

Abstracts

Invited Speakers

S1

An introduction to genomic instability and chromosome structure

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The stability of our genomes is tightly regulated to prevent excessive mutations which can lead to genetic disease and cancer. However, some mutation must occur, as evolution relies on it. Hence a delicate balance is required.

Each human body is subjected to 1018 DNA damages per day, and unrepaired damage results in mutation. As a result, all organisms have a range of mechanisms to repair such damage to reduce the potential mutational load. These pathways have been highly conserved throughout evolution. They include the reversal of DNA damage, its removal from DNA by excision repair processes, the tolerance of DNA damage during DNA replication and recombination mechanisms. The absolute need for such mechanisms is highlighted by the fact that defects in DNA repair are related to a number of human cancer-prone and immune deficient conditions.

We know a considerable amount about how these pathways operate on damage in naked DNA. However, in humans there is 2 metres of DNA packaged into each cell nucleus; we know far less about how these mechanisms access the packaged genome and whether they can operate equally on different regions of our genome where the packaging varies. Furthermore, to date we have focussed on the extreme effects of DNA repair defects, yet we have little idea as to whether there are lesser variations in capacity amongst the so called "normal" population. These events have implications for molecular diagnostics related to mutational risk assessment and to the efficacy of cancer therapeutics that damage DNA.

S2

Replication of damaged DNA and cancer protection

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Although cells have many ways of removing different lesions from their DNA, damage often persists until the cells replicate their DNA. An important pathway by which cells are able to tolerate this unrepaired DNA damage during replication is translesion synthesis (TLS). In this process DNA is synthesized past the damaged bases by specialized DNA polymerases, most of which belong to the Y-family. These polymerases have an open structure which allows them to accommodate damaged DNA bases in their active sites. Deficiency in one of these polymerases, pol eta, is responsible for the variant form of the highly skin cancer-prone disorder xeroderma pigmentosum. Regulation and control of the Y-family polymerases is mediated by important motifs in the C-terminal third of the polymerases. These motifs are required for their correct localisation and for protein-protein interactions. The sliding clamp accessory protein PCNA plays a crucial role in regulating TLS. When the replication fork is blocked, PCNA becomes ubiquitinated. This increases the affinity of Y-family polymerases for PCNA, because they all contain both PCNA-binding and ubiquitin-binding motifs. Localisation of the polymerases in replication factories and interaction with PCNA are complex and highly dynamic processes.

S3

Telomeres, genome stability and human disease

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Telomeres provide the end-capping function that allows the chromosomal terminus to be distinguished from double-stranded DNA breaks. Telomere erosion acts as a cell division "counter", imposing a proliferative lifespan barrier that must be overcome to allow the progression to malignancy. This tumour suppressive function is paralleled by the ability of dysfunctional telomeres to trigger genomic instability, whereby the loss of the capping function leads to telomere fusion events; the resulting cycles of anaphase-bridging, breakage and fusion result in genomic rearrangements, such as non-reciprocal translocations, that typify early-stage neoplasia. We have developed single-molecule approaches to characterise telomere length and fusion. We have been using these technologies to study in detail the dynamics of telomeres and the mechanisms underlying telomeric instability and fusion.

We have shown that gradual telomere erosion is consistent with the end-replication problem and that this is superimposed by additional mutational mechanisms that create severely truncated telomeres. We have also provided a definition of the length at which telomeres become dysfunctional and undergo fusion, as well as mechanistic insights into the fusion process.

We are now applying our understanding of telomere dynamics gained in vitro, to examine the role that telomere dynamics may play in the progression of neoplastic conditions. Data concerning these aspects of our work will be presented, focusing in particular on chronic lymphocytic leukaemia.

S4

Clinical aspects of human genomic instability syndromes: common cancer predisposition

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Inherited predisposition to cancer has been recognised for many years. In particular rare dominantly inherited families have been described caused by genes that often show a syndromic predisposition such as those that cause neurofibromatosis or familial adenomatous polyposis. It was assumed that the apparently more common predisposition to common cancer such as breast/ovarian cancer and Lynch syndrome would also be due to typical tumour suppressor genes. However, discovery of BRCA1 and BRCA2 that cause a high risk of breast and ovarian cancer and the mismatch repair genes that underlie Lynch (Hereditary non Polyposis Colorectal cancer-HNPCC) syndrome has shown that these genes are actually DNA repair genes and the mechanism of action is loss of a vital repair pathway that leads to tissue specific accumulation of DNA damage when gene function is lost. The combined frequency of inherited mutations is about 0.5% for the BRCA genes and 0.1% for the MMR genes. They still have their action by loss of the normal copy (recessive at cellular level), but their action in families is shown by a high penetrance tumour predisposition. Intriguingly targeting either DNA damage or disabling another repair pathway is now showing immense promise in tumour therapy for these disorders. Methotrexate is being in trials for MMR deficiency and PARP inhibitors for BRCA deficiency.

S5

Defective biological responses to DNA damage and human disease

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Humans are endowed with multiple mechanisms for coping with the potentially mutagenic and lethal effects of DNA damage. These protective mechanisms include (i) several distinct DNA repair modes collectively referred to as excision repair, during which various types of genomic insult are physically removed from the genome, (ii) several repair processes collectively referred to as DNA damage reversal that reverse damage, thereby restoring affected nucleotides in DNA to their normal chemistry, (iii) several distinct modes by which arrested DNA replication is relieved without removing or reversing the damage, collectively referred to as DNA damage tolerance. Inherited defects in these biological responses to DNA damage can lead to several hereditary diseases, including xeroderma pigmentosum (XP), trichothiodystrophy (TTD), Cockayne syndrome (CS) and the combined CS and XP complex. This lecture will briefly review these biological responses to DNA damage and describe the principal features of these diseases.

S6

Lymphoma diagnosis and classification – WHO and beyond

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Lymphoma classification developed through frequent changes ultimately resulting in entity based, “REAL” classification in 1994. This new approach relied on recognition of morphological features, immunophenotypes, genetic markers and specific clinical characteristics. This necessitated application of immunocytochemistry, flow cytometry, conventional cytogenetics, interphase FISH and molecular clonality studies in an integrated laboratory setting, together with wider clinicopathological correlation. The subsequent WHO classification, based on the same principles, has recently been updated. Diagnostic criteria are clarified and new entities are introduced highlighting particularly the significance of the clinical presentation (e.g. cutaneous follicular lymphoma, primary diffuse large B-cell lymphoma (DLBCL) of the CNS, primary cutaneous DLBCL of leg type, LBCL arising in HHV8 associated multicentric Castleman disease). New categories are introduced to highlight diagnostic “grey zones” between DLBCL and Burkitt lymphoma and DLBCL and classical Hodgkin lymphoma respectively, rationalising management of these contentious cases. Better understanding of EBV and immunosuppression associated lymphoproliferations resulted in the addition of entities such as EBV positive T-cell lymphoproliferative disorders of childhood and EBV positive DLBCL of the elderly. New entities are still emerging and classification changes will continue with a greater influence of gene expression analysis and requirement for its routine diagnostic application. Further classification changes might be based around common therapeutically relevant cellular pathways, facilitating targeted, entity specific clinical management. To keep pace with the new changes, proactive preparation with appropriate training and timely introduction of new technology is needed. However, “good old” morphology will remain, for still some time, the key to diagnosis.

S7

Airborne mineral particles, properties, deposition patterns in the lung and disease

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The size, shape and density of airborne mineral particles are the properties which will determine their ability to be inhaled and penetrate deeply into the lungs. They also influence the pattern of deposition throughout the respiratory tract but more importantly the lung parenchyma. This presentation will illustrate how mineral particles as different as coal and asbestos can be inhaled, deposited in the lung and retained to produce deposition patterns which can be related to the aerodynamic size distribution of each dust. The results illustrate how variations in the size of specific mineral particles can alter their potential to cause disease. The health hazard represented by an exposure to a mixed mineral dust can be assessed from a characterisation of the particles it contains. This will enable strategies to be designed for atmospheric dust control purposes and reduce the health effects produced as a result of exposure to airborne mineral particles.

S8

Pathways in colorectal carcinogenesis

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Colorectal carcinogenesis involves transit from normal mucosa via adenomas to carcinomas. Cancer formation is associated with characteristic genetic changes that occur at a relatively high frequency, including mutations to APC (~80%), p53 (40-50%), K-ras (30-50%) and PI3K (15-35%), or deletions of SMAD4/2 on chromosome 18 (~60%), or alterations to the DNA mismatch repair (MMR) genes (~15%), amongst others. Recent large-scale sequencing studies have identified a much larger number of genes that are mutated in colorectal cancer, but at a lower frequency. Mutation of APC (inherited in familial adenomatous polyposis) and/or loss of APC are seen in adenomas and this represents the major pathway of adenoma formation. Progression to carcinoma is often associated with chromosomal instability and acquired genetic and epigenetic alterations. A second pathway involves transition from hyperplastic polyps to serrated adenomas to carcinomas, usually with evidence of microsatellite instability (MSI) due to MMR deficiency. MSI is associated with mutations to repetitive sequences in other genes (e.g. Bax, TGFBR2, etc) and accounts for cancer susceptibility in Lynch (HNPCC) syndrome patients with germline MSH2 or MLH1 mutations. Around 15% sporadic colorectal cancers show MSI mostly due to MLH1 promoter methylation. Overlapping with these two major carcinogenic pathways is the phenomenon of CpG Island Methylator Phenotype (CIMP), whereby promoter methylation transcriptionally silences certain genes (e.g. MLH1, MGMT, CDKN2A/p16, MINT31, etc) and associates with BRAF mutation. The molecular changes in the different carcinogenic pathways often affect a similar range of signalling pathways.

S9

Getting the most from a resection specimen

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Quality assessment and assurance are important issues in modern health care. For the evaluation of surgical procedures, there are indirect parameters such as complication, recurrence, and survival rates. These parameters are of limited value for the individual surgeon, and there is an obvious need for direct parameters. In addition to the established factors such as circumferential margin involvement and involvement of the distal margin, macroscopic evaluation of colorectal resection specimens has found its place in the pathological workup. The value of the evaluation of the planes of mesorectal resection in rectal cancer specimens has recently been confirmed in a large trial with 1156 patients. When a superior plane of surgery was achieved, local recurrence risks were significantly lower.

Recently, a macroscopic evaluation method for colon cancer has been described. In the single centre study of 400 patients, the plane of surgery was associated with survival, this was especially marked in stage III tumours. These developments have further established the important role of the pathologist in the multidisciplinary evaluation of colorectal cancer patients.

S11

DNA methylation in the progression and early detection of colorectal neoplasia

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Colorectal cancer is the second leading cause of cancer-related deaths in the UK. Its evolution from histologically recognizable pre-invasive lesions to carcinoma is now known to be associated with stepwise changes in gene function, and to involve several pathways and a variety of genetic and epigenetic mechanisms¹⁻⁴. Understanding these events is crucial for the development of better strategies for both treatment and screening.

In the colorectal group at Addenbrooke's hospital, I have mapped DNA methylation changes across the spectrum of pre-invasive and invasive colorectal neoplasia, using both low and high throughput approaches to reproducibly and quantitatively assess DNA methylation at the single CpG dinucleotide level.

CpG methylation of targets identified is shown to be quantitatively cumulative during neoplastic progression from normal mucosa to adenoma to carcinoma and these DNA methylation changes are correlated with changes in expression of the genes examined as well as a DNA methyltransferase. Two of these DNA methylation targets are potential biomarkers and are shown to discriminate normal DNA from neoplastic DNA with 100% sensitivity (95% CI: 93.2-100.0) and 90.5% specificity (95% CI: 69.6-98.8). These findings have implications for and are discussed in the context of bowel cancer screening strategies.

References:

- [1] Nagasaka, T. et al. (2008), *Gastroenterology*, 134,1950–1960.
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- [3] Jass, J. R. (2007), *Surg Oncol*, 16 Suppl 1, S7–9.
- [4] Kim, Y. et al. (2006), *Genes Chromosomes Cancer*, 45, 781–789.

S10

Pathology-guided colorectal cancer treatment

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The pathologist plays a central role in management of colorectal cancer by making the diagnosis, identifying background disease states, staging the tumour and identification of molecular features which guide therapy. Identification of background disease gives insight into causation and guides the need for more extensive surgical resection or more intensive surveillance. The mismatch repair phenotype is important as a prognostic marker of survival, a predictive marker of response to 5-FU chemotherapy and a screening tool for HNPCC.

In the earliest T stage tumours the Kikuchi staging system correlates most closely with risk of nodal involvement. Poor risk Stage II disease can be identified from serosal involvement, extramural vascular invasion, perforation through tumour and incomplete excision. The presence of adequate (>12 nodes) nodal harvest is also crucial to be certain that occult nodal involvement has not been overlooked. Microarray signatures to detect poor risk stage II disease from FFPE are not yet in widespread clinical use to select patients for chemotherapy.

The finding that *kras* mutation identifies patients who gain no benefit from inhibition of EGFR signalling has opened the door to molecular geneticists in colorectal cancer diagnostics. Prediction of response to conventional chemotherapy agents is more difficult. Identification of germline polymorphisms (in *UGT1A1*) has not been confirmed. The presence of topoisomerase I (the molecular target of irinotecan) has been associated with benefit from both irinotecan and oxaliplatin, while low topo-I expression showed no significant benefit. The era of molecularly guided therapy in colorectal cancer is just beginning.

S12

“So when I am an Accredited Specialist Expert ...”

S Dicken¹

¹*Walsall Hospitals NHS Trust*

In 2008, the Department of Health published a consultation document entitled: *The Future of the Scientific Workforce*.

Modernising Scientific Careers: The Next Steps.

The document reviews the key contribution of science to healthcare; The challenges of modern healthcare; Highlights the for change in the health care science workforce, and details the vision for healthcare science. Proposals for modernising scientific careers and the benefits for these proposals are described.

This presentation reviews the *Modernising Scientific Careers* publication. The proposed changes to training and careers are designed to allow employers to optimise the skill mix and effectiveness of their workforce.

The stages of training and career pathways for healthcare science are defined as Healthcare Science Assistants (HCSA); Healthcare Scientist Practitioners (HCSP) and Healthcare Scientists (HCS).

The MSC publication identifies the educational pathway for healthcare science workers and identifies the educational framework which will accompany its implementation.

There will be development of a BMedSci which will be available for the healthcare science workforce.

These changes have implications on the biomedical science so it is appropriate that the MSC proposal takes notice of the views expressed during consultation.

These issues include concerns regarding lack of clarity about implementation; the need to completely review training pathways especially when well established, successful programmes are already in place, i.e with Biomedical Science. There are also concerns with regard to the introduction of a broad based, "2one size fits all" BMedSci degree.

S13

Cancer causing lipids

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We have previously shown that Hodgkin's lymphoma (HL) cells produce two small bioactive lipids, sphingosine-1-phosphate (S1P) and lysophosphatidic acid (LPA). We have also shown that infection with the lymphomagenic Epstein-Barr virus (EBV) is sufficient to increase S1P and LPA levels; an effect that is mediated by at least three latent viral genes through distinct cellular pathways. S1P and LPA are potent mediators of the transformed phenotype in a variety of cancer types and have recently been implicated in the development of several lymphomas and leukaemias. These observations are of interest because monoclonal antibodies directed against S1P and LPA are now being evaluated in mice and non-human primates having been shown to be effective in vitro against several cancer cell types. More recently, we have investigated the expression of several enzyme regulators of these small lipids in primary tissues. Importantly, we have also established the methodology for the demonstration by immunohistochemistry of both S1P and LPA in histological sections. This will enable us to determine for the first time the impact of their detection on clinical outcome and treatment response in malignant lymphomas

S14

Bone marrow trephine biopsies - time for a national technical EQA?

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Bone marrow trephine biopsies are commonly used for the identification, staging and detection of relapse in haematolymphoid malignancy. The histological findings in these biopsies can be critical to patient care, particularly where there is a dry tap or a haemodilute aspirate. Since trephine biopsies contain bone, soft tissue and haemopoietic elements, the latter having several different components, analysis requires sections with well-preserved morphology that can also be used for immunohistochemical analysis.

Despite the critical nature of such biopsies, there is little consensus on the technical aspects. Fixation can be in 5% formalin or aceto-zinc formalin. Decalcification may use weak organic acids, Gooding and Stewart's decalcification fluid, calcium chelation or even inorganic acids. The tissue can be embedded in paraffin wax or in plastic, sections can be cut at a variety of thicknesses and different strategies are used for taking levels and sections for immunohistochemistry. Many laboratories are moving to rapid processors or short processing schedules, and on the diagnostic side there is an increasing requirement for both PCR and FISH-based molecular techniques.

The situation seems to be calling out for a national EQA scheme that would operate in a similar manner to the current UK NEQAS schemes for cellular pathology techniques and immunohistochemistry. By collecting data on the methods used in UK laboratories and by independently assessing the results, both for morphology and immunohistochemistry, it should be possible to identify the best practice for the technical aspects of bone marrow trephine biopsies.

S15

Pathology: the patients' champion in bowel cancer

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Large bowel cancer is the third commonest malignancy in the UK with around 35,000 new cases annually and the second commonest cause of cancer-related mortality with 16,000 deaths. Pathologists play a major role in bowel cancer care and provide important quality control feedback to other members of the multidisciplinary team. This has contributed to a marked increase in survival over the last 30 years following subsequent improvements in surgery, radiology, oncology and pathological reporting.

Studies have shown that large differences in outcome exist between different surgeons and different centres suggesting that surgical differences account for a proportion of this effect. Pathologists led the discovery of the importance of the circumferential resection margin and plane of surgery in rectal cancer and have provided important evidence to generate a change in practice.

Pathologists continue to drive further improvements in survival through better reporting, earlier detection, identification of predictive markers and by influencing the type and quality of surgery. The new NHS bowel cancer screening programme is estimated to reduce mortality by 10% through the earlier detection of tumours. The identification of patients with wild type k-ras allows us to select those more likely to respond to anti-EGFR therapy. New evidence generated by our unit demonstrates that changing the way surgeons operate in colonic and low rectal cancer may further improve outcomes by around 10%. These measures could lead to over 3000 extra lives saved per year in the UK and many more if translated around the world.

S16

Muscle histochemistry pilot scheme for EQA

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Muscle biopsies are performed to confirm a clinical diagnosis, distinguish between nerve and muscle disorders, identify a metabolic defect of muscle, diagnose disease of connective tissue and blood vessels, detect inflammation of muscle or rule out muscle disease. With occasional exceptions it is the essential element in the assessment of patients with suspected myopathy. Although the British Neuropathological Society run an EQA scheme for Neuropathologists whose primary aims are both educational and to identify poor performance in interpretive skills by comparison with their peers, departments operate without a specific technical EQA scheme. Following discussion at PATHSOC in 2007, support was given to initiate a pilot scheme in order to ascertain whether or not there was a genuine requirement.

Assessments were based on predetermined criteria against four chosen histochemical techniques which were H&E, GMT, NADH, and COX. Up to 14 centres submitted slides for each assessment. These were assessed by volunteer assessors which included Biomedical Scientists and Neuropathologists.

In conclusion it is obvious that the preparation of muscle biopsies is technically demanding. The pilot scheme emphasised this as marks were deducted for preparation, cryotomy, staining and finishing. Poorly prepared tissue can lead to difficulties in subsequent reporting. The aim of the presentiaion is to ascertain a need for an established scheme. It will allow for specialised departments to be confident that the standard and quality of work being produced is equal to that of their peers and allows regular scrutiny of performance.

S17

Osteoarticular pathology: pilot EQA scheme

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Hard-tissue histology (bone and cartilage) poses significant difficulty to the laboratory as blocks selected for microscopy must often be sawn from the gross specimen, and virtually all must be decalcified pre-processing to paraffin wax. Decalcification, usually by exposure to acid, affects the staining properties of the cut section, be the stain tinctorial, empirical or immuno-histochemical.

At the outset the primary question addressed by the six participating labs was whether bone work was sufficiently different to "the rest" to merit its own scheme; on the basis of the processes used to produce sections that are fit for purpose in every way it was agreed that OA work did merit its own scheme.

Four H&Es, two from the participants archive, and two from material supplied from a pool assembled by participants are assessed each run; sections submitted are assessed using the General Scheme criteria, plus an additional microscopical assessment of adequacy of decalcification. Sections are assessed by two persons, one of whom has expertise in OA histology.

Following the first run, agreement was reached as to the nature of the material supplied from pool; each lab would receive two fixed blocks of bone from different specimens, to decalcify, process, section and stain H&E. This change was introduced to eliminate variation in staining occurring as a result of differences in decalcification procedures between participants.

Results of the first run: range 5/10 to 9/10, mean scores varying from 6.50 to 8.00

S18

Lister: Pathologist

R MacSween¹

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Lister is universally recognised for introducing the concept of antisepsis (asepsis) into surgical practice in the 1860s. He held pathology in high regard and wrote in 1857 "We stand in need of the beacon light of correct pathology to steer a safe course amid the various conflicting opinions which assail us". He was an accomplished microscopist, taught by his father who was elected FRS for his contributions to the development of achromatic lenses. During his time as a lecturer in Professor Symes Dept. of Surgery at Edinburgh in the 1850s Lister prepared a series of 'case reports' now archived in the library of the Royal College of Surgeons of England. These contain clinical details and drawings, extended macroscopic descriptions of resected specimens highlighted by superb water colour illustrations and with some cytological details derived from unstained free-hand sections examined by camera lucida. I will demonstrate some of this material which should be of interest to all Society members. These reports show that Lister was a surgical pathologist of distinction and an excellent artist.

S19

Was Homer a pathologist?

R Marshall¹

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This study looks at descriptions of wounding and death in Homer's Iliad and considers what relevance they have to modern medical practice.

Much scholarship has been expended in assessing how anatomically and pathologically accurate Homer's descriptions are – could a spear thrust to the centre of the forehead dislodge both eyeballs? Could the shaft of a spear lodged in the mediastinum quiver with each beat of the heart? The detail and accuracy of some of Homer's descriptions even led to the suggestion that he was Surgeon-General in the Agamemnon army.

A different reading of the text would ask whether the question of accuracy is relevant. Homer constructed his story within the knowledge and narrative demands of his age. Today, we are not practising pathology that is in some sense 'right' but are bound by our own age's constructs of knowledge. Our students' knowledge of the internal body is one based on books, models and the web. Their knowledge will be of virtual bodies and virtual pathology, probably well-suited to practising medicine that will also be largely virtual.

Where Homer's descriptions seem wrong – the warrior killed by a blow severing the large vessel running from the neck down the centre of the back, for example, - we want to ask 'Surely, he had the opportunity to examine cadavers?' We find ourselves asking the same question today. Perhaps the question will seem as inappropriate in a hundred years time as it is of Homer now.

S20

An international evidence-based clinicopathological classification of IgA nephropathy: the Oxford Classification

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A new classification for IgA nephropathy has been developed by an international working group. Clinical data were obtained on 206 adults and 59 children with IgA nephropathy, median follow-up 5 years. Patients were from 8 countries on 4 continents; Asia (62), Europe (94), Americas (109). Renal biopsies were scored by multiple renal pathologists for 24 histological variables.

Six reproducible histological lesions were identified. Four predicted renal outcome independent of clinical variables: mesangial hypercellularity, segmental glomerulosclerosis, endocapillary hypercellularity, and tubular atrophy/interstitial fibrosis.

The predictive value of pathology variables on rate of renal function decline was not influenced by age. Endocapillary proliferation (ECP) was the only lesion influenced by immunosuppression; in patients with ECP who received immunosuppression, the rate of renal function decline was -1.5 ± 8.3 ml/min/1.73m²/yr, versus -5.4 ± 1.1 ml/min/1.73m²/yr in those who did not receive immunosuppression ($p=0.006$). There was no interaction with ethnicity, other than ECP. The rate of renal function decline associated with ECP in Asian subjects was significantly better compared to Caucasians. However, Asian patients with ECP were more likely to receive immunosuppressive therapy (42% versus 22% in Caucasians, $p=0.002$).

An approach to reporting biopsies with IgA nephropathy is recommended. The summary line should include a MEST score: mesangial proliferation ≤ 0.5 (M0), >0.5 (M1); endocapillary hypercellularity absent (E0), present (E1); segmental glomerulosclerosis absent (S0), present (S1); tubular atrophy/interstitial fibrosis $<25\%$ (T0), 26-50% (T1), $>50\%$ (T2).

S21

When should a renal pathologist order electron microscopy? The evidence

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The crucial role that electron microscopy plays in renal pathology continues undisputed. By allowing recognition of findings not identifiable under the light microscope, electron microscopy has contributed immensely to the understanding of medical renal diseases and has proven to be of unquestionable diagnostic value. Customarily, a sample is submitted for ultrastructural evaluation in every diagnostic renal biopsy that is performed. The role of electron microscopic evaluation in transplant renal biopsies is still debatable but many have agreed that even in that setting ultrastructure can provide useful information at least in selected situations. The percentage of cases in which electron microscopic evaluation adds important information, that is either key for establishing or confirming a diagnosis or provides additional important data that affects patients' management has remained quite stable over the years, as reported by several authors analyzing large numbers of renal biopsies over a span of 30 years. This figure; however, changes depending on the renal biopsy service that is surveyed. Another important factor that needs to be recognized is that in approximately 10% of the cases, the renal pathologist makes a diagnosis based on light and immunofluorescence data felt to be sound, only to find out later, when the electron microscopic findings become available that the initial impression was totally or partially incorrect, or that additional clinically useful information needs to be added to the initial interpretation.

S22

Application of electron microscopy within drug discovery

A Bigley¹

¹*AstraZeneca*

Transmission electron microscopy is employed as an important complementary tool to the light microscope, providing significant data for the pathological assessment of safety in both drug discovery and development.

To further elucidate on changes observed at light microscope level, for example in staining characteristics, such as basophilia or eosinophilia, or morphology, such as vacuolation or hypertrophy, electron microscopy is required to relate these to a target organelle or early toxicological features.

Electron microscopy is valuable in the characterisation of ostensibly minor changes to cell organelles, such as peroxisomes, mitochondria and endoplasmic reticulum when correlated with alterations in metabolic enzymes, where early toxicological effects may be related to drug class and action. Indeed, electron microscopy is employed as the 'gold standard' in the identification of xenobiotic induced phospholipidosis, especially in relation to the evaluation of cationic amphiphilic compounds.

Immuno-electron microscopy may also be employed in the evaluation of tissue antigens, for example, in the validation of safety biomarkers, which is an important adjunct to normal tissue identification and protein localisation by immunohistochemistry.

S23

Advances in clinical electron microscopy, 1963–2000: a personal view

R Griffin¹

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A diagnostic electron microscope (EM) service commenced at Southampton in 1964. The service expanded. Advances critical to the rise of the EM as a diagnostic tool are considered in this presentation. The EM of the 1960's demanded a high level of operator expertise and service support to maintain its optical performance and function. Improvements were made and instruments became easier to use. Specimen preparation became easier and surer.

Crucial to the advancement of EM as a service was the introduction, in 1965, of machines to make glass knives used to cut ultrathin sections. Previously, glass knives were made by hand using hand tools. Knife production was unreliable. In another critical step, glutaraldehyde replaced osmium tetroxide as the primary fixative. Fixatives containing osmium tetroxide are hazardous and require laboratory containment. Ultramicrotomes improved with thermal regulation of section thickness and superior optical equipment. Gradually, resins for embedding improved until consistent results were possible.

Alongside technical improvements were advances in the recognition of the value of EM. Particularly in renal pathology, neuropathology, classification of tumours especially lymphomas and disorders of blood cells and platelets. Negative staining methods made rapid identification of viruses possible. The usefulness of the EM was extended by the techniques of x-ray microanalysis and immunocytochemistry.

Qualifications in Electron Microscopy awarded by the Institute of Biomedical Science have advanced career development for Biomedical Scientists in EM laboratories. Previously, those working in EM laboratories were disadvantaged. Career and promotion prospects for Biomedical Scientists were linked to the large major pathology disciplines .

S24

Diploma of Expert Practice in ultrastructural pathology

P Tarpey¹

¹*Central Manchester Foundation Trust*

2008 saw the first submissions for the Diploma of Expert Practice in Ultrastructural Pathology from Biomedical Scientists working in the field of electron microscopy.

Prior to examination, the candidates are required to submit a portfolio. The Institute expect that this portfolio "will demonstrate a range of competencies, skills, experience and an overall reflective approach". As a member of the Examining Board for this award I am presenting my views on the required content of submitted portfolios to demonstrate completion of training in ultrastructural pathology. The presentation also has examples of evidence, which I would expect to see, to demonstrate knowledge and skills in the areas of Personal and Professional Development, Education and Training and Management

S25

External quality assurance for diagnostic electron microscopy: the Australian experience

J Stirling¹

¹SA Pathology, The Flinders Medical Centre, Bedford Park, Australia

Diagnostic electron microscopy (EM) is in a difficult phase due to closure of laboratories and staff retirements; monitoring of quality is critical if negative impacts on patient care are to be avoided. RCPA Quality Assurance Programs Pty Ltd (RCPA QAP) undertook an EM pilot survey in 2006, now an internationally accredited (ILAC G13) annual fee-for-service program with 20 participants. The program has two sections. The first covers technical-scientific proficiency: specimen preparation, artefact recognition, microscope performance, imaging and micrograph interpretation. Participants submit micrographs, complete a technical questionnaire and identify artefacts. The second tests diagnostic accuracy and is aimed at pathologists; micrographs are provided for diagnostic interpretation.

In 2006, 87% of responses in the technical-scientific section were 'satisfactory'; results for 2007 were similar. In 2008 the spread of scores was broader with 74% 'satisfactory'. Diagnostic accuracy scores varied (concordance of interpretation with an expected diagnosis): (2006) thin basement membrane nephropathy 79%; (2007) post infectious glomerulonephritis 100% and cryptosporidiosis 95%; (2008) diffuse diabetic glomerulopathy with Kimmelstiel-Wilson nodules 68% and primary ciliary dyskinesia 76%, ~50% of respondents failed to identify the tip and base of a normal cilium.

Results indicate that technical-scientific expertise is declining. Anecdotal evidence suggests this may be because inadequately trained junior staff are being given responsibility for EM laboratories. In respect to diagnostics, 'classic' features are identified, more unusual cases are problematic.

QAP is an essential part of responsible practice with a significant educational role that can improve outputs.

S27

Tissue proteomics: a way forward to quantitative biomarker analysis

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Molecular medicine is currently migrating from genomics to proteomics. Recently, much progress has been made for protein analysis of archival tissues, including formalin fixed and paraffin embedded (FFPE) material. We focused on utility of full length proteins extracted from FFPE tissues for Western blot and reverse phase protein microarray analysis. In a pilot study for applying the new technology, we asked whether the p38-mitogen activated protein kinase (MAPK) and/or the AKT - glycogen synthase kinase-3 (GSK-3beta) pathways are involved in the regulation of the E-cadherin repressor Snail in human endometrial carcinomas.

Proteins were extracted from 17 FFPE primary endometrioid endometrial carcinomas. 25 antibodies were used to precisely quantify signalling end points using protein lysate microarrays spotted onto nitrocellulose-coated glass slides. All antibody specificities were validated by Western blot analysis. Expression of activated EGFR (epidermal growth factor receptor, Tyr1086) and p38-MAPK (Thr180/Tyr182) correlated with increased levels of Snail protein. These data suggest that EGFR and p38 MAPK activation may be involved in the stabilisation of Snail protein in primary endometrial cancers, possibly resulting in down-regulation of E-cadherin.

Our vision is that monitoring phosphorylated proteins in archival clinical tissues may allow us in the near future to infer the activity levels of proteins in a particular pathway as starting point for the design of individual therapy regimens without changing the routine clinical workflow for tissue analysis.

S26

Molecular morphology employing quantifiable internal reference standards

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In order fully to realize the potential of immunohistochemistry (IHC) it will be necessary radically to change the mindset of pathologists, from the current practice of regarding IHC as a 'special stain', to accepting it as a tissue based immunoassay, having the characteristics of precision and quantification of an enzyme-linked-immunosorbent assay (ELISA), with which it is analogous. As an essential step to accomplishing this goal it is proposed to develop a system of Quantifiable Internal Reference Standards (QIRS).

An IHC stain, in principle, is identical to an ELISA test performed in the clinical laboratory. It is a curious oversight of pathologists, that principles and reagents used in one environment (serum-ELISA) are universally accepted as providing a quantitative result, but when applied to formalin paraffin tissue sections (IHC), constitute only a qualitative stain, that at best may be subject to a crude semi-quantitative score.

The key difference, comparing IHC and ELISA, is lack of 'proper performance' of the former. The missing elements in IHC, as currently practiced, are lack of control of sample preparation (including fixation), inconsistent validation of reagents and protocols, incomplete automation, non-standardized image analysis, and absence of calibration or reference standards. Of these deficiencies the last is most critical. The proposed development of Quantifiable Internal Reference Standards (QIRS) is described, as providing a viable approach to establishing IHC as a reproducible, quantitative, tissue based immunoassay.

S28

The Human Tissue Act and research

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The Human Tissue Act 2004 makes it a criminal offence to use human tissue in research, or to store human tissue for research use, unless certain conditions are met. Human tissue is defined as anything containing human cells, but excludes human cells that have proliferated in vitro.

To use human tissue is lawful if 'appropriate consent' for use is available, or if the sample was obtained from a living person, the person from whom the sample came is 'not identifiable' to the researcher and an appropriately constituted research ethics committee has approved its use in research without consent.

Storage for research use is lawful either if a Human Tissue Authority licence for such storage is available or if the sample is being stored as part of an ongoing REC-approved project.

There are special provisions in relation to the analysis of DNA without consent. Remarkably, these do not apply to collections of DNA after extraction from human cells.

Unfortunately, these superficially straightforward statements are complicated by the need to define terms such as 'research', 'appropriate consent', 'not identifiable', 'appropriately constituted research ethics committee' and 'storage'. Questions arise as to whether some preparations contain human cells or not. There are uncertainties around storage that is incidental to acquisition and transportation. These problems will be explored in the light of Human Tissue Authority guidance on the subject.

The law in Scotland is different and will not be addressed, but problems in relation to the import and export of tissues will be considered.

S29

Ethics in research

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This presentation will concentrate exclusively on the ethics of research using human tissue, and mainly on the arguments that are relevant to an REC approving the use of human tissue samples in research where 'appropriate consent' is not available.

The different ethical status of pre-mortem and post-mortem samples will be reviewed.

In relation to pre-mortem samples, an argument will be proposed based on the assumption that tissue removed from the living may be regarded as 'discarded' by the patient once diagnostic procedures are completed. This stance will be justified by the observation of universal NHS practice.

The need for consent will then be analysed not as a method to enhance patient autonomy, but as a basis which can legitimise actions that might otherwise be regarded as causing an insult or injury. On this basis it is possible to construct a logical justification to distinguish situations where the use of human tissue in research does and does not require consent, and to identify situations where consent must be study-specific or may be generic. Arguments will also be considered around the durability of consent, the amount of information that must be given if consent is to be valid and the ethics of removing fresh tissue for research before there has been time for laboratory examination for the benefit of the patient.

These arguments have been accepted by several NRES committees, so it is to be hoped that their inclusion in research ethics applications will facilitate REC approval.

S31

Prostate cancer: from screening the masses to tailored treatment for the individual

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A decade ago, the uncertainties around prostate cancer management stemmed from the knowledge that many men had indolent disease that would not threaten their health, while some men had aggressive disease that was incurable. The roles of screening and of attempted curative treatment were unproven, and opinions were held with disproportionate conviction. The biology of prostate cancer was ill-understood, despite over 50 years' of experience with androgen suppression. Are we any further on today? We now believe that radical surgery may, sometimes, improve survival, but does it need to be performed immediately, or only on disease progression? Recent randomised trials of PSA screening have just been reported; their results are discordant, but current calculations suggest that 48 men must be treated in order to save 1 life. Specific chromosomal aberrations such as the TMPRSS-2/Erg gene fusion may both drive the development of the disease, and also define its behavior. New markers, such as sarcosine could have utility in diagnosis, prognosis, and even as therapeutic targets. Recent clinical data with drugs such as Abiraterone teach us that many men with so-called *hormone-refractory* disease may actually have *hormone super-sensitive* disease. The major challenges in prostate cancer management are to learn how to better target screening and curative treatment to those men who need it, and to develop better treatments for men with advanced disease. Understanding the biology will be a pre-requisite to achieving these goals.

S30

Normal biology and new insights into breast cancer

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Studies of normal development have had enormous impact on our understanding of cancer. The discovery of nerve growth factor by Rita Levi-Montalcini and epidermal growth factor by Stanley Cohen, led to *in vitro* culturing of skin for burns patients and the blocking of growth factor signalling pathways in targeted treatments of cancer with drugs such as herceptin and gefitinib.

The main development of the breast takes place during puberty, when in the mouse the rate of migration of the epithelium is 1mm/day. Isolated epithelial cells from a virgin adult have the capacity to develop into a complete mammary gland. This post-natal development produces a unique system in which to study the control of morphogenesis, epithelial migration and stem cells. Studies of the normal cell lineages have important implications to current hypotheses on the molecular classification of breast cancer. BRCA1 is important in mammary development where it may act as a regulator of the stem cell compartment, whilst mutations result in a morphology similar to sporadic breast cancers that lack ER/PGR and HER2. This has raised the possibility that some sporadic triple negative cancers may be more responsive to DNA damaging agents and EGFR inhibitors.

Recent data suggests that the normal breast and breast cancers share similar migratory mechanisms to those in brain development. Using both studies of individual molecules, such as reelin and pathways analysis of the pubertal and involuting gland have revealed novel insights into invasion and metastasis.

S32

Quality control of the diagnostic process: the pivotal role of histopathology

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There is no formal prostate cancer screening program in the UK, nevertheless the majority of biopsies and subsequent resections seen by histopathologists are the result of opportunistic or self referred PSA screening. This process should be subject to the same quality control as a planned screening program; histopathology is the source of a number of key variables that can assess the possible effectiveness of screening; and some of these depend on the quality of the histopathological process itself.

The proportion of cancer to benign diagnoses in biopsies (controlled for presentation PSA) is a key indicator. Evidence suggests that if this is below 0.35 it may indicate poor biopsy method or inadequate laboratory processing. Measuring the total length of prostate core examined on the histological slide can assess this. Routine measurement of this could act as a running quality measure to intercept problems before they become apparent as adverse outcomes.

A number of quality measures are available on the examination of radical prostatectomy specimen including nature of resection at posterior fascia, incidence of capsular incisions, the relative proportions of 'insignificant' cancers, significant T2 cancers and T3 cancers, and the rate of margin positivity and lymph node metastasis. These are all dependant on consistent processing and can indicate both the quality of surgery and the appropriateness of selection for treatment.

These pathological variables; together with clinical and biochemical covariates can offer a screening program a robust quality management system.

Processing and reporting the radical prostatectomy specimen

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The International Society of Urological Pathology (ISUP) organized a Consensus Conference on Handling and Staging of Radical Prostatectomy Specimens in Boston, USA in March 2009 with 116 voting delegates from 23 countries.

The meeting recommended that the prostate should be weighed independent of seminal vesicles and measured in three dimensions. The sagittal cone method should be used both at apex and base. Cases with biopsy diagnosed cancer but no cancer found in the radical prostatectomy specimen should be staged pT0. Substaging of pT2 was controversial and although no specific recommendations were issued, many speakers argued for a revision. Zonal location of index tumour should be reported and also a measure of tumour size (such as volume, diameter or percentage).

Location of extra-prostatic extension (EPE) and margin positivity should be categorized as Posterior, posterolateral, lateral, anterior at either the apex, mid, base. EPE should be quantitated, but there was no consensus on the method. Bladder neck involvement is best considered pT3a, in line with the upcoming TNM revision. Cancer invasion into the intra-prostatic portion of the seminal vesicles should not be considered seminal vesicle invasion. The diameter of the largest lymph node metastasis is the best outcome predictor for metastatic deposits and should thus be reported. The extent of a positive margin should be given as millimeters of linear involvement.

These recommendations will serve as an important aid for future revisions of staging systems and local guidelines but also emphasize the need of further studies in some controversial areas.

Morphological and molecular techniques for identifying the tigers in the cattery: current status and future directions

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The prediction of the behaviour of low grade/stage prostate cancer remains challenging. Data can be gathered from retrospective conservatively treated cohort studies and current active-surveillance cohorts. New biomarkers must give information in excess of currently known parameters to improve the prognostic model. Nearly all studies show that Gleason grading remains the most powerful predictor of behaviour. However as a result of immunochemistry for basal cell markers and the ISUP 2005 consensus conference, there have been recent proposed changes to Gleason grading which need to be applied in a consistent fashion.

Further morphological observations may refine our ability to predict disease progression. Evidence based reviews have shown that the presence of peri-neural invasion and cancer extent may give prognostic information. Numerous immunohistochemical markers have shown promise, but only a few have been assessed in pre-treatment biopsies, at the point when therapeutic decisions are made. Ki-67 is the most promising marker at present, which has the advantage of being in widespread use. However translation into clinical use for any immunochemical biomarker is a huge challenge and has to achieve intra-laboratory and intra-observer consistency.

The recently described translocation TMPRSS/ERG may be studied in formalin fixed tissue. Data on the prognostic significance of this translocation is contradictory, and it may be that overall genetic instability is the best molecular marker for outcome.

The use of standardised retrospective cohorts with long outcome data or mature prospective studies with sufficient material for assessment are vital for biomarker development.

The histological assessment of the prostate biopsy

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Recent years have seen an inexorable increase in the number of prostate needle biopsies due to increases in both the number of men undergoing biopsy as well as the number of biopsies performed on each patient. Despite advances in special techniques such as immunohistochemistry, morphology remains the cornerstone of prostate biopsy assessment. Hence, a thorough understanding of the rationale behind prostate biopsy reporting protocols as well as a systematic approach to evaluating prostate needle biopsies is essential.

This presentation will highlight the unique features of prostate needle biopsy interpretation, explaining how it differs from assessment of TURP specimens as well as needle biopsies from other sites such as the breast. A practical approach to reporting prostate biopsies will be outlined with emphasis on the clinical significance of various morphological diagnoses. The nebulous diagnostic category "suspicious for malignancy" will be shown to be heterogeneous with at least three different groups that differ greatly in their clinical significance. The current raging controversy regarding the classification of atypical intraductal proliferations including intraductal carcinoma of the prostate will be addressed. Various malignant mimics of benign prostatic lesions and benign mimics of prostate cancer will be discussed with particular emphasis on newer "entities" such as PIN-like prostate cancer, partial atrophy and diffuse adenosis. A morphology based approach to the use of immunohistochemistry to establish the diagnosis of prostate cancer will also be described.

