# Invited Speaker Abstracts

## WINTER MEETING
## 7–8 JANUARY 2016

**205th Meeting of the Pathological Society of Great Britain & Ireland**
**Hosted by the Department of Pathology · University College London**
**Venue: Guoman Tower Hotel · London**

UCL

Pathological Society
*Understanding Disease*

## KEY

Ⓟ = Presenter

## PRESENTER'S INDEX

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## PROGRAMME ACKNOWLEDGEMENTS



**This Programme is published by The Pathological Society of Great Britain & Ireland.**

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# S1

## Liquid Biopsy – Diagnosis and Stratification

Ⓟ T Forshew

*UCL Cancer Institute, London, UK*

There is growing evidence that many different cancer types release their DNA into a patient's blood stream. Detection and quantification of this circulating and cell free tumour DNA (ctDNA) has a broad range of potential applications such as non-invasive cancer detection, molecular stratification, disease monitoring and the non invasive analysis of tumour evolution. The main challenges with the analysis of ctDNA are its dilute and fragmented nature and the often low tumour DNA fractions. Advances in methods including digital PCR and next generation sequencing have recently made the detailed analysis of this DNA possible. Based on experience from some of the largest ctDNA studies to date, I will outline the challenges of ctDNA analysis and highlight some of the key components to successful ctDNA research. I will describe recent clinical ctDNA studies demonstrating some of the potential applications of ctDNA as a biomarker as well as some of its limitations.

# S2

## How to Get the Most From FFPE

Ⓟ M Gupta; H Costa; V Rathbone; G Gerrard; M Rodriguez-Justo; A Flanagan

*University College London, Research Dept. of Pathology, London, UK*

A number of genetic markers of diagnostic and therapeutic relevance have been identified in the last decade in cancer e.g. mutations in KRAS, BRAF and EGFR. Testing of the above markers starts with nucleic acids, notably DNA, from pathological tissues. Given that formalin-fixed and paraffin-embedded (FFPE) material forms a large part of archives in hospitals globally, researchers have tapped into this resource to identify genetic markers and to test for clinical purposes.

However, FFPE-derived DNA is fragmented, has protein-protein and protein-nucleic acid cross-links and has further damage due to formalin treatment. As a result, FFPE-derived DNA is one of the most difficult matrix with which to work. Extracted DNA is generally of suitable quality when used in simple experiments such as direct PCR-based applications. However, for wider analysis of a sample, involving greater number of genes, either through capture-panels or whole-genome sequencing, the quality of DNA is not adequate. Hence the quality of DNA needs to be improved if we are to benefit from sequencing technologies.

We, as a part of 100,000 Genomes Project, have established a DNA extraction pipeline that through the use of 1mm cores of FFPE-based tissue almost completely removes the risk of contamination between samples on the microtome and offers most efficient deparafinisation through 'Adaptive Focused Acoustics™' sonication (Covaris®). Further, the purification is performed on a bead-based automated instrument (Chemagic Prepito®-D, Perkin Elmer). In comparison with various other methods, this pipeline has delivered the best quality DNA that we have generated to date as assessed by shallow whole genome sequencing as a quality control metric. It is now a routine method of DNA extraction in our laboratories.

# S3

## Risk Stratification for Childhood Brain Tumours

Ⓟ TS Jacques

*UCL Institute of Child Health, London, UK*

Brain tumours are the commonest solid tumour in children and the commonest cancer-related cause of death in children. For many of the more common tumours, survival rates exceed 70% at 5-years but neurological and endocrine complications are frequent amongst the survivors and are responsible for considerable disability. As these children may only be in the first decade of life, this is a considerable life-long burden of disability. Therefore, there is a very critical balance between offering sufficient treatment to maintain survival rates, while at the same time not over-treating children in order to maximize their quality of survival. A major goal in pediatric neuropathology is, therefore, to identify accurately, children with high-risk disease who require escalated treatment (or in whom further treatment is futile) and to distinguish them from children with good prognosis disease for whom reduced treatment would be safe. This has lead to very rapid implementation of upfront molecular and pathological stratification in trials and routine clinical pathology, which may have implications in broader clinical practice.

# S4

## PI 3-Kinase, from the Bench to the Clinic

Ⓟ B Vanhaesebroeck

*UCL Cancer Institute, London, UK*

The PI 3-kinase (PI3K) signal transduction pathway has been implicated in a variety of physiological responses, and is a therapeutic target in amongst other cancer and inflammation. Mammals have eight distinct isoforms of PI3K. In order to gain insight into the physiological roles of PI3K isoforms, we have created 'kinase knockin' mice that have germline inactivating point mutations in the ATP-binding site of PI3K isoforms. This knockin strategy more faithfully mimics pharmacological inhibition than the classical knock-out approach. These knockin mice have allowed us to uncover new roles for PI3K isoforms in physiology, disease and therapy. We have recently also generated mice which allow to temporal and spatial induction of PI3K mutations found in human disease. Importantly, the careful modelling of PI3K inactivation or activation has led to (pathological) phenotypes in mice that have turned out to be remarkably predictive of the human situation, both at the level of disease induction and drug responses.

# S5

## Activated PI3-kinase Delta Syndrome (APDS): Genetics, Immunodeficiency, Diagnosis and Related Pathology

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Gain-of-function mutations in the genes encoding either the catalytic or regulatory subunits of PI3K delta lead to the Activated PI3K Delta Syndrome (APDS). The clinical phenotype is of B and T cell dysfunction with recurrent bacterial and viral infections, benign and malignant lymphoproliferative disease (encompassing reactive lymphadenopathy, mucosal lymphoid hyperplasia and B cell lymphomas), and features suggestive of autoimmunity (immune cytopenias and solid organ disease).
In a cohort of 53 patients, 64% developed chronic lymphadenopathy, often associated with local or disseminated infection. Histologically lymph nodes showed atypical follicular hyperplasia, with attenuated mantle zones and germinal centers disrupted by a follicular helper T-cell infiltrate. Mucosal nodular lymphoid hyperplasia affected 32%, with pathological features similar to those seen in lymph nodes. Six patients developed lymphoma, with 3 fatalities (2 with EBV-positive disease). There were 2 cases of diffuse large B-cell lymphoma, and single cases of nodular sclerosis Hodgkin lymphoma, marginal zone lymphoma, lymphoplasmacytic lymphoma, and post-transplant lymphoproliferative disease. Glomerulonephritis affected 3 children, with proliferative, membranoproliferative, and focal and segmental changes. Three patients developed cirrhosis, 1 of whom also had sclerosing cholangitis; sclerosing cholangitis affected a further, non-cirrhotic patient.
Insights into disease pathogenesis have also come from mouse models; for example, the demonstration of abundant PI3K delta in the developing mouse CNS and in the adult murine hippocampus and cerebral cortex strengthens an observed association with neurodevelopmental delay and autism-spectrum disorder.
Thus histopathological analysis has helped to define the range of lymphoproliferative and inflammatory disease in APDS, and further analysis of pathology in mouse models will give insights into the immunopathogenesis of this condition.

# S6

## Rare Types of Breast Cancer: Deconstructing the Complexity and Heterogeneity of Breast Cancer

Ⓟ B Weigelt

*Memorial Sloan Kettering Cancer Center, New York, USA*

Large-scale massively parallel sequencing studies have revealed the complexity and genetic heterogeneity of common types of cancer. To date, large-scale breast cancer sequencing efforts have mainly focused on the most prevalent histological subtypes, the invasive ductal carcinomas of no special type (IDC-NSTs) and invasive lobular carcinomas. These studies have demonstrated that there is a limited constellation of recurrently mutated genes and a vast number of genes that is mutated in a small subset (<2%) of cases. Through a combination of pathology and transcriptomic analysis, we have demonstrated that tumours from each special histological type of breast cancer are phenotypically and molecularly more homogeneous than IDC-NSTs. Furthermore, we have shown that some special histological types of breast cancer may constitute entities distinct from IDC-NSTs of the same grade and oestrogen receptor status, such as mucinous carcinomas. Our studies have demonstrated that there are special histological types of triple-negative phenotype that have a more indolent clinical behaviour than the common forms of high-grade triple-negative breast cancer (TNBC), and display distinctive patterns of genetic alterations, such as adenoid cystic carcinomas. Finally, through massively parallel sequencing analysis of polymorphous low-grade adenocarcinoma (PLGA), another vanishingly rare TNBC also present in the salivary glands, we have identified a hotspot somatic mutation affecting PRKD1 (i.e. E710D) in >70% of PLGAs. In this lecture, I will discuss how a combination of pathology with genetics is resulting in the development of a taxonomy for breast cancers that is more representative of the biology of the disease, and illustrate that investigating the genetic underpinning of rare cancer types provides an efficient approach for the identification of new driver genes in cancer.

# S7

## Introducing Digital Pathology

Ⓟ IA Cree

*University Hospitals Coventry and Warwickshire, Coventry, UK*

Technological advances have rarely troubled histopathologists over the last century, with the exception of improvements in microscope design and ergonomics. Automation of staining procedures and tissue processing in the laboratory still result in glass slides that would be familiar to pathologists working in the 1930s. Digital pathology represents a complete paradigm shift as it has the capacity to replace microscopes almost completely over the next few years. Just as radiologists adapted rapidly to the loss of x-ray films, histopathologists will adapt equally rapidly to the new realities of working with screens. There is little to lose, and much to gain. Not only does Digital Pathology offer a better workflow within histopathology departments, but it makes consultation with colleagues much easier. It also opens up the possibility of using image analysis tools to provide quantitate of information in addition to the diagnosis. Comparisons of glass slide versus digital diagnosis show excellent concordance. Digital pathology image analysis tools will be far better than the pathologist at estimating Her2, ER, PR and other immunohistochemistry results. These new tools are arriving rapidly to join the existing simple planimetry applications on Digital Pathology systems which already permit accurate measurements of tumour size to be made with a single sweep of the mouse. It is almost certain that pattern recognition tools will be developed which can help pathologists determine neoplastic cell percentage for molecular pathology, grade tumours, and assist diagnosis. The equipment required for digital pathology is not cheap, and represents a major investment for most pathology department. This is perhaps the biggest challenge, and the cost effectiveness of the systems requires evidence.

# S8

## The Genetic Pathology of Lung Cancer – Applying the Knowledge to Everyday Practice

Ⓟ JR Gosney

*Royal Liverpool University Hospital, Liverpool, UK*

The approach to the diagnosis, classification and analysis of lung cancer has changed beyond all recognition in the last decade. This revolution, which is still gathering momentum, is being driven by the continuing development of precisely-targeted drugs that are active against particular sub-groups of lung cancer defined more by their genetic pathology or their protein expression than by their morphology. The timely, rational and cost-effective use of these 'tailored therapies', that include anti-folates, tyrosine kinase inhibitors and, most recently, immune modulators, depends crucially on the rapid, accurate and integrated analysis of diagnostic histology or cytology specimens in particular, and poses major challenges for pathologists handling specimens of pulmonary tumours.

# S9

## Multigene Prognostic Assays in Breast Cancer — An Update

Ⓟ EA Rakha

*University of Nottingham, Nottingham, UK*

Breast cancer (BC) is a heterogeneous disease with variable presentation, morphology, behaviour and response to therapy. Management of BC relies on well-established clinicopathological variables and few validated molecular markers namely ER and HER2. However in the era of personalised medicine these variables seem insufficient to reflect BC biological and clinical heterogeneity. For instance, more than half of early-stage BC patients are classified as clinically indeterminate. Subsequently, decision to offer chemotherapy is often problematic. Advances in molecular techniques and bioinformatics have contributed to our understanding of BC biology and to the development of multigene prognostic molecular assays (MGPA) that can refine prediction of disease recurrence and determine benefits of chemotherapy. These assays were developed based on supervised clustering analysis of gene expression microarray data. Several MGPA have been developed and their component genes vary from 2 genes to more than 100 genes with little overlap. In MGPA, data on component genes are used to generate a score and cutoffs are applied to stratify patients into risk groups. The majority of MGPA contained genes related to proliferation, ER and HER2 pathways but some were developed based on genes involved in a biological process such as wound and immune response signatures. The first MGPA developed was MammaPrint which is a 70-gene microarray-based assay that requires fresh tissue. Others include Oncotype Dx, EndoPredict, Genomic Grade Index and 2-gene ratio which utilise fixed tissue and RT-PCR. Mammostrat, IHC4 and NPI+ are immunohistochemistry-based while Prosigna test utilises the NanoString nCounter technology. Although MGPA are useful in the clinically indeterminate ER+/HER2- BC class to determine the use of chemotherapy they can be applied to other classes. Several prospective clinical trials such as TAILORx, MINDACT and OPTIMA are underway to assess the clinical utility of various MGPA.

# S10

## The Role of Molecular Pathology in the Diagnosis and Treatment of Ovarian Tumours

Ⓟ CS Herrington

*Edinburgh Cancer Research Centre, Edinburgh, UK*

There are 5 distinct predominant types of ovarian carcinoma, with different origins and molecular profiles. High-grade serous carcinoma typically arises in the Fallopian tube and is associated with *TP53* mutation and homologous recombination deficiency. Diagnostically, the combination of WT1 positivity and an aberrant pattern (indicating gene mutation) of p53 expression is extremely useful. Many tumours previously categorised as high-grade endometrioid carcinoma, clear cell carcinoma or transitional cell carcinoma have been re-classified as high-grade serous carcinoma. Low-grade serous carcinoma is a distinct tumour type characterised by *BRAF* or *KRAS* mutation without *TP53* mutation. The tumour cells are low grade and, although WT1 positive, do not show an aberrant p53 pattern. Low-grade ovarian endometrioid carcinomas typically arise in association with ovarian endometriosis. These show a similar molecular profile to endometrial endometrioid carcinomas, with mutations in *CTNNB1*, *ARID1A* and *PIK3CA* being most frequent. They have a similar immunoprofile to endometrioid endometrial carcinomas. *PTEN* mutation is also found but may be less prevalent in ovarian, compared with endometrial, endometrioid carcinomas. Clear cell carcinomas of the ovary show abnormalities particularly in *ARID1A* and *PIK3CA* and are typically negative for ER and WT1, and show a wild-type p53 pattern. They also arise in association with endometriosis. Primary mucinous carcinomas of the ovary are uncommon and often show a mixture of benign, borderline and invasive components. They are associated with *KRAS* mutation and approximately 20% also show HER2 overexpression/amplification. Mucinous metastases to the ovary are more common and tend to be more often bilateral and of small size. Molecular analysis is less involved in therapeutic decision-making, although the stratification of patients for PARP inhibitor therapy, and the identification of HER2 overexpression/amplification, may have an emerging role.

# S11

## Clonal Evolution and Cancer Medicine: Implications for Pathologists

Ⓟ SAJ Aparicio

*BC Cancer Agency & UBC, Vancouver, Canada*

The notion that most cancers are ecosystems of evolving clones has implications for biological understanding and clinical application. The evolution of clonal composition has particular significance when evidence of positive or negative selection can be associated with the clonal genotype or epigenotype. Over the last 5 years next generation sequencing (NGS) of tumours and methods for single cell analysis have opened up this approach for solid epithelial malignancies. I will discuss the implications of clonal evolution for cancer medicine and biological studies of cancer with special reference to breast cancers. We have developed informatics approaches to population level clonal analysis using NGS methods and we have extended these to single cell measurements of genotypes. I shall discuss our more recent data from single cell sequencing and clonal analysis applied to clonal evolution of patient derived tumour xenografts, to illustrate the impact of clonal evolution on biological studies of cancer in model systems. Finally I will address some implications of clonality for tissue assessment and NGS quality control in molecular diagnostics of cancer.