

Joint Meeting of the Pathological Society of Great Britain and Ireland and the Dutch Pathological Society (NVvP)

Leeds 1 - 4 July 2008



Hosted by the
Department of Pathology and Tumour Biology,
Leeds Institute of Molecular Medicine,
University of Leeds

Venue
Conference Auditorium, University of Leeds,
Leeds, England, LS2 9JT

PROGRAMME ACKNOWLEDGEMENTS

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PROGRAMME QUICK REFERENCE TABLES – LEEDS 2008

Tuesday 1 July 2008

SPORTS HALL 1	
10.00	Registration and Coffee
SPORTS HALL BALCONY	
11.00–17.00	Slide Seminar Viewing: <i>Soft tissue</i>
CONFERENCE AUDITORIUM 1	
11.00–11.15	Welcome Address
11.15–13.00	Symposium: <i>Pathology informatics</i>
12.15–13.00	Keynote Lecture Prof F Ponten, Uppsala
SPORTS HALL 1	
13.00–14.00	Lunch, Poster Viewing and Trade Stands
CONFERENCE AUDITORIUM 1 AND CONFERENCE AUDITORIUM 2	
14.00–17.00	Oral Presentations
SPORTS HALL 1	
15.30–16.10	Tea
CONFERENCE AUDITORIUM 1	
17.00–18.00	Public Lecture Prof TH Pennington, Aberdeen
PARKINSON COURT, PARKINSON BUILDING, UNIVERSITY OF LEEDS	
18.30–21.30	Welcome Reception – Sponsor: Leica Microsystems

Wednesday 2 July 2008

SPORTS HALL 1		
07.30	Registration and Coffee	
SENIOR COMMON ROOM		
07.45–09.15	Trainees' Breakfast Meeting	
SPORTS HALL BALCONY		
09.00–17.00	Slide Seminar Viewing: <i>Soft tissue</i>	
CONFERENCE AUDITORIUM 1	CONFERENCE AUDITORIUM 2	
09.20–12.10	Symposium: <i>Incipient neoplasia</i>	09.20–12.10 Symposium: <i>Pathology of regeneration and degeneration</i>
SPORTS HALL 1		
10.30–11.10	Coffee	
CONFERENCE AUDITORIUM 1		
12.10–13.00	Japanese Pathology Society Guest Lecture Prof T Arai, Tokyo	
SPORTS HALL 1	CONFERENCE AUDITORIUM 1	CONFERENCE AUDITORIUM 2
12.30–14.00	13.30–15.00	13.00–15.00
Lunch and Trade Stands	Meets the Experts: <i>Colorectal polyps and the new Colorectal Cancer Screening Programme</i>	Liver EQA
SPORTS HALL 1		
14.00–15.00	Poster Rounds	
CONFERENCE AUDITORIUM 1		
15.00–17.30	Plenary Oral Presentations	
SPORTS HALL 1		
16.00–16.40	Tea	

PROGRAMME QUICK REFERENCE TABLES – LEEDS 2008

Thursday 3 July 2008

SPORTS HALL 1 08.00 Registration and Coffee		
SPORTS HALL BALCONY 09.00–15.00 Slide Seminar Viewing: <i>Soft tissue</i>		
CONFERENCE AUDITORIUM 1 09.00–12.00 Symposium: <i>Pathology and targeted treatment</i> Sponsor: International Society for Cellular Oncology	CONFERENCE AUDITORIUM 2 09.00–12.00 Oral Presentations	ROGER STEVENS BUILDING – ROOM 19 09.30–17.00 Association of Clinical Electron Microscopists
SPORTS HALL 1 10.30–11.10 Coffee		SPORTS HALL 1 10.30–11.00 Coffee
CONFERENCE AUDITORIUM 1 12.00–13.00 Pathological Society Business Meeting	CONFERENCE AUDITORIUM 2 12.00–13.30 Renal EQA	
SPORTS HALL 1 13.00–14.00 Lunch and Trade Stands		SPORTS HALL 1 13.00–14.00 Lunch and Trade Stands
SPORTS HALL 1 13.30–14.30 Poster Rounds		
CONFERENCE AUDITORIUM 1 14.30–17.00 Oral Presentations	CONFERENCE AUDITORIUM 2 14.30–15.30 Trainees Session: <i>Translational aspects of disease</i>	
SPORTS HALL 1 15.30–16.10 Tea		SPORTS HALL 1 15.30–16.00 Tea
	CONFERENCE AUDITORIUM 2 16.10–17.00 Trainees Session – Panel Based Forum Discussion: <i>Achieving excellence in research, teaching and diagnostics work whilst maintaining work-life balance</i>	
CONFERENCE AUDITORIUM 1 17.00–18.00 Pathological Society's Doniach Lecture Prof Sir JCE Underwood, Sheffield		
NATIONAL RAILWAY MUSEUM, YORK 19.30–22.30 Conference Dinner. <i>Please note buses depart at 18.15</i>		

Friday 4 July 2008

SPORTS HALL 1 08.30 Registration and Coffee		
CONFERENCE AUDITORIUM 2 09.30–13.30 Symposium: <i>The genome and cancer</i>		
SPORTS HALL 1 10.45–11.25 Coffee		

SCIENTIFIC SESSIONS INFORMATION

ORAL COMMUNICATIONS

Sessions will be held as follows:

Tuesday 1 July, 14.00–17.00	[CONFERENCE AUDITORIUM 1 & 2]
Thursday 3 July, 09.00–12.00	[CONFERENCE AUDITORIUM 2]
Thursday 3 July, 14.00–17.00	[CONFERENCE AUDITORIUM 1]

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

PLENARY ORAL SESSION [CONFERENCE AUDITORIUM 1]

The seven highest-ranked submitted oral abstracts will be presented on Wednesday 2 July, 15.00–17.30.

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

POSTERS [SPORTS HALL 1]

Viewing:	Tuesday 1 July, 13.00–14.00
Viewing and Formal Poster Rounds:	Wednesday 2 July, 14.00–15.00
	Thursday 3 July, 13.30–14.30

Note to presenters: Ideally, posters should be in place by 10.00 hrs on Tuesday 1 July and removed by 17.00 hrs on Thursday 3 July. At least one of the contributors must be in attendance during the formal viewing period, as indicated in the programme synopsis.

Prizes: The Pathological Society Sir Alastair Currie Prize and second and third poster prizes will be presented at the Society Dinner.

SYMPOSIA

Tuesday 1 July

11.15–13.00 *Pathology informatics* [CONFERENCE AUDITORIUM 1]

Wednesday 2 July

09.20–12.10 *Incipient neoplasia* [CONFERENCE AUDITORIUM 1]
Pathology of regeneration and degeneration [CONFERENCE AUDITORIUM 2]

Thursday 3 July

09.00–12.00 *Pathology and targeted treatment* [CONFERENCE AUDITORIUM 1]
Sponsor: International Society for Cellular Oncology

Friday 4 July

09.30–13.30 *The genome and cancer* [CONFERENCE AUDITORIUM 2]

TRAINEES' PROGRAMME

Wednesday 2 July

07.45–09.15 *Workplace-based assessment and MRCPath Autopsy Examination*
[SENIOR COMMON ROOM]
13.30–15.00 *Meet the Experts – Colorectal polyps and the new Colorectal Cancer Screening Programme* [CONFERENCE AUDITORIUM 2]

Thursday 3 July

14.30–15.30 *Translational aspects of disease* [CONFERENCE AUDITORIUM 2]
16.10–17.00 *Panel based Forum discussion: Achieving excellence in research, teaching and diagnostics work whilst maintaining work-life balance* [CONFERENCE AUDITORIUM 2]

SCIENTIFIC SESSIONS INFORMATION

SLIDE SEMINAR VIEWING/COMPETITION – *Soft Tissue* [SPORTS HALL BALCONY]

Competition: There will be a slide competition using virtual slides, which will be available for viewing on:
Tuesday 1 July, 11.00–17.00
Wednesday 2 July, 09.00–17.00
Thursday 3 July, 09.00–15.00

KEYNOTE AND NAMED LECTURES [CONFERENCE AUDITORIUM 1]

Tuesday 1 July

12.15–13.00 **Keynote Lecture:** *A human protein atlas*
Prof F Ponten, Uppsala
17.00–18.00 **Public Lecture:** *MRSA/Hospital acquired infection*
Prof TH Pennington, Aberdeen

Wednesday 2 July

12.10–13.00 **Japanese Pathological Society Guest Lecture:** *The pathology of colorectal and gastric carcinomas in the elderly: Clinicopathological characteristics and molecular mechanisms*
Prof T Arai, Tokyo

Thursday 3 July

17.00–18.00 **Doniach Lecture:** *Sights unseen, truths untold: Pathology and the modern medical curriculum*
Prof Sir JCE Underwood, Sheffield

COMPANION MEETINGS

Wednesday 2 July

13.00–15.00 Liver EQA [CONFERENCE AUDITORIUM 2]

Thursday 3 July

09.30–17.00 Association of Clinical Electron Microscopists [R STEVENS BUILDING LT19]
12.00–13.30 Renal EQA [CONFERENCE AUDITORIUM 2]

TRADE EXHIBITION [SPORTS HALL 1]

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

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:

For each full day 7 points
For each half day 3 points

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

GENERAL ARRANGEMENTS

PRESENTATION CHECKING AND PREVIEW [*SPORTS HALL BALCONY*]

ORAL PRESENTATIONS AND LECTURES

Presentation format:

Powerpoint only

Must be PC compatible

Must be on memory sticks or CD

Presenters must attend their nominated lecture theatre 30 minutes before their presentation time.

POSTERS

Poster boards will be size A0 (841 x 1189 mm – *landscape*). Please do not exceed these dimensions. Velcro will be provided.

INTERNET ACCESS [*SPORTS HALL BALCONY*]

Wireless Internet access will be available for all delegates who will be provided with personal usernames at the registration desk. Alternatively, the on-site internet café can be used.

MESSAGES

During the meeting, messages for delegates may be left at the following number:

+44 (0)113 343 8818

There will also be a message board located beside the Registration Desk.

REFRESHMENTS

All refreshments will be served in the Sports Hall unless stated otherwise in the Detailed Programme.

BADGES

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

COATS AND BAGS [*TABLE TENNIS ROOM*]

Secure facilities will be provided for coats and bags.

TRAVEL

See website for information.

ACCOMMODATION

See website for information.

DISCLAIMER

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

GENERAL ARRANGEMENTS

SOCIAL ACTIVITIES

Welcome Reception [PARKINSON COURT, PARKINSON BUILDING, UNIVERSITY OF LEEDS]

Tuesday 1 July, 18.30–21.30

Please reserve your free ticket when registering – places are limited.

Sponsor: Leica Microsystems

Conference Dinner [NATIONAL RAILWAY MUSEUM, YORK]

Thursday 3 July, 19.30 for 20.00–22.30. *Please note buses depart at 18.15.*

Please reserve your ticket (cost £50) when registering.

Local places of interest

Please visit this website for information: www.leedsliveitloveit.com

FUTURE MEETINGS

2008

1–4 September

Second Summer School, Cambridge

Molecular pathology for the uninitiated: a conceptual and practical guide

2009

8–9 January

Winter Meeting including Trainees' Programme, King's College, London

26–30 January

Winter School (formerly Newcastle Pathology Course), London

30 June–3 July

Cardiff Pathology 2009 (Fifth Joint Meeting of the BDIAP and the Pathological Society)

2010

6–8 January

Winter Meeting including Trainees' Programme, Imperial College, London

29 June–2 July

Summer Meeting, St Andrews

2011

January

Winter Meeting including Trainees' Programme
(dates and venue to be confirmed)

10–13 May

Ghent Pathology 2011 (Sixth Joint Meeting of the BDIAP and the Pathological Society)

REGISTRATION

Registration is via our on-line facility found on our website: www.pathsoc.org
An email acknowledgement will be sent automatically.

FEES					
Delegate Type	Fee Categories	Whole Meeting	Day or Part Day	Whole Meeting	Day or Part Day
		<i>EARLY BIRD</i> <i>Up to and including 2 June</i>	<i>EARLY BIRD</i> <i>Up to and including 2 June</i>	<i>From 3 June</i>	<i>From 3 June</i>
Pathological Society and Dutch Pathological Society Members	Ordinary Membership, Consultant and/or equivalent position	£ 225	Tue–Thu £ 90 Fri £ 55	£ 315	Tue–Thu £ 130 Fri £ 75
Pathological Society and Dutch Pathological Society Concessionary Members	Biomedical Scientists; Honorary or Senior Members; PhD Students; Post-Doctoral Fellows, Technicians and Trainees	£ 55	Tue–Thu £ 30 Fri £ 20	£ 80	Tue–Thu £ 45 Fri £ 30
Undergraduate Students *		£ 55	Tue–Thu £ 30 Fri £ 20	£ 80	Tue–Thu £ 45 Fri £ 30
Non-Members	Consultant and/or equivalent position	£ 315	Tue–Thu £ 155 Fri £ 55	£ 440	Tue–Thu £ 215 Fri £ 75
Non-Members Concessionary *	Biomedical Scientists; PhD Students; Post-Doctoral Fellows, Technicians and Trainees	£ 80	Tue–Thu £ 45 Fri £ 20	£ 110	Tue–Thu £ 65 Fri £ 30
All delegates and fee categories		Welcome Reception (Tuesday 1 July)			Free
All delegates and fee categories		Society Dinner			£ 50

* CONCESSIONS

Delegates from categories:

UNDERGRADUATE STUDENTS

NON-MEMBERS CONCESSIONARY

must provide an identification document as proof of their student or trainee status, including NTN's where applicable. Proof must be by way of a statement from the Head of Department.

Please email to: julie@pathsoc.org

ADVANCE REGISTRATION

Advance registration will close on **Friday 20 June**. Thereafter delegates may only register on-site in Leeds.

CANCELLATIONS

Please note that we are unable to refund registration fees for cancellations received after **Monday 16 June**.

ENQUIRIES

Enquiries should be addressed to: Pathological Society of Great Britain & Ireland.

Tel: +44 (0)20 7976 1260 Fax: +44 (0)20 7426 0047 Email: admin@pathsoc.org

Detailed Programme – Tuesday 1 July 2008

Presenter = {P} · Abstract numbers are shown in bold and square brackets eg [S123]

- 10.00 Sports Hall 1**
REGISTRATION and COFFEE
- 11.00–17.00 Sports Hall Balcony**
SLIDE SEMINAR CASE VIEWING: *Soft Tissue*
Cases submitted by Dr W Merchant, Department of Histopathology and Molecular Pathology, St James's Institute of Oncology
- 11.00–11.15 Conference Auditorium 1**
WELCOME ADDRESS
Dr H Grabsch, University of Leeds
- 11.15–13.00 Conference Auditorium 1**
SYMPOSIUM: *Pathology informatics*
Chair: Dr H Grabsch, University of Leeds
Dr D Treanor, University of Leeds
- 11.15–11.45 [S1] *Virtual slides in research and diagnosis*
Dr D Treanor, University of Leeds
- 11.45–12.15 [S2] *NCRI/NCI Bioinformatics initiative*
Dr S Bell, National Cancer Research Institute, Informatics Initiative, London
- 12.15–13.00 **KEYNOTE LECTURE:**
[S3] *A human protein atlas*
Prof F Ponten, Department of Genetics and Pathology, Uppsala University Hospital, Uppsala, Sweden
- 13.00–14.00 Sports Hall 1**
LUNCH
POSTER VIEWING and TRADE EXHIBITION
- 14.00–17.00 Conference Auditorium 1**
ORAL COMMUNICATIONS: *Breast*
Chair: Prof LB Jones, Queen Mary's School of Medicine and Dentistry, London
Dr A Shaaban, St James's Institute of Oncology, Leeds
- 14.00–14.15 [O1] *FABP7 Expression predicts better outcome in basal-like breast cancer*
{P} H Zhang, EA Rakha, GR Ball, EC Paish, C Caldas, IO Ellis, AR Green
- 14.15–14.30 [O2] *Tiling path genomic profiling of grade III invasive ductal breast carcinomas*
{P} R Natrajan, SM Rodriguez-Pinilla, C Marchio, R Vatcheva, MB Lambros, LG Fulford, A Grigoriadis, A Mackay, K Fenwick, N Tamber, D Hungermann, J Palacios, H Buerger, A Ashworth, JS Reis-Filho
- 14.30–14.45 [O3] *Downregulation of 15-hydroxyprostaglandin dehydrogenase contributes to tamoxifen resistance in breast cancer*
{P} M Cummings, L Maraqa, MB Peter, V Speirs

Detailed Programme – Tuesday 1 July 2008

Presenter = {P} · Abstract numbers are shown in bold and square brackets eg [S123]

- 14.45–15.00 [O4] *Predicting outcomes in sporadic versus hereditary breast cancer: a limited immunohistochemical panel does not identify BRCA1 and BRCA2 carriers*
{P} DL Holliday, C Chelala, P Goinath, DM Eccles, JL Jones
- 15.00–15.15 [O5] *An altered myoepithelial cell phenotype in DCIS influences tumour-associated angiogenesis*
{P} SJL Payne, M Allen, I Hart, JL Jones
- 15.15–15.30 [O6] *Genomic and immunophenotypical characterisation of pure micropapillary carcinomas of the breast*
{P} C Marchio, M Iravani, R Natrajan, MB Lambros, K Savage, S Di Palma, FC Schmitt, G Bussolati, IO Ellis, A Ashworth, A Sapino, JS Reis-Filho
- 15.30–16.10 TEA [SPORTS HALL 1]
- 16.10–16.25 [O7] *Neural networks identify gene profiles that predict clinical outcome with high accuracy in breast cancer: CA IX is identified as the principal prognostic indicator*
LJ Lancashire, {P} DG Powe, E Rakha, AR Green, R Mukta, EC Paish, RC Rees, IO Ellis, GR Ball
- 16.25–16.40 [O8] *Clear cells of the nipple epidermis: a reserve of motile mammary precursor cells?*
{P} J Going, M Currie
- 16.40–16.55 [O9] *Development and characterisation of a new breast cancer cell line of basal phenotype*
{P} V Speirs, CA Green, RH Partanen, AM Shaaban, AM Hanby
- 14.00–17.00 Conference Auditorium 2**
ORAL COMMUNICATIONS: Gastrointestinal
Chair: Dr MJ Arends, University of Cambridge
Dr N Scott, St James's Institute of Oncology, Leeds
- 14.00–14.15 [O10] *Methylation pattern of high risk flat adenomas in CRC*
{P} QJM Voorham, B Carvalho, AJ Spiertz, NCT Van Grieken, S Derks, H Grabsch, B Rembacken, AP De Bruïne, M Van Engeland, GA Meijer
- 14.15–14.30 [O11] *Combining proteomics and genomics to identify biomarkers for colorectal cancer*
{P} M De Wit, CR Jimenez, B Carvalho, S Piersma, R Lamerichs, GA Meijer, RJA Fijneman
- 14.30–14.45 [O12] *Chromosomal aberrations and APC promoter methylation in sporadic and coeliac disease related small intestinal adenocarcinomas*
{P} B Diosdado, TE Buffart, M Tijssen, BJ Bolijn, B Ylstra, B Carvalho, R Watkins, F Lewis, ID Nagtegaal, CJJ Mulder, K Maude, H Grabsch, P Quirke, P Howdle, GA Meijer
- 14.45–15.00 [O13] *K-Ras and B-Raf mutations in the MRC CR08 FOCUS Trial*
{P} S Richman, P Chambers, F Elliott, C Daly, M Braun, J Barrett, G Taylor, P Quirke, M Seymour, and MRC Focus Investigators
- 15.00–15.15 [O14] *Patient survival according to the quality of colonic cancer surgery: time for action?*
{P} NP West, EJA Morris, O Rotimi, A Cairns, PJ Finan, P Quirke

Detailed Programme – Tuesday 1 July 2008

Presenter = {P} · Abstract numbers are shown in bold and square brackets eg [S123]

- 15.15–15.30 [O15] *The prognosis of oesophageal carcinoma depends on the number of lymph nodes examined in the resection specimen*
{P} CP Twine, WG Lewis, A Casbard, MA Morgan, D Chan, GWB Clark, T Havard, TD Crosby, SA Roberts, GT Williams
- 15.30–16.10 TEA [*SPORTS HALL 1*]
- 16.10–16.25 [O16] *MAL is silenced by promoter hypermethylation in gastric cancer*
TE Buffart, RM Overmeer, RDM Steenbergen, M Tijssen, NCT Van Grieken, PJF Snijders, H Grabsch, CJH Van De Velde, {P} B Carvalho, GA Meijer
- 16.25–16.40 [O17] *Regulation of the adenomatous polyposis coli gene by the miR-135 family in colorectal cancer*
{P} B Diosdado, R Nagel, C Le Sage, M Van Der Waal, JAF Oude Vrielink, A Bolijn, GA Meijer, R Agami
- 16.40–16.55 [O18] *An interobserver study of mandard grading in patients receiving preoperative chemoradiotherapy for advanced rectal cancer*
I Craven, {P} N Scott, O Rotimi, C Verbeke, D Sebag-Montefiore

17.00–18.00 **Conference Auditorium 1**

PUBLIC LECTURE

Chair: Prof DA Levison, President, Pathological Society

MRSA/Hospital acquired infection

Prof PH Pennington, University of Aberdeen

18.30–21.30 **Parkinson Court, Parkinson Building, University of Leeds**

WELCOME RECEPTION

Hosted by the Section of Pathology and Tumour Biology, Leeds Institute of Molecular Medicine, University of Leeds

Sponsor: **Leica Microsystems**

The Right Worshipful the Lord Mayor of Leeds, Councillor Frank Robinson, accompanied by the Lady Mayoress, Mrs Sheila Robinson, will be attending.

Detailed Programme – Wednesday 2 July 2008

Presenter = {P} · Abstract numbers are shown in bold and square brackets eg [S123]

- 07.30 Sports Hall 1**
REGISTRATION and COFFEE
- 07.45–09.15 Senior Common Room**
TRAINEES' BREAKFAST SESSION
(BREAKFAST WILL BE PROVIDED)
Chair: Dr KE Robertson, University of Dundee
- 07.45–08.15 [S4] *Workplace-based assessment*
Dr EW Benbow, University of Manchester
- 08.15–09.15 [S5] *The MRCPATH autopsy examination*
Prof SB Lucas, Guy's, King's & St Thomas' School of Medicine
- 09.00–17.00 Sports Hall Balcony**
SLIDE SEMINAR CASE VIEWING: *Soft tissue*
Cases submitted by Dr W Merchant, Department of Histopathology and Molecular Pathology, St James's Institute of Oncology
- 09.20–12.10 Conference Auditorium 1**
SYMPOSIUM 1: *Incipient neoplasia*
Chair: Prof AM Hanby, University of Leeds
Prof GA Meijer, VU University Medical Centre, Amsterdam
- 09.20–09.45 [S6] *Chromosomal instability in colorectal adenoma to carcinoma progression and early detection of colorectal cancer*
Prof GA Meijer, VU University Medical Centre, Amsterdam, NL
- 09.45–10.10 [S28] *Incipient pulmonary neoplasia*
Prof FB Thunnissen, VU University Medical Centre, Amsterdam, NL
- 10.10–10.30 [S7] *The molecular pathology of cervical neoplasia*
Prof CS Herrington, University of St Andrews
- 10.30–11.10 COFFEE [SPORTS HALL 1]
- 11.10–11.30 [S8] *Occult lymphoproliferative disorders*
Dr AS Jack, St James's Institute of Oncology, Leeds
- 11.30–11.50 [S9] *Incipient testicular germ cell neoplasia: biology and possible clinical intervention*
Dr LHJ Looijenga, Erasmus University Medical Centre Rotterdam, NL
- 11.50–12.10 [S26] *Evidence for a stem cell hierarchy in prostate cancer*
Dr A Collins, University of York

Detailed Programme – Wednesday 2 July 2008

Presenter = {P} · Abstract numbers are shown in bold and square brackets eg [S123]

- 09.20–12.10 Conference Auditorium 2**
SYMPOSIUM 2: Pathology of regeneration and degeneration
Chair: Dr D Treanor, University of Leeds
Prof P van der Valk, VU University Medical Centre, Amsterdam, NL
- 09.20–09.45 [S10] *Stem cells*
Prof R Poulsom, Cancer Research UK, London Research Institute
- 09.45–10.05 [S11] *Organ regeneration*
Prof J Southgate, University of York
- 10.05–10.30 [S12] *Spinal degenerative disease*
Prof AJ Freemont, University of Manchester
- 10.30–11.10 COFFEE [SPORTS HALL 1]
- 11.10–11.40 [S24] *Hypoxia as an inducer of leaky microvessels and growth of atherosclerotic plaques*
Prof MJAP Daemen, Academic Hospital, Maastricht, NL
- 11.40–12.10 [S13] *Liver regeneration: from start to finish*
Prof MR Alison, Queen Mary University of London
- 12.10–13.00 Conference Auditorium 1**
JAPANESE PATHOLOGICAL SOCIETY GUEST LECTURE
Chair: Dr H Grabsch, University of Leeds
- [S14] *The pathology of colorectal and gastric carcinomas in the elderly: clinicopathological characteristics and molecular mechanisms*
Prof T Arai, Tokyo Metropolitan Geriatric Hospital, Japan
- 12.30–14.00 Sports Hall 1**
LUNCH and TRADE EXHIBITION
- 13.00–15.00 Conference Auditorium 2**
LIVER EQA
- 13.30–15.00 Conference Auditorium 1**
TRAINEES' PROGRAMME: Meet the Experts
Chair: Dr A Treacy, Mater Misericordiae University Hospital, Dublin, Ireland
- [S15] *Colorectal polyps and the new Colorectal Cancer Screening Programme*
Dr N Scott, St James's Institute of Oncology, Leeds

Detailed Programme – Wednesday 2 July 2008

Presenter = {P} · Abstract numbers are shown in bold and square brackets eg [S123]

14.00–15.00

Sports Hall 1

POSTER VIEWING and CHAIRMAN'S ROUNDS

CATEGORIES

Breast (1)	[P1–P22] ¹
Gastrointestinal (1)	[P45–P59] ²
Genitourinary/Renal	[P75–P88] ³
Gynaecological	[P89–P101] ⁴
Lymphoreticular	[P102–P112] ⁵
Neuropathology/Ophthalmic	[P113–P115] ⁵
Osteoarticular/Soft Tissue	[P116–P119] ⁵

Chair: ¹ Dr A Shaaban, Leeds and Prof LB Jones, London
² Dr C Verbeke, Leeds and Prof GA Meijer, Amsterdam
³ Dr SS Cross, Sheffield and Dr L Looijenga, Rotterdam
⁴ Dr N Wilkinson, Leeds and Prof WG McCluggage, Belfast
⁵ Prof DA Levison, Dundee and Prof P van der Valk, Amsterdam

15.00–17.30

Conference Auditorium 1

PLENARY ORAL SESSION

Chair: Prof IO Ellis, University of Nottingham
Dr H Grabsch, University of Leeds

- 15.00–15.15 [PL1] *Chemical carcinogenesis in K-ras exon 4A knockout mice shows that mutationally activated K-ras 4A and 4B both mediate lung tumour formation*
{P} MJ Arends, CE Patek, WA Wallace, F Luo, S Hagan, DG Brownstein, SJ Plowman, RL Berry, W Kolch, OJ Sansom, DJ Harrison, ML Hooper
- 15.15–15.30 [PL2] *Identification of PPM1D as a novel therapeutic target in ovarian clear cell adenocarcinomas*
{P} DSP Tan, MBK Lambros, S Rayter, C Marchio, C Jameson, A Williams, WG McCluggage, M El-Bahrawy, AJW Paige, SB Kaye, A Ashworth, JS Reis-Filho
- 15.30–15.45 [PL3] *Expression pattern of DNA double strand break repair proteins and response to therapy in gastric cancer*
{P} M Hollings, C Beaumont, T Ivanova, L Ling Cheng, K Ganesan, Y Zhu, J Lee, P Tan, H Grabsch
- 15.45–16.00 [PL4] *Inhibition of PKC-zeta expression in human prostate cancer cells*
S Yao, Y Ke, C Gosden, {P} CS Foster
- 16.00–16.40 TEA [SPORTS HALL 1]
- 16.40–16.55 [PL5] *Identification of key breast cancer phenotypes*
{P} AR Green, JM Garibaldi, D Soria, F Ambrogi, D Powe, E Rakha, GR Ball, P Lisboa, T Etchells, P Boracchi, E Biganzoli, IO Ellis
- 16.55–17.10 [PL6] *The potential role of 3' alternate splicing events in the regulation of SEPT9 protein expression*
{P} GC Crossan, N Pentland, SEH Russell, PA Hall
- 17.10–17.25 [PL7] *Nanog, a putative stem cell gene, stimulates cell proliferation in colorectal cancer cell lines but is enriched in the CD133 negative sub-population of tumour cells.*
S Sandhu, R Seth, S Crook, D Jackson, {P} M Ilyas

Detailed Programme – Thursday 3 July 2008

Presenter = {P} · Abstract numbers are shown in bold and square brackets eg [S123]

- 08.00 Sports Hall 1**
REGISTRATION and COFFEE
- 09.00–15.00 Sports Hall Balcony**
SLIDE SEMINAR CASE VIEWING: *Soft tissue*
Cases submitted by Dr W Merchant, Department of Histopathology and Molecular Pathology, St James's Institute of Oncology
- 09.00–12.00 Conference Auditorium 1**
SYMPOSIUM: *Pathology and targeted treatment*
Chair: Prof PW Hamilton, Queen's University Belfast
Dr V Speirs, University of Leeds
Sponsor: **International Society for Cellular Oncology**
- 09.00–09.30 [S16] *Basics of the epidermal growth factor receptor: amplifications, mutations and transcriptional regulation*
Prof B Brandt, University Medical Centre Hamburg-Eppendorf, Germany
- 09.30–10.00 [S17] *Pitfalls in immunohistochemistry of the epidermal growth factor receptor*
Prof PJ van Diest, University Medical Center Utrecht, NL
- 10.00–10.30 [S18] *Targeting EGFR in clinical oncology*
Dr F Eskens, Erasmus University Medical Centre Rotterdam, NL
- 10.30–11.10 COFFEE [SPORTS HALL 1]
- 11.10–11.30 [S19] *Oestrogen receptors and targeted therapy*
Dr V Speirs, University of Leeds
- 11.30–12.00 [S27] *The role of pathology in the development of antiangiogenic therapies*
Dr H Koeppen, MD Anderson Cancer Centre, Houston, USA
- 09.00–12.00 Conference Auditorium 2**
ORAL COMMUNICATIONS
Categories: Autopsy/Forensic; Education & Audit; Technical Advances
Chair: Dr NR Griffin, St James's Institute of Oncology, Leeds
DR RFT McMahon, University of Manchester
- 09.00–09.15 [O19] *Changes in autopsy practice following cardiac surgery over 17 years*
{P} MF Alikhan, PJ Gallagher, DE Pontefract
- 09.15–09.30 [O20] *An audit of autopsy toxicology requests*
{P} H Brownlow, M Tripathi, J Biswas, CP Dorris, SK Suvarna
- 09.30–09.45 [O21] *Undergraduate exposure to clinical pathology and autopsies: potential effects on junior doctor recruitment into histopathology*
{P} L Browning, J Lortan, E Soilleux
- 09.45–10.00 [O22] *An evaluation of the impact of trainees on a large histopathology department*
{P} J Dormer, L Wheatley, S Carvell, K West, A McGregor

Detailed Programme – Thursday 3 July 2008

Presenter = {P} · Abstract numbers are shown in bold and square brackets eg [S123]

- 10.00–10.15 [O23] *Dutch carcinoma audits over 2005 and 2006: a consistent 40% had no smear while detection failures were 25% in 2005 and 29% in 2006*
{P} F Van Kemenade, MK Casparie
- 10.15–10.30 [O24] *Use of RNAi in cell lines to generate optimal controls for optimisation of antibodies for immunohistochemical analysis on formalin-fixed paraffin embedded samples*
{P} K Savage, S Parry, E Iorns, N Johnson, CJ Lord, A Ashworth, JS Reis-Filho
- 10.30–11.10 COFFEE [SPORTS HALL 1]

ORAL COMMUNICATIONS

Categories: Technical Advances; Cardiovascular/Pulmonary; Skin

- 11.10–11.25 [O25] *3-Dimensional pathology with virtual slides*
{P} D Treanor, K Brabazon, D Magee, P Quirke
- 11.25–11.40 [O26] *The immunohistochemical differentiation between metastatic breast cancer and malignant pleural mesothelioma*
{P} GR Powell, WR Roche
- 11.40–11.55 [O27] *Immunohistochemical expression of CD10 in cutaneous basal and squamous cell carcinoma*
{P} H Aiad, H Hanout

12.00–13.00 **Conference Auditorium 1**
PATHOLOGICAL SOCIETY OF GREAT BRITAIN & IRELAND
ANNUAL BUSINESS MEETING
(Members will have received an Agenda)

12.00–13.30 **Conference Auditorium 2**
RENAL EQA

13.00–14.00 **Sports Hall 1**
LUNCH and TRADE EXHIBITION

Detailed Programme – Thursday 3 July 2008

Presenter = {P} · Abstract numbers are shown in bold and square brackets eg [S123]

13.30–14.30

Sports Hall 1

POSTER VIEWING and CHAIRMAN'S ROUNDS

CATEGORIES

Autopsy & Forensic	[P120–P125] ¹
Breast (2)	[P23–P44] ²
Cardiovascular/Pulmonary	[P126–P129] ¹
Cellular/Molecular	[P130–P139] ³
Education & Audit	[P140–P156] ¹ (Note: P150 and P153 withdrawn)
Endocrine	[P157] ⁴
Experimental Tumour Path	[P158–P161] ³
Gastrointestinal (2)	[P60–P74] ⁵
Head & Neck	[P162–P164] ⁴
Hepatobiliary/Pancreas	[P165–P167] ⁵
Skin	[P168–P172] ⁴
Technical Advances	[P173–P178] ³

Chair: ¹ Dr PJ Gallagher, Southampton and Prof SB Lucas, London
² Prof AM Hanby, Leeds and Prof RA Walker, Leicester
³ Prof M Ilyas, Nottingham and Dr JS Reis-Filho, London
⁴ Dr N Kirkham, Newcastle and Prof KA MacLennan, Leeds
⁵ Dr MJ Arends, Cambridge and Prof M Pignatelli, Bristol

14.30–17.00

Conference Auditorium 1

ORAL COMMUNICATIONS

Categories: Lymphoreticular; Osteoarticular/Soft Tissue

Chair: Prof KA MacLennan, St James's Institute of Oncology, Leeds
Prof F van Kemenade, VU University Medical Centre, Amsterdam

14.30–14.45

[O28] *Genomic analysis of follicular lymphoma subgroups*
{P} D Marjenberg, PJ Batstone, KE Robertson, DA Levison, JR Goodlad

14.45–15.00

[O29] *Genomic instability of haemopoietic cells in newly diagnosed Myelodysplastic Syndrome (MDS) and Acute Myeloid Leukemia (AML) assessed by G-banding, multicolour FISH and array-CGH*
{P} M Yusuf, N Rooney, A Whiteway, T Davies, M Williams, N Affara, R Furlong, P Case

15.00–15.15

[O30] *PI3K/AKT/mTOR pathway implicated in pathogenesis of non-skull based chordomas*
{P} A Shalaby, N Presneau, B Idowu, L Thompson, AM Flanagan

15.15–15.30

[O31] *Activating Gs(alpha) mutational analysis can be used as a diagnostic tool for Intramuscular Myxomas (IM)*
{P} D Delaney, N Presneau, S Hing, BD Idowu, R Tirabosco, AM Flanagan

15.30–16.10

TEA [SPORTS HALL 1]

Detailed Programme – Thursday 3 July 2008

Presenter = {P} · Abstract numbers are shown in bold and square brackets eg [S123]

ORAL COMMUNICATIONS

Categories: **Genitourinary/Renal; Gynaecological; Head & Neck**

- 16.10–16.25 [O32] *pS2 (TFF1) expression in prostate carcinoma, correlation with steroid receptor status*
{P} A Abdou, H Aiad, S Sultan
- 16.25–16.40 [O33] *Molecular genetic profiling of ovarian clear cell adenocarcinomas*
{P} DSP Tan, M Iravani, B Mahler-Araujo, MBK Lambros, R Natrajan, N Tamber, K Fenwick, A Mackay, C Jameson, A Williams, WG McClugage, M El-Bahrawy, A Ashworth, SB Kaye, JS Reis-Filho
- 16.40–16.55 [O34] *Pure salivary duct carcinomas can be classified into luminal, her2 and basal-like phenotypes*
{P} S Di Palma, A Skalova, M Ungari, A Sandison, R Simpson, C Marchio, JS Reis-Filho
- 14.30–15.30 Conference Auditorium 2**
TRAINEES PROGRAMME: *Translational aspects of disease*
Chair: Dr H El-Daly, Imperial College, London
Dr J Le Quesne, CRUK, Cambridge Research Institute
- 14.30–15.00 [S25] *Understanding molecular pathogenesis of Barrett's adenocarcinoma and application to the clinic*
Dr R Fitzgerald, Hutchison-MRC Research Centre, Cambridge
- 15.00–15.30 [S20] *The role of DNA methylation in colorectal neoplasia*
Dr A Ibrahim, University of Cambridge
- 15.30–16.10 TEA [SPORTS HALL 1]**
- 16.10–17.00 Conference Auditorium 2**
TRAINEES PROGRAMME: *Panel based forum discussion: achieving excellence in research, teaching and diagnostics work whilst maintaining work-life balance*
Chair: Dr M Deheragoda, London
Dr KE Robertson, University of Dundee
- Panel: Dr R Fitzgerald, Cambridge
Dr A Ibrahim, Cambridge
Dr KA Oien, Glasgow
Dr JS Reis-Filho, London

Detailed Programme – Thursday 3 July 2008

Presenter = {P} · Abstract numbers are shown in bold and square brackets eg [S123]

17.00–18.00 Conference Auditorium 1
PATHOLOGICAL SOCIETY OF GREAT BRITAIN & IRELAND'S
6TH DONIACH LECTURE

Chair: Prof DA Levison, President, Pathological Society

[S21] *Sights unseen, truths untold: pathology and the modern medical curriculum*
Prof Sir James Underwood, University of Sheffield

19.30–22.30 National Railway Museum, York
SOCIETY DINNER

(PLEASE NOTE: BUSES DEPART FOR SOCIETY DINNER AT 18.15)

Detailed Programme – Thursday 3 July 2008

Presenter = {P} · Abstract numbers are shown in bold and square brackets eg [S123]

09.30–17.00 Roger Stevens Building – Room 19
ASSOCIATION OF CLINICAL ELECTRON MICROSCOPISTS
11TH ANNUAL SCIENTIFIC MEETING

09.30–10.20 *The Liver Institute – what it does, & where EM fits in*
Chair: Mr B Wagner
Dr A Knisely, King's College Hospital, London

10.20–10.30 GROUP PHOTO

10.30–11.00 COFFEE [SPORTS HALL 1]

11.10–11.50 *CADASIL diagnosis – ultrastructural and immunohistochemical*
Chair: Mr I Shore
Mr R Moss, St George's Hospital, London

12.00–13.00 *Technical EM – EQA feed back*
Chair: Mr B Wagner
Mrs T de Haro, Leicester Royal Infirmary

13.00–14.00 LUNCH [SPORTS HALL 1]

14.05–14.50 *Gut dysmotility – histopathology and ultrastructure*
Chair: Mr G Anderson
Prof JE Martin, The Royal London Hospital

14.55–15.30 *Renal biopsy case presentation with review of literature*
Chair: Mrs T Dopping-Hepenstal
Mr B Wagner, Northern General Hospital, Sheffield

15.30–16.00 TEA [SPORTS HALL 1]

16.00–17.00 AGM
(Open to ACEM members and non-members)
Chair: Dr T Ryder, Charing Cross Hospital, London

Detailed Programme – Friday 4 July 2008

Presenter = {P} · Abstract numbers are shown in bold and square brackets eg [S123]

- 08.30 **Sports Hall 1**
REGISTRATION and COFFEE
- 09.30–13.30 **Conference Auditorium 2**
SYMPOSIUM: *The genome and cancer*
Chair: Prof KJ Hillan, Genentech Inc, San Francisco, USA
 Prof G Taylor, Cancer Research UK Genomic Services, Leeds
- 09.30–10.15 *Insights from the genome*
 Prof S Aparicio, Vancouver, Canada
- 10.15–10.45 *The cancer genome*
 Prof Sir A Markham, University of Leeds
- 10.45–11.25 COFFEE [*SPORTS HALL 1*]
- TALKS AND PANEL DISCUSSION: *Applications in Humans***
- 11.25–11.55 [**S22**] *Genomic approaches to understanding gastric cancer progression*
 Prof P Tan, Duke-NUS Graduate Medical School, Singapore
- 11.55–12.25 [**S23**] *Molecular prediction: making the most of treatments for bowel cancer*
 Prof MT Seymour, St James's Institute of Oncology, Leeds
- 12.25–12.55 *Some problems with sample size*
 Prof M Bland, University of York
- 12.55–13.30 Panel discussion
- 13.30 Close of meeting

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Abstracts

Plenary

Note: Presenter's name is shown in **bold**

PL1

Chemical carcinogenesis in K-ras exon 4A knockout mice shows that mutationally activated K-ras 4A and 4B both mediate lung tumour formation

MJ Arends¹, CE Patek², WA Wallace³, F Luo¹, S Hagan⁴, DG Brownstein⁵, SJ Plowman², RL Berry², W Kolch⁴, OJ Sansom⁴, DJ Harrison², ML Hooper².

¹Pathology Department, University of Cambridge, Cambridge, ²Sir Alastair Currie CRUK Lab, Molecular Medicine Centre, University of Edinburgh, Edinburgh, ³Division of Pathology, University of Edinburgh, New Royal Infirmary of Edinburgh, Edinburgh, ⁴Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Glasgow ⁵Research Animal Pathology Core Lab, Queens Medical Research Institute, University of Edinburgh, Edinburgh

Mice with K-ras exon 4A deleted have a normal lifespan with no increase in sporadic tumour susceptibility. Lung carcinogenesis was induced by N-methyl-N-nitrosourea (MNU) in mice with combinations of K-ras exon 4A-knockout and K-ras whole gene-knockout, to investigate the roles of K-ras4A and K-ras4B isoforms in lung tumorigenesis. MNU induced K-ras G12D mutations that jointly affect both isoforms. Compared with K-ras-4A- mice (where tumours can express mutationally activated K-ras4B only), tumour number and size were significantly higher in K-ras+/- mice (where tumours can express mutationally activated K-ras4A and K-ras4B), and significantly lower in K-ras4A-4A- mice (where tumours can express both wild-type and activated K-ras4B). MNU induced significantly more and larger tumours in wild-type than K-ras4A-4A- mice which differ in that only tumours in wild-type mice can express wild-type and activated K-ras4A. Lung tumours from K-ras+/- and K-ras-4A- mice exhibited phospho-Erk1/2 and phospho-Akt immunostaining. We conclude that: (1) mutationally activated K-ras4B is sufficient to activate the Raf/MEK/ERK (MAPK) and PI3-K/Akt pathways and initiate lung tumorigenesis; (2) when expressed with activated K-ras4B, mutationally activated K-ras4A further enhances lung tumour formation and growth (both in the presence and absence of its wild-type isoform); and (3) wild-type K-ras4B shows tumour suppressor activity by reducing lung tumour number and size.

PL2

Identification of PPM1D as a Novel Therapeutic Target in Ovarian Clear Cell Adenocarcinomas

DSP Tan^{1,2}, MBK Lambros¹, S Rayer¹, C Marchio¹, C Jameson³, A Williams⁴, WG McCluggage⁵, M El-Bahrawy⁶, AJW Paige⁶, SB Kaye², A Ashworth¹, JS Reis-Filho¹.

¹The Breakthrough Breast Cancer Research Centre, Institute of Cancer Research, London, ²Section of Medicine, Institute of Cancer Research, Royal Marsden Hospital, Sutton, Surrey, ³Department of Pathology Royal Marsden Hospital, London UK, ⁴Department of Pathology, University of Edinburgh, Edinburgh, ⁵Department of Pathology, Royal Group of Hospitals Trust, Belfast, ⁶Imperial College School of Medicine, Hammersmith Hospital, London

The genetic and transcriptomic profiles of 12 ovarian clear cell adenocarcinoma (OCCA) cell lines were analysed using a 32K tiling path microarray CGH platform and the Illumina human ref6 gene expression array respectively. Our aims were to characterise the molecular genetic profiles of OCCAs and identify potential therapeutic targets from a list of candidate amplicon drivers. Recurrent gains/amplifications containing a number of putative oncogenes were identified including a focal amplification of 17q23.2, whose smallest region of overlap harbours PPM1D, APPBP2 and CA4, in one cell line (SMOV2). Mann Whitney U analysis between the levels of gene expression for PPM1D, APPBP2 and CA4 for OCCA cell lines with and without gains/amplifications in this region revealed a trend for higher levels of PPM1D expression in cell lines with gains/amplifications of 17q23.2. This was confirmed at the protein level by western blot analysis. Colony formation assays using a recently developed chemical inhibitor of PPM1D (CT007093) and a short hairpin RNA (shRNA) against PPM1D demonstrated that PPM1D signalling is selectively essential for the survival of SMOV2 cells. Chromogenic in situ hybridisation with an in-house generated probe for PPM1D revealed high level gain/amplification of this locus in 6 out of 59 (10.2%) primary OCCAs. Our study suggests that PPM1D is a potential novel therapeutic target for a subgroup of OCCAs and provides a model for the integration of high throughput genetic, genomic and shRNA data for the identification of novel therapeutic targets for these chemotherapy resistant tumours.

PL3

Expression Pattern of DNA Double Strand Break Repair Proteins and Response to Therapy in Gastric Cancer

M Hollings¹, C Beaumont¹, T Ivanova², L Ling Cheng², K Ganesan², Y Zhu², J Lee², P Tan², H Grabsch¹.

¹Pathology and Tumour Biology, Leeds Institute of Molecular Medicine, University of Leeds, UK, ²Cellular and Molecular Research, National Cancer Centre, Singapore

DNA double strand breaks (DSB) are the most dangerous form of DNA damage. pH2AX has a crucial role in the immediate cellular response to DSBs. We hypothesised that the expression pattern of pH2AX and downstream DNA DSB repair proteins is related to response to therapy in gastric cancer (GC) cell lines.

26 GC cell lines were either processed into paraffin for tissue microarray (TMA) construction or challenged with oxaliplatin (OX), cisplatin (CIS) or 5-fluorouracil (5FU). GI50 (50% growth inhibition) was used to discriminate between sensitive and resistant cell lines. Immunocytochemistry (ICC) with antibodies against pH2AX, H2AX, p53, RAD51, MRE11, BRCA1, ATM, Ku70, Ku80 and DNA-PKc was performed on TMA sections and percentage of positive cells was scored.

Differential expression was seen for pH2AX, H2AX, p53, Ku70 and RAD51. Cell lines with high pH2AX were more likely resistant to OX and CIS whereas 5FU resistant cell lines were more likely to have low pH2AX and low p53. Hierarchical clustering of all ICC scores revealed a cluster of cell lines which are both, sensitive to OX and resistant to 5FU.

This is the first study investigating the association between the expression pattern of proteins of the DNA DSB repair pathway and response to therapy in a large set of GC cell lines. Further studies are justified to characterise the expression pattern of all proteins involved in DNA DSB repair, to identify the most important molecular player by functional studies and to validate the findings in clinical trial material in parallel.

PL4

Inhibition of PKC-zeta Expression in Human Prostate Cancer Cells

S Yao¹, Y Ke¹, C Gosden¹, CS Foster¹.

¹University of Liverpool

Protein Kinase C-zeta (PKC- ζ) independently predicts poor clinical outcome in prostate cancer. Several variants of PKC- ζ are potentially encoded by the PKC- ζ gene, although their functions remain unclear. After showing that antisense inhibition of PKC- ζ gene expression diminished the motility of prostate cancer cells, we hypothesised that PKC- ζ might be responsible for modulating prostate cancer cell apoptosis, invasion and metastasis. Using RNA-i directed to three different exonic sites in variant "b" (wild-type) of the PKC- ζ genome, we have generated both transient and stable transfectants of the prostate cancer cell-line PC3-M. These genetic knockdowns expressed reduced levels of PKC- ζ at, or below, that of benign PNT-2 prostate cells, as confirmed by qPCR. Cell invasion assays in-vitro confirmed altered morphology of the cells with maintained levels of proliferation but almost complete arrest of invasive capacity into collagen. However, apoptosis was not significantly affected. We now believe we have strong evidence that expression of PKC- ζ variant "b" in human prostate cancer cells is differentially involved in promoting cell invasion and hence metastasis. Conversely, expression of this gene does not affect either cell proliferation or apoptosis. Analysis of downstream genes modulated following PKC- ζ knockdown may identify new therapeutic targets to inhibit prostate cancer cell invasion and metastasis.

PL5

Identification of Key Breast Cancer Phenotypes

AR Green¹, JM Garibaldi², D Soria², F Ambrogi³, D Powe¹, E Rakha¹, GR Ball⁴, P Lisboa⁵, T Etchells⁵, P Boracchi³, E Biganzoli³, IO Ellis¹.

¹Pathology, School of Molecular Medical Sciences, Nottingham University Hospitals and University of Nottingham, ²School of Computer Science, University of Nottingham, ³Institute of Medical Statistics and Biometry, University of Milan, ⁴School of Biomedical and Natural Sciences, Nottingham Trent University, ⁵School of Computing and Mathematical Sciences, Liverpool John Moores University

Breast cancer is a heterogeneous disease, of which several forms have been identified on the basis of their gene expression characteristics but translation of this into routine clinical practice remains elusive or prohibitively expensive. We have previously used protein expression characteristics to identify comparable classes. In this study, we extend this approach and further define the key criteria for class membership.

Expression of twenty-five proteins, with known relevance to breast cancer, have been assessed in a series of 1,076 patients. This large data set has been examined by four alternative data clustering techniques: Hierarchical, K-means, Partitioning around medoids, Adaptive resonance theory. Concordance between techniques was used to elucidate 'core classes' of patients.

A total of 663 (62%) of the 1076 patients were assigned to six core classes, while 413 (38%) patients were of mixed class. Three core classes correspond to well known clinical phenotypes (luminal A/B and HER2). Two classes correspond to the well known basal phenotype, but exhibit a novel differentiation into two sub-groups. The last class appears to characterise a novel luminal subgroup.

Key clinical phenotypes of breast cancer can be identified using standard, widely available immunocytochemistry technology. The main luminal and basal breast cancer phenotypes appear to be heterogeneous, containing distinct sub-groups. The six clinical phenotypes determined in this study are a new luminal group, luminal-N, new basal sub-groups, basal p53 altered and basal p53 normal, as well as the well-established luminal A/B and HER2 groups.

PL6

The potential role of 3' alternate splicing events in the regulation of SEPT9 protein expression

GC Crossan¹, N Pentland¹, SEH Russell¹, PA Hall¹.

¹Queen's University Belfast, Belfast, Northern Ireland

We have shown that the 5'UTRs of *SEPT9_v4* and *v4** contribute to the regulation of *SEPT9_v4* levels (*Hum Mol Gen* 2007;16:742-52). We have now addressed the role of the 3'UTR in regulating *SEPT9* expression. Bioinformatic analysis indicates that there are three 3' ends encoding 3 carboxy termini ('a', 'b' & 'c') with two different 3' UTRs (*version 1* in 'a' & *version 2* in 'b' & 'c') due to non-consensus alternate splicing within exon 12. This was confirmed by a comprehensive set of RT-PCR experiments coupled with sequencing. Furthermore by RT-PCR we find differential expression of the 3 distinct 3' ends in a panel of samples and cell lines with the 'a' form being predominant in malignant tumours. We hypothesise that the different 3' UTRs might contribute to the translational efficiency of *SEPT9* transcripts. One UTR is short (32 bp *version 2*) while *version 1* is 1938 bp and has considerable predicted secondary structure as defined by the *mFOLD* algorithm ($\Delta G = -816.10$). Further bioinformatic analysis shows that the splicing events that create *version 2* UTR remove a set of potential microRNA binding sites present in *version 1* UTR. We have created luciferase reporter constructs where the luc gene has either the *version 1* or *version 2* UTR, or mutants forms of the *version 1* UTR. Using these tools we are investigating the role of sequences within the UTRs to regulate luciferase transgene expression.

GPC received a Pathological Society Intercalated BSc Award

PL7

Nanog, a putative stem cell gene, stimulates cell proliferation in colorectal cancer cell lines but is enriched in the CD133 negative sub-population of tumour cells.

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Introduction: Nanog is a transcription factor associated with maintenance of embryonic stem cells. It is reported that Wnt signalling can stimulate Nanog expression and since most colorectal cancers arise as a consequence of aberrant Wnt signalling, it may represent a mechanism of maintaining stem cells in these tumours. We sought to evaluate Nanog expression and function in colorectal cancer (CRC). **Methods:** 24 human CRC cell lines were evaluated for Nanog expression using real-time QPCR. In addition, Nanog expression was tested in CD133+ and CD133- sub-populations of the cell line SW480. Nanog function was tested using siRNA followed by proliferation and apoptosis assays.

Results: All 24 CRC cell lines showed Nanog expression. Variable expression with up to 4 orders of magnitude difference between the highest and the lowest expressing cell line was found. HT29 was chosen for functional analysis. Gene knockdown resulted in an increase in cell number ($p = 0.01$) but there was no difference in apoptotic activity. Quantitative analysis of CD133+ and CD133- sub-populations of SW480 showed that Nanog is enriched (approximately two-fold) in the CD133- cells.

Conclusions: Nanog expression is classically associated with ES cells although our data show that it is also expressed in CRC cell lines. In CRC, Nanog may drive cell proliferation but it does not appear to influence apoptosis. Although purportedly involved in stem cell maintenance, Nanog expression does not correlate with expression of CD133 (another stem cell marker) and the relative roles of these two genes is unclear.

Abstracts

Posters

Note: Presenter's name is shown in **bold**

P1

Distinction of Fibroadenoma & Phyllodes Tumour in Needle Core Biopsy: Analysis of Discriminant Features

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Background and Aim: Needle core biopsy (NCB) has largely supplanted Fine Needle Aspiration Cytology (FNAC) in pre-operative diagnosis of breast lesions but distinction between benign phyllodes and fibroadenoma remains problematic. The aim of this study was to confirm and analyse histological features, which allow the pre-operative distinction between the fibroepithelial lesions phyllodes tumour (PT) and fibroadenoma (FA) of the breast on needle core biopsy. **Method:** Previously identified and defined histological criteria were applied to 112 core biopsies of fibroepithelial lesions of the breast without knowledge of final diagnosis. The results were analysed using contingency tables for frequency analysis, logistic regression, receiver operating characteristic (ROC) and linear discriminant analysis. **Results:** Frequency analysis identifying significant differences on NCB agreed with published data. Logistic regression analysis using age and mitotic rate allowed 95% of cases to be correctly categorised and discriminant analysis for age, mitoses and % stroma allowed 93.8% correct pre-operative diagnosis. ROC analysis identified cut off values (between FA and PT) for age (50-55yrs), %stroma (85-90) and mitoses (>1 / 2.2mm²). **Conclusion:** The results provide predictive tools, which need to be tested prospectively.

P2

Audit of Results of Routine Her-2 Testing by Immunohistochemistry and FISH

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Herceptin was initially licenced as a treatment for metastatic breast cancer and has subsequently been approved for adjuvant use requiring the routine testing of all new breast cancers. The results of routine Her-2 testing using immunohistochemistry (IHC) and Fluorescent in situ Hybridisation (FISH) were audited over a 3 year period. 2046 cases were analysed by IHC with 1028 (50%) grade 0, 324 (15%) grade 1+, 421 (20%) grade 2+ and 273 (13%) grade 3+. 421 cases (at grade 2+) required FISH analysis of which 83 (25%) were FISH positive. The overall Her-2 positivity rate was 17.8%. Analysis by year shows a reduction in the Her-2 positive rate (from 22.7% to 12%) reflecting the initial testing of aggressive metastatic disease only. Later data includes testing of all breast cancers after the introduction of adjuvant treatment. Turn-around time for analysis reduced from 13 days to 7.6 days due to more frequent batching of tests. Many patients with metastatic Her 2 + tumours did not receive Herceptin because of co morbidity. Audit of clinical response of metastatic disease to Herceptin treatment proved difficult (some patients had concurrent chemotherapy) but this data should be gathered routinely to validate the Her 2 assay results.

P3

EpCAM Expression is an Indicator of Recurrence in Breast Cancer

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Recent evidence suggests the transmembrane adhesion molecule EpCAM is associated with invasive and metastatic behaviour in gastro-intestinal, ovary, bladder and breast cancer. However, the biological role and prognostic significance of EpCAM in breast cancer currently remains poorly understood. We have therefore analysed and characterised EpCAM expression in a large cohort (n=880) of breast tumours with long-term follow-up using tissue microarray and immunohistochemistry in order to determine the prognostic and diagnostic significance of EpCAM in breast cancer. A total 729 tumours were analysed after the uninformative cores were excluded. A total of 7% of tumours showed strong EpCAM membranous reactivity, whilst 17.7% were negative. EpCAM expression was associated with higher histological grade, larger tumour size, presence of vascular invasion, distant metastasis (particularly to bone and brain), and medullary carcinoma type (all, p<0.05). EpCAM expression showed an inverse correlation with ER, PgR and positive association with cytokeratin (CK) CK5/6, CK14, smooth muscle actin, p53 and P-cadherin (all p<0.05). EpCAM expression was associated with a poorer overall survival and shorter disease free interval (DFI) (p<0.01). In multivariate analysis, EpCAM expression remained a prognostic factor for DFI, independent of tumour grade, size and lymph node stage (p=0.023). EpCAM expression is associated with basal-like breast cancer and a poorer prognosis. Furthermore, EpCAM is an independent predictor of clinical outcome in breast cancer, particularly shorter DFI. Further studies analysing EpCAM as a potential therapeutic target for breast cancer patients are therefore warranted.

P4

Is Cytokeratin 19 (CK19) an appropriate marker for circulating tumour cell detection?

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Many studies have shown the presence of circulating tumour cells (CTCs) in the blood of breast cancer patients. They have been shown to be of prognostic significance in the metastatic setting. Robust detection of CTCs could have considerable impact on cancer management. However the rarity of CTCs, even patients with relatively high levels have up to 10 cells per millilitre of blood, presents a substantial challenge for their reproducible analysis. Detection methods must allow detection with sufficient sensitivity, while maintaining specificity to disregard the vast excess of haematopoietic cells. Reverse transcriptase-polymerase chain reaction (RT-PCR) assays provide an obvious choice for a balance of high-sensitivity with appropriate specificity. Cytokeratin 19(CK19) is the most frequently used marker being predominately expressed in cells of epithelial origin but rarely in other tissues, therefore CK19 expression within the blood has been taken to equate to the presence of CTCs. Here we show that specific detection of CK19 expression in blood is problematic. It has been accepted that two CK19 pseudogenes, CK19a and CK19b, are potential sources of false positives; products can potentially be amplified from pseudogene DNA, rather than CK19 mRNA if blood RNA preparations are contaminated with any genomic DNA. We highlight another important difficulty - that pseudogene CK19a is expressed as a non-functional mRNA, therefore false positives can occur in the absence of any contaminating genomic DNA. We conclude that CK19 may not be a suitable marker for detecting circulating tumour cells in peripheral blood and discuss alternatives.

P5

Cell type specific localisation of ERbeta isoforms in mammary epithelial cells and fibroblasts

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Five estrogen receptor β (ER β) isoforms have been identified, of which ER β 1 and ER β 2 are important in the breast. In breast tissue sections, both nuclear and cytoplasmic immunolocalisation of ER β 1, but in particular, ER β 2 has been observed in normal and tumour epithelial cells. The aim of this study was to establish specific localisation of ER β and in establish if this was cell type specific using immunofluorescence on cultured mammary epithelial cells and fibroblasts. ER β 1 was predominantly nuclear in a range breast epithelial cells; benign and both malignant invasive/non-and tamoxifen-resistant. However, ER β 2, showed intense cytoplasmic fluorescence in these cells with only moderate nuclear positivity. In fibroblasts ER β 1 was mostly nuclear, whereas both the nuclear and cytoplasmic expression of ER β 2 was observed. Nuclear speckling was also observed with ER β 2. Using a specific mitochondrial marker, ER β 2 was shown to colocalise in mitochondria. Additionally ER β 2 showed a punctate nuclear expression pattern, and several large nuclear speckles were consistently observed.

In order to characterise these speckles antibodies directed against three nuclear proteins, nucleolin, nuclear speckle and p80 coilin, were used to determine if their expression correlated with ER β 2 in MCF7 cells. The p80 coilin protein is localised in Cajal bodies and using immunofluorescence showed a similar expression pattern to ER β 2, with predominantly 1-2 large 0.5 μ m speckles per cell. However, when we performed colocalisation studies using antibodies against ER β 2 and p80coilin no colocalisation was observed. Currently we are continuing to explore the functional significance of ER β 2 nuclear speckles.

P6

In-transit Lymph Node Metastases in Breast Cancer: a Possible Source of Local Recurrence after Sentinel Node Procedure

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Background: In-transit lymph node metastases are a common phenomenon in patients with melanoma, raising more attention since the introduction of the Sentinel Node (SN) procedure. To which extent this also occurs in breast cancer patients has not been studied yet.

Aim: To explore the occurrence of in-transit lymph nodes metastases in breast cancer.

Methods: Between 1998 and 2001, afferent lymph vessels to the SN identified by blue dye were removed from 18 breast cancer patients during regular SN procedure.

Results: Three out of 18 patients showed a lymph node associated with the afferent lymph vessels. One of these lymph nodes (6%) showed a breast cancer metastasis, to be regarded as an in-transit metastasis. This metastasis would normally have been left in situ and could thereby have been a source of local recurrence.

Conclusion: In-transit lymph nodes associated with the afferent SN lymph vessels seem to occur in a significant proportion in breast cancer patients, and may contain metastases. As these are a potential source of local recurrence when left in situ, there may be an indication to remove the SN afferent lymph vessels during the SN procedure.

P7

The Microanatomic Location of Metastatic Breast Cancer in Sentinel Lymph Nodes Predicts Non-sentinel Lymph Node Involvement

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Background: The majority of sentinel node (SN) positive breast cancer patients do not have additional non-SN involvement and may not benefit from axillary lymph node dissection (ALND). Previous studies in melanoma have suggested that microanatomic localization of SN metastases may predict non-SN involvement. The present study was designed to assess whether these criteria might also be used to be more restrictive in selecting breast cancer patients who would benefit from an ALND.

Methods: A consecutive series of 357 patients with invasive breast cancer and a tumour positive axillary SN, followed by an ALND, was reviewed.

Microanatomic SN tumour features (subcapsular, combined subcapsular and parenchymal, parenchymal or extensive localization, and the penetrative depth from the SN capsule) were evaluated for their predictive value for non-SN involvement.

Results: Non-SN metastases were found in 136/357 cases (38%). A subcapsular localisation of tumour deposits and limited penetrative depth were associated with a low frequency of non-SN involvement (10%).

Conclusions: Microanatomic location and penetrative depth of breast cancer SN metastases predict non-SN involvement. However, based on these features no subgroup of patients could be selected with less than 10% non-SN involvement.

P8

Morphometry of Isolated Tumour Cells in Breast Cancer Sentinel Lymph Nodes: Metastases or Displacement?

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Iatrogenic displacement of epithelial cells to the sentinel node (SN) may result in false positive findings, but little biological evidence has yet been presented for this. As malignant nuclei are larger than benign ones, nuclear morphometry of SN isolated tumour cells (ITC) could provide relevant information with regard to the malignant origin-or-not of epithelial cells in the SN.

Methods: In patients with invasive breast cancer and SN ITC with (N=16) or without (N=45) non-SN involvement after axillary lymph node dissection, nuclear morphometry was performed on the primary tumour as well as on the ITC in the SN.

Results: Patients with SN micro- (N=30) and macrometastases (N=30) served as controls. Nuclear size of ITC was significantly smaller compared to that of the corresponding primary tumour (P<0.0001). In contrast, there were no differences in nuclear size between SN micro- and macrometastases on the one hand and their corresponding primary tumours on the other. In addition, a subgroup of cases (10/61, 16%) with benign morphometric features of SN ITC nuclei (small size, isomorphic) could be discerned that had no non-SN metastases.

Conclusion: a subgroup of breast cancer patients have significantly smaller nuclei in the SN ITC compared to the corresponding primary tumour, often not associated with non-SN metastases, indicating that some of these deposits could represent benign epithelium or degenerated malignant cells lacking outgrowth potential.

P9

Mistletoe should be reserved for Christmas only and not used as anti-cancer drug

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A 42 year old women with previous history of breast cancer, presented with chest wall subcutaneous mass. The possibility of metastatic breast cancer was raised.

Core biopsy was performed and two cores of pale brown tissue was send to the histopathology department. Microscopically, we identified cores of adipose tissue and part of lymph node with focal necrosis and perinodal acute inflammation (lymphadenitis). These changes were interpreted as secondary to chemotherapy and radiotherapy treatment. In summary we found no evidence of malignancy, confirmed by immunohistochemistry. After discussion the case at the multidisciplinary meeting, it transpired that the patient had been receiving subcutaneous injections of mistletoe extract as complementary therapy.

Discussion:

Mistletoe is a semi parasitic perennial, it is used as a Christmas decoration with the traditional of kissing any person who lingers under it.

Mistletoe has been tested extensively as a treatment for cancer, but the most reliable randomised controlled trails fail to show benefit, and some reports show considerable potential harm. Today, despite the lack of robust data supporting the use of mistletoe as an anticancer drug, it is widely used in middle Europe. Wide ranges of serious adverse reactions have been noted, such as anaphylaxis, dyspnoea and lymphangitis.

This case taught us the importance of good communication, and the honest working relationship between the pathologist and the surgeon, and between the surgeon and the patient. This story also shows that patients sometime withhold information from us and may assume that alternative therapies have no relevance in conventional medical consultation.

P10

Reduced transforming growth factor beta induced gene and protein in breast cancer

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We have previously reported that breast cancers in women ≤ 35 yrs have more aggressive features than those of older women. To examine this further a pilot cDNA microarray comparing cancers from women 35 yrs and normal breast was performed. Of the genes upregulated in cancers several were selected for further study, including Transforming Growth Factor Beta Induced (TGFBI). There is conflicting evidence about the role of TGFBI in tumourigenesis, and its adhesive and proliferative functions.

Expression of TGFBI in 6 breast cell lines, normal breast organoids and 21 frozen breast cancers (10 \leq 35 yrs; 11 > 35 yrs) was analysed by quantitative RT-PCR. Protein analysis was by western blotting of the same cell lines and further organoids, and by immunohistochemistry of normal breast and 55 cancers (17 \leq 35 yrs; 17 36-49 yrs; 21 \geq 50 yrs).

In contrast to the microarray data there was reduced expression by qPCR in younger (P=0.026) and older (P=0.001) breast cancer cases compared to normal. HBL100 had high RNA expression but low level protein, whereas both were absent in cancer cell lines. TGFBI was present in normal breast organoids and by immunohistochemistry as strong nuclear staining. 37/55 cancers had no or low level staining. There was a significant correlation between lack of staining and grade III (P=0.008). The cancers from women 36-49 yrs had lower staining in comparison to ≥ 50 yrs group (P=0.039). Further studies are needed to determine the role of TGFBI in breast cancer.

P11

Beta-catenin is Expressed within the Nuclei of Fibroblasts in Breast Cancer

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Background

β -catenin, when located within the nucleus, acts as an oncoprotein by activating TCF/LEF transcription factors. Nuclear β -catenin is frequently seen in epithelial cancer cells. However, breast tumours are unusual in that nuclear β -catenin is relatively rare in epithelial breast cancer cells. On the other hand, nuclear β -catenin expression has been documented in fibroblasts within breast fibroadenomas and benign phyllodes tumours. Preliminary observations within our laboratory indicated that stromal fibroblast in and around breast carcinomas also frequently express nuclear β -catenin. Our aim in this work was to validate this observation, and correlate its expression with other biological parameters and survival.

Methods

We performed immunohistochemistry for β -catenin on whole sections of breast cancers from 200 individual cases. A scoring system based on the number of fibroblasts expressing nuclear β -catenin was devised and fibroblasts within tumour and normal breast tissue were scored by two observers. Survival data and biological indicators of prognosis including size, grade and lymph node status were obtained. Data were analysed using SPSS statistical software.

Results

We found that fibroblasts expressing nuclear β -catenin are frequent in and around breast tumours, while they are very rare in normal breast tissue. Initial survival analysis has shown that an increased number of fibroblast expressing nuclear beta-catenin correlated with a decrease survival. Further multivariate analysis will be undertaken to investigate the relationship between nuclear beta-catenin expression in fibroblast and tumour size, grade and lymph node status.

P12

The Prognostic Significance of Medullary-Like Histological Type and Inflammation in Invasive Carcinoma of The Breast

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The molecular classification of breast cancer recognises medullary carcinoma as a member of the basal group. The basal group is considered to have a poor prognosis, but medullary carcinoma is believed to have a better prognosis than other grade 3 carcinomas. Furthermore the prognostic significance of inflammation, an important feature of medullary carcinomas, is controversial. This study analysed a large well-characterised series of breast cancers from 1974 to 1988 with up to 30 years follow. No patients received adjuvant systemic treatment. The aim was to assess the prognostic importance of medullary histological type and inflammation. Typical and atypical medullary carcinomas (n = 140) had similar survival, so were grouped together. Almost all recurrences and deaths from medullary carcinoma occurred in the first 10 years. Medullary carcinoma had a better prognosis than grade 3 ductal carcinoma with prominent inflammation, which in turn had a better prognosis than grade 3 ductal carcinoma without prominent inflammation. These differences were independent of other prognostic factors. These results suggest that typical and atypical medullary carcinomas have a similar prognosis, which questions the current strict diagnostic criteria. Prominent inflammation is associated with a better prognosis, but it cannot explain the better prognosis in medullary carcinoma compared with grade 3 ductal carcinoma with prominent inflammation. The good prognosis of medullary carcinoma emphasises the heterogeneity of basal type carcinomas. Further studies are needed to investigate the these issues.

P13

Can we Improve our Margin Involvement Rate in Breast Cancer Excisions? An Audit.

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We audited our reporting of breast cancer excision specimens with particular reference to tumour involvement of margins. As our gold standard we used national figures for Scotland from the British Association of Surgical Oncologists which indicate a re-excision rate of 14.5% following breast conservation surgery.

All breast excision specimens with a diagnosis of invasive carcinoma and / or ductal carcinoma in-situ (DCIS) over a six month period (July to December 2007) were identified from our computer records (n=287) and their reports reviewed. 273 of these had had a pre-operative core biopsy in our department the results of which were reviewed similarly.

While 22.6% (65/287) of our excision specimens had one or more involved radial margin, 18.5% (53/287) underwent subsequent re-excision which compared favourably with the gold standard.

On excision specimens the whole tumour size (p<0.001) and specimen weight (p<0.001) were significantly associated with involvement of radial margins on multivariate analysis. On core biopsy only DCIS remained significantly associated with margin involvement (p<0.001) on multivariate analysis (although its presence or absence was only recorded in 74% of reports).

We conclude that our re-excision rate was comparable to the national average but additional emphasis on recording the presence or absence of DCIS on core biopsy may help improve our rate of margin involvement.

P14

FOXA1 Expression in Breast Cancer and its Prognostic Significance

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BACKGROUND: FOXA1 controls downstream transcription of estrogen receptor (ER)-regulated genes and has been proposed as a growth stimulator and repressor. FOXA1 enhances binding of (ER α) to its target genes. As a repressor, FOXA1 promotes E-cadherin expression, and may block metastatic progression via the BRCA1 associated cell cycle inhibitor, p27.

AIM: We assessed the prognostic value of FOXA1 expression in a large series of invasive breast carcinoma cases, and a subset of ER-positive patients.

METHOD: FOXA1 protein expression was quantified in malignant breast tissue using the H-score technique on paraffin-processed tissue microarrays (696 cases). The association between FOXA1 and pathoclinical markers was investigated to determine the clinical outcome.

RESULTS: FOXA1 expression was significantly increased in low grade carcinomas and was positively associated with variables of good prognosis including ER α , PgR, AR, smaller tumour size, lower grade, luminal cytokeratins (CK18 and CK7/8), BRCA1 and E-cadherin. In contrast, FOXA1 showed an inverse relation with basal CKs (CK14 and CK5/6) and P-cadherin. In ER-positive patients FOXA1 expression retained its association with smaller tumour size, lower grade and positive expression of AR and PgR. High FOXA1 associated with better survival in the whole series but was not found to be an independent prognostic marker.

CONCLUSION: In breast cancer, FOXA1 protein is associated with good prognosis supporting its role as a growth repressor but because it was not found to be an independent prognostic marker, its clinical relevance in stratifying ER-positive tumours appears limited.

P15

Transferrin Receptor (CD71) Expression in Breast Cancer and its Prognostic Significance

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BACKGROUND: Iron is essential for rapidly growing cells and its uptake and internalisation is performed by the transferrin receptor, CD71. It has been proposed that CD71 is associated with tumour growth and has its over-expression has been described in various cancers.

AIM: We have assessed the prognostic value of CD71 expression in a large series of invasive breast carcinoma (853 cases) and a subset of ER-positive patients.

MATERIALS AND METHODS: The CD71 protein expression was quantified in malignant breast tissue using an H-score technique on paraffin-processed tissue microarrays. The association was investigated between CD71 and other pathoclinical markers to determine clinical outcome.

RESULTS: High CD71 expression was significantly associated tumours aggressive characteristics including low steroid receptor expression, high proliferative activity (mitotic activity and MIB-1 expression), basal cytokeratins (CK5/6 & CK14), p53, EGFR and HER2 over-expression. Similar findings were found in the ER-positive patient cohort. CD71 expression was associated with shorter breast cancer specific survival in whole series, in ER-positive cohort and in Tamoxifen-only treated patients. A multivariate Cox models showed CD71 is an independent prognostic factor in the whole series (HR=1.372) and in the ER-positive cohort (HR=1.539).

CONCLUSION: We conclude that CD71 expression may have applications in determining patient follow-up, and for designing new therapeutic strategies targeting highly proliferating malignant cells in breast cancer. CD71 expression could be used to characterize a subset of ER-positive luminal tumours with poor prognosis and high proliferation.

P16

Using LNA-ISH to detect miRNAs in archival breast tumour tissue

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Micro-RNAs (miRNAs) are a class of at least 500-600 small (21-23nt) non-coding RNAs that are expressed in a tissue-dependent manner. They function by down-regulating the expression of target mRNAs via complementarity with the 3' untranslated region (UTR). They are implicated in diverse biological processes, and their dysregulation is a key step in many cancers.

Previously, using miR expression data from a library of 93 frozen breast tumours, we have shown that several miRNAs correlate with clinicopathological features of breast cancer including molecular subtype, ER status and grade. Thus miRNAs hold the potential to act as diagnostic or prognostic markers.

We are now able to detect and quantitate miRNAs in archival tumour samples using a combination of locked nucleic acid (LNA) probe *in situ* hybridization (ISH), tyramide signal amplification and fluorescence microscopy. We have applied this technology to a large set of breast tumours in the form of tissue micro-arrays (TMAs), and have identified several potentially useful markers.

Furthermore, in an effort to improve and streamline the assessment of marker expression in TMAs, we have developed an entirely automated method of signal quantitation. The TMAs are scanned by a robot, and core images are analysed using a novel algorithm that quantifies signal intensity within the cytoplasmic compartment of the epithelial cells. This method compares very favourably with manual quantitation, and has great advantages of speed and repeatability.

We present both our novel histological markers and the automated method of cytoplasmic signal quantitation.

P17

Specimen Handling of Screen-Detected Ductal Carcinoma-In-Situ (DCIS) by Surgeons and Pathologists: Could We Do Better?

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The management of DCIS requires close cooperation between, surgeon, pathologist and radiologist in order to identify the size and surgical clearance of DCIS accurately and optimise the effectiveness of subsequent therapy. We have analysed results from 2099 cases of screen-detected DCIS recorded in the Sloane database of screen-detected DCIS to assess current practice.

70.6% had 1 operation (wide local excision (WLE) or mastectomy), and 2.8% had 3 or more operations. In 12% of WLEs specimen orientation markers were either absent or uninterpretable. Whereas 96% cases had specimen radiology as part of the surgical procedure, only 31.5% had subsequent specimen radiology undertaken by the pathologist and practice in individual laboratories ranged from 0% specimens imaged to 100% specimens imaged. Cases that were not imaged by a pathologist had significantly fewer blocks of tissues sampled and the size of the DCIS was probably underestimated in some of these cases.

11.3% of local excisions specimens undergoing only 1 therapeutic excision had radial surgical margins of less than 1mm, 27% cases had radial margins of 1.1-5mm and 50.8% cases had radial margins of >5mm.

Conclusion: The results suggest that there is still considerable scope for improved specimen handling by surgeons and pathologists and current therapeutic decisions may be based on less than optimal information in a significant proportion of cases.

P18

Is Acinic Cell Carcinoma a Variant of Secretory Carcinoma?

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Secretory carcinomas (SCs) and acinic cell carcinomas (ACCs) of the breast are rare, low grade malignancies that preferentially affect young female patients. These lesions are reported to have overlapping morphological and immunohistochemical features, which have led some to propose that they would be two morphological variants of the same entity. It has been demonstrated that SCs of the breast consistently harbour the t(12;15)ETV6-NTRK3 translocation. We hypothesised that if ACCs were variants of SCs, it would be reasonable to expect that ACCs would also harbour ETV6 gene rearrangements. Using the ETV6 FISH DNA Probe Split Signal (Dako), we investigated the presence of ETV6 rearrangements in 3 SCs and 6 ACCs. Cases were considered as harbouring an ETV6 gene rearrangement if >10% nuclei displayed 'split apart signals' (i.e. red and green signals were separated by a distance greater than the size of two hybridisation signals). Whilst the three SCs displayed ETV6 split apart signals in >10% of the neoplastic cells, no ACC showed any definite evidence of ETV6 gene rearrangement. Using in-house probes to investigate the presence of fusion between ETV6-NTRK3, we confirmed the presence of the t(12;15) in SCs, but not in ACCs. Based on the lack of ETV6 rearrangements in ACCs, our results strongly support the concept that SCs and ACCs are distinct entities and should be recorded separately in breast cancer taxonomy schemes.

P19

Successful oxytocin supported harvesting of nipple fluid

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Background: New non-invasive breast cancer screening modalities are required. Nipple fluid, that contains breast epithelial cells, is produced in small amounts in the breast ducts of non-lactating women and can be collected by non-invasive vacuum-aspiration. Previous studies failed to obtain nipple fluid in a considerable proportion of women.

Aim: feasibility of performing (intranasal) oxytocin supported nipple aspiration (by vacuum) was assessed on 67 healthy female volunteers, age 18-60, 12% postmenopausal.

Results: Nipple aspiration was successful in 63/67 women (94%); in 13 women (19%) unilaterally and in 50 (75%) bilaterally. The only predictor for fluid yielding during aspiration was history of nipple discharge (p<0.005 and p=0.05 for right and left breast, respectively). Previous breastfeeding, age, family history, history of breast lumps or surgery, hormonal and reproductive factors showed no predictive value for either the ability to yield fluid or the volume of nipple fluid. Volumes ranged from 5 to >100 μ l, containing 50-200 ng/ μ l DNA, which showed to be largely enough for performing a quantitative methylation specific PCR for multiple genes. The procedure was very well endured. Mean discomfort-rating during different stages of the procedure was 1.3 (on a 0-10 scale), compared to 1.9 for breastfeeding and 4.3 for mammography.

Conclusion: Oxytocin supported nipple aspiration provides a valuable tool for accessing mammary epithelium, providing sufficient DNA for a broad spectrum of analysis in the large majority of women.

P20

Expression of Estrogen Receptor Beta is Regulated by Alternative 5'-Untranslated Regions in Breast Carcinogenesis

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Estrogen receptor (ER) expression is a key determinant of breast tumour behaviour, although the role of ER β , unlike the well-studied ER α , remains uncertain. This is partly because analyses have been confused by discrepancies between ER β mRNA and protein levels. We demonstrate that alternative 5'-untranslated regions (5'UTRs) allow differential post-transcriptional regulation of ER β expression, which may be responsible for non-concordance of mRNA and protein and could provide an important level of modulation of ER activity. We studied three alternative ER β 5'UTRs that are produced from alternative promoters and/or transcriptional start sites. We show that these ER β 5'UTRs are differentially expressed between normal and tumour tissues. Furthermore, each 5'UTR has profound and differential influences on mRNA translational efficiency and stability. We investigated sequence determinants of these effects and influences of single nucleotide polymorphisms. We also demonstrate that each 5'UTR is preferentially associated with mRNA variants containing different 3' splicing patterns, which code for different functions. Finally, using a 442-case breast cancer TMA, we identified a positive correlation between expression of ER β 1 and eIF4E, a known regulator of 5'UTR function, thereby suggesting a role for cross-talk between 5'UTRs and cellular factors in defining ER β expression.

In conclusion, post-transcriptional regulation plays an important role in determining ER β expression and function. This may have an overall influence on ER activity and could have important implications on breast cancer biology and treatment.

P21

Audit of HER2 Status of Core Biopsies and Excisions of Invasive Breast Cancer

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HER2 assays are routinely performed on both core biopsy and excision specimens in each breast cancer case. Debate exists as to the reliability and precise clinical role of core biopsy assay.

In order to establish the degree of concordance, an audit of all breast core biopsy HER2 assays on confirmed breast cancer cases between January 2005 and February 2008 was performed. Cases were identified by searching the pathology database for all patients with a core HER2 result and identifying any corresponding excision testing. Discordant cases were reviewed in detail.

Of 185 cases, 80 went on to have a HER2 assay on excision samples. Of these, six assays were discordant between core and excision. Four cases had positive cores with negative excision HER2 status, and two had negative cores with positive excision results. All of the discordant cases had borderline immunohistochemistry scores on the core specimens and proceeded to FISH detection of HER2 gene amplification. No heterogeneity in HER2 expression was identified on review of the excision samples.

It is suggested that better fixation of core biopsies compared to some excision specimens, perhaps together with neo-adjuvant chemotherapy, will account for most core-positive discordant results. As in other areas of pathology, transcription errors may exist in some reports. However, this series indicates that it is generally safe to rely on core HER2 results without need for a routine excision specimen assay, in the context of ongoing audit of these tests.

P22

DCIS in Triple Negative and Basal-Like Breast Cancer

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The primary objective of this study was to determine whether basal-like breast cancer has a DCIS precursor. The secondary objective was to compare patient characteristics between basal and non-basal invasive tumours. Archives of the Western Infirmary Pathology Department were searched for triple-negative tumours with a DCIS and invasive component. Immunohistochemistry was performed for oestrogen receptor (ER), progesterone receptor (PgR), epidermal growth factor receptor (EGFR), epidermal growth factor receptor-2 (HER2) and cytokeratin 5/6 (CK5/6). Both DCIS and invasive lesions were scored. Positive cutoffs were: Allred >3 (ER and PgR), Herceptest score = 3+ (HER2) and any cells positive (EGFR or CK5/6). Basal status was determined by negativity for ER and HER2 with positivity for EGFR and/or CK5/6. Data regarding age, invasive tumour size, lymph node status, DCIS grade, invasive grade and presence of lymphovascular invasion were obtained from the database search. Sixty-six cases were analysed. Differences in percentages between DCIS and invasive components were: ER=1.51%, PgR=6.06%, HER2 = 0%, EGFR=3.03% and CK5/6=0%. There was no significant difference between DCIS and invasive tumours for basal-like status in this cohort (p=0.48). No significant difference was observed for age, tumour size, lymph node status, DCIS grade, invasive grade or presence of lymphovascular invasion after adjustment for multiple analyses in this small cohort. We conclude there is evidence of a DCIS precursor to invasive basal-like cancer, within a triple negative cohort.

P23

Duct Diversity in the Human Nipple

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Background: To explain the small number of nipple ducts (< 10) which can be cannulated in human breast, compared with a median of 27 ducts in the nipple duct bundle, Going and Moffat [J Pathol 2004] proposed that a few 'type A' ducts open on the nipple surface while more smaller-calibre 'type B' ducts originate from skin appendages. Subsequently, Rusby [Breast Cancer Res Treat 2007] proposed that many ducts share a few common openings on the nipple surface.

Aims and methods: To explore these competing explanations by examining duct immunophenotypes in the nipple duct bundle and duct morphology in the apex of the papilla. Coronal sections through the duct bundle at the nipple base in 10 mastectomies were stained with haematoxylin/eosin and immunostained for oestrogen and progesterone receptor; proliferation markers Ki-67 and mcm-3; E-cadherin; basal markers p63, CD10 and smooth muscle actin; basal cytokeratins 5, 14 and 17; luminal keratins 7/8 and 19; and Cox 2. Sagittal sections stained for haematoxylin/eosin, ck 7/8, ck 14 and PR were examined for 30 more cases.

Results: There was no size or immunophenotype dichotomy of ducts in the main duct bundle. Some 'type A' ducts communicated directly via a 'waist' (diameter ~50µ) with an infundibulum opening onto the nipple surface, while other ducts tapered to a pinpoint lumen (<10µ) in nipple epidermis. Such ducts did sometimes cluster around a common opening.

Conclusions: Further clarification of human nipple duct structure is still required.

P24

CD109 expression in basal-like breast carcinoma

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Aims: Breast cancer can be classified into several subtypes based on gene expression profiling. Basal-like carcinoma (BLC) has a triple negative phenotype that lacks the estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2, and has myoepithelial properties. We have recently reported that CD109, a glycosylphosphatidylinositol (GPI)-anchored protein, is a new breast myoepithelial marker. CD109 expression was investigated in invasive ductal carcinomas (IDCs) of the breast by immunohistochemistry.

Methods and results: 88 breast carcinomas of formalin-fixed, paraffin-embedded were obtained and immunostained with anti-CD109, high molecular weight cytokeratin (HMW-CK: CK15/10/14), CK5/6, calponin, Vimentin and p63 antibodies respectively and statistically analyzed. CD109 was detected in 18 of 30 basal-like breast carcinomas (BLCs) but not in other types of 53 IDCs (non-BLCs). Statistical analysis revealed that the CD109-positive group in all IDCs cases was significantly associated with a high histological grade, high frequency of cell mitosis, high nuclear atypia, poor tubule formation, high MIB-1 positive ratio. The percentage of CD109-positive tissues (18/30, 60%) in BLCs was higher than those of other myoepithelial markers including p63 (7/30, 23%), calponin (10/30, 33%) and vimentin (10/30, 33%). Although it was lower than that of HMW-CK (28/30, 93%), CK5/6 (19/30, 63%), HMW-CK was also positive in 28% of non-BLCs and CK5/6 positive in 2% of non-BLCs.

Conclusion: These findings indicate that CD109 is a useful marker for BLCs.

P25

Mib1 Tumour Growth Fraction Assessment in Lymph Node Negative Breast Cancer

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Better characterisation and prognostication forms the core of current breast cancer pathology research, aiming at improving outcome of this globally rising disease. In this study, tumour growth fraction has been evaluated in a large series of operable invasive breast carcinoma and compared with clinicopathological variables of known clinical importance.

Immunohistochemical expression of MIB1 was used to study the growth fraction of 921 lymph node negative operable invasive breast carcinomas, (median follow-up of 126 month). This series was previously evaluated regarding the expression of a panel of biomarkers closely relevant to breast cancer, including ER, PR, p53, luminal and basal cytokeratins.

Using the median growth fraction of 15% a highly significant positive correlation ($p < 0.001$) between the percentage MIB1 expression (growth fraction) and tumour grade, histologic tumour type, larger tumour size, poorer Nottingham Prognostic Index, and basal cytokeratins (CK5/6, and CK14) was observed. Inverse associations ($p < 0.0001$) were observed between tumour growth fraction and patients' age, menopausal status, ER, PR, p53, P-cadherin, and luminal cytokeratins (CK18 and CK19). Outcome analysis confirmed a relationship between higher growth fraction and liability for disease recurrence ($p = 0.04$), distant metastasis occurrence ($p = 0.02$), shorter disease free interval ($p = 0.03$), distant metastatic pattern ($p = 0.004$), and overall patients' survival ($p < 0.001$)

Tumour growth fraction assessment using MIB1 immunohistochemical expression provides an objective method for breast cancer stratification into high and low risk categories which could potentially provide clinically important information in lymph node negative breast cancer patients.

P26

Primary Tumour Growth Fraction: a Predictor of Chemotherapeutic Response in Lymph Node Negative Breast Cancer Patients

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Breast cancer; a heterogeneous group of tumours with diverse behaviour, outcome, and response to therapy, is nowadays treated locally; using surgery if operable, and systematically through adjuvant therapy. Today, no validated markers exist for prediction of chemotherapeutic sensitivity/resistance in early breast cancer patients. Nevertheless, proliferative fraction is one assay being currently investigated as a predictor for response to chemotherapy in breast cancer. The purpose of the present study was to test the hypothesis that tumour growth fraction could predict patients' response to such mode of therapy.

We investigated the association between primary tumour immunohistochemical expression levels of MIB1 and the objective response to chemotherapy in 100 primary operable breast carcinomas with median follow up of 124 months.

Increasing tumour growth fraction was significantly associated with longer patient survival, delayed loco-regional recurrence, and longer time to distant metastasis, indicating a better response to adjuvant chemotherapy more than tumours of low proliferative fractions ($p = 0.03$, $\chi^2 = 0.05$, $\chi^2 = 0.02$ respectively)

In the present exploratory study, high primary tumour growth fractions were significantly associated with a good response to chemotherapy in a subset of operable lymph node negative breast cancer patients treated with cyclophosphamide/methotrexate/5-fluorouracil regimen. Patients with very highly proliferative tumours (growth fraction $\geq 90\%$) should be offered systemic chemotherapy, to which their response would expectedly be excellent. On the contrary, those with 10% or less proliferative fraction are not likely to respond, thus could be excluded, and for whom alternative methods of therapy to be searched for.

P27

Genomic Profile of a Secretory Breast Cancer With an ETV6-NTRK3 Duplication

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Secretory breast cancer (SBC) is a rare entity characterised by an indolent clinical behaviour, distinctive histological features and the presence of a recurrent chromosomal translocation $t(12;15)(p13;q25)$, leading to the formation of the ETV6-NTRK3 fusion gene. In this study we present a case of SBC which harbours a duplication of the ETV6-NTRK3 translocation, $der(15)t(12;15)$, retention of one normal copy of both ETV6 and NTRK3 genes but has lost the derivative translocation $der(12)t(12;15)$. Tiling path array CGH (aCGH) analysis confirmed the presence of losses of the region centromeric to ETV6 and the region telomeric to NTRK3 observed from FISH analysis due to the loss of the derivative translocation. Additional regions of copy number changes included gains of 10q21, 10q26.3, 12p13.3-p13.31 and 15q11-q25.3 and losses of 6q24.1-q27, 12p13.2-q12 and 15q25.3-q26.3. Gain of the whole of chromosome 16 was also observed. To the best of our knowledge, this is the first time a carcinoma is shown to harbour a duplication of ETV6-NTRK3 translocation. The presence of an additional copy of $der(15)t(12;15)$ coupled with deletion of the other derivative $der(12)t(12;15)$ in the modal population of cancer cells suggest that this was either an early phenomenon or conferred additional growth advantage to neoplastic cells.

P28

High Grade Triple Negative Phenotype characterises Breast Cancer in Nigeria

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Introduction: Breast Cancer is the commonest malignancy in Nigerian women with an increasing incidence, high mortality rate, late presentation and early age occurrence. The aim of this study was to study the histological and immunohistochemical characteristics of breast carcinoma in a Nigerian cohort.

Methods: A total of 89 cases of invasive breast cancer seen in our hospital between 2004 and 2007 were identified. Histological review was performed and immunohistochemical staining for estrogen receptor (ER α), progesterone receptor (PR) and Her2 was done on representative sections.

Results: Patients age ranged from 22-82 years (mean = 48 years.) More than half of the patients were aged below 50 years and 25.7% fell within the 30-39 years group. Infiltrating ductal carcinoma NST was the commonest histological variant. Sixty two cases (69.7%) were grade 3, 23 cases (25.8%) were grade 2 and 4 cases (4.5%) were grade 1. Out of the 73 cases analyzed for ER immunocytochemistry using the Allred Score, 41 cases (62.1%) were completely negative. Seventy nine percent were PR negative and only 3 cases (4.5%) showed Her2 overexpression. Out of 58 cases with complete immunohistochemical data, 50% were triple negative.

Conclusion: Our data suggest that breast cancer in Nigeria occurs at younger age, is of higher histological grade and is more likely to be triple negative when compared with Western breast carcinomas. A larger study with comprehensive immunohistochemical analysis is underway to further characterize breast cancer in Africa.

P29

Biological Characteristics of ER Positive Operable Breast Cancer in Elderly Patients Treated with Primary Endocrine Therapy

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Primary endocrine therapy (PET) with tamoxifen has been used in the elderly with oestrogen receptor positive (ER+) primary breast cancer (PBC) who refuse or are unfit for surgery. However little is known about their biological characteristics, which may be important in therapeutic strategies such as selecting endocrine agents as primary, neoadjuvant or adjuvant therapy.

We therefore analysed a cohort of 133 patients aged ≥ 70 years with ER+ positive early operable PBC receiving endocrine therapy and assessed for response to treatment (UICC criteria). Median duration of follow-up was 17 months (range=1-150) and. Core biopsies were analysed for expression of PgR, ER β , HER2, EGFR and Ki-67, using immunohistochemistry, to determine if any of biological tumour characteristics could predict treatment efficacy.

The biological characteristics of the tumours were:

PgR		ER β		HER2		EGFR		Ki-67	
+	-	+	-	+	-	+	-	+	-
71	61	132	1	10	123	6	127	49	84

There was no association between PgR, ER β , EGFR expression and response to treatment, time to progression or duration of response (DoR) However, there was a significantly shorter DoR with HER-2 positive tumours ($p=0.003$) and those with higher growth fraction ($p=0.042$).

Expression of HER2 and growth fraction could therefore potentially be markers of a shorter duration of response to endocrine therapy in elderly patients. Clinical follow-up for longer-term efficacy assessment is continuing in order to further elucidate the relationship between endocrine sensitivity and these biological characteristics.

P30

SLC12A2 is an Oestrogen-regulated Gene that is Expressed in Breast Cancer

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Introduction: Oestrogens are critical agents that mediate diverse aspects of breast tumourigenesis via ER α -mediated regulation of the expression of select genes. SLC12A2 encodes an electroneutral Na⁺K⁺-2Cl⁻ cotransporter and was identified as an oestrogen-regulated gene in the present study. SLC12A2 is expressed in secretory epithelial cells and diverse tissues. SLC12A2 is a marker of murine breast epithelial cells and has a role in breast morphogenesis.

Methods: Human breast cancer cells (EFF-3, EFM-19 & MCF-7) were grown to 70% confluence and deprived of oestrogens for 7 days before treatment with 10^{-8} M oestradiol. RNA was extracted according to Affymetrix protocols prior to hybridization to Affymetrix Hu U133 Plus 2.0 GeneChips. Quantitative real-time PCR (qPCR) was then performed to validate SLC12A2 oestrogen-regulation in breast cancer cell lines and expression in snap-frozen invasive breast carcinoma specimens ($n=18$).

Results: 2800 oestrogen-regulated transcripts were identified, including PgR, TFF1, IGF1R and IRS1. SLC12A2 is a novel oestrogen-regulated gene that was confirmed by qPCR to be down-regulated upon oestradiol treatment.

Differential gene expression was observed to be time and oestradiol dose responsive. SLC12A2 mRNA was expressed in a panel of invasive breast carcinoma samples at mean higher levels in ER α + tumours compared with ER α - tumours ($p=0.0872$).

Conclusions: SLC12A2 mRNA expression is decreased by oestradiol in oestrogen-responsive breast cancer cells. SLC12A2 mRNA is expressed in invasive breast carcinoma tissue but this does not show a statistically significant association with ER α -status.

P31

Tiling Path Array CGH Analysis of HER2 Amplified Cancers: The Influence of ER Status

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The molecular genetic profiles of breast cancers have been shown to be strongly correlated with histological grade and oestrogen receptor (ER) expression. Expression profiling analysis has demonstrated that HER2 amplified breast carcinomas fall into two distinct molecular subgroups: those that express ER, usually cluster together with luminal B cancers, whereas HER2 amplified/ ER-negative cancers form a distinct group. We hypothesised that these transcriptomic differences could be driven by distinct patterns of genomic changes in ER positive vs ER negative HER2 amplified tumours. To test this hypothesis, we profiled 26 HER2 amplified cancers, of which 11 were ER-positive, with tiling path microarray based comparative genomic hybridisation analysis. Surprisingly, supervised categorical analysis revealed that 1.42% of the genome was significantly differentially gained, lost or amplified in these two subgroups of HER2 amplified carcinomas. Two regions were significantly more frequently amplified in ER positive HER2 amplified tumours: 11q13.3 (69.18-69.31Mb) and 17q21 (35.72-35.89Mb). Using probes specific to these regions, chromogenic in situ hybridisation analysis confirmed the presence of amplifications at these two loci. No amplification of ESR1 gene was found in ER positive cancers. HER2 amplified breast carcinomas constitute a distinct yet heterogeneous group of tumours at the genetic level, however the differences in the genetic profiles do not seem to be driven by the ER status of the tumours.

P32

Expression of the Co-activator NCOA3 in Breast Cancer

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A cDNA microarray comparing cancers from women of 35 yrs and normal breast identified genes that were upregulated in cancers. One of these was NCOA3 which has been found to have increased expression in breast cancer. Although it is a steroid receptor co-activator, there are conflicting reports as to whether increased expression occurs in oestrogen receptor (ER) positive or negative cancers. This study examined expression in breast cancers from different age groups with known ER status.

Expression of NCOA3 in 6 breast cell lines, normal breast organoids and 21 frozen breast cancers (10 < 35 yrs, 11 > 35 yrs) was analysed by quantitative RT-PCR. Protein analysis was by western blotting of the same cell lines and organoids, and by immunohistochemistry of normal breast and 56 breast cancers (20 < 35 yrs, 15 36-49, 21 50 > yrs).

There was higher expression by qPCR in the breast cancers from younger women compared to those >35 ($P=0.006$). Higher mRNA expression was found in the ER positive cancer cells, with western blotting showing protein in the same cells. Immunohistochemistry showed NCOA3 to be nuclear with some cytoplasmic staining. 25/56 had staining for NCOA3. In contrast to the qPCR there were fewer positive cases in the <35 group but this was not significant ($P=0.06$). 70% of cases were ER positive but there was no relationship to NCOA3 ($P=0.15$).

Expression studies suggest that NCOA3 may be of significance in breast cancers in young women, and not relate to ER.

P33

What is the Real Incidence of HER2 Positivity in Breast Cancer in the Neo-Adjuvant Setting?

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Prior to 2005, Herceptin was only used to treat breast cancer patients with HER2 positive metastatic disease and 25 - 30% of breast cancers were reported HER2 positive; pN0/M0 breast cancers were not assessed for HER2 status routinely. Despite the recent introduction of mandatory HER2 testing of all newly diagnosed breast cancers the real incidence of HER2 positivity in the adjuvant setting is not well established yet.

We have reviewed the results of 1107 newly diagnosed breast cancer patients routinely tested for Herceptin eligibility using an automated, gene-based, silver in-situ hybridisation assay (SISH) from four NHS Trusts.

The rate of positivity for HER2 in this group of patients was 13.3% (147/1107) which is approximately half that previously reported. This is likely to be due to at least two reasons; one being the fact that we are now testing all breast cancer cases and as a breast cancer screening centre receive a significant number of T1 cancers and also up to 80% ER positive luminal phenotype rather than HER2 positive phenotype cancers. We also speculate that the gene-based test we are using is more accurate in identifying HER2 positive cancers. This therefore reduces the number of HER2 positive cancers compared to that generated when using an immunohistochemistry assay.

P34

Uparap/Endo180 Expression in Invasive Breast Carcinoma and Its Relation to Patient Outcome

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Introduction: Local growth, invasion, and metastasis of malignancies of invasive breast carcinoma involve extensive degradation and remodelling of the surrounding, collagen-rich connective tissue. Urokinase plasminogen activator receptor-associated protein (uPARAP)/Endo180 is an endocytic receptor recently shown to play a critical role in the uptake and intracellular degradation of collagen by mesenchymal cells. However, the expression of this protein and its clinical significance in breast cancer is unknown

Methods: immunohistochemistry was used to investigate the expression of (uPARAP)/Endo180 in tissue microarrays of a large (n=880) well-characterized series of human breast carcinomas using blinded semiquantitative scoring, in addition to a set of well known biological markers in breast cancer.

Results: (uPARAP) was expressed in (5.7%) of invasive breast cancer, and in (78.8%) in the stroma surrounding these tumours. Positive expression of Endo 180 in the tumour cells was significantly correlated with negative steroid receptor as, ER (P=.013), and AR (P=.001), negative luminal cytokeratins like CK7/8 (P=.041). further more, a positive correlation was found between Endo180 expression and basal subtype of breast carcinoma (P=.003).

In addition to the association between its expression and shorter disease free interval (P=.01).

Conclusion: The association between (uPARAP)/Endo180 expression in malignant cancer cells with basal phenotype and its association with poor patient outcome could explain the aggressive behaviour of these types of tumour.

P35

Stromal CD8+ T Lymphocytes are Associated with Better Clinical Outcome in Breast Cancer

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Cytotoxic T-cells have the potential to destroy neoplastic cells. Recently, the association between a better prognosis and the number of tumour-infiltrating lymphocytes (including CD8+ T-cells) and tumour progression has been determined in colorectal, ovarian, and renal carcinomas.

We have analysed and characterised the expression of tumour-infiltrating CD8+ T-cells in a large cohort of breast tumours (n=1200) with long-term follow up using TMA and immunohistochemistry and investigated the relationship with clinical outcome.

A total 957 tumours were analysed after the uninformative cores were excluded. 427 cases (45%) showed CD8+ lymphocytes, 392 cases (41%) were positive for intratumoural (tumour touching) CD8+ cells (mean 6.94cells/core, range 1-136) and 320 tumours (33%) were positive for stromal (not-touching) tumour cells, mean 4.49cells/core, range 1-100). Total number of intratumoural CD8+ T-cells showed no association with overall survival (OS) or disease free interval (DFI). However, stromal CD8+ T-cell expression was significantly associated a better OS and longer DFI (all p<0.002). There was a significant association between presence of CD8+ lymphocytes touching tumour cells and higher tumour stage (p=0.001) and tumour grade (<0.001), and larger tumour size (p=0.043). Tumour touching CD8+ cells were also associated with ER and PgR negative tumours (p<0.001 and p=0.023, respectively). Stromal CD8+ lymphocytes were not associated with tumour stage, grade, or size.

This study highlights the importance of stromal CD8+ T-cell infiltrate in breast cancer patient survival.

P36

TWIST1 Expression and Methylation in Breast Cancer

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TWIST1 is an anti-apoptotic and pro-metastatic transcription factor involved in regulation of epithelial to mesenchymal transition (EMT). It is expressed in a number of epithelial cancers. In breast cancer, TWIST1 expression has been found to relate to metastases formation. Interestingly, methylation of the TWIST1 promoter that generally silences gene expression frequently occurs in breast cancer. This does not fit with the paradigm of an oncogene. We therefore studied TWIST1 methylation in relation to its expression in normal breast tissue and invasive breast cancers. We found that TWIST1 promoter methylation is significantly enhanced in malignant compared to healthy breast tissue. Furthermore, TWIST1 protein expression was significantly higher in breast malignancy compared to matched healthy tissue from the same patients. There was no correlation between TWIST1 promoter methylation and protein or RNA expression. This might point to a biphasic role of TWIST1 in breast cancer development.⁴

P37

Eosinophilic Mastitis Associated with Montelukast Treatment of Asthma

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Montelukast is a cysteinyl-leukotriene antagonist that significantly improves asthma control in corticosteroid-treated asthmatic patients. Some patients who are treated with montelukast develop eosinophilia, pulmonary infiltrates and vasculitis.

A 14-year-old girl with severe asthma, treated with high dose steroids, was started on treatment with montelukast in the summer of 2007 at the same time that steroid treatment was reduced. She has no other known immunological disorders. By the end of 2007, she had developed a swelling measuring 10x7 cm in the left breast, accompanied by a smaller lesion in the right breast. Clinical evaluation suggested the presence of a phyllodes tumour or fibroadenoma. Fine needle aspiration cytology showed numerous eosinophils. Wide bore needle core biopsies showed dilated ducts containing necrotic eosinophilic debris, surrounded by an intense infiltrate of eosinophils, plasma cells and mast cells. There was no evidence of granulomatous inflammation or vasculitis. Montelukast was discontinued and there has been partial regression of the swelling over a two month period.

These very unusual findings could represent an idiopathic eosinophilic mastitis but, in the clinical context, a side effect of montelukast seems more likely. Eosinophilic mastitis appears to be a novel observation in association with montelukast treatment. Whether the eosinophilic infiltration represents a true side effect of the drug or reflects unmasking of a pre-existing Churg-Strauss syndrome following steroid withdrawal, is uncertain.

P38

Caveolin 1 and Caveolin 2 are associated with breast cancer basal-like and triple negative immunophenotype

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Background:

Caveolin-1 (CAV1) and caveolin 2 (CAV2) are the principal structural proteins of caveolae, sphingolipid and cholesterol-rich invaginations of the plasma membrane involved in vesicular trafficking and signal transduction. Over the recent years there has been controversy about their role in breast cancer and their suitability as markers of basal-like phenotype.

Material and Methods:

CAV1 and CAV2 protein expressions were assessed on a tissue microarray containing 880 unselected invasive breast cancer cases, by means of immunohistochemistry.

Results:

CAV1 and CAV2 expression was observed in 13.4% and 5.9% of all breast cancer, respectively. Their expression was strongly associated with high histological grade, lack of steroid hormone receptor positivity (ER and PR), and expression of basal markers (basal cytokeratins, P63, P-cadherin). Furthermore there was a significant association between CAV1 and CAV2 expression and basal-like phenotype. On univariate analysis only CAV2 had a prognostic impact on breast cancer-specific survival; however, this was not independent from other traditional markers on multivariate analysis.

Conclusion:

Our results demonstrate that both CAV1 and CAV2 are associated with basal-like phenotype. Further studies are warranted to determine whether they play an oncogenic role in basal-like/ triple negative breast cancer development or are just surrogate markers for this subgroup.

P39

The Clinical and Histopathological Significance of Performing Surgical Cavity Random Biopsies in Breast Conserving Surgery

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Background

Surgical treatment of breast cancer has changed dramatically in recent years. The National Institutes of Health Consensus Panel confirmed that breast-conservation surgery (BCS) is as effective as mastectomy in overall patients' survival. Our aim was to assess the clinical and pathological value of performing surgical cavity random biopsies (SCRb) in BCS and their impact on the subsequent management.

Materials and Methods

A retrospective study was conducted on 494 patients who had BCS in our firm between 2001 and 2006. Outcome measures were examined at 3,4,5 years follow up period for each patient. Tumour immunohistochemistry, adjuvant therapy, recurrence rate and Demographic data were collected and represented with Pearson's chi-squared test.

Results

The median age was 59 years. Out of 494, 23 patients (4.65%) had positive SCRb whom all had subsequent surgical intervention plus adjuvant therapy. 7 patients had total mastectomy, 13 had re-excision of positive margins and only 3 had axillary node clearance. Recurrence rate was reported in 7 patients only (1.41%) and the overall mean survival time for all patients was 74.585 months (95% C.I 73.839-75.332).

Conclusion

Our practice of performing SCRb had changed the management of 23 patients and revealed an excellent recurrence rate of 1.41% which is below the national figures reported in the literatures (7-9%). SCRb is a reliable method of determining margin status, minimising re-excision and reducing overall recurrence rate.

P40

The Clinical and Histopathological Impact of Performing Surgical Cavity Random Biopsies On The Long-Term Outcome Of Breast Conserving Surgery, Literatures Review.

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Background

Surgical treatment of breast cancer has changed significantly in recent years. The significance of recurrence rate following breast-conserving surgery (BCS) and its relationship to long-term outcome remains a matter of debate. The aim of this literature review was to assess the impact of performing tumour-bed sampling to ensure the completeness of surgical excision after BCS.

Materials and Methods

A structured literature review was performed from 1987-2007 using Medline, Embase, Cochrane Library and also retrieving original papers cited from related references. Full papers were compared together with regard to demographics, incidence of positive biopsies, rate of re-operation, adjuvant therapy and recurrence rate.

Results

Literatures search has identified a total of 27 papers, 13 of those related to the resection margins, hence only 14 papers were included in this search involving the surgical cavity random biopsy. The total number of patients reviewed were 2862 with a mean of 204.4. The median age was 56.23 years. 770 patients (26.89%) had residual tumour and further surgery was performed in 365 patients (47.46%) with the rest having adjuvant therapy. Regional and distal recurrence was noted in 9.35% of patients after tumour resection.

Conclusion

The aim of breast-conserving surgery is to excise the tumour completely and achieve an excellent cosmetic result without compromising the degree of local disease clearance offered by mastectomy. Cavity shaving is a practical and sensitive method of assessing completeness of excision as well as providing useful prognostic information in BCS.

P41

MCPH1, a potential predictor for response to cancer chemotherapy

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We have shown MCPH1, a DNA damage response protein involved in the regulation of BRCA1, is defective in one form of microcephaly. The MCPH1 locus (8p23) is frequently deleted in many tumour types including breast cancer and this depletion is associated with a poor prognosis.

Reduced expression of MCPH1 causes premature chromosome condensation (PCC). Using time-lapse imaging we have identified further mitotic defects including slower mitotic progression, aberrant chromosomal congression and micronuclei formation in MCPH1 deficient cells. This mitotic phenotype suggests that loss of MCPH1 function could cause mitotic errors with resultant aneuploidy.

Chemotherapeutic agents such as Taxol require a functional spindle checkpoint for the induction of apoptosis in cancer cells. Our data indicates that MCPH1 plays a role in the spindle checkpoint. We hypothesise that, while germ line defects in MCPH1 cause microcephaly, somatic defects may cause aneuploidy development and resistance to some chemotherapeutic agents in breast cancers.

We have demonstrated reduced MCPH1 expression in 17 out of 54 (32%) breast cancers, particularly in higher grade tumours. Interestingly we also identified high levels of MCPH1 expression in 8 out of 36 (22%) low grade breast cancers, which was associated with a good prognosis. We are now studying a larger series of breast cancer samples using tissue micro arrays to investigate MCPH1 in a larger cohort in order to validate our initial results and relate them to patient outcome.

P42

Case Report – Tubular Adenoma Colonised by LCIS

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We present the case of a 52 year old woman with a screen detected 10mm well-defined rounded mass in her right breast. Ultrasound examination was compatible with a fibroadenoma (U2). Core biopsy showed crowded acinar structures surrounded by P63 positive myoepithelial cells but expanded by lobular carcinoma in-situ (LCIS).

Excision biopsy was performed to establish the nature of the mass lesion. Excision biopsy showed an ovoid 10 x 7mm breast tumour composed of closely packed acini with minimal intervening stroma with the architecture of tubular adenoma. 99% of the acini were distended by monotonous cells with rounded nuclei and clear cytoplasm containing acidic and neutral mucins. The morphological appearance of LCIS was confirmed by lack of E-Cadherin membrane positivity. The acini were surrounded by SMA positive epithelial cells and no stromal invasion could be identified (confirmed with cytokeratin immunohistochemistry).

Fibroadenomas in which there is focal LCIS replacing the epithelial element are described. This case is a well-defined mass composed of closely packed tubules with minimal intervening stroma with the architecture of a tubular adenoma. The epithelial component appears to have been replaced by LCIS without invasion. Tubular adenoma is a rare lesion (comprising 0.01%) of benign well-defined fibroepithelial lesions and diffuse replacement of the epithelial element by LCIS has not been previously described.

P43

New Highlights on the Clinical, Biological and Prognostic Implications of p53 Transcriptional Status in Breast Cancers

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Aim

To investigate p53 transcriptional activity in determining pathoclinical, prognostic and treatment response in breast carcinoma (BC)

Methods

Immunohistochemistry and quantitative scoring was employed to determine p53 transcriptional activity using three of its downstream targets (MDM2, p21 and Bcl-2) on 615 successive surgical BC cases with clinical follow up (> 20 years). Statistical analysis was performed on the whole patient series, in node-negative and node-positive subgroups, and those that had/had not received adjuvant treatment.

Results

Six p53 phenotypes were identified that exhibited 2 main clinical outcomes: (a) **Good prognosis group** including an MDM2-overexpression phenotype (p53+/MDM2+), and active wild-type p53 phenotype (p53-/MDM2+/Bcl2+). These showed favorable clinical parameters (ER-alpha+), occurred in low nuclear grade breast neoplasia (LNGBN), and had significantly reduced tumour recurrence/distant metastasis after hormonal-therapy (p=0.035). (b) **Poor prognosis group** including the mutational phenotypes: point mutation (p53+/MDM2-), null mutation (p53-/MDM2-/p21-), and inactive wild phenotype (p53-/MDM2+/Bcl2-). These tumours were characterised by aggressive features and did not benefit from adjuvant therapy. The p53+/MDM2-/p21+ phenotype had the worst prognosis especially in ER-α positive patients given adjuvant chemotherapy (p=0.02). Compared to the point mutation phenotype, p53 null mutation phenotype showed increased ER and PgR-expression, and occurred more frequently in LNGBN with low tumour stage.

Conclusion

p53 status determined by transcriptional activity has prognostic and treatment predictive significance in breast carcinoma and could assist routine clinical decision-making regarding optimum treatment selection.

P44

An Audit of Pathology Reporting of Breast Biopsies in Kent

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Introduction: Pathology plays an essential role in the assessment of risk, diagnosis, and management of breast cancer. This audit reviews the laboratory handling and pathological reporting of 500 breast biopsy specimens across Kent. High quality and cost effective pathology reports are vital for the delivery of a fast and accurate diagnostic component to the patient pathway and delivery of 31/62 day National Cancer wait targets.

Method: Consecutive batches of 100 needle core biopsy reports from each of the 5 Kent histopathology departments are being retrospectively reviewed. We examine adherence to agreed national and local guidelines. Conformity with the minimum dataset of diagnostic information as defined by the Royal College of Pathologists is being investigated. In addition, we perform an analysis of turnaround times in view of a 4 day target as defined by the Kent Cancer Network Breast Pathway.

Results: The audit results are to be presented. This audit will identify variations in the handling and pathological reporting of breast biopsy specimens across Kent. The aim is to identify the most efficient and effective means of providing a high quality diagnostic service and achieving a standardised approach to the handling and reporting of these specimens. The audit has relevance, not only for Breast Pathology Services, but also Breast Surgical and Radiological Practice.

P45

Evidence Supporting an Ischaemic Mechanism for NSAID Injury to the Small Bowel

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Background: Experimental studies have identified an early acute phase of pre-ulcerative small intestinal injury induced by the non-steroidal anti-inflammatory drug (NSAID) indomethacin. This phase included villous shortening and buckling, capillary occlusion with congestion, smooth muscle and endothelial cell crowding all in the absence of significant inflammatory cell infiltration. This phase, as well as the subsequent mucosal necrosis, was considered to arise as a consequence of ischaemia induced by the drug.

Aim: To report changes described above in histopathological specimens of human small bowel reported as having ischaemic injury.

Methods and results: Routine human surgical specimens with (i) acute small bowel infarction or ischaemic enteritis (n=8) and (ii), ileum from right hemicolectomy colorectal cancer controls (n=10) were scored according to a grading system defining progressive early NSAID damage in the rat. All eight of the human infarction cases, and only two of the controls, showed mucosal changes identical to early experimental indomethacin injury (P=0.0011).

Conclusions: A pattern of ischaemic change observed in small bowel infarction specimens that is identical to experimental NSAID small bowel injury, is described. The findings support the hypothesis that NSAIDs damage the small bowel by primary mechanisms that involve ischaemia to the bowel wall.

P47

Audit of colorectal cancer histopathology staging at Maidstone and Tunbridge wells NHS Trust.

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Method:

The national minimum data sets for all colorectal cancer cases over 12 month period (Jan.2007 to Dec.2007) were examined. The stage, lymph node count and presence of extramural vascular invasions were analysed. The reporting practice is sub-specialised, two consultants report colorectal cancer excision at Maidstone Hospital (Pathologist A and B), while at Pembury Hospital, only one consultant with another consultant to cover for any leaves.

Results:

	PATHOLOGIST			
	A	B	C	D
Total number of cases	45	27	84	18
Colon	35	18	68	12
Rectum	10	9	16	6
T stage				
pT4 (overall)	18 (40%)	9 (33.3%)	21 (25%)	5 (27.7%)
pT4 (rectum)	2 (20%)	1 (11.1%)	2 (12.5%)	1 (11%)
pT4 (colon)	16 (45.7%)	8 (44.4%)	21 (30.8%)	4 (33%)
Lymph node range	7-39	9-42	9-41	10-29
Mean lymph node count	17.3	20.6	15.5	16.3
EMVI	16 (35.5%)	9 (33.3%)	39 (46%)	8 (44.4%)

Discussion:

New national guidance has been published with audit standards suggested for EMVI (at least 25%) and T4 rate (at least 20% for colonic and 10% for rectal tumours). The existing guidance of a mean of 12 lymph nodes remains. All four pathologists are achieving all three of these audit standards.

P46

Management of colorectal liver metastases: a single institution experience

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Introduction: Metastatic colorectal cancer (CRC) to liver has been associated with poor outcome. As CRC is a common disease with approximately 25% of patients presenting with liver metastases, and an additional 25% developing liver metastases, management of these patients has been the focus of much research.

Methods: We identified all new patients with CRC who presented with/developed liver metastases from July 1st 2005-June 30th 2007. Resectable patients had upfront liver resections, and unresectable patients were assessed for 'neoadjuvant' chemotherapy and targeted therapy. Response to chemotherapy was evaluated using response evaluation criteria in solid tumours (RECIST) and then liver resection was revisited. All findings at surgery were discussed and concordance with radiology assessed.

Results: 185 patients were diagnosed with CRC. 29 (73%) of these patients were unresectable due to poor performance score, excess number or location of metastases, and presence of extra-hepatic disease. 18 patients with unresectable disease received 'neo-adjuvant' treatment with FOLFOX6 and Bevacizumab. 13 of these patients went for resection with 11 having a partial response (PR) and 2 patients having a complete response (CR) on imaging. 11 patients had 'curative' resections. Discordance between surgery and radiology was seen in 3 cases. Chemotherapy was well tolerated in the majority of cases and there was no significant postoperative morbidity.

Conclusion: Liver is the most common site of colorectal metastases. Our experience in the management of liver metastases from CRC shows that 38% of unresectable liver disease is rendered resectable with modern chemotherapy.

P48

MYH-Associated Hyperplastic Polyposis Syndrome – A Case Report

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Hyperplastic Polyposis Syndrome (HPS) is rare, usually characterized by multiple, large hyperplastic polyps. MutY-human-homolog (MYH) associated HPS (MAP) is a recently characterized autosomal recessive variant usually causing adenomatous polyposis with fewer polyps than seen in classical FAP caused by APC mutation. Rarely, MYH-mutations have been described in association with hyperplastic and adenomatous polyps fulfilling current criteria for hyperplastic polyposis.

A 64-year-old female presented with simple fresh per rectal bleeding over a two-month period. Colonoscopy revealed 100 colonic hyperplastic polyps and 30-50 colonic adenomas, histology was highly suspicious of HPS. Following discussion at MDT she underwent a panproctocolectomy and formation of ileostomy. Approximately 250 polyps were identified in the proctocolectomy specimen. The majority of the polyps were identified as hyperplastic polyps, although larger than typical sporadic hyperplastic polyps and demonstrated unusual architectural features including inversion. Tubular and tubulovillous adenomas were predominantly found in the proximal colon while hyperplastic polyps and serrated adenomas were mainly present in the distal colon. Mutation analysis of APC and the MYH genes identified 2 MYH variants and a diagnosis of MAP was made. Screening colonoscopy of her two children revealed no evidence of polyposis.

In conclusion, MAP is a rare variant of HPS. Responsible for 1.4% of all adenomatous polyposis, it is the first human cancer predisposition disorder to be linked to defects in the BER-pathway with mutations occurring in the MUTYH gene. This syndrome has defined pathological and genetic properties, raising important questions for screening with 1:100 of the population being affected by the mutation.

P49

CTEN positively regulates cell migration in colorectal cancer

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INTRODUCTION

C-Terminal Tensin like (CTEN) is a recently cloned gene which has homology with the tensin family of genes. These genes localized at focal adhesion molecules and appear to be involved in the regulation of cell motility. We have previously found that CTEN is over expressed in adenoma in Min (Multiple Intestinal Adenoma) mice. We aimed to evaluate the expression and function of CTEN in human colorectal carcinoma.

METHODS

Expression of CTEN gene in 26 human colorectal carcinoma cell lines was detected by QR-PCR. This was used to identify cell lines with low expression of CTEN for further functional studies. One of these (HCT116) was selected for transfection with a GFP tagged CTEN expression vector. Establishment of a stably transfected cell line was verified by Western blotting for both GFP and CTEN. This cell line was then tested for changes in proliferation, apoptosis and migration compared with stable transfected empty GFP vector.

RESULTS

CTEN mRNA was expressed with varying levels in 26 human colorectal cell lines but levels were very low in HCT116. Evaluation of proliferation and apoptosis showed no difference between the cell lines transfected with GFP-CTEN and GFP alone. The migration results showed that cells expressing GFP-CTEN showed a significantly greater level of transwell migration than GFP control ($p < 0.01$).

DISCUSSION

In this study, we have demonstrated, for the first time that CTEN is able to promote cell migration in HCT116 colorectal carcinoma cell line when it is expressed.

P50

An Unusual Stomach Ulcer: Inflammatory Myofibroblastic Tumour

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A 52 year old lady presented with anaemia, she had numerous endoscopies where an ulcer was visualised, repeat biopsies showed fibrous tissue and inflammatory cells only with no definitive diagnosis. As the ulcer persisted a gastrectomy was performed and on the greater curve a 6cm ulcerated tumour with rolled edges was identified. The tumour was white, extremely firm and extended through the full thickness of the stomach into the mesocolon. The surrounding lymph nodes were strikingly enlarged.

Microscopically the tumour extended into the mesocolon and was composed mainly of acellular connective tissue. Numerous lymphoid aggregates with prominent germinal centres were scattered throughout, as were collections of eosinophils and plasma cells. The lymph nodes all showed marked follicular hyperplasia without malignancy.

The diagnosis of an inflammatory myofibroblastic tumour was made. This tumour falls within the spectrum of lesions formerly known as inflammatory pseudotumours. It is composed of myofibroblastic spindle cells accompanied by a mixed inflammatory infiltrate of plasma cells, eosinophils and lymphocytes. It frequently recurs (25% of abdominopelvic tumours) but rarely metastasises. It usually occurs in children and young adults (mean age 10 years) but can occur throughout adulthood, as in this case.

P51

RKIP Expression Levels Predict Survival in a Large Cohort of Colo-Rectal Cancer Patients

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Raf Kinase Inhibitor Protein (RKIP) plays a role in numerous cell signalling pathways which are important in cancer. Decreased expression has been shown in a number of human cancers, including breast, prostate and colo-rectal carcinoma (CRC). In a previous study of 200 cases of Dukes B CRC we showed that decreased RKIP expression correlates with a poor prognosis, independent of peritoneal involvement, lympho-vascular invasion and tumour size. Here we set out to validate this result in a larger cohort of CRC patients. In this study we used immunohistochemistry to stain a tissue microarray, consisting of 1034 patients with CRC (kindly provided by Dr. Nik Zeps, Western Australia Research Tissue Network). The slides were stained for RKIP protein and scored using a semi-quantitative system combining staining intensity and area stained. Using this system RKIP expression was divided into 3 groups; negative, weakly positive and strongly positive.

As no difference was seen between the negative and weakly positive group, these were combined and compared with the strongly positive group. Log-Rank test showed a significant difference between the 2 groups in both overall ($p=0.0007$) and disease-specific survival ($p=0.0024$). Median overall survival in patients with high levels of RKIP expression was 72% longer than in those with low levels of RKIP expression (107.6 v 62.5 months).

This large study confirms the results of our previous work showing RKIP to be a useful prognostic marker which may aid in risk-stratifying patients with CRC.

P52

Anal Melanoma: an Intriguing Case Report

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Anorectal melanoma comprises 0.25% to 1.25% of all the malignancies originating in this anatomic region and is often mistaken clinically for benign conditions as either haemorrhoids or rectal polyp. We report a case of an anal polyp from an elderly female which showed a pleomorphic spindle cell morphology. Immunohistochemistry was performed for this malignant tumour with a panel of markers to differentiate a poorly differentiated anorectal carcinoma from GIST and a spindle cell melanoma. The tumour cells were strongly positive for S100, melan-A and CD117 whilst being negative for pancytokeratins. This is a case of anal melanoma expressing CD117/c-kit protein and it highlights an unexpected finding by immunohistochemistry with a potential of being misdiagnosed as a GIST/ GANT. Previously some studies have reported CD117 positivity in a small number of anorectal melanomas of which a minority have shown c-kit mutation.

Anorectal melanomas are highly aggressive and unresponsive to both radical and local control with a poor 5 yr survival of 10-15%. Given the resistance of this tumour to conventional chemotherapy and radiation, the incidence of the c-kit alteration may represent a novel approach to a gene-directed treatment using a c-kit inhibitor (Glivec/ Imatinib) similar to that which has been proposed in GISTs. Thus on the basis of detection of CD 117 expression by immunohistochemistry in anorectal melanomas, c-kit mutational analysis is warranted to determine the eligibility of this tumour for imatinib targeted therapy.

P53

Assessment of peritumoural inflammatory infiltrate provides independent prognostic information when compared with current pathological criteria in colorectal cancer

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Provision of adjuvant chemotherapy in colorectal cancer is guided by pathological criteria. The prognostic value of the Petersen Index (PI) has been validated (Morris, Gut 2007;56:1419-1425). Inflammatory cell response at the invasive edge also confers prognostic value (Jass, Lancet 1987;1:1303-1306). The Finnish inflammatory infiltrate score (FS) has recently been validated (Roxburgh2008 unpublished), however whether this is independent of Dukes stage, PI or Jass classification remains unclear.

Aim: To investigate the prognostic value of the FS in colorectal cancer, compared with existing validated pathological criteria.

Methods: 207 patients from 1997-2004. 2 observers scored 3 H+E slides per specimen. FS was assessed as low or high grade at the invasive front. Specimens were grouped by Jass' classification. Dukes stage and PI were constructed from pathology reports.

Results: Median follow-up for survivors was 72 months, during which 81 died, 50 of cancer. Most were >65 years (60%), colonic tumours (61%) and Dukes A/B disease (59%). Univariate analysis of A/B disease: age ($p<0.05$), PI ($p=0.001$), Jass group ($p<0.005$) and FS ($p<0.005$) were all significantly associated with cancer-specific survival; Dukes stage was not ($p=0.866$). Multivariate analysis of Dukes A/Bs: age (HR2.82 $p<0.005$), PI (HR6.31 $p=0.001$), and FS (HR0.35 $p<0.005$) were independently associated with cancer-specific survival.

Conclusion: FS offers prognostic information independent of currently assessed pathological variables. FS should be considered for inclusion in routine pathological assessment of colorectal cancer.

P54

Can we identify putative stem cell markers in formalin fixed paraffin embedded gastrointestinal tissues?

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Background. Many studies have attempted to identify gastrointestinal stem cells using various methods. To be of value, immunohistochemistry using putative stem cell markers needs to be applicable in routine, formalin fixed paraffin embedded material.

Methods. Tissue microarrays (TMAs) were constructed using oesophageal, gastric, ileal and colonic tissues. Brain tissue and tissue from germ cell tumours were used as positive controls. Immunohistochemistry was performed using antibodies against CD133, Musashi-1, Oct-4, Sox-2, Ssea-4, Gpr49 and Dcamk-1.

Results. All antibodies showed expected staining patterns in control tissue. However, none of the antibodies produced a staining pattern consistent with the predicted frequency and location of putative stem cells in the GI tissues. Whilst a variable staining pattern was observed using Oct-4, Sox-2, Ssea-4, Gpr49 and Dcamk-1, cytoplasmic Musashi-1 staining was restricted to a small sub-population at the base of ileal and colonic crypts. Additionally, membranous CD133 positivity was seen in a subpopulation of cells at the base of the ileal crypt and in the lower half of the colonic crypts. No distinct staining pattern was observed in gastric or oesophageal tissues for any antibody.

Conclusions. This study demonstrates the current problematic nature of immunohistochemical identification of putative gastrointestinal stem cells in FFPE material. Despite appropriately staining controls, none of the markers appeared to stain a putative stem cell population in the correct anatomical location in FFPE GI tissues.

P55

Is Routine Histology Required for all Gall Bladders?

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Background: The debate over routine histological assessment of gall bladders continues despite the Royal College of Pathologists guidance(1). Studies suggest this practice is superfluous (2) however the focus has been surgical assessment.

Aim: To identify whether macroscopic examination by a histopathologist together with clinical findings can accurately evaluate the need for histology. **Method:** Retrospective review of pathology reports of all gall bladder cancer, dysplasia, benign polyps and adenomas over 5 years. Clinical details correlated with macroscopic and microscopic findings. Identical criteria applied retrospectively to a 10% sample and prospectively to gall bladders for 6 months.

Results:

Microscopic diagnosis	No clinical details	Clinical correlation	Macroscopic abnormality	Clinical & macroscopic	No correlation
Malignant ¹ n=33	2/33 6.1%	29/31 93.5%	28/33 84.8%	30/31 96.8%	1/31 3.2%
Dysplasia n=27	5/27 18.5%	8/22 36.0%	8/27 30.0%	13/27 48.1%	11/27 40.7%
Adenoma n=7	2/7 28.6%	4/5 80.0%	7/7 100%	7/7 100%	0/7 0%
Polyps n=8	1/8 12.5%	5/7 71.4%	8/8 100%	8/8 100%	0/8 0%

¹ primary or metastatic

Conclusion: Combining macroscopic and clinical findings accurately predicts the need for gall bladder histology in most cases except dysplasia.

References:

1. Histopathology and cytopathology of limited or no clinical value. Royal College of Pathologists 2005
2. Focus on: Cholecystectomy. NHS Institute for innovation and improvement 2007.

P56

Expression of the iron export protein Ferroportin is associated with increased survival in oesophageal adenocarcinoma

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Introduction: The development of oesophageal adenocarcinoma is associated with the dysregulation of a variety of proteins involved in iron transport. Accumulation of intracellular iron secondary to these disturbances is thought to modulate cellular proliferation via a variety of mechanisms and may also induce oxidative stress. We undertook an analysis of the relationship between survival in oesophageal adenocarcinoma, and tumour expression levels of 3 proteins involved in iron transport and metabolism. **Methods:** Triplicate cores from 40 formalin-fixed paraffin-embedded samples of oesophageal adenocarcinoma were mounted in a tissue microarray. Arrays were stained for Duodenal Cytochrome B (DCytB), Ferroportin, and Transferrin Receptor 1 (TRF1). Immunohistochemical expression levels were assessed using a semi-quantitative scale and averaged across the three cores. **Results:** Cox regression analysis demonstrated a significant correlation between Ferroportin expression and survival, with higher levels of expression associated with increased survival ($p=0.021$). There was no association between survival and expression of TRF1 or DcytB. Dividing the study population into approximately equal groups on the basis of Ferroportin staining intensity demonstrated a 2-fold increase in median survival duration in the those with higher expression (862 days v 376 days; $p=0.04$; Kaplan-Meier log-rank). **Discussion:** These data emphasise the potential clinical relevance of the association between intracellular iron levels and cell proliferation. The identification of a mechanistically plausible and statistically significant effect in this relatively small dataset supports the case for larger studies of the prognostic significance iron transport proteins.

P57

Meta-clustering of Small Intestinal, Gastric and Colorectal Adenocarcinomas Based on DNA Copy Number Profiles.

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Adenocarcinomas of the stomach, small intestine and colorectum share several clinical and phenotypical characteristics. In addition, the majority of these adenocarcinomas show chromosomal instability, which results in DNA copy number aberrations that can be measured by array comparative genomic hybridization (aCGH). To which extent these three tumour types share DNA copy number profiles is unknown. We and others evaluated the chromosomal gains and losses by array CGH of colorectal and gastric tumours. Small intestinal cancer is rare in comparison to colorectal and gastric cancer and high resolution aCGH data were not previously reported to our knowledge. Previously, our group succeeded to classify 373 epithelial tumours according to their organ of origin based on their aCGH profiles by hierarchical clustering (Jong et al., *Oncogene* 2007). Within this meta-cluster of primarily epithelial tumours, the gastrointestinal profiles split into a cluster with primarily colon tumours and one cluster with both gastric and colon tumours. No small intestinal tumours were included.

The aim of the present study is to investigate DNA copy number profiles of small intestinal tumours in comparison to gastric and colorectal.

Materials: aCGH data from 35 adenocarcinomas of each organ were selected from different in house datasets, based on array quality and clinical data. Smoothed data were called by the calling algorithm CGHcall. Called data were analyzed with hierarchical clustering and with frequencies of aberrations per organ

Results: First results indicate substantial overlap of DNA copy number profiles between small intestinal and colorectal cancer and less overlap with gastric cancer.

P58

Validation of a Prognostic Inflammatory Infiltrate Score in Colorectal Cancer

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Recently, a prognostic score based on inflammatory cell reaction at the tumour's invasive edge was reported in Dukes A/B colorectal cancer in Finland (Klintrup *EJC*2005;41:2645-2654).

Aim: To validate the Finnish inflammatory infiltrate score (FS) in patients undergoing potentially curative resection for colorectal cancer.

Methods: 207 patients between 1997-2004. 2 observers scored 3 H+E slides per specimen independently. FS was assessed as low or high grade at the deepest point of invasion.

Results: Median follow-up for survivors was 72 months. During this time 81 died, 50 of their cancer. Most were >65 years (60%), colonic tumours (61%) and Dukes A/B disease (59%). Characteristics were similar to Klintrup's population (DukesA/Bs 61%). Univariate analysis: age ($p<0.05$), Dukes stage ($p<0.001$) and FS ($p<0.001$) were significantly associated with cancer-specific survival. Multivariate analysis: age (HR1.56 $p=0.010$), Dukes stage (HR2.31 $p<0.005$) and FS (HR0.50 $p<0.001$) were independently associated with cancer-specific survival.

In Dukes A/Bs, age ($p<0.05$) and FS ($p<0.005$) were associated with cancer-specific-survival, however Dukes stage was not ($p=0.866$). Multivariate analysis: age (HR2.61 $p=0.005$) and FS (HR0.33 $p=0.001$) were independently associated with cancer-specific-survival. In Dukes Cs only FS was associated with cancer-specific-survival ($p<0.05$).

Conclusion: FS is an independent indicator of cancer-specific survival in colorectal cancer. Assessment of the FS should be considered for inclusion in routine pathological assessment of colorectal cancer.

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Fragile Histidine Triad (Fhit) and Ki67 Expression in Aberrant Crypts and Colorectal Carcinomas.

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Background: Abnormal Expression of Fhit Protein is Observed in Different Types of Human Malignancies. There Are Limited Literature Analysing Fhit and Ki67 Co-expression in Colorectal Lesions. Objective: Comparative Analysis of Fhit and Ki67 Expressions by Colorectal Aberrant Crypts (ACF) and Adeno Carcinoma (Adeno Ca). by Immunohistochemistry. Methods: Ninety-five Colonic Adeno Ca were Examined to Identify ACF in Mucosa Adjacent to Tumour. Sections Bearing ACF and Tumour were Used for the Study. Immunohistochemistry for Fhit and Ki67.

Results: All had Hyperplastic ACF (HACF) in Adjoining Mucosa, 31% had Dysplastic ACF (DACF). Normal Crypt Epithelium Showed Strong Cytoplasmic Expression for Fhit, Stronger along Surface Epithelium, Similar Pattern Observed in HACF, with Stronger Expression by Surface Epithelium. 28.57% of DACF were Negative and 71.43% Showed Weak to Moderate Intensity. Six of Adeno Ca Showed Faint Cytoplasmic Positivity. HACF, DACF and Adeno Ca had Significantly Different Fhit Expression ($p<0.05$). HACF Showed Ki67 Positivity Extending up to Neck Region, and DACF having Positive Surface Epithelium. Adeno Ca Showed Strong Ki67 Expressions. Significant Differences Between Normal, HACF, DACF and Adeno Ca ($p<0.05$). No Correlation between Cigarette Smoking and Fhit Expression. DACF were Older with Predominant Male Sex ($p>0.05$).

Conclusion: Gradually Decreasing Fhit Expression From Normal to HACF to DACF to Adeno Ca with a Reverse Pattern for Ki67, Suggesting Gradational loss of Fhit Protein in Colorectal Carcinogenesis by a Release Phenomenon Over Cell Proliferation.

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Audit of Lymph Node Retrieval From Colorectal Cancer Specimens at a District General Hospital

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Diligent lymph node retrieval is essential for colorectal cancer specimens as Dukes' stage B cancers have a significantly better prognosis than stage C tumours and they are considered adequately treated by surgery alone and not offered neoadjuvant therapy. Accepted guidance stipulates a minimum number of 12 lymph nodes should be retrieved from a colorectal cancer specimen but many publications demonstrate that average node retrievals are frequently less than this.

We audited lymph node retrieval from colorectal cancer specimens in a busy district general hospital. 245 resections for colorectal cancer were identified. 47% of these cases were Dukes' stage A or B. These figures compare favourably with published figures for node-negative cancers. The median number of lymph nodes retrieved from the 245 specimens was 12 (range 1-39, mean 13). In 122 (50%) specimens less than 12 lymph nodes were identified and in 37 (15%) 6 or fewer lymph nodes were identified. These results may indicate that some Dukes' C cancers are being missed. Although the evidence from the proportion of node-negative cases suggests that our hospital is performing as well as other hospitals with respect to node retrieval, the further breakdown of data shows that it should be doing better and implies this is true of many other institutions as well. The importance of adequate lymph node retrieval cannot be overestimated and we have suggested a number of recommendations with the aim of highlighting the importance of lymph node retrieval and providing strategies for increasing lymph node harvest.

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Proportion of Tumour Cells and Stroma – An Inexpensive but Reliable Predictor of Patient Survival in Colorectal Cancer

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Recent studies indicate that tumour growth is determined by cancer cells themselves as well as by tumour stroma. Although expression studies have shown that stroma-like gene expression patterns are related to cancer progression, tumour components have not been quantified in a large series of colorectal cancer (CRC).

Performing point counting on virtual HE stained slides, we analysed the relative proportion of tumour and stroma in two independent CRC series: CRC-G (n=147, consecutive cases from one German hospital) and CLASICC (n=150, cases from a UK randomised clinical trial). Results were compared with clinicopathological parameters and patient survival. Cut offs for Kaplan-Meier analyses were established using ROC curve analyses.

High tumour cellularity was associated with prolonged survival in CRC-G (p=0.011) and in CLASICC (p=0.033) and high proportion of stroma was related to poor patient survival in CRC-G (p=0.002) and in CLASICC (p=0.015). No significant associations were found with any other clinicopathological parameters.

This is the first study that objectively quantified the morphological tumour components in a large series of CRC. It demonstrates that this inexpensive morphometric method can reproducibly predict patient prognosis in CRC confirming data from a previous much smaller study in CRC. Although, we currently do not fully understand the molecular mechanisms that regulate the proportion of stroma in a tumour, the morphological quantification of tumour components may be a useful marker for patient stratification to therapies targeting tumour stroma.

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A Novel SNP in the 5'UTR of ATP5A1 is Associated with Reduced Gene Expression in Colorectal Cancer Cell Lines

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Introduction

Allelic imbalance (AI) at 18q is a common event in colorectal cancer (CRC). Recently, a loss of function mutation in the gene Atp5a1 has been found to be associated with increased risk of tumour progression in Min (Multiple Intestinal Neoplasia) mice. The human ortholog maps to chromosome 18q and thus we investigated whether ATP5A1 could be a target of 18q allelic loss.

Methods

Eighteen CRC cell lines were studied for ATP5A1 mutation using PCR and High resolution Melting analysis. Samples showing aberrant melting curves on the HRM were sequenced. The expression of ATP5A1 mRNA was investigated using real-time quantitative PCR.

Results

HRM analysis identified several previously described SNPs but loss of function somatic mutations were not found within ATP5A1. A novel A to G transition was discovered in exon 1. This change is located in the 5'UTR of ATP5A1 and is present in LOVO/HCT116 (heterozygous) and RKO/SW480/SW620 (homozygous). All cell lines expressed ATP5A1 mRNA and further analysis demonstrated that cell lines carrying this change expressed lower levels of ATP5A1 mRNA than those with the wild type sequence (p = 0.05).

Conclusion

Somatic mutations of ATP5A1 were not found in this study. The identical nature of the sequence change in the 5'UTR in 5 different cell lines suggest that this is more likely a novel SNP than a somatic change. The lower level of gene expression associated with this change is of uncertain significance but may give an increased risk of tumour progression.

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Routine Staining of Gastrointestinal Biopsies in the United Kingdom

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A survey was conducted into the routine staining of gastrointestinal biopsies in histopathology departments within the NHS. We aimed to compare the sole use of HE staining with the use of HE combined with special stains according to the biopsy site. This project has been endorsed by the Pathology Section of the British Society of Gastroenterologists.

167 histopathology departments in the United Kingdom were contacted using an email questionnaire. The following question was asked: Which stains are routinely used in your department when staining biopsies from the oesophagus, stomach, duodenum, small bowel and large bowel?

Valid return rate was 92 of 167 departments (55%). Approximately two thirds of departments only employ HE for assessment of oesophageal biopsies. The distribution with regard to staining of gastric biopsies was approximately equal with 50% HE-only to 50% HE combined with special stains (mostly AB-PAS and Giemsa). Duodenal, small and large bowel biopsies are mostly stained with HE-only. No significant difference could be identified between the use of special stains in district general hospitals as opposed to university, teaching or expert centres.

The results of the survey show routine use of special stains to be variable, especially for oesophageal and gastric biopsies. A review of the literature has shown special stains to enhance sensitivity and specificity for the detection of pathological abnormalities, especially metaplasia and infections. The diversity of routine staining practice within the NHS highlights the need to discuss guidelines. A consensus on best practice is desirable.

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Perforated Amoebic Colitis Complicating Cortico-steroid and Chemotherapy for Metastatic Adenocarcinoma of Unknown Origin

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Fulminant amoebic colitis is a rare form of amoebiasis in which the trophozoites of *Entamoeba histolytica* invade the colonic wall resulting in massive necrosis of wide segments of large bowel. It begins as a mucosal ulcer, which expands within the submucosa.

Fulminant amoebic colitis is associated with a high mortality despite aggressive treatment with anti-microbial agents. It has been reported in patients with asymptomatic amoebiasis who are given corticosteroid for another indication and should be considered in the differential diagnosis of patients who develop fever and diarrhoea after starting such therapy. In fact, corticosteroid use is the one factor that predicted the likelihood of multi-organ failure in patients with fulminant amoebic colitis. Despite a high incidence of colonic perforation (some series reporting incidences as high as 87%, the majority of these having multiple perforations), the finding of a soft compressible abdomen is present in as many as 65% of cases of fulminant amoebic colitis. Early laparotomy may decrease mortality from nearly 100% to 40%. Demonstration of erythrophagocytic amoebae in the stool by microscopic examination is 100% specific for invasive amoebiasis.

We hereby present a case that developed fulminant amoebic colitis and colonic perforation after receiving corticosteroid and chemotherapy for metastatic adenocarcinoma of unknown origin.

Colovesical Fistula - Complicated Diverticular Disease or Invasive Disease?

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A colovesical fistula is an abnormal communication between the bladder and colon. Initially described by Cripps in 1888, is a well recognized complication of diverticulitis. However the exclusion of coexisting malignant disease may present a diagnostic challenge.

A 69 year old gentleman presented with recurrent urinary tract infections, pneumaturia and faecaluria. CT demonstrated thickened sigmoid colon with associated diverticular disease. A 6cm air containing diverticular abscess extended from the sigmoid colon onto the fundus of the bladder. Air in the bladder confirmed fistulation. Subsequent colonoscopy revealed a large pedunculated polyp, confirmed on histology as a tubular adenoma.

Anterior resection, partial cystectomy and reimplantation of left ureter was performed to resect the large sigmoid mass fistulating into the bladder and confirm histology. A 19cm section of large bowel was excised containing a 3.5cm stricture connected anteriorly to inflamed bladder mucosa. Serial sectioning of the stricture showed bowel wall effacement by a circumferential soft, white lesion associated with prominent fibrosis raising the concern of malignant disease. Interestingly intra-operative frozen sections confirmed benign disease.

Histological appearance of the resected specimen demonstrated a moderately differentiated, infiltrative adenocarcinoma arising from the colonic mucosa invading through muscularis propria into the pericolic connective tissue with an inflamed fistula tract (pT4). The tumour was accompanied by florid active chronic inflammation with abscess formation and foci of granulomatous inflammation.

This case highlights diagnostic challenges associated with the diagnosis and management of colovesical fistulation and importance of exclusion of malignant disease in the presence of complicated diverticular disease.

Pathological Outcomes of Neoadjuvant Chemoradiotherapy for Locally Advanced Rectal Cancer at Oldchurch and Harold Wood Hospitals. A retrospective study.

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Aim: To assess the effectiveness of neoadjuvant treatment for locally advanced rectal cancer at our institution, we present the pathological outcomes from a cohort of 43 patients that received neoadjuvant chemoradiotherapy prior to total mesorectal excision.

Methods: Patients with locally advanced, biopsy proven rectal carcinoma treated with capecitabine and long course radiotherapy prior to TME, between 2001-2006 were identified. Pre-treatment stage and tumour position as assessed by pelvic MRI were collated. Resection specimens were reviewed and tumour type, yp-stage and status of resection margins were recorded. The pre and post-treatment T-stages were compared and extent of tumour regression in resection specimens assessed (Dworak regression grading).

Results: Forty-two patients had adenocarcinoma and one patient had cloacogenic carcinoma. At presentation 42 patients had T3 disease and 1 patient had T2 disease with clinically involved regional nodes. Surgical outcomes are summarized in Table 1. Sixteen patients were down-staged, 6 were sterilised of disease and 4 had minimal residual disease, Table 2.

Discussion: The frequency of down-staging was less than that achieved in other studies (37% v 47% Gavioli et al and 47.6% Yoon et al 2007). Sterilised disease (14%) and minimal residual disease (9%) were also marginally lower. These differences more likely reflect the smaller size of the study population in this cohort (a typical workload of a district general hospital in the U.K) than variations in the regimens used. Significant down-staging and sterilised disease are associated with improved long-term outcomes; however, there remains a small but significant risk of distant metastasis in patients with minimal residual disease.

Table: 1

Surgery	Number	R0	R1	R2
Hartman's Procedure	1 (2%)	1	0	0
Anterior Resection	27 (63%)	25	2	0
Abdominoperineal Resection	15 (35%)	14	0	1

Table: 2

Change in T stage	Number of cases (%)	Dworak Tumour Regression			
		1	2	3	4
T3-T4	2 (5%)	1	1	0	0
T3-T3	25 (58%)	19	4	2	0
T3-T2	8 (19%)	4	3	1	0
T3-T1	2 (5%)	1	0	1	0
T3-T0	5 (12%)	0	0	0	5
T2-T2	0	0	0	0	0
T2-T1	0	0	0	0	0
T2-T0	1 (2%)	0	0	0	1

Sterilised disease= Dworak 4, Minimal residual disease= Dworak 3

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Tumour Cellularity in Rectal Cancer with and without Neoadjuvant Therapy – What is The Relationship?

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The RCPATH Colorectal Cancer minimum data set requires categorisation of the histological response to treatment into 'no residual tumour cells', 'minimal residual tumour' or 'no marked regression'. Terms such as 'minimal' and 'marked' seem to be highly subjective. The aim of our study was to establish whether the tumour composition differs in rectal cancer (RC) with and without neoadjuvant therapy and whether the morphometrically measured minimum tumour proportion of the surgery alone group could potentially be a more objective and better reproducible marker to identify treatment responders.

The proportion of tumour cells (Tu) was quantified by point counting using virtual H&E stained slides in 2 independent RC series: A (n = 45, from a single hospital, treated by surgery alone) and B (n = 27, from multiple centres, treated by chemoradiation before surgery). Each series was scored by two independent observers.

Establishing tumour components by point counting proved to be reproducible between observers. Tu was significantly different between series A (median: 55%, range: 22 to 76%) and B (5%, 0-38%), p<0.001. 88% of RC's from series B were considered 'responders' as their Tu was below 22%, the minimum Tu of series A.

The findings from this pilot study warrant validation in a larger post-treatment series. Further studies are needed to investigate whether the suggested threshold of 20% tumour cells in the post-treatment specimen is a suitable and better reproducible marker to predict patient survival compared to 'minimal residual tumour'.

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Audit of Colorectal Cancers: The Aberdeen Experience

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Introduction: The Royal College of Pathologists outlined a set of audit standards in their recently published "Standards and datasets for colorectal cancer" for reporting colorectal cancers. Subsequently, we audited a large series of colorectal cancer specimens resected at Aberdeen Royal Infirmary which is a regional cancer centre. It has also been a pilot centre for the national bowel screening programme. **Methods:** All colorectal cancers (n=741) resected during the three year period (2005-2007) were audited against the standards recommended by The Royal College of Pathologists. The audit parameters are (1) The mean number of lymph nodes examined is 12. (2) The frequency of serosal involvement is at least 20% for colonic cancers. (3) The frequency of extramural venous invasion is at least 25%. The core colorectal cancer histopathology data items were extracted from the histopathology reports and entered into a database. The data was analysed using SPSS. **Results:** (1) The mean number of lymph nodes examined was 15, the median being 14. A minimum of 12 lymph nodes were retrieved in 72% of cases. (2) The frequency of serosal involvement was 26%. (3) The frequency of extramural venous invasion was 23%. **Conclusions:** This audit of colorectal cancers shows that there is evidence of good lymph node yield at our centre and also the rates of serosal involvement and extramural venous invasion are comparable to the standards proposed by The Royal College of Pathologists.

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Give GIST a miss

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Spindle cell tumours in the upper GI tract are not uncommon and their histogenesis is usually evident by performing immunohistochemistry which almost always includes C-Kit. We present two cases of spindle cell lesions in the upper GI tract with unusual immunohistochemistry.

The first case was a 45 year old man who presented with multiple plaque like lesions in the duodenum with ulcerative oesophagitis. Biopsies showed a spindle cell lesion in the duodenum and a poorly differentiated neoplasm in the oesophagus. Immunomarkers (S100, SMA, desmin, MNF116, C-Kit) were all negative in the spindle cell lesion. The oesophageal neoplasm showed positive staining with VS38c and CD45 markers consistent with a plasmablastic lymphoma. This alerted us to the possibility of Kaposi's Sarcoma in the duodenum which was confirmed by immunohistochemistry with vascular (CD31, CD34, Factor8) and KSHV-1 markers.

The second case was a 54 year old man who developed a gastric polyp while on a trial drug (Sunitinib) for metastatic renal cell carcinoma. Biopsies from the polyp showed a neoplastic spindle cell polyp. The immunomarkers as in the previous case were all negative. Metastatic renal cell carcinoma was also excluded. A polypectomy was performed and results were identical to the first instance. Tissue molecular studies confirmed a primary gastric neoplasm with no specific molecular differentiation. The polyp was regarded as a primary spindle cell sarcoma, NOS.

Both these cases illustrate the value of lateral thinking in the work-up of spindle cell lesions in the upper GI tract.

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Adenocarcinoma of the anal gland(s) – a twist to the usual fare

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Adenocarcinoma of the anal glands is distinctly uncommon. It accounts for only 3-10% of tumours at this site, as basaloid and squamous carcinomas of the anal canal predominate. We present a case of this entity with its distinctive features and review the literature.

Our case was an 83 year old lady who presented with a polypoidal lesion in the anal canal suspected to be a squamous cell carcinoma. Biopsies were performed from the lesion for a histological diagnosis. Microscopy showed features of a high-grade dysplastic villous glandular lesion in the transitional zone with extra-mammary Paget's Disease in the anal squamous mucosa. In-situ glandular dysplasia extended into the deep glandular crypts and deeper levels showed invasion. A diagnosis of adenocarcinoma of the rectum extending into the anal canal with Paget's Disease was considered but an obvious intraluminal tumour was not seen. CK7/20 Immunohistochemistry showed a distinctive lack of CK20 staining and positive staining for CK7 marker only in both the invasive and in-situ dysplastic glandular components. CK7 also highlighted native anal glands and it was evident that the dysplastic process originated from rather than merely extended into the anal crypts. This formed the basis of the diagnosis of the entity of anal gland(s) adenocarcinoma.

The difficulty in identifying the site of origin and the natural conclusion that the carcinoma may be metastatic in the anal gland may result in an erroneous diagnosis of an otherwise rare but an important entity.

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Ciliated foregut cyst of gallbladder with extensive squamous dysplasia

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Ciliated foregut cysts of the gallbladder are rare congenital lesions derived from the embryological foregut. We report a case of a 62-year-old woman who presented with symptoms of acute cholecystitis and had a laparoscopic cholecystectomy. Histology revealed parts of a cyst wall adjacent to the cystic duct. The cyst contained bile concretions and was lined by metaplastic squamous and transitional type epithelium with focal superficial ciliated columnar cells. The metaplastic squamous epithelium showed variable cytological atypia in areas amounting to high grade dysplasia. Within the cyst wall there were seromucinous glands and smaller squamous lined outpouchings with dysplasia. The subepithelial tissues show a marked chronic inflammatory cell infiltrate with lymphoid follicles beneath which there was a well formed smooth muscle layer. The metaplastic squamous epithelium showed variable cytological atypia in areas amounting to high grade dysplasia. There was no evidence of invasive carcinoma. The cyst appeared to focally communicate with the gallbladder which showed features of chronic cholecystitis. Ciliated foregut cysts are rare cysts that, when discovered, deserve careful sampling to identify possible squamous metaplasia, dysplasia or foci of squamous cell carcinoma. As a few cases of squamous cell carcinoma have been described in ciliated foregut cysts, complete surgical removal of the cyst is therefore appropriate.

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Is histology required in resections for diverticular disease?

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The Royal College of Pathologists document "Histopathology and cytopathology of limited or no clinical value" describes an approach to reduce workload related to pressures in consultant staffing. Colonic resections for diverticular disease represent specimens where the pathology is usually straightforward, and while not formally discussed in the College document represents an area in which this approach may be useful. We audited reporting of resections for diverticular disease.

350 colonic resections for diverticular disease from 2003-2007 were identified from the laboratory IT system. Information was collected including the length of resected bowel, number of blocks taken, macroscopic and microscopic findings and any additional pathology.

20 cases showed significant other pathology (e.g. colonic carcinoma, inflammatory bowel disease) that was either clinically known or obvious on macroscopic examination: these cases were excluded from further study. No cases showed unexpected pathology on microscopic evaluation. The mean number of blocks has reduced significantly over the period of study (1.45 in 2003, 7.52 in 2007); the mean length of bowel resected has not changed.

Diverticular disease is usually obvious on macroscopic examination of the bowel, and this study has not identified any cases with unexpected microscopic findings. While the mean amount of work per case has reduced (less blocks taken) there is no evidence that the quality of reports is reduced. There is no evidence to support extensive block-taking in diverticular disease: it may be possible to take minimal or no blocks in cases of otherwise straightforward diverticular disease.

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The Putative Stem Cell Marker CD133 is Variably and Reversibly Expressed in Colorectal Cancer Cell Lines

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Introduction:

New cancer model postulated that only a minority of cells, called "cancer stem cells" (CSC), have the ability to generate new clones. Recently, data have identified CD133 as a marker for CSCs in many tumours including colorectal cancer. We sought to investigate the population size and biological characteristics of CD133+ cells in colon cancer.

Methods

CD133 expression was evaluated by flow cytometry using two different antibodies CD133/1 and CD133/2 to evaluate the size of the CD133+ population. For functional analysis, one cell line was sorted into CD133+/- sub-populations and each sub-population tested for their proliferation and colony formation capacity.

Results

Out of 14 cell lines, two lacked CD133 expression; one showed CD133 expression in 100% of cells and the remaining ones showed 2 - 70% of a CD133+ cell population. Sorted CD133+ cells from the SW480 colon cancer cell line showed a slight but significant increase in proliferative index (36%) and higher colony forming activity (25%) than CD133- cells. PCR analysis revealed that the CD133- cells still contained CD133 mRNA and that after 3 weeks culture, CD133 surface expression could be detected in 18% of the sorted CD133- cells.

Conclusion

Most but not all cell lines have a dichotomous CD133+/- population. CD133- cells show proliferative and colony forming ability and so CD133 is not necessary for this. The presence of CD133 mRNA and unexpected levels of emergent CD133+ populations in long term cultured CD133- cells raises the possibility of re-induction of CD133 in culture.

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The pathological and radiological findings of mesorectal excision specimens after radical pre-operative chemo-radiation therapy: The Harlow experience

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We describe the clinical and pathological findings of 30 of 200 patients with rectal carcinoma treated with pre-operative radical chemo-radiation therapy (CRT) at PAH. Pre-operative MRIs have been carried out before and after CRT with T2 to T4 disease, some with clear evidence of nodal metastasis. Here, we present data to indicate evidence of clinical down staging of disease and in some cases evidence of complete cure. A majority of cases however, have residual disease with a variety of histo-pathological changes, ranging from mucosal ulceration, remnants of pools of mucin, fibrosis and residual disease. This range of histo-pathological changes will be quantified. In addition, EGFR expression in the index biopsies, a previously described predictive marker of response to CRT, will be correlated with tumour down-staging by MRI and pathological assessment.

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Intrarenal multiple and multilocular epidermoid cysts presented as an end stage kidney disease

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This report describes a case of large multiple and multilocular epidermoid cysts affecting the left kidney of 67 old male. The condition is accidentally discovered during investigations for left loin pain complaint. Ultrasonography revealed enlarged left kidney with the picture of hydronephrosis. Grossly, the kidney is distorted by these cysts that were filled by cheesy like material. The histologic picture of an end stage kidney disease was apparent in the compressed renal parenchyma by these cysts that were identical to an epidermoid cyst elsewhere. In conclusion, although of the rarity of an epidermoid cyst of the kidney, it could cause serious kidney damage leading to eventual end stage kidney disease. Epidermoid cyst of the kidney could be presented as hydronephrosis at least for nephrologists and radiologists so, awareness of the occurrence of this cyst would broaden the differential diagnostic categories.

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Three Genetic Developmental Stages of Papillary Renal Cell Tumours: Duplication of Chromosome 1q Marks Fatal Progression

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Clinico-pathological studies suggested that papillary renal cell carcinomas (RCC) have a good prognosis. However, no markers distinguishing between papillary renal cell adenomas (RCA) and RCCs were available. We have now analysed the genetic alterations in 60 papillary renal cell tumours (RCT) with at least 8 years follow-up. Based on the complexity of genetic changes we separated three groups of tumours. In the first group (18 cases), all tumours display a combined trisomy of chromosomes 7 and 17. Tumours of the second group (33 cases) showed the alterations characteristic for the first group and trisomies of chromosome 3q, 8q, 12q, 16q and 20q as well. In addition to the genetic alterations found in the first two groups, the third group of papillary RCCs (9 cases) displayed duplication/gain of chromosome 1q, deletion/loss of 6q, 9p and 14q. Only one of the patients died due to disease in the second group, whereas 7 of the 9 tumours in the third group showed fatal clinical progression. We suggest that the first group of tumours are papillary RCAs, the second group are papillary RCCs and the third group of tumours are papillary RCCs with an aggressive clinical behaviour. As the cellular phenotype of papillary RCTs is variable from case to case and also within a given tumour, the genetic analysis offer a better system to classify the developmental stages of clinical significance.

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Testicular Cancer Reporting Before and After Introduction of the RCPATH Minimum Dataset

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Introduction: Pathological reporting of orchidectomy specimens provides important information affecting treatment and prognosis of testicular cancer. The Royal College of Pathologist's (RCPATH) Minimum Dataset for the reporting of testicular cancer was first published in April 2000. This retrospective audit was performed to examine whether the publication of this dataset has been associated with an improvement in the reporting of dataset items relevant to clinical management of testicular cancer.

Methods: An APEX database search was performed to identify all testicular germ cell tumours reported in our Department in the year 1999 (prior to publication of the dataset) and the year 2006. Inclusion in reports of the following dataset items were audited: tumour type, tumour size, completeness of excision, vascular space invasion, invasion of adjacent structures, ITGCN, British / WHO classification, TNM Stage.

Results: 7 out of 8 of the dataset items were mentioned in over 95% of reports from 2006 (n=25), compared with only 4 out of 8 from 1999 (n=24). The number of Consultants reporting testicular tumours decreased from 6 in 1999 to 3 in 2006, with the number of cases double-reported increasing from 17% (n=4) to 32% (n=8).

Conclusion: Following publication of the original (2000) RCPATH minimum dataset for reporting testicular cancer, there has been an improvement in the number of dataset items recorded in our Pathology reports. During this time there has been a move towards specialist reporting and the number of cases double-reported has also increased.

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Histopathological Evaluation of Residual Tumour in Cystectomy Specimens

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Background: Improved survival rates have been reported in the absence of residual carcinoma in cystectomy specimens. However, there are no formal guidelines specifying the block number required to identify residual tumour not visible on macroscopic examination.

Aims: To assess the proportion of cystectomy specimens with no residual tumour, and the number of blocks examined; also to compare the stage of residual malignancy in cystectomies with the highest stage in previous biopsies.

Methods: Eighty-two cystectomies for primary bladder carcinoma received by Department of Cellular Pathology, Royal Victoria Infirmary in 2005-2006 were identified by SNOMED coding. Data were analysed using Excel.

Results: Fifty-two (64%) cystectomies had residual invasive carcinoma and five (6%) showed carcinoma-in-situ; 11/52 patients with residual invasive tumour were excluded from staging correlation due to missing biopsy data. In thirty-eight (93%) the previous biopsy showed the same (15/41) or lower tumour stage (23/41) than in the cystectomy; in 3 (7%) the biopsy showed tumour one stage higher than in the cystectomy. Twenty-five (30%) cystectomies showed no residual invasive or in-situ tumour. Median block number was 31 (range 13-60).

Conclusion:

- Guidelines specifying the minimum block number needed to confirm presence or absence of residual tumour are required.
- Staging of bladder biopsies was predictive of high stage tumour in the cystectomy in most (93%) cases. Inadequate sampling/sampling errors may explain the few cases (7%), where tumour stage was higher on previous biopsy than in the cystectomy.

P79

Evaluation of survivin expression in testicular tissues of infertile males

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Normal spermatogenesis represents a precisely regulated balance between continuous cell proliferation and apoptosis. Survivin is one of the inhibitors of apoptosis. We aimed to examine the testicular survivin expression in infertile men and to evaluate its possible role in defective spermatogenesis. 55 infertile male patients were included. Testicular biopsies were taken and were divided into 3 groups; normal spermatogenesis (NS), maturation arrest (MA) and sertoli cell only (SCO) groups. Immunohistochemical expression of survivin was detected in all groups but varied in degree of intensity. Only two specimens from SCO group were devoid of survivin expression. Survivin expression was detected in the cytoplasm of not only spermatogenic cells but also sertoli cells and interstitial cells of leydig. Most of patients of NS group (70%) and MA group (75%) showed marked survivin expression. In SCO group, 22% showed negative expression, 67% showed mild expression and 11% showed marked expression. The intensity of survivin expression was significantly higher in NS and MA groups when compared to SCO group ($P=0.0198$ & $P=0.0008$) respectively. However, there was no statistical significant difference in intensity of survivin expression between NS and spermatogenic failure (SCO and MA) ($p=1$). Also, there was no statistical difference between early and late MA ($P=1$).

Conclusion: Survivin expression is not a specific marker for germ cells. Survivin expression is significantly reduced in sertoli cell only patients when compared to patients with maturation arrest or normal spermatogenesis however, survivin expression couldn't correlate with the stage of maturation arrest.

P80

An Audit of The Staining Patterns of P504S, CK34BE12 and p63 in Benign and Malignant Prostatic Glands.

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Aims: To audit and compare staining of prostatic adenocarcinoma with P504S, CK34BE12 and p63 on core biopsies.

Methods: Prostatic cores containing adenocarcinoma from 51 patients were retrospectively reviewed. All had sextant biopsies and 23 patients had >1 positive core. Immunohistochemical stains for p63, CK34BE12 and P504S (AMACR) were performed. Staining was recorded as either positive or negative for CK34BE12 and p63, and either strong, weak or absent luminal or cytoplasmic staining for P504S.

Results: P504S showed cytoplasmic and / or luminal positivity in both benign and malignant glands. Forty-five percent of biopsies showed positivity in benign glands and 90% showed positivity in malignant glands. The sensitivity was 90%, specificity 55%, and positive predictive value 67%. There was some variation in staining pattern between benign and malignant glands with malignant glands more likely to show both luminal and cytoplasmic staining or cytoplasmic staining alone, and benign glands more likely to show luminal staining alone ($p=0.0012 \chi^2$). Cytoplasmic staining was more likely to be 'strong' in malignant glands ($p=0.0134$, Fisher's Exact). There was no significant difference in intensity of luminal staining between benign and malignant glands ($p=0.2300$).

Conclusion: Although the sensitivity of P504S as a marker of prostatic malignancy is 90%, specificity and positive predictive value are poor. There is some variation in intensity and staining pattern between malignant and benign glands but there is considerable overlap. Used alone, P504S has significant limitations and it should rather be used as an adjunct to CK34BE12 and p63.

P81

Urothelial Carcinoma with Rhabdoid Features: A Case Report

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Extrarenal rhabdoid tumours have been described in a variety of primary sites with only rare case reports of urothelial carcinomas with rhabdoid features in the literature.

We describe a rare case of poorly differentiated urothelial carcinoma with rhabdoid features, confirmed on immunohistochemistry. Bladder biopsies from three different sites were received from a large solid mass, all of which showed similar features. The tumour was poorly differentiated invasive urothelial carcinoma infiltrating the deep muscle and composed of high grade pleomorphic nuclei with frequent mitosis. Also present was the rhabdoid component composed of large rhabdoid cells with abundant pink cytoplasm, vesicular nuclei, and a prominent nucleolus in a myxoid background. Rhabdoid cells were discohesive and were present singly, in clusters, or in nests. Immunohistochemical stains for cytokeratin CK7, CK20, CD10, AE1/AE3 showed either dotlike positivity or diffuse cytoplasmic staining in the rhabdoid component of the tumours. The tumour cells with the rhabdoid features showed positivity with desmin and vimentin.

Malignant tumours with rhabdoid features in adults in extrarenal locations are considered to be phenotypic variants and have aggressive outcome. These have been described in all age groups and from multiple sites, including the extremities, brain, liver, mediastinum, orbit, heart, and is now accepted as a discrete entity. The tumours generally have a histological appearance similar to that of renal rhabdoid tumours. Immunohistochemical analysis usually demonstrates reactivity to vimentin, desmin, and keratin. Extrarenal rhabdoid tumours do not demonstrate skeletal muscle components by immunohistochemical analysis.

P82

Tale of Two Urinary Bladder tumours – Myeloid Sarcoma as a Primary manifestation of Acute Myeloid Leukemia and Extranodal Marginal Zone Lymphoma

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Case 1: Myeloid sarcoma (MS) of the lower urinary tract is rare.

We describe a 47-year-old man with pyuria, who underwent TURBT for a suspected bladder tumour and was found to have acute myeloid leukemia. Fragments of ulcerated bladder mucosa with underlying distinct monomorphic population of cells with granular and clear cytoplasm were noted. Immunohistochemistry revealed strong expression of myeloperoxidase and weak IRF4(MUM1) positivity. Expression of transcription factor PU-1 was accompanied by weak CD45. CD3, CD20, CD138, CD34, CD68, IRF8, MNF116 and Cam5.2 were negative. Blood count after one month was WBC-99.0x10⁹/L, with predominant population of leukemic blasts.

Case 2: Malignant lymphoma of the bladder can be classified into 3 groups: 1) Primary lymphoma localized to the bladder; 2) Lymphoma in the bladder as disseminated disease (non-localized lymphoma); 3) Recurrent bladder involvement by lymphoma(secondary lymphoma). Primary extranodal marginal zone lymphoma of MALT type of the urinary bladder is rare and generally has excellent prognosis. We present a 70 yr female with a newly diagnosed bladder tumour comprising of neoplastic lymphoid infiltrate with focal nodular pattern and occasional lymphoid follicles. The focal nodular growth pattern was as a result of colonization of the germinal centres by the neoplastic lymphoid infiltrate.

Immunohistochemistry showed these lymphoid cells to be CD20, CD79a and bcl-10 positive and CD5, CD43, CD10, cyclin D1 negative. Demonstration of light chain restriction was not possible. Bone marrow aspirate and trephine biopsy showed no evidence of lymphoma infiltration.

P83

Adenocarcinoma Arising in an Endocervicosis of the Urinary Bladder

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Endocervicosis is a non-neoplastic lesion exhibiting müllerian differentiation. A malignant tumour arising in endocervicosis has not been reported, except for a case of adenocarcinoma originating from a lesion of the vagina.

Herein, we describe a case of adenocarcinoma arising in endocervicosis of the urinary bladder. The patient is a 58-year-old woman. She had a history of endometriosis, for which a total hysterectomy was performed 16 years previously. Cystoscopy revealed an elevated nodular mass, 4*4 cm in size, in the triangle area. Total cystectomy was performed. The tumour was composed of two different histological components. The dominant component was proliferation of multiple cystic glands lined with cuboidal epithelium. The lining epithelium had clear cytoplasm, which resembled an endocervical gland. The other histological component was proliferation of atypical cells with large nuclei, which infiltrated the surrounding connective tissue. This histological finding was the same as the biopsy specimen taken previously. From these findings, we suggest that this is the first case of adenocarcinoma arising in endocervicosis of the urinary bladder.

To determine the histochemical nature of the mucus of this tumour, we performed various histochemical stainings, including Alcian Blue (AB)-PAS, High iron diamine (HID)-AB. We then compared the results with four control populations (normal cervical gland, normal endometrial gland, adenocarcinoma of cervix, adenocarcinoma of endometrium). It is difficult to differentiate these groups based only on their staining characteristics, but endocervicosis is shown to have a similar histochemical nature to a normal endocervical gland.

P84

High-Resolution SNP-Array Analysis of Chromophobe Renal Cell Carcinomas and Renal Oncocytomas

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Benign renal oncocytomas (RO) and chromophobe renal cell carcinomas (RCC) make up approximately 10% of renal cell tumours. The histological diagnosis remains uncertain in several cases due to their overlapping phenotype.

Previously, we have detected complex losses of whole chromosomes 1, 2, 6, 10, 13, 17 and 21 in 70%-90% of chRCCs, whereas the loss of chromosomes 1 and 14 and the Y chromosome or translocation between chromosome 11q13 and another chromosomes or random genetic changes have been described in ROs. Now, we applied a high density 250 K SNP oligoarray to detect loss of small DNA segments at specific chromosomal regions in chromophobe RCCs and ROs, which may mark the loci of genes involved in the tumorigenesis. We have detected several genetic alterations up to 1Mb in size in both types of tumours, most of them occurring only in a single case. Although, we failed to achieve our goal of identifying tumour suppressor gene loci, we have confirmed that the loss of chromosomes 2, 13, 17 and 21 occurs exclusively in chromophobe RCCs and therefore, the complex loss of these chromosomes can be used for the differential diagnosis in cases with overlapping phenotype. As we used a high density array for analysing an appropriate number of cases, we can exclude the possibility of the presence of recurrent small genetic alterations in these two types of renal cancers.

P85

The role of urine cytology in the diagnosis of bladder cancer at University College London Hospitals during one year

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We have seen an increase in the number of urine specimens for cytological examination in our department derived from a wide range of clinical specialities. We audited the number of urine cytology specimens received over one year (1st December 2006 to 30th November 2007) to determine the ability of a urine examination to diagnosis cancer.

In total we found 1522 urine cytology specimens had been reported during this year. A histology report was found in relation to 216 (24%) specimens. Out of these 216 histology reports, 113 (52%) were biopsies from bladder, prostate, kidney, ureter and urethra (urology) of which 82 (73%) were bladder biopsies. A histological diagnosis of papillary TCC was made in 50 and a TCC (NOS) in 10 reports.

The cytological and histological diagnoses were categorized into three categories; cancer, atypia and no malignancy.

There was a complete agreement in 8 cases of cancer and 22 cases of no malignancy.

In 11 cases atypia was reported cytologically and TCC was reported in histology.

In 41 (50%) samples, no evidence of malignancy on cytology but a carcinoma was diagnosed on histology.

In summary, in our department, a cytological diagnosis of malignancy is indicative of a malignant lesion of urinary bladder but a negative cytology does not exclude bladder carcinoma. It is essential that clinical and radiological data are considered in every case of a negative cytology to triage patients that would require further investigation. In addition, our laboratory exploring ways of increasing sensitivity without compromising the specificity.

P86

Analysis of the Appropriateness of Urine Cytology Specimens

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Urine specimens are commonly sent for cytological examination. There are some valid indications for this such as follow up of previous urothelial neoplasia. Unfortunately, some specimens are sent for inappropriate clinical reasons creating unnecessary work for laboratory staff, cytopathologists and secretarial staff. We conducted an audit of urine cytology specimens received in our department to determine the proportion of cases sent for inappropriate clinical indications with a final aim of producing guidelines outlining the appropriate indications for urine cytology requests.

Twenty two percent of the specimens were sent for follow up of urothelial neoplasia, 39% were sent for haematuria, 15% were sent as "?? malignancy" and 24% were sent for a range of miscellaneous reasons including infective symptoms, chest pain and vomiting. One percent of all the specimens had malignant cytology and 10% had atypical cytology. All of these malignant and atypical cases were sent for follow up of previous neoplasia or as part of the investigation of haematuria.

We conclude that outside of the setting of follow up and haematuria, there is a strong argument that urine specimens should not be routinely sent for cytological examination. If this had been done during this audit period, no cases of neoplasia would have been missed and there would have been an 40% reduction in the number of specimens saving time and money.

P87

Incompletely Differentiated (Unclassified) Sex Cord / Gonadal Stromal Tumour of the Testis with "Pure" Spindle Cell Component: A Case Report

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The group of incompletely differentiated (unclassified) sex cord/gonadal stromal tumours includes rare cases with predominant spindle cell morphology. We report a rare case of a "pure" spindle cell tumour of the testis with morphological and immunohistochemical features consistent with the diagnosis of "incompletely differentiated sex cord / gonadal stromal tumour".

A 39-year old male presented with a lumpy/hard left testis, clinically suspected as a tumour on ultrasound. A left radical orchidectomy was performed. We received a testis with attached spermatic cord containing a 5 mm nodule at the periphery. Microscopically, the nodule was predominantly composed of benign spindle cells arranged in fascicles with no evidence of mitoses or necrosis. These were admixed with steroid cells with epithelioid and signet ring morphology. Immunostaining for S100, inhibin and SMA was positive, a pattern also seen in both adult and juvenile granulosa cell tumours; whereas desmin, CD34, c-kit and cytokeratin was negative. This concurrent presence of some morphological and immunohistochemical features of both steroid and granulosa cell lines in the tumour suggests its origin from a stromal stem cell, possibly capable of dual differentiation, but with an arrest of maturation at an early phase of differentiation.

Unclassified sex cord stromal tumours (SCSTs) of the testis can occur at all ages, but most common in children (30% in < 1 yr). The behavior is mostly benign in prepubertal (<10yrs) children, but these have malignant potential in older individuals. Prognosis depends on the age, size, invasion beyond testis, necrosis, pleomorphism and mitosis.

P88

Seminoma with Exclusive Intertubular Growth: Report of a Clinically and Grossly Inconspicuous Testicular Tumour

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Seminoma with intertubular growth pattern represents a rare but distinct clinicopathological subset of testicular seminomas. Such tumours do not present as mass lesions, and may be discovered during investigation of infertility, pain or metastatic disease. No distinct tumour is grossly apparent in most of these cases. Microscopic identification is difficult, as the tumour cells can be obscured by lymphocytic reaction and hyperplastic Leydig cells.

We present the case of a 43-year old man who had right orchidectomy for chronic pain. On gross examination, testis measured 45x35x25 mm. No tumour was apparent macroscopically. Cut surface showed atrophic testicular tissue.

Microscopic examination revealed mainly atrophic testis, but there was intertubular germ cell neoplasia (ITGCN), with pagetoid spread to the rete testis and epididymis. More interestingly, there were seminoma cells with a rather unusual pattern of intertubular invasion with some of the cells encircling the seminiferous tubules. The seminoma was very subtle and after submitting the entire testis, a small aggregate of seminoma cells was noted. There were also few multifocal dispersed individual tumour cells which were obscured by lymphocytes and hyperplastic Leydig cells.

Despite the inconspicuousness of purely intertubular seminomas, there is evidence that this pattern may represent a more aggressive seminoma variant. The only way to avoid the pitfall of overlooking these cases is to be aware of the possibility of intertubular seminoma whenever a lymphocytic infiltrate is identified in the testis or there are large cells scattered in the interstitium.

P89

Neurofibroma of the uterine cervix, case report and literature review

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Introduction

Neurofibromatosis type 1 (Von Recklinghausen's disease) is an autosomal dominant condition caused by a mutation in the NF1 gene (chromosome 17) and has a prevalence of approximately 1 : 3000. Neurofibromas rarely involve the urogenital organs. There is a 12 - 29% risk of malignant transformation and therefore, long-term follow-up is warranted.

Case report

A 46-year old Caucasian woman referred to the colposcopy clinic with abnormal looking cervix. She was previously diagnosed with type I neurofibromatosis and had undergone 2 previous excisions of neurofibromas from her upper lip. She suffered from a small congenital ventricular septal defect, which was managed conservatively. Her smear history was unremarkable.

Colposcopy revealed a 3-4 cm mass at the left lateral aspect of the cervix with warty features. Two directed punch biopsies were obtained to establish the diagnosis. Histology showed cervical stromal lesion consisting of bland spindle cells with serpiginous nuclei, covered with cervical squamous epithelium. Occasional attempts at Meissner-body formation were noted.

Immunohistochemistry showed the lesion to strongly express S100 protein. A diagnosis of a diffuse neurofibroma of the uterine cervix was made. No further management was suggested to this lady who remained well on follow-up.

Conclusion

The female genital system is rarely affected in neurofibromatosis. The vulva is the most common part of the female genital system to be involved. Few cases of cervical neurofibroma have been described in literature. Despite its rarity, clinicians should consider the possibility of genitourinary involvement in patients with history of neurofibromatosis.

P90

Cervical Cytology Suggestive of Glandular Neoplasia: An Audit of Outcomes

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Seventy-seven cases having a cervical smear result showing abnormal glandular cells between 1st January 2005 and 31st Dec 2006 were identified and matched with the diagnostic histology result.

The number of glandular smears has doubled in Cardiff since introduction of liquid based cytology in 2001. The mean age of women with a glandular abnormality was 39 years. The positive predictive value of a glandular smear was 56%, the negative predictive value 99.8%, sensitivity 0.08% and specificity 99.9%.

Correlation with histology revealed the following results: ten cases (12%) were associated with invasive malignancy; one case of cervical squamous carcinoma, four cases of cervical adenocarcinoma and five cases of endometrial adenocarcinoma. In-situ neoplasia of the cervix was present in 41 (54%) of those with positive smears; fourteen (18%) had cervical squamous intra-epithelial neoplasia (CIN) only and twenty-seven (36%) had cervical glandular intra-epithelial neoplasia (CGIN) +/-CIN. Eleven (14%) patients had negative histology, two were inadequate and thirteen (17%) had other non-neoplastic diagnoses including inflammation, metaplasia and reactive atypia.

The majority of women were asymptomatic (84%). Women who were post-menopausal and symptomatic were more likely to have an endometrial lesion than a glandular abnormality of the cervix.

P91

Immunohistochemical study on cell cycle related proteins (PTEN, p27, p53, cyclin D1, cyclin E) of ovarian cancers

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The PI3K (phosphatidylinositol 3-kinase) / pAkt pathway is an important regulator of cell cycle progression and cell survival. PTEN (phosphatase and tensin homologue deleted on chromosome 10) with phosphatase activity regulates this PI3K / pAkt pathway, thereby functioning as a tumour suppressor. This study was conducted to clarify the significance of PTEN and its down-stream proteins, such as p27, p53, cyclin D1 and cyclin E, that relate to the cell cycle, with a special focus on the relationship among these proteins in ovarian carcinomas. We examined immunohistochemical expression in 64 cases of primary ovarian carcinomas comprised of 17 serous, 8 mucinous, 21 endometrioid, and 18 clear cell carcinomas. Immunohistochemical data were analyzed with a co-relation of histologic subtypes, nuclear grades and clinical stages. Significant differences of PTEN expression were identified in different histological subtypes. Percentage of positive staining of PTEN was lower in endometrioid carcinoma (38.1%) than those of other histologic subtypes (55.6-82.4%). PTEN expression did not correlate statistically with expression of p27, p53, cyclin D1 and cyclin E in Fisher's exact probability test. In all histologic subtypes of ovarian carcinoma except for clear cell carcinoma, high-level cyclin D1 expression was more frequently found in stage I, II tumours than in stage III, IV tumours ($p < 0.01$), and grade 1 tumours than grade 2, 3 tumours ($p < 0.05$). The results suggest that activation of cyclin D1 might be an early event in ovarian carcinogenesis.

P92

Primary Mucinous Carcinoid, a rare entity and a diagnostic dilemma

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A previously well 34 year old woman presented with a large pelvic mass, found to be ovarian at laparoscopy. The resected ovarian mass was composed of small well formed acini with focal goblet cells, mucin containing signet ring cells that focally expressed chromogranin and contained sub nuclear argyrophil granules. A small epidermoid cyst was seen adjacent to the tumour. The case was diagnosed as a primary mucinous carcinoid of ovary and with a high mitotic rate (up to 6/10hpf), it was regarded as an atypical carcinoid. The other ovary was also involved. The tumour first recurred as a pelvic mass 3 years later and after another 2 years in the form of ileal and sigmoid metastasis. These cases present challenges for diagnosis and prediction outcome having an immunoprofile that overlaps with their gastrointestinal counterpart.

Reference:

Baker PM, Oliva E, Young R H et al. 2001 Ovarian mucinous carcinoids including some with a carcinomatous component – A report of 17 cases. *Am J Surg Pathol* 25:557-568

P93

Comparison of Mucin and CD10 expression among ovarian serous adenocarcinoma, clear cell adenocarcinoma, endometrioid adenocarcinoma, mucinous adenoma, mucinous borderline tumour, and mucinous adenocarcinoma

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The aim of this study is to evaluate the immunohistochemical expression of mucin and CD10 in ovarian serous adenocarcinoma (SC), clear cell adenocarcinoma (CC), endometrioid adenocarcinoma (EC), mucinous adenoma (MA), mucinous borderline tumour (MB), and mucinous adenocarcinoma (MC), and to analyze the relationship between prognosis and these expressions. Method: Formalin-fixed paraffin-embedded tissue sections from 35 cases of SC, 45 cases of CC, 25 cases of EC, and 26 cases of MC were immunostained using antibodies for MUC1, MUC2, MUC4, MUC5AC, MUC6, and CD10. The staining results were evaluated according to the proportion of positive cells. Results: MUC1 was more frequently expressed in EC, CC and SC than MC. MUC2, MUC5AC, and MUC6 were mainly expressed in MC and EC. In contrast, SC and CC revealed negative or low expression for MUC2, MUC4, MUC5AC, MUC6, and CD10. MC revealed more frequent expression for MUC2 and MUC5AC than the other histological types. MUC1, MUC2, and CD10 were increased from MA to MC. MUC5AC were decreased from MA to MC. Low proportion of positive cells for MUC2 and MUC4 in MC, high proportion of positive cells for MUC1 in EC, and CD10-negative SC showed better long-term survival rate.

Conclusions: These results suggest that the difference in mucin and CD10 expression among ovarian carcinomas may be useful for the prediction of clinical outcome. In mucinous tumour, the expression patterns of MUC2, MUC5AC, and CD10 suggest that there is close association between carcinogenesis and intestinal metaplasia in ovarian mucinous tumours.

P94

The Origin of Gliomatosis Peritonei

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Gliomatosis Peritonei (GP), Characterised by Peritoneal and Omental Glial Implants (GI), Occurs Almost Exclusively in Association With Ovarian Teratomas (OTs). Some Investigators Have Suggested That GI Originates Through Capsular Rupture of the Teratoma With Subsequent Implantation, or Via Angiolymphatic Spread. Others Advocate That GP Arises From Glial Differentiation of Pluripotent Peritoneal Stem Cells. In Contrast to Normal Tissue DNA Which Contains Maternal and Paternal Genetic Material, and Thus is Heterozygous at Many at Polymorphic Microsatellite Loci (PML), OTs Often Contain a Duplicated Set of Maternal Chromosomes, Demonstrating Homozygosity of Alleles.

Twelve OTs Associated With GP Were Identified. DNA Was Extracted From Paraffin-Embedded Tumour Tissue, and Polymerase Chain Reaction (PCR) Was Used to Determine Their Genetic Pattern at Various PML. Eight Teratomas Arose Post-Meiosis I, Displaying a Predominantly Heterozygous Microsatellite Pattern. These Cases, Considered Non-Informative, Were Excluded From Further Investigation. Four Teratomas Arose Pre-Meiosis I, Showing a Predominantly Homozygous Microsatellite Pattern. In These Informative Cases, DNA Was Extracted From Paraffin-Embedded Matched Normal Tissue and GI, and Analysed Using PCR. In Two Cases, the Matched Normal Tissue and Associated GI Demonstrated a Predominantly Heterozygous Microsatellite Pattern in Contrast to the OT. DNA Analysis of the Other Two Cases is Being Performed.

Thus, Our Preliminary Results Support the Theory That GP is Genetically Unrelated to the OT, Most Likely Arising From Pluripotential Cells Within the Peritoneum. Factors Responsible For Glial Differentiation of Peritoneal Stem Cells in This Setting Are Poorly Established, Being an Important Area of Future Investigation.

P95

Ovarian Neuroblastoma Arising Within a Mature Cystic Teratoma: A Rare Pathological Entity

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Mature cystic teratomata (MCT) are common ovarian neoplasms which undergo malignant transformation in 1-2% of cases with squamous cell carcinomas accounting for the vast majority. Malignant neural tumours arising from MCT are exceptionally rare. Here we describe a case of neuroblastoma developing within an ovarian teratoma in a 30 year old female.

Macroscopically the specimen was received from theatre in pieces containing part of a cyst wall lined by hair bearing skin. Solid areas were identified showing a variegated appearance with firm white tumour and foci of haemorrhage.

Histologically the cyst was lined by stratified squamous epithelium with appendiceal structures, adipose tissue and mature neural elements. The solid areas consisted of sheets and nests of small pleomorphic blue cells, neurofibrillary stroma, focal Homer-Wright rosettes and multifocal anaplasia with bizarre uninucleated and multinucleated giant cells. No ganglion cell differentiation was identified.

Immunohistochemistry showed these cells to be neuroblastic in origin being positive for CD56, synaptophysin and NB84. The tumour was classified according to the International Neuroblastoma Pathology Classification (INPC) as stroma poor with an intermediate MKI (mitotic-karyorrhectic index). Fluorescent in-situ hybridization was carried out on paraffin sections and showed N-myc amplification and a relative 17q gain in the neuroblastic tumour.

Only a handful of cases of neuroblastoma arising in ovarian MCT have been described to date and all previous reports were in patients under 20 years of age. According to the age-linked INPC, this tumour is thought to carry an unfavourable prognosis.

P96

Morules in endometrioid proliferations of the uterus and ovary consistently express the intestinal transcription factor CDX2

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Aims: To undertake an immunohistochemical analysis of squamous elements in endometrioid proliferations of the uterus and ovary and to compare the immunophenotype of typical squamous elements and so-called squamous morules.

Methods+Results: Cases of uterine or ovarian endometrioid glandular lesions with squamous elements were stained with CDX2, β catenin, ER, CD10, p63 and highmolecularweight cytokeratin LP34. Thirteen cases had typical squamous elements and 18 cases morules. Morules typically exhibited diffuse nuclear CDX2 and β catenin immunoreactivity and were positive with CD10 and LP34. They were usually ER and p63 negative. In contrast, typical squamous elements were usually positive with ER, CD10, p63 and LP34. They were usually CDX2 negative or focally positive and exhibited no nuclear staining with β catenin. Ten endometrioid carcinomas not exhibiting squamous differentiation were stained with CDX2; one was focally positive. Electron microscopy in two ovarian endometrioid adenocarcinomas with extensive morular differentiation showed that the morules exhibited epithelial features but no overt evidence of squamous differentiation.

Conclusions: Typical squamous elements and morules have an overlapping but differing immunophenotype. Morules exhibit no firm immunohistochemical or ultrastructural evidence of squamous differentiation, although immature squamous differentiation cannot be excluded. Nuclear β catenin positivity is in keeping with the observation that endometrioid glandular lesions with morules are often associated with β catenin gene mutation. The explanation for diffuse nuclear positivity with the intestinal transcription factor CDX2 in morules is not clear but may be a result of overexpression of nuclear β catenin. We suggest that the term morular metaplasia is used instead of squamous morules.

P97

Clear Cell Carcinomas of the Female Genital Tract- A Clinicopathological Analysis of 73 Cases

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Clear cell carcinomas (CCC) arise from the female genital tract and from the peritoneum. Studies indicate that they lack oestrogen receptors. The genetic association for these tumours have been described, and various associations have been proposed ranging from BRCA1, p53 to HER2-neu. These tumours in particular have a distinct molecular signature with simple hierarchical clustering. We have retrieved 73 consecutive cases from archives of Royal Surrey County Hospital, Guildford from 1997 to present. Surgical pathology reports and original histology slides have been retrieved. The aim of this study is to update our knowledge of these tumours in view of gene expression profile data suggesting a "distinct" molecular signature of clear cell carcinoma. Immunohistochemical staining for oestrogen receptors, both alpha and beta, androgen receptors, Her-2 protein (4B5) and Her2 gene, MIB-1, p53, CD 10 and p16 are being performed at Royal Surrey County Hospital. Amplification by silver in situ hybridisation (SISH) techniques is also in progress.

P98

Increased p16 Expression in High Grade Serous Carcinoma Compared to Other Morphological Types of Ovarian Carcinoma

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It has been previously shown that p16 is overexpressed in high grade serous carcinoma but there has been little detailed comparison of p16 expression in common types of ovarian carcinoma. This study aimed to compare p16 expression in ovarian carcinomas of serous, endometrioid, clear cell and mucinous type and ascertain whether high expression in a primary ovarian carcinoma is specific for a serous neoplasm. Problematic cases which are difficult to type, such as poorly differentiated and undifferentiated carcinomas and serous carcinomas with clear cells were also included. In these problematic groups, p16 expression was compared with that of WT1, which is known to be relatively specific for serous phenotype. Cases of ovarian high grade serous (n=38), endometrioid (n=15), clear cell (n=12) and mucinous carcinomas (n=10) were stained with p16. Cases were scored with respect to distribution of immunoreactivity and intensity, and an immunohistochemical composite score calculated. Serous carcinomas typically exhibited high p16 expression; there was statistically significant higher p16 expression in serous carcinomas compared to other morphological types. High p16 and WT1 expression was identified in undifferentiated carcinomas and in serous carcinomas with clear cells, suggesting that these represent variants of serous carcinoma. We have demonstrated that p16 is highly expressed in high grade serous carcinomas compared to other morphological types of ovarian carcinoma. This may be useful, in conjunction with WT1, in the diagnosis of problematic cases. p16 may be involved in the pathogenesis of high grade ovarian serous carcinomas, through inactivation of retinoblastoma protein.

P99

Cystic Monodermal Teratoma of Neurogenic Type

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We report a case of a 28 years old lady, found to have a left ovarian cyst which was removed laproscopically. The specimen was received as two small fragments of a cyst wall of 3mm thickness. The microscopic appearance was that of a cyst wall lined by flattened, or cuboid columnar ciliated epithelium which form simple papillary structures in areas. The underlying stroma was poorly cellular, delicate and fibrillary in nature. The lining epithelium and stroma were strongly GFAP positive and WT1 negative. No atypical or immature neural elements were present and there was no evidence of malignancy. The features are consistent with a benign cystic monodermal teratoma of neurogenic type and this is a rare case with only two reports in the literature in the last 20 years. The appearances of ciliated epithelial lining and the papillary choroid structures can easily be misdiagnosed as a benign or borderline serous cyst if the underlying fibrillary stroma is not appreciated.

Reference:

International Journal of Gynecology Pathology 1990,9:283-290

Histopathology Journal 1994, 24 : 477-480

P101

Regulation of MUC16 in Ovarian Cancer by Micro RNA

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CA125 is a tumour antigen used to monitor progression and response to therapy in epithelial ovarian cancer, and is encoded by the MUC16 gene. Micro RNA's (miRNA) are short non-coding RNA strands that can regulate gene expression through regulation of the stability or translation of mRNA. We investigated the possible regulation of MUC16 in ovarian cancer cell lines by four miRNAs predicted to target this gene.

Relative quantification real time PCR was performed on mRNA extracted from 22 primary ovarian tumour cell lines grown from ascitic fluid samples taken from 17 ovarian cancer patients. The established ovarian tumour cell line OVCA433 was used as a calibrator. The levels of miR-92, miR-193a, miR-452 and miR-651 miRNAs and MUC16 mRNA were measured relative to a standard internal control, β -actin. MUC16 mRNA levels were found to vary by three orders of magnitude between the 22 cell lines, compared to a variation of only 30-fold in the levels of CA125 found in the serum of the 17 patients at the time of ascitic fluid removal. We found very high (> 0.8) or high (> 0.6) positive correlations of 0.869, 0.773, 0.713 and 0.690 when MUC16 mRNA levels were compared to miR-92, miR-453, miR-193a and miR-651 levels respectively. These observations are consistent with the negative regulation of MUC16 mRNA translation, rather than stability, by each of four miRNAs predicted to target this gene transcript.

P100

A Case of Cervical Squamous Cell Carcinoma and Schistosomiasis

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We report the case of a 26 year old female who presented with mild dyskaryosis on a cervical smear. At colposcopy examination, she was thought to have high grade cervical intraepithelial neoplasia (CIN) and a cervical biopsy was performed. The biopsy showed CIN II, CIN III and spherical bodies with terminal spines consistent with *Schistosoma haematobium* eggs. A LLETZ was performed and this showed high grade CIN, well-differentiated invasive squamous cell carcinoma and further schistosome eggs.

When schistosomiasis affects the female genital tract, it is most commonly seen in the cervix and is usually *Schistosoma haematobium*. The presence of *Schistosoma haematobium* in the bladder is known to be associated with the development of squamous cell carcinoma due to long-standing chronic inflammation and it is feasible that it plays a similar role in the cervix. We review the published literature looking at the role schistosomiasis of the cervix might play in the development of cervical squamous cell carcinoma.

P102

The Diagnostic Utility of PCR in a Specialist Haematopathology Unit

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Molecular analysis of tissue using the polymerase chain reaction (PCR) is an important adjunct to diagnosis in haematopathology. PCR is used for the demonstrating T or B cell clonality and for detecting specific chromosomal translocations. A PCR technique is also available for the detection of tuberculosis (TB). The diagnostic contribution of PCR in our department was assessed by a twelve-month retrospective analysis of its use.

Our haematopathology unit receives around 2300 biopsies annually. In 2006 PCR was used on 118 biopsies (5%) and on 15 cytology specimens. In 19 cases (14%) PCR could not be assessed due to insufficient or poor quality DNA; this was seen most frequently in skin biopsies suspicious for mycosis fungoides (7 cases). Where DNA was successfully extracted, PCR was performed for suspected lymphoma in 92 cases (81%) and for TB in 22 cases (19%).

In no case did TB PCR yield a positive result. In suspected lymphoma PCR made an important contribution to the final diagnosis in 84 cases (91%), and amended an initial diagnosis in 7 cases (8%). In 13 cases (14%) PCR did not corroborate the provisional diagnosis, and was deemed non-contributory.

Our results show that PCR is a valuable tool in lymphoma diagnosis, but is less helpful in identifying TB or confirming the diagnosis of mycosis fungoides in skin biopsies, where the DNA extraction can be problematic. These findings may be useful in planning the appropriate use of PCR in haematopathology units.

P103

Biopsy Pathology in HIV in the Era of HAART

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The introduction of highly active anti-retroviral therapy (HAART) has markedly improved patient survival in human immunodeficiency virus (HIV) infection. HAART prevents many opportunistic infections, but with increased survival times patients can go on to develop a range of other pathological conditions. To assess these conditions, we have examined the spectrum of disease seen in recent tissue biopsies from patients with HIV.

In 2006 we received 151 biopsies from HIV positive patients. The most common specimens (50%) were haematological in nature, and included 41 bone marrow trephines (27%), 29 needle core biopsies of lymph node (19%) and 21 whole lymph node specimens (14%). 25 biopsies (17%) were from the GI tract, and the remainder were from a variety of sites including skin, salivary gland, chest wall, breast and nasopharynx.

Lymphoproliferative disease was the commonest diagnosis, and was found in 22% of biopsies. Diagnoses in this category included classical Hodgkin lymphoma, diffuse large B cell lymphoma, plasma cell Castleman's disease, Burkitt lymphoma and primary effusion lymphoma. In 20% of the biopsies (mainly in bone marrows) there were non-specific HIV related changes, and in 15% of the specimens the histology was non-diagnostic. Opportunistic infections were seen in only 9 cases (6%).

Our findings suggest that the most likely positive finding in biopsy specimens from HIV patients is lymphoproliferative disease, and that infectious disease is now relatively rare. This should be taken into account when determining reporting strategies for biopsies from HIV positive patients.

P104

Atypical Morphology in Plasma Cell Myeloma

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Plasma cell myeloma (PCM) represents 15% of all haematological malignancies. PCM is a bone marrow-based multifocal plasma cell neoplasm characterised by a serum monoclonal protein and skeletal destruction with osteolytic lesions, pathological fractures, hypercalcaemia and anaemia. The disease results from the expansion of single clone of immunoglobulin secreting, terminally differentiated end stage B cells (plasma cells). Histologically plasma cells characteristically show eccentric nuclei, "clock face" nuclear chromatin and abundant basophilic cytoplasm with focal perinuclear clearing (hof). We present a series of 6 cases of PCM where the plasma cells lacked this typical morphology, so that the diagnosis depended on the clinical history supplied and the immunohistochemical findings.

In 2007 we received 220 biopsies with a diagnosis of PCM. Whilst the majority showed characteristic plasma cells, we identified 6 cases (2.7%) that showed atypical morphology. In these cases the neoplastic cells variously resembled mast cells, histiocytic/monocytic cells, hairy cells, or melanoma. One case showed pleomorphic bizarre cells suggestive of a poorly differentiated carcinoma. Immunohistochemistry revealed positive CD138 staining and immunoglobulin light chain restriction in all cases, confirming the diagnosis of PCM. A history of previous myeloma was present in four of the cases, raising the possibility that the atypical features were treatment-induced.

Pathologists should be aware that neoplastic plasma cells can show an abnormal histological appearance, and where there is a history or clinical suspicion of PCM should always include CD138 and light chains in their immunohistochemistry panel.

P105

Rapid Reporting of Bone Marrow Trephine Biopsies – an Assessment of the Effects on Diagnostic Accuracy

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A departmental audit in 2006 identified a turnaround time (TAT) for bone marrow trephine biopsies (BMT) of 7 days. Streamlining fixation and decalcification and the use of a rapid processor reduced the technical time, but waiting for immunohistochemistry (IHC) remained a source of delay. At the clinicians' request we instituted a new BMT reporting protocol whereby the Consultant issued a rapid initial report based solely on the H&E morphology. A supplementary report with the IHC findings was added later. These measures reduced the TAT to 2.5 days.

To assess the diagnostic effects of this strategy, all cases subject to rapid reporting between October 2006 and October 2007 were retrieved and the initial and supplementary reports compared.

Over the year 73 cases were reported under this protocol, and 61 (84%) had IHC. The initial reports were classed as diagnostically useful in 67 cases (92%); the remaining 6 reports (8%) were purely descriptive. In 12/67 cases (14%) a firm diagnosis was made on morphology alone. In the remaining 55 biopsies, IHC confirmed the morphological diagnosis in 44 cases (80%), added information in 2 cases (4%), and in 9 cases (16%) resulted in significant change to the initial diagnosis.

Overall approximately 20% of the rapid initial reports were diagnostically unhelpful, either being purely descriptive (6/73, 8%) or later turning out to be inaccurate (9/73, 12%). We and the clinicians now need to decide if this level of diagnostic inaccuracy is acceptable in order to produce faster turnaround times.

P106

An Unusual Case of Granulomatous Lymphadenitis

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We describe an unusual case of a 61 year old man who initially presented with axillary lymphadenopathy, which was reported as granulomatous lymphadenitis (special stains for micro-organisms and a lymphoma panel were negative). Approximately one year later the patient presented with lethargy, abdominal pain, weight loss and subcutaneous nodules over his trunk and limbs. CT scan revealed persistent axillary lymphadenopathy and also splenomegaly.

A second lymph node biopsy was performed and again histology showed granulomatous lymphadenitis (special stains and a lymphoma panel were negative). However, a biopsy of one of the subcutaneous skin nodules revealed a heavy dermal infiltrate of immature and atypical myeloid cells, which was confirmed by immunohistochemistry. Further immunohistochemistry was performed on the lymph node and this highlighted a heavy infiltrate of atypical myeloid cells that was not initially obvious on the H&E sections. A diagnosis of a myeloid neoplasm was then suggested and a recommendation made to exclude acute myeloid leukaemia (AML). Bone marrow aspiration and trephine were performed and the appearances were consistent with a myelodysplastic syndrome and acute myeloid leukaemia transformation.

Granulomatous inflammation can be associated with Hodgkin's lymphoma and some T cell lymphomas. This case describes an unusual presentation of AML involving axillary lymph nodes which presented as granulomatous lymphadenitis.

P107

Type and maturational status of dendritic cells in cutaneous B-cell lymphoproliferative disorders

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Emerging evidence suggests that cutaneous B-cell lymphoproliferations represent a continuous spectrum of disease, with polyclonal B-cell cutaneous lymphoid hyperplasia (BCLH) at one end and monoclonal cutaneous marginal zone lymphoma (CMZL) at the other. We believe it probable that dendritic cells (DCs) play a central role in the genesis and evolution of such cutaneous lymphoproliferations. As a preliminary step towards testing this hypothesis, we sought to compare the number, subtype and maturational status of DCs in biopsy specimens showing features of BCLH and CMZL.

Immunohistochemistry was used to identify Langerhan's cells (Langerin), dermal DCs (DC-SIGN) and plasmacytoid DCs (BDCA2), and to differentiate between mature (CD83) and immature (CD1a) DCs. The mean number of positive cells/mm² was calculated for each antibody in all cases.

There were significantly more Langerhan's cells and dermal DCs in BCLH than CMZL, and significantly more mature DCs in BCLH than CMZL. No significant difference was seen in the number of plasmacytoid DCs or immature DCs.

Mature DCs function as antigen presenting cells and are involved in priming T-cells, suggesting that these functions are important in the genesis of BCLH, consistent with the hypothesis that persistent localised antigenic stimulation is the cause of these lesions. Conversely, immature DCs may play a more important role in maintenance of established CMZL through modulation of the anti-tumour immune host response, since persistence of DCs with an immature phenotype, as shown here, has been shown to lead to a state of immune tolerance.

P108

Identifying HIV Infection in Diagnostic Histopathology Tissue Samples

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Using immunocytochemistry, HIV-1 infection can be visualised in fixed tissue samples.

AIM: To investigate the practicalities and utilities of routine HIV-1 p24 immunocytochemistry on tissue samples received in a London histopathology laboratory.

METHODS: Over a 3-year period, 2005-7, fixed tissue samples from patients (age 2-65yrs) with and without known HIV infection, including primary diagnostic biopsies, consultation referrals and autopsy material, were stained with Dako anti-HIV-1-p24 antibodies. Cases were selected that, on H&E stains, morphologically suggested HIV infection, eg lymphoid hyperplasia in non-lymphoid organs, atypical germinal centres in lymphoid tissue (PGL), and encephalitis.

RESULTS: Of 123 cases, 36 were HIV-1 p24+ve: 24/87 lymph node, 7/13 Waldeyer's ring, 1/3 parotid, 1/2 anus, 2/5 lung, and 1/2 brain samples. In 10/36 cases (28%), the p24+ staining was the first indication to clinicians that the patient was HIV infected, and was reported as such. These included 6 lymph node and 3 Waldeyer's ring and 1 parotid samples. The p24+ patients had blood viral loads, where known, from <50-151462 (median 16026) copies/ml. Of the known HIV+ve cases (45), 9 were p24-ve. The p24+ was characteristically dense clustered staining within CD21+ dendritic cells or microglial cells.

CONCLUSION: Diagnostic histopathology can identify HIV infection directly in tissue samples when the local viral load is sufficiently high. As well as confirming specific pathologies such as PGL and HIV encephalitis, p24 staining identifies patients not previously known to be HIV infected. This technique should be applied more widely to diagnose HIV+ve patients earlier and bring them into treatment programmes.

P109

Role of Tissue Biopsy in the Rapid Diagnosis of Nodal & Extranodal Lymphomas

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AIM OF THE WORK: To find out reliable means for rapid and solid diagnosis of lymphomas that can allow quick therapeutic intervention and save patient lives.

MATERIAL AND METHODS: Specimens from 10 patients : 2 children and 8 adults, were received in the Department of Pathology during December 2007, January and February 2008; all with first presentation of their disease and requiring rapid and solid diagnosis to allow immediate intervention. Specimens were subjected to full pathologic examination including immunophenotyping.

RESULTS: F.N.A. Cytology specimens from 3 cases, TRU-CUT BIOPSIES from 6 cases, and Multiple Endoscopic biopsies from 1 case, were received. Accurate diagnosis and typing of lymphoma was reached in 8 cases (i.e. 80% of cases), and was suggested in 2 cases which required open tissue biopsy for confirmation and immuno-phenotyping of the disease (micrographs of the findings will be presented). Mean period required for initial reporting in each case ranged from 1 hour to 24 hours; and diagnosis was completed in upto 4days, including immunophenotyping.

CONCLUSION: F.N.A. CYTOLOGY specimens, TRU-CUT BIOPSIES, and ENDOSCOPIC BIOPSIES can be used reliably to allow rapid and solid definitive diagnosis of different types of lymphomas if two conditions are present : 1-Availability of ample amount of submitted specimen, e.g. multiple long intact cores of tissue, and enough aspirated fluid to allow multiple smear and/or cell block preparation. 2- Close clinico-pathologic communication before specimen taking.

P110

TTF-1 Positive Diffuse Large B-cell Lymphoma: An Unusual Finding Causing Diagnostic Dilemma

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Thyroid transcription factor-1 (TTF-1) is expressed in most thyroid carcinomas and in vast majority of lung carcinomas; hence it is one of the most useful markers in differentiating these carcinomas from other malignancies. Diffuse large B-cell lymphomas (DLBCL) form a heterogeneous group of high grade non-Hodgkin's lymphomas which show a variety of immunologic staining patterns. They are typically positive with LCA (CD45) and B-cell antigens (e.g. CD20, CD79a), but negative with Cytokeratins and TTF-1; which forms the basis of its differentiation from many carcinomas. Here we present a case of DLBCL that stains positively with TTF-1.

A 73-year old lady presented with a left supraclavicular node. The lymph node architecture was completely effaced and replaced by a diffuse infiltrate of large cells having pleomorphic nuclei, vesicular chromatin, prominent nucleoli and scant amount of cytoplasm. Initial run of immunohistochemistry showed strong positivity with TTF-1. EMA stained a few cells, but HMWCK, S100, thyroglobulin, CD30, PLAP, CK20, CK7 and LCA were all negative; and it was thought to be a metastatic carcinoma. But further IHC revealed AE1/AE3 to be negative. Repeat IHC showed LCA, CD79a and CD20 to be positive. Therefore, the final diagnosis was a DLBCL.

The diagnostic dilemma created by TTF-1 positivity in this case of DLBCL has not been addressed in the literature. We do not know the significance of this finding, but it may be of concern if more cases are reported in future.

P111

YY1 is a Prognostic Marker in Follicular Lymphoma

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Follicular lymphoma is the second most common type of Non-Hodgkin's Lymphoma worldwide. The majority of patients diagnosed with follicular lymphoma have an indolent, incurable form of the disease, however a subset of patients have aggressive disease with a shorter survival interval, but are potentially curable. In order for the correct treatment options to be considered it is important to distinguish between these patients.

The transcription factor YY1 has been shown to play an important role in cancer biology. PolyA RT-PCR has recently identified YY1 mRNA as being over-expressed in follicular lymphoma patients with a shorter survival interval. This study aimed to validate these findings at the protein level.

Quantification of the YY1 protein was carried out on 26 follicular lymphoma biopsy samples using Quantum Dot labelled immunohistochemistry. Expression levels of the YY1 protein were significantly increased in those patients alive after follow-up, in comparison to those dead ($P < 0.025$). Kaplan-Meier survival analysis identified that higher expression levels of YY1 were associated with longer survival times ($P < 0.01$). In order to identify the expression pattern of YY1 within follicular lymphoma tissue, co-localisation with CD20 was carried out. The results demonstrated that YY1 is expressed in CD20 positive and CD20 negative cells.

These observations indicate the presence of a negative feedback loop controlling YY1 protein and mRNA expression. They also support the hypothesis that YY1 can predict survival in follicular lymphoma, and indicate that YY1 could be a novel target in future anti-cancer therapies.

P112

Grading Follicular Lymphomas by Counting and "Eye-balling"

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Grading follicular lymphomas (FL) is recommended in current WHO guidance but the perceived accuracy of this process is controversial. This pilot study compares grading by eye – qualitatively assessing numbers of centroblasts in FLs – with formal counting.

FL cases from 2003-2004 ($n = 34$) were retrieved from Departmental files. Three cases were excluded owing to missing material. Slides were reviewed independently by an SpR and two consultants and the results of their analyses correlated. These results were then compared with the formal grading of the tumours carried out at the time of reporting.

Of the 31 cases assessed, 28 were nodal and 3 were extra-nodal. Overall, results show significant correlation ($p < 0.01$) between the three reviewers' eye-ball grading. In 8 cases, all grades were identical; in 15, two agreed and the third varied by one grade. In 5 low grade cases there was no agreement apart from classification as "low grade". Comparison with the formal grading showed complete agreement by eyeball and counting about the one case graded formally as 3B. In three cases one of us disagreed by eye between Grades 3A and 3B - formal counting graded each of these as 3A. Cases graded 1, 2 and 3A by eye-ball were all graded by counting as "low grade".

This pilot study suggests there is reasonable consensus in eye-ball and counted grading of low grade FLs but with variation in that range. "Eye-ball" 3A or 3B cases would benefit from formal counting.

P113

Microphthalmia in the Himalayan Rabbit: a Teratogenic Effect

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Forty pregnant Himalayan does were exposed to a New Chemical Entity (NCE), a mitochondrial complex II inhibitor, as part of a GLP Regulatory Study to investigate teratogenic potential. Exposure was from Day 6 to Day 30 of gestation at doses of 0, 600, 800 and 1000 ppm. All animals gained weight, increased their food consumption and survived to the end of pregnancy, with no adverse clinical signs. Examination of the pre-term foetuses (Day 30) revealed gross evidence of a reduction in eye size in all treated groups, but not in control animals. The reduction in eye size varied from 25-50%; no dose relationship was observed (0/33, 10/37, 5/22, 14/44 respectively). The heads were fixed in Bouin's for 3 weeks and then processed to wax blocks. Coronal sections of each head were cut in a rostro-caudal direction through the level of the eyes and stained with hematoxylin and eosin. The number of sections obtained per foetus varied from 11-18. The sections for examination were chosen on the basis of sectional symmetry, presence of all eye structures and proximity to the middle of the globe. Histopathological examination (13/33, 24/37, 16/22, 27/44 respectively) revealed retinal dysplasia, choroidal hypoplasia, cataract and persistence of the lens vesicle (0/13, 15/24, 9/16, 17/27 respectively). Such findings are considered to represent an unusual teratogenic effect. The relationship to the mode of action of the NCE is to date unclear.

P114

A three-year retrospective diagnostic review of CNS metastatic tumours

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We reviewed the pathological/ neurosurgery records of last 3 years (2005-2007) for metastatic tumour removal. Forty-six cases were identified including 42 craniotomies and 4 vertebral decompressions. Age of patients was 34-80 years (median = 60 years). In 12 cases the original primary was not known and by use of a panel of immunohistochemical stains the most likely primary site was identified in 10 of these cases. In two cases the patients had 2 separate known primaries. Breast cancer was the most common primary closely followed by lung. (Chart 1)

Chart 1 Site of primary cancer

Breast	15*	
Lung	13	
Colorectal	03	
Stomach	02	* 1 patient also had ovarian cancer
Oesophagus	02	1 patient also had lung cancer
GI Tract	02	
Kidney	02	
Bladder	01	
Unknown	02	

A total of 16 different immuno stains were used and were tailored for sex and previous diagnosis.

In conclusion a variety of distant metastasis of cancer are treated by surgical removal and the neuropathologist has a role in confirming the diagnosis and also identifying the site of primary when clinically not known.

P115

A Meningeal-Based Neural Epithelioid Fibroblastic Tumour; a Neoplasm Related to Solitary Fibrous Tumour and Showing Neuroblastic Transformation

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This article describes a meningeal-based tumour with fibroblastic and epithelioid elements, arising in the anterior cranial fossa. The tumour cells were positive for CD34 and bcl-2, and negative for EMA, an immunophenotype suggesting relationship to solitary fibrous tumour, and effectively excluding meningioma. The tumour subsequently recurred at the same site, with more aggressive clinical course, invaded into the nasopharynx, and resulting in the death of the patient. Histological examination of the recurrent tumour showed a component similar to the previous sampling, admixed with high grade tumour with neuroblastic features consistent with olfactory neuroblastoma. The radiological and histological co-localisation of the high grade neuroblastic recurrence raises the possibility of neuroblastic transformation of the original tumour.

P116

Ischaemic fasciitis, unusual location and presentation

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We present a case of ischaemic fasciitis in a female patient aged 45 years old with unusual site and history. She presented with a hard infiltrating mass in the anterior axillary fold with a history of modified radical mastectomy and completion of chemotherapy and radiotherapy courses. The clinical presentation has led to the assumption that the case could be recurrent breast carcinoma or even sarcoma. However, the microscopic picture was typical of ischaemic fasciitis by its characteristic central necrosis, vascular and fibroblastic proliferation, in addition to the presence of ganglion like cells, inflammatory cells and hemosiderin granules. Ischaemic fasciitis should be considered in the differential diagnosis of anterior chest wall masses and it could be induced by previous surgical trauma.

P117

A Large Extraperitoneal Myolipoma

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Myolipoma is a rare neoplasm, mostly occurring in adults, with female preponderance. It is characterised by the admixture of mature adipose tissue and smooth muscle tissue in varying proportions; most often the muscular component being predominant. Myolipoma have been described in the round ligament, eyelid, subcutaneous, pericardium, retroperitoneum, rectus sheath and abdominal cavity. In deeply situated tumours it is likely to be confused with a well-differentiated liposarcoma, extrarenal angiomyolipoma and leiomyoma with fatty change.

A 44-year-old lady presented with gradual abdominal distension for 15 months. The CT scan raised a suspicion of an ovarian tumour with liposarcoma as a differential diagnosis. She underwent a debulking laparotomy, which surprisingly revealed a well-demarcated large extraperitoneal mass measuring 24x24x10cm, weighing 2.7kg and extending between the urinary bladder and the epigastrium. This partly cystic and partly solid mass was completely excised. The pelvic organs including the ovaries were normal.

Histologically, the tumour was encapsulated, composing of smooth muscle elements with rather oedematous and myxoid areas together with islands of inactive adipose connective tissue. The blood vessels within the tumour were not predominant. No mitotic activity was seen. A diagnosis of myolipoma was made. Immunohistochemistry revealed a positive staining for ER, PR, desmin and smooth muscle actin.

Myolipoma may be clinically or radiologically mistaken as a malignant lesion. Myolipoma is a benign tumour with good prognosis and pathologists should consider it in the differential diagnosis of fat-containing extraperitoneal masses. These tumours may show positive staining for ER and PR.

P118

Case Report - Intra-abdominal Ossification

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We present a case of a 76 year old man admitted for the surgical repair of an abdominal aortic aneurysm (AAA). Post-operatively he required six laparotomies; day 6: adhesionolysis for small bowel obstruction; day 11: abscess drainage secondary to pancreatitis; days 31 and 35: unexplained bleeding; day 52: two entero-cutaneous fistulae; day 162: closure of abdominal wound and fistulae. At wound closure hard, calcified deposits were found in the omentum and on the peritoneum of the anterior abdominal wall. These were sent for pathological examination.

Macroscopically, six solid, white nodules, labelled omentum, were sent together with two pale, brown peritoneal nodules. Histologically, the omental specimen actually contained skin with underlying scar tissue, foreign body granulomas and a 5mm focus of ossification with bone marrow elements. The peritoneal nodules contained cancellous bone with bone marrow elements.

Intra-abdominal ossification is an unusual finding. Mature bone with haemopoietic elements present within the small bowel mesentery has been described as heterotopic mesenteric ossification (HMO), typically occurring after AAA repair. Mesenteric lesions were lacking in this case, however some reports include omental lesions as HMO. Heterotopic bone formation has been described in abdominal wounds; a strong possibility given the number of laparotomies performed. The most important differential is with an extra-skeletal osteosarcoma. Given the lack of cellularity and pleomorphism, this is unlikely.

The role of acute pancreatitis in this case is uncertain. Although saponification of fat with calcium deposition is well known, ossification with haematopoiesis has not been described.

P119

Ossifying Fibromyxoid Tumour of the Parapharynx: A Case Report of a Rare Tumour at an Unusual Site

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Ossifying fibromyxoid tumour of soft tissue (OFMT) is a rare tumour that typically occurs in the extremities of adults with cases located in the head and neck being recognised as unusual.

We describe a case of OFMT of the parapharyngeal space in a 53-year-old female that initially presented as a lump in the right cheek. The tumour was resected and histopathological examination showed bland cells with ovoid nuclei in a fibromyxoid stroma and a focus of central ossification. Mitotic activity was absent (0 per 50 hpf). Immunohistochemistry was focally positive for S100 and CD68 but was negative for epithelial, vascular, smooth muscle and other neural markers. Cytogenetic analysis of the tumour using fluorescent in-situ hybridisation found no rearrangement of FUS [associated with a t(7;16) translocation], excluding the diagnosis of low-grade fibromyxoid sarcoma.

A diagnosis of OFMT was made. Because of the low nuclear grade, low cellularity and absent mitotic activity, the tumour was graded as a benign typical OFMT.

P120

Localised Crush Injury to the Cricoid Cartilage: Vital Evidence Requiring a Meticulous Autopsy for Recognition

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Fractures of the cricoid cartilage are uncommon but when present, they are almost always indicative of significant violence to the neck. Injury usually occurs when the structure is crushed by antero-posterior force, such as a blow with the edge of the hand, a kick or forceful compression.

We present a case of an elderly male, found dead after a four week post-mortem interval, where the identification of cricoid trauma was critical in establishing the nature of the death. Examination of the neck structures revealed fractures of the thyroid cartilage and vertical, paramedian, undisplaced fractures of the cricoid cartilage. Subtle associated bruising was revealed only on reflection of the cricothyroid muscles. A transverse cut, inferior to the cricoid cartilage in the fixed laryngeal specimen, revealed localised bruising that was otherwise not visible. Histological examination of the cricoid cartilage revealed five cartilaginous fractures, with a well-preserved haemorrhagic and fibrinous reaction, despite the post-mortem interval.

Fractures to the cricoid cartilage may be easily overlooked at autopsy, particularly if the associated bruising is not great. This case highlights the need for meticulous identification and histological sampling of neck injuries in order to provide maximum forensic evidence, especially where subtle findings may be obscured by decompositional change.

P121

Audit of Post-Mortem Histology

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The 2006 NCEPOD report on the coroner's autopsy raised concerns regarding the frequency of tissue sampling for histopathological assessment. We reviewed our current practice with regard to histological sampling in coronial autopsies.

The Royal College of pathologists' guidelines recommend the sampling of all major organs in all autopsies. However, with the constraints which exist between the coronial system and pathologists we felt a standard of 100% unrealistic. The frequency of histopathological sampling in the NCEPOD report was 19% (baseline for early 2005) however the advisers raised concerns that this was not enough, therefore a standard of 19% could be considered too low. As a compromise we chose a standard of 30% for this audit.

We also looked at the following questions: How do we word the preliminary report when histology is pending? Which cases do we take histology from? How often does the histology confirm the macroscopic appearances? How often does the histology bring a new unexpected pathology to light that was relevant to the cause of death? What disposal options are relatives choosing for handling retained tissues? How do we document tissue retention, consent and arrangements for disposal?

Results & Conclusion: We sampled tissue for histology in 6% of cases and so did not meet the standard for this audit of 30%. Do we consider this enough bearing in mind the constraints? As a result of this audit we will be reviewing our criteria for selecting cases for histopathological assessment.

P122

Disseminated sarcoidosis and cardiac involvement: report of a post-mortem case

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A 46 year old lady with disseminated sarcoidosis died of cardiorespiratory arrest in the hospital.

At autopsy there was serous pleural (2000mls each side) effusion and pericardial adhesions. There was no significant cardiomegaly (weight 263grams) and both atrial and ventricular wall thicknesses were within normal limits (15mm and 3mm respectively). On slicing there were focal areas of ill-defined scarring in the myocardium. The coronary arteries and valves were normal. There was no lymphadenopathy or organomegaly.

Histology of the lungs confirmed the established diagnosis of sarcoidosis by presence of multiple non-caseating granulomata. The areas of scarring within the myocardium showed fibrosis and non-caseating granulomata.

Sarcoidosis is a multisystemic disease with an unclear aetiology. Direct cardiac involvement is seen in 20 to 27% of post-mortem cases of sarcoidosis. Cardiac sarcoidosis is mostly identified by impaired systolic left ventricular (LV) function, a feature of more advanced disease. Once symptomatic cardiac sarcoidosis develops in pulmonary sarcoidosis patients, the prognosis becomes very grim. In contrast, the prognosis in asymptomatic cardiac involvement in pulmonary sarcoidosis patients is good. In patients with sarcoidosis regular screening for cardiac involvement with regular methods is advised.

P123

After NCEPOD: A Potential Solution For Coroners' Post Mortems?

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A previous study showed difficulty in predicting the cause of death from the history alone in coronial post mortem cases in an unselected population. Our hypothesis is that a large proportion of community deaths can safely be categorised as "atherosclerotic cardiovascular disease" (ischaemic heart disease, cerebrovascular disease, ruptured aneurysms) and may not require post mortem. All coroner's cases over a 2 month period were evaluated and after exclusion of cases that would definitely or probably require a post mortem (e.g. possible unnatural deaths, deaths in hospital), 132 community deaths were included in the study. Six pathologists read the coroner's clinical summaries and predicted causes of death: these were then compared with the cause of death at post mortem.

None of the cases had an unnatural cause of death. In 38% of cases the pathologist correctly predicted the cause of death from the history alone. If allowed to use the term "atherosclerotic cardiovascular disease" (ACD) as described above, then this correctly categorised the cause of death in 73% of cases. There were a small number of false positive and false negative predictions of ACD as the cause of death.

Use of the term ACD increases the accuracy of prediction of the cause of death from history alone. Less precise categorisation of the cause of death within a group of diseases with similar presentation, aetiology and risk factors may not be important. With better coroner's histories, the cause of death in certain cases may be predicted without need for post mortem examination.

P124

Sudden Death in Undiagnosed Graves' Disease

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A 54 year old lady was admitted to A&E with sudden breathlessness. She had tachycardia at rest and bilateral proptosis. Investigations revealed supraventricular tachycardia and cardiomegaly. The findings suggested thyrotoxicosis but the patient died before a definite diagnosis could be established. A coroner's postmortem was ordered.

At autopsy the thyroid showed subtle enlargement. There was a thrombus in the left atrial appendage and an infarct in the right kidney. The lungs were congested and the liver had a nutmeg appearance. Tissues were sampled for histology. The thyroid showed characteristic features of Graves' disease. Histology confirmed heart failure cells in the lung and chronic passive congestion in the liver. The A&E blood sample revealed a raised freeT4 confirming thyrotoxicosis. The renal infarct was probably a result of an embolus from the thrombus in the left atrial appendage. Formation and dislodgement of thromboemboli are known to be associated with supraventricular tachycardia.

This case highlights some important points. It emphasises the importance of clinical information in order to interpret the autopsy findings in the correct context. It reiterates the value of postmortem histology. This is relevant in the current climate where postmortem histology is becoming increasingly difficult to take. It highlights the opportunity the antemortem A&E blood samples offer to carry out relevant investigations. Finally this was an opportunity to see the characteristic histological features of untreated Graves' disease. These are rarely seen in surgical pathology specimens because of prior treatment.

P125

Coronary artery thrombosis associated with a ruptured atheromatous plaque and sarcoid granulomas of the vasa vasorum. An autopsy case report

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Sarcoidosis is a multisystem disease of unknown aetiology and approximately 20% of sarcoid patients have involvement of the heart at autopsy. The most involved area is the myocardium and the coronary arteries are only very rarely affected. We present a case of a 63 year old woman who died from acute myocardial infarction as a result of a ruptured atheromatous coronary artery plaque with a superimposed thrombus. An unusual incidental finding was the presence of giant cell granulomas associated with the vasa vasorum of the coronary arteries underlying the ruptured atheromatous plaque. Non-caseating epithelioid granulomas were also seen elsewhere in the coronary arteries and in the lungs and liver. An infectious aetiology was excluded by special stains and culture studies and the appearances were consistent with sarcoidosis. Our unusual case demonstrates involvement of the coronary arteries by sarcoidosis in a case that would appear clinically straightforward. It is speculation whether granulomas associated with vasa vasorum could contribute to plaque instability.

P126

Blake and Rocket Drains, Which is Better in Cardiothoracic Surgery?

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BACKGROUND

It has been a standard teaching that drainage of the mediastinum is best accomplished using rigid large-bore Rocket drains. Recent trends in cardiothoracic surgery have suggested using Blake drains (thin, flexible, fluted with sideholes). Our aim was to compare Rocket and Blake drains with regards to pain score, amount of pericardial effusion and risk of atrial fibrillation after drains removal.

METHODS

Retrospective audit on 100 patients with major cardiothoracic operations divided into two groups, Group-A (50 patients, Blake drain, Consultant SG) and Group-B (50 patients, Rocket drain, Consultant MC).

Measured parameters: amount of pericardial effusion, incidence rate of atrial fibrillation during drains-removal and pain-scores with a descriptive scale (1-10) before, during and after drain removal. Statistical analysis was carried out using the Wilcoxon signed ranks test.

RESULTS

Group-A had a median pain score of 2, 6, 2 while Group-B had a median pain score of 3, 8, 4 before, during and after drains removal respectively. This is clinically and statistically significant with $p=0.02$. The mean effusion drainage was 89ml/day, 92ml/day while the incidence rate of atrial fibrillation was 8%, 12% for Group-A and Group-B respectively.

CONCLUSIONS

Larger chest tubes are not necessarily better when it comes to draining the mediastinum. We believe that Blake system can replace standard chest tubes as it is significantly less painful, associated with less risk of atrial fibrillation and drains the same amount of effusion compared to Rocket drains.

P127

The effect of inhaled carbon dioxide on constricted airways in an animal model of asthma

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The objective of this study was to determine the effects of inhaled CO₂ on constricted airways in a rat model of allergic asthma and to determine the mechanism of CO₂-induced bronchial smooth muscle relaxation.

An animal model of chronic asthma was developed using Brown Norway rats immunized and challenged with ovalbumin. Histologic examination of the airways showed most of the features of human asthma. The response to allergen and treatment was monitored using non-invasive whole body plethysmography.

Inhaled CO₂ caused a rapid, reversible and dose-dependent relaxation of constricted airways during the late phase of the asthmatic response. The percent drop of the enhanced pause (an index of bronchoconstriction) was 6.9% ± 5.29, 15.8% ± 5.83, 43.7% ± 6.37 and 68.5% ± 11.1 (mean ± SE) with 2%, 5%, 8% and 20% CO₂ respectively. Arterial blood gases during the late phase response in ovalbumin-challenged rats showed a significant increase in PaCO₂, decreased arterial blood pH and decreased PaO₂ compared with saline challenged rats.

CO₂ caused rapid dose-dependent relaxation of constricted airways in an allergic model of asthma. Our results suggest that CO₂ is a bronchial smooth muscle relaxant and support the notion that hypocapnia (low PCO₂) seen in some asthmatics may play a role in the pathogenesis of their asthma.

P128

Frequency and significance of atypical histological features in pulmonary inflammatory myofibroblastic tumours

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Inflammatory myofibroblastic tumour (IMT) is a rare mesenchymal neoplasm which may present in many organs including lung. Tumour behaviour is unpredictable although the WHO states 'intermediate biological potential'. However, some pulmonary cases are still being classified as inflammatory pseudotumour (IPT), a term that likely includes varying reactive mass lesions with similar morphology, confounding prognostic data. Furthermore, within IMTs overall, atypical features (high cellularity, rounded/large polygonal cells, abundant ganglion-like cells, necrosis and giant cells) and lack of ALK-1 expression have recently been suggested to be associated with more aggressive behaviour.

We have retrospectively reviewed 38 cases reported as IPT, plasma cell granuloma or IMT between 1992 and 2008. Of these, 24 cases were classified as lesions other than IMT. In the remaining 14 cases, ages ranged from 5 to 70 years with 8 females. Tumour sizes ranged between 11-110mm. All lesions showed ill-defined borders, with 8/14 showing extrapulmonary spread into either chest wall or mediastinal structures. 9/14 cases show atypical features including high cellularity, rounded/pleomorphic cells and necrosis, including all those with extrapulmonary extension. Where tissue was available, 8/9 cases were SMA positive. Staining for ALK-1 was negative in 4/4 cases, all of which showed atypical histological features, two with extrapulmonary extension. Pulmonary IMTs lack the frequency of ALK-1 expression seen in IMTs presenting at other sites and frequently show 'atypical' features that may be associated with behaviour more aggressive than those presenting at other sites.

P129

Secondary vascular changes in pulmonary sequestrations

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Pulmonary sequestrations, both intralobar and extralobar, are localised lesions comprising lung parenchyma receiving their blood supply via aberrant systemic arteries and lacking continuity with the upper respiratory tract. This study retrospectively reviews parenchymal changes within 27 resected cases, in particular concentrating on secondary vascular changes.

All 27 cases (1982-2008) had H&E and EVG stains for review. There were 11 females and 16 males, with an age range of 2 months to 60 years (average = 13 years). There were 22 cases with intralobar and 5 cases with extralobar sequestration. A specific feeding vessel was identified in all but one extralobar case. 15 of 27 cases showed intimal fibrosis and/or medial hypertrophy characteristic of plexogenic changes in the pulmonary vasculature with additional plexiform changes seen in 6 cases. Vascular changes such as medial hypertrophy were also seen within otherwise normal lung adjacent to the mass lesions in 4/22 intralobar cases. In addition, one case each of dissection of systemic vessels and lymphangiomatosis were seen, both in extrapulmonary lesions. In terms of the parenchyma within the sequestrations, 17 showed features of type 2 congenital cystic adenomatoid lesions.

Marked hypertensive vascular changes may be seen within the pulmonary vessels in sequestrations with lesser changes more rarely seen in adjacent lung, likely due to prolonged increased pressure from the systemic blood supply. In addition, dissection and coexistent lymphangiomatosis are reported in these lesions.

P130

Automated Scoring of Tissue Microarrays using Virtual Slides

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Tissue microarrays (TMAs) are a widely used tool in medical research to rapidly compare protein expression across hundreds of samples. However scoring tissue microarrays with a conventional microscope is a laborious task which is prone to error.

Virtual slides are an excellent tool for capturing TMA images for later analysis as they allow manual scoring of TMAs at a computer console. Furthermore they introduce the possibility of rapid automatic scoring of entire tissue microarrays using image analysis.

We describe a system developed to automatically score immunohistochemical staining on tissue microarrays with virtual slides.

The system analyses the slide at low resolution to identify cores (and identifies damaged or missing cores). It then analyses each core individually to calculate the amount of positive staining in the tissue. The results are presented to the user together with the virtual slide image of the cores for quality control and further study. This system has been used to score a TMA series of over 3,000 cores in less than 24 hours, significantly faster than a human scorer would be able to achieve.

P131

Immunophenotype of Ductal Carcinoma in Situ in BRCA Germline Mutation Carriers

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Background: Germline BRCA1 related breast cancers have a distinct basal/triple negative immunophenotype, and show EGFR and HIF1 expression. Little is known about the immunophenotype of precursor lesions in BRCA1/2 germline mutation carriers. The aim of this study was to examine whether this characteristic phenotype is already present in the pre-invasive stage.

Material and Methods: DCIS of 6 proven BRCA1 and 4 BRCA2 germline mutation carriers were stained by immunohistochemistry for ER, PR, HER-2/neu, Ck5/6, Ck14, EGFR and Ki67.

Results: 4/11 cases (36%) were ER positive, 0/7 (0%) were PR positive, 0/10 (0%) were HER2 positive, 5/10 (50%) were CK5/6 positive, 1/9 (11%) were CK14 positive, and 6/10 (60%) were EGFR positive. Mean percentage Ki67 nuclear staining was 30% (range 0-100). These percentages are similar to those that have been reported for invasive cancers in BRCA1/2 mutation carriers, except for ER that is generally even lower in BRCA1/2 related cancers.

Discussion: DCIS in BRCA1/2 germline mutation carriers shows a so called basal immunophenotype with high proliferation and EGFR positivity similar to that of invasive cancers in such patients. This may be useful to identify "BRCA-ness" in cases of DCIS in diagnostic pathology, and opens up new ways for targeted therapy against EGFR to prevent development of invasive cancer in case of a germline mutation.

P132

Hypoxia-Inducible Factor 1 α is Essential for Hypoxic p27 Induction in Endometrioid Endometrial Carcinoma

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Hypoxia-inducible factor 1 α (HIF-1 α) plays an essential role in the cellular adaptive hypoxia response. The cyclin-dependent kinase inhibitor p27(Kip1) is highly expressed in the normal endometrium but is lost during endometrial carcinogenesis. However, in high-grade cancers, p27 re-expression is observed. We analysed the role of HIF-1 α in hypoxia-induced expression of p27 in endometrial cancer. Paraffin-embedded specimens from 39 endometrioid endometrial carcinomas were immunohistochemically stained for HIF-1 α , p27, and Ki67. HEC1B, an endometrial carcinoma cell line, was cultured under normoxic or hypoxic conditions in the presence or absence of transiently expressed shRNAs targeting HIF-1 α . Protein expression of p27 and HIF-1 α was assessed by western blotting.

Immunohistochemical staining revealed perinecrotic HIF-1 α expression in 67% of the cases and p27 staining centrally in the tumour islands, mostly around necrosis, in 46% of the cases. In 50% of the tumours with perinecrotic HIF-1 α expression, p27 and HIF-1 α perinecrotic/central co-localization was observed. Hypoxia-associated p27 expression showed less proliferation around necrosis. In HEC1B, p27 protein expression was induced by hypoxia. This induction was abrogated by transient knockdown of HIF-1 α using RNAi. Furthermore, hypoxia induced cell cycle arrest in HEC1B cells. We conclude that, in endometrioid endometrial carcinoma, p27 re-expression by hypoxia is HIF-1 α -dependent and leads to cell cycle arrest. This may contribute to the survival of cancer cells in hypoxic parts of the tumour.

P133

Perinecrotic HIF-1 α Expression and Necrosis Predict Prognosis in Patients with Endometrioid Endometrial Carcinoma

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Background. Hypoxia-inducible factor 1 α (HIF-1 α) plays an essential role in the adaptive response of cells to hypoxia, triggering biologic events associated with aggressive tumour behaviour. Hypoxia and its key regulator HIF-1 α play an important role in endometrial carcinogenesis, but contradictory results have been published as to the prognostic value of HIF-1 α expression in endometrial carcinoma. We therefore re-evaluated the prognostic value of HIF-1 α expression in a large representative group of endometrioid endometrial cancer using well-established methodology.

Methods. In 98 patients with endometrioid endometrial cancer, expression levels of HIF-1 α and p27 were analyzed by immunohistochemistry. Presence of necrosis, and type of HIF-1 α expression (perinecrotic, diffuse, or mixed) were noted.

Results. Stage, grade and depth of invasion showed prognostic value as expected. Indicators of poor prognosis were presence of necrosis (p=0.05) and perinecrotic type of HIF-1 α expression (p=0.03). In patients with perinecrotic type of HIF-1 α expression, high p27 expression was an additional prognostic factor. In Cox regression, HIF-1 α was an additional prognostic factor to stage.

Conclusion. In patients with endometrioid endometrial cancer, necrosis and necrosis related expression of HIF-1 α are important prognostic factors. In view of the proposed role of hypoxia and HIF-1 α in endometrial cancer, HIF-1 α is thereby an attractive therapeutic target.

P134

Nitric oxide down-regulates expression of the haemoglobin-haptoglobin scavenger receptor (CD163) on human monocyte / macrophages

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Nitric oxide (NO) mediates many effects on immune system function. Although exaggerated NO production is well-characterised in many pathological states, its effect on the expression and function of the haemoglobin-haptoglobin scavenger receptor (CD163) is unknown.

Human monocytes were isolated by density centrifugation and subsequently exposed in 18-24 hour cultures to the NO generator DETA-NONOate. Co-incubation with factors known to promote CD163 expression was also performed. Metalloproteinase inhibitors were utilised to assess shedding as possible regulatory mechanism. CD163 expression was quantified by flow cytometry with supernatant soluble CD163 concentrations determined by ELISA. Post-incubatory cell viability was confirmed by metabolic capacity and CD14 expression. CD163 expression was also evaluated following exposure to the guanylate cyclase activator 8-Br-cGMP.

Nitric oxide downregulated monocyte CD163 expression by upto 70% at maximal concentrations. Similar attenuation was observed following co-exposure to both NO and interleukin-10 or dexamethasone. CD163 expression was downregulated by 24% through NO exposure following super-induction of CD163 expression by co-incubation with IL-10 and dexamethasone. Nitric oxide had no effect on cell viability but did induce a reduction in soluble CD163 detected in the culture supernatants relative to controls, thus excluding shedding as a downregulatory mechanism. The guanylate cyclase activator, 8-Br-cGMP, also induced downregulation of CD163, indicating a possible role of guanylyl cyclase in the downregulatory process.

This study has established a role of nitric oxide in regulating expression of CD163, possibly through activation of guanylate cyclase.

P135

Clinical significance of miR-21 and miR-145 expression in Colorectal Cancer

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Colorectal cancer (CRC) is the third most common cancer worldwide. The pathogenesis of CRC may involve aberrant expression of microRNA's (miRNAs) which are endogenously expressed short non-coding ~23nt regulatory RNAs that control the translation of many genes. Previous studies have shown that several miRNAs have altered expression in CRC including mir21 and mir145. In this study, the expression of these two miRNAs was quantified relative to 18s rRNA by real time RT-PCR in primary CRC. Total RNA was extracted from paraffin embedded formalin fixed sections of 30 primary CRCs, their flanking non-tumour tissues and 6 cases of adenoma by manual microdissection. The relative level of expression of both miRNAs was correlated to clinico-pathological features including; tumour size, Duke' stage, differentiation and lymph node metastasis. mir21 and mir145 were significantly over-expressed in tumours; mir21 (p<0.001) and mir145 (p=0.004). Both microRNAs also showed a significant association with Dukes' stage, mir21 (p=0.018) and mir145 (p=0.029), and lymph node metastasis, mir21 (p=0.017) and mir145 (p=0.029). Adenomas showed higher expression of mir21 than normal tissue suggesting that mir21 amplification is an early event in cancer development. Recent studies have shown that mir21 targets important tumour suppressor genes including Pdc4, tropomyosin1, Pten and maspain thus increasing the ability of the cancer to invade. The target for mir145 is currently not known. Our results support the view that mir21 and mir145 may have a role in the pathogenesis of CRC.

P136

CSMD1, a prognostic factor in gastric cancer

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We have identified a large candidate tumour suppressor gene with CUB and sushi domains (CSMD1) on chromosome 8p23, a region frequently deleted in many tumours including up to 30% of gastric cancers (GC). Allelic loss at this locus has been associated with poor prognosis and metastases in many cancers.

CSMD1 was investigated by immunohistochemistry in a series of 48 GC. In addition, the RNA expression pattern of CSMD1 was quantified in 47 GC by q RT-PCR.

CSMD1 staining was heterogenous in positive cases. Samples with less than 30% of positive cells were considered to contain reduced CSMD1 expression. Reduced CSMD1 staining was observed in 17/47 (40%) of GC. CSMD1 RNA expression was reduced in 10/46 (22%) GC and amplified in 5/46 (11%) GC. CSMD1 protein expression was related to Laurén classification (p = 0.067) and higher in the well differentiated GC. A significant association between CSMD1 transcript expression and tumour stage was observed (p = 0.040). Moreover, the mean survival time for the patients with loss of either CSMD1 protein or transcript was shorter than that of patients with amplified or unaltered CSMD1.

The data of this pilot study suggest that CSMD1 may play a role in GC progression and prognosis. Further studies in a larger number of cases are warranted to validate these results.

P137

CSMD1, a novel tumour suppressor gene in breast cancer

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We have identified a large candidate tumour suppressor gene with CUB and sushi domains (CSMD1) on chromosome 8p23, a region frequently deleted in many tumours including 50% of breast cancers. CSMD1 has homologies to proteins implicated in cancer and cell adhesion.

We studied CSMD1 expression in breast cancer samples (n = 50) by immunohistochemistry. CSMD1 was silenced using siRNA to investigate the biological consequences of reduced CSMD1 expression in 4 breast cancer cell lines.

Down regulation of CSMD1 expression was identified in 26/52 (50%) of breast cancers. Down regulation of CSMD1 expression was more frequent in ductal cancers (P < 0.05) and in poorly differentiated tumours (P < 0.0007). Similarly CSMD1 expression was less frequent in large tumours compared to small tumours. Reduced CSMD1 expression in the cell lines BT20 and T47D decreased adhesion to fibronectin and/or vitronectin. No change was detected in either the proliferation or the invasiveness of these cells. However, there was a 15% increase in the proliferation of the benign MCF-10A cells, while the invasive MDA-MB-435 cells showed little change.

We conclude that CSMD1 is an adhesion molecule due to its protein domain homology, cell surface localization and effect on adhesion. Moreover, like integrins, CSMD1 has the ability to influence cell proliferation.

P138

Expression array analysis of RPL19 gene silencing in prostate cancer

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Expression Array Analysis of RPL19 Gene Silencing in Prostate Cancer. Ribosomal protein-19 (RPL19) mRNA is differentially expressed in benign and malignant prostatic cell lines and tissues. In-situ hybridization confirmed it to be a powerful biomarker of prostatic malignancy and as accurate as Gleason score in predicting patient outcome. As part of the ribosomal protein complex, RPL19 modulates the synthesis of cellular proteins, hence contributing to the malignant phenotype. RNAi silencing directed against exon 11 of RPL19 variant "c" inhibited expression of the gene in PC3M prostatic cancer cells. Exon 11 was chosen as the target for suppression since it is in seven of eight potential RPL19 splice variants. Stable RPL19-silenced transfectants were constructed and cloned. Quantitative PCR of the transfectants confirmed a 70% reduction in expression in the malignant cells to levels at, or below, those of benign prostatic PNT2 cells. Transfected RPL19 cells remained viable but were less adhesive than their parental cells. Invasion and collagen activity assays were performed on the RPL19 silenced clones. Transfectants RNA expression was analysed by two-colour Human Genome DNA Expression Microarray (Agilent Technologies) against control PC3M cells. GO term enrichment analysis was performed separately with lists of significantly up- and down-regulated genes to identify significant functional terms. RPL19 suppression in malignant PC3M cells had greatest effect on depleting the expression of groups of genes within pathways, particularly collagenases and the Rho pathway, indicating possible genetic mechanisms responsible for prostate cancer dissemination and metastasis.

P139

Expression of Pim Kinases in Non-Hodgkin's Lymphomas: Evidence For a Role in the Inactivation of Wild Type p53

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Two murine models of cancer development are associated with a striking propensity to develop lymphomas. Homozygous deletion of the p53 gene in mice is associated with an increased risk of developing lymphoma and in another murine model it has been shown that overexpression of the Pim-1 kinase is associated with development of lymphomas of B and T cell type. However, inactivating mutation of the p53 gene is uncommon in cases of lymphoma in man, despite p53 mutation being a frequent molecular event in the pathogenesis of many other forms of malignant disease. Inactivation of p53 may be achieved by other means, for example through interaction with cellular proteins such as MDM2, the activity of which has been found to be enhanced in the presence of Pim-1. Pim-1 overexpression and consequent increase in MDM2 activity and/or levels could provide an alternative mechanism of p53 inactivation and therefore reconcile the experimental and clinical data with regard to the role of p53 in the pathogenesis of lymphoma. In this study immunohistochemical analysis of a series of 35 mantle cell lymphomas found Pim-1 expression to be elevated in 46% of cases. In addition there was a significant association between Pim kinase overexpression and elevated levels of MDM2 ($p = 0.0031$), which would support the theory that Pim may play a role in the inactivation of p53 through MDM2 activation.

P140

National E-learning Survey Among Histopathology ST1 Trainees

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Electronic learning or e-learning is an all-encompassing term to describe learning supported by the use of information and communication technology. Some emerging e-learning projects in pathology are already delivering high-quality learning solutions on a national scale, and are driving up standards by providing greater consistency and reliability in terms of quality learning and experience. The RCPATH is hoping to work with DH to develop a comprehensive e-learning training application along the lines of that already developed for radiology.

On behalf of the Histopathology training school board a survey was carried out among the ST1 trainees in the histopathology in the UK. The survey mainly focused on the views of the trainees on Histopathnet (the current ST1 e-learning resource) and the upcoming RCPATH developments in e-learning.

Responses were received from 51/84 (61%) trainees. 57% of trainees used on-line education facilities on a daily basis, with a further 24% weekly. 82% reported on-line study was mainly at the workplace. Popular sites included Histopathnet and RCPATH. Online facilities were used mostly for literature reviews, case reviews and text book access. 84% rated computer facilities at the workplace as excellent or satisfactory. Within Histopathnet, the 'Cases' section was most popular with 69% rating this excellent or satisfactory. There was support (75%) for development of virtual microscopy images to within the RCPATH development.

ST1 trainees are clearly IT literate and actively using e-learning resources. This should encourage the further development of high quality resources.

P141

The Value of the Black Box Meeting

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The "black box" meeting is a common training tool in histopathology departments. Trainees view pre-selected cases, getting a chance to see rarer entities and broaden their diagnostic experience. Despite its widespread use there is little literature on the content and overall relevance of these meetings. We have audited the black box meetings held in our teaching hospital department between November 2005 and November 2007, with the aim of quantifying the contribution that the meetings make to SpR training. Over the audit period we recorded the number of meetings, the number of cases presented per meeting and the diagnosis and speciality of each case. The gold standard was set at one meeting per week, at least four cases per meeting, and for all specialities listed in the RCPATH curriculum for specialist training in histopathology 2007 to be represented.

There were 65 meetings (0.6 per week) with 242 cases (3.7 per meeting) over 2 years. The number of meetings and cases per meeting declined in the second year. All the specialities in the curriculum were represented; gastrointestinal (19%) and skin cases (16%) were the most frequent. Malignant tumours were the commonest diagnostic category (40%) followed by non-tumour pathology (31%). The most frequent individual case type was malignant melanoma.

We concluded that the black box does make a significant contribution to SpR training and provides a sufficiently wide range of specialities. Following this audit the department has implemented measures to counteract the declining frequency of meetings.

P142

Lymph node yields in colorectal cancer – pathologists, patients or procedures?

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Variations in lymph node yields in colorectal cancer might be dependent on pathologists, patients or surgical procedure. Low yields could reflect diligence in finding small lymph nodes. This audit, therefore, assesses lymph node numbers and size in relation to grade of pathologist, patient age and surgical procedure. One hundred consecutive colorectal cancer resections were identified. Cases having neo-adjuvant treatment, concomitant inflammatory bowel disease or synchronous tumours were excluded leaving 81 cases. Small lymph nodes were defined as <5mm diameter. Mean (median) yield for trainees was 16.6 (15.0) and for consultants 14.2 (14.0). Mean (median) proportion of small lymph nodes was 74.0% (77.4%) for trainees and 72.7% (75.0%) for consultants. Mean (median) yields for procedures were: anterior resection 13.9 (13.5); right hemicolectomy 13.3 (14.5); sigmoid colectomy 14.6(14.5). Mean (median) yields by age were; up to 60 years 17.6 (17); 61-70 years 16.4 (13); 71-80 years 15.8 (15); 81+ years 11.5 (11.5). Mean (median) proportion of small lymph nodes by age was: up to 60 years 68.1% (70.6%); 61-70 years 64.4% (68.0%); 71-80 years 70.6% (73.3%); 81+ years 89.4% (92.9%). These results suggest that older patients have fewer and smaller lymph nodes and this should be taken into account when comparing lymph node yields for different pathologists.

P143

Streamed Internet Video for Pathology Research and Education: The Pathology Video Journal www.PathLab.org

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Introduction: The amount of internet information available in video format is expanding rapidly. Most content is not peer reviewed or assessed for quality or veracity. No other pathology website currently publishes in pure video format.

Methods: User generated content is submitted to the website moderator/editor and assessed with direct feedback to the author requesting video edits, or amendments. After approval by the website editor/peer reviewer each video clip has a written abstract posted on the website which can then be retrieved by searching Google. The video clip is uploaded onto the Google Video server.

Results: The 5 videos posted have already received a total of 468 viewings and 81 downloads with very positive user feedback. A single hour-long video describing how to take a FNA received 358 viewings and 76 downloads. www.PathLab.org is now hypertext linked to RCEngland, BSCC and BASO websites as the FNA video is also of interest to surgeons and radiologists.

Discussion: Internet video-on-demand will be an increasingly important means of teaching and training in biomedicine and science, particularly when describing a practical technique which is complex, difficult to apply or to replicate, and which would traditionally be taught 'hands-on' in the laboratory or clinic or by tutorials or seminars involving small groups of students or other professionals. Caveats include suitability for public display, patient informed consent, and a requirement to limit access via password protection for certain video footage.

P144

Prostate biopsy workload: are Histopathologists working harder?

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Background: Extended prostate biopsy strategies have been shown to enhance diagnostic sensitivity for malignancy, compared with the classical sextant approach, and have been incorporated into current guidelines for biopsy sampling. Few studies to date have examined the workload implications of these changes in biopsy practice on Histopathology services.

Materials and Methods: All needle core prostate biopsies submitted for histological examination to Leicester University Hospitals NHS Trust over a ten-year period from Jan 1998 - Dec 2007 were identified from the APEX computer system, using standard SNOMED codes. Chi-square and linear regression analyses were performed to examine temporal trends in study variables.

Results: Results of preliminary analyses demonstrate positive linear trends in number of biopsies reported (+272%; $P < 0.001$), paraffin blocks per case (+46%; $P < 0.001$), percentage of cases requiring additional work (+24%; $P < 0.001$) and mean cores per case (+63%; $P < 0.001$). The malignant diagnosis rate has shown no significant change over this time.

Conclusions: Prostate biopsy workload for the Histopathologist (total number of biopsies, blocks and cores per case, and additional work performed) have all increased significantly over the study period. A substantial proportion of this increase reflects changes in clinical biopsy practice. These trends are likely to have major resource implications for Histopathology services in the future.

P145

Gynecological malignancy referrals within the Yorkshire Cancer Network: An audit of diagnostic discrepancies

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Background: Yorkshire Cancer Network (YCN) Standard Operating Procedures dictate most gynecological malignancies encountered by peripheral Cancer Units should be referred to the Leeds Cancer Centre gynecological MDT for specialist management. As part of the referral process, histological diagnoses are reviewed by specialist gynecological pathologists to ensure diagnostic integrity. To date, the degree of diagnostic correlation between the pathology departments of the Cancer Units and the Cancer Centre is unknown.

Methods: Histopathology reports of 534 gynaecological malignancies of Cancer Unit origin were compared against corresponding review reports issued by the Cancer Centre gynaecology MDT over a 5 year period (2002–2006). Diagnostic discrepancies were classified according to RCPATH guidelines: C1–Diagnostic error with major clinical impact, C2–Diagnostic error with minor clinical impact, C3–Diagnostic error with no clinical impact, N–No discrepancies.

Results: Of the 534 reports assessed, 230 (43.1%) contained diagnostic errors. 36 (6.7%) were classified as C1, 71 (13.3%) were classified as C2 and 123 (23%) were classified as C3. Complete agreement was noted in 304 (56.9%) cases. Of the 230 cases with diagnostic errors (C1,2,3), 63% occurred within cases of uterine malignancy (cervix / uterus).

Conclusions: This audit highlights a significant level of diagnostic error within Cancer Unit reporting of gynaecological malignancies. In the majority of such cases, errors were of a minor nature. Only a very small minority of cases revealed errors of potentially major clinical impact. In such cases the need for specialist review of referred gynecological malignancies remains important for adequate patient care.

P146

Accuracy in Death Certification – An Audit of Current Practice

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Background: Current legislation on death certification in England and Wales requires that a doctor who has attended a deceased person must issue a medical certificate of cause of death (MCCD). Accurate certification of death allows statistical information to be gathered and used for health care monitoring, planning and provision as well as epidemiological research. Advisory notes for doctors are provided in the front of all blank books of death certificates together with an expanded form published by the Office for National Statistics' Death Certification Advisory Group.

Aims: The purpose of this audit was to establish whether death certificates are being completed to a minimally acceptable standard in accordance with published guidelines.

Methods: Counterfoils from death certificates completed in a district general hospital over a three month period were analysed retrospectively for completion errors and logical sequencing of cause of death.

Results and Conclusions: A total of 306 certificates were completed during this time period. Of these 47% were completed to a minimally acceptable standard in accordance with published advisory notes. This figure increased to 76% if errors due to incomplete information and use of abbreviations were removed since these were felt to be spuriously high due to the use of counterfoils in data gathering. The findings are comparable to those of similar studies.

Accuracy of death certification may be increased with greater education of junior doctors and greater trust emphasis on following published advisory notes to doctors.

P147

Patient Understanding of Hospital Pathology Services: A Questionnaire Based Audit

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Pathologists traditionally carry out much their work behind closed doors and out of the public eye. We aimed to determine the current level of patient understanding of hospital pathology services.

Forty semi-qualitative questionnaires were completed by patients admitted to a hepatology unit between January and March 2008. The study group consisted of 23 males and 17 females with a mean age of 46.6 years (range 16-71 years). 22 patients were admitted for post-transplantation issues, 12 for pre-transplantation liver biopsy and 6 for transplantation. 31 patients had undergone at least one previous tissue biopsy.

80% of respondents had previously heard of a pathologist but only 38% recognised that they analysed their biopsy or explant. 60% thought pathologists were somehow involved in the diagnosis of liver disorders but only 50% thought they could influence treatment. An additional 15% believed that pathologists were only involved in the examination of dead bodies. 13% recognised that disciplines such as haematology and microbiology also fell under 'pathology services'. When asked to rate the importance in their care, pathologists fared the worst behind surgeons, physicians and radiologists. 95% had no concerns about tissue retention and 78% were happy for their tissue to be used for research purposes.

This study highlights the current lack of patient understanding of hospital pathology services, even in those who have undergone previous tissue biopsies. We believe this re-enforces the need for education and interaction through events such as the forthcoming Royal College of Pathologists 'Pathology Week'.

P149

An Audit of Colorectal Cancer Specimens – What is the Best Measure for the Adequate Numbers of Lymph Nodes Examined?

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Aim:

To perform an audit on colorectal cancer specimens for the 3 main standards as recommended in the Royal College of Pathologists' Minimum Dataset, 2nd edition, September 2007.

Methods:

The reports of 120 consecutive colorectal specimens between December 2006 - August 2007 were analysed.

Results:

- frequency of serosal involvement was 26.9% for colonic tumours and 25% for rectal tumours.
- frequency of extramural venous invasion was 26.7%.
- mean number of lymph nodes examined was 12.75.

All of these results are within the range recommended by the Royal College.

Observations:

In regards to the lymph node yield, is the calculation of the mean the best value for assessing adequacy?

Our results showed the range of lymph nodes examined was 0 – 40. the data is positively skewed, with 58.3% of the cases having lymph node numbers less than the mean.

The median may be a better central tendency measurement as it is used for asymmetrically distributed data. In our audit the median was 11.5, the value at which 50% of the cases have lower values.

It may be prudent to calculate the median as well as (or instead of) the mean in future audits, to remove the false assurance that achieving a mean number of lymph nodes above 12 is adequate, when more than half the cases may fall below this value.

P148

Scottish Histopathology Specialty Training Recruitment 2008: Evaluation of Process

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The Scottish Histopathology recruitment process has evolved over three years, offering 8 ST1 posts in 2008. The person specification and application form were developed from templates. The application required factual information and evidence of reflective learning with eligibility criteria and 9 scoring fields. The forms were scored by 8 paired, trained selectors over one day. The interview was structured in advance, mixing behavioural and scenario questions that assessed understanding of a histopathology report, clinical prioritisation, dealing with an error, interpreting a picture, preparing a presentation and commitment to the specialty. Communication, language skills and submitted portfolios were also assessed. Shortlister scores were correlated (Pearson). Reliability of the shortlisting and interview tools was tested (Cronbach's). An anonymous exit questionnaire was administered to interviewed candidates. Focus groups provided insight to assessors' views of shortlisting and interview.

The 41 from 88 applicants who scored well at shortlisting were invited to interview. Interview candidates (n = 40) were assessed by two of four panels of two selectors providing 20 "appointable" individuals. Shortlisters' scores correlated well (0.964, p<0.01). The shortlisting and interview tools were reliable (Cronbach's alpha 0.859, 0.815). There was no correlation between shortlisting and interview scores (0.158). Candidates were positive about the interviews, considering the process fair, providing opportunities to display relevant attributes. Assessors highlighted shortlisting as better than 2007 and aspired to develop interviews towards a more sophisticated selection centre. The evidence suggests a successful recruitment episode that has the confidence of assessors and is acceptable to candidates.

P150

This abstract as been withdrawn
prior to the Meeting

P151

The Central & Northern Research Collaborative

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The Royal College of Pathologists and the Pathological Society are supporting collaboratives in the UK to stimulate academic and research activity amongst established researchers and trainees. There are four collaboratives which cover the UK.

The Central & Northern Research Collaborative (CNRC, covering East Midlands/ Northern England) has been active in developing a research-supportive infrastructure. There have been two CNRC meetings (November 2007 and May 2008). In addition to stimulating collaborative research, agreed desired objectives were mentorship for trainees, sharing of teaching materials and establishment of training courses. The meetings' consensus was that a means of easily gathering/disseminating information was essential to achieving these objectives. This could be best achieved via a website containing all the information essential to the activities of the Collaborative (available projects, techniques, biological resources, lecturing materials etc.).

The CNRC has taken a number of steps forward. The architecture of the CNRC is Specialist Groups (centred around organ systems) with a chosen lead for each group to oversee activity. A community based website (www.ipath.org.uk) has been established with each Specialist Group forming a community. All persons are invited to register their interests/expertise and tag clusters are allowing new communities to emerge. Collaborative projects between Nottingham and Leeds have been developed together with some exchange of teaching materials. Training courses in PCR and Laser Capture Microdissection are planned. Ultimately, it is hoped that joint tissue resource will develop to expedite development of multi-centre projects.

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This abstract as been withdrawn
prior to the Meeting

P152

The impact of the european working time directives on modern surgical trainees

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Introduction

The European working time directive (EWTd) was introduced to establish minimum safety and health requirements for employment. The effects EWTd will have on surgeons in training are unknown. The aim of this study was to evaluate the effects of EWTd implementation on the quality of life and training of surgical speciality trainees.

Materials and Methods

Questionnaires were posted to all 250 surgical trainees in Yorkshire. Subjective evaluation of training and theatre exposure in the three months before and the three months after enforcement of the EWTd were investigated, substantiated by objective logbook records. Groups were compared using the Wilcoxon signed rank test.

Results

50 responses were received. 30 trainees (60%) were working more hours than permitted, with 19 claiming to working >6 hours per week extra. EWTd did not appear to effect overall patient management ($p=0.423$), continuity of care ($p=0.954$), teamwork ($p=0.647$) or theatre exposure ($p=0.677$) as subjectively assessed by the trainees. Objective logbook data, however, showed that trainees were operating significantly less ($p=0.025$) after EWTd implementation, tending to act more as assistants and less as operating surgeons. Overall, 62% of trainees felt that derogation should be made with EWTd.

Conclusions

There are early signs that the EWTd is subjectively and objectively detrimental to the well being and training requirements of future surgeons, with indicators of increased job dissatisfaction and stress, along with poorer training opportunities. Assessments were hampered because logbooks are no longer mandatory for speciality membership examinations.

P154

Audit of Correlation Between Respiratory Cytology and Histology

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A retrospective audit covering a one year period was undertaken to assess the correlation between respiratory cytology and histology diagnoses and the reasons for mismatches with calculation of the diagnostic accuracy of respiratory cytology.

There were 258 respiratory cytology specimens from 174 patients, 58 (33.3%) of whom had corresponding histology specimens. Inadequate cytology samples totalled 17 (6.6%), 11 of which were lung fine needle aspirates (FNA). There was diagnostic correlation in 44 cases (75.8%). In 12 cases there was a mismatch with negative cytology and positive histology, 2 of which were true false negatives with the other 10 cases negative due to sampling error or poor preservation. In 2 cases with positive cytology and negative histology, the cytology diagnoses were considered true positives on review, while the histology diagnoses were altered on review.

The sensitivities of the adequate cytology specimens were 80% for lavage, 84.5% for bronchial brushings, 74.1% for bronchial trap/washing, 100% for lung FNA and 50% for sputum. This represented an improvement for all specimen types from a previous audit and is comparable to figures in the literature.

Overall there was good correlation between respiratory cytology and histology diagnoses. The majority of false negative cytology specimens were due to non representative sampling. The high level of inadequate lung FNA samples has been evaluated in a recent audit and recommendations made for improvement.

P155

How Do Histopathology Trainees Choose their Training School?

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There is a national appointment process for applicants for histopathology training schemes in England and Wales. A combination of applicant choice, interview ranking and special circumstances is used to match appointees to training schools. This increases the best candidates' chances of gaining a training post, but decreases their control over where they work. The geographies offered were related to the Deaneries co-ordinating training programmes at Yr 2 – 5 level.

We surveyed all 1st year Histopathology run-through trainees in England and Wales to enquire about the information they used to choose a training school, the factors they considered and their opinions of the process.

The response rate was 78%, most gaining their information from the Histopathology Training Schools' website. The commonest factors in choosing a training school were the location of a partner (75%), and the Histopathology Training School's reputation (59%). Children were a factor for 18%, and 24% expressed an interest in research. For 52%, the location of their family was the most important factor. Encouragingly, 84% felt that the matching process used was fair.

Our survey has shown an acceptable matching scheme with a website (histopathsho.34sp.com) which is a valuable resource for applicants. While geography is an important factor, almost a quarter of trainees chose their training school, at least in part, because of the research opportunities available. This is encouraging for a potential resurgence in histopathology research and underline the importance of the newly created academic pathology training posts in many training programmes.

P156

The Value of One Stop Head and Neck Clinics

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Background: One stop head and neck clinics were introduced in response to NICE guidelines 2004 with the aim to provide rapid assessment and provisional diagnosis of suspected cancer patients. They comprise clinical review followed by ultrasound-guided fine needle aspirate with immediate cytological reporting by the pathologist. At our centre this clinic was established two years ago. The aim of this audit was to study the impact of the 'One Stop' method by investigating the correlation between clinical impression, radiological findings, cytology and histology reports, and subsequently comparing this data with cases prior to the set up. Methods: FNA and histology reports were collected from approximately 100 patients who had attended the clinic between 2005 and 2007. We combined this data with the clinical details and radiology reports provided by the surgical team. This information was then analysed and compared to a similar cohort prior to this set up. Discussion: Our data shows a good overall correlation between FNA cytology and histology reports. Furthermore the number of inadequate samples has significantly reduced over the past two years. In this centre the introduction of the one stop head and neck clinic has had a significant impact on management of cancer cases by allowing rapid accurate diagnosis and thereby permitting early treatment. In addition, it has benefited patients through fewer hospital visits, prompt reassurance and reduced anxiety overall.

P157

Intrathyroid lymph node tissue in multinodular goiter, case report in Egyptian female.

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We report a case of intrathyroid lymph node tissue in 40 years old Egyptian female. Collections of lymphoid follicles surrounded by mature fat cells were intimately associated with normal thyroid follicles. The presence of this heterotopic tissue could arise by the process of heteroplasia or it could be just deviation of the normal anatomy of cervical lymph node groups.

P158

Engaging Histopathologists in Pharmaceutical Research and Development

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A major part of the practice of most histopathologists is to continue to provide the "gold standard" for cancer diagnosis. Their trained observations also contribute significantly to information that is linked to prognosis and are used in cancer therapy planning. Histopathologists can also play an important role in pharmaceutical research and clinical trials though very few are employed full-time in industry.

In our practice we have identified the following areas where application of histopathologists' expertise adds value to the drug discovery process:

- Provision of the general and specialist, diagnostic and research skills, in basic and molecular pathology used in hospital practice and university laboratories, to pharma as "key opinion leaders" and as consultants for individual projects. This area has the greatest potential for public/industry collaborative research.
- Histopathologists are custodians of tissue that is surplus to diagnostic requirements that would normally be archived or discarded.
- Histopathologists are key to the laboratory collection, processing and review of samples taken as part of clinical trials as well as allowing access to archival human tissue and pathology data for patient inclusion.
- Provision of histopathology services to a pharma company as external consultants or as employees for histopathological and biomarker analyses including the evaluation of new technology platforms.

In clinical research histopathologists are integral to translational science, for proof of mechanism and/or principle, and also play an important role in drug development in Phase III trials.

P159

Immunohistochemistry Biomarker Validation for Use in Clinical Trials – Case Study with Src Inhibitor AZD0530

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Biomarkers of Src and small-molecule drug inhibitory activity have been critical in supporting early clinical development of AZD0530. We describe preclinical development and clinical implementation of tumour tissue biomarker assays of Src activity utilizing immunohistochemistry (IHC) endpoints.

Immunohistochemical assays utilizing phosphospecific antibodies to integrin-linked Src substrates focal adhesion kinase (FAK) and paxillin were developed in the laboratory using AZD0530-treated cell lines and xenograft models. Biopsies were used to develop assay protocols in a clinical setting before implementation in a Phase I study in patients with advanced solid malignancies. IHC was performed on formalin fixed, paraffin embedded tissue samples from patients where pre- and post-treatment tumour biopsies were collected.

In this Phase I study, AZD0530 reduced the levels of p-FAK and p-paxillin in some but not all patients. Biomarker changes were seen at all doses and in all tumour types. Paired pre- and post-treatment biopsies were evaluable for blinded IHC analysis in 26 and 22 out of 51 patients for p-FAK and p-paxillin, respectively. Concordance was significant for both biomarkers: 21/26 samples (81%) were correctly assigned based on p-FAK staining (P=0.001, 80% exact CI: 0.672–0.899) and 15/22 (68%) based on p-paxillin staining (P=0.067, 80% exact CI: 0.523–0.813).

These data describe the validation of IHC biomarkers to demonstrate in early clinical development the drug inhibition of Src pathway signalling, as proof of mechanism for AZD0530 in target tissues of cancer patients.

P160

Thymoma associated with hypergammaglobulinemia: A case report

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Thymoma is a term that should be restricted to neoplasms of thymic epithelial cells, irrespective of the presence or the number of lymphocytes.

The usual location of thymoma is the anterosuperior mediastinum; however, this tumour can also occur in other mediastinal compartments, in the neck, within the thyroid gland, in the pericardial cavity, the pulmonary hilum, within the lung parenchyma, or the pleura itself.

The Association of thymoma with hypogammaglobulinemia, mucocutaneous candidiasis and SLE is well known. Here we present a patient admitted in our hospital with buttock rashes regarded to be zoster of the buttock.

In this past medical history he had recurrent cutaneous infections, lupus erythematosus, hypergammaglobulinemia, recurrent oral candidiasis and B-cell dysfunction.

During the patient evaluation, a mass was found in the mediastinum and percutaneous needle biopsy confirmed the diagnosis of thymoma (type B3).

This is the second case reported having thymoma in association with hypergammaglobulinemia.

Key word: Thymoma- hypergammaglobulinemia- systemic lupus erythematosus

P161

Differential expression of microRNA92 and one of its gene targets, ER beta, in normal and malignant breast tissues and cell lines

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ERβ expression in breast cancer is lower than in normal breast, suggesting ERβ is downregulated during carcinogenesis. Silencing of ERβ in breast tumours may be mediated by one or more specific microRNAs. The aim of this study was to identify expression profiles of ERβ mRNA and to correlate this with miR92, a predicted ERβ target, in normal and malignant breast tissues and cell lines. Interrogation of the Sanger miRBase database revealed a conserved target-site for miR92 within the ERβ 3'-UTR at 955–976 nt, confirming its potential role as a regulator of ERβ. Total RNA was extracted from breast tumours (n=10), paired normal/ tumour samples (n=11) and breast cancer cell lines. TaqMan® MicroRNA Assay hsamiR92 was used to estimate miR92 expression and SYBR green QRT-PCR was used to detect ERβ expression in all samples. We observed that miR92 was differentially expressed in normal breast tissues and cancers. Breast tumours with abundant miR92 expression displayed a significant decrease in ERβ expression (n=14; p=0.005). The reverse was observed in tumours with low miR92 expression (n=7; p=0.01). In breast cancer cell lines with low ERβ expression (BT20 and MDAMB453), miR92 expression was also high. In addition, there was a statistically significant difference in ERβ expression between normal and breast tumours (p=0.004) but not miR92. Our results suggest that our observed downregulation of ERβ expression in breast tumours and cell lines may be mediated by miR92. Current work is confirming this.

P162

EGFR, Her-2/neu and Cyclin D1 Gene Copy Number and Protein Expression in Head and Neck Squamous Cell Carcinoma

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This study has examined the associations between, and prognostic value of, changes in chromosome number, gene copy number and protein expression of EGFR, HER2/neu and cyclin-D1 by fluorescence in situ hybridization (FISH) and immunocytochemistry on tissue microarray sections of 200 cases of head and neck squamous cell carcinoma (HNSCC). Gene amplification was regarded as a ratio of 2 or more between the signals for the genes and the chromosomal centromeres.

Amplification of the cyclin-D1 gene was present in 31.3% cases with chromosome 11 trisomy in 41.9% cases and polysomy in 22.2% cases. Amplification correlated with high (>median) protein expression (p<0.000). Amplification of the EGFR gene was present in 6.9% cases with chromosome 7 trisomy in 57.4% cases and polysomy in 26.6% cases. Amplification correlated with high protein expression (p<0.002). Amplification of the HER2/neu gene was present in 5.9% cases with chromosome 17 trisomy in 48% cases and polysomy in 5.2% cases. Amplification correlated with high protein expression (p<0.000). Both cyclin-D1 gene amplification and protein overexpression were associated with pharyngeal primary tumours (p<0.000, <0.001 respectively), high grade primary tumours (p<0.001, 0.037) and with nodal metastasis (p<0.001, <0.013). There was no association between gene amplification, protein expression and survival.

These results suggest that amplification of EGFR and Her-2/neu genes are relatively rare events in HNSCC. Cyclin-D1 amplification is associated with tumour progression and poor prognosis.

P163

Benign Vascular Invasion in Pleomorphic Adenoma

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Pleomorphic adenoma is the most common benign neoplasm of the salivary glands. Although the histological diagnosis is relatively easy, the presence of atypical features such as vascular invasion may pose considerable diagnostic difficulty. Vascular invasion is a hallmark of malignancy but more recently a sub-group of pleomorphic adenomas have been described exhibiting vascular invasion without any evidence of malignancy. The significance of vascular invasion in pleomorphic adenoma is not clear.

Here we present 6 cases of pleomorphic adenomas from the parotid gland that shows tumour emboli in vessels within and outside the capsule. There is a history of fine needle aspiration (FNA) in 4 cases and incisional biopsy in 2 cases. We speculate that the intra-vascular tumour is a result of the traumatic diagnostic procedure that may have dislodged the tumour cells into the vessels rather than true vascular invasion. Furthermore pleomorphic adenomas with a predominant myoepithelial/myxoid component may be more susceptible to this complication. Review of data from the literature also appears to support our observation.

P164

Primary olfactory neuroblastoma of the oropharynx - Case report of a unique pathological entity

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Olfactory neuroblastoma, also termed esthesioneuroblastoma, is a rare malignant tumour of neuroectodermal origin that arises from progenitor cells of the olfactory neuroepithelium. Rarely, ectopic occurrences have been reported in the maxillary sinus, sphenoid sinus and pituitary gland. Here we present a unique case of olfactory neuroblastoma arising within the oropharynx of a middle-aged patient.

A middle-aged female presented with chronic throat pain. Clinical examination revealed a mass localised to the left palatine fossa with involvement of the adjacent soft palate. Computer tomography confirmed an ill-defined tumour of the left tonsillar bed with encroachment into adjacent tissues. The nasal cavity and paranasal sinuses appeared normal with no other abnormality identified. Surgical resection followed, yielding a 2.5 cm irregular grey / brown tumour.

Histological analysis revealed a lobular tumour consisting of small cells possessing scant cytoplasm. Marked nuclear atypia was noted in association with prominent nucleoli. Mitotic activity was brisk and necrosis was evident. A neurofibrillary background was absent and rosettes were not identified.

Immunohistochemistry revealed diffuse positivity of neuron specific enolase with focal positivity for synaptophysin. All other markers were negative. Cytogenetic analysis highlighted losses at 1p36 and 11q23. No EWSR1 gene rearrangements or MYCN amplifications were identified. Electron microscopy showed intra-cytoplasmic neurosecretory granules and confirmed the presence of true epithelial desmosomes.

The histological, cytogenetic and ultrastructural findings confirmed the diagnosis of high grade olfactory neuroblastoma. This represents the first case of such an entity arising within palatine fossa of the oropharynx.

P165

Liver Histopathology in the Yorkshire Region: a Network Model

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The national plan for liver services UK 2004 states "each hepatology centre should have access to a specialised liver pathologist. Ideally this would be in the site of the lead centre providing expertise and training for the local pathology departments within the managed clinical network."

Methods: Questionnaire survey about liver biopsy reporting was sent to histopathologists in 11 hospitals in Yorkshire.

Results: Replies from 23/31 (74%) pathologists in 9 non-specialised DGH departments and 3/4 liver pathologists in 2 larger specialised departments (Bradford, Hull). DGH departments receive average 70 liver biopsies/year (30–150; 67% medical, 33% for tumour) representing 0.55% (0.33–1.1%) histology requests. Reporting is shared by all 2–6 pathologists/department. Estimated 35% (5–100%) cases are discussed with the clinician during a CPC (5/9) and/or by phone (5/9). 5–10% biopsies are reviewed in Leeds. The two larger departments receive 120 and 170 biopsies/year shared by 2 sub-specialists. 2–5% biopsies are reviewed in Leeds. Of 26 pathologists 20 said they enjoyed liver work, 23 often read up about biopsies, and 20 discussed them with their colleagues. 18 would like to do liver CPD, 9 had done liver CPD in the last 3 years. 5 would like to refer more biopsies centrally. Only one said they would prefer not to do any liver work.

Conclusion: Liver biopsies account for 0.5% Yorkshire DGH histopathology specimens. The results support a more formalised network, supporting pathologists working with their local clinicians.

P166

Review of liver biopsies in a hepatology centre: how often and why is the diagnosis changed?

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Introduction: 160 liver biopsies/year are reviewed in Leeds.

Aim: To determine the proportion of medical biopsies where review had resulted in a change of diagnosis.

Methods: Referral reports for 100 consecutive cases were reviewed.

Inclusion criteria: Referred adult medical liver biopsies where original pathology report available. Original diagnosis was compared retrospectively with centre histology report (JIW). Original clinical information in referred report was noted, together with correspondence and CPC discussion. Cases categorised as major change in category (e.g. chronic hepatitis to chronic biliary disease), minor change in interpretation within category (eg likely aetiology, change in stage, second diagnosis), or no change.

Results: Major changes occurred in 25% and minor change in 24% diagnoses. Biliary disease accounted for 40% revised diagnoses.

Conclusion: Review revised the diagnosis in 49% cases. Review usually included clinic-pathological discussion, and this study is not a direct audit of the initial diagnosis. The study highlights areas which should be targeted in liver histopathology CPD for non-specialist histopathologists.

Main biopsy diagnosis (number of final diagnoses)	Major change	% of major	Minor change	% of minor	No change
Acute Hepatitis (13)	4	16%	2	8%	7
Granulomatous (4)	0	0%	3	13%	1
Normal/Non-Specific (8)	3	12%	0	0%	5
Biliary (32)	10	40%	10	42%	12
Chronic Hepatitis (23)	1	4%	3	13%	19
Fatty Liver Disease (16)	5	20%	5	21%	6
Vascular Outflow (4)	2	8%	1	4%	1
Total (100)	25	100%	24	100%	51

P167

Presence of stem cell traits in human pancreatic cancer cell lines

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Cancer stem cells (CSCs) have been identified in leukaemia and some solid tumours and retain properties and markers of normal adult stem cells. However, a definitive stem cell remains unidentified within normal or malignant pancreas.

The presence of four reported features of adult stem cells was evaluated in 4 human PDAC cell lines (Panc-1, Psn1, COLO 357 and Capan-2): aldehyde dehydrogenase (ALDH) activity was evaluated by flow cytometric (FACS) analysis using Aldefluor, ABC-transporter activity (Side Population analysis) was investigated by Hoechst 33342 efflux assay, and the presence of CD133 and CD44 evaluated by immunocytochemistry (IHC) and FACS. The colony-forming potentials of subpopulations thus isolated were assessed in vitro.

An ALDHbright subpopulation and CD44+ cells were present in all lines. A side population (SP) was present in Panc-1 and Capan-2 lines. CD133+ cells were present in Panc-1, Psn-1 and COLO 357. Colony forming ability (CFA) was significantly higher for ALDHbright and SP cells versus controls in Panc-1 and Capan-2 respectively. However, increased SP CFA may be artefactual, as Hoechst exposure significantly affects proliferation and CFA in the non-SP fraction of Capan-2. CD133+ Psn-1 cells showed increased CFA versus unsorted controls, whilst this trend was reversed for Panc-1. CD133+ Colo357 cells did not show elevated CFA but formed holoclone-like colonies, and progeny of these CD133+ cells were heterogeneous for CD133 expression, suggestive of asymmetric division. Membranous CD44 expression did not correlate with enhanced CFA in any line.

P168

Helicobacter pylori infection in patients with chronic urticaria, correlation with the pathological findings in their gastric biopsies

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Background: Chronic urticaria is a persistent urticaria for longer than six weeks affecting 20 % of general populations. Various infectious agents have been reported as causes for urticaria including helicobacter pylori, which is considered as a common worldwide bacterial infection. Its role in inducing allergic conditions such as chronic urticaria has been suggested in some reports and refused in others. This study aimed to assess the prevalence of HP infection in patients with chronic urticaria and explore the possible etiopathogenetic link between them.

Methods: Thirty five patients suffering from chronic urticaria and ten normal control individuals were subjected to upper endoscopic gastric biopsies to assess and semi-quantify HP infection and address other pathological abnormalities using routine Hematoxylin & Eosin staining and Giemsa staining.

Results: Forty percent of control subjects and 57% of patients revealed positivity for HP infection but the difference did not reach a significant level (P=0.47). The severity of urticarial symptoms was higher in HP positive group than negative one (P=0.019). Heavy bacterial colonization (p=0.008) and intense gastric inflammation (p<0.0001) were significantly associated with severe clinical manifestations.

Conclusions: HP may have a role in exacerbation of urticarial symptoms even if it is not directly involved in its etiology. The severity of symptoms will depend on the density of bacterial infection and the intensity of inflammatory infiltrate in the gastric biopsy.

P169

Metaplastic Ossification in Soft-tissue Angiomatosis: A Case Report with Review of Literature

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Angiomatosis is defined as a haemangioma that affects a large segment of the body in a contiguous fashion, either by vertical extension, to involve multiple tissue planes (eg. Skin, subcutis, muscle, bone) or by crossing muscle compartments to involve similar tissue types (eg. Multiple muscles). Such lesions usually present in the first two decades of life and have a highly characteristic but not totally specific histologic pattern. Histology usually shows a haphazard mixture of small and medium sized vessels, fat, connective tissue and lymphatics. Large amounts of mature fat frequently accompany these lesions; suggesting that these lesions may be more generalised mesenchymal proliferation rather than exclusive vascular lesions. But metaplastic ossification has not been described in these lesions. Here we present a rare example of angiomatosis showing metaplastic bone formation.

A 41-year old lady presented with swelling of the right thigh. Specimen was a lump of fatty tissue with ellipse of skin, containing nodular grey areas with foci of bleeding and calcified areas. Histology revealed a remarkable vascular proliferation showing vertical extension involving skin and subcutaneous tissue. The proliferation was a mixture of venous, cavernous and capillary sized vessels scattered haphazardly throughout soft/ fatty tissue. An unusual feature was mature bone formation close by the vascular proliferation.

This case not only demonstrates a rare example of osseous metaplasia in angiomatosis, but also supports the contention that angiomatosis may be a more generalised mesenchymal proliferation.

P170

Nodular "Fasciitis" Presenting as a Polypoid Dermal Mass

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This case report describes a variant of "nodular fasciitis" presenting as a cutaneous polyp on the upper arm of an 8 year old girl. Nodular fasciitis is a reactive fibroblastic proliferation that can be mistaken clinically as sarcoma given its rapid growth. As its name implies, nodular fasciitis was originally described involving the fascia. Although very rare dermal cases have been described, this is the first report of a dermal polypoid variant known to us.

P171

Audit of GP Histopathology Requests for Skin Biopsies

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Most histopathology departments receive numerous primary care skin biopsies. In many cases the lesions biopsied are non-serious in nature. However, some of the specimens will contain important/serious diagnoses. Clinical information is important for many histopathological diagnoses. The aim of this audit was to assess the information provided by GPs on skin biopsy request forms, to determine what kinds of lesions GPs are removing and to compare any clinical diagnosis provided with the histological diagnosis.

We examined 100 primary care skin biopsy request forms received in January 2006 at a district general hospital. GPs were excellent at reporting the site of the lesion but only 54% gave a description of the lesion. They were very poor at providing any relevant clinical history and poor at reporting the type of biopsy performed. 41% offered a clinical diagnosis, and of these 76% were found to have given the correct diagnosis. 18 different histological diagnoses were made, with most being non-serious lesions. Seborrhoeic keratosis was the most commonly diagnosed lesion (29 cases), with intradermal naevus the next most frequent diagnosis (17 cases). All other diagnoses were made on 8 or less occasions. 5 basal cell carcinomas, 2 squamous cell carcinomas, and 2 malignant melanomas were removed by the GPs. The malignant melanomas, squamous cell carcinomas and possibly the basal cell carcinomas should have been referred to a dermatologist for removal. We have made a number of recommendations that we hope would encourage GPs to provide all the required clinical information.

P172

Visceral Leishmaniasis Presenting in Kaposi's Sarcoma of the Skin

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A 40 year old man - HIV positive - presented with an 11 month history of multiple asymptomatic purple papules, weight loss and night sweats. Histology of the skin showed features of coexistent Leishmaniasis and Kaposi's sarcoma. Bone marrow aspirate confirmed visceral Leishmaniasis.

There is a high prevalence of visceral leishmaniasis among HIV positive patients. In parallel with this are reports of the co-existence of leishmania parasites in a variety of skin eruptions including Kaposi's sarcoma, dermatofibroma, psoriasiform dermatitis, tattoo infiltration, dermatomyositis and herpes zoster. In our patient the finding of Leishmania parasites within a Kaposi sarcoma enabled a diagnosis of unsuspected visceral Leishmaniasis. The fact that vascular lesions regressed completely after anti-protozoal therapy could support the idea Kaposi sarcoma might be a reactive condition rather than a true neoplasm.

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P173

Comparison of Different Waxes for the Construction of Tissue Microarrays

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Tissue microarrays (TMAs) are important in pathology research. Their construction and the small size and large number of tissue cores used renders them more fragile than whole blocks. The type and quality of wax used to make TMAs may reduce this fragility. Our aim was to assess different waxes to compare their performance in TMA production.

Ten waxes were tested: Histowax (Cellpath), Blue Ribbon, Histowax (Surgipath), Tissue Tek III, Lambwax, Purewax, VA5, Paraplast, Paraplast X-tra and Precision Cut. Tissue was embedded in each wax to create donor blocks and sectioning performed. Separate recipient blocks were made. TMAs were constructed and sections cut and stained. At each step the waxes were assessed, compared and ranked.

In the generation, sectioning and staining of whole blocks, most waxes performed well with only slight cracking. Three waxes showed some tissue separation when sectioning. When making recipient blocks, waxes ranged in firmness, with some rather brittle or soft. In TMA construction, the most brittle waxes impaired tissue coring. For TMA sectioning and staining, most waxes performed well but two were difficult to section.

Overall, three waxes performed consistently well, ranking in the top half for each step assessed, particularly for the TMA-related criteria: Paraplast, Tissue Tek and Lambwax. All three contain polymers, which provide elasticity and are postulated to minimise wax cracking and separation, and are of similar cost. Although the remaining seven waxes were of good quality, we considered that Paraplast, Tissue Tek and Lambwax would be particularly suitable for TMA production.

P174

A Virtual Reality Powerwall Compared to the Conventional Light Microscope: Results of a Pilot Study

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BACKGROUND: Previous work presented to the Pathological society showed that virtual slides are up to 60% less efficient than the conventional light microscope. We sought to develop and test a system to improve the efficiency of virtual slides.

METHODS: A Powerwall is a novel user interface device which allows display of large high resolution images. It comprises an array of 28 LCD screens controlled by an 8 node computer cluster. The resulting image is over 5 by 3 metres in size, but has the same resolution as a conventional computer screen. This allows, for example, a whole gastrointestinal biopsy to be seen at once at a resolution which shows every individual cell in detail. Custom-made software was written to render virtual slides on a 50 megapixel virtual reality powerwall. A controlled experiment was performed with 8 pathologist subjects and 4 standard tasks to compare the efficiency and acceptability of the two modalities. RESULTS: Users had no previous experience of using a powerwall. Efficiency and diagnostic confidence were equivalent - mean time to complete diagnostic tasks was 86s for the conventional microscope and 88s for the powerwall; self-reported diagnostic confidence was over 90% for both modalities.

CONCLUSIONS: This pilot study suggests that a powerwall has the potential to be as efficient as the conventional microscope, and despite the lack of familiarity of subjects with the system their diagnostic confidence was maintained using this new interface.

P175

Systematic Random Sampling with Virtual Slides: A New Software Tool for Tissue Research

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Systematic random sampling is a powerful tool in tissue based research. It allows accurate quantification of tissue elements on pathology slides while minimising the number of samples needed to measure – for example the ratio of cancer to stroma, or the distribution of blood vessels.

Historically optical graticules with conventional light microscopes have been used for systematic randomised sampling, but this method has significant drawbacks in terms of flexibility and user acceptability. Commercial software is available which adds grids to captured micrographs, but this requires individual snapshots of tissue to be taken manually. Using virtual slides for this task is simpler, less labour-intensive, and allows recording of results directly into computer software.

We describe a system using custom made software developed locally which allows users to select arbitrary areas of any virtual slide and add any number of sampling points using a systematic grid. The system is web based, allowing researchers to apply a systematic random grid to any sample from their personal computer. Once the sampling points are generated they can be viewed locally using virtual slide viewing software and the scores entered rapidly and efficiently by the user for export and further analysis.

P176

Tracking with virtual slides: a tool to study diagnostic error in pathology

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BACKGROUND: Diagnostic error in pathology is a significant problem. Studying the reasons for error is difficult because of a lack of data on the diagnostic process - virtual slides are a useful tool to study error in pathology as they allow unsupervised study of diagnosis.

METHODS: Custom software was developed to produce visualisations of the diagnostic track followed by pathologists as they viewed virtual slides. These showed the diagnostic path in 3 dimensions, areas studied for >1000ms, and subject's comments about the areas viewed. The system was used to study 2 trainee and 2 expert pathologists diagnosing 60 oesophageal biopsies showing Barrett's oesophagus. Comparisons of the diagnostic tracks between experts and trainees were studied to classify the reason for errors.

RESULTS: 46 cases had an expert consensus diagnosis. The two trainees made errors in 21 cases (46%) and 15 cases (33%) respectively, of which 11 and 9 were clinically significant errors. Errors were made across the whole spectrum from normal biopsies to intramucosal carcinoma, though there was a tendency for serious undercalls to be more common than serious overcalls. Detailed examination of the tracks showed that in all 36 errors there was an error in interpretation of information; in 3 errors there was an additional component of failure to identify diagnostic features.

CONCLUSIONS: Tracking with virtual slides and the resulting visualisations are a useful tool in studying diagnosis. The tool has the potential for use in training and assessment in order to improve the quality of diagnosis in pathology.

P177

The Digital Pathology Archive: the UMCU initiative

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Background:

Our Pathology Department produces yearly about 100.000 slides. After initial diagnosis, slides often have to be retrieved. Which takes a lot of time, and slide quality deteriorates in time. We have therefore set up infrastructure to create a fully digital archive.

Methods: Two Aperio scanners processes batches of each 120 slides, recognize the tissue on low resolution, auto focus on a defined number of spots, and then scan the tissue in true colour at x20. Logistics to maximize scanning capacity have been worked out. File size is about 500 megabytes on average. The images are stored on a Sun HSM system consisting of a storage server, 6 TB fast hard disks and a tape robot of 120 TB. Access time for the images varies from a few seconds for the hard disk to several minutes for the tape storage.

Conclusions: It is technically and practically feasible to digitize all slides produced by an average size pathology lab, and store them on a SAN. Doing clinicopathological conferences digitally will gradually be implemented which will save a lot of preparation time and increase flexibility in location of the conferences. Further, consultations will be more efficient. Slide images will be integrated within the Electronic Patient File, and practical slide sessions for teaching purposes will be done digitally using the Slidepath application.

P178

Non-Side Population Cell Cycle Suppression by Hoechst 33342 Occurs in the Absence of Significant Cell Death in the Pancreatic Cancer Line Capan-2

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Isolation of a "side population" (SP) via Hoechst (Ho) 33342 efflux assay is a popular tool for adult stem cell identification. High activity of ABC-transporters confers the enhanced dye efflux capacity of SP cells and cytotoxic drug resistance. However, Ho33342 is known to be capable of inducing cytotoxic and cytostatic effects, raising the possibility that enhanced clonogenicity and proliferative capacities exhibited by SP cells versus non-SP cells isolated in this way may be an artefact of Ho33342 overload in the non-SP population.

SP and non-SP cells were isolated from two human pancreatic adenocarcinoma lines (Panc-1 and Capan-2) via standard Ho33342 efflux assay. Viability, colony forming ability (CFA) and proliferation of SP, non-SP and unsorted populations exposed to Ho33342 ± reserpine (an inhibitor of ABC-transporter activity), reserpine or vehicle alone were examined.

Capan-2 SP cells showed elevated CFA versus unsorted Ho-exposed controls. However, in unsorted samples, Ho33342 exposure produced significant decreases in CFA and population doubling rate, exacerbated by co-incubation with reserpine. Conversely, Panc-1 cells showed no significant differences in CFA or proliferation rate between treatments or sub-populations. These differing responses to Ho33342 exposure could not be explained by differences in cell cycle position, DNA content or apoptosis levels. Furthermore, the percentage of propidium iodide-negative viable cells did not vary between lines or treatments.

Therefore, elevated CFA of Capan-2 SP cells may be an artefact of dye-induced suppression of proliferation of the non-SP fraction.

Abstracts

Oral

Note: Presenter's name is shown in **bold**

01

FABP7 Expression Predicts Better Outcome in Basal-Like Breast Cancer

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The recently identified basal-like cancers (BP) have attracted attention as a poor prognostic class of breast cancer. However, BP appear to encompass biologically and clinically heterogeneous tumours; resulting in the lack of a consensus definition of BP.

In order to refine BP, we analysed expression of 48,000 gene transcripts in 132 breast carcinomas using Artificial Neural Network analysis and identified two genes (OATP2 and FABP7) associated with BP (defined by cytokeratin (CK)5/6 and/or CK14 positivity). Using a large invasive breast carcinoma cohort (n=900), prepared as TMA, we assessed the immunohistochemical expression of OATP2 and FABP7 and investigated their association with clinicopathological variables, patients' outcome and ability to refine BP classification.

A total of 8.9% and 15.6% cases were OATP2 and FABP7 positive respectively. OATP2 was associated with high grade tumours (p<0.01), ER, PgR and HER2 negativity (p<0.01) and shorter breast cancer specific survival (BCSS; p=0.04). FABP7 expression was associated with lower lymph node stage (p<0.01), ER, PgR and HER2 negativity (p<0.01), longer BCSS (p=0.05) and disease-free survival (DFS; p=0.01). BP tumours which were FABP7 positive had a significantly longer BCSS and DFS compared with FABP7 negative basal tumours (p<0.01).

This study confirms the biological and clinical heterogeneity of BP in breast cancer. We have identified a novel subgroup of basal tumours showing FABP7 expression that have significantly better clinical outcome. Further studies analysing the role of FABP7 are therefore warranted.

02

Tiling Path Genomic Profiling of Grade III Invasive Ductal Breast Carcinomas

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Grade III Invasive Ductal Cancers of no special type (IDC-NST) comprise up to 60% of all breast cancers. Here we report on genome-wide analysis of 95 Grade III Invasive ductal cancers and copy number alterations specific to Basal-like, HER2 amplified and Luminal tumour groups, utilising a tiling path 32K BAC array platform. We demonstrate that basal-like tumours are predominantly characterised by a 'sawtooth' pattern of alterations and HER2 cancers by a 'firestorm' profile. HER2 and basal-like cancers were found to harbour lower frequencies of 16q whole arm loss than luminal tumours (24% HER2 of 20% Basal-like, 42% luminal). This provides evidence in support of the concept that the majority of GIII HER2 and basal-like cancers do not stem from low grade IDC. Apart from known amplifications associated with basal-like (1q21, 10p, 12p), luminal (8p12, 11q13, 11q14) and HER2 (17q12) cancers, we identified previously unreported recurrent amplifications associated with each molecular subgroups: 19q12 in basal-like; 1q32.1 in luminal, and 14q12 and 17q23 in HER2 cancers. These amplifications harbour genes that may constitute some of the molecular drivers of specific breast cancer subtypes and/ or can be explored as potential therapeutic targets.

03

Downregulation of 15-Hydroxyprostaglandin Dehydrogenase Contributes to Tamoxifen Resistance in Breast Cancer

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Tamoxifen (TAM) is the principal endocrine therapy for premenopausal ER α -positive breast cancer. However, resistance and recurrence remain serious problems. Our previous work indicated that 15-hydroxyprostaglandin dehydrogenase (15-PGDH) was significantly downregulated in tamoxifen resistant (TAMr) MCF7 derivatives compared to sensitive controls. 15-PGDH is the key enzyme for biological inactivation of prostaglandins, including prostaglandin E2 (PGE2), and has been shown to be a tumour suppressor in breast cancer. However, a role for 15-PGDH downregulation in endocrine resistance has not previously been identified. Downregulation of 15-PGDH mRNA and protein in TAMr MCF7 was confirmed by qPCR and Western blotting. To determine the role of 15-PGDH in TAMr, we stably transfected TAMr MCF7 cells with human 15-PGDH cDNA. Overexpression of 15-PGDH in TAMr MCF7 cells partially restored sensitivity of TAMr cells to 4-Hydroxytamoxifen (4HT) by MTT assay, demonstrating that 15-PGDH downregulation plays a functional role in the acquisition of TAMr. Moreover, treatment of TAM sensitive MCF7 cells with PGE2 increased their resistance to 4HT, suggesting that 15-PGDH downregulation contributes to TAMr by increasing PGE2 levels. Treatment of TAMr MCF7 cells with a DNA methyltransferase inhibitor (5-azacytidine), and a histone deacetylase inhibitor (trichostatin A), led to re-expression of 15-PGDH, suggesting that 15-PGDH is epigenetically silenced during the acquisition of TAMr. In conclusion, our data suggest that acquisition of TAMr in vitro involves epigenetic silencing of 15-PGDH, and that 15-PGDH downregulation has a novel, functional role in TAMr.

04

Predicting Outcomes in Sporadic versus Hereditary Breast Cancer: a limited immunohistochemical panel does not identify BRCA1 and BRCA2 carriers

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One aim of the POSH (Predicting Outcomes in Sporadic versus Hereditary) breast cancer study is to identify molecular signatures to predict BRCA status in young women with breast cancer. TMAs were constructed using a training set comprising 144 cases [40 BRCA1, 44 BRCA2 and 60 non-BRCA controls (<40 yrs at presentation)] and immunohistochemistry carried out for ER, Her-2, CK14 and beta6 integrin. For analysis, data were classified as negative or positive and an unsupervised hierarchical cluster analysis performed. Across the cohort, 37% of tumours were ER+, 7% Her-2 positive, 25% CK14 positive and 15% beta6 integrin positive. Significant correlation between ER negativity and CK14 or beta6 positivity was observed (p<0.001 and p=0.04 respectively). Linking expression profile to BRCA status revealed a significant association between BRCA1 mutation and ER negative tumours (34/38 BRCA1 tumours, 17/36 BRCA2 and 32/57 controls, p<0.001). Furthermore no tumours from BRCA1/2 patients expressed Her-2 compared with 10/50 controls (p<0.001). However, heterogeneity within the BRCA1 group was evident, with CK14 positive, beta6 positive and CK14/beta6 negative subsets identified with similar frequency to the non-BRCA controls. This preliminary analysis of the POSH Training set confirms that the majority of BRCA1-related tumours are ER and Her-2 negative, but demonstrates heterogeneity of the group in terms of basal and other markers, and shows that a limited IHC panel will not discriminate BRCA-1 associated tumours in a young patient cohort.

O5

An Altered Myoepithelial Cell Phenotype in DCIS Influences Tumour-Associated Angiogenesis

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The process of angiogenesis is a critical factor in breast tumour progression and has also been demonstrated in preneoplastic breast lesions.

Limited published data suggests that myoepithelial cells are anti-angiogenic. This observation is based on 'normal' myoepithelial cells. It is recognised, however, that myoepithelial cells are altered in DCIS. We have previously demonstrated a difference characterised by the up-regulation of $\alpha\beta6$. This study aimed to investigate whether expression of $\alpha\beta6$ in DCIS is associated with angiogenesis, and to establish if $\alpha\beta6$ expression could contribute to enhanced angiogenesis.

Immunohistochemistry for $\alpha\beta6$ and the vascular marker CD31 was carried out on tissue microarrays (TMAs) of 148 cases of pure DCIS, and on whole sections from 36 of these cases. TMAs were found to be of limited use in analysis. In whole sections, 47% expressed $\alpha\beta6$ with 53% negative for $\alpha\beta6$. The angiogenic pattern was scored as rim pattern, where vessels are adjacent to the DCIS ducts and diffuse pattern, where vessels are present in the surrounding stroma. A rim pattern of staining was only seen in the $\alpha\beta6$ positive group. Using an immortalised myoepithelial cell line (1089) and an $\alpha\beta6$ over-expressing counterpart ($\beta6$ -1089), we demonstrated that $\beta6$ -1089 cells release higher levels of VEGF than 1089 cells and also stimulated more vascular sprouting in a mouse aortic ring assay. This supports the hypothesis that up-regulation of $\alpha\beta6$ on myoepithelial cells in DCIS is of functional significance and may contribute to invasive progression.

O6

Genomic and Immunophenotypical Characterisation of Pure Micropapillary Carcinomas of the Breast

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Pure invasive micropapillary carcinoma (MPC) is a special type of breast cancer characterised by a distinctive growth pattern and a more aggressive clinical behaviour than invasive ductal carcinomas of no special type (IDC-NSTs). To define the molecular characteristics of MPCs, we profiled 12 MPCs and 24 grade and oestrogen receptor (ER)-matched IDC-NSTs using microarray comparative genomic hybridisation (aCGH). In addition, we generated a tissue microarray containing a series of 24 MPCs and performed immunohistochemical analysis with ER, PR, Ki67, HER2, CK5/6, CK14, CK17, EGFR, topoisomerase-II α , cyclin D1, caveolin-1, E-cadherin and β -catenin antibodies. In situ hybridisation probes were employed to evaluate the prevalence of amplification of HER2, TOP2A, EGFR, CCND1, MYC, ESR1 and FGFR1 genes. aCGH analysis demonstrated that MPCs significantly differed from IDC-NSTs at the genomic level. Gains of 1q, 2q, 4p, 6p, 6q23, 7p, 7q, 8p, 8q, 9p, 10p, 11q, 12p, 12q, 16p, 17p, 17q, 19p, 20p, 20q and 21q and losses of 1p, 2p, 6q11, 6q21, 9p, 11p, 15q and 19q were more prevalent in MPCs. Amplifications of 8p12, 8q12, 8q13, 8q21, 8q23, 8q24, 17q21, 17q23 and 20q13 were significantly associated with MPCs. A comparison between 24 MPCs and a series of 48 grade and ER matched IDC-NSTs revealed that high cyclin D1 expression, high proliferation rates and MYC amplification were significantly associated with MPCs. Our results demonstrate that MPCs have distinctive histological features and molecular genetic profiles supporting the contention that they constitute a distinct pathological entity.

O7

Neural networks identify gene profiles that predict clinical outcome with high accuracy in breast cancer: CA IX is identified as the principal prognostic indicator

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Gene expression microarrays allow for the high throughput analysis of hundreds of thousands of gene transcripts. This technology has been applied to the molecular classification of cancer patients. Subsequently, it is recognised that different patient subgroups show distinct molecular signatures corresponding to cancer-associated disease pathways. Importantly, these gene profiles provide information on clinical outcome. A potential handicap of such high throughput technologies is the unprecedented amount of data generated, often of high complexity. This has created a demand for novel data analysis methodologies. Here, we present an Artificial Neural Network based methodology that has identified a prognostic gene expression signature in breast cancer. In applying this approach to a previously published seminal data set we have identified nine key genes whose expression profiles are capable of predicting development of distant metastases to accuracies of 98%. The principal prognostic indicator identified was carbonic anhydrase IX (CA IX), a component of the hypoxia-pathway. We subsequently tested the suitability of CA IX for predicting survival outcome using semi-quantitative immunohistochemical morphometry on a large number of unselected breast cancers. Interestingly, our results showed that membranous CA IX expression is predictive of shortened overall survival, reduced disease free interval, and lymph node involvement.

O8

Clear Cells of the Nipple Epidermis: a Reserve of Motile Mammary Precursor Cells?

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Background: Clear cells of the nipple epidermis [Toker, Cancer 1970] have no known function but, with a relationship to mammary duct ostia and ultrastructural features in common with light cells of the mammary epithelium, it has been proposed that they migrate into nipple epidermis from subjacent ducts [Marucci, Virchows Archiv 2002].

Aims and methods: We examined clear cell morphology and relationships in the nipple in 30 mastectomy breasts. Paraffin sections were immunostained for cytokeratins 7+8, 14 and progesterone receptor (PR).

Results: Ck 7/8 and PR positive clear cells clustered around duct ostia were abundant in 6, fairly numerous in 7, scanty in 10 and absent in 7 cases. They may be single, clumped or form hollow spheroids. Some have a 'foot' on basement membrane. A subset show structural features implying motility. A thin protrusion inserted with the aid of a leading filopodium (microspike) between adjacent keratinocytes is anchored by a rounded, club-like termination. The protrusion thickens, most strikingly at its far end, and adopts a flattened, lamellipodium-like contact profile with adjacent keratinocytes before shortening to draw the cell body in the direction of travel.

Conclusions: Clear cells of the nipple epidermis are associated with duct openings and combine a lack of differentiated features with apparent motility. We propose that they represent a reserve of mammary progenitor cells, and that trafficking of precursor cells between separate mammary lobes via the nipple epidermis is a possible mode of mammary gland maintenance over repeated cycles of pregnancy and lactation.

Development and characterisation of a new breast cancer cell line of basal phenotype

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Breast cancer is heterogeneous and current cell line models cannot adequately reflect all the phenotypic and genotypic changes observed clinically. During our routine development of short term primary cultures of breast cancer epithelial cells we observed spontaneous immortalisation of one of these cultures after 12 months in vitro. These cells, termed LG11T, were isolated in 2002 from a recurrent breast tumour from an elderly patient who received tamoxifen as primary therapy and grew as a monolayer with cobblestone morphology. The primary tumour was classified as a lymph node, ER α and PR negative squamous cell carcinoma but expressed ER β . Phenotypic characterisation by immunofluorescence revealed expression of basal cytokeratins CK5/6 and CK14, the hormone receptors ER β 1 and ER β 2 but not ER α or HER2 or the luminal markers CK18 and CK19, which is consistent with basal breast cancer. As with the primary tumour, LG11T cells remained unresponsive to tamoxifen in vitro, however there was some evidence of hypersensitivity at low doses (>10⁻¹⁰M). Telomerase activity was assessed by a PCR-based ELISA and remained high in LG11T cells which is indicative of escape from senescence. In summary we have developed and characterised a new breast cancer cell line of basal phenotype. As there is currently considerable interest in understanding the biology of basal breast cancer this cell line represents a powerful new tool for investigating this further.

Methylation Pattern of High Risk Flat Adenomas in CRC

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Introduction

Flat colorectal adenomas are considered to have a different molecular pathogenesis than polypoid-shaped lesions and are associated with more aggressive clinical behaviour. In CRC development methylation is an early event, but little is known about promoter hypermethylation in flat lesions.

Aim

To analyze the methylation status of 16 CRC related genes in flat adenomas and compare this to established methylation patterns of polypoid lesions.

Materials and methods

44 FFPE flat adenomas, (classified according to the Paris classification) and 11 flat carcinomas were used. Promoter methylation status of 16 genes (O⁶MGMT, hMLH1, APC, p14^{ARF}, p16^{INK4A}, RASSF1A, RASSF2A, GATA-4, GATA-5, CHFR, NEUROG1, IGF2, HLF, CACNA1G, RUNX3 and SOCS1) was studied by methylation-specific PCR.

Results

The methylation frequency of these genes in flat adenomas was comparable to that observed in polypoid adenomas, except for CHFR (P=0.02) and GATA-5 (P=0.03) which were significantly less frequently methylated in flat adenomas. In the panel of flat carcinomas the promoter regions of GATA-4 (73%) and GATA-5 (100%) were most frequently methylated. Similar methylation patterns were found for flat and polypoid carcinomas, except for CHFR (P=0.03), which shows less methylation in flat carcinomas.

Conclusion

For these 16 genes methylation status was similar for flat and polypoid lesions, except for lower promoter methylation for GATA-5 and CHFR in flat adenomas and for CHFR in flat carcinomas. This is consistent with methylation being an early event in pathogenesis of both flat and polypoid colorectal carcinoma.

Combining Proteomics and Genomics to Identify Biomarkers for Colorectal Cancer

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Introduction and Aim: Detection of colorectal cancer (CRC) at an early stage of disease is a realistic approach to reduce cancer death. The aim of this study is to identify biomarkers that discriminate low-risk colon adenomas from high-risk adenomas and CRC and that can be applied for molecular imaging. By microarray expression analysis, we obtained a list of genes of which mRNA levels are increased in CRC compared to low-risk adenomas. Genes encoding proteins with extracellular domains are the most promising candidates for molecular imaging. Therefore we set out to identify cell surface proteins. **Methods:** CRC cell lines were cultured until 70-80% confluency and incubated with Biotin to biotinylate cell surface proteins, which were isolated from the whole cell lysate. Protein mixtures were fractionated by gradient 1D SDS-PAGE and further processed for in-depth proteomics analysis by liquid-chromatography followed by tandem mass spectrometry (LC-MS/MS). **Results:** A total of 1046 proteins were identified upon analysis of three biological replicates of colo 205 cells, 563 of which were reproducibly detected in all three samples. Integration of the cell-surface proteomics data with the microarray expression data showed that 98 of the 1046 proteins matched with genes that showed mRNA overexpression in CRC compared to low-risk adenomas.

Conclusion: These preliminary data indicate that this strategy resulted in the successful identification of cell surface biomarkers. Currently we are extending the experiments to other CRC cell lines and the results of these experiments shall be presented.

Chromosomal aberrations and APC promoter methylation in sporadic and coeliac disease related small intestinal adenocarcinomas

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Background: Small intestinal adenocarcinoma (SIAC) is a rare malignancy accounting for less than 2% of all gastrointestinal cancers. The molecular mechanisms involved in SIAC pathogenesis are not completely characterized. Coeliac disease (CD) is a chronic disorder caused by intolerance to gluten in genetically predisposed individuals. CD patients show 80-fold increased risk of developing SIAC. Despite this evidence, DNA copy number and methylation changes of CD-related to non-CD SIAC have not been compared.

Aim: To determine the patterns of DNA copy number alterations in non-CD and CD-related SIAC.

Methods: Array Comparative Genome Hybridization, microsatellite instability and APC promoter hypermethylation status were determined in 35 non-CD and 15 CD-related SIAC.

Results: Gains of 7p22-p22, 7q22-q34, 8q24, 20q11-q13 overlapped between CD-related and non-CD SIAC. CD-related SIAC showed frequent (>15%) losses of 5q15-q23 and gains of 5p13-p15, 7p36, 9q34, 13q12-q13 and 20p13-20q11, whereas non-CD-related SIAC showed frequent losses of 4q12-q35. To further investigate the association between 5q15-q23 loss and the APC gene, we determined the methylation status of the APC promoter region. 58% of the CD-related and 32% of the non-CD SIAC showed APC promoter hypermethylation (p=0.009).

Conclusions: this is the largest study comparing CD-related and non-CD SIAC, which show similar patterns of chromosomal aberrations, however, CD-related SIAC present increased frequency of 5q15-q23 losses, APC promoter hypermethylation and decreased frequency of 4q12-q35 losses.

O13

K-Ras and B-Raf mutations in the MRC CR08 FOCUS Trial

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Epidermal Growth Factor Receptor (EGFR) activation by ligand binding, with subsequent activation of Ras/Raf/MAPK pathways, is important for survival, proliferation, angiogenesis and metastasis in some cancers. Monoclonal antibodies to EGFR are used in colorectal cancer (CRC), but tumours with activating KRas mutations may be resistant to EGFR inhibition, due to downstream activation of the Ras/Raf pathway. In FOCUS, advanced CRC patients were randomised to 5FU, 5FU/irinotecan or 5FU/oxaliplatin. We previously showed that benefit from irinotecan/oxaliplatin was associated with topoisomerase-1 (Topo1) expression, assessed immunohistochemically. We investigated whether K-Ras (or B-Raf) status is also predictive of benefit from these drugs.

KRas/B-Raf pyrosequencing and Topo1 immunohistochemistry were performed in 801 patients. There was no correlation between Topo1 expression and Ras/Raf mutations. Mutation rates were consistent with previous literature:

	KRas (codons 12,13, 61)	B-Raf (V600E)	Either
Mutation	336 (41.9%)	60 (7.5%)	392 (48.9%)
No Mutation	449 (56.1%)	729 (91.0%)	406 (50.7%)
No Result	16 (2.0%)	12 (1.5%)	3 (0.4%)

The impact of oxaliplatin/irinotecan on Progression-Free and Overall Survival were analysed according to Ras/Raf status. No association was found: wild-type and mutant patients are equally likely to benefit from these drugs (interaction $p=0.4-0.8$).

KRas/B-Raf mutations are independent of Topo1 and are not predictive biomarkers for irinotecan/oxaliplatin. FOCUS3 will prospectively assess Topo1 and KRas to select optimum combinations of anti-EGFR and irinotecan/oxaliplatin therapy.

O14

Patient Survival According to the Quality of Colonic Cancer Surgery: Time for Action?

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We have previously shown that the quality of colonic cancer surgery varies widely when retrospectively assessing the plane of mesocolic dissection. Until now its relationship to patient survival has remained unknown.

400 colonic cancer resections performed between 1997-2002, which had adequate photographic images, were collected and studied. Cases were graded as having surgery in the mesocolic plane, intramesocolic plane or muscularis propria plane. All cases were assessed by two independent observers with agreement in 85.5% of cases. Additionally, tissue morphometry was performed on the cross sectional images from 252 cases using the Leica QWin image analyzer. Patients were retrospectively followed up for a period of five years to determine overall survival.

32% of cases were resected in the mesocolic plane, 44% in the intramesocolic and 24% in the muscularis propria plane. Better quality surgery removed more tissue around the tumour ($p=0.0003$), with a greater distance to the resection margins ($p<0.0001$). There was a significant relationship between the plane of surgery and survival with a 15% difference between the mesocolic and the muscularis propria grades at five years ($p=0.023$). This effect was particularly strong in TNM stage III disease rising to a 30% difference at five years ($p=0.004$).

We have shown in a retrospective observational study that there is marked variability in the quality of colonic cancer surgery. Improving the plane of dissection through educational master classes may improve outcome, particularly in stage III disease, gaining as much benefit as adjuvant chemotherapy.

O15

The Prognosis of Oesophageal Carcinoma Depends on the Number of Lymph Nodes Examined in the Resection Specimen

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The prognosis in surgically resected oesophageal carcinoma (OC) is dependent on the number of regional lymph nodes involved. In colorectal cancer prognosis is also dependent on the number of lymph nodes examined in the resection specimen. We have investigated whether this phenomenon occurs in OC. 237 consecutive patients undergoing oesophagectomy for OC (median age 61 yr, 184 male, 189 adenocarcinoma, 42 squamous carcinoma, 123 following neoadjuvant chemotherapy) by a regional network were studied. Data obtained from routinely generated pathology reports were analysed in relation to patient survival.

17% of tumours were pT1, 14% pT2, 61% pT3 and 8% pT4. The median lymph node count (LNC) was 12 (range 1-38). LNC correlated strongly with outcome; a plateau was reached after a count of 10. The median, 2 year and 5 year survival was 42 months, 52% and 29% respectively when <10 nodes were examined ($n=88$) compared with 61 months, 68% and 48% when ≥ 10 nodes were examined ($n=149$), $P=0.005$. In 105 patients classified as pN0 the cumulative 2 yr survival was 67% when <10 nodes were examined ($n=45$) compared with 88% when ≥ 10 nodes were examined. On forward conditional multivariate Cox regression analysis LNC was independently associated with survival (HR 0.95, 95% CI 0.91-0.99, $P=0.01$), as was pT stage and the absolute number of positive lymph nodes. The effect of LNC was independent of neoadjuvant chemotherapy.

These results demonstrate the importance of careful pathological examination and lymph node retrieval in OC resections. At least 10 nodes should be examined to designate an OC as pN0.

O16

MAL is Silenced by Promoter Hypermethylation in Gastric Cancer

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Background: T-lymphocyte maturation associated protein MAL has been described as a tumour suppressor gene with diagnostic value in colorectal and oesophageal cancers. Moreover, previous studies have shown inactivation of this gene by promoter hypermethylation. The aim of the present study was to analyse promoter hypermethylation of MAL in gastric cancers and to correlate it to clinicopathological data.

Material and methods: Bisulphite-treated DNA isolated from formalin-fixed and paraffin embedded of 202 gastric adenocarcinomas and 24 normal gastric mucosa samples was subjected to real-time quantitative methylation-specific PCR (Q-MSP). Two regions (M1: -680 to -573 bp and M2: -92 to -7 bp before the transcription start site) within the MAL promoter were analysed. In addition, 17 frozen gastric carcinomas were analysed both by Q-MSP and quantitative RT-PCR.

Results: Methylation of M1 and M2 occurred in 71% and 80% of the gastric cancers, respectively. Concurrent methylation of both promoter regions was observed in 66% of the gastric adenocarcinomas. In normal gastric mucosa methylation of both regions was found only in one sample (4%). Hypermethylation of M2 region correlated with significantly better survival ($p=0.03$) and with downregulation of expression ($p=0.04$).

Conclusion: Promoter hypermethylation of MAL is frequently observed in gastric cancers but not in normal gastric mucosa. These results indicate that MAL is a putative tumour suppressor gene involved in gastric cancer, which may be used as a diagnostic and prognostic marker.

O17

Regulation of the Adenomatous Polyposis Coli gene by the miR-135 family in colorectal cancer

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Background: The canonical Wnt pathway maintains the proliferative compartment at the crypts of the intestine and cell fate and position of the epithelial cells. Inactivation of the tumour suppressor gene Adenomatous Polyposis Coli (APC), a key component of the Wnt pathway, causes formation of adenomas. MicroRNAs (miRNAs) are non-coding RNAs which silence the expression of their target genes. MiRNAs act as oncogenes and tumour suppressors by regulating the expression of genes involved in central mechanisms of tumorigenesis.

Aim: To determine miRNAs involved in CRC initiation by elucidating miRNAs regulating the Wnt pathway.

Methods: A miRNA expression library was used to identify miRNAs regulating the APC gene. Functional relevance of the APC-miRNA interactions was assessed using TOPFLASH reporters and transcriptional induction of c-myc. Expression of the APC gene and the miRNAs was measured by qRT-PCR in 20 adenocarcinomas, 23 adenomas and 18 controls.

Results: We showed that miR-135a&b target the 3'UTR of APC, suppress its expression and induce Wnt pathway activity. We found significant up-regulation of miR-135a&b from controls, through adenomas to adenocarcinomas, which is correlated with decreasing expression of APC.

Conclusion: Our results uncovered a miRNA-mediated mechanism for the control of the Wnt pathway activity and suggest its contribution to CRC pathogenesis.

O19

Changes in Autopsy Practice Following Cardiac Surgery Over 17 Years

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BACKGROUND: There has been a decline in general autopsy frequency and the use of histology in recent years. Previous studies at our centre have demonstrated a constant autopsy rate (86-88%) but declining use of histology after cardiac surgery.

AIMS: To assess changes in the autopsy rate, use of histology and cause of death following adult cardiac surgery in a UK teaching hospital.

METHODS/RESULTS: A retrospective analysis of the local cardiac surgery database identified 5089 patients over the age of 16 who underwent a total of 5885 procedures between April 2002 and October 2007. Deaths in this group were identified by automated database integration with Office of National Statistics data. Autopsy reports from this institution and surrounding hospitals were sought. 226/5089 (4.4%) patients died prior to post-operative discharge with 141(62%) undergoing autopsy. 307/4863 (6.3%) patients died after post-operative discharge. In contrast to those patients who died in hospital only 21 (7%) had an autopsy (p<0.001). Histology was performed in 101/141(72%) of autopsies before post-op discharges, significantly greater than autopsies from 1999-2002 (46%, p<0.0001). Cardiac disease (74%) and multi-organ failure (16%) were the most frequent causes of death.

CONCLUSIONS: Autopsy frequency following death during initial post-operative recovery has declined over the last 17 years yet remains higher than the national average. It is of particular interest that histology has increased significantly compared to 1999 – 2002 and has remained unchanged during the current study period.

O18

An Interobserver Study of Mandard Grading in Patients Receiving Preoperative Chemoradiotherapy for Advanced Rectal Cancer

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Introduction With increasing use of pre-operative chemoradiotherapy (CRT) in advanced rectal cancer, a pathological measure of efficacy would be an attractive method of comparing different chemotherapy regimens (eg Mandard Grading system or pathological complete response (pCR)). The subjective nature of the Mandard system requires prospective evaluation of the degree of interobserver variation using this approach.

Methods 100 histopathological slides of rectal cancer resection specimens were selected for Mandard Grading and were scored by 3 experienced colorectal pathologists. All patients received preoperative fluoropyrimidine CRT in a large Teaching Hospital Trust.

Results 97 slides were included in the study. All 3 pathologists agreed in 58% of the slides. Where interobserver variation was identified the disagreement was by a single point except for 1 slide.

Table 1 shows the agreement between the pathologists. The Kappa statistics between the pathologists varied between $\kappa = 0.61$ and $\kappa = 0.65$ depending on which two pathologists were compared.

		Mandard grade assigned by 2 pathologists				
		1	2	3	4	5
Mandard grade assigned by other pathologist	1	15	4			
	2	6	6	4		
	3		3	19	5	1
	4			6	15	5
	5				7	1

Interestingly in 10 slides there was interobserver variation in allocating a Mandard grade 1 (pCR) to the specimen.

Discussion Using the Mandard Grading system, a moderate degree of interobserver variation is seen amongst experienced colorectal pathologists. This may have implications for the future design of studies that include a pathological regression grading system as part of the assessment of efficacy of CRT regimens.

O20

An Audit of Autopsy Toxicology Requests

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A review of autopsy cases within a single coronial jurisdiction prompted an audit of the toxicology requests. During a two-year period, 213 autopsies had toxicology testing. The cases (age range 5/52 - 95, M 159: F 54) covered a variety of different death scenarios - accident (10), alcohol-related (24), fire (4), head injury (7), drug-related (39), potential homicide/manslaughter (11), vehicle-related (24), sudden death (25), SUDI (8), suicide (56), other(5). Clearly, many are protocol/policy-driven requests, required as part of the autopsy. However, 9 cases were identified (4.2%) where toxicology requests, in retrospect, appeared inappropriate - comprising: other 2/5, vehicle-related 2/24, sudden death 5/25. These included deaths of passengers in motor accidents, some cases with clearly defined macroscopic pathology and one case of inadvertent testing of retained samples. This would suggest that toxicology requesting by a range of hospital and forensic consultants appears appropriate for Coronial purposes and a reasonable use of resources.

O21

Undergraduate exposure to clinical pathology and autopsies: potential effects on junior doctor recruitment into histopathology

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There has been a gradual erosion of clinical pathology teaching from undergraduate medical curricula. Our medical school is the only UK medical school that has retained a block laboratory medicine course, which takes place close to the beginning of the first year of clinical training. At this medical school, which retains a traditional 3-year preclinical and 3-year clinical structure, we accept students from 3 different preclinical courses, as well as from our own one-year graduate entry preclinical course. These students have, therefore, had varying previous exposure to pathology.

Methods: Our 161 first year clinical students were asked to complete an anonymous questionnaire at the beginning and end of the 9-week block course detailing their perception of the role of the histopathologist and their attitudes towards a future career in histopathology.

Results: Prior to the block laboratory medicine course 142/161 (88.2%) students responded, while 124/161 (77.0%) students responded at the end of the course. Prior to the course, 23/142 (16.1%) students had witnessed an autopsy, 119/142 (83.8%) students believed pathologists were medically qualified, 91/142 (64.1%) students were sure that being a pathologist did not entail predominantly forensic work, 88/142 (62.0%) students believed that pathologists had an input into patient management and 87/142 (61.3%) students believed a pathologist might see live patients (e.g., in a fine needle aspirate clinic). Following the block course, 104/124 (83.9%) students had witnessed an autopsy, 117/124 (94.4%) students believed pathologists were medically qualified, 115/124 (92.7%) students were sure that being a pathologist did not entail predominantly forensic work, 112/124 (90.3%) students believed that pathologists had an input into patient management and 113/124 (91.1%) students believed a pathologist might see live patients. Crucially, prior to the course only 6/142 (4.2%) students said they would consider a career in histopathology, while, following the course, this figure had risen to 29/124 (23.4%), $p < 0.0001$.

Conclusions: We are aware that, of UK graduates subsequently entering histopathology training, a significant proportion have undertaken the laboratory medicine course at our medical school. We believe that the provision of such a course plays a crucial role in this recruitment process, both by stimulating an interest in histopathology and by providing the opportunity to gain an insight into what the job entails.

O22

An Evaluation of the Impact of Trainees on a Large Histopathology Department

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Histopathology training schools were introduced in 2001 to address the histopathology workforce crisis, resulting in large cohorts of junior trainees, and a slight increase in the total number of trainees nationally. The possible impact of this has been predicted but not formally investigated.

The laboratory IT system was interrogated from 1999-2007 for numbers of specimens and turnaround time (TAT). A qualitative questionnaire was used to evaluate the impact of trainees in all areas of the department.

Trainees cut up 27-54% and reported 13-41% of surgical cases per annum. As expected the amount of clinical work completed by a trainee increases with experience. On average, TAT was a little over one day slower on cases reported by a trainee compared with those reported by consultants alone. The proportion of cases trainees reported independently varied from 0.6-13%; audit of these reports revealed no major errors. Subjectively trainees were deemed to enhance the department in terms of improved morale, completion of audits, research and a substantial amount of undergraduate teaching.

We have evaluated the impact of trainees on a histopathology department by quantitative and qualitative means. Trainees carry out a significant amount of service work, varying depending on experience, with a modest impact on TAT, and non-service work particularly teaching. Subjectively, trainees improve morale and may improve quality by delivering completed clinical audits. Training histopathologists is not an option; its impact needs accurate assessment to balance appropriately the needs of trainees with the needs of the service.

O23

Dutch carcinoma audits over 2005 and 2006: a consistent 40% had no smear while detection failures were 25% in 2005 and 29% in 2006

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Lack of attendance and low test sensitivity hamper cervical screening programmes. We measured all Dutch cervical carcinomas and its smears prior to diagnosis. We compared results over 2006 with those of 2005.

Using the National Pathology Database (PALGA) we tabulated all cases of squamous- and adenocarcinomas of the cervix uteri in invited age cohorts and controlled 1) whether there was any smear history, 2) whether the smear was an invitational smear and 3) whether the smear diagnoses were coherent with the histological diagnoses (97% of programmed smears over 2006 could be analysed).

Of the 634 women with carcinoma of the cervix in 2006, 224 (35%) had never been screened prior to the diagnosis of cancer. This percentage was similar to 2005 (35%). The remaining 410 women with carcinoma had a smear prior to diagnosis, 294 of whom (46% of all adeno- and squamous cell carcinomas) had been invited by the programme, whereas 116 (19% of all cases) had undergone a smear for other reasons ('spontaneous' or symptoms). The 'advice profile' of the 294 invitational smears from 2006 could be analysed in 291 cases and was as follows: 119 of 291 (41%) had a smear within normal limits, whereas 172 of 291 (59%) either elicited an indirect or a direct referral. 3 cases (1%) could not be tabulated.

The percentage of women that had not been reached by the programme remained stable for two years (35%). Sensitivity of invitational smears remained stable as well 59% in 2006 compared to 60% in 2005.

O24

Use of RNAi in Cell Lines to Generate Optimal Controls for Optimisation of Antibodies for Immunohistochemical Analysis on Formalin-Fixed Paraffin Embedded Samples

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Immunohistochemistry (IHC) is a valuable tool in surgical pathology, with important applications in translational research and basic science studies. Optimisation of antibodies on formalin fixed, paraffin embedded (FFPE) tissues is technically challenging, owing to differences in antigen conformation caused by fixation. Western blotting data, used to establish specificity of antibody binding, is not readily translatable to IHC. It is therefore crucial to use optimally validated positive and negative FFPE controls. We describe the use of FFPE cell line pellets and RNA interference technology to develop optimal controls for IHC. Cell lines known to express high levels of a given gene at mRNA and protein levels were transfected with short interfering RNA (siRNA). Gene silencing was confirmed by RT-PCR and western blot. Pellets of parental (positive control) and siRNA-transfected cells (negative control) were fixed in formalin overnight. Paraffin-embedded cell blocks were produced. Sections from cell blocks were cut and tested by IHC using a 'test battery' approach, in which a number of standardised antigen retrieval techniques were compared to define whether any yield specific staining, by differentiating between the positive and negative FFPE cell preparations. Using this approach, we have successfully optimised antibodies for numerous research uses, including an anti-CRK7 antibody. This antibody was subsequently validated on control tissues and applied to test samples. This approach offers a more objective assessment of the specificity and sensitivity of a given antibody directly on FFPE tissues, and a more rapid translation of expression profiling results into immunohistochemical tests.

O25

3 Dimensional Pathology with Virtual Slides

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Visualisation of tissue in 3 dimensions (3D) has many potential uses in tissue based research. Previously, obtaining 3D models of tissue has been a laborious process requiring manual photomicrography of standard pathology slides or the use of alternative acquisition methods which result in a loss of cellular information.

We describe a method for 3D reconstruction of tissue using virtual slides which allows reconstruction of structures at high resolution (that of tissue micro-architecture) using standard pathology slides. The method uses novel custom-developed software to align (register) virtual slides and extract 3D information from them.

Our methods differ from previous research in this area in that they allow a high-throughput process minimising the need for manual intervention. Virtual slides can be scanned automatically under standard scanning conditions, easily producing sequential images to use in 3D construction. Once low resolution images have been registered, a further novel aspect of our approach is that small tissue micro-architectural details can be reconstructed in 3D from any part of the registered 3D volume without the need to re-scan the area at higher resolution.

Using this method we have produced demonstrator 3D reconstructions of a mouse embryo and cirrhotic human liver with 50-200 sections, and an entire glomerulus with over 200 semi-thin sections.

This technique can be used to study disease processes in 3 dimensions using standard histochemical and immunohistochemical techniques.

O26

The Immunohistochemical Differentiation Between Metastatic Breast Cancer and Malignant Pleural Mesothelioma

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Background. The mesothelioma specific markers, calretinin and cytokeratin 5/6, and the breast cancer marker oestrogen receptor are frequently used to differentiate metastatic breast cancer in the pleura from mesothelioma. However, some metastatic breast cancers may mimic the immunohistochemical profile of mesothelioma. The objectives of this study were to firstly examine the expression of these markers in invasive breast cancer, and secondly, to audit the immunostaining of archived pleural malignancies.

Methods. 64 cases of primary invasive breast cancer were immunostained for calretinin. Tumours that expressed calretinin were additionally immunostained for cytokeratin 5/6. Pleural malignancies diagnosed in biopsies from patients with a history of breast cancer but without asbestos exposure were retrieved and re-examined.

Results. 5 cases of breast cancer (8%) expressed calretinin and 4 of these 5 tumours also expressed cytokeratin 5/6. The pattern of expression of both markers was indistinguishable from that seen in epithelioid mesothelioma. All of the 5 tumours were Blooms grade 3 invasive ductal carcinomas and none expressed oestrogen receptor. The audit found 2 patients had been diagnosed with mesothelioma despite a past history of breast cancer and no history of asbestos exposure. These tumours expressed calretinin but not oestrogen receptor.

Conclusions. Concurrent expression of calretinin and cytokeratin 5/6 and negative expression of oestrogen receptor in high-grade breast tumours is a potential diagnostic problem in pleural biopsies. This may lead to patients with metastatic breast cancer being misdiagnosed with malignant mesothelioma.

O27

Immunohistochemical Expression of CD10 in Cutaneous Basal and Squamous Cell Carcinoma

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CD10 is a zinc-dependent metalloproteinase. CD10 expression has been investigated in some cutaneous tumours, however, data regarding its expression in cutaneous epithelial neoplasms are very limited. We aimed to examine the immunohistochemical expression of CD10 in basal cell carcinoma (BCC) (21 cases) and squamous cell carcinoma (SCC) (16 cases). Positive CD10 staining was identified as brown cytoplasmic with or without cell membrane staining. In all the 16 SCC cases, tumour cells failed to stain to CD10 in contrast to the stromal cells that showed CD10 expression in 13 cases (81%). In BCC cases, the expression of CD10 was noted in tumour cells in 47.6% and stromal cells in 95.24%. Most of CD10+ 7/10 BCC showed well-circumscribed deep margin, however, most of CD10- cases 9/11 showed infiltrating base (P=0.030). BCCs with infiltrating deep margins (12 cases) tended to show CD10 negative basaloid cells (9/12) and CD10 positive stromal cells (12/12) (P=0.0003). There was no significant relation between CD10 and other clinicopathological factors in SCC or BCC. From our results we suggest that CD10 might be a useful immunohistochemical marker to differentiate between BCC and SCC. At least, if tumour cells were CD10 positive, this would favor BCC over SCC. Absence of CD10 in all the SCC and in infiltrating BCC together with its overexpression in the surrounding stromal cells might confer invasive properties to such tumours. However, its relation to other poor prognostic factors needs larger studies to be confirmed.

O28

Genomic Analysis of Follicular Lymphoma Subgroups

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Background: The characteristic genetic feature of follicular lymphoma (FL) is a t(14;18)(q32;q21) present in 85% of cases, leading to inhibition of apoptosis and an indolent phenotype. However, recent studies suggest that there may be a t(14;18)(q32;q21)-negative subgroup of FL that is more closely related to, and better treated as, diffuse large B-cell lymphoma (DLBCL). We sought to determine if there were genetic abnormalities to support such a hypothesis, by studying the genomic profile of a large cohort of t(14;18)(q32;q21)-positive and negative lymphomas of follicle centre origin.

Methods: A combination of morphology, immunohistochemistry and fluorescence in situ hybridization was used to identify groups of translocation positive and negative FL, and DLBCL of germinal centre type. Karyotypic analysis (N=100) and microarray-based comparative genomic hybridisation (aCGH) (N=40) were then used to determine the chromosomal aberrations in each group.

Results: t(14;18)(q32;q21)-positive and negative FL were found to have almost identical genetic profiles, with the exception of a loss of 1p36 found in 70% of t(14;18)(q32;q21)-positive FL but only 10% of t(14;18)(q32;q21)-negative FL. A greater difference in chromosomal profiles was witnessed when t(14;18)(q32;q21) negative FL was compared with the cohort of DLBCL.

Conclusion: The results indicate that t(14;18)(q32;q21)-negative FL is genetically more closely related to t(14;18)(q32;q21)-positive FL than to DLBCL. Further investigations are required to determine whether there are more subtle genetic abnormalities or epigenetic phenomena that might explain the pathogenesis of t(14;18)(q32;q21)-negative FL and account for the aggressive behaviour of a subset of cases.

O29

Genomic instability of haemopoietic cells in newly diagnosed Myelodysplastic syndrome (MDS) and Acute myeloid Leukemia (AML) assessed by G-banding, multicolour FISH and array-CGH

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We hypothesised that if haemopoietic cells are genetically unstable during MDS-AML transformation in-vivo, that this instability might express itself in tissue culture during cell divisions, in-vitro, over 4 days. Therefore we have followed bone marrow cells from 12 patients looking at different cell divisions (0hrs, 24hrs, 48hrs and 96hrs) in tissue culture. We employed conventional cytogenetics, multicolour FISH and array based comparative genomic hybridization (array-CGH) to identify all aberrations and to search for genomic imbalances. We detected copy number changes by array-CGH even in patients who had a normal karyotype. Data from 9 patients (each with data from 0hrs, 24hrs, 48hrs and 96hrs) were grouped and a mixed model ANOVA was applied. 180 clones showed a significant and progressive change ($p < 0.001$); (Test/Reference Ratios) with culture progression. Of the clones that showed the greatest change with culture progression ($p < 0.001$), 10% were identified as genes relating to MDS/AML (e.g. APOC1, PRDM16, APC), 47% related to cancer in general and 43% were not related in cancer. In contrast of the clones that showed the least change with culture progression only 3% were related to MDS/AML, 65% related to cancer and 32% were not related to cancer. Chromosome 5q deletion is an important prognostic marker in MDS-AML. Interestingly, 5q was the only chromosome that showed loss of DNA over time (0hrs to 96hrs). Array CGH is a powerful tool and could potentially provide important prognostic and pathogenic information in MDS-AML.

O30

PI3K/AKT/mTOR Pathway Implicated in Pathogenesis of Non-Skull Based Chordomas

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Chordomas are radio and chemoresistant tumours and upto 40% of patients develop metastatic disease. The aim of this project was to identify molecular therapeutic targets for chordoma. Reports of chordoma occurring in patients with tuberous sclerosis complex implicate the tumour suppressor genes TSC1 and TSC2 in addition to other molecules involved in this signaling pathway, including mTOR and its effector molecules 4E-BP1 and eIF-4E, in the pathogenesis of chordoma. Study of this tumour is hampered by its rarity and absence of cell lines. Method and results: A tissue microarray containing 50 conventional chordomas was analysed for expression of active molecules involved in the PI3K/AKT/TSC/mTOR transduction pathway by immunohistochemistry: results were confirmed by western blot analysis. We found that PTEN expression was lost in 16% of cases: chordomas were positive for the following molecules: pAKT (91%), pTSC2 (95%), pS6K (61%), p4EBP-1 (96%), eIF4 (97%), cyclin D1 (38%), P27 (59.5%) and P53 (54%). Mutations failed to be identified in PIK3CA, KRAS, and RHEB1 (genes involved in activation of this pathway) using genomic DNA from tumours. Finally we had limited success in generating xenografts for testing inhibitors to this pathway.

Conclusion: Our data indicate that the PI3K/AKT/mTOR pathway is activated in chordomas, and that somatic mutations in TSC2 only rarely account for this disease. These data provide a rational basis on which to consider patients with chordoma for treatment with rapamycin, its analogues and/or the newly described eIF-4E antisense oligonucleotides.

O31

Activating Gs(alpha) Mutational Analysis Can be Used as a Diagnostic Tool for Intramuscular Myxomas (IM)

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IM is a rare benign soft tissue neoplasm that occurs either in isolation, or less commonly in association with fibrous dysplasia (Mazabraud's syndrome). Fibrous dysplasia (FD) is a benign intramedullary osteofibrous neoplasm which affects either one (monostotic) or multiple (polyostotic) bones. FD, and endocrine neoplasms developing on a background of McCune Albright syndrome, result from post-zygotic activating Gs α mutations resulting in a mosaic distribution of tumour-bearing cells. These mutations have also been identified in a small number of IM. IM can be difficult to distinguish from other myxoid neoplasm especially on needle core biopsy and it would be valuable to have a molecular marker. The aim of this study was to ascertain the incidence of Gs α mutations in a large number of IM.

Materials and Results: Using mutation-specific restriction enzyme digest (MSRED) 28 cases of IM, including cellular variants, were investigated for the 3 commonest Gs α mutations: all negative cases were subsequently tested for 7 rarer mutations. Eight (29%) revealed point mutations in the 2 most commonly affected codons. Single nucleotide polymorphism array analysis was also performed on 5 cases revealing no chromosomal losses or gains. Chart review revealed that only 2 cases had co-existing FD and none of the lesions recurred following excision.

Conclusion: This is the largest study into the presence of Gs α mutations in IM and demonstrates that 29% of tumours harbour this mutation. Does this tumour represent a mosaic disorder?

O32

pS2 (TFF1) expression in prostate carcinoma, correlation with steroid receptor status

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pS2 or TFF1 is a member of trefoil factor family which is distributed throughout the gastrointestinal tract in both normal and diseased tissues. It is also considered as one of the major estrogen regulated proteins and an indicator of estrogen receptor (ER) functionality. Our purpose was to correlate pS2 expression to steroid receptor status (ER alpha and progesterone receptor) and other pathological variables in prostate carcinoma. Fifteen benign prostate hyperplasia (BPH) and 47 prostate carcinoma cases were investigated by means of immunohistochemistry for pS2, ER and PR expression. Eighty percent of BPH showed cytoplasmic immunoreactivity for pS2 in hyperplastic acini and about half of these cases also exhibited nuclear staining decorating basal or both basal and luminal nuclei. pS2 was highly expressed in prostate carcinoma (91.4%) with both cytoplasmic and nuclear patterns of staining. This last pattern was significantly associated with carcinoma of low Gleason score ($p = 0.02$). pS2 lacked any significant correlation with steroid receptor status, stage or grade. The diagnostic value of pS2 expression in prostate carcinoma validated 74.19% accuracy, 91.48% sensitivity and 78.18% positive predictive value. The absence of correlation and dissimilarity in immunolocalization between pS2 and ER alpha leads to the assumption that ER alpha could not be the regulatory protein for pS2 and may raise questions about the functionality of estrogen receptor alpha in prostate. Nuclear pattern of pS2 immunoreactivity either in benign or malignant prostatic lesions is similar to the published data on ER beta distribution.

Molecular Genetic Profiling of Ovarian Clear Cell Adenocarcinomas

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Ovarian clear cell adenocarcinomas (OCCAs) are relatively resistant to platinum-based chemotherapy and associated with a poorer prognosis when compared to other subtypes of ovarian cancer. Hence, the identification of therapeutic targets for OCCAs would be invaluable. To characterise the molecular genetic profiles of OCCAs and identify potential therapeutic targets, the genetic profiles of 52 OCCA tumours were analysed using a 32K tiling-path microarray CGH (aCGH) platform. Genome-wide aCGH analysis revealed that OCCAs could be classified into “simplex” (62%), “complex” (23%) and “amplifier” (15%). Recurrent amplifications (in >20% of OCCAs) included 1p36.32, 8q24.21, 8q24.3, 10q26.3, 17q25.3, 19p13.3, 20q13.33, and 21q22.3. Of particular interest is a region of recurrent amplification on 20q13.33 with a frequency of 50%. The smallest region of overlap within this amplicon maps to a 543kb region on chromosome 20q13.33 between 61178kb to 61721kb and includes YTHDF1, BIRC7, ARFGAP1, COL20A1, EEF1A2, PTK6, SRMS, PDIP1 and GMEB2. We are currently optimising in house generated chromogenic in situ hybridisation probes to validate this amplicon in a larger series of OCCAs. Amplification and overexpression of PTK6 has been described in high-grade serous ovarian carcinomas and is associated with a poor prognosis, while expression of the antiapoptotic BIRC7/Livin gene is associated with chemoresistance in renal clear cell carcinomas. Our study provides the first high-resolution molecular genetic analysis of OCCA tumours and identifies putative amplicon drivers that may be exploited as therapeutic targets.

Pure Salivary Duct Carcinomas Can Be Classified Into Luminal, Her2 and Basal-Like Phenotypes

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Salivary duct carcinomas and invasive ductal carcinomas of the breast have similar morphological features. It has recently been demonstrated that invasive breast cancers can be subclassified into luminal, HER2 and basal-like cancers and that these groups have distinct molecular characteristics and clinical behaviour. The aim of this study was to apply an immunohistochemical panel previously validated for breast cancer to determine whether salivary duct carcinomas could also be classified into these similar molecular groups (i.e. luminal, HER2 and basal-like). 27 pure salivary duct carcinomas were reviewed by one of the authors, typed and graded according to the WHO classification, and stained with antibodies against oestrogen receptor (ER), androgen receptor (AR), HER2, epidermal growth factor receptor (EGFR) and cytokeratin (Ck) 5/6. Cases were classified as of HER2 phenotype if they expressed HER2 3+. HER2 negative cases were classified as of ‘luminal’ phenotype if positive for ER or AR. Cases that lacked HER2, ER/ AR and expressed EGFR and/or Ck 5/6 were considered of ‘basal-like’ phenotype. Cases lacking all markers were considered of indeterminate phenotype. 15% were of HER2 phenotype, 70% were of luminal phenotype, 3% were of basal-like phenotype and 11% were of indeterminate phenotype. In a way akin to breast cancer, salivary duct carcinomas can also be classified according to the molecular subgroups. Most importantly, we report for the first time the existence of a subgroup of pure salivary duct carcinomas that have the typical immunohistochemical profile of ‘basal-like’ carcinomas.

Abstracts

Speakers

Note: Presenter's name is shown in **bold**

S1

Virtual slides in research and diagnosis

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Virtual slides are rapidly becoming an essential technology in pathology research and in teaching. While they are less commonly used in diagnosis the technology is surely heading in that direction – the integration of digital imaging in radiology is now complete in the UK.

This talk gives an overview of the technology, describes the use of virtual slides in diagnosis and research, and discusses the advantages and the challenges – technical and otherwise – of virtual slides. Aspects of the virtual slide project at Leeds will be described.

In particular aspects of integrating virtual slides into laboratory workflows, user acceptability, and usability will be covered. Further work needed to develop the technology in future will also be discussed.

S2

NCRI/NCI Bioinformatics Initiative

S Bell¹

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At present, data, services and tools generated via individual cancer research endeavours are frequently unavailable to the wider research community. Utilising IT 'grid' technology, the UK National Cancer Research Institute (NCRI) Informatics Initiative and the National Cancer Institute (NCI) are working to provide the means to locate and access these datasets, services and tools.

The NCRI's 'Informatics Platform' and the NCI's cancer Biomedical Informatics Grid™ (caBIG™) are demonstrating the potential of interoperable technologies to enhance research, allow re-use of data and potentially allow faster translation of basic research to new treatments.

To exemplify how the Informatics Platform can provide real benefits to clinicians the NCRI Informatics Initiative supported an 'Imaging and Pathology' demonstrator project.

This project drew on the combined expertise of a consortium of leading UK research centres to integrate pathology and multi-modal medical image data drawn from clinical trials of colorectal cancer. The project allowed imaging techniques to relate diagnostic parameters and features within the images and data sets to transform that data into clinically relevant information of direct benefit in diagnosis and treatment. The project successfully proved that linkage of imaging data sets was feasible and provided real value to researchers, and is now being extended further in collaboration with the NCI.

The NCRI continues to make progress in the development of a publicly-available Informatics Platform to facilitate cancer research. To this end the NCRI Informatics Initiative has the support of the UK Government, charities and industry, amongst others.

S3

A Human Protein Atlas

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The completion of the human genome sequence has opened up a possibility for global expression profiling of human tissues and cells, allowing for comparative studies between normal and disease tissues. A multi-disciplinary research program to create a "Human Proteome Resource" was started in July 2003. The aim of the program was to allow for systematic exploration of the human proteome using antibody-based tissue proteomics, combining high-throughput generation of mono-specific antibodies (affinity-purified) with protein profiling in human tissues and cells using tissue microarrays. Recombinant protein fragments selected from unique regions called Protein Epitope Signatures Tags (PrESTs) were used as immunogens to generate antibodies. Analysis of protein expression patterns was performed on tissue and cell microarrays containing >700 spots of normal and cancer tissues as well as in vitro cultured cells.

We have used this strategy to construct a comprehensive, antibody-based protein atlas for expression and localization profiles in 48 normal human tissues and 20 different cancers. The results are presented in a publicly available database (www.proteinatlas.org) containing images and data from protein profiling using over 3,000 antibodies. Each image has been manually annotated and curated by a certified pathologist to provide a knowledge base for functional studies and to allow searches and queries about protein profiles in normal and disease tissue. Our results suggest that it should be possible to extend this analysis to a majority of all human proteins thus providing a valuable tool for medical and biological research.

S4

Workplace-Based Assessment

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The Royal College of Pathologists set up a Working Party on Workplace Based Assessment (WPBA) in May 2006, recognising the importance of WPBA in specialist medical training, and fulfilling the Postgraduate Medical and Education Training Board's (PMETB) standards. WPBAs for pathology trainees have been under-utilised and poorly recorded, but can provide robust evidence for the Record of In Training Assessment (RITA) process, and for the End of Stage Reviews (ESRs) for Senior House Officers, and will continue to support the Annual Review of Competence Progression (ARCP) system that has replaced them.

For histopathology trainees, methods include Case-based Discussion (CbD), Direct Observation of Practical Skills (DOPS) and Evaluation of Clinical Events (ECE), together with multi-source feedback gathered on-line with e-PATH SPRAT. CbDs assess decision making, and the application of medical knowledge in individual cases. DOPS evaluate the capacity of trainees to complete day-to-day routine tasks, with immediate feedback about strengths and weaknesses. ECEs look at integration of simple skills into complex tasks, such as presentations at multidisciplinary team meetings or clinicopathological correlation. The e-PATH SPRAT is 3600 appraisal, where characteristics are rated on a standard form, completed on line by several raters; this includes self-assessment.

Education research in clinical disciplines shows that formative WPBA can change trainees' behaviour. The commitment of trainers, properly trained, is crucial: anything less risks generating a lot of pointless box-ticking.

S5

The MRCPATH Autopsy Examination

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The MRCPATH examination changed radically in 2005, with the Autopsy and Histopathology/Cytopathology modules separated and becoming non-contingent. The revised full-day autopsy exam enabled a fuller evaluation of candidates, but suffers from lack of standardisation in all the three sections (autopsy dissection & presentation, laboratory diagnosis, viva). Along with problems from 1) increasing difficulties in providing training in autopsy procedures and the associated 2) introduction of Autopsy Optional Training (AOT) in the near future, and 3) organising autopsy centres within the exam time frame, a new format has been consulted upon and (in general) agreed. The new exam is for those trainees not opting out of autopsy practice. In outline this will be:

A. a workplace based assessment (WPBA) of actual autopsy dissection competence

- undertaken after stages A+B of training, at the mutual convenience of trainee and assessors
- at the trainee's home mortuary, or the regional examiner's if appropriate
- assessed by the local educational supervisor plus a visiting regional examiner
- to be completed within 3 hours, including write up and documentation of cause of death

B. Once a trainee has satisfied this WPBA, a centralised part 2 examination with all candidates in one place, undertaken twice a year. This includes:

- critical evaluation of autopsy reports
- viva on communication skills & medico-legal aspects
- microscopy and gross diagnosis of autopsy material
- biochemical, toxicological and microbiological evaluation
- questions on autopsy practice
- case interpretation and cause of death

S6

Chromosomal Instability in Colorectal Adenoma to Carcinoma Progression & Early Detection of Colorectal Cancer

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Colorectal adenomas are commonly found in individuals over 60 years of age, and only a minority of colorectal adenomas progress to adenocarcinomas. This progression is mostly associated with overt chromosomal instability (CIN). In contrast to the less frequently occurring microsatellite instability, which is caused by failing DNA mismatch repair, the mechanisms underlying CIN in colorectal adenoma to carcinoma progression are still largely obscure.

We have investigated the patterns of CIN in colorectal adenoma to carcinoma progression by measuring DNA copy number changes with arrayCGH. Amongst others, frequent DNA copy number alterations on chromosomes 13q and 20q turned out to affect mRNA and miRNA expression of multiple genes like AURKA and the miRNA cluster mir-17-92.

Apart from mutations and DNA copy number alterations, also promoter hypermethylation is an important factor in colorectal carcinogenesis. When comparing promoter hypermethylation and DNA copy number changes in different stages of colorectal carcinogenesis, promoter hypermethylation occurred earlier. In addition, also specific associations between DNA copy number alterations and promoter hypermethylation occurred like promoter methylation of GATA-4 and p16INKA4 that were inversely related to chromosomal loss at 15q11-21 and gain at 20q13 respectively (P values: 3.8×10^{-2} and 4.5×10^{-2} respectively).

Current challenge is to translate this knowledge into approaches for molecular early diagnosis of colorectal cancer, like stool DNA based tests. Methylation markers already have yielded promising results, but we also have generated pilot data for detecting DNA copy number alterations in stool derived DNA

S7

The Molecular Pathology of Cervical Neoplasia

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It is now accepted that almost all cervical neoplasia is causally associated with high-risk human papillomavirus (HPV) infection. However, HPV infection is not sufficient for the development of neoplasia and other factors are involved. HPV integration has emerged as an important factor in the progression of HPV-associated neoplasia and the effects of integration, notably loss of HPV E2 expression and up-regulation of HPV E6 and E7 expression, can lead to many of the molecular features associated with the development of high-grade intraepithelial and invasive squamous and glandular lesions, for example telomerase activation and chromosome abnormalities. Sites of frequent chromosome abnormality include 3q, 5p and 20q, all of which have been associated with gain and/or elevated expression of specific genes, for example the telomerase gene TERC, the microRNA processor Drosha, and DNA methyltransferase 3B (DNMT3B) respectively. This is consistent with the sequential methylation of several tumour suppressor gene promoters, notably MGMT, during the progression of HPV-transfected keratinocytes to anchorage independence. Thus, both genetic and epigenetic changes appear to be important in the progression of HPV-associated neoplasia, driven by the effects of HPV infection and integration.

S8

Occult Lymphoproliferative Disorders

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The use of sensitive flow cytometric and molecular diagnostic tests has led to the detection of low level lymphoproliferative disorders in increasing numbers of healthy individuals. In the case of CLL it is estimated that around 3% of the adult population may be affected and a similar proportion can be shown to have a paraprotein. There is less information on the prevalence of other forms of occult lymphoproliferative disorder, but it is clear that low levels of B-cell lymphoproliferative disorders with non-CLL phenotypes are also relatively common. This is consistent with the frequent detection of t(14;18) by sensitive PCR in healthy individuals. Clonal T-cell lymphoproliferative disorders, usually with a cytotoxic T-cell large granular lymphocyte (LGL) phenotype, are also frequently encountered in the investigation of lymphocytosis detected on routine blood counts.

The detection of these conditions in the course of other forms of medical investigation and treatment raises a number of important issues. If the patient has Diffuse Large B-cell Lymphoma the possibility that disease has arisen by transformation of an underlying indolent lymphoma will need to be addressed and this may have prognostic implications. In other patients the detection of an occult lymphoproliferative disorder may cause significant anxiety and require follow up commensurate with the risks of disease progression. Finally, the clinical consequences and disease associations of clonal T-LGL disorders are well recognised. In contrast, the possible health effects of the more numerous B-cell disorders remains poorly understood.

S9

Incipient testicular germ cell neoplasia: biology and possible clinical intervention

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Within the human testis, various germ cell lineage-derived cancers can be found. In neonates and infants, teratomas and yolk sac tumours are diagnosed, i.e., type I Germ Cell Tumours (GCTs), for which no precursor cells are identified so far. The adult testes predominantly develop type II GCTs, i.e., seminomas and nonseminomas. The III GCTs of elderly are rare and benign, of which the precursor cell is likely a primary spermatocyte.

Type II GCTs originate from carcinoma in situ (CIS). CIS and seminoma cells mimic primordial germ cells/gonocytes, a maturation stage normally absent in adult testis. The embryonic stage is demonstrated by expression of OCT3/4. OCT3/4 is proven to be a highly informative diagnostic marker for CIS, seminoma and embryonal carcinoma. In addition, it can be used as target for a non-invasive screen, in which the diagnosis is based on semen investigation. Most recently, SOX2 is identified as a diagnostic marker for embryonal carcinoma and SOX17 for seminoma (and CIS). This provides an informative diagnostic profile based on three genes.

Besides undescended testis, infertility (combined with microlithiasis) and familial predisposition, disorders of sex development (DSD) is a risk factor for type II GCTs. Specifically, hypovirilization and various forms of gonadal dysgenesis, in the presence of the so-called GBY region, result in an increased risk. In case of gonadal dysgenesis, gonadoblastoma can also be the precursor lesion. Knowledge about the pathobiology allows development of methods for early diagnosis in males with an increased risk.

S10

Stem Cells

R Poulson¹

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Stem cells have been claimed to offer the basis for cell-based therapies for a wide range of diseases, although significant problems exist. Therapeutic transfer of haematopoietic stem cells is now well established and effective, but attaining this has required considerable experimentation in animal models and in patients over several decades. Our understanding of other adult stem cell populations is nowhere near so complete. There is good evidence for their existence in gut and skin, yet there is debate over their existence in the liver and kidney, and over the value and reproducibility of putative stem cell markers such as CD133 and Igr5 (GPR49). Therapies relying on cells that exhibit certain stem cell characteristics (such as clonogenicity and the ability to generate differentiated daughter cells) are being developed, with some notable progress in regeneration of corneal epithelium (from cultured limbal stem cells) and of the skin (from cultured and genetically engineered epidermal stem cells). Retrovirus-induced overexpression of key stem cell proteins induces a pluripotent stem (iPS) cell phenotype in cultured human and mouse fibroblasts. iPS cells have the potential to replace embryonic stem (ES) cell cultures for the isolation of tissue-specific stem cells for regenerative medicine. There are significant inherent risks from multiple retroviral integrations, yet iPS cells have already been used to generate haematopoietic stem cells capable of rescuing mice that model human sickle cell disease. Better understanding of stem cells? behaviour, normally and during tissue regeneration, may help develop safer future therapies.

S11

Organ Regeneration

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¹University of York

A shortage of donor organs, the ageing population and growing quality-of-life expectations are all driving research to find novel solutions for organ regeneration. Tissue-engineering is a multidisciplinary approach that incorporates cell biology, materials sciences and engineering into the construction of tissues suitable for transplantation. Although the objectives are clear, there are still a number of major scientific and technical obstacles to progress, including how to manipulate in vitro-expanded cells to form organised, functional tissues. The urinary bladder will be used as an example to indicate the range of strategies being pursued and to discuss progress and unresolved issues in the field.

S12

Spinal degenerative disease

T Freemont¹

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It is now recognised that back pain is caused by degeneration of the intervertebral disc. The analysis of degeneration by molecular pathology has shown that this is an active disorder and very different from that implied by the term "degeneration". Work from our laboratory has shown that the processes of degeneration are triggered by a change in the normal cytokine biology of the disc, to one that favours catabolic rather than anabolic processes. All the available data indicate that the key cytokine is IL-1 and the degeneration mechanism is driven by MMPs, and related degradative enzymes.

The resulting back pain is caused by loss of disc height and nerve ingrowth. Demonstrating that these mechanisms are key to degeneration, has also allowed the identification of molecular and other targets, to reverse the processes of degeneration. Amongst these are the use of stem cells, novel biomaterials and delivery of regulatory biologics to regenerate normal disc tissue in the site of previous degeneration.

S13

Liver Regeneration: From start to finish

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Liver regeneration is normally achieved by the recruitment of formerly quiescent hepatocytes into the cell cycle; in the rat after a two-thirds partial hepatectomy (PH) hepatocytes begin entry into S phase within 15 hours. In young animals all hepatocytes traverse the cell cycle at least once after PH, thus no distinct stem/progenitor cell compartment is involved. The response is initiated by the inflammation-triggered release of cytokines such as TNF α and IL-6 from Kupffer cells, priming hepatocytes to respond to mitogens such as HGF and the EGFR ligands. TGF β 1 produced by stellate cells, and suppressor of cytokine signalling 3 (SOCS3) preventing Stat3 phosphorylation, are both implicated in the curtailment of the response. In human liver, expression of the embryonic transcription factors Nanog and Oct4, together with Stat3, may identify hepatocytic stem cells, and loss of sensitivity to TGF β negative signalling may herald their transformation to cancer-initiating cells. Searching for patches of cells expressing an identical mutation in mtDNA-encoded cytochrome c oxidase, we have found strong evidence for clonal expansion within normal human liver.

In many chronic liver diseases (e.g. cirrhosis) hepatocyte replicative senescence ensues, resulting in the activation of a facultative stem cell compartment located within the smallest branches of the intrahepatic biliary tree – the canals of Hering. This results in a ductular response producing hepatic progenitor cells (HPCs) that are at least bipotential, generating hepatocytes and cholangiocytes. In rodents, HPCs are known as oval cells, and hepatocyte-derived SDF-1 and T cell-derived TWEAK are involved in their activation.

S14

The pathology of colorectal and gastric carcinomas in the elderly: clinicopathological characteristics and molecular mechanisms

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The occurrence of malignant neoplasms increases with advancing age. Although aging and carcinogenesis are basically different processes, there are phenomena common to each such as accumulation of DNA damage and abnormal proteins. Colorectal and gastric carcinomas are representative tumours in which the prevalence and the number of patients increase significantly with age, as well as lung and prostatic carcinomas. Compared with colorectal and gastric cancers occurring in younger patients, those occurring in older patients have clinicopathological differences in tumour location, gender distribution, histological type, histological diversity, multiplicity, incidence of lymph node metastasis, and favorable prognosis. In the elderly, there are peculiar types of carcinoma such as medullary-type poorly differentiated colorectal adenocarcinoma and solid-type poorly differentiated gastric adenocarcinoma, both of which prefer to occur in older women. These tumours have characteristic features such as hypermethylation of the promoter region of hMLH1 gene with absent hMLH1 expression and microsatellite instability. Generally, methylation status of the genomic DNA gradually changes with aging. Aging-related genome-wide decreases in methylation have been observed along with both hypermethylation and hypomethylation. Methylation depends on the tissue and the gene. In the development of colorectal and gastric carcinomas, hypermethylation of the hMLH1 promoter increases with age and may contribute to the induction of 15% of all carcinomas of the large bowel and stomach in elderly patients. Other mechanisms, e.g., chromosomal instability due to telomere dysfunction, are responsible for the development of most carcinomas in the elderly. Methylation, apoptosis, and telomere dysfunction play important roles in the development of colorectal and gastric cancers in the elderly.

S15

Colorectal polyps and the new Colorectal Cancer Screening Programme

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Colorectal polyps, particularly adenomas and hyperplastic polyps, are common in the ageing population. With the introduction of the national bowel cancer screening programme (BCSP) pathologists are likely to see large numbers of polyps, some of which will cause diagnostic problems. The recent recognition of new types of serrated polyp (traditional serrated adenoma and sessile serrated adenoma) is especially likely to cause diagnostic confusion. Malignant polyps will be seen more often than previously and must be differentiated from adenomas showing epithelial misplacement (pseudoinvasion). An evidence based approach to the management of these polyp cancers is very important since the pathology report will strongly influence whether the patient proceeds to surgery or is managed conservatively. Finally the BCSP represents a unique opportunity to improve national standards in typing and grading of adenomas, subjective features which may determine subsequent surveillance of the colon.

S16

Basics of the epidermal growth factor receptor: Amplifications, mutations and transcriptional regulation

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The epidermal growth factor receptor as a tyrosine kinase couples binding of extracellular growth factors to intracellular signal transduction pathways, mediating physiological environmental response for embryogenesis, organogenesis, tissue regeneration and wound healing, as well as pathological processes in carcinogenesis and tumour progression. Abnormal function of the members of EGFR resulting in receptor hyper-activation due to gene amplification, protein overexpression or abnormal transcriptional regulation has been detected in breast, lung, bladder, oral and ovarian cancer. Antibodies inhibiting EGFR and small-molecule inhibitors impairing EGFR tyrosine kinase activity have already been applied in clinical therapy regimens. Disappointingly, the results from these studies have noted that the level of expression of EGFR cannot predict the sensitivity of cells to the inhibitors *in vivo*. Applying only in a small subset of advanced NSCLC patients mutations in the ATP-binding domain of the EGFR predict for complete responses. A novel transcriptional regulation mechanism has been described that depends on the length of a CA repeat in intron 1 of the EGFR gene. Thereby, the number of CA repeats is inversely correlated to pre-mRNA synthesis. Indirect evidence for the importance of this mechanism includes the preferential occurrence of amplifications in that sequence in premalignant and cancer tissues. From a practical perspective, assessment of the CA repeats as a predictor for clinical outcome, therapy side-effects and response can be easily measured in normal and cancer tissues (blood cells, skin, tumour biopsies), in an assay that is technically simple, objective, and even quantitative.

S17

Pitfalls in Immunohistochemistry of the Epidermal Growth Factor Receptor

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The Epidermal Growth Factor Receptor (EGFR, HER1) is a prognostic factor and therapeutic target in various types of cancers. Besides looking at genetic alterations in EGFR such as amplifications and mutations, immunohistochemistry has been employed to assess overexpression. There are many commercially available antibodies against EGFR raised against different epitopes in EGFR that provide varying results with regard to robustness and pattern of staining, varying from cytoplasmic to membrane staining, and clinical value. Besides, antibodies have been raised that specifically recognize phosphorylated EGFR. All this has caused much confusion and may explain the varying results between studies. This presentation will give an overview the specifics of these various antibodies, critically reviews the results obtained and will try to come up with some guidelines with regard to their usefulness for clinical practice or research applications.

S18

Targeting EGFR in Clinical Oncology

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The Epidermal Growth Factor Receptor is being expressed on numerous human epithelial tumours.

Constitutive activation of the EGFR, more than just simple immunohistochemical expression, is a hallmark of these tumours, and therefore the EGFR is an important target in anticancer treatment.

To inhibit or stop the sequelae of the activated EGFR, two approaches have been explored;

Monoclonal antibodies (MoAb) (trastuzumab, cetuximab and panitumumab) are given intravenously on an intermittent basis and block the extracellular domain of EGFR. Results of randomised studies have led to regulatory approval for various indications. Clinical activity has been observed as single-agent treatment and in combination with chemotherapy.

Small molecule tyrosine kinase inhibitors (TKI) are given orally usually on a continuous basis. Here a 'tsunami' of compounds has been developed, of which some (gefitinib, erlotinib, lapatinib) have gained regulatory approval. Clinical activity has been observed as single-agent and in combination with chemotherapy.

Whereas the current MoAbs target either HER-1 / EGFR or HER-2, increasingly novel TKIs inhibit both receptors. The underlying mechanism of cross-talk between HER-1 and -2 is underlying the principal thought that a small spectrum target inhibition might probably lead to unsatisfactory results when using TKI.

Clinical data of the currently registered MoAbs and TKIs will be discussed, and some of the newer compounds will be mentioned. Epidemiology and potential treatment options for toxicities will be discussed.

S19

Oestrogen receptors and targeted therapy

V Speirs¹

¹Leeds Institute of Molecular Medicine

Targeted cancer therapy is aimed at targeting critical molecular pathways which are necessary for cancer cell survival and has received much attention in recent years. Targeted therapy is much more refined than conventional regimes which simply target rapidly dividing cells - unsatisfactory as this can also affect some rapidly dividing normal cells, causing multiple side effects. In recent years, targeted therapies for breast cancer have been developed. These include trastuzumab (HerceptinTM), lapatinib (TykerbTM) and bevacizumab (AvastinTM). However, the first true therapeutic target in breast cancer was the oestrogen receptor (ER) which mediates the action of oestrogens and antioestrogens. ER is expressed by 2/3 of breast tumours, can be measured reproducibly and correlates with clinical benefit - all hallmarks of a robust clinical target. Subsequently, two ERs have been described; ER α and ER β . Oestrogen action is mediated by these receptors and also by non-receptor mechanisms. In this lecture I shall outline the cell and molecular mechanisms surrounding oestrogen action and discuss how these could provide new opportunities for targeted cancer therapy of hormone-dependent cancers.

S20

The Role of DNA Methylation in Colorectal Neoplasia

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¹Department of Pathology, University of Cambridge, ²Cancer Research UK, Cambridge Research Institute.

There is abundant evidence that human carcinogenesis (and particularly colorectal neoplasia) is linked to disruption of normal patterns of DNA methylation (1,2,3). Using Pyrosequencing and microarray technology (4), we show that CpG-island hypermethylation of genes is an early cumulative event during the multi-step pathogenesis of colorectal cancer. We also confirm findings that aberrant DNA methylation of CpG islands "CpG Island Methylator Phenotype" (CIMP) is a critical pathway for inactivation of multiple cancer-related genes by promoter hypermethylation in colorectal cancer (1,2). Our findings also provide a useful platform for the use DNA methylation in early detection and screening of colorectal cancer.

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S21

Sights Unseen, Truths Untold: Pathology and the Modern Medical Curriculum

JCE Underwood¹

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Particularly among pathologists, there is widespread and deep concern about the decline of pathology in modern medical curricula. Until the late 20th century, medical students benefited from a wide range of learning opportunities in pathology — lectures, tutorials, practical classes, museum collections and autopsy demonstrations. Pathology was regarded as core knowledge, examined separately from other subjects and serving as a vital educational bridge between the preclinical sciences and the clinical years.

During the last few decades, in the UK and elsewhere, there have been fundamental changes in medical education: adoption of problem-based learning; a shift in emphasis away from science towards clinical and communication skills; and curricular organisation aligned with body systems rather than academic disciplines. Academic departments (where they survive) have ceded their curricular control to curriculum committees. Pathology museums (where they survive) are rarely visited by students. Most doctors now graduate without learning from autopsies. Where formerly students had to pass an examination in pathology before proceeding, the subject is diluted by other disciplines and is much less visible.

Evidence is now emerging of serious risks to patients, possibly as a consequence of some of these changes in medical education. Many surgeons are worried about significant gaps in anatomical knowledge. A recent study reveals poor understanding of pathology investigations. Despite all UK medical schools satisfying the General Medical Council, recent graduates now differ markedly in their knowledge according to which medical school they attended.

With evidence and advocacy, it is vitally important — for patients — that pathologists actively engage in curricular organisation and delivery. Pathological knowledge elevates medicine from a caring profession to a curing profession.

S22

Genomic Approaches to Understanding Gastric Cancer Progression

P Tan¹

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Gastric cancer (GC) is the second highest cause of worldwide cancer mortality, yet comparatively little is known about its underlying genetics and key oncogenic pathways. In this talk, I will describe our attempts to understand GC from a genomics-oriented perspective, in order to better identify cellular interactions involved in this disease, and potential nodes for pharmacologic intervention. I will present our results in establishing the "gastrome", a consensus gene co-expression metanetwork of GC derived from hundreds of gastric tissues and tumours, and describe how a systematic analysis of this metanetwork can provide unique insights into various topological and systems properties of GC. I will further describe how we were able to use the gastrome to uncover a novel cellular pathway for GC invasion and metastasis, involving the survival-related gene PLA2G2A.

S23

Molecular prediction: making the most of treatments for bowel cancer

MT Seymour¹

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We now have an increasing range of drug therapies with proven efficacy in bowel cancer. However, each drug typically produces major benefits in less than one-third of patients receiving it, whilst the toxicity and costs of treatment are incurred by all. Predictive biomarkers have the potential to identify subpopulations of patients with higher or lower probability of benefit or toxicity with specific drugs. If validated and incorporated into practice, they could allow drugs to be targeted to patients most likely to benefit, thereby improving both clinical and cost effectiveness. Ascertainment and validation of predictive biomarkers requires large, statistically robust studies attached to randomised clinical trials (RCTs). The UK NCRI and NCRN provide the infrastructure and opportunities for such studies.

MRC FOCUS is the largest RCT ever reported in advanced colorectal cancer. We received FFPE tumour material from over 1600 FOCUS patients and have investigated several candidate biomarkers. Topoisomerase-I (TopoI) protein emerged as a strong candidate predictor of irinotecan and oxaliplatin efficacy, and is now undergoing independent validation. Meanwhile, other trials have identified *KRAS* oncogene status as predictive of benefit from anti-EGFR-antibody therapies. A new NCRI trial, FOCUS-3, will now prospectively compare standard therapy or TopoI/*KRAS*-directed drug choices.

S24

Hypoxia as an inducer of leaky microvessels and growth of atherosclerotic plaques

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Atherosclerosis is considered to be a chronic inflammatory disease with a prominent role for lipids and macrophages. Since atherogenesis is also associated with intraplaque neovascularization, which is thought to be stimulated by hypoxia, the presence of hypoxia and hypoxia-inducible transcription factors (HIF) was studied in human carotid atherosclerosis. To show hypoxia in atherosclerotic plaques, the hypoxia marker pimonidazole was infused immediately prior to carotid endarterectomy of 7 symptomatic patients. Subsequent immunohistochemistry demonstrated the presence of hypoxia especially in the macrophage-rich centre of the lesions. Hypoxia was associated with the presence of a thrombus, neovascularization and expression of HIF1A, 2A and responsive genes. Microvessels appeared to be very thin walled, both in the adventitia as well as in the intima, and contained endothelial cells with many membrane blebs and vacuoles, which may explain their leaky phenotype.

Hypoxia was most prominent in the macrophage-rich centre and was associated with the presence of a thrombus, neovascularization and the expression of HIF1A, 2A and responsive genes. Also, the expression of proteins in the HIF pathway was associated with lesion progression and microvessel density, suggesting the involvement of the HIF pathway in the response to hypoxia and the regulation of neovascularization in human atherosclerosis. Microvessel (ultra)structure in plaques may explain their leaky phenotype.

S25

Understanding molecular pathogenesis of Barrett's adenocarcinoma and application to the clinic

R Fitzgerald¹

¹MRC Cancer Cell Unit, Cambridge

Barrett's oesophagus is important due to its association with oesophageal adenocarcinoma. Any management strategy for patients with Barrett's oesophagus needs to take into account the low annual cancer conversion risk of 0.5 to 1% per annum. The current standard of care involves endoscopic surveillance with the assessment of multiple biopsies for histopathological evidence for dysplasia. This is a subjective diagnosis. Ongoing research aims to better identify the patients at greatest risk for malignant progression using molecular techniques. Despite efforts to identify biomarkers they have not progressed beyond early phase trials. This may be because progression from metaplasia to dysplasia and adenocarcinoma is a result of multiple molecular abnormalities without a predictable linear sequence. The complex molecular pathways to cancer may be explained as a result of the chronic injury from duodeno-gastro-oesophageal refluxate resulting in a stochastic accumulation of genetic and epigenetic changes. The stromal microenvironment may also have profound influences on the epithelial phenotype. With this in mind some success is being made in utilising combinations of genetic biomarkers for endpoints which reflect a myriad of specific genetic derangements such as proliferative indices and copy number changes. In the future successful clinical application of biomarkers will also depend on the development of effective and feasible assays.

S26

Evidence for a stem cell hierarchy in prostate cancer

A Collins¹

¹York University

The prostate is renewed throughout adult life and this depends upon a subpopulation of epithelial stem cells. Stem cells have the ability to perpetuate themselves through self-renewal and their longevity makes them excellent candidates for tumour-initiation. Despite the potential importance of stem cells in the genesis and perpetuation of cancer, little is currently known about the biological and molecular properties that make cancer stem cells distinct from normal stem cells.

Research from our laboratory identified the cancer stem cell (CSC) from human prostate tumours as phenotypically integrin $\alpha_2\beta_1$ hi/CD133⁺. The CSC population possesses a capacity for self-renewal and differentiation to a secretory luminal lineage. Tumour incidence is greater from this population, whilst differentiated phenotypes form only rare tumours, even with an input of 10^6 cells. The identification of CSCs in leukemia and solid tumours raises the question as to the extent to which these cells have a different gene expression profile from non-tumour initiating cancer cells. To date, cDNA microarrays have failed to account for cellular heterogeneity and differences in proliferative potential within tumours. By directing expression analysis to enriched populations of tumorigenic cells, we have identified a gene expression signature for the CSC which is distinct from normal stem cells and differentiated progenitors. By further analyzing this expression signature in the context of biologically connected genes, key pathways have been identified that are associated with inflammation, ECM and stem cell self-renewal. The goal now is to determine whether these targets are effective therapeutically.

S27

The Role of Pathology in the Development of Antiangiogenic Therapies

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Numerous antiangiogenic agents have recently entered clinical trials to evaluate safety and efficacy against a variety of solid and hematolymphoid malignancies. It appears that antiangiogenic therapies can be associated with the risk for significant toxicities. However, the search for predictive markers has so far been unsuccessful. The identification of such markers would allow the selection of patients who are most likely to respond while avoiding exposure of patients with little chance to respond to potentially toxic therapies.

The humanized anti-VEGF antibody bevacizumab showed efficacy in patients with metastatic colorectal cancer in a phase III trial when used in combination with standard-of-care chemotherapy and gained approval for clinical use by the Food and Drug Administration in this indication. We attempted to identify predictive markers on tissues collected during this trial by assessing the expression levels of angiogenic factors as well as performing a mutational analysis for k-ras, b-raf and p53.

Our results indicate that neither the mutational status of k-ras, b-raf or p53 nor the expression level of vascular endothelial growth factor (VEGF) appear to be predictive for response to anti-VEGF therapy. However, the analysis was limited to the evaluation of paraffin-embedded tissues of the primary tumour in these patients, which may only be poorly reflective of the metastatic disease present at the time of therapy.

Strategies that would ensure adequate downstream analysis of trials evaluating antiangiogenic therapies will be discussed.

S28

Incipient pulmonary neoplasia

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Incipient pulmonary neoplasia has three morphologic directions. In squamous metaplasia polysomy 3, or amplification is occasionally found in high grade dysplasia, but not in lower grades. Immunohistochemical reaction in metaplasia/ dysplasia for p53, chromosome 17p13, is consistently associated with increased chance for the occurrence of squamous cell carcinoma. The earliest molecular changes (LOH) which are also present in invasive cancer may be found in normal bronchial epithelium.

In adenocarcinomas a difference exists between Asiatic and western countries in the occurrence and timing of molecular abnormalities in AAH, in-situ carcinoma and invasive adenocarcinoma. In Asiatic countries EGFR and K-ras mutations are present in >40 and <15%, respectively; while in western countries the fraction of EGFR and K-ras mutations is <15% and > 20%. Mutations in EGFR and K-ras, but not amplification, occur already in atypical adenomatous hyperplasia, a benign precursor lesion, while amplification of these genes is associated with progression in already malignant tumours, but not necessarily with metastases.

DIPNECH is a rare lesion characterized by diffuse pulmonary neuroendocrine cell hyperplasia in de bronchial wall and multiple pulmonary nodules consisting of tumour lets and usual typical, and occasionally also atypical carcinoids. Many cases remain stable over a long period of time. The prevalence of DIPNECH is much lower than for carcinoids, emphasizing that the neuroendocrine precursor lesion is not well known.

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