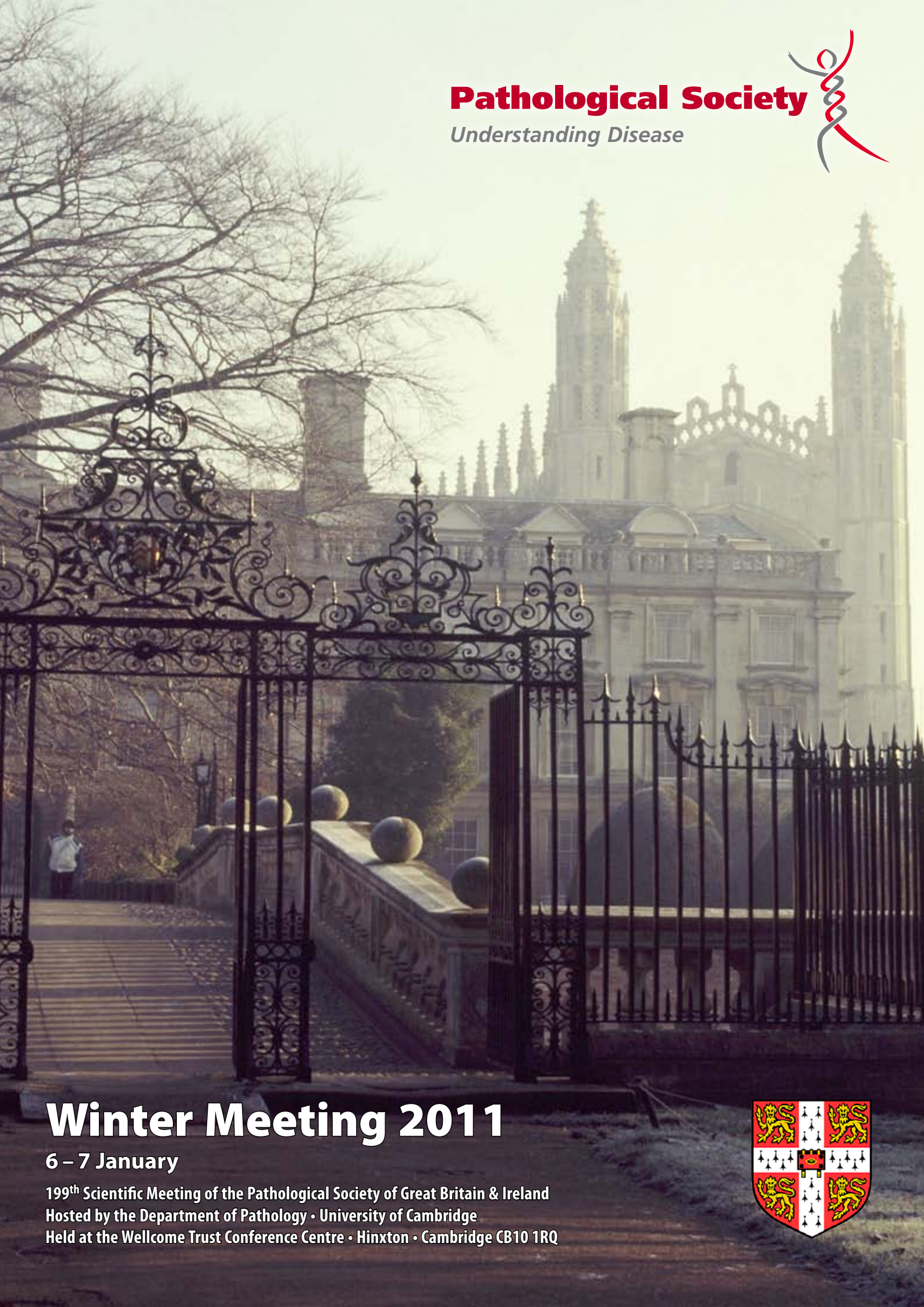


Pathological Society

*Understanding Disease*

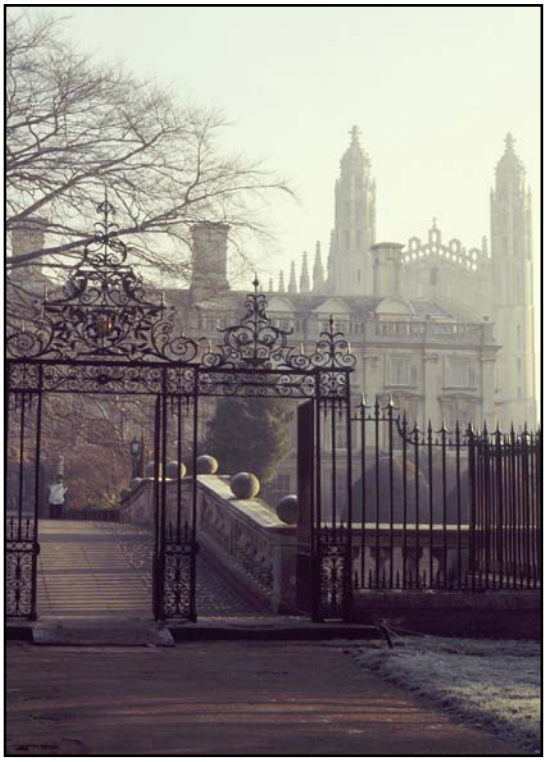
# Winter Meeting 2011

## 6 – 7 January

199th Scientific Meeting of the Pathological Society of Great Britain & Ireland
Hosted by the Department of Pathology • University of Cambridge
Held at the Wellcome Trust Conference Centre • Hinxton • Cambridge CB10 1RQ

Pathological Society

*Understanding Disease*



# Winter Meeting Programme

❁

## 6 – 7 January 2011

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**199th Scientific Meeting of the**
**Pathological Society of Great Britain & Ireland**

**Hosted by:**
**The Department of Pathology**
**University of Cambridge**

**Venue:**
**The Wellcome Trust Conference Centre**
**Genome Campus • Hinxton • Cambridge • CB10 1RQ**

**PROGRAMME ACKNOWLEDGEMENTS**

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**Back cover:** Wellcome Trust Conference Centre, Hinxton

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# Contents

# Programme Quick Reference Tables

## THURSDAY 6 JANUARY 2011

**FOYER**

**08.00** **Registration and Coffee**

**ROSALIND FRANKLIN PAVILION**

**09.00–18.00** **Slide Seminar Case Viewing:** ***The Partnership between Molecular and Conventional Histopathology***
*Competition closes at* ***16.30***

**FRANCIS CRICK AUDITORIUM**

**09.00–12.00** **Symposium:** ***Molecular Pathology for Today and Tomorrow — 1***

**CLOISTERS**

**10.30–11.00** **Coffee / Poster Viewing / Trade Exhibition**

**CLOISTERS**

**11.00–11.10** **Welcome – Sir L Borysiewicz, Vice-Chancellor, University of Cambridge**

**CLOISTERS**

**12.00–13.00** **Lunch / Trade Exhibition**

**CLOISTERS**

**13.00–15.00** **Poster Viewing and Chairman's Rounds** *(all categories)*

**FRANCIS CRICK AUDITORIUM**

**15.00–17.30** **Plenary Oral Presentations**

**CLOISTERS**

**16.00–16.30** **Tea / Trade Exhibition**

**FRANCIS CRICK AUDITORIUM**

**17.30–17.35** **Presentations**

**1 Pathological Society Undergraduate Essay Prize Competition Winner 2010**
Mr C Yang Koo, University College London

**2 Journal of Pathological Jeremy Jass Prize for Research Excellence in Pathology 2009**
Dr T Forshew, Cambridge Research Institute, and
Dr R Tatevossian, St Jude's Children's Research Hospital, Memphis, USA

**FRANCIS CRICK AUDITORIUM**

**17.35–18.35** **Pathological Society's Goudie Lecture**
Prof M Smith, Cleveland, USA: *The Pathology of Alzheimer Disease: Pathogenic or Pathognomonic?*

**DOWNING COLLEGE, CAMBRIDGE**

**19.15** **Buses depart for Downing College**

**19.45** **Society Dinner**

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

# Programme Quick Reference Tables

## FRIDAY 7 JANUARY 2011

**FOYER**

**08.00** **Registration and Coffee**

**ROSALIND FRANKLIN PAVILION**

**09.00–17.00** **Slide Seminar Case Viewing:** ***The Partnership between Molecular and Conventional Histopathology***

**FRANCIS CRICK AUDITORIUM**

**09.00–12.15** **Symposium:** ***The Involution of Normal and Cancer Tissue***

**CLOISTERS**

**10.30–11.00** **Coffee / Poster Viewing / Trade Exhibition**

**CLOISTERS**

**12.15–13.15** **Lunch / Trade Exhibition**

**FRANCIS CRICK AUDITORIUM**

**12.45–13.30** **Meet the Experts Trainees Session:** ***Liver Pathology***
Dr SE Davies, Cambridge

**JAMES WATSON PAVILION**

**12.45–14.15** **Satellite Session:** ***HER2 Testing for Neoadjuvant Patients: Challenges and Opportunities***
*Academic Workshop organised by NCRI/BCSG*

**FRANCIS CRICK AUDITORIUM**

**13.30–14.15** **Slide Seminar Discussion Session:** ***The Partnership between Molecular and Conventional Histopathology***

**FRANCIS CRICK AUDITORIUM**

**14.15–17.15** **Symposium:** ***Molecular Pathology for Today and Tomorrow — 2***

**CLOISTERS**

**15.45–16.15** **Tea / Poster Viewing / Trade Exhibition**

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

## PLENARY ORAL SESSION [FRANCIS CRICK AUDITORIUM]

The eight highest-ranked submitted oral abstracts will be presented on Thursday 6 January, 15.00–17.30.

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

## POSTERS, VIEWING AND CHAIRMAN'S ROUNDS [CLOISTERS]

**POSTER SIZE**
Poster boards will be size 1m x 1m. Please ***do not*** exceed these dimensions. Velcro will be provided.

**VIEWING**
**Thursday 6 January** 10.30–11.00 and 16.00–16.30
**Friday 7 January** 10.30–11.00 and 15.45–16.15

**CHAIRMAN'S FORMAL POSTER ROUNDS**
**Thursday 6 January** 13.00–15.00

**PRIZES**
Poster round chairs will be circulating on Thursday 6 January 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 Thursday 6 January.

**NOTE TO PRESENTERS**
Posters should be in place by 09.00 on Thursday 6 January and **must be removed by 17.00 on Friday 7 January**.

**PRESENTATION**
The presenting author (or another author) 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 — *The Partnership between Molecular and Conventional Histopathology*

### SLIDE CASE COMPETITION AND VIEWING *(via PCs)* [ROSALIND FRANKLIN PAVILION]

**Thursday 6 January** 09.00–18.00 *(Note: Competition closes at 16.30)*
**Friday 7 January** 09.00–17.00

**PRIZE**
The winner will be announced at the Society Dinner on Thursday 6 January, the prize being a case of champagne *(which at the discretion of the winner, by tradition, is shared amongst those present at the dinner!).*

### REVIEW SESSION [FRANCIS CRICK AUDITORIUM]

**Friday 7 January** 13.30–14.15

## SATELLITE SESSION [JAMES WATSON PAVILION]

**Friday 7 January** 12.45–14.15
***HER2 Testing for Neoadjuvant Patients: Challenges and Opportunities***
*Academic Workshop organised by NCRI/BCSG*

## CONTINUING PROFESSIONAL DEVELOPMENT [CPD]

This Meeting has been approved by the **Royal College of Pathologists** for the purpose of Continuing Professional Development. Credits can be accrued as follows:

| | FULL DAY | HALF DAY |
|---|---|---|
| **Thursday 6 January** | 8 credits | 4 credits |
| **Friday 7 January** | 7 credits | 3 credits |

Delegates who are eligible for CPD points should complete the CPD Certificate Request form which will be provided in delegate packs at the meeting.

## SOCIETY DINNER [THE HALL, DOWNING COLLEGE, CAMBRIDGE]

**Thursday 6 January.**
Tickets are £55 – please book your ticket(s) when registering on-line.
For information on The Hall, Downing College, go to their **website**.

## TRADE EXHIBITION [CLOISTERS]

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

## PRESENTATION CHECKING AND PREVIEW [ROSALIND FRANKLIN PAVILION]

## INTERNET ACCESS [ROSALIND FRANKLIN PAVILION]

Delegates will be issued with usernames and passwords at the Registration Desk. Wireless access will be available.

## MESSAGES

During the Meeting, messages for delegates may be left at the following telephone number: **+44 (0)7818 640887**
There will also be a message board located beside the Registration Desk.

## REFRESHMENTS [CLOISTERS]

All refreshments will be served in the Cloisters.

## BADGES

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

## COATS AND BAGS [FOYER]

Secure facilities will be provided for coats and luggage.

## TRAVEL, ACCOMMODATION AND VENUE INFORMATION

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

## 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

## DISCLAIMER

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

## FUTURE MEETINGS

**2011**

**24–28 January** — **Pathological Society Winter School for Trainees**
Holiday Inn Hotel, Kings Cross/Bloomsbury, London

**10–13 May** — **Ghent Pathology 2011**
*6th Joint Meeting of the British Division of the IAP and the Pathological Society*
Het Pand, Ghent University, Belgium

**2012**

**5–6 January** — **Pathological Society Winter Meeting**
Hosted by Barts and the London, Queen Mary University
Guoman Tower Hotel, Tower Hill, London

**3–6 July** — **Pathological Society Summer Meeting**
University of Sheffield

**2013**

**January** — **Joint Meeting with the Dutch Pathological Society** (NVvP)
Utrecht, The Netherlands *(to be confirmed)*

**18–21 June** — **Edinburgh Pathology 2013**
*7th Joint Meeting of the British Division of the IAP and the Pathological Society*

# Fees and Registration

| REGISTRATION FEES<br>FEES INCLUDE REFRESHMENTS AND LUNCH | | | | |
|---|---|---|---|---|
| DELEGATE TYPE | FEE CATEGORIES | DAY or PART DAY | | SOCIETY DINNER |
| | | UP TO AND INCLUDING 22 NOV 2010 | AFTER 22 NOV 2010 | |
| Pathological Society Members | Ordinary Members, Consultant and/or equivalent position | £ 95 | £ 150 | £ 55 |
| Pathological Society Concessionary Members | Biomedical Scientists; Honorary or Senior Members; PhD Students; Post-Doctoral Fellows, Technicians and Trainees | £ 40 | £ 60 | £ 55 |
| Undergraduate Students ❇ | | £ 40 | £ 60 | £ 55 |
| Non-Members | Consultant and/or equivalent position | £ 150 | £ 220 | £ 55 |
| Non-Members Concessionary ❇ | Biomedical Scientists; PhD Students; Post-Doctoral Fellows, Technicians and Trainees | £ 50 | £ 80 | £ 55 |

## ADVANCE REGISTRATION

Registration is via our on-line facility found on our website: **www.pathsoc.org**
**Advance registration will close at midnight on Monday 13 December 2010.**
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 **Wednesday 1 December 2010**. Thereafter a 25% charge will be made for cancellations received in writing before **Friday 10 December 2010**, thereafter no refunds will be made.

## DELEGATE ENROLMENT (AT THE MEETING)

The Registration Desk will be open from 08.00 each day.

**FOYER**

**08.00** **REGISTRATION AND COFFEE**

**ROSALIND FRANKLIN PAVILION**

**09.00–18.00*** **SLIDE SEMINAR COMPETITION CASE VIEWING: *The Partnership between Molecular and Conventional Histopathology***

** Note: Competition closes at 16.30.*

**FRANCIS CRICK AUDITORIUM**

**09.00–12.00** **SYMPOSUM: *Molecular Pathology for Today and Tomorrow – 1***

Chair: Prof VP Collins, University of Cambridge, Addenbrooke's Hospital, Cambridge
Prof AL Børresen-Dale, Oslo University Hospital Radiumhospitalet, Oslo, Norway

09.00–09.15 ***Introduction***
Prof AH Wyllie, University of Cambridge

09.15–09.45 ***The Lessons of GIST***
Dr R Bulusu, Addenbrooke's Hospital, Cambridge

09.45–10.30 **[S1]** ***Molecular Classification of Breast Cancer; A Systems Pathology Approach***
Ⓟ Prof AL Børresen-Dale

*Oslo University Hospital Radiumhospitalet, Oslo, Norway*

Microarray technologies, applied to the study of DNA/mRNA/miRNA, can be used to portray a tumour's detailed phenotype in its unique context, and to generate molecular signatures that will improve our understanding of the causes and progression of the disease, for the discovery of new molecular markers, for therapeutic intervention and for developing new prevention strategies.
Two platform independent algorithms were developed to explore genomic architectural distortion using aCGH data to measure whole arm gains and losses (WAAI) and complex rearrangements (CAAI). By applying this to >500 bc cases relationship between structural genomic alterations, expression subtypes and clinical behaviour could be found.
Using SNP arrays and a novel bioinformatic approach, ASCAT, we could accurately dissect the allele-specific copy number in each tumour, simultaneously estimating and adjusting for both tumour ploidy and non-aberrant cell admixture. This enabled us to construct genome-wide map of allelic skewness, identifying loci where one allele is preferentially lost/gained, indicating different influence on bc development. By integrating data from the patient's own genotype with data from the tumour at the DNA level, (copynumber, mutations, methylation), mRNA and miRNA level as well as metabiloc profiles revealed from HR-MAS MR analyses of the tumour, we seek to reach a more fundamental understanding of the biological dynamics of bc. This will facilitate identification of risk factors, search for novel cancer diagnostics, prediction of therapeutic effects and prognosis and identification of new targets for therapy. Perou C et al Nature 2000 Sorlie T et al PNAS, 2001 and 2003 Bergamaschi A et al GCC, 2006 Hicks J et al. Gen. Res. 2006 Russnes H et al ScienceTM, 2010 Van Loo P et al PNAS, 2010.

10.30–11.00 **COFFEE / POSTER VIEWING / TRADE EXHIBITION [CLOISTERS]**

11.00–11.10 **WELCOME**
From Sir L Borysiewicz, Vice-Chancellor, University of Cambridge

11.10–11.30 **[S2]** ***The Histopathologist and Breast Cancer Trials***
Ⓟ Dr E Provenzano

*Addenbrooke's Hospital, Cambridge, United Kingdom*

Neoadjuvant therapy is being increasingly utilised in the treatment of patients with breast cancer. The neoadjuvant setting provides an excellent opportunity for evaluation of response to newer chemotherapeutic and hormonal agents with complete pathological response (pCR) acting as a surrogate outcome for disease free survival. Neoadjuvant clinical trials can be used to answer both clinical and prospective translational molecular questions, with detailed biological assessment of tumour tissue for molecular profiling, biomarker discovery and candidate gene analysis. The accuracy of the results from these subsequent biomarker and molecular genetic analyses is dependent upon thorough handling of the surgical specimen with the detection of any residual disease. Surgical specimens post neoadjuvant therapy can be very difficult to handle, especially when there has been a good response to therapy with no macroscopically detectable residual lesion. Accurate clinical details and correlation with

radiology is essential, as is thorough sampling of the tumour bed. However, there is huge variation in the literature as to what constitutes adequate sampling and in the way neoadjuvant specimens are reported between labs. In addition, traditional histological factors such as tumour growth pattern and histological grade can be altered by chemotherapy, and their validity in this context remains to be determined. Issues in the handling and reporting of neoadjuvant specimens will be discussed, including grading of response and the use of sentinel lymph node biopsy in this setting. Along with the emergence of new technologies and molecular diagnostic techniques, accurate detailed macroscopic and histological assessment remains of vital importance in patient management and in guiding future research.

11.30–12.00 **[S3]** ***Prospects in Glioma Diagnosis and Treatment***


Ⓟ Prof VP Collins

*University of Cambridge, Dept. of Pathology, Addenbrooke's Hospital, Cambridge, United Kingdom*


Over the last 25 years we have learnt a considerable amount about the genetic abnormalities found in the common brain tumours of man. Some of the findings have provided diagnostic markers while others provide prognostic information, therapy response indicators or even potential therapeutic targets. Many of the genes identified belong to cellular pathways that are more or less well understood. The major pathways involved include those transmitting signals from the external environment (signal transduction pathways) and those controlling the cell cycle and apoptosis. While the pattern of genetic abnormalities found may be distinct to a particular tumour, the pathways disrupted by these genetic abnormalities are often the same as found in tumours of other organs. As a consequence quite a number of targeted therapies produced for the more common malignancies may be applicable to brain tumours. The problems targeting lesions behind the blood brain barrier will be briefly summarized and the various pathways identified to date, and potentially targetable, in the common paediatric and adult brain tumours, will be reviewed.

**CLOISTERS**

**12.00–13.00 LUNCH / POSTER VIEWING / TRADE EXHIBITION**

**CLOISTERS**

**13.00–15.00 POSTER VIEWING AND CHAIRMAN'S ROUNDS**

| CATEGORY | POSTER NUMBERS |
|---|---|
| Autopsy & Forensic | P1[1] |
| Breast | P2–P17[2] *(Note: P6 withdrawn)* |
| Cardiovascular/Pulmonary | P18–P20[1] |
| Cellular/Molecular Pathology | P21–P27[6] |
| Education & Audit | P28–P40[3] |
| Endocrine | P41[1] |
| Experimental Tumour Pathology | P42[6] |
| Gastrointestinal | P43–P57[4] *(Note: P46 withdrawn)* |
| Genitourinary/Renal | P58–P62[5] |
| Gynaecological | P63–P64[5] |
| Head & Neck | P65–P66[5] |
| Hepatobiliary/Pancreas | P67–P70[4] |
| Lymphoreticular | P71–P74[7] |
| Neonatal/Paediatric | P75–P76[7] |
| Osteoarticular/Soft Tissue | P77–P82[7] |
| Skin | P83–P87[1] |
| Technical Advances | P88–P90[6] |

Chair:
[1] Dr EW Benbow, Manchester; Dr N Kirkham, Newcastle; Dr AJ Marker, Cambridge
[2] Dr R Liebmann, Kent; Dr E Provenzano, Cambridge
[3] Dr Dr JWM Chow, London; Prof AH Wyllie, Cambridge
[4] Prof M Pignatelli, Bristol; Dr SE Davies, Cambridge
[5] Prof JE Martin, London; Dr MJ Arends, Cambridge; Dr TR Helliwell, Liverpool
[6] Prof M Ilyas, Nottingham; Dr MJ Arends, Cambridge; Prof GI Murray, Aberdeen
[7] Dr RJ Byers, Manchester; Dr F Jessop, Cambridge; Dr P Ramani, Bristol

## FRANCIS CRICK AUDITORIUM

**15.00–17.30 PLENARY ORAL PRESENTATIONS**

Chair: Prof IO Ellis, University of Nottingham
Prof R Poulsom, Cancer Research UK – London Research Institute

15.00–15.15 [**PL1**] ***Mammary Gland Involution: Characterization of the Switch to Irreversibility***


Ⓟ K Hughes; CJ Watson

*University of Cambridge, Cambridge, United Kingdom*


STUDY PURPOSE: Mammary gland involution comprises the organ's retrograde change to its pre-pregnant state following weaning. Involution has an initial reversible phase characterized by epithelial apoptosis and the ability to recommence lactation if the pups are returned to the dam. A subsequent transition to irreversibility heralds further cell death, basement membrane degradation, stromal remodelling and the inability to lactate. Mice with a mammary-specific deletion of the transcription factor Stat3 exhibit a pronounced delay in involution, and have an extended period during which lactation may be restored. Comparison of these mice with controls offers the opportunity to study factors controlling the switch to irreversible involution.
METHODS AND RESULTS: Utilizing microarray data and RT-PCR analysis of murine tissue at specific involution time points, we have identified a panel of 'switch marker genes'. Of particular interest is CXCL14, a chemokine which is involved in macrophage recruitment and fibroblast stimulation. We demonstrate that CXCL14 exhibits a pronounced increase in expression at the switch in control animals. In the Stat3 reversible model CXCL14 expression is lower, and reduces as involution progresses. In vitro, mammary epithelial EpH4 cells exhibit a marked increase in CXCL14 expression following oncostatin M (OSM) stimulation. OSM is a potent stimulator of Stat3 activity in the second phase of involution. In vivo, a large influx of macrophages can be seen in control glands at 96 hours of involution, with reduced numbers observed in Stat3 knockout glands.
CONCLUSIONS: Our data suggests that the chemokine CXCL14 is a robust marker of the switch to irreversible involution, and may be a direct target of Stat3. The role of CXCL14 in the switch to irreversible involution merits further investigation, particularly given its poorly defined role in breast tumour biology.

15.15–15.30 [**PL2**] ***The Tumour Suppressor ARF Can Promote Invasion in a p53 Null Setting***


Ⓟ B Doyle[1]; EH Tan[2]; P Timpson[2]; LM Machesky[2]; RA Ridgway[2]; RE Jeffery[3]; R Poulsom[3]; JP Morton[2]; OJ Sansom[2]

*[1]St. James's Hospital, Dublin, Ireland; [2]Beatson Institute for Cancer Research, Glasgow, United Kingdom; [3]Histopathology Lab, Cancer Research UK – London Research Institute, London, United Kingdom*


We have previously shown that a combination of APC and p53 mutation results in a dramatic increase in invasion, compared to APC mutation alone in a mouse model of intestinal carcinoma. This model closely mimics the human disease, including the potential to metastasise. We now go on to investigate the mechanism of this increased invasion. In doing this we have examined the β-catenin paradox and studied a potentially novel pro-invasive role for ARF.
In the invasive carcinomas that developed in the p53 mutant mice we could detect nuclear β-catenin throughout the tumours, however there was a massive increase at the invasive front. This increase in nuclear β-catenin was associated with an increase in Wnt target genes, including Myc, Sox-9, Lgr5 and the pro-invasive actin regulator Fascin. As Myc is known to induce ARF expression we examined levels of this protein and found that it is indeed upregulated, specifically at the invasive front. This increase in ARF expression was specific to tumours in which p53 function was lost. In the few tumours with wild-type p53 that did show invasion no increase in ARF was seen at the invasive front.
In order to assess if this increase in ARF was functional, rather than merely marking aberrant Myc levels, we overexpressed ARF in p53 null HCT-116 colorectal cancer cells. We showed that overexpression of ARF resulted in a survival advantage for p53 null cells and moreover cells overexpressing ARF showed an increase in invasive behaviour in both in-vitro and in-vivo assays.
In this study we have shown two levels of β-catenin activation in invasive tumours, with higher levels of expression seen at the invasive front of the tumours. We have also shown that ARF levels are increased at the invasive front. Although ARF is generally thought of a tumour suppressor, we have shown a pro-invasive role for ARF in the setting of p53 loss.

15.30–15.45 [**PL3**] ***A Kras-Cten-FAK Axis Promotes Cell Motility in Pancreatic Cancer***


Ⓟ S Al-Ghamdi; J Cachat; M Ahmed; D Jackson; K Kindle; M Ilyas

*Nottingham University, Nottingham, United Kingdom*


Cten is a protein located at focal adhesions and involved in cell motility. The role of Cten in neoplasia is context dependent – while it acts as a tumour suppressor in prostate cancer, it acts as an oncogene in colon, breast, lung and gastric cancer. In this study we evaluated the role of Cten in the pancreas by a dual approach of knockdown in high expressing cell lines and ectopic expression in low Cten level cells. Firstly, Cten was knocked down in PSN-1 and ectopic expressed in Panc-1 and this was associated with inhibition and promotion of cell migration ($p<0.006$ and $P<0.029$ respectively).There was no change in cell proliferation in any of the cell lines following alteration of Cten expression although Cten did seem to stimulate colony formation. These data show that Cten alters cell motility in pancreatic cell lines and, since it localises to focal adhesions, we tested whether it was signalling through either Integrin-linked kinase (ILK) or Focal Adhesion Kinase (FAK). Forced changes of Cten expression were

mirrored by alterations in FAK although ILK expression did not seem to be perturbed. Information about Cten regulation is sparse but we have recently shown that Cten is downstream of Kras in colon cancer. Since around 80% of pancreatic cancers possess a Kras mutation, we hypothesised that Kras may regulate Cten. Knockdown of Kras resulted in down-regulation of Cten and inhibition of cell motility ($p<0.001$). It was also associated with down-regulation of FAK expression. Restoring Cten in cells which are depleted in Kras restored cell motility and FAK expression. This is the first description of Cten in pancreatic cancer. Our data suggest that it has similar functions to those we have demonstrated in colorectal cancer. Similar to our result in colon cancer, we found that Cten is downstream of K-ras upstream of FAK. Our data suggest a Kras-Cten-FAK axis regulating cell motility in pancreatic cancer.

15.45–16.00 **[PL4]** ***CD95 Targetted Microparticles Enhance Intracellular Drug Delivery and Increase the Efficacy of Paclitaxel Therapy in an Orthotopic Ovarian Cancer Model***

DD Ateh[1]; VH Leinster[1]; SR Lambert[1]; A Shah[1]; A Khan[1]; H Walklin[1]; J Johnstone[1]; N Ibrahim[1]; M Kadam[1]; Z Malik[1]; M Girones[2]; G Veldhuis[2]; G Warnes[3]; S Marino[4]; I Mcneish[5]; Ⓟ JE Martin[1]

*[1]BICMS Pathology, Barts and the London School of Medicine and Dentistry, London, United Kingdom; [2]Nanomi, Oldenzaal, Netherlands; [3]ICMS Flow Cytometry, Barts and the London School of Medicine and Dentistry, London, United Kingdom; [4]BICMS Neurioscience and Trauma, Barts and the London School of Medicine and Dentistry, London, United Kingdom; [5]Institute of Cancer, Barts and the London School of Medicine and Dentistry, London, United Kingdom*

CD95 induces apoptosis after binding with its ligand CD95L. Expression of CD95L enables killing of activated CD95-positive immunocytes. Tumour cells expressing CD95L include brain tumours, ovarian cancers and many others. There is significantly increased CD95L expression in malignant ovarian tumours compared to benign tumours and experimental cell lines IGROV-1 and SKOV-3 express the ligand in 85.1% and 70.5% of cells (Knox et al., 2003; Xu et al., 2002). There is also recent data suggesting an alternative role for the CD95/CD95L system as tumour growth promoter (Chen et al., 2010) rather than suppressor. We describe for the first time the use of CD95/CD95L enhanced phagocytosis to deliver drug-loaded microparticles into cells. We challenged medulloblastoma Daoy, ovarian IGROV-1 and breast cancer cell lines with unmodified and CD95 surface coated microparticles and measured uptake using microscopy and flow cytometry. In general, uptake of CD95 modified microparticles was superior and microparticle uptake was size and cell type dependent. Increased uptake of CD95 modified microparticles could be knocked down by blocking cell surface CD95L by CD95-Fc pre-exposure. Using this approach we show increased efficacy of cytotoxic cell killing in cell lines, and show drug delivery into neurons. We show treatment efficacy in medulloblastoma in vivo xenografts, and an in vivo orthotopic ovarian cancer model, where greater than 65 fold reduction in tumour bioluminescence was achieved, compared with conventional paclitaxel therapy at the same dose. Target modified phagocytosis as a delivery platform can contribute to improved function of cytotoxics in cancer, potentially increasing drug efficacy whilst reducing toxicity. The packaging of the agent provides a protective environment which could also provide a delivery option for promising intracellular therapeutics not currently considered viable for development.

16.00–16.30 **TEA / POSTER VIEWING / TRADE EXHIBITION [CLOISTERS]**

16.30–16.45 **[PL5]** ***Deletion of* PARK2 *Frequently Occurs in Colorectal Cancer and Promotes Adenoma Development in* Apc *Mutant Mice***

G Poulogiannis[1]; RE McIntyre[2]; M Dimitriadi[1]; JR Apps[1]; CH Wilson[2]; AH Wyllie[1]; DJ Adams[2]; Ⓟ MJ Arends[1]

*[1]University of Cambridge, Cambridge, United Kingdom; [2]Sanger Institute, Wellcome Trust Genome Campus,, Cambridge, United Kingdom*

Purpose: Colorectal cancers (CRC) frequently display gains and losses of large parts of chromosomes, but may also show such changes restricted to short genomic sections. Short regions of consistent DNA copy number (DCN) loss in cancers are relevant as they may be used to identify previously unrecognised tumour suppressor genes. Chromosome 6 was selected for systematic analysis for such short regions, because of the low frequency of DCN alterations relative to most other chromosomes. Methods: 100 primary colorectal cancers were analysed for consistent short regions of DCN loss on chromosome 6 by tiling-path array comparative genomic hybridization. A potential tumour suppressor gene was studied for functional effects. Results: Several consistent short regions of DCN loss on chromosome 6 were identified. One of these regions included PARK2, a gene encoding an E3 ubiquitin ligase, mutations in which are associated with a hereditary form of Parkinson's Disease. Overall, 33% of CRC exhibited PARK2 DCN loss, mostly as a result of heterozygous, intragenic deletions involving exons 3 and 4. As this region spans FRA6E, a well-known Common Fragile Site, we sought evidence that the PARK2 deletion caused authentic functional effects, rather than merely reflecting damage secondary to enforced high proliferation in tumours. First, using a bioinformatic approach, we showed that reduced expression of PARK2 is significantly associated with APC deficiency in human CRC. Second, engineered over-expression of PARK2 in cultured CRC cell lines dramatically suppressed cell proliferation. Third, crossing Park2+/- heterozygous knockout mice with Apc-Min mice resulted in a four-fold increase in intestinal adenoma prevalence compared with Park2+/+, Apc-Min siblings. Conclusion: PARK2 is a tumour suppressor gene whose haploinsufficiency cooperates with mutant APC in around one third of human colorectal cancers.

16.45–17.00 [**PL6**] ***K-ras Mutation is a Negative Prognostic Marker, and Does Not Preclude Benefit From 5-FU in Stage II/III Colorectal Cancer***


Ⓟ K Southward[1]; G Hutchins[1]; K Handley[2]; L Magill[2]; C Beaumont[1]; J Stalhschmidt[3]; S Richman[1]; P Chambers[1]; M Seymour[4]; D Kerr[5]; R Gray[2]; P Quirke[1]

*[1]Leeds Institute of Molecular Medicine, Leeds, United Kingdom; [2]Birmingham Clinical Trials Unit, Birmingham, United Kingdom; [3]Histopathology and Molecular Pathology, Leeds, United Kingdom; [4]CRUK Cancer Centre, Leeds, United Kingdom; [5]Sidra Medical and Research Centre, Doha, Qatar*


There is currently uncertainty whether the efficacy of 5-FU adjuvant therapy in stage II colorectal cancer is sufficient to justify the cost and toxicity. Tumour markers that could distinguish patients who will benefit from adjuvant chemotherapy from those that won't, or that were strongly associated with disease prognosis, are desirable both to save money and to avoid treating patients who would derive little or no benefit from chemotherapy. Mutations of the Kirsten-ras gene occur in 30–40% of sporadic colorectal cancers and are established as clinically useful predictors of lack of response to EGF receptor targeted treatment in stage IV disease.

We assessed the Kirsten-ras gene as a predictor of response to chemotherapy and risk of recurrence in patients with stage II/III colorectal cancer randomised between fluorouracil/folinic acid (FUFA) chemotherapy and control in the Quasar clinical trial. K-ras mutation status was assessed by pyrosequencing for 1583 tumours and was then correlated to prognosis and treatment benefit from FUFA therapy.

The risk of recurrence for K-ras mutant tumours was significantly higher than that of wildtype: 28% vs 21% (RR=1.40, 95% CI 1.13-1.74). The proportional increase in risk of recurrence was larger in rectal cancer and in men but similar in the presence and absence of chemotherapy. K-ras did not predict response to FUFA treatment

K-ras mutation is a useful predictor of recurrence risk in stage II/III colorectal cancer. The greater prognostic value in rectal cancer and men needs independent confirmation.

17.00–17.15 [**PL7**] ***Activating Autophagocytosis Decreases Fat Within the Liver***


Ⓟ A Levene[1]; H Kudo[1]; M Thursz[1]; QM Anstee[2]; RD Goldin[1]

*[1]Imperial College Faculty of Medicine at St Mary's Hospital, London, United Kingdom; [2]The Institute of Cellular Medicine, Newcastle University, Newcastle, United Kingdom*


*Background*: Autophagy is the degradation of intracellular components by the lysosome system. Macroautophagy is when content is sequestered in a double membrane structure which fuses with a lysosome and subsequently the contents are degraded. Recently it has been suggested that macroautophagy plays a role in fat metabolism within the liver. Our aim was to activate autophagocytosis in liver cells and assess the fat levels and amount of autophagocytosis.

*Methods*: HUH7 cells, a liver differentiated cell line, were grown in normal or high fat medium with and without rapamcyin, an autophagocytosis activator for 24 hours. The cells were stained with Oil Red-O (ORO) and the percentage fat calculated using digital image analysis (DIA). Triglyceride levels within the cells were measured biochemically as a true reflection of cell lipid content. Autophagocytosis was measured in the cells using co-localistation of LAMP1 (a lysosomal marker) and BODIPY (a neutral lipid marker) by confocal immunofluorescence microscopy.

*Results*: ORO staining identified significantly more fat in the cells grown in oleate medium compared to normal medium ($p<0.01$). There was significantly less fat in the cells grown in oleate/normal medium with rapamycin than those without rapamycin ($p<0.01$). These results correlated extremely well with the triglyceride concentration in the cells showing ORO DIA accurately reflected triglyceride concentration (Pearson correlation $R=0.995$, $p<0.01$). Confocal immunofluorescence demonstrated a significant increase in co-localisation of lysosomes and fat in cells grown in oleate/normal medium with rapamycin compared to those grown without rapamycin ($p<0.01$).

*Conclusions*: Activating autophagocytosis decreased fat within liver cells. This is a novel method of fat metabolism within liver cells and this pathway provides a possible target for the development of therapeutic agents in non-alcoholic fatty liver disease.

17.15–17.30 [**PL8**] ***Microarray Gene Expression Profiling Identifies ABC and GCB Subtypes of DLBCL in Paired Fixed (FFPE) and Unfixed Samples***


Ⓟ C Howarth[1]; K Linton[2]; RJ Byers[2]; J Chan[3]; S Pepper[4]; M Wappett[4]; JA Radford[2]

*[1]The Medical School, Manchester, United Kingdom; [2]University of Manchester, Manchester, United Kingdom; [3]University of Nebraska Medical Center, Omaha, United States; [4]Paterson Institute for Cancer Research, Manchester, United Kingdom*


Microarray gene expression studies have radically changed understanding of cancer, and of lymphoma in particular. However, almost all studies have relied on fresh/frozen tissue whilst formalin-fixed, paraffin embedded tissue (FFPET) is, and is likely to remain, the standard for processing routine clinical biopsies. Ability to use FFPET for microarray analysis would open a vast archive for study but RNA from FFPET is badly degraded and is widely considered unsuitable for microarray gene expression profiling. This study assessed the feasibility of re-profiling diffuse large B-cell lymphoma using routinely processed archival FFPET samples. Twenty paired FFPET and unfixed lymphoma biopsies were processed for gene expression microarrays; the unfixed samples had previously been assigned as either ABC or GCB molecular subtype. The paired biopsies showed good global correspondence of present (61%) and absent (83%) transcripts. The FFPET samples were clustered using both a published 90 gene list discriminatory for ABC/GCB subtypes (Alizadeh et al) and a new list of the top 100 differentially-expressed genes within the new data set and an unsupervised approach. Both lists of genes (previous ABC/GCB and differentially

expressed genes) clustered samples according their previously determined ABC/GCB subtypes, despite only 5 genes being common to both gene lists. These results support the robust nature of the ABC/GCB subtypes in that: a) the new list of differentially expressed genes recapitulated ABC/GCB clustering and b) the ABC/GCB list clustered appropriately when applied to a new sample set. Furthermore the results indicate the feasibility of gene expression profiling of FFPET. These findings have promising implications for the use of archival samples in research, and specifically for producing better prognostic models of diffuse large B-cell lymphoma.

**17.30–17.35 PRESENTATIONS**

**1 Pathological Society Undergraduate Essay Prize Competition Winner 2010**
Mr C Yang Koo, University College London

**2 Journal of Pathology Jeremy Jass Prize for Research Excellence in Pathology 2009**
Dr T Forshew, Cambridge Research Institute, and
Dr R Tatevossian, St Jude's Children's Research Hospital, Memphis, USA

**17.35–18.35 PATHOLOGICAL SOCIETY OF GREAT BRITAIN & IRELAND'S 7th GOUDIE LECTURE**

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

**[S12]** ***The Pathology of Alzheimer Disease: Pathogenic or Pathognomonic?***

Ⓟ Prof MA Smith[1]; HG Lee[2]; SL Siedlak[2]; A Nunomura[3]; T Hayashi[4]; M Nakamura[5]; X Zhu[2]; G Perry[6]; RJ Castellani[7]

*[1]Case Western Reserve University, Department of Pathology, Cleveland, Ohio, United States; [2]Case Western Reserve University, Cleveland, Ohio, United States; [3]University of Yamanashi, Chuo, Yamanashi, Japan; [4]Hokkaido Institute of Public Health, Sapporo, Japan; [5]Asahikawa Medical College, Asahikawa, United Kingdom; [6]University of Texas at San Antonio, San Antonio, Texas, United States; [7]University of Maryland, Baltimore, Maryland, United States*

Alzheimer's disease (AD) is an age-related neurodegenerative disease characterized clinically by cognitive decline and pathologically by the accumulation of amyloid-β-containing senile plaques and neurofibrillary tangles. A great deal of attention has focused on amyloid-β as the major pathogenic mechanisms with the ultimate goal of using amyloid-β lowering therapies as an avenue of treatment. Unfortunately, nearly a quarter century later, no tangible progress has been made and, most recently, clinical trials have spectacularly failed. We have long contended, as has substantial literature, that amyloid-β is a downstream response to the disease and that therapeutically targeting such an adaptive response would be futile if not detrimental. Current research examining amyloid oligomers, therefore, will add copious details to what is, in essence, a reductionist distraction from upstream pleiotrophic processes such as oxidative stress, cell cycle dysfunction, and metabolic dysfunction. It is now long overdue that pathologists avoid the pitfall of perseverating on "proteinopathies" and recognize that the continued targeting of end stage lesions in the face of repeated failure, or worse, is a losing proposition.

**19.15 BUSES DEPART FOR DOWNING COLLEGE**

**THE HALL, DOWNING COLLEGE, CAMBRIDGE**

**19.45 SOCIETY DINNER**

## FOYER

**08.00** **REGISTRATION AND COFFEE**

## ROSALIND FRANKLIN PAVILION

**09.00–17.00** **SLIDE SEMINAR CASE VIEWING:** ***The Partnership between Molecular and Conventional Histopathology***

## FRANCIS CRICK AUDITORIUM

**09.00–12.15** **SYMPOSUM:** ***The Involution of Normal and Cancer Tissue***

Chair: Dr MJ Arends, University of Cambridge
Dr A Ibrahim, Addenbrooke's Hospital, Cambridge *(to be confirmed)*

09.00–09.30 [**S4**] ***How do Tissues React to Injury?***

Ⓟ Prof AH Wyllie

*University of Cambridge, Cambridge, United Kingdom*

This symposium draws together several speakers who, collectvely, are unfolding the still poorly-understood story of tissue involution. The now familiar intracellular events of apoptosis account for some, but not all of this process, and even this part of the story continues to recruit new molecular players. Just as significant as death through apoptosis and other routes is the process of autophagy, an adaptive response to restriction in energy and nutrient supply that frequently occurs in injured tissues but, unlike apoptosis, is reversible. How these processes interlock in whole tissues, where stromal and parenchymal cells constantly signal to each other, presents a further level of complexity. This relatively new field is of great importance to pathologists and oncologists today, as it encompasses the increasingly accessible goal of therapy-induced tumour regression.

09.30–10.00 [**S5**] ***Many Roads to Apoptosis***

Ⓟ Prof S Grimm

*Imperial College London, London, United Kingdom*

As apoptosis is genetically regulated, many efforts have been made to isolate the components of apoptosis signalling pathways. Our own approach was motivated by a conspicuous correlation: most of the genes involved in apoptosis exert their activity also upon over-expression. At first, it seemed impractical to use this dominant effect for isolating such genes because their activity leads to cell death instead of cell proliferation as in the case of oncogenes. However, using a genetic screen we have been able to discover dominant, apoptosis-inducing genes. This assay, which we named RISCI (robotic single cDNA investigation), is now executed in a high-throughput format by custom-made transfection- and DNA isolation-robots. Using additional information from extensive literature studies and careful sequence analysis we have chosen several genes from the screen for further studies. The components of the mitochondrial "permeability transition pore", which is important for cell death by anti-cancer drugs, is a focus of the laboratory as is the respiratory chain complex II, which contains tumour suppressor proteins such as cybL, whose gene was isolated in the screen. With one of the mitochondrial fission genes we have detected a novel caspase-activating complex at the interface between mitochondria and the ER. Moreover, the metastasis suppressor KAI1 at the plasma membrane is studied in our group. How the ubiquitin/proteasome system regulates cell death is explored with a gene that functions as a ubiquitin-specific protease for apoptosis induction. Besides this, an interesting aspect of the screen is presently another focus of our work: we have found a tumour-specific gene, i.e. a gene that induces apoptosis only in transformed tumour cells. This indicates that our screen provides a systematic way to uncover such synthetic lethal "anti-cancer genes".

10.00–10.30 [**S6**] ***Cell Death Pathways in the Mammary Gland***

Ⓟ Prof C Watson[1]; PA Kreuzaler[1]; AS Staniszewska[1]; W Li[1]; RA Flavell[2]

*[1]University of Cambridge, Cambridge, United Kingdom; [2]Yale University, New Haven, United Kingdom*

Post-lactational regression of the mammary gland epithelium (involution) is one of the major cell death events in the adult mammalian organism. Extensive cell death occurs within 12 hours of forced weaning and is nearing completion by 6 days in the mouse. Using genetically engineered mice, we have identified a number of the essential mediators of involution including the transcription factors Stat3 and NF-κB and their upstream regulators such as the cytokines LIF, OSM and TWEAK. Since activation of executioner caspases and translocation of cytochrome C into the cytosol have been reported during involution, the modality of programmed cell death is presumed to be apoptosis. However, so far this has not been unequivocally demonstrated. Contrary to this view, we have recently discovered that mammary gland epithelium undergoes cell death through a non classical, lysosomal mediated pathway. During involution, lysosomes in the mammary epithelium undergo widespread lysosomal membrane permeabilisation (LMP), releasing cysteine cathepsins into the cytosol. This cell death is independent of executioner caspases 3, 6 and 7 but requires Stat3, which up-regulates the expression of cathepsins B and L while down regulating the cathepsin inhibitor Serine Protease Inhibitor 2A (Spi2A). This is the first time that such a lysosomal mediated programmed cell death pathway (LM-PCD) pathway has been shown to control physiological cell death

in vivo. Since tumours often have an increased lysosomal compartment, this could render them more susceptible to drugs that induce LM-PCD. Thus, our findings will be of major importance in the design of treatments for cancers such as breast, colon and liver where cathepsins are commonly over-expressed and Stat3 is hyper-activated.

10.30–11.00 **COFFEE / POSTER VIEWING / TRADE EXHIBITION [CLOISTERS]**

11.00–11.30 [**S7**] ***How Tumour Suppression Works to Kill Cancer Cells***

Ⓟ Prof G Evan[1]; L Soucek[2]; C Martins[2]; K Shchors[2]; D Murphy[2]; M Junttila[2]; D Garcia[2]

*[1]University of Cambridge, Cambridge, United Kingdom; [2]UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, United States*

Myc and p53 are pleiotropic transcription factors that, respectively, drive and suppress cancer. Myc levels are elevated and deregulated in most cancers, implicating a pivotal role for Myc in oncogenic signaling. However, Myc mutations are relatively rare and the aberrant Myc in most tumours appears to be a consequence of aberrant upstream signals. The extent to which such endogenous Myc, acting as a mere client of upstream oncogenes, might be a therapeutic target depends on the degree to which it coordinates functions essential for tumour maintenance. To investigate the therapeutic potential of Myc inhibition we have constructed switchable genetic mouse models in which endogenous Myc can be systemically and reversibly inhibited in normal and tumour tissues in vivo. Our data indicate that inhibiting Myc has a remarkably efficacious therapeutic impact on multiple cancer types, triggering widespread tumour cell apoptosis while eliciting surprisingly only mild, reversible and non-cytotoxic side effects in normal tissues. The p53 tumour suppressor, or its attendant pathway, is functionally inactivated in almost all human cancers. However, the mechanism by which p53 suppresses tumorigenesis remains obscure. p53 mediates the cellular apoptotic and senescence response to DNA damage, and this is thought to be critical for both tumour suppression and for the progressive erosion of somatic regenerative capacity that characterizes aging. However, using a unique mouse model in which the endogenous p53 gene is replaced by one encoding a ligand-dependent, reversibly switchable variant of p53, we show that the p53-mediated DNA damage response is dispensable for tumour suppression. Hence, by selectively manipulating the DNA damage and tumour suppressor functions of p53 it may be possible to potentiate tumour suppression and cancer therapy whilst simultaneously extending life-span.

11.30–12.15 [**S8**] ***Autophagy in the Involution of Normal Tissue***

Ⓟ Prof B Levine[1]; KJ Jia[2]; SH Huang[1]; XQ Qu[3]

*[1]UT Southwestern Medical Center, Dallas, United States; [2]Florida Atlantic University, Boca Raton, United States; [3]Genentech, San Francisco, United States*

Autophagy, a lysosomal degradation pathway, is commonly observed in metazoan organisms during programmed cell death (PCD). However, its function in dying cells has been controversial. Our laboratory has used genetic approaches in mouse embryonic stem cells and in *C. elegans* to address this question. In published studies, we examined the role of autophagy in embryonic cavitation, the earliest PCD process in mammalian development. We found that embryoid bodies (EBs) derived from cells lacking the autophagy genes, *Atg5* or *beclin 1*, failed to cavitate. This defect was due to persistence of cell corpses, rather than an impairment of PCD. Dying cells in autophagy gene null EBs failed to express the 'eat-me' signal, phosphatidylserine exposure, and secreted lower levels of the 'come-get-me' signal, lysophosphatidylcholine. These defects were associated with low levels of cellular ATP and were reversed by treatment with the metabolic substrate, methylpyruvate. Moreover, mice lacking *Atg5* displayed a defect in apoptotic corpse engulfment during embryonic development. In unpublished studies, we also found that autophagy genes are essential in the clearance of apoptotic corpses during nematode embryonic development. The defects observed in apoptotic corpse clearance in *C. elegans* with null mutations in the autophagy genes, *bec-1* or *atg18*, can be rescued by re-introducing the autophagy genes in both the dying and engulfing cells, but not in only the dying cells. Thus, taken together, our results in mammal and nematode systems suggest that the autophagy pathway is important in both dying and phagocytic cells for normal tissue involution during development.

**CLOISTERS**

**12.15–13.15 LUNCH / POSTER VIEWING / TRADE EXHIBITION**

**FRANCIS CRICK AUDITORIUM**

**12.45–13.30 MEET THE EXPERTS – TRAINEES' SESSION: *Liver Pathology***

Chair: Dr I Proctor, University College London

Speaker: Dr SE Davies, Addenbrooke's Hospital, Cambridge University NHS Trust

## JAMES WATSON PAVILION

**12.45–14.15** **SATELLITE SESSION:** ***HER2 Testing for Neoadjuvant Patients: Challenges and Opportunities***
*Academic Workshop organised by NCRI/BCSG*

12.45–12.50 ***Introduction***
Prof J Bartlett, University of Edinburgh

12.50–13.05 ***Neoadjuvant Treatment and Trials – The Drive for Early HER2 Testing?***
Dr L Hayward, University of Edinburgh

13.05–13.20 ***Fast HER2 Turnaround – The Leeds Experience***
Dr A Shaaban, St James's University Hospital, Leeds

13.20–13.35 ***Fast HER2 Testing – The Dundee Experience***
Dr C Purdie, University of Dundee

13.35–13.45 ***Birmingham Heartlands – An Audit of Barriers to Fast HER2 Testing***
Dr J Starczynski, Birmingham Heartlands Hospital

13.45–14.10 ***Discussion and Audience Perspective: Can we "fix it for you"***

## FRANCIS CRICK AUDITORIUM

**13.30–14.15** **SLIDE SEMINAR DISCUSSION SESSION:** ***The Partnership between Molecular and Conventional Histopathology***

Chair: Prof VP Collins, University of Cambridge, Addenbrooke's Hospital, Cambridge
Prof M-Q Du, University of Cambridge

Speakers: Dr MJ Arends, University of Cambridge
Dr N Coleman, Medical Research Council Cancer Cell Unit, Cambridge
Prof VP Collins, University of Cambridge, Addenbrooke's Hospital, Cambridge
Prof M-Q Du, University of Cambridge
Dr E Provenzano, Addenbrooke's Hospital, Cambridge

## FRANCIS CRICK AUDITORIUM

**14.15–17.15** **SYMPOSUM:** ***Molecular Pathology for Today and Tomorrow – 2***

Chair: Prof M-Q Du, University of Cambridge
Dr AC Wotherspoon, Royal Marsden Hospital, London

14.15–14.45 ***The Diagnostic Approach to Paediatric Bone and Soft Tissue Sarcomas***
Prof LG Kindblom, Royal Orthopaedic Hospital, Birmingham

14.45–15.15 [**S9**] ***An Overview of Molecular Diagnostic Haematopathology***
Prof M-Q Du

*Department of Pathology, Univerity of Cambridge, Cambridge, United Kingdom*

Lymphomas are a very large group of highly heterogeneous tumours, and their accurate diagnosis is essential for successful patient management. The diagnosis and classification of lymphoproliferative disorders depend on a careful integrated investigation of clinical, morphological, immunophenotypic and genetic features. Molecular and molecular genetic investigations play an important role not only in lymphoma diagnosis, but also in guidance of treatment choice, prediction of treatment response and disease follow up. I will review our current applications of molecular and molecular genetic investigations in routine diagnostic haematopathology, as well as in research and development.

15.15–15.45 [**S10**] ***Diffuse Large B-cell Lymphomas: Molecular Biology and Sub-classification***

Prof A Rosenwald

*Institute of Pathology, University of Würzburg, Germany, Josef-Schneider-Str. 2, Würzburg, Germany*

The clinical heterogeneity of diffuse large B-cell lymphoma (DLBCL) is mirrored by a large variation of its underlying genetic and molecular features. The gene expression based distinction into the germinal centre B-like (GCB) and activated B-like (ABC) subtypes was shown to have significant prognostic impact in the CHOP treatment era and appears to remain prognostically relevant when patients are treated with R-CHOP. Moreover, GCB and ABC DLBCL tumours have different genetic alterations. The translocation t(14;18) and PTEN deletions are predominantly detected in GCB DLBCL, whereas ABC DLBCL are characterized by deletions of p16 and amplification of the BCL2 locus. A major pathogenetic feature of ABC DLBCL is the constitutive activation of the oncogenic NFkB signaling pathway that may be targeted therapeutically in the future. Recent work identified mutations in several genes associated with NFkB leading to deregulation and aberrant activation of this pathway. For example, activating mutations of CARD 11, a protein that physiologically assembles with BCL10 and MALT1 in B-cells upon antigen engagement, are present in approximately 10% of ABC DLBCL tumours. Likewise, inactivation of A20, a negative regulator of NFkB, is also frequently detected in DLBCL. The B-cell receptor associated signaling subunits CD79A and CD79B carry mutations in approximately 20% of ABC DLBCL which result in increased expression of the B-cell receptor leading to chronic active B-cell receptor signaling. It is likely that additional molecular alterations of the NFkB pathway in DLBCL will be identified in the near future.

15.45–16.15 **TEA / POSTER VIEWING / TRADE EXHIBITION [CLOISTERS]**

16.15–16.45 ***Intermediate Lymphoma between Burkitt Lymphoma and DLBCL***

Prof H Stein, Institute for PathDiagnostic, Berlin, Germany

16.45–17.15 [**S11**] ***Selection of Therapy According to Molecular Phenotype in Diffuse Large B-Cell Lymphoma***

Ⓟ Prof P Johnson[1]; AJ Davies[1]; P Fields[2]; M-Q Du[3]; A Jack[4]

*[1]CR UK Centre, Southampton, Southampton, United Kingdom; [2]Guy's Hospital, London, United Kingdom; [3]University Department of Pathology, Cambridge, United Kingdom; [4]HMDS, Leeds, United Kingdom*

Several oncogenic mechanisms distinguish the two sub-groups of DLBL. In particular, constitutive activation of the nuclear factor-kB (NF-kB) signalling pathway appears central to cell survival in ABC-like lymphomas. These rely upon the CARD11/MALT1/BCL10 signalling complex to activate IkB kinase. More than half of ABC-DLBL carry somatic mutations in multiple genes, including negative (A20) and positive (CARD11, TRAF2, TRAF5, MAP3K7 and TNFRSF11A) regulators of NF-kB. Mutations in B-cell receptor components such as CD79B may be responsible for abnormal retention of the BCR on the cell membrane following stimulation. Induction of the NF-kB pathway appears to suppress the apoptotic effect of cytotoxic chemotherapy and this may contribute to the observed differences in outcome. These findings provide the rationale for exploration of small molecule therapeutics to target elements of the dysregulated pathways. This can be done downstream, for example by proteosome inhibitors to reduce degradation of inhibitory IkB, or closer to the start of the pathway, for example with inhibitors of Bruton's tyrosine kinase. The challenge is to design clinical trials based upon real-time determination of molecular phenotype, since immunohistochemistry remains imprecise in distinguishing biologically distinct sub-groups. The NCRI Lymphoma Group is conducting a study based upon gene expression profiling on formalin fixed paraffin embedded tumour material, in which patients will be randomised to receive Bortezomib in addition to standard R-CHOP chemotherapy. Randomisation is stratified according to the molecular phenotype, with stopping rules to exclude sub-groups according to the interaction of the gene expression profile and the targeted therapy.

**17.15 CLOSE OF MEETING**

# Acknowledgments (Trade Exhibition)

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# Poster Abstracts

Presenter = Ⓟ

# P1

## The Retention and Storage of Post-mortem Tissue

Ⓟ CVL Randall; D Hilton

*Derriford Hospital, Plymouth, United Kingdom*

Following increased public and media awareness the Human Tissue Act 2004 was implemented to protect families from unnecessary distress by providing a clear legal framework for the correct retention and storage of post-mortem tissues. In our department post-mortem tissue is taken with the coroner's permission and retained under their authority until released. The family then has 3 months to give their consent for either storage, disposal or return of the tissue. If consent is not returned, there is a legal obligation to dispose of the tissue.

The aim of the study was to ensure that our Trust was achieving the correct standards in tissue retention and storage, in relation to the HTAct, and to highlight areas for improvement.

We reviewed 194 post-mortems from April to June 2008. The post-mortem reports, the post-mortem histology forms and returned relatives consent forms were reviewed and compared with the laboratory files of slides, blocks and wet tissue.

We found that 26% of cases had histology taken, approximately 600 blocks of tissue. One case (2%) indicated that 4 lung blocks were taken when in fact only 3 were taken. Six cases (12%) had some inaccuracies of exactly which samples were retained stated on the consent form sent to the relatives by the Coroner. All returned consent instructions were followed correctly. More than 50% requested storage in the hospital. 3 cases (6%) were misfiled due to mislabelling. There were no slides, blocks or wet tissue in file that should have been disposed of or returned to families.

In a busy department where many people are involved in a complicated multi-step process errors are likely to occur. However our study showed a high level of performance with only minor errors requiring improvement in filing, coding and correct form details. Several of these areas have already been addressed with alterations to the forms for recording post-mortem histology and relative's consent.

# P2

## Gene Copy Number Analysis of Paired Myo and Luminal Epithelial Breast Cancer Cell Lines Isolated from a Primary Breast Tumour

Ⓟ DL Holliday[1]; MB Lambros[2]; JS Reis-Filho[2]; C Toomes[1]; M Cummings[1]; AM Hanby[1]; V Speirs[1]

*Leeds Institute of Molecular Medicine, University of Leeds, Leeds, United Kingdom; [2]The Breakthrough Breast Cancer Research Centre, Institute of Cancer Research, London, United Kingdom*

We previously (Path Soc 2008) reported development and characterisation of a new breast cancer cell line of basal phenotype, LGI1T, derived from an ERα, PR and LN negative squamous cell carcinoma. Subsequently, we observed 2 morphologically distinct cell populations in culture; cobblestone and spindle-shaped cells. We separated these using β4-integrin labelled immunomagnetic beads which yielded LGILUM and LGIMYO cells. By immunofluorescence, LGIMYO expressed the basal cytokeratins CK17 and CK5/6 and were negative for the luminal epithelial marker EMA. LGILUM expressed EMA and negative for basal cytokeratins. To define the patterns of gene copy number aberrations in LGILUM and LGIMYO, these were subjected to high resolution comparative genomic hybridisation (aCGH) using a 32K bacterial artificial chromosome array platform (approx resolution 50kb). Both LGILUM and LGIMYO displayed common gene copy number aberrations, including loss of 1p, 9p21-p11 and chromosome 3 and gain of 19q and chromosome 20. However, some genetic aberrations were unique to each cell type; LGILUM harboured loss of 19p13.3-p13.2, 15q, 22q, and gain of 5q33-q35 and 16q, whereas loss of 18q and 10q, and gain of 7q and 9q were only found in LGIMYO. Differential patterns of genetic aberrations in these breast cancer cell types may contribute to their biological behaviour. Furthermore, these paired cell lines provide a powerful tool to investigate breast cancer cell biology without the complication of donor-donor variation seen in standard breast cancer cell lines.

# P3

## Contribution of Stroma to Breast Cancer Outcome

Ⓟ SA Simpkins; AM Hanby; D Treanor; V Speirs; DL Holliday

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Cancer associated fibroblasts (CAFs) form a major component of breast cancer stroma. Altered protein expression in CAFs can alter tumour cell behaviour and impact on tumour progression and patient prognosis. Recently a role for caveolin-1 (cav-1) in predicting breast cancer progression was identified with loss of expression associated with increased metastasis and poor prognosis. Activation of fibroblasts demonstrated by α-smooth muscle actin (α-SMA) expression is linked to the cancer invasion-promoting phenotype. In addition the presence of more stroma (hence more CAFs), determined by measuring the stroma-tumour ratio has been associated with poor prognosis in breast cancer. The aim of this study was to test the hypothesis that CAF phenotype and their proportional relationship to tumour epithelial cells predict breast cancer progression and prognosis. With ethical approval, immunohistochemistry for cav-1 and α-SMA was performed on 140 cases of breast cancer with known clinico-pathological data and follow up (mean follow up: 91 months). Using α-SMA staining to identify CAFs cav-1 staining was scored as strong, weak or absent. Stromal content was assessed by point counting on virtual tissue sections and tumour-stroma ratio calculated using an in-house computer algorithm. Cases with >50% stroma were classed as stroma-rich; those with <50% were stroma-poor and results were correlated to clinico-pathological data. In this relatively small cohort, we observed no differences in survival between stromal rich or stroma-poor cases however there was a non significant trend for better survival in cases with stromal cav-1 expression. We are currently expanding the number of cases. These data suggest that consideration of stromal characteristics may be of value in predicting breast cancer outcome.

# P4

***This abstract is not available for publication before the Meeting.***

# P5

### Targeting the HMG-CoA Synthase-Mevalonate Pathway Sensitises Breast Cancer Cells to Tamoxifen

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Tamoxifen is the mainstay endocrine therapy for premenopausal women with breast cancer, yet resistance and recurrence remain significant clinical problems. We identified Hydroxymethyl Glutaryl CoA Synthase 2 (HMGS2) mRNA as highly upregulated in a tamoxifen resistant (TAMr) MCF7 model of breast cancer. HMGCS2 is a mitochondrial ketogenic enzyme in the liver but also functions in the mevalonate pathway in other tissues. The purpose of this study was to investigate the role of upregulated HMGCS2 in TAMr, and the utility of the HMG-CoA synthase step of the mevalonate pathway as a tamoxifen-sensitising target. HMGCS2 mRNA and protein upregulation in TAMr MCF7 relative to TAM-sensitive controls was confirmed by qRT-PCR and Western blotting, and immunofluorescence indicated mitochondrial localisation. TAMr MCF7 cells were modestly but significantly ($P < 0.01$) more sensitive than controls to growth inhibition by hymeglusin, a specific inhibitor of HMG-CoA synthases 1 and 2 (by MTT assay). This growth inhibition was completely reversed by supplementation with mevalonate but not squalene, the immediate precursor of cholesterol biosynthesis. These results suggest that i)TAMr cells may be more dependent on the mevalonate pathway for growth/survival than TAMs cells as a consequence of upregulated HMGCS2 and ii) this is likely mediated through the isoprenoid synthesis/protein prenylation branch of the mevalonate pathway, not cholesterol biosynthesis. The combined effects of hymeglusin with 4-hydroxytamoxifen (4HT) were greater than that of either drug alone in both TAMr and TAMs cells ($P <0.01$). However, this sensitising effect was achieved with lower doses of hymeglusin in TAMr compared to TAMs cells. In conclusion, blockade of the mevalonate-isoprenoid pathway through HMG-CoA synthase inhibition is able to re-sensitise TAMr cells to 4HT, and may represent a future therapeutic target for the treatment of TAMr breast cancer.

# P6

***This abstract has been withdrawn.***

# P7

***This abstract is not available for publication before the Meeting.***

# P8

### Breast Needle Core Biopsy Diagnosis of Papillary Carcinoma - Implications of a B5c Categorisation

Ⓟ PA Bennett; M Singh; R Linforth; PJ Carder

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Papillary carcinoma of the breast may be diagnosed on needle core biopsy but it may be difficult, if not impossible, to decide if the tumour is invasive. A "B5c" designation may be appropriate if invasion is "not assessable".

We reviewed 20 core biopsy-diagnosed "B5c" papillary carcinomas from our unit from between 2005 and 2010 in order to further understand the implications of this diagnosis. Findings on needle core biopsy and subsequent histology were correlated to determine the likelihood of invasive malignancy and lymph node metastasis.

Follow-up histology was available for 17 cases. Of these 9 (53%) had a final diagnosis of invasive or likely invasive malignancy. Subsequent diagnoses were invasive papillary carcinoma (3), invasive carcinoma arising on a background of solid papillary carcinoma (3), encapsulated papillary carcinoma (EPC) with foci suspicious of microinvasion / invasion (2), and invasive tubular & lobular carcinoma adjacent to an EPC. Seven cases (41%) had a final diagnosis of in-situ disease, including EPC (4) and papillary ductal carcinoma in-situ (DCIS) (3). One case (6%) had no residual disease at excision. Nodal disease was identified in 4 of the 11 patients who underwent axillary surgery. Three cases had isolated tumour cells (ITC) only. Macrometastatic disease was identified in a single case, associated with a 70mm invasive papillary carcinoma.

In summary, there was 53% chance of concurrent invasive malignancy following a core biopsy diagnosis of a "B5c" papillary carcinoma. The likelihood of significant nodal disease was very low. The decision to perform an axillary procedure should follow detailed clinico-pathological correlation at the multi-disciplinary team meeting.

# P9

## Audit of B3 Breast Core Biopsies

Ⓟ L Moore[1]; A Graham[1]; L Carson[1]; SD Heys[2]; M Fuller[3]; I Miller[1]

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*Introduction*: Core needle biopsy is an accurate and cheap test for the diagnosis of breast lesions, and can be safely performed in the out-patient setting. However, borderline histology lesions or "lesions of uncertain malignant potential" (B3) are a significant sub-group.
*Aims*: The aim of this study was to correlate B3 needle core biopsy findings with those in the surgical excision specimens to determine associated rates of malignancy.
*Methods*: We identified all B3 core needle biopsies performed at Aberdeen Royal Infirmary over a 6-year period from 2004-2009. The needle core biopsy pathology reports were reviewed and correlated with the diagnosis in the excision specimens. Cases where no subsequent excision reports were available or those where multiple biopsies had been obtained, were excluded.
*Results*: The total number of cases was 181. All the patients were women. The most frequent lesions on needle core biopsy were: atypical intraductal epithelial proliferation (AIDEP) 46; papillary lesions (PL) 45; Radial scar/complex sclerosing lesion (RS/CSL) 33 and phyllodes tumour (PT) 17. The final diagnosis was malignant in 43 patients (overall malignancy rate 24 %). Nine of the patients with a malignant diagnosis had invasive carcinoma. The lesion specific rates of malignancy were: lobular in-situ neoplasia (LISN) 57%; flat epithelial atypia (FEA) 37.5%; AIDEP 30%; PL 20%; PT 6 % and RS/CSL 0%.
*Conclusions*: Nearly a quarter of all B3 needle core biopsies proved to be malignant on excision. Specific lesion types are associated with highly variable rates of malignancy. Further research is required to investigate lesion specific malignancy rates particularly in the recently emerged groups of lesions such as FEA.

# P10

## A Falsely Positive (C5) FNAc from a Lymph Node with Benign Vascular Transformation of the Sinuses (VTLNS)

Ⓟ T Grigor; H Jones

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A 70 year old woman presented with a symptomatic breast lump while part of the breast screening programme. Mammography demonstrated a calcified mass, 30mm diameter and a 9mm, radiologically indeterminate, ipsilateral axillary lymph node. Ultrasonography of the symptomatic mass was sonographically malignant (U5). The axillary tail lymph node had an sonographically indeterminate echogenic centre. Ultrasound guided needle core biopsy showed Grade 2 IDC. The cytology smear from the axillary lymph node was reported as falsely positive for carcinoma cells (C5). A right mastectomy and axillary node clearance was performed. Histological examination demonstrated a Grade 3 IDC with high grade comedo ductal carcinoma in situ. Pathological node status of the specimen was ascertained from 19 lymph nodes in the tail of the mastectomy specimen (level I nodes), nine in a separate piece of tissue incorporating level II nodes (largest 13mm) and 4 level III nodes in a piece of apical tissue. All 32 lymph nodes examined were free of tumour (pT2, pN0, pMx). However several showed characteristic VTLNS with an intra-sinusoidal proliferation of endothelial cells stainable for VWF accompanied by an intra-sinusoidal fibrous reaction. Pre-operative staging of the axilla using FNAc can triage women with operable breast cancer prior to an initial nodal surgical procedure. VTLNS is an example of a benign process that can simulate metastatic involvement of a lymph node by carcinoma diminishing the accuracy of this test.

# P11

## Core Biopsy Predictors for Positive Retroareolar Tissue in Nipple-Sparing Surgery for Breast Malignancy

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*Purpose* In selected breast cancer cases, nipple-sparing surgery results in improved cosmesis and recurrence rates. We aim to evaluate the pathological features on core biopsy and whether they are predictive of involvement of retroareolar tissue at subsequent resection.
*Methods* All 78 cases of therapeutic nipple-sparing procedures from 2004 to 2009 were retrieved from a prospectively maintained database. Slides of initial core biopsies and subsequent retroareolar frozen sections or fixed retroareolar tissue sections were reviewed by two pathologists. Presence or absence of malignancy in the retroareolar tissue was correlated with core biopsy features.
*Results* Fifty-five biopsies showed invasive carcinoma (39 ductal, 9 lobular, 7 mixed and 1 micropapillary types), and 23 showed in situ carcinoma. In 67 cases the retroareolar tissue showed benign histology; 4 cases showed ductal carcinoma in situ; 3 cases showed invasive carcinoma; 1 was suspicious for malignancy; 2 cases showed lobular in situ neoplasia and 1 case showed ductal atypia. Of the 7 malignant cases, the biopsy diagnoses were invasive ductal carcinoma (5), lobular carcinoma (1) and DCIS (1). 2/8 (25%) cases with lymphovascular invasion (LVI) on biopsy had positive retroareolar tissue, compared to 4/47 (8.5%) cases without LVI. 2/12 (16.7%) oestrogen receptor (ER) negative biopsies had positive retroareolar tissue, compared to 5/65 (7.7%) of ER positive cases. These findings were not statistically significant. There was no significant association between tumour type, grade or presence of DCIS on core biopsy and presence of malignancy in retroareolar tissue.
*Conclusion* The rate of retroareolar tissue involvement in nipple-sparing procedures was low (8.9%). Lymphovascular invasion and ER negativity on biopsy were associated with positive cases, although numbers were small. These features may be useful in patient selection in addition to current criteria.

# P12

## Investigating the Re-excision Rate in Patients with Breast Carcinoma

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Introduction: Infiltrating lobular breast carcinoma is associated with an increased rate of multifocality. It has been suggested that pre-operative MRI may identify patients with multifocal disease and stratify those requiring primary mastectomy. The aim of this study was to determine the re-excision rate in patients undergoing curative surgery for breast carcinoma.
Methods: We analysed clinico-pathological data from 2816 consecutive patients who underwent curative surgery for breast carcinoma in a single centre between January 2004 and December 2009.
Results: We found that 12% of patients with unifocal ductal carcinoma required re-excision compared with 19% of patients with unifocal lobular carcinoma. Fifteen percent of patients with multifocal ductal disease required a re-excision. In contrast, 28% of patients with multifocal lobular carcinoma required a re-excision for residual invasive disease. We noted that 34% of patients with multifocal lobular carcinoma (n=12) had primary conservation and of these patients, 75% required a second operation with the majority of those patients requiring a completion mastectomy for residual invasive disease. Of note, only 33% of patients with multifocal lobular carcinoma were known to have multifocal disease prior to primary surgery compared with 50% of patients with multifocal ductal carcinoma.
Conclusion: In conclusion, 19% of unifocal invasive lobular carcinoma patients required re-excision compared with 12% of unifocal ductal type. Of the multifocal carcinomas, 28% of lobular type and 15% of ductal type required re-excision. Only 33% of all multifocal lobular cancers were known to be multifocal pre-operatively compared with 50% of cases of multifocal ductal disease. This suggests that MRI may be helpful in detecting multifocality and in reducing re-excision rates, particularly in cases of invasive lobular carcinoma.

# P13

## Papillary Carcinoma of the Breast - Predicting Behaviour

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Papillary carcinomas are a rare morphological type of breast cancer. Controversy remains over whether these lesions are in-situ or invasive cancers, and the role of myoepithelial cell in differentiating between them. In this study, we reviewed 167 cases of papillary carcinoma diagnosed at the Department of Histopathology Nottingham, UK. Of these cases, 106 were consultation cases in which lymph node status and/or follow-up data were unavailable. In the 61 cases where clinical and follow-up data were available, 48 lesions were pure papillary carcinomas while 13 cases showed associated invasive carcinoma of different histologic type; the latter were excluded from the analysis. Lymph nodes were sampled in 18 of the 48 pure papillary cancers and in 3 of these cases micrometastatic disease was identified (with 1 involved node per case). In an additional case, regional recurrence in the lymph nodes was identified after a period of 2 years (2 out of 13 nodes were positive). None of the cases were reported to have distant metastases during the period of follow-up (average 10 years). In all 4 cases of papillary carcinoma with positive nodes, the primary tumour showed a high nuclear grade. Importantly, the absence of myoepithelial cells cannot differentiate between papillary carcinomas which have the potential for metastatic disease and those which remain localised. In conclusion, our study supports the concept that some forms of papillary carcinoma, particularly those of high cytonuclear grade behave as an invasive variant of breast carcinoma which is associated with an excellent prognosis. Sentinel node biopsy may be warranted in these cases.

# P14

## Comparison of ER Assessment Using Three Antibodies Optimised for use with an Automated IHC Stainer System

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Purpose of the study: Immunohistochemical (IHC) assessment of oestrogen receptor (ER) in breast cancer has a significant predictive value in identifying poor patient response to tamoxifen or aromatase inhibitor therapy. Recent changes in guidelines have suggested that patients with very low positive ER status determined by IHC (1% of cells) could still benefit from treatment. We have evaluated three ER clones (1D5, 6F11, SP1) in invasive breast cancers to determine any difference in staining patterns and subsequent effect on clinical treatment.
Method: A consecutive series of invasive breast carcinomas (n= 250) were used to construct tissue microarrays (TMAs). IHC was carried out using all three clones on an automated staining system. Two observers scored TMAs independently using the Allred ER scoring method. Clinical cut-offs (Allred < 3 = negative) were evaluated with positive scores subdivided into low and high ER expression levels (Allred 3-6 & 7-8 respectively).
Summary of results. Mean Allred scores for each antibody were 5.23 (6F11), 4.80 (1D5) & 4.77 (SP1). Negative ER values were: 1D5 = 22.6%, 6F11 = 25.4%, SP1 = 26.9%. Low ER expression levels: 1D5 = 5.6%, 6F11 = 7.3 %, SP1 = 13.3%. High ER expression levels: 1D5 = 71.8%, 6F11 = 67.3%, SP1 = 59.8%.
Conclusions: Most ER values are at Allred score extremes (0,7,8) and result in no significant difference in current clinical practice. However, variation exists, in small numbers, between clones at current clinical cut-off thresholds (Allred <3) and low expression levels. These require further study.

# P15

## Immunohistochemical Profiling of Ductal Carcinoma In Situ of the Breast

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Purpose of the study: Molecular profiling has given rise to distinct groups of invasive breast cancer. Basal-like, Luminal A, Luminal B and Her2 cancers can be identified using immunohistiochemistry (IHC) as a surrogate protocol to these genomic methods. Ductal carcinoma in situ (DCIS) is a precursor lesion that may or may not result in progression into these cancer subtypes. Like invasive breast cancer, it is becoming apparent that DCIS is not a single entity but multiple disease subtypes. We have applied a panel of antibodies (ER, PR, Her2, EGFR, CK5, CK14) to cases of DCIS to determine if IHC can be used to assign these precursor lesions into similar cancer subgroups.
Methods: A consecutive series of DCIS (n= 280) was used to construct tissue microarrays (TMAs) comprised of 2.00mm cores to retain tissue morphology. IHC was carried out using seven antibodies on an automated staining system. Two observers scored TMAs. ER and PR were scored using the Allred method, Her2 with the Dako Herceptest recommendations. All other antibodies were scored as percentage of positive cells.
Summary of Results: DCIS positive cases: ER 70%, PR 52%, Her2 22%, CK5 33% & CK14 36% EGFR 2%. Frequency distributions were calculated and non parametric tests gave significant P-values for ER v Her2 (< 0.0001), PR v Her2 (< 0.0001), and EGFR v ER and PR (< 0.0001, 0.0017), CK14 v Ck5 (< 0.0001), ER v PR (< 0.0001).
Conclusions: DCIS profiling using an IHC panel show some features common to molecular profiling of invasive breast cancers. Our series shows similarities with other published profiling data on DCIS. Further studies will determine the clinical relevance of this immunoprofile.

# P16

## Pseudoangiomatous Stromal Hyperplasia: A Case Report

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INTRODUCTION: Pseudoangiomatous Stromal Hyperplasia (PASH) is a rare benign proliferating breast condition that was reported in 1986. We describe a large PASH, mimicking inflammatory carcinoma in a young lady that was excised with excellent cosmetic results.
CASE REPORT: A 22-year-old lady presented with one year history of a progressive large lump in the right breast. Clinically, the breast was diffusely erythematous with a 10x8cm suspicious mass mimicking inflammatory carcinoma. Radiologically the appearances suggestive of a fibroadenoma or Phyllodes tumour. Fine needle aspiration cytology results were equivocal showing cohesive cells with focal mild nuclear atypia and numerous bare nuclei. Histopathology showed the presence of anastomosing groups of spindle cells in the stromal fibrous tissue in a pseudoangiomatous pattern which fitted the diagnosis of PASH. Immunohistochemistry was positive for CD34 and Vimentin while Factor VIII, cytokeratin, progesterone and oestrogen receptors were negative. Though the mass was large, surgical excision was done using an inferior circumareolar incisionwith excellent cosmetic results.
DISCUSSION: PASH is a benign proliferation of the mammary stromal tissue. It is rare for a discrete mass to have PASH as the main pathological. These tumours are classified into either simple or fascicular/proliferative subtypes. It is important to differentiate the lesion from low grade angiosarcoma. Core biopsy or Mammotome biopsy is needed for diagnosis preoperatively, although in some cases the diagnosis is made only after excision. On immunohistochemistry, PASH is positive for CD34 and vimentin and negative for factor VIII-related antigen and cytokeratin. If diagnosis is confirmed on biopsy and the lesion is small, surgical excision can be avoided but for the larger lesions, and especially if there are suspicious features then excision is indicated.

# P17

## Potential Roles of Corticotropin-Releasing Hormone (CRH) in Breast Cancer

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Carcinoma of the breast is the most frequently occurring malignant tumour in women and one of the leading causes of cancer-related deaths. It has recently been proposed that corticotropin-releasing hormone (CRH) might act as a negative regulator of oestrogen-induced breast cancer cell growth. Through characterisation of downstream signalling events, it might be possible to find new targets for breast cancer therapy.
To explore the role of CRH in breast cancer in vitro, we used the MCF-7 cell line as a model for oestrogen-receptor positive breast cancer. Here we show that CRH receptor mRNA is expressed in MCF-7 cells, with functional CRH receptor proteins expressed primarily in the plasma membrane of these cells. We identified that CRH actions are mediated via activation of multiple kinases, suggesting a complex signalling network. We demonstrate that CRH stimulates p38MAPK phosphorylation. CRH was found to inhibit basal ERK1/2 activity whilst having no effect on E2-stimulated ERK1/2 activity. Furthermore, we observed that CRH stimulates GSK-3β activation and downstream β-catenin phosphorylation. These findings provide new insights into a number of molecules which may have a role in breast cancer cell growth, and specifically provides further understanding of the mechanisms by which CRH is able to influence breast cancer cell growth, with the evidence suggesting involvement of the Wnt/β-catenin signalling pathway.
This research was supported by a Pathological Society Undergraduate Elective Grant

# P18

## Aetiology of Myocardial Infarction with Normal Coronary Arteries in Sudden Cardiac Death (SCD)

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Most myocardial infarcts (MI) are due to atheroma. However, MI that presents with normal coronary arteries poses a challenge to both clinicians and pathologists. Clinicians define this entity as myocardial infarction with angiographically normal coronary arteries or MINCA, which has been shown to occur with a higher prevalence in those <50 years of age and is associated with coronary artery spasm. It is impossible to prove coronary artery spasm at autopsy and it is diagnosed by exclusion of other causes. In our database we identified 18 SCD cases (10 males and 8 females) with mean age of 36.1±17.5 years (range 16-84) and four cases were ≤18 years. Risk factors that may have predisposed a coronary artery spasm were identified in 13 cases; these included a history of alcohol and/or drugs (n=6), exertion with sport or restraint (n=2), pregnancy (n=1), general anaesthesia (n=1), epilepsy (n=1), hypertension (n=1) and smoking (n=1). The regional location of the infarcts varied but affected mostly the anteroseptal wall (left anterior descending territory). Other cases that will be presented include a posterobasal infarction (right coronary artery territory) and a complete left ventricular and subendocardial infarction. Pathologists need to be aware of this entity and the risk factors that can trigger it particularly alcohol/drugs or exertion. Therefore, toxicology and the historical information including the circumstances of death are particularly important in these cases.

# P19

## Benign Primary Cardiac Tumours associated with Sudden Death

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Background Sudden cardiac death (SCD) accounts for 50% of cardiovascular mortality with an estimated annual toll of 300 000 deaths in the USA and 60 000 deaths in the UK. Benign primary cardiac tumours are extremely rare and although many of these entities are clinically silent, a few can cause significant morbidity or even sudden death. Objective To raise awareness of benign primary cardiac tumours as a possible cause of SCD so that more cases are identified by pathologists during life. Methods and Results Between 1994 and 2009, the hearts of more than 1600 people undergoing SCD were referred for pathological assessment to ascertain the precise aetiology of SCD. Thirteen cases of SCD associated with benign primary cardiac tumours were identified; four were cystic tumours of the atrioventricular (AV) node, two rhabdomyomas, two lipomas, two inflammatory myofibroblastic tumours (IMT), one fibroma, one haemangioma and one paraganglioma. Conclusion This study highlights that benign primary cardiac tumours are exceptionally uncommon yet potentially lethal. Careful examination of the heart will identify most of these lesions. A major exception, however, is cystic tumour of the AV node, the most common primary cardiac tumour causing sudden death. Since the gross findings of this lesion may be minimal, we recommend taking a routine section of the conducting system, including the AV node, in all cases of sudden death.

# P20

## Malignant Mesothelioma Presenting as an Anterior Abdominal Wall Mass: An Unusual Presentation.

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Malignant mesothelioma is a rare and aggressive malignancy of the pleura. Primary presentation outside the pleural space is extremely rare with this being only the third case reported within the literature.
A 54 year old former plumber with a history of asbestos exposure was referred to the surgical outpatient department by his GP, after having refractory left anterior abdominal wall pain for 2 years. Clinical examination revealed a firm, nodular, superficial mass extending from the left sub-costal region to iliac crest and from the midline to anterior axillary line. Radiological imaging with CT and MRI scans showed an oedematous, haemorrhagic 19×14.5×2.7 cm soft tissue mass infiltrating all the muscle layers of the anterior and lateral abdominal wall. There was mild ascites, but no radiological evidence of thoracic or peritoneal disease or invasion. The initial core biopsy showed only features suggestive of fibrois and inflammation. Subsequently, he had 2 further biopsies before we could confirm the diagnosis of malignant epitheloid mesothelioma based on the morphology and immunohistochemical staining.
The patient is presently undergoing chemotherapy with Pemetrexed and Cisplatin as the lesion was considered unresectable. Being large and closely related to the small bowel radiotherapy was not felt to be suitable.
This is the first reported case of malignant mesothelioma without any associated pleural or peritoneal disease treated with primary chemotherapy.Maligant mesothelioma is rather difficult to manage with surgery, radiotherapy and chemotherapy alone or in combination. The emphasis should be on identification of such unusual presentations early enough to optimise the clinical outcome.

# P21

## Synchronous DNA Methylation Changes of Wnt Signalling Antagonists Occur During Colorectal Cancer Progression

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Background: Colorectal cancer (CRC) is the third most common cancer in the UK. Wnt signalling plays an important role in initiation and maintenance of CRC. DNA methylation of Wnt pathway antagonists has been proposed to increase Wnt activity. No data are available to define DNA methylation changes of known Wnt antagonists during CRC progression from normal to adenoma to adenocarcinoma. We therefore analysed DNA methylation changes of known Wnt antagonists in matched set of normal, hyperplastic or adenomatous polyps and carcinoma tissue samples obtained from colectomy specimens.

Method: Using pyrosequencing assays we profiled methylation changes of a set of CpG islands associated with the Wnt signalling antagonists *SFRP1*, *SFRP2*, *SFRP4*, *SFRP5*, *WIF1*, *DKK1*, *DKK2*, *DKK3*. In addition, we examined DNA methylation status of two other non-antagonist genes involved in Wnt siganlling (*CDH1* and *DVL2*) for comparison. We analysed a set of 48 cases comprising matched normal (n=73), hyperplastic polyps (n=7), adenomas (n=44) and adenocarcinoma (n=48) tissue samples.

Results: We identified significant widespread hypermethylation changes of the above Wnt antagonists in the transition from normal to hyperplastic polyp to carcinoma and from normal to adenoma to carcinoma mostly in a stepwise pattern ($p<0.05$, Wilcoxon rank sum test). *DKK1* showed no significant change, SFRP4 was not significantly hypermethylated during the adenoma to adenocarcinoma progression and *DKK3* did not show significant methylation changes during the hyperplastic polyp to carcinoma stage. The non-antagonist Wnt genes, *CDH1* and *DVL2* showed consistent normal levels of methylation throughout CRC progression.

Conclusion: Synchronous widespread stepwise hypermethylation selectively targets Wnt antagonists during CRC progression. CpG island hypermethylation of Wnt antagonists could be a biomarker for early detection and progression of CRC.

# P22

## Contribution of the BRCA/FA Pathway to the Radiation-induced DNA Damage Response in Non-targeted Cells

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Purpose: This study aims to identify potential molecular targets for differential modulation of radiation responses in directly irradiated and non-targeted cells.

Methods: Filtered medium from X-irradiated human cell cultures was transferred to non-irradiated cultures to determine DNA damage signalling and colony formation in bystander cells. Samples were analysed by immunofluorescence microscopy and flow cytometry.

Results: BRCA1 and FANCD2 formed foci and co-localised with gamma-H2AX/53BP1 foci in non-targeted T98G cells, suggesting the activation of the BRCA/FA DNA repair pathway in the bystander response. Formation of FANCD2 foci in non-targeted ATM-mutant GM05849 but not in ATR-deficient F02-98 hTERT cells demonstrated ATR but not ATM dependency of FANCD2 foci in bystander cells. BRCA1 bystander foci were ATM dependent. BrdU pulse labelling showed accumulation of BrdU positive bystander cells at an intra-S-phase checkpoint in T98G and 48BR hTERT cells but not in ATR and ATM mutants. Phospho-Chk1 foci formation was observed in T98G bystander cells, in keeping with the known function of Chk1 as a main downstream target of ATR which is involved in the activation of the BRCA/FA pathway. Clonogenic survival assays showed moderate radiosensitisation of directly irradiated cells by the Chk1 inhibitor UCN-01 but increased radioresistance of bystander cells similar to observations in ATR deficient cells reported previously.

Conclusion: In addition to ATR, BRCA1, FANCD2 and Chk1 are further potential molecular targets for differential modulation of radiation responses in directly irradiated and non-targeted cells.

# P23

***This abstract is not available for publication before the Meeting.***

# P24

## In Vitro Generation of Mature Human Osteoclasts

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Osteoclasts form from $CD14^+$ mononuclear precursors in the presence of macrophage-colony stimulating factor (MCSF) and receptor activator for nuclear factor κB ligand (RANKL). Osteoclasts can be generated *in vitro* from peripheral blood mononuclear cells (PBMCs) cultured with MCSF and RANKL but it is not possible to distinguish the effect of a specific agent on osteoclast activity as opposed to osteoclast differentiation using this system. We have therefore developed a method of obtaining a population of mature human osteoclasts to study specifically osteoclast resorption activity.

$CD14^+$ PBMCs from human buffy coat blood were cultured on Lumox hydrophobic dishes with MCSF (25ng/ml) + RANKL (50ng/ml). After 19 days the cells were then seeded onto coverslips and dentine slices and cultured for a further 1-4 days (+/- osteoclast resorption inhibitors). After culture the coverslips were stained for osteoclast markers and the dentine slices were assessed for evidence of lacunar resorption. Multinucleated cells (10-50 nuclei) expressing TRAP, VNR, and Cathepsin K formed on hydrophobic dishes between day 7 and day 10 and increased in number until day 19. These cells stained for F-actin rings and were capable of resorption after 24 hours in culture. The addition of calcitonin, osteoprotegerin, and zoledronate directly inhibited lacunar resorption pit formation by these cells.

This method of mature human osteoclast generation should provide a valuable means of assessing the specific effect of molecular factors (e.g. cytokines, growth factors, hormones) and therapeutic agents on mature human osteoclast activity. It should also permit analysis of differences in the resorbing activity of abnormal osteoclasts in conditions such as Paget's disease and giant cell tumours of bone.

# P25

## *MGMT* Methylation is Strongly Associated with and May Precede *IDH1/IDH2* Mutations in Adult Astrocytic and Oligodendroglial Tumours

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Gliomas are the most common group of primary brain tumours. They include astrocytic (including glioblastoma), oligodendroglial and mixed gliomas. *IDH1/IDH2* mutations are found in more than 50% of astrocytic and oligodendroglial tumours of grade II and III and considered to be an early change. Presence of *MGMT* methylation has been shown to predict a good response to temozolomide and improved survival. A problem for clinical *MGMT* methylation testing is the lack of consensus on what area of the *MGMT* CpG island (CGI) should be analysed. The aim of this study was to 1) define the optimal target region for *MGMT* methylation-testing by bisulfite modification and pyrosequencing 2) profile methylation at each CpG in *MGMT* CGI in a large cohort of gliomas and 3) correlate *MGMT* methylation with other genetic abnormalities including *IDH1/IDH2* mutations.

The methylation status of each CpG in *MGMT* CGI was determined and compared with *MGMT* mRNA expression in 22 glioblastoma xenografts, 13 glioma cell lines and 6 normal brain tissues. A luciferase assay was used to investigate selected CpGs for their role in transcription. The optimised pyrosequencing assay was then used to investigate 406 astrocytic and oligodendroglial tumours of all major types.

We identified a 120 bp region containing 16 CpG sites to be most critical in transcriptional control. Methylation in this region was present in 58% of 406 gliomas. We found that *MGMT* methylation was 100% concordant with *IDH1/IDH2* mutations in astrocytoma grade II and all oligodendrogliomas. All glioblastomas with *IDH1/IDH2* mutations also had *MGMT* methylation, while the majority of glioblastomas with *MGMT* methylation did not have *IDH1/IDH2* mutations.

We propose a robust pyrosequencing assay to accurately assess *MGMT* methylation. Our data suggests that *MGMT* methylation is the earliest change in the development of astrocytomas and oligodendrogliomas, preceding *IDH1/IDH2* mutations.

# P26

## The Expression of Thyroid Hormone Receptors in Colorectal Carcinoma

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Background & Aims: Among the aetiological factors of colorectal cancers (CRCs), hormones have also been known to play a role. Thyroid hormones have also been indirectly linked to the development of CRCs. They exert their effects through receptors (THRs) and in previous studies, upregulated THR-β1 was found to be associated with polypoid growth and K-ras mutation. The aim of this study was to investigate the expression of THRs (α & β) in CRCs, their normal adjacent bowel and lymph node metastasis. Comparisons were also made with the cell proliferation markers Ki-67 and PCNA.

Methodology & Results: Standard immunohistochemistry techniques using commercially available antibodies were used to determine receptor immunoreactivity (IR). A 'Modified Quickscore' technique was used to score the intensity and density separately and a multiple was obtained. These were statistically evaluated using t-test to obtain P-values. Comparisons were made by gender of the patients, differentiation and Dukes stage to evaluate if this had any effect on receptor expression.

THR IR (both α and β) was significantly reduced in the tumour tissue, adjacent normal bowel and lymph node metastasis compared to normal controls ($P<0.01$). There was a significant direct correlation between THR-α IR and gender of the patient, differentiation of the tumour and Dukes stage ($r=0.4$, $P<0.001$). THR-β IR however, had a significant direct correlation with tumours in females only. There was a significant direct correlation between Ki-67 IR and THR-α IR in the lymph nodes ($r= 0.32$, $P<0.03$), but not in cancer tissue ($P>0.2$). No correlation was found between Ki-67 and THR-β in any tissue ($P>0.2$). No correlation was found between PCNA and THR-α or THR-β ($P>0.1$).

Conclusion: There is no evidence to support the hypothesis that thyroid hormone receptors have a role in the pathogenesis of colorectal cancers.

# P27

## The Expression of Growth Hormone Receptors in Colorectal Carcinoma

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Background and Aims: Among the aetiological factors of colorectal cancers (CRCs), hormones have also been known to play a role and growth hormone has been indirectly linked to the development of CRCs. It exerts its effects through receptors (GHRs) and previously, upregulation of GHRs has been found. Except for one, these studies did not use normal controls. The aim of this study was to investigate the expression of GHRs in CRCs, their normal adjacent bowel and lymph node metastasis. Comparisons were also made with the cell proliferation markers Ki-67 and PCNA.

Methodology and Results: Standard immunohistochemistry techniques using commercially available antibodies were used to determine receptor immunoreactivity (IR). A 'Modified Quickscore' technique was used to score the intensity and density separately and a multiple was obtained. These were statistically evaluated using t-test to obtain P-values. Comparisons were made by gender of the patients, differentiation and Dukes' stage to evaluate if this had any effect on receptor expression.

GHR IR was significantly reduced in tumour and adjacent normal bowel compared to normal controls ($P<0.001$). The metastatic lymph nodes however showed an increased GHR IR ($P<0.001$). There was a significant direct correlation between GHR IR and well-to-moderate differentiation of the tumour and Dukes' stages B and C ($r=0.3$ &0.4, $P<0.02$). There was no correlation of GHR IR with gender of the patient ($P>0.1$). There was a significant direct correlation between Ki-67 IR and GHR IR in the cancer tissue ($r= 0.33$, $P<0.001$). No correlation was found between Ki-67 and GHR IR in the lymph nodes ($P>0.2$), or between PCNA and GHR in any tissue ($P>0.1$).

Conclusion: There is no evidence to support the hypothesis that growth hormone receptors have a role in the pathogenesis of colorectal cancers. The changes in IR within lymph node metastases may be without a cause and effect relationship.

# P28

## Audit of Sentinel Node Handling and Reporting in Kent

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Sentinel lymph node biopsy (SLNB) is rapidly becoming the standard of care in staging the axilla for early stage, clinically node negative breast cancer. This audit reviews the laboratory handling & pathological reporting of sentinel nodes (SNs) across Kent and has been performed at the request of the Kent Cancer Network Breast Disease Oriented Group (DOG).

A retrospective analysis of 20 consecutive breast SN cases from each of the 5 Kent hospitals was carried out. All reports & slides of positive SNs were reviewed. Detailed structured questionnaire based enquiries to lead breast pathologists were made. Data was extracted in Microsoft Excel & analysed in relation to the standards. All aspects of the pathology service were examined, including macroscopic data (number of SNs / case, dimensions, sampling and slicing details) and microscopic data (performance of levels & immunohistochemistry , TNM staging & SNOMED coding).

One site was unaware of radioactivity guidelines while another was unaware of national guidelines. 28% to 84% of SNs were bisected longitudinally and levels were sparingly used, except at 1 site. TNM staging and SNOMED coding in the reports were varied.

This audit demonstrates substantial variation in SN handling and reporting practice across Kent. Since the quality of the pathology service in breast cancer is of paramount importance, it is imperative that a standardised approach is achieved in the handling and reporting of SNs. To achieve this, recommendations have been suggested and agreed upon, the success of which will be measured in a re-audit next year.

# P29

## An Audit on the Reporting of Prognostic Factors in Basal Cell Carcinoma

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Basal cell carcinoma (BCC) is the most common invasive malignancy in Europe. In our hospital some punch biopsies as well as excision specimens are performed with a therapeutic intent. Reporting should include all known prognostic factors which can be accurately deduced. In 2009, the North London Cancer Network (NLCN) issued evidence-based minimum dataset guidelines for BCC reporting. Although subtype is an important prognostic factor, there is no universally agreed subtype classification system. The aim of the audit was to identify the percentages of BCC reports which included the minimum prognostic factors with respect to the NLCN Guidelines. The diagnostic consistency of subtyping into high and low risk categories was also assessed. 60 cases of BCCs reported in 2008 and 2009 were selected via a SNOMED code search of the database. Pathology reports and H&E slides were reviewed with the lead consultant for skin pathology.

The sample included 28 excisions and 26 punches reported by 7 consultants. Subtype was reported in 82% of excisions and 65% of punches. Distances to both margins were reported in 75% of excisions and 46% of punches. For excisions, presence/absence of perineural invasion was reported in 46% (no cases missed) and size was reported in 96%. Histological review showed concordance for all cases reported as a high risk subtype (19/19). 10% (2/21) of cases reported as a low risk subtype had a high risk component on review.

In conclusion, size, subtype and margins were reported in a high percentage of excisions. Subtype and margins were less frequently reported for punches. This partly reflects difficulty in accurately determining features in small specimens and lack of clarity from the clinicians as to which punches are therapeutic. Histological review showed high concordance for reporting of high and low risk subtypes, suggesting this important distinction is consistent and reproducible within our department.

# P30

## Can Cervical Sampling be Reduced in Benign Hysterectomy Specimens?

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In the current climate of budgetary constraints we must rationalise our handling of surgical specimens in order to reduce workload and expenditure. Current recommended practice is to sample both the anterior and posterior cervical lips of non malignant hysterectomy specimens. This retrospective audit sought to determine whether there is a justification for reducing this to a single cervical block. A sample of 100 hysterectomy specimens received by our department during 2009 were reviewed. In each case there was no stated clinical suspicion of malignancy. Our findings indicate that unexpected clinically significant disease occurred in 2% of specimens and may have been missed in 1% had sampling of the cervix been reduced to a single block. We therefore support the current guidelines.

# P31

## An Audit on the Diagnostic Accuracy of Cervical Biopsies in Determining Cervical Lesions

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Purpose of study: This audit was carried out to assess the accuracy of the cervical biopsy done during colposcopy in comparison with the diagnosis from the subsequent cone excision. In addition, the impact of specialist gynaecological histopathology reporting, as compared with general histopathology reporting was analysed. Methods: Retrospective analysis was performed by examining the paired cervical biopsies and cones histopathology reports for cases reported from April 2004 to March 2005 (when cervical biopsies and cones were reported by general pathologists) and from January to December 2008 (when reporting by specialist gynaecological pathologists was instituted). The rates of consistent diagnosis, overdiagnosis and underdiagnosis between cervical biopsies and cone excisions were studied. Sensitivity and specificity rates were also estimated. Results: 150 women had both cervical punch and cone biopsies performed in the 2004-2005 period. In comparison, 149 women had both biopsies performed in the 2008 period. In 2004-5, the rate of consistent diagnosis was 68.7%, compared with 75.8% in 2008. This was due to a decrease in both the rates of overdiagnosis (16.7% vs. 14.8%) and underdiagnosis (14.7% vs. 9.4%), which was statistically significant. The sensitivity rates for 2004-5 and 2008 were 87.5% and 89.7%, and the specificity rates for the same periods were 39.8% and 39.4% respectively. Conclusions: Cervical biopsy is essential but has its limitations. The audit highlights the importance of the recommendation of the National Health Service Cervical Screening Programme that patient management should be based on co-ordinated information of smear results, history, colposcopy and cervical biopsies. The introduction of specialist gynaecological histopathology reporting has improved the rates of consistent diagnosis significantly, as well as improving the rates of sensitivity of cervical biopsy reporting.

# P32

## Is Routine Double Reporting Justified? An Audit of Negative Prostate Core Biopsies

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Purpose of the Study: Review of slides by a second operator as a means of reducing error is widely used in cytopathology, but less so in histopathology. Although blinded review by a second pathologist has been reported to reduce the rate of clinically significant error, the time and monetary costs associated with this are not insignificant.

The 2008 NICE guideline 'Prostate cancer: Diagnosis and treatment' recommends that all prostate biopsies be reviewed by a urological cancer MDT. NICE state that they do not envisage that all negative biopsies will be reviewed by the MDT pathologist, but do not explicitly state what form they expect this review to take.

The purpose of this audit was to determine whether routine double reporting of negative prostate core biopsies was justified in terms of additional workload and improved diagnosis of prostatic adenocarcinoma and prostatic intraepithelial neoplasia (PIN).

Methods: A total of fifty negative prostate core biopsies reported between September 2007 and December 2009 were reviewed by a consultant histopathologist and specialty registrar. Where there was disagreement between the original reporting pathologist and the reviewer, the case was shown to a second consultant pathologist. Additional immunohistochemistry was performed where necessary.

Summary of results: Three of fifty cases reviewed were re-classified: One contained high grade PIN, one contained an atypical small acinar proliferation and one contained both high grade PIN and an atypical focus suspicious for adenocarcinoma.

Reviewing one set of biopsies took at least ten minutes, not including interpreting immunohistochemistry or obtaining a second opinion.

Conclusions: Review of fifty cases resulted in change of diagnosis in three. In all Cases this would potentially have changed patient management. However double reporting is demanding of consultant time and there may be practical barriers to its institution.

# P33

## 'Brainless' Autopsy – an Audit

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The aim of this audit was too see how often and in what circumstances the brain is not examined at post-mortem.
Method: All adult post-mortem cases performed between January 1st and December 31st 2009 were reviewed. The number of cases in which the brain was not examined was noted together with the stated cause of death. After careful discussion we considered that brain examination was not strictly necessary in (1) acute myocardial infarction, especially with haemopericardium, (2) fresh coronary thrombosis, (3) ruptured aortic aneurysm, (4) large pulmonary embolus, (5) unequivocal bronchopneumonia in the elderly, (6) cancer patients with disseminated malignancy or recent chemotherapy, (7) patients who have spent a long period in intensive care.
Results: In 101 of 764 autopsies the brain was not examined. 65 cases (64%) fell into one of the seven categories described above. The other 36 cases (36%) did not fall into any of these categories and should, according to this remit, have had the brain examined. In contrast in 139 of the 663 cases (21%) in which the brain was examined had a cause of death that indicated that the brain need not be examined.
Conclusion: Relatives dislike brain examination and many ask for a limited examination. Our results show that brain examination could have been avoided in 26.7% of all autopsies. However in 36 cases (4.7%) the brain was not examined when according to our guidelines it should have been.

# P34

## Audit of Duodenal Biopsy Subsequent to Positive Coeliac Serology, and Reaudit of Coeliac Serology Testing after Histopathological Diagnosis of Lymphocytic Duodenosis

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Purpose of the study: To identify the rate of duodenal biopsy following positive serological test for coeliac disease as per the NICE guidelines, identify whether coeliac disease serology testing is done following a diagnosis of lymphocytic duodenosis on a duodenal biopsy, and to compare the results with a previous audit.
Methods: A list of patients who had a positive result on IgA tTG testing for coeliac disease and all duodenal biopsies done in 2009 was obtained. The pathology reporting system was checked to see if a duodenal biopsy was done in those who tested positive for coeliac disease on serology, and if serology for coeliac disease was done on those patients diagnosed with lymphocytic duodenosis.
Summary of results: 88 patients tested positive for coeliac disease on serological testing, with duodenal biopsy done in 43% of cases subsequently. 72 patients were diagnosed with lymphocytic duodenosis or features suspicious for coeliac disease on duodenal biopsy. Serology for coeliac disease was done in only 56% of these cases, of whom 15% had a positive result for coeliac disease. The previous audit showed that 66% of 55 patients with lymphocytic duodenosis had serological test for coeliac disease, of which 23% were found to be positive.
Conclusions: 57% of patients with a positive serological test for coeliac disease did not have a duodenal biopsy and 44% of patients diagnosed with lymphocytic duodenosis did not have a serological test for coeliac disease. The aim should be to do serological testing in all cases diagnosed with lymphocytic duodenosis. Improvement needs to be made on adherence to guidelines for the diagnosis of coeliac disease. A proposed algorithm for the approach to diagnosis of coeliac disease based on the NICE guidelines is being developed. A reaudit is proposed after 1 year to evaluate any improvement in following the guidelines to complete the audit cycle.

# P35

## Do Cell Blocks Add Value to Cytological Diagnosis?

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*Purpose of the Study:*The use of cell blocks for processing cytology fluids has been reported since 1947 when Chapman and Whalen (N. Eng.Med 237;15,192) first described the technique for serous fluids. They are a valuable ancillary tool for evaluation of non-gynaecological cytology specimens by enabling the cytopathologist to study morphological detail. They also allow for the evaluation of ancillary studies such as immunocytochemistry, in-situ hybridisation tests (FISH/cISH) and in-situ PCR. This study aims to assess the value of cell blocks in cytological diagnosis.
*Methods:*All non-gynaecological specimens for cytopathology that consisted of both smears and cell blocks over a three month period were reviewed and analysed. The 190 specimens comprised 119 FNAs from various sites, 28 EBUS FNAs and 43 body fluid and washing specimens. We retrieved the cell block slides and cytology reports for the 190 cases. The slides were reviewed by a cytopathologist for assessment of material, and after correlation with the cytology report, a conclusion was made regarding the contribution of the cell block e.g. confirmed cytological diagnosis but did not add new information, confirmed primary origin of tumour, confirmed subtype of lymphoma or confirmed benign nature of cells.
*Summary of results:*Cell blocks were essential for diagnosis in 26% of cases. For the different specimen sites their utility was as follows: body fluid and washing specimens (43%), EBUS FNAs(57%) , FNAs (9%) and thyroid FNAs (0%).
*Conclusion:*Overall, the cell block technique was contributory to the final cytological diagnosis, especially for fluid and EBUS FNA specimens. This supports the view that cell block preparation should be considered for most cytologic specimens after morphologic review. This study did not find cell blocks to be useful in the evaluation of benign thyroid nodules.

# P36

## THY Categorisation of Thyroid FNA's from 2008

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Purpose of the study:To highlight the importance of the guidelines issued by the British Thyroid Association for fine needle aspiration of the thyroid and to compare our practice to these guidelines.
Methods:Gathering retrospecitve data of all patients who underwent fine needle aspiration of the thyoid for the year 2008/2009 at Imperial College Hospitals. The data included information on the request form, the performer, and the method in which results were released.
Summary of results:98.8% were designated a THY category 179 cases (99.4%) were aspirated by competent aspirators. All THY 3, 4 & 5 are discussed through the weekly thyroid MDT. The majority of thyroid FNA's are performed by consultants that regularly perform FNA's. Only one case was performed by an aspirator not regularly perfoming this technique. There were only 2 cases that were not given a THY category. One of these cases was referred to the thyroid MDT for discussion. The side of the thyroid nodule being aspirated was not provided in the majority of cases.
Conclusions: The guidelines issued by the British Thyroid Association were followed to a high standard. All thyroid aspirates were reported by pathologists with a specialist interest in cytopathology. All THY 3, 4 & 5 cases are discussed at the weekly MDT. The majority of thyroid FNA's are performed by consultants that regularly perform FNA's. We conclude and suggest that request forms need to include the side being aspirated and to re-audit the changes for 3-6 months and close the audit loop.

# P37

## Paediatric Post-mortem Consent Audit

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Introduction: Paediatric and perinatal post-mortems are carried out by specialist Pathologists, predominantly in tertiary referral centres. The post-mortems and their interpretation are time and resource consuming but provide answers to important questions for parents and clinicians and are a rich resource for research, education, audit and quality assurance.
Given the opportunities that paediatric post-mortems provide and within the legal requirements of the Human Tissue Act 2004, it is important that the recently bereaved parents, consenting to a post-mortem, are given the opportunity to consent to donate diagnostic material for research, education, audit and quality assurance.
Method: A retrospective audit of consented hospital post-mortems carried out by the Paediatric and Perinatal Pathologists, over a one year period, at a London tertiary referral centre. The consent forms used provide information regarding the use of blocks and slides for diagnostic and non-diagnostic activities and give the parents the option to opt out of consent for retention and use in education, audit and quality assurance but asks specifically for consent for use in research.
Results: 98% of parents consented to retention of blocks and slides. Of these, 99% consented to use of blocks and slides for teaching, audit and quality assurance and 70% consented to their use for research. In 85% of cases, consent was taken by a doctor (predominantly Registrar level). Parents from a mixed or minority ethnic background had lower consent rates, especially in regards to research.
Conclusion: The findings of the review show encouragingly high rates of consent to retention of blocks and slides and for their use in a non-diagnostic setting. This information, especially that highlighting groups with low consent rates, is to be fed back to the clinicians as a tool for modifying clinical practice.

# P38

## Reliability of Histological Classification of Screen-detected Colorectal Polyps

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Background: The UK Bowel Cancer Screening Programme (BCSP) was introduced in 2007 to improve detection and management of early bowel cancers and pre-invasive lesions. Reporting guidelines were published to encourage diagnostic uniformity and ensure comparability of data between screening centres.
Aims: 1. To compare the histological characterisation of screen-detected colorectal polyps between two centres participating in the BCSP.
2. To explore the impact of inter-observer variation on tumour classification.
Method: A retrospective series of 1329 screen-detected polyps (2008-2010) was identified from computerised records at two histopathology departments participating in the BCSP. Reports were reviewed and differences in histological features assessed using chi square analyses. Slides from a sample of 239 polyps were exchanged between centres for histological review and measurement of inter-rater (kappa) agreement.
Results: There were significant between-centre differences in reported frequencies of tubular (57% v 43%) tubulovillous (18% v 16%) and villous (2% v 6%) adenomas, high grade dysplasia (5% v 16%) and suspected stromal invasion (3% v 9%). Histological review confirmed relatively low inter-rater agreement with respect to histological type (65%). Levels of concordance were highest for grade of dysplasia (77%) and the presence of invasive malignancy (77%).
Conclusions:
•There is marked inter-observer variation in the histological classification of colorectal polyps.
•In some instances, concordance may have been reduced by failure to provide macroscopic details to assist reviewers' assessments.
•Other differences, such as those observed for histological type, are unlikely to be of clinical significance.
•Appropriately, the highest levels of inter-rater agreement were seen for prognostic factors which guide clinical management.

# P39

## An Audit of Urgent Histopathology Requests

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Histopathological specimens marked as 'urgent' are prioritised. With increased work volume and pressure on turnaround times, requests inappropriately marked urgent may adversely affect overall service provision. An audit was performed to define the extent of the problem in terms of the volume and appropriateness of 'urgent' requests, with a focus on the adequacy of supplied clinical details.
*Methods:* No published guidelines were identified, but certain rudimentary standards were considered *de facto* requirements. These standards were: requester's name and contact details should be present and legible, request should ideally be made by consultant or general practitioner, request should indicate the clinical situation necessitating the urgency, suspected disease process should be life-threatening or serious enough to require immediate therapeutic intervention or cessation of treatment, and, the request should give a date by when the result is needed. All histology requests received during October 2009 within Maidstone and Tunbridge Wells NHS Trust were retrospectively analysed using paper and computer records with reference to the defined standards.
*Results:* Urgent cases accounted for 99 of 2517 cases (3.9%) and 251 of 8700 (2.9%) tissue blocks. 57% of forms had no legible name, 76% had no contact details and in 70% the grade of requesting doctor was unknown. All requests supplied clinical details, but the reason for urgency was often assumed rather than explicitly stated. Suspected or established cancer was the most common justification for urgency. Only 7 of 99 cases specified a date by which the report was required.
*Conclusion:* Adherence to the standards was poor. Using strict criteria, none of the requests were judged to be clinically urgent. The design of the request form was felt to be a contributory factor. Dissemination of the results to end-users and redesign of the form is planned prior to a second audit cycle.

# P40

## The Predictive Value of Thyroid Fine Needle Aspiration

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Background In 2009 the Royal College of Pathologists (RCPath) published the document *'Guidance of the Reporting of Thyroid Cytology Specimens'*. This includes a modification of the 2007 British Thyroid Association 'Thy' scoring system and the expected rates of malignancy for each Thy category (1-5).
Aims 1. To audit the use of the Thy scoring system for thyroid fine needle aspiration (FNA) specimens in our department.
2. To compare the rates of malignancy of each Thy category for thyroid FNA samples reported in our department to the expected ranges published by the RCPath.
Methods A SNOMED search identified thyroid FNA samples from September 2008-December 2009. The Thy score assigned to each case was recorded. Subsequent histology was used to calculate the risk of malignancy for each Thy category. Results were compared to the published RCPath guidance.
Results 465 cases were identified. 83.8% had a Thy score assigned. 27.3% of cases were given an unsatisfactory Thy score (Thy 1). Based on the Thy scores assigned in our department, the predicted rate of malignancy for each Thy category were: Thy 1 - 1.9% (RCPath range 0-10%), Thy 2 - 0.42% (RCPath range 0-3%), Thy 3 - 5.6% (RCPath range 5-15%), Thy 4 - 62.5% (RCPath range 60-75%), Thy 5 - 100% (RCPath range 97-100%)
Conclusions 1.Two main areas were identified to improve the reporting of thyroid FNAs in our department. Firstly, to increase the use of the Thy score in the routine reporting of thyroid FNA specimens (No score was assigned in 16.2% of cases). Secondly, to reduce the inadequacy (Thy 1) rate of 27.3%.
2.The predicted rates of malignancy for each Thy category of samples reported in our department were all within the expected ranges published in the 2009 RCPath guidance.
Recommendations 1. Continue using the Thy scoring system as per 2009 RCPath guidance
2. Discuss the technique with radiologists
3. Reduce the number of people performing the technique
4. Re-audit annually

# P41

## Epithelioid Angiosarcoma Involving the Thyroid

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BACKGROUND: Angiosarcomas usually arise in the skin and soft tissue. Angiosarcomas involving the thyroid are rare and are aggressive tumours mostly described in people living in mountainous Alpine regions.
CASE: A 73 year old man with a longstanding history of goitre presented with a mass in the left thyroid lobe. This mass extended below the suprasternal notch, displacing the trachea. Cytology showed occasional clusters of neoplastic cells with eccentrically placed nuclei within a bloodstained background. Some of these cells were binucleate, recapitulating the Reed Sternberg cells of lymphoid neoplasms. A tissue biopsy revealed a vascular tumor infiltrating the thyroid gland. The tumour was composed of large, round, epithelioid cells lining vascular spaces. Some of these cells had a plasmacytoid appearance. These neoplastic cells were immunoreactive for CD31 and focally for AE1.AE3 but negative for thyroglobulin, TTF1 and S100.
CONCLUSION: Angiosarcomas are difficult to recognize on FNA cytology especially when they occur in organs where carcinomas occur commonly (as in this case) and when cytology may simulate those of other tumours (here, a lymphoma). As they can have similar features to that of anaplastic carcinomas and can also co-express epithelial markers, an immunohistochemical panel should include vascular markers to prevent potentially erroneous diagnoses.

# P42

## Development of a Model to Study NG2/CSPG4 Roles in Human Chondrocytes

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Introduction: NG2/CSPG4 transmembrane chondroitin sulphate proteoglycan, expressed by human cartilage where less is known of its function. Aims were to investigate whether different types of chondrocytes show variation in NG2/CSPG4 expression and through a knock down approach develop a model system to facilitate study of NG2/CSPG in chondrocytes.
Materials and Methods: Primary chondrosarcoma cells, JJ012, chondrosarcoma cell line, primary osteoarthritic (OA) chondrocytes, and chondrocyte cell line C20A4 were used. NG2/CSPG4 expression was investigated by RT-PCR, western blotting, flowcytometry and immunohistochemistry. NG2/CSPG knockdown was carried out by viral transduction using 5 different constructs. The effect of knock-down was investigated by qPCR, western blotting and flowcytometry.
Results: NG2/CSPG4 mRNA was detectable in all cells tested. Western blotting showed expression of only 270kD core protein in JJ012 and C20A4 cells; OA chondrocytes expressed three forms: a 400kD, 270kD with a further as yet unidentified band at 117kD. Flow-cytometry showed expression in C20A4 cells>JJ012 cells>OA chondrocytes. Expression in JJ012 cells was predominantly membrane associated whilst in OA chondrocytes and C20A4 additional, predominant punctuate cytoplasmic distribution was evident. NG2/CSPG4 gene knock down was achieved in JJ012 chondrosracoma cell line with gene expression being reduced to 2.9 and 1.1 % of maximum in two constructs (B3 and D1 respectively). NG2/CSPG4 protein expression was undetectable in B3 by western blotting.
Discussion: Altered expression of NG2/CSPG4 in normal and transformed chondrocytes may relate to different functions. Creation of a chondrocyte cell line that has stable knock down of NG2/CSPG4 will allow further investigation of NG2/CSPG function in chondrocytes.

# P43

## B-raf Mutation and Mismatch Repair Deficiency are Significantly Correlated and Both are Commonly Found in Right-sided Stage II Colorectal Cancers.

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Molecular biomarkers that could predict risk of colorectal cancer recurrence and/or sensitivity to chemotherapy would usefully complement existing histopathological prognosticators and improve patient management. B-raf mutation is associated with poor outcome in stage IV colorectal cancer whereas mismatch repair deficiency (dMMR) is a marker of good prognosis in early disease. We investigated the prognostic and predictive value of these two markers in QUASAR, a large prospective randomised trial of 5-FU/folinic acid chemotherapy versus control in stage II/III disease.
B-raf codon 600 was determined by pyrosequencing and mismatch repair status by immunohistochemistry for 1354 patients. Overall 11% of tumours were dMMR and 8% B-raf mutant, consistent with previous studies.
B-raf mutations occur more frequently in dMMR than MMR proficient tumours; 37% (58/156) compared to 5% (54/1194); p<0.0001. Both B-raf mutations and dMMR are significantly more common in right–sided than left-sided or rectal cancers: (17% (98/570) v 2% (10/513) vs 2% (8/345) for B-raf and 26% (179/695) v 3% (22/682) v 1% (3/407) for mismatch repair deficiency.
Recurrence risk for dMMR tumours was half that for MMR-proficient tumours [11% (25/218) v 26% (438/1695) recurred; Risk ratio (RR)= 0.53, 95%CI 0.40 to 0.70: p<0.0001] but B-raf mutation was not predictive of recurrence. Neither biomarker predicted response to chemotherapy.

# P44

## Expression of the Phosphorylated ERK and MEK MAPKs and Upstream Growth Factor Receptors in Adenomas and Carcinomas of Patients with Familial Adenomatous Polyposis

Ⓟ JRF Hollingshead[1]; J Wang[2]; N El-Masry[3]; P Trivedi[4]; D Horncastle[2]; I Talbot[5]; MR Alison[6]; I Tomlinson[7]; M El-Bahrawy[2]

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Familial Adenomatous Polyposis (FAP) is an autosomal dominant condition characterised by the development of multiple colonic adenomas with likely progression of some to invasive cancer. The ERK MAPK pathway is a signalling pathway activated by the Epidermal Growth Factor Receptor-1 (EGFR). The aim of this study is to investigate the expression of key members of this pathway in colonic tumours of FAP patients. Fifteen patients with FAP who had developed colorectal cancer were identified and colectomy specimens reviewed. We studied the expression of EGFR, HER2, phosphorylated ERK (pERK) and phosphorylated MEK (pMEK) by immunohistochemistry on formalin fixed paraffin embedded tissue from normal tissue, multiple adenomas, and carcinomas from each patient. There was a statistically significant increase in nuclear staining intensity for pERK in adenomas (p=0.0017) and carcinomas (p=0.0067) compared to normal tissue. There was a statistically significant increase in nuclear staining intensity for pMEK in carcionomas compared to normal tissue (p=0.001). EGFR expression correlated with pERK nuclear staining (p=0.0015) but not with pMEK nuclear staining. HER2 staining was weak in all tumours examined, and no significant correlations were found between HER2 expression and other markers. These data suggest a progressive increase in activity in the ERK MAPK pathway from normal tissue through adenoma to carcinoma in FAP tumours, which correlates with EGFR, but not with HER-2 expression. The activation of this pathway appears to be an early event in the pathogenesis of these tumours, suggesting members of this pathway can be therapeutic targets for control of development and progression of colonic tumours in FAP patients.

# P45

## Case Report of a Primary Undifferentiated Sarcoma of Small Bowel

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Primary undifferentiated sarcoma of the small intestine is an extremely rare disease, often diagnosed at an advanced stage, and displays an aggressive clinical course. Despite treatment by means of optimized surgical procedures, patients who have undergone complete tumour resection have a poor prognosis. Review of articles yields only limited reports. This case report presents the clinical presentation, investigation including the histopathological features, surgical treatment and lastly the prognosis of this very rare entity.

# P46

***This abstract has been withdrawn.***

# P47

## Modelling the Effects of Endoscopic Screening for Barrett's Oesophagus in a Geographically-Defined Population

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We have constructed a system dynamics model of endoscopic screening for Barrett's oesophagus for the population of Sheffield. The screening process was an invitation to endoscopy for the entire population over 40 years (model I) or the population as they reached the age of 40 (model II). If Barrett's was detected then 2 yearly surveillance was instituted. The incidence of Barrett's, progression to cancer, survival at screen-detected stage and survival at symptomatic presentation were derived from a systematic search of the published literature. The model was run over a 40 year time period. Outputs included deaths due to Barrett's related oesophageal adenocarcinoma, cost, number of endoscopies, complications and deaths due to endoscopy. Each input parameter was varied across a feasible range to provide sensitivity testing of the model. With full uptake of screening 1,657 deaths would be prevented in 40 years for model I and 820 prevented deaths in model II. The cost per prevented death was very similar in each model with a range of £100,000 to £200,000. If population uptake fell to 50% the costs rose to £150,000 to £250,000. These costs lie within the ranges that NICE set as being economically feasible however the logistics required to implement the programme (e.g. training/recruitment of 50 extra endoscopists in Sheffield for the first 5 years of model I) are challenging. The logistics and cost could be considerably reduced by the introduction of a screening step before endoscopy, such as sponge cytology, and this requires further modelling. This work was supported by a BSc grant from the BDIAP to RC and an educational grant from the Pathological Society of Great Britain & Ireland.

# P48

## A Model of Mutation in Intestinal Crypts Based on Evolutionary Graph Theory

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Nowak's evolutionary graph theory (Nature 2005; 433:312-316) is a method of describing and analysing quantal changes in networked populations. We have used this to produce a quantitative simulation model of neutral mutations in intestinal crypts. The network is based on the known structure of intestinal crypts with a stem cell niche at the base and differentiated epithelial cells above this. In the initial model best known estimates of the number of stem cells and number of columns and height of columns of epithelial cells in the colon were used. The model was constructed as a directed network with niche succession of stem cells. The model was run as a quantitative simulation. We initially ran the entire model to determine the probability of a neutral mutation in any crypt cell fixing across the entire crypt but a single run of the model required several days computation, with as many as 70 million cellular events to produce a completely mutated crypt. We then simplified the model to include only the stem cells since no other cells will produce a completely mutated crypt and the epithelial cells act only as reporters of stem cell mutations. Using this model we showed that the fixation probability of a stem cell mutation was $1/n$ where $n$ is the number of stem cells. The number or direction of connections between the stem cells (above one connection) had no effect on this. If the number of stem cells in small intestinal crypts is greater than that in colonic crypts then this could account, in some part, for the lower rate of neoplasia in the small intestine. Further developments of the model will include non-neutral mutations and time series analysis of the spread of mutations.

# P49

## An Analysis of Microsatellite Instability in Colorectal Cancers Using Immunohistochemistry for DNA Mismatch Repair Deficiency

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According to the WHO, 100 new cases of colorectal cancer are diagnosed per day in the UK. Lynch syndrome is the most common form of hereditary colorectal cancer and around 90% of these cancers exhibit microsatellite instability (MSI). 5-FU is the mainstay chemotherapeutic agent used for treatment and there is evidence that cancers with a defective mismatch repair system do not benefit from 5-FU. Therefore testing for MSI could be used to determine whether 5-FU chemotherapy would be effective.
The aim of this study was to determine if testing for MSI in right sided colonic cancers was simple, reproducible and cost-effective in a DGH setting and possibly across the region.
A total of 204 patients with right-sided tumours, which were 'poor prognosis' Dukes' B (extramural venous invasion or serosal involvement) or Dukes' C, were identified. Tumour sections from each case were stained with antibodies against MLH1 and MSH2. Intensity of immunoreactivity and percentage positivity was recorded and a semiquantitative score was given for each slide.
These antibodies were found to be easy to use, clean staining, and easily interpreted. The cost per slide for each antibody was £11.02 for MLH1 and £12.11 for MSH2.
When comparing MSI high and microsatellite stable tumours, a number of prognostic variables were found to be statistically significant. These included: tumour differentiation (p=<0.001), gender (p=0.034), age (p=0.012) and Dukes' stage (p=0.012). Interestingly, MSI status when compared with survival rates was found to be statistically insignificant (p=0.873).
This study could feasibly represent a model for using IHC to test for MSI in colonic tumours, however the actual implementation costs required to roll this out across the region is unknown.

# P50

## Histopathological Changes in Resected Gastrointestinal Stromal Tumours (GISTS) Treated with Tyrosine Kinase Inhibitor Imatinib Mesylate

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Background: GISTs are the most common mesenchymal tumours of the gastrointestinal tract. Imatinib mesylate (IM), a c-kit tyrosine kinase inhibitor remains the standard treatment for metastatic GISTs. Material & methods: This study looked at 12 GISTs which were resected post IM treatment. The histology of the resected lesion was compared with the histology taken prior to IM exposure (biopsy obtained from either the primary tumour or metastatic disease). The tumour architecture, cell morphology and nuclear characteristics on H&E were recorded. The pre IM treatment immunohistochemical profile was compared with that of the post IM treated resected specimen. CD117, CD34, desmin, actin, SMA, DOG-1 and MIB-1 staining patterns were analysed. Results: A wide range of appearances were noted in lesions resected post IM treatment. These included: necrosis and cystic changes; haemorrhage; hyalinization; variable mitotic count and MIB-1 expression; and altered cell morphology with more epithelioid or smooth muscle appearance. Typical changes in the immunohistochemical profile included loss of CD117 and CD34 expression, and acquisition of desmin and actin positivity. Some non responding lesions retained the same phenotype of the pre IM treated lesion. Conclusions: The histological changes in IM treated GISTs may reflect the heterogeneity of the GISTs in their response to IM treatment. In responding patients, cell necrosis, hyalinisation and loss of CD 117 are the typical features. Differentiation into a smooth muscle phenotype is rare. Intra and inter lesional differences in the secondary mutations in c-kit may account for some of these changes and further research is clearly needed.

# P51

## An Audit of Compliance with the RCPATH Minimum Dataset for Colorectal Cancer Resections: Comparison Between Specialist Trainees and Consultants

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*Background & Methods:* The minimum datasets (MDS) published by the RCPath aim to provide evidence-based definitions to set a nationwide standard for ensuring accurate reporting in colorectal cancer resections. Our aim was to compare the compliance with these quality indicators when the primary cut up was done by specialist registrars (SpRs, 170 cases at a teaching hospital) or consultants (110 cases at a private laboratory).
*Results:* Compliance with reporting of the macro- and microscopic core data items was 100% for both SpRs and consultants (Cs). For rectal excisions, reporting of the plane of excision (Cs=100%, SpRs=85%) and the relation to the peritoneal reflection (Cs=100%, SpRs =79%) was better when cut up was done by consultants. The percentage of cases in which less than 12 lymph nodes were harvested was the same for groups ((15%). The average number of lymph nodes harvested was similar for SpRs and consultants and above the minimum required (Cs= 16, range = 5-43; SpRs = 18, range = 3-55). The percentage of cases with reported positive vascular invasion was above the recommended figure for both groups (28% for SpRs and 29% for Cs; recommended national figure = 25%). The percentage of colon cancer cases with serosal involvement was 23% for consultants and 24% for SpRs (recommended = 20%). The percentage of rectal cancer cases with serosal involvement was 11% for consultants and 11% for SpRs (recommended figure = 10%).
*Conclusion:* This study shows that block selection for both colon and rectal cancers was good for both SpRs and consultants. Overall, there was good adherence to the minimum dataset regardless of whether consultants working in the private sector or SpRs working in NHS Hospital performed the initial cut up. There was good concordance between SpRs and consultants. Overall, the reporting in our services is of good standard and meets the requirements set by the RCPath.

# P52

## A Thickened Subepithelial Collagen Band is Specific for Collagenous Colitis and is not a Non-Specific Response to Injury

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*Introduction:* Collagenous colitis (CC) is characterized by a thickened subepithelial collagen band and may be accompanied by an increased number of intraepithelial lymphocytes (IEL). Some of its features may overlap with other entities such as inflammatory bowel disease (IBD).
*Aims and Objectives:* To study whether a thickened subepithelial band is specific for CC or whether it is a non-specific response to injury and to assess the relationship between a thickened subepithelial collagen band and the number of IEL in CC.
*Materials and Methods:* 150 cases were divided into 4 cohorts; *Group 1 (control):* patients with normal random colonic mucosal biopsy (n=30), *Group 2:* patients with a clinical and histopathological diagnosis of CC on biopsy (n=40), *Group 3:* patients with a clinical suggestion of CC but negative biopsy (n=40), *Group 4:* patients with IBD (Ulcerative Colitis/Crohn's Disease, n=40). Each case was stained with Masson Trichrome, CD79a and CD3. A subepithelial collagen band was measured using NIS Elements Imaging Software and the number of IEL/ 100 enterocytes counted. The mean collagen band thickness and mean CD3 positive IEL count between the groups were compared and assessed using Kruskall-Wallis test. The association between band thickness and IEL count was examined using Spearman's rank correlation.
*Results:* There was a significant overall difference in mean collagen band thickness between the four groups (p<0.001); highest in the CC group (median = 17µm). There was a significant overall difference in the CD3+ IEL count between the four groups (p<0.001); highest in the CC group (median = 9/100 enterocytes). There was no strong association between band thickness and CD3+ IEL count for any of the groups.
*Conclusion:* A thickened subepithelial collagen band was specific for CC and was not a non-specific response to injury with no strong association between band thickness and IEL count identified in this entity.

# P53

## Reporting Paneth Cell Metaplasia in Paediatric GI Biopsies: An Audit on Current Practice and Review of Guidelines

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Purpose of the study: Paneth cells (PCs) play a key role in regulating intestinal defense and paneth cell metaplasia (PCM) is becoming an increasingly important histopatholgical feature of inflammatory bowel disease (IBD). The definition of when PCs are 'metaplastic' is uncertain as they are found normally in the proximal large bowel. This audit aims to evaluate the current practice of reporting PCM in paediatric GI biopsies. Methodology: 22 random IBD and 22 normal control cases were selected. The reports were reviewed and histological examination of biopsy slides was performed. For each biopsy site the first 10 well-orientated crypts were examined and the number of PCs, presence and degree of acute/chronic inflammation and architectural distortion were recorded. Results: 22 patients with IBD and 22 normal colonic series were examined. 10 control identified PC's in the proximal colon therefore PC's in the distal colon in the IBD group were interpreted as PCM. 17 CD and all 4 UC cases had PCM. There was a 100% correlation between PCM and chronic inflammation. In all cases of UC and 14 out of 17 CD cases with PCM there was associated acute inflammation. In 13 CD cases and all UC cases PCM was associated with architectural distortion. PCM was never mentioned in the report. Conclusion: These results show PCs in the distal colon (PCM) are abnormal and PCM is never seen in the absence of other histological changes of IBD. PCM in biopsies coincided with acute and chronic inflammation as well as architectural distortion. PCM was almost never mentioned in the report which is not surprising as guidance for reporting PCM in GI biopsies is limited. Little is to be gained by recording the presence of PCM if a diagnosis of IBD is made. In equivocal cases, or if features of IBD are not present, we recommend that PCM should always be reported, since it is not seen in the normal colon.

# P54

## Gastrointestinal Stromal Tumours Associated with Other Cancers

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Gastrointestinal stromal tumours (GISTs) may coexist with non-GIST tumours (NGTs) either metachronously or synchronously. This conjunctive occurrence has not been sufficiently analysed in the literature. We report our five year prospective data from our GIST archive.

METHOD 150 patients diagnosed with GIST were collected from Cambridge GIST database between 2005 and 2009. These records were reviewed and those cases associated with NGTs were selected. Data were collected on age, gender, type of coexisting malignancy, site of GIST and NGT, risk group (according to National Institutes of Health criteria), family history and survival.

RESULTS 21 patients out of 150 had dual malignancies. Median age was 69 years (46-88 years) with slight male dominance (1.3:1). Mean tumour size was 5 cm (0.13-12cm). The patients were divided into three groups: (1) 62% of patients were incidentally diagnosed with GISTs during evaluation of NGTs, (2) 28% of patients had pre-existing NGTs, (3) one patient with inoperable GIST was found to have pancreatic cancer with liver metastasis during imatinib treatment. 71% of the GISTs were in the low risk group. Majority of GISTs were diagnosed in the gastrointestinal tract (stomach 66%, duodenum 4.5%, other small bowel 4.5%, oesophagus 4.5%, colorectum 4.5%), followed by extra-gastrointestinal sites (14%). The gastrointestinal tract was the most frequent site for the coexisting NGTs (colorectum 19%, stomach 14%, oesophagus 4.5%). Occasionally rare malignancies were associated with GISTs, including oestrogen positive male breast cancer. 76% of patients are still alive without recurrence of GISTs or NGTs.

CONCLUSION 14% incidence of coexisting GIST and NGT was revealed in our cases. Majority of both malignancies were diagnosed in the gastrointestinal tract, but other rare associations were also found. These scenarios have important diagnostic and therapeutic implications in patients' management.

# P55

## An Audit of BCSP Polyp Cancer Reporting in the Pennine Acute Hospitals NHS Trust

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The BCSP started to be rolled out in July 2006 and achieved national coverage this year. It aims to detect bowel cancer at an early stage when treatment is more likely to be effective. Inevitably, the programme was likely to detect many pT1 stage tumours, including polyp cancers. Since the current evidence base for the management of these cancers is poor, the RCPath produced clear guidelines for reporting these cancers in September 2007.

At Pennine Acute Hospitals NHS Trust, a screening centre, the programme started in March 2008. The aim of this audit was to assess the current reporting practices against the RCPath guidelines.

In the Pennine screening centre, 870 colonoscopies were untaken between 01/03/08 and 10/05/10 with a 95.0% colonoscopy completion rate. In 51.5% of cases, one or more polyps were seen and 94.4% of polyps were removed for pathological examination.

We received samples from a total of 385 patients, of which 75 specimens contained adenocarcinoma, and of these 18 were polyp cancers. These polyp cancers were further analysed.

The results show that 72% of these polyp cancers were in males and 88.9% were in the sigmoid colon. 66.7% were pedunculated and of these 72.7% were given a Haggitt level. 33.3% were sessile and of these 28.6% were given a Kikuchi level. In 3 cases, inappropriate grading systems were used, with Haggitt being used for 2 sessile polyps and Dukes' staging was used in 1 case.

Completeness of excision was documented in 83.3% of cases. The distance to the deep margin was documented in 77.7% of cases and vascular invasion was documented in 55.6% of cases.

*Conclusion* – Improvement is required in the reporting of BCSP polyp cancers within our department. Some of the recommended key features are frequently omitted. Improvement may be achieved by proforma/tabular reporting.

# P56

## Utility of Faecal DNA as a Potential Test for Bowel Cancer Screening

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Early detection of colorectal cancer (CRC) often leads to cure. Screening strategies for early detection include Faecal Occult Blood Test (FOBT) and colonoscopy. FOBT low sensitivity results in a large number of unnecessary colonoscopies. Colonoscopy is a reliable screening test, but its invasive nature and cost are major disadvantages for its use as the primary test in population screening. These limitations highlight the need for a non-invasive screening test with better predictive value. Analysis of human DNA content in stool and its correlation with presence of adenoma and adenocarcinoma has been proposed as an alternative strategy for non-invasive detection of CRC.

We extracted DNA from faeces using the QIAamp DNA Stool Mini Kit (Qiagen, UK) from 144 patients from the NHS Bowel cancer screening programme. To quantify the relative amount of E.coli and human DNA we targeted ~150bp fragments within the E coli *ClpP* gene and the human *SYT10* gene by quantitative PCR.

Human DNA content relative to total DNA in the low-risk (normal volunteers) group ranged from 0.0000091% to 3.83% (mean=0.21%, n=58), in the intermediate-risk (patients with adenomas) group from 0.0021% to 90% (mean=0.46%, n=61), and in the high-risk (patients with adenocarcinomas) group from 0.000025% to 9.3% (mean=0.64%, n=25). The correlation between the amount of human DNA and the CRC risk group was significant ($p$=0.029, Spearman's correlation test) but weak ($r$=0.183). The sensitivity of human DNA quantification to detect CRC risk was 23% and specificity was 43%.

The weak correlation between human DNA content in stool and CRC risk implies that quantification does not provide a sensitive nor specific test for CRC screening. This large variation in human faecal DNA content highlights the importance of faecal human DNA quantification for downstream analysis of potential biomarkers such as known mutations or DNA methylation targets.

# P57

## Collision Tumour of the Sigmoid Colon with Lymphoma and Adenocarcinoma with Concomitant Chronic Lymphocytic Leukemia: A Rare Case

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Introduction: Non-Hodgkin's lymphoma of the sigmoid colon is very rare. There are very few case reports in literature of non-Hodgkin's lymphoma co-existing with adenocarcinoma in the sigmoid colon. We present a case of this rare combination with detailed histopathology and immunohistochemical profile.
Case report: A 69-year old Caucasian male presented as an emergency with clinical features of large bowel obstruction. CT scan revealed an obstructing sigmoid tumour. On emergency laparotomy there was localized perforation of the sigmoid tumour.The patient underwent a sigmoid colectomy and Hartman's procedure. The post-operative course was complicated by pulmonary embolism, stroke and wound infection. The patient recovered after prolonged rehabilitation.
Histopathology: Sections showed large bowel mucosa with evidence of a moderately differentiated adenocarcinoma. None of the 16 lymph nodes had metastatic involvement. In addition, a dense lymphoid infiltrate of small lymphocytes was seen surrounding the tumour and involving the lymph nodes. Immunohistochemistry was positive for CD20, CD79a, CD5 and Bcl 2 while it was negative for CD3, CD23, CD10 and Cyclin D1. Ki67 was weaky positive. These features were suggestive of Low Grade Non-Hodgkin's B cell lymphoma. Concomitant peripheral blood analysis confirmed the diagnosis of chronic lymphocytic leukaemia which was also first diagnosed during the same admission.
Conclusion: The presence of lymphomas in colon is quite rare but its coexistence with adenocarcinoma and CLL is extremely rare. It's crucial to identify the different pathologies on the same specimen which can be challenging since it has significant impact on further adjuvant treatment, investigations and follow up.

# P58

## Identification of Lesions Indicating Rejection in Kidney Transplant Biopsies: Observational Non-comparative Study of 100 Cases

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Background: In Banff classification of kidney transplant rejection, tubulitis and intimal arteritis are regarded as the key histological features of acute rejection. Just one lymphocyte in these locations can change the classification of a biopsy. Mild tubulitis can sometimes be seen in biopsies that do not represent acute rejection; but in case of intimal arteritis, just one lymphocyte can justify anti-rejection treatment.
Objectives: To audit the reliability and accuracy of recognizing early tubulitis and intimal arteritis in conventionally stained sections in the analysis of kidney transplant biopsies and to correlate any discrepancies with subsequent graft function.
Methods: Retrospective review of kidney transplant biopsy reports from 1st January 2009 to 31st December 2009 to tabulate the reported presence or absence of tubulitis and arteritis. One hundred reported negative biopsies were stained with CD3 and PAS as a counterstain. These were reviewed to detect missed intimal arteritis and/ tubulitis. Discrepancies between the report and the immunostain results were broken down by biopsy type and reporting consultant. The graft function of any patient with missed intimal arteritis was checked to test for adverse impact on the patient.
Results: Missed tubulitis was found in 68% of biopsies reported as negative. Only one case of missed intimal arteritis was found (1%) and the subsequent clinical course suggested that this was genuinely early rejection. There was no significant discrepancy between consultants.
Conclusion: We concluded that tubulitis is missed frequently, but the Banff classification seems to have been 'calibrated' to allow for this and it does not adversely affect the identification of clinically significant acute rejection. Immunostaining to detect missed tubulitis is therefore not indicated in routine practice. Intimal arteritis is indicative of acute rejection even if extremely mild.

# P59

## Immunohistochemical and Real-time PCR Measurement of the Field Effect in Prostate Cancer

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The field cancerisation effect is a novel concept in prostate cancer that may have significant clinical implications for diagnosis and treatment. It hypothesises that benign tissue adjacent to cancer shares some molecular characteristics of the latter. To test this hypothesis we investigated the expression of postulated markers of the field effect in benign and malignant prostatic tissue by immunohistochemistry (IHC) and real time PCR (RT-PCR). Fifty nine patients under investigation for prostate cancer were recruited into the study (40 archival samples and 19 prospective samples). Archival samples (n=40) were evaluated using quantitative IHC for 5 antigens. The prospective samples were subject to poly(A) PCR and RT-PCR for 10 genes. IHC demonstrated significant differences between cancer and benign tissue for HEPSIN (P=0.003). Benign tissue distant from or adjacent to cancer tissue showed differential expression of AMACR and GSTP1 (P=0.002) and (P=0.01). HEPSIN also differed in cancer tissue compared to adjacent benign tissue (P=0.004). HEPSIN staining in cancer was correlated with disease stage (P=0.006) and AMACR staining in adjacent benign tissue correlated with survival (P=0.006). Of the 19 prospective samples 18 contained benign tissue only but gene expression in these samples correlated with the presence of cancer elsewhere in the prostate. RT-PCR, demonstrated association of ERG expression with Gleason grade (P=0.021), HEPSIN with the presence of carcinoma in situ (P=0.0054) and PCA3 with percentage cancer in the prostate (P=0.047). This study provides evidence that the field effect is measurable in prostate cancer and that it has valuable clinical implications for the prediction of prognosis. Further research in this area may yield better diagnostic and prognostic tests for prostate cancer.

# P60

## Reporting of Suspected Renal Cell Carcinoma Specimens in Pathology.

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The pathologist plays a crucial role in the urological multidisciplinary team by reporting on renal cell carcinoma specimens and providing prognostic information that can be used by the team to determine which patients might benefit from further therapy. It is therefore important that pathologists' reports of the specimens are accurate so that the risk and prognosis associated with the tumour can be determined and an appropriate management plan made early.
The Royal College of Pathologists (RCP) set out a dataset for adult renal parenchymal cancer histopathology reporting in November 2006. This dataset lists the core data items that should be included in reports of renal cell carcinoma specimens. An audit of thirty renal cell carcinoma histopathology reports made in 2009-10 was undertaken at a district general hospital in the North West of England. The reports were audited against the RCP dataset to determine compliance with their reporting standards.
Findings show that compliance was 100% with all standards except for sarcomatoid change where only 50% of reports included information about this. In three of these cases (10% of the total) the tumour was grade 4 but evidence of sarcomatoid change was not reported. Such high compliance with other items of the RCP dataset is likely due to the use of a standardised proforma which acts as an aid memoir when reporting. We aim to disseminate these findings within the pathology community to improve awareness of the importance of reporting on sarcomatoid change in high grade renal cell carcinoma tumours.

# P61

## Waldenströms Macroglobulinaemia – A rare Cause of Obstructive Nephropathy

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Case
We report a 78 year old man with a history of stable Waldenströms Macroglobulinaemia (also known as lymphoplasmacytic lymphoma) who presented with obstructive uropathy secondary to a retroperitoneal mass which extrinsically compressed both ureters.
The CT findings resulted in significant diagnostic uncertainty. The differential diagnoses considered included retroperitoneal fibrosis, lymphoma and metastatic malignancy.
Post mortem examination revealed localised retroperitoneal amyloidosis which proved on immunohistochemistry to label positive for anti-IgM antibody consistent with the deposition secondary to Waldenströms Macroglobulinaemia.
Discussion
Amyloidosis, resulting from IgM paraprotein deposition, is a rare complication of Waldenströms Macroglobulinaemia and poses an adverse survival factor. It can be seen in a wide variety of patterns and locations throughout the body; retroperitoneal involvement however, is infrequent and has to our knowledge not been reported before.
Since early diagnosis is the key for effective treatment and prevention of complications we would like to emphasis that localised retroperitoneal amyloidosis should be added to the list of causes of ureteric obstruction especially in the background of Waldenströms Macroglobulinaemia.

# P62

## Diffuse p63 Positivity in Prostate Cancer: A Case Report

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Prostatic atrophy can closely mimic atrophic pattern of prostate cancer due to the combination of a pseudoinfiltrative architecture and abnormal cytology with prominent nucleoli in the basal cells. In morphologically equivocal cases, immunohistochemistry using antibodies to basal cell markers, high molecular weight cytokeratin (HMWCK) and p63 is widely used to aid in the distinction. The presence of basal cell marker immunoreactivity in the suspect glands would strongly suggest a benign diagnosis. We describe a case of atrophic prostate cancer that showed diffuse p63 immunoreactivity in the malignant glands. The prostate needle biopsy showed a 4mm focus composed of small irregular atrophic acini with scant cytoplasm and macronucleoli, infiltrating between morphologically benign glands. On immunostaining, the atypical glands were diffusely p63 positive with a non-basal cell cell distribution. No immunoreactivity was seen in the suspect glands for HMWCK CK5. Despite the p63 positivity a diagnosis of prostatic adenocarcinoma was made in view of the distinctive morphology and HMWCK immunonegativity. Subsequent radical prostatectomy revealed prostatic adenocarcinoma, which in some areas showed diffuse p63 positivity. Diffuse p63 immunoreactivity in prostate cancer is very rare and only a single series of such cases has been reported to date. However, awareness of this phenomenon is important to avoid misdiagnosing prostate cancer as benign. It is noteworthy that 19 of the 21 cancers in the published series showed an atrophic appearance as in the current case. Misdiagnosis can be avoided by using p63 in conjunction with a HMWCK antibody such as CK5, CK5/6 or 34betaE12 either individually or as a cocktail and recognising the non-basal cell distribution the pattern of p63 immunoreactivity in these rare cancers.

# P63

## Lipoprotein Lipase is a Candidate Oncogene in Cervical Squamous Cell Carcinoma

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Host genomic abnormalities that drive progression of cervical squamous cell carcinoma (SCC) remain poorly understood. In a systematic molecular cytogenetic analysis of cervical carcinoma cells, we identified a novel reciprocal translocation, t(8:12)(p21.3:p13.31), present in 100% of metaphases of the SCC cell line MS751. We undertook detailed mapping of the chromosomal breakpoints using tiling BAC metaphase-FISH and fosmid fibre-FISH, followed by long-range PCR and sequencing across the fusions. We identified that the rearrangement involved lipoprotein lipase (LPL) on chromosome 8 and peroxisome biogenesis factor 5 (PEX5) on chromosome 12, resulting in two novel fusion genes. Whereas LPL-PEX5 was expressed at low levels and contained a premature stop codon, PEX5-LPL was highly expressed and encoded a full length chimeric protein (including the majority of the LPL coding region) that most likely drove selection of the translocation. Consistent with these findings, reverse transcription PCR (RTPCR) showed that PEX5 was constitutively expressed in normal cervical squamous cells, whereas LPL expression was negligible. In a tissue microarray, LPL rearrangement was seen in only 1/151 cervical SCCs using interphase FISH. However, over-expression of wild type LPL was common, being detected by quantitative RTPCR in 14/38 tissue samples and cell lines. Forced over-expression of both wild type LPL and the PEX5-LPL fusion gene resulted in increased motility and invasiveness in cervical SCC cells, attributable to the C terminal non-catalytic domain of LPL, which was retained in the fusion gene. We conclude that over-expressed wild type or translocated LPL may serve as an oncogene in cervical SCC and thereby represent a novel candidate for targeted therapy.

# P64

## Primary Pseudomyxoma Peritoneii in Benign Ovarian Lesions

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Background: Psuedomyxoma peritonei is an uncommon fatal disease often associated with primary appendiceal or gastrointestinal tumour. It is characterised by accumulation of gelatinous material in the pelvis and abdominal cavity and mainly affects elderly females with a mean age of 58years at presentation.
Cases: We report two cases of pseudomyxoma ovarii with mucinous cystadenoma. Two females aged 25years and 42years with clinical diagnosis of advanced ovarian carcinoma presented respectively with a year and 5years history of abdominal pain, swelling and mass, menstrual irregularity and weight loss. Exploratory laparotomy revealed unilateral multi-lobulated ovarian masses, mucinous peritoneal and omental deposits. There were no obvious appendiceal and gastrointestinal lesions. Both had oophrectomy and peritoneal clearance of the mucinous deposits. Grossly, the excised lesions showed papillary multi-locular cystic masses containing gelatinous fluid. The tissues were processed in paraffin wax and stained with haematoxylin & eosin, periodic acid Schiff, diastase and mucicarmine. Histology revealed thin strips of ovarian stoma overlying numerous cystic cavities containing mucin and lined by epithelial cells with intracellular mucin.
Conclusion: The diagnosis of pseudomyxoma peritonei is often incidental and there are no specific clinical symptoms to aid diagnosis. Mucinous ovarian cystadenocarcinoma may be associated with pseudomyxoma peritonei with or without appendiceal or gastrointestinal malignancy. However, neither of the two presented cases had malignant focus and were categorized as disseminated peritoneal adenomucinosis which is characterized by abundant extracellular mucin and scanty mucinous epithelium with little atypia or mitotic activity. Pseudomyxoma may occur in younger females in association with primary benign ovarian neoplasm.

# P65

## Palatal Malignant Melanoma in a Middle -Aged Female

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Background:Malignant melanoma of the oral cavity is an uncommon aggressive tumour that preferentially affects elderly males. Its pathological classification is unclear and the diagnosis is often fraught with delay. Case:A 45 year old emanciated Fulani woman with a 6-month history of left palatal swelling and loosened teeth is presented. She had no history of pre-existing palatal or oral cavity lesion. Examination revealed a 9x4cm sessile nodular dark grey mass involving the anterior two-thirds of the hard palate and a 5x5cm palpable submandibular lymph node. The mass bled spontaneously and extended to the posterior gingiva. Palatal radioragh showed an osteolytic lesion with destruction of the left maxillary bone. She had a hemi-maxillectomy with nodal excision. Tissue histology of both specimens showed infiltrating sheets of tumour cells composed of spindle cells having ovoid nuclei with prominent eosinophilic nucleolus and moderate cytoplasm containing melanin pigments. Extensive melanin incontinence was seen within the stroma and the resection margins showed tumour involvement. She was diagnosed as Clark's level IV and stage III melanoma. Conclusion:Palatal melanoma has a rapid growth rate and commonly arises from pre-existing dysplastic lesions. It may be seen in younger females as in the index case. Most patients present with advanced disease which has a poorer prognosis because early disease is often asymptomatic. Its classification and treatment is still based on the cutaneous disease and requires a combination of surgery and chemotherapy. Awareness and knowledge of its biologic behaviour by attending clinicians should aid early diagnosis and reduce morbidity and mortality from this aggressive tumour.

# P66

## Do Papillary Carcinomas arise in Thyroglossal Cysts?

Ⓟ T Grigor; J Mathews

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A 68 year old male presented with a longstanding, non-tender, midline swelling of his neck that appeared to move on swallowing. Serum biochemical analysis was unremarkable (TSH 1.15 mIU/L). Pre-operative imaging or cytological assessment were not obtained. An excision biopsy was performed. Macroscopically the specimen comprised of scanty fatty tissue surrounding a smooth, thin-walled 16mm diameter cyst with a smooth inner lining containing a small amount of straw coloured fluid. Light microscopic examination showed a typical thyroglossal duct cyst architecture. Focally there were intra-luminal papillary projections covered by CK-19 and thyroglobulin positive cells that had overlapping, occasionally grooved nuclei, without the typical 'Orphan Annie' appearance. Intracystic aggregates of pigmented macrophages admixed with reactive fibrosis and a lymphocytic inflammatory infiltrate were also a feature. There were two lymph nodes in the wall of the cyst; one appeared to contain crushed epithelial cells in a sub-sinus location that were stainable for thyroglobulin antibody. Specialist review confirmed a diagnosis of Cystic Papillary Carcinoma arising in thyroglossal duct remnants with a micrometastasis to a lymph node adjacent to it. This patient's case is interesting because of its rare and unusual mode of clinical presentation and the necessary exclusion of benign papillary hyperplasia as a possible alternate diagnosis. Specific light microscopic features, immunohistochemistry and the fortuitous inclusion of an adjacent 2mm lymph node containing a micrometastasis were the keys to this case. Kusunoki et al. Thyroid. 2007 Jun; Vol 17 (6):591-2. Carcinoma arising in thyroglossal duct remnants.

# P67

## Cell Surface Markers in Alcoholic Hepatitis: A Pilot Study of Targets for Monoclonal Antibody Therapy

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Background: Alcoholic hepatitis is a serious life-threatening condition with a short-term mortality of 30-50%. Current treatment is largely inadequate with controversy surrounding the benefits of corticosteroid treatment and a shortage of donor organs for liver transplantation. Aims: The aim of this project was to investigate the presence of cell surface markers using immunohistochemistry in liver biopsies from patients with clinically evident alcoholic hepatitis and compare them with normal controls. Identifying the cell surface markers present may indicate a potential role for biological therapy in the treatment of alcoholic liver disease. Methods: We identified 22 patients with clinically and histologically evident alcoholic hepatitis and 20 biopsies of normal liver tissue. Sections were stained with 4 antibodies: anti-CD20 (mouse monoclonal 1:75 Novocastra), anti-CD52 (rat monoclonal 1:40 Serotec), anti-TNF-R1 (rabbit polyclonal 1:150 Abcam) and anti-IL-1 R1 (rabbit polyclonal 1:50 Abcam) and staining patterns were compared between the two groups. Results: B cells were sparsely distributed in liver tissue from both the test and control groups. There were slightly more B cells (not statistically significant) in diseased liver. Anti-CD52 antibody intensely stained Kupffer cells and macrophages, which were easier to identify in normal tissue. Anti-IL-1 R1 antibody bound intracellular Mallory's hyaline and also produced background nuclear staining of hepatocytes. Anti-TNF-R1 diffusely stained hepatocytes; there was no difference in staining patterns or intensity between the test and control groups. Conclusions: Of the antibodies tested, anti-CD52 seems to be the most promising as it intensely stained Kupffer cells, however, a possible down-regulation of CD52 in advanced disease could be a concern. Further studies with more patients are required.

# P68

## Perisinusoidal and Kupffer Cell Numbers in Autoimmune Chronic Hepatitis

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Introduction: 40% of autoimmune chronic hepatitis (AICH) patients eventually develop liver cirrhosis. Liver fibrosis, a consequence of the interaction between Kupffer cells (KC) and perisinusoidal cell (PSC), is thought to be "irreversible" but there is evidence that AICH-related fibrosis may regress following corticosteroid and immunosuppressant therapy.
Aim:To determine perisinusoidal cell (PSC) and Kupffer cell (KC) numbers during the acute (presenting) (AP) and the late (post-treatment; resolved) phase (LP) of AICH.
Method: 45 AICH AP-LP matched biopsies identified from our database using SNOMED search criteria (T56* and D4700). 4µm sections of each phase were stained with antibodies to Smooth Muscle Actin (SMA) (clone 1A4, Dako, dilution 1:400) and CD68 (clone KP1, Dako, dilution 1:400) with a Bondmax® automated immunostainer. 10 images at x400 magnification were taken from periseptal or periportal areas of each biopsy using a BX61 Olympus microscope and a DP70 camera with Cell$^P$ imaging software. PSCs and KCs were counted using the softwares' touch count facility in $\mu m^2$, and converted to cells/$mm^2$.
Results: The difference in the numbers of PSCs in AP (mean = 392cells/$mm^2$; 95% CI, 349.20 - 435.35) to PSCs in LP (mean = 317 cells/$mm^2$;95% CI, 283.18 - 352.03) was significant (Wilcoxon, Z = -2.489, p=0.013). Similarly, the difference in the numbers of KCs in AP (mean = 728 cells/$mm^2$; 95% CI, 621.18 - 835.93) to KCs in LP (mean = 439 cells/$mm^2$; 95% CI, 392.84 - 486.48) was significant (Wilcoxon, Z=-4.534 , p= <0.00).
Conclusion: There is a significant decline in the numbers of PSCs and KCs during the AP and LP of AICH following treatment. This supports the contention that the interaction of these two cell types is important in the development of AICH related fibrosis and "resolution", although other mechanisms are probably important in the latter.

# P69

## Investigation of the Fate of Cholangiocytes in Cholestatic Liver Disease

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During cholestatic liver disease, cholangiocytes are exposed to oxidative stress and may become senescent. Senescent renal epithelial cells can secrete cytokines, such as TGF-β, which promote fibrosis in kidneys by activation of epithelial to mesenchymal transition (EMT). The current study was designed to examine the relationship between the induction of senescence and EMT in human liver after transplantation.
Human cholangiocytes (H69 cell line) were subjected to oxidative stress by treatment with 600μM H2O2 for 1h. After 24, 48, 72 and 120 hours the treated cells were immunostained to allow quantification of the EMT markers S100A4, αSMA and ZO-1 and the senescence marker p21. Analysis by immunofluorescence confocal microscopy showed an increase in S100A4 and αSMA ($p=0.034$) expression after 48h, with decreasing expression of ZO-1 to background after 120h ($p<0.001$) and acquisition of fibroblast-like morphology; p21 was upregulated at 48h ($p<0.001$) before returning to baseline. A parallel study analysed expression of S100A4, p21 and the cholangiocyte marker CK7 in liver transplant biopsies (34 biopsies: 25 rejection, 9 controls). The expression of S100A4 and p21 correlated in acute rejection ($p=0.001$) and both antigens were expressed at higher levels in moderate/severe than normal/mild rejection ($p=0.003$ and $p=0.02$ respectively).
In conclusion, oxidative stress causes cholangiocytes to express the senescence marker p21 transiently before induction of a fibrotic EMT phenotype. Parallel expression of p21 and S100A4 in bile ducts in acute rejection biopsies suggests a mechanism in which senescent cells can induce fibrogenic EMT within human bile ducts.

# P70

## A Tale From the Pancreatic Tail

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CASE: A 79 year old woman was found on computer tomography scanning to have a 2.5cm mass lesion in the tail of the pancreas, which was positive on octreotide scan and was thought to be a non-functioning neuroendocrine tumour. A laparoscopic distal pancreatectomy was performed.
PATHOLOGY: Within the pancreas there was a dark tan coloured, well circumscribed 25mm lesion. Microscopically, the lesion was composed of normal splenic tissue. The lesion was confirmed to be an intrapancreatic accessory spleen (splenunculus).
DISCUSSION: Accessory spleens have been reported in just over 10% of cases in a post mortem series. The pancreas is the second most common site of occurrence. They are usually asymptomatic, but may be misdiagnosed as a pancreatic neoplasm on imaging, as described in 14 case reports of intrapancreatic accessory spleens and 25 case reports of epithelial or epidermoid cysts arising within intrapancreatic accessory spleens. Non-invasive diagnosis of these lesions has been described using endoscopic ultrasound guided fine needle aspiration and additional imaging techniques (splenic scintigraphy using Tc-99m heat-damaged red blood cells, contrast enhanced ultrasound imaging and superparamagnetic iron oxide-enhanced MRI). Post mortem studies provide valuable information on the incidence of benign congenital anomalies, and hence enabling correlation with imaging findings, and guiding investigations in patients.

# P71

## A20 and ABIN-1/2 Inactivation in Ocular Adnexal MALT Lymphoma

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Background: Recent studies showed that A20, an NF-kB inhibitor, was inactivated by gene deletion, mutation and promoter methylation in ocular adnexal MALT lymphoma. However, the number of cases investigated in each of the previous studies is rather small and the true incidences of A20 abnormalities and their clinical impacts are largely unknown. In addition, it is unknown whether other NF-kB inhibitors, such as ABIN-1 and ABIN-2, are targeted for inactivation in ocular adnexal MALT lymphoma.
Design and methods: A total of 105 cases of ocular adnexal MALT lymphoma were investigated for A20 mutation/deletion, ABIN-1 and ABIN-2 mutation, MALT1 and IGH involved chromosome translocation. On selected cases, the expression of the NF-kB target genes CCR2, TLR6, BCL2 and CD69 was measured by quantitative reverse transcription PCR. Correlation among genetic changes and clinicopathological parameters was comprehensively investigated.
Results: Somatic mutation was seen frequently in A20 (8.6%), but rarely in ABIN-1 (1%) and ABIN-2 (1%). A20 mutations were significantly associated with A20 heterozygous deletion, and both were mutually exclusive from MALT1 or IGH involved translocations. A20 mutation/deletion was also significantly associated with increased expression of the NF-kB target genes CCR2, TLR6 and BCL2. The cases with A20 mutation/deletion required significantly higher radiation dosages to achieve complete remission than those without these abnormalities.
Conclusion: A20 is frequently inactivated by gene mutation/deletion in translocation-negative ocular adnexal MALT lymphomas. A20 inactivation was associated with enhanced NF-kB activities and the use of higher radiation dosages to achieve complete regression.

# P72

## Genomic, Epigenomic and Transcriptomic Molecular Characterization of Splenic Marginal Zone Lymphoma Reveals Recurrent Abnormalities in miR-182

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Splenic marginal zone lymphoma (SMZL) is a low grade B-cell non-Hodgkin's lymphoma. The molecular pathology of this entity remains poorly understood. To characterise this lymphoma at the molecular level, we performed an integrated analysis of 1) genome wide genetic copy number alterations 2) gene expression profiles and 3) epigenetic DNA methylation profiles.
We have previously shown that SMZL is characterised by recurrent alterations of chromosomes 7q, 6q, 3q, 9q and 18; however, gene resolution oligonucleotide array comparative genomic hybridisation did not reveal evidence of cryptic amplification or deletion in these regions. The most frequently lost 7q32 region contains a cluster of miRNAs. qRT-PCR revealed that three of these (miR-182/96/183) show underexpression in SMZL, and miR-182 is somatically mutated in >20% of cases of SMZL, as well as in >20% of cases of follicular lymphoma, and between 5-15% of cases of chronic lymphocytic leukaemia, MALT-lymphoma and hairy cell leukaemia. We conclude that miR-182 is a strong candidate novel tumour suppressor miRNA in lymphoma.
The overall gene expression signature of SMZL was found to be strongly distinct from those of other lymphomas. Functional analysis of gene expression data revealed SMZL to be characterised by abnormalities in B-cell receptor signalling (especially through the CD19/21-PI3K/AKT pathway) and apoptotic pathways. In addition, genes involved in the response to viral infection appeared upregulated. SMZL shows a unique epigenetic profile, but analysis of differentially methylated genes showed few with methylation related transcriptional deregulation, suggesting that DNA methylation abnormalities are not a critical component of the SMZL malignant phenotype.

# P73

## Skp2 Expression in Diffuse Large B-cell lymphoma: Correlation with Prognosis

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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. Skp2 is a member of mammalian F box proteins, which displays S-phase-promoting function through ubiquitin-mediated proteolysis of the cyclin dependent kinase (CDK) inhibitor, p27. The aim of this study is to evaluate the prognostic value of skp2 in DLCBL (70 cases) by immunohistochemical staining technique, and its correlation with the clinicopathological features and survival. Five (25%) control cases showed high skp2 expression compared to 52.9% of DLCBL using 10% as a cut off point with a significant difference (p=0.04). Skp2 was seen staining the large cells in proliferating germinal centers of control group. High skp2 expression in DLCBL was associated with several progressive parameters such as advanced stage (p=0.036), involvement of more than one extranodal site (p=0.05), presence (p=0.007) and extent (p=0.002) of necrosis. It was also significantly associated with Ki-67 (p=0.0001) and inversely correlated with p27 expression (p=0.0001). Skp2 expression in DLBCL identified subset of cases characterized by aggressive features like advanced stage, increased number of extranodal sites, presence of necrosis and high proliferation. Hypoxia resulted from necrosis could have a role in up regulation of skp2 which in turn may be responsible for induction of this necrosis by promoting proliferative tumour capacity.

# P74

## Primary cardiac lymphoma : A post mortem case report

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This is a case of a 77 year old man who was admitted to hospital complaining of palpitations, dizziness and sweating. During his hospital admission he was treated for various arrhythmias and an echocardiogram was performed which was initially abnormal raising the possibility of an infiltrative process within the right ventricle. A subsequent echocardiogram was reported as showing right ventricular hypertrophy. The patient self discharged and was awaiting further investigations but unfortunately died suddenly at home. An autopsy was conducted and the findings showed a large tumour infiltrating the full wall thickness of the right ventricle with extension towards the right atria. The remaining heart structures were otherwise normal. Histological examination of the cardiac tumour showed sheets of diffuse abnormal and atypical lymphoid cells with pleomorphic features. Immunohistochemistry was performed and the majority of the lymphoid cells were positive for CD45 and CD20, suggesting a high grade B cell lymphoma most probably representing a diffuse large B cell lymphoma. Primary cardiac lymphomas are rare and are associated with a high mortality, although some case reports have highlighted that early diagnosis and management can result in prolonged survival of more than 12 months.

# P75

## Invasive Group A Streptococcal Infection and Sudden Unexpected Death in Children.

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Three cases of sudden unexpected death in children aged between 18 months and 4 years revealed infection with invasive group A streptococcus (iGAS), between December 2009 and May 2010 in South Central England. All cases grew iGAS from blood and lung swabs, had significant empyemas and were M/emm type 1.

Reports of increases in iGAS infections have been documented since the 1980's; this prompted the Pan European (strep-EURO) surveillance programme (2003-2004). In January 2009 a National Incident Management Team was convened in England due to an increase in reported infection rates and over-representation in both fatalities and lower respiratory tract infections.

iGAS shows a diverse clinical spectrum of disease type and severity, causing death in 20% of cases within 7 days of infection.

This is now a statutory 'notifiable' disease (since April 2010). iGAS infection has public health implications with household contacts needing information about early signs and symptoms and evaluation for antibiotic prophylaxis.

The Health Protection Unit Briefing Document, 2009, has recommended that histopathologists are made aware of the wide modes of presentation and maintain a high index of suspicion as they can present as sudden unexpected deaths in the community. They stress the need for appropriate samples for culture and that all iGAS positive samples are referred to the HPA Streptococcus and Diphtheria Reference Unit for typing. Information from this type of surveillance is important for many reasons including new vaccine developments.

# P76

## Neonatal Intrapericardial Immature Teratoma – Surgical Emergency

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Teratomas are solid/cystic tumours arising from totipotential cells and are composed of tissues representing all 3 embryonic layers, typically presenting as a sacrococcygeal mass. We present a case of an intrapericardial neonatal teratoma arising from the adventitia of the ascending aorta which presented as a surgical emergency. Prenatal ultrasound at 20 weeks did not show fetal structural abnormalities. A male neonate (normal male karyotype 46XY) was delivered at 37 weeks gestation but was tachypnoeic/tachycardiac and floppy/pale at birth (APGAR scores 4 & 8). A chest radiograph revealed complete opacification of both lung fields and an enlarged cardiac silhouette. An echocardiogram showed a large pericardial effusion with CT imaging demonstrating a soft tissue mass. The patient had cardiac tamponade on day 3 and required urgent exploration/excision. Macroscopically, the tumour was an encapsulated solid/cystic mass weighing 33g and 5cm in maximum dimension. Histologically, the tumour was an immature teratoma (because of immature neuroectodermal elements) with no malignant yolk sac tumour identified. Excision appeared complete histologically and no further treatment was required. Post-resection monitoring showed a decrease in alpha fetoprotein levels to the normal range. The mediastinum is an uncommon anatomical extragonadal site for a teratoma which was undiagnosed antenatally. Most cases present with hydrops and successful in utero pericardiocentesis has been reported. This case highlights the clinical challenges of managing a neonate with a life-threatening intrathoracic tumour as well as the histological difficulties of using the grading system (originally used to classify adult ovarian tumours) and the identification of malignant elements within an immature teratoma.

# P77

## P63 Does Not Regulate Brachyury in Chordomas and Osteosarcomas

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Background: Chordomas are rare tumours sited along the axis and characterised by the expression of brachyury, a transcription factor, which under physiological conditions is only expressed in the developing skeleton. The mechanism by which brachyury expression is regulated in mammalian cells is unknown. It was therefore of interest to read recently that the deltaNP63 isoform is responsible for the transcriptional regulation of brachyury in murine development and osteosarcomas. Purpose: The aim of this study was to investigate whether expression of P63 accounted for the aberrant expression of brachyury in human chordomas and to determine if brachyury were expressed in osteosarcomas. Methods:We screened 26 chordomas and the UCH1 human chordoma cell line for P63 mRNA expression by RT-PCR initially using a PAN P63 primer set, and thereafter algorithmically using sets of transcript specific primers for the TAP63 and deltaNP63 transcripts. All 27 cases showed expression of pan P63 but were negative for the deltaNP63 transcript. Immunohistochemistry using the panP63 antibody4A4 was performed on a tissue microarray with 50 chordomas including the 26 cases that were analysed by RT-PCR. None of the cases showed P63 expression. In addition we screened a tissue microarray, containing sections of 200 osteosarcomas, for the expression of both brachyury and deltaNP63. None of the cases showed immunoreactivity for these antibodies. Conclusion: While it is intriguing that P63 is involved in the transcriptional regulation of brachyury in murine embryos we have excluded this molecule as a transcriptional regulator of brachyury in chordomas. Furthermore, neither brachyury nor P63 are expressed in human osteosarcomas. In summary, the finding that the deltaNP63 isoform in mice regulates the expression of brachyury cannot be translated to humans in the context of chordoma and osteosarcoma development. Supported by PathSoc grant.

# P78

## Is It Possible to Diagnose Septic Arthritis with High Specificity and Sensitivity by Using Procalcitonin in a Novel Way?

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PURPOSE OF THE STUDY: Septic arthritis is a challenging medical emergency which needs an urgent diagnosis. No gold standard is available for diagnosing septic arthritis. A novel diagnostic test with high specificity and sensitivity is required. Serum procalcitonin (PCT) is a biomarker which has been used widely to diagnose infection but although present in the serum of patients with septic arthritis it is not present in high levels in synovial fluid. There are controversial results regarding its efficacy in diagnosing septic arthritis. This work investigates a novel aspect of PCT expression in septic arthritis and reviews the literature around PCT expression and septic arthritis.
METHODS: 64 synovial biopsies from patients with established diagnosis of different arthropathies were studied by PCT immunohistochemistry. The number of neutrophils expressing PCT together, with other parameters, were assessed in each biopsy and used to generate receiver operating characteristic (ROC) curves that enable septic arthritis to be distinguished from other arthropathies.
SUMMARY OF RESULTS: PCT is expressed by cells in synovium, and (ROC) curves derived data have produced a diagnostic parameter of septic arthritis with 100% sensitivity and 80% specificity. In addition, within infected joints fibrin attached to the synovial surface stains for PCT, suggesting that sequestration of PCT by fibrin might explain why in septic arthritis PCT can be identified in serum but not in synovial fluid.
CONCLUSION: PCT immunohistochemistry could be used as a diagnostic test for septic arthritis. Fibrin binding of PCT may explain the discrepant results around the utility of serum and synovial fluid PCT as diagnostic test in septic arthritis.

# P79

## Diseased Intervertebral Discs Tissues Express a marker of Infection

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PURPOSE OF THE STUDY: Low Back Pain affects around 40% of people in the UK at some point in their lives. It is an expensive disease which costs the UK alone over £5-12 billion per annum and 180 million working days. Some studies reported a link between infection with Propionibacterium acnes and low back pain, but this has not been proven. We found Procalcitonin (PCT) protein which is a well known infection marker and widely produced from different tissues during infection to be a helpful diagnostic marker for osteoarticular infections. We hypothesised that were intervertebral disc degeneration (IVDD) an osteoarticular infection disc cells would express procalcitonin.
METHODS: We studied 13 cases of lumbar IVD disease by procalcitonin immunohistochemistery. Immunoelectron microscopic studies were done to detect the subcellular localization of PCT in IVD tissues. Results were compared to procalcitonin expression in other chondroid tissues as chondrocytes in diseased synovium and in recent fractures.
SUMMARY OF RESULTS: We found that PCT is widely expressed in IVD cells and matrix. There was an expression of PCT in nucleus pulposus cells and its pericellular matrix that we have not seen with other cells. This distribution has been studied by immunoelectron microscopy which showed that PCT was found in intracellular vesicles and had a zonal distribution in matrix.
CONCLUSION: This unexpected result could indicate that IVDD is caused by infection but in the absence of any other supporting evidence it is more likely that PCT expression in IVD tissues may indicate that PCT has a role in IVD matrix and cellular degeneration. These findings need further study to find out if PCT has a role in IVD cell metabolism or a specific role in IVDD.

# P80

## Podoplanin Expression in Adamantinoma of Long Bones and Osteofibrous Dysplasia

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Adamantinoma of long bones (ALB) and osteofibrous dysplasia (OFD) are rare osteolytic bone tumours that principally arise in the tibia. Both ALB and OFD contain epithelial and stromal elements. ALB needs to be distinguished from metastatic cancer and OFD contains fibrous dysplasia (FD)-like areas. Podoplanin, a transmembrane glycoprotein expressed by osteocytes, has been reported in a number of biphasic tumours including adamantinoma of the jaw. In this study we assessed podoplanin expression in ALB, OFD, fibrous dysplasia (FD) and metastatic cancer.
42 cases of ALB and OFD were stained by immunohistochemistry for expression of podoplanin and epithelial cell markers.
Podoplanin was detected in epithelial cells of ALB as well as in scattered fibroblast-like stromal cells in both ALB and OFD. Podoplanin expression in FD was not seen intertrabecular fibrous tissue. Podoplanin expression was not seen in epithelial cells in (non-squamous) metastatic carcinomas. Podoplanin expression was noted in osteocytes but not in osteoblasts of bone trabeculae in ALB, OFD, FD and metastatic carcinoma.
The finding of a common osteocyte marker on OFD/ALB stromal cells is in keeping with a close histogenetic relationship between OFD and ALB. This may reflect the prominence of fibro-osseous proliferation in these tumours. Expression of podoplanin in an osteolytic tumour of the tibia may be a useful diagnostic discriminant in distinguishing in ALB from metastatic carcinoma and in the differential diagnosis of OFD from FD.

# P81

## Desmoplastic Small Round Cell Tumour of the Ilium associated with a Variant EWS-WT1 Transcript

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Desmoplastic small round cell tumour is a rare aggressive neoplasm which typically arises within the abdominal cavity of children and young adults. It is characterised by a t(11;22)(p13;q12) translocation which results in the formation of an EWS-WT1 fusion transcript. We report an unusual case of desmoplastic small round cell tumour arising in the left ilium of a 31 year old man. Imaging showed diffuse sclerotic involvement of the iliac wing with extension into the soft tissue to form masses at the medial and lateral aspects. The patient underwent a core biopsy which revealed a high-grade 'small round cell tumour' composed of cords and nests of cells surrounded by a desmoplastic stroma. Immunohistochemistry showed that the tumour cells were positive for MNF116 and desmin (dot-like positivity). EWS gene rearrangement was demonstrated by interphase FISH but the most common EWS-WT1 7/8 fusion transcript was not detected by RT-PCR. A variant EWS-WT1 fusion transcript was subsequently detected using primers for EWS exon 9 and WT1 exon 8. Primary desmoplastic small round cell tumour of bone is extremely rare with only three previously reported cases. Molecular confirmation of the histological diagnosis is particularly important in cases arising in unusual sites. However, it should be noted that variant fusion transcripts represent a potential pitfall in diagnosis.

# P82

## Telomerase and Related Molecules in Soft Tissue Neoplasia

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This study has investigated the relationship between the expression of the catalytic subunit of telomerase (h-TERT), cell proliferation and hypoxia in a range of soft tissue neoplasms. A tissue microarray of 122 neoplasms (91 sarcomas) was constructed and sections stained by immunocytochemistry for h-TERT, p53, Rb, p21, CD31, HIF-1 alpha, MDM2 and Ki67 using heat-induced epitope retrieval and the Dako EnVision FLEX+ detection system.

h-TERT was expressed in 16% of benign and 65% of malignant neoplasms (p= <0.005) with higher expression in leiomyosarcomas, synovial sarcomas, undifferentiated sarcomas and myxofibrosarcomas, and lower expression in liposarcomas. Higher expression of h-TERT, HIF-1 alpha, and Ki-67, and loss of Rb expression were associated with higher grade sarcomas (all p<0.004). Expression of p53 and MDM2, p21 and tumour vascularity were not associated with grade. Higher expression of h-TERT was associated with higher levels of expression of p53, HIF-1 alpha, Ki-67, and MDM2 (all p<0.003) and there were significant positive correlations between Ki67, HIF-1 alpha, and CD31.

h-TERT is expressed in a wide range of high grade sarcomas. The positive correlations between proliferation, hypoxia and vascularity support the view that mesenchymal cells are adapted to proliferate in a hypoxic environment. Increased expression of h-TERT may be part of this adaptive response.

# P83

## Diagnostic and Prognostic Role of Galectin 3 Expression in Cutaneous Melanoma

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Many of the histopathological criteria used to diagnose melanoma overlap with atypical but otherwise benign naevi such as dysplastic or Spitz naevi. Galectin-3 is a member of the galectin gene family and is expressed at elevated levels in a variety of neoplastic cell types. The aim of the present study was to investigate the diagnostic value of galectin-3 expression compared to HMB-45 (one of the established and widely used immunohistochemical melanocytic markers) together with assessment of its prognostic value in melanoma lesions. This study was carried out on 21 cases of melanoma and 20 benign pigmented naevi. Galectin-3 was expressed in all the examined benign and malignant melanocytic lesions. The nucleocytoplasmic pattern of galectin-3 appeared in malignant cases only with 42.86% sensitivity, 100% specificity and 70.73 % accuracy. This pattern tended to be associated with thick melanoma (p=0.08) and reduced survival (p=0.22). The intensity of galectin-3 assessed by H score was significantly of higher values in malignant lesions compared to benign lesions (P<0.0001). The best cut-off value for discrimination between benign and malignant melanocytic lesions was 295 with 95% sensitivity, 70 % specificity and 83 % accuracy. The diagnostic power of galectin-3 in distinguishing between benign and malignant melanocytic lesions relies on the pattern and intensity of its expression. The nucleocytoplasmic pattern of galectin-3 expression carries greater probability of a malignant phenotype and a poor prognostic impact on patients' outcome.

# P84

## Case Report of Primary Subcutaneous Mucormycosis in an Immunocompetent Man

Ⓟ M Emami

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An 18 year old male was admitted with a history of face cellulitis for one month. Initially the lesion appeared like a furuncle on face in left side of nose and gradually its swelling and tenderness got worse. The patient was afebrile, non-toxic without lymphadenopathy. He was non-diabetic, non-neutropenic, non-immunocompromised and not previously on immunosuppressive drugs .There was no history of erosion or rupture on the face skin. Outpatient therapy included various topical or oral antibiotics and corticosteroids. At last, triamcinolone was injected subcutaneously that made it deteriorated. As inpatient therapy, several days of wide-spectrum systemic antimicrobial therapy (Imipenem, Vancomycin & etc) failed to have any advantage and the face cellulitis grew larger, caused left eye to get semiopen.Incisional drainage of abscess was done to reduce the swelling and skin biopsy was taken based on 3 early diagnoses (Leishmaniasis, Anthrax & Tumours). Spiral CT-Scan: Soft tissue density with cystic changes (abscess formation) was seen at left side of face from inferior rim of orbit extended to inferior of maxillary sinus, this was seen anterior to nasal ridge too. Mucosal thickening of ethmoidal air cells was detected. Laboratory data- WBC: 8600, Neut: 70%, Lymph: 22%, CRP: Neg., ESR: 10, Blood Culture: Negative .Other biochemical parameters were also in normal range. Abscess drain direct smear: Gram negative bacilli, Abscess drain culture: Pseudomonas aeruginosa. Microbiological findings were not useful in treatment and caused misdiagnosis. Pathology: Microscopic sections revealed broad branching nonseptate hyphae on a necrotic background infiltrated by giant cells and neutrophils in hypodermis and subcutaneous tissue. So the definite diagnosis was mucormycosis (Zygomycosis) infection. Finally, the patient treated successfully with antifungal therapy.

# P85

## Anti phospho-histone H3, an Emerging Diagnostic Tool for Counting Mitoses in Thin Melanomas

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The 7th edition of American Joint Committee on Cancer (AJCC) staging manual outlines some important changes from 6th edition in melanoma staging system notably for T1 tumours where depth of invasion has been replaced by the presence of mitotic index/mm2. It has emerged as a primary criteria along with the presence or absence of ulceration. If the mitotic count is more than 1.0/ mm2 the staging is upgraded from T1a to T1b. This modification was recommended after statistical survival analysis revealed that mitotic rate is a stronger predictive of increased disease free survival than depth of dermal invasion as assessed by Clark's level.Accurate mitotic count is therefore essential for patient management and any tool which can remove inter- and intraobserver variability should be searched.Aim of this study is to identify a means of assessing mitoses in a more reproducible manner than conventional assessment on H&E alone.
We studied the antibody to phospho-histone H3 (PHH3) which detects phosphorylation of amino acids at histone 3, a phenomenon strictly correlated with condensation of chromatin during mitosis and meiosis and thus only labels cells in the actual phase of cell division. Instead of MIB-1 immunostaining which detects cells in all phase of replication (G1, S, G2 and M),we employed PH3 immunostaining to count mitoses using 40x objective in tissue sections from 30 melanoma patients with thin melanomas and compared results with routine hematoxylin and eosin (H&E) in a double-blind fashion. The study concluded that mitotic figures were not only readily visible by anti-PHH3 immunostain but in certain cases also upgraded the stage.
Our results indicate that anti-pHH3 is an emerging tool for labeling mitoses in melanocytes. It can prove to be a staging technique in the future which allows accurate and time effective assessment of mitoses in thin melanomas with reliable differentiation from apoptotic figures.

# P86

## BRAF V600E Mutations in Melanoma: Should All Malignant Melanomas Be Tested ?

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BRAF gene mutations are present in 50-60% of melanomas. Ninety percent of these mutations are located at position 600 (V„{sup3}E). BRAF V600E mutations lead to continuous stimulation of the MAP kinase signalling pathway which in turn confers a proliferative phenotype on the cell.
A Phase I clinical trial for a drug, PLX 4032, that targets tumours of melanoma patients with the BRAF V600E mutation, has recently been reported and shown promising results (70% response rate in patients with this mutation). Tumour shrinkage has been observed at metastatic sites including liver, bone and lung.
We have implemented a real-time PCR assay for the BRAF V600E mutation and begun testing melanoma samples. To date 18 patients with metastatic malignant melanoma, 5 women and 13 men, aged between 24 and 98 have been tested. The most common site was the back, but samples from the foot, finger, abdomen and lymph node metastasis were also included. Of these 18 patients, 9 (50%), were positive for BRAF V600E mutations. 7 patients with other tumours acted as controls, all of which were BRAF negative.
Our results confirm the literature to date; that BRAF mutations are present in approximately 50% of melanomas. We suggest that BRAF status should be part of the diagnostic work up for all melanomas in vertical growth phase to predict eligibility to clinical trials with molecular inhibitors such as PLX 4032, should the patient develop metastatic melanoma.

# P87

## Pagetoid Variant of Actinic Keratosis: Can Immunohistochemistry Help in the Differential Diagnosis?

Ⓟ V Willis; M Taylor

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Background: Actinic keratosis is a commonly diagnosed skin lesion associated with chronic sun exposure. Pagetoid variant of actinic keratosis is an important entity as its differential diagnosis includes extra-mammary Paget's disease and melanoma. Currently there is little in the literature regarding useful immunostains to address this issue. In this study we have reviewed the immunohistochemical profile of pagetoid actinic keratosis and attempted to identify a panel to aid in accurate diagnosis.
Methods: Eight skin specimens reported as pagetoid actinic keratosis over a five month period (January to May 2010) were retrospectively identified. The local electronic database was used to search for clinical details. The pattern of immunohistochemical staining was reviewed in each case.
Results: CK20, S100 and GCDFP-15 immunostaining was uniformly negative whilst EMA was uniformly positive. Six in eight cases were negative with CK7. Pancytokeratins (CAM5.2 and MNF116) showed marked variability, as did histochemical staining with DPAS. Interpretation with CK14 was difficult due to membranous staining and background positivity.
Discussion: All lesions in actinically damaged skin demonstrating pagetoid spread of atypical keratinocytes should be differentiated from extra-mammary Paget's disease and melanoma. We have used a panel of staining comprising CK7, CK14, CK20, S100, EMA and GCDFP-15 together with DPAS. Although no immunohistochemical stain is entirely specific, we find these stains helpful and recommend they be part of a standard panel. Correlation with clinical information such as biopsy site, previous history and clinical impression is vital in these cases. Lesions occurring in anogenital skin may require additional testing with uroplakin and CEA. Clearly further cases need to be studied to improve consistency in histological reporting.

# P88

## Optimising Parameters in an Agent Based Image Analysis System

Ⓟ SS Cross; OTK Lobo; RF Harrison

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We have previously presented a proof of concept agent based image analysis system that has the potential to identify sparse regions of interest (ROI) in histological images e.g. mitotic figures. The system consists of hundreds of individual agents that exhibit collective behaviour when presented with image 'landscapes'. We are now seeking to optimise variable agent parameters for fast and efficient identification of image features. One key parameter is the threshold above which an agent will follow the trail of another agent. We systematically varied this parameter across a range of values and ran the system 50 times for each value on a test image. Analysis of these results showed that at high threshold values (>1.4) the agents did not cluster on the ROI. At lower values there was clustering on the ROI and the speed of clustering was related to the threshold value. Initial systematic testing showed that a value of 0.5 produced the most efficient clustering on the test image, however the testing was computer and operator intensive requiring 23 three hour batches of computing time on a fast dual processor machine. We therefore tried another strategy where we used a 'genetic' search algorithm to examine the parameter space. Although this was also computationally intensive it was much quicker because it was not an exhaustive search of the entire parameter space and did not require any user intervention. This method produced a threshold value of 0.4 for efficient clustering which below our initial range used in the systematic method. Further systematic testing showed 0.2 to be the optimal value. We conclude that variation of certain parameters in our agent based image analysis system produces radical change in the system behaviour and that genetic search algorithms are useful for finding the optimal values of such parameters.

# P89

## A Generic Self-Learning Agent-Based Image Feature Detection System

Ⓟ SS Cross; RF Harrison

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Most image analysis systems have a strong element of top-down design. A target feature, e.g. a mitotic figure, is selected and *a priori* decisions are made about the software strategies to be used to detect it. We have previously presented a proof of concept agent-based image analysis system. In the course of developing this we have found it is very difficult to make any decisions about parameter optimisation to improve the detection of selected features. We have therefore redesigned the system to produce a completely generic self-learning system. The initial stage is to take a test image and identify the pixels that contain the feature to be identified. The co-ordinates of these pixels are entered into the programme and the measure of success is the proportion of the total agents in that area at the end of a programme run. The programme contains a wide range of parameters that extract certain features of the image (e.g. absolute value of red, ratio of blue to green etc.). All these parameters are set to neutral at the start. A genetic algorithm programme is then used to search the parameter space using the previously defined measure of success averaged over 10 model runs for each parameter combination, each of 5000 programme steps. The number of combinations of possible parameter states is close to a sextillion so an exhaustive search is not feasible. A standard genetic algorithm using grey binary chromosome encoding, mutation rate of 0.03, crossover rate of 0.07 and 5000 model runs produced a set of parameters that successfully identified the targets in simple test images. We have demonstrated a generic self-learning image feature detection system that produces robust and efficient identification of selected features. Further development will include scaling the system up to full field microscopy images and running the system on a high performance cluster to speed up the development process.

# P90

## Evaluation of Nucleic Acid Quality From Long-Time Stored Fresh-Frozen Breast Cancer Tissues After Non-Automated Needle Micro-Dissection.

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The fundamental basis for high throughput technologies (HTH) such as microarrays or next generation sequencing is the isolation of sufficient amounts of good quality mRNA and DNA from the cells of interest. We have evaluated whether mRNA and/or DNA extracted from non-automated needle dissected frozen tissue, which has been stored for a prolonged period (>20ys), is still suitable for microarray profiling. 263 frozen samples of invasive breast carcinoma collected between 1984 and 2002 were reviewed. 215/263 (82%) cases contained at least 70% malignant cells. These were stained, needle micro-dissection and nucleic acids extracted with Qiagen kits. Quantities were assessed using Agilent Nano Kit (RNA), gel electrophoresis (DNA) and spectrophotometric analysis (both). Latest HTH profiling techniques require a minimum spectrophotometric 260/280 ratio of 2 and 260/230 ratio > 1.5. This was achieved for 199/215 (93%) RNAs and 210/215 (98%) DNAs. Mean Bioanalyzer RNA Integrity Number (RIN) was 6.8, RIN ≥8 23/215 (11%); ≥7 62/215 (29%); ≥6 69/215 (32%); ≥5 7/215 (3%) and <5 54/215 (25%). RIN value was not affected by length of storage (Pearson's correlation coefficient 0.072). Gel electrophoresis of DNA samples did not present any extensive degradation. Approximately 88% samples with RIN ≥5 have so far been used for expression and genomic profiling. Only 2 RNAs and 1 DNA samples have not performed well. We have demonstrated that long-term storage of fresh-frozen breast tumour that have been manually needle micro-dissected does not affect the quality of nucleic acids, and can therefore successfully be used for high throughput technologies.

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