



# Belfast Pathology 2017

## Plenary Oral and Oral Abstracts

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**Companion Sessions**

AIDPATH • Association of Clinical Electron Microscopists  
British Association of Cytopathology  
British Association of Gynaecological Pathology  
British Association of Urological Pathologists  
Dutch Irish English (DIE) Cardiac Group • Renal EQA  
Renal Transplant EQA • 100,000 Genomes Project



**KEY**

Ⓟ = Presenter

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**Front** — **Top:** Belfast City Hall <sup>1</sup> **Middle:** Queens University Belfast <sup>2</sup> **Bottom:** The Titanic Centre <sup>1</sup>

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## PL1

**Thioredoxin Interacting Protein (TXNIP) is an Independent Prognostic Factor in Breast Ductal Carcinoma In Situ**

© I Miligy<sup>1</sup>; A Gaber<sup>2</sup>; M Diez Rodriguez<sup>1</sup>; CC Nolan<sup>1</sup>; AR Green<sup>1</sup>; IO Ellis<sup>1</sup>; EA Rakha<sup>1</sup>

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**Background:** Current clinico-pathological parameters are useful predictors of recurrences in breast ductal carcinoma in situ (DCIS) but they are insufficient to reflect its molecular heterogeneity and a proportion of DCIS patients are over-treated. Biomarker expression can be useful in individualising therapy for DCIS. Thioredoxin interacting protein (TXNIP), located at 1q21.1, shows copy number alteration during DCIS progression to invasive disease and is a key player in oxidative stress. This study aims to investigate the role of TXNIP in DCIS progression.

**Patients and methods:** 776 consecutive DCIS patients treated in Nottingham between 1990 and 2012 were included into this study. Following histological review of the slides, tissue microarrays were constructed from representative tumour blocks. Patients' information, treatment and follow-up data were collected. The expression of TXNIP was assessed immunohistochemically after validation of specificity using Western blotting technique.

**Results:** Low/absent cytoplasmic expression of TXNIP was associated with features of aggressiveness including high nuclear grade ( $p=0.000004$ ), presence of comedo necrosis ( $p=0.0003$ ) and solid histological type ( $p=0.000001$ ). Univariate outcome analysis showed an inverse association with development of local recurrences ( $p=0.013$ ). Multivariable analyses showed that independent predictors of recurrence are low TXNIP expression ( $p=0.005$ , HR=0.51 and CI 0.32-0.81), larger size  $>43\text{mm}$  ( $p=0.009$ , HR= 0.6 and CI 0.46-0.89) and high tumour grade ( $p=0.003$ , HR= 1.78 and CI 1.2-2.6).

**Conclusion:** TXNIP expression predicts local recurrence in DCIS patients and is potentially useful is prognostic stratification of DCIS patients for management decisions.

## PL3

**Adaptive Clonal Heterogeneity During Oesophageal Cancer Progression**

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**Purpose of the study:** The development of columnar metaplasia in Barrett's oesophagus in response to gastro-oesophageal reflux is thought to represent the product of natural selection acting at the level of stem cells. Mucin production is the key trait that connects 'fitness' to clonal expansion of metaplastic columnar epithelium in the distal oesophagus. This mucin acts as a public resource by protecting the distal oesophagus from acid reflux-mediated damage. The development of dysplasia is accompanied by a loss of cellular differentiation and decreased protective mucin production. It is unknown how expanding dysplastic clones cope with the loss of mucin production and avoid extinction due to erosion in this harsh reflux environment.

**Methods:** We created three-dimensional reconstructions of BE progression to single cell resolution using a combination of spatial modelling, genetic lineage tracing and expression studies. Results were evaluated across a large cohort of patients ( $n=60$ ) and correlated to mucin production and differentiation status.

**Summary of results:** We found that mucin-depleted BE dysplasia co-opts the pre-existent glandular network by forming extensive heterotypic glandular networks with pre-existent non-dysplastic Barrett's glands. We show that these heterotypic glandular networks functionally mimic normal BE tissue organisation and that the degree of mucin production within BE dysplasia predicts formation of these heterotypic gland structures.

**Conclusions:** Acid-biliary reflux is traditionally seen solely as a driver of tumor progression in the distal oesophagus. Our data show that the caustic micro-environment actively constrains evolution to cancer. Recognising these heterotypic glandular networks will aid in the histopathologic classification of Barrett's dysplasia in routine practice.

## PL2

**Survey of UK Pathology Consultants' Attitudes Towards Academic and Molecular Pathology**

© SF Brockmoeller<sup>1</sup>; © C Young<sup>1</sup>; JL Lee<sup>2</sup>; M Arends<sup>3</sup>; JL Jones<sup>4</sup>; M Salto-Tellez<sup>5</sup>; GJ Thomas<sup>6</sup>; KA Oien<sup>7</sup>

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Over the past 15 years, the Medical Schools Council (MSC) has documented an ongoing decline in the number of academic pathologists within the UK, posing a major threat to cellular pathology's capacity to innovate and gain molecular skills. The National Cancer Research Institute (NCRI) initiative in cellular and molecular pathology (CM-Path) was launched in June 2016 to reinvigorate UK academic pathology and promote the adoption of molecular pathology. The CM-Path initiative distributed a survey to UK-based consultant pathologists via the Pathological Society and the Royal College of Pathologists email networks. The survey, which examined attitudes towards academic and molecular pathology, was distributed over a 4 month period with completion on 1st March 2017. 347 consultants responded to the survey, 77% of whom were histopathologists. Regarding involvement in academic research, only 10% of respondents had never been involved in research; 41% were currently involved, on an informal basis; 24% were formally undertaking research; and 23% had previously been involved. Major barriers to undertaking research were "lack of time" and "lack of training". Regarding molecular pathology, 38% cited that they used molecular pathology "a lot" and 34% were beginning to use molecular pathology in NHS diagnostic work, however, only 10% of respondents had received formal training. Major barriers to training in molecular pathology were "lack of time" and "lack of available training opportunities". Despite MSC data showing a decline in the number of UK academic pathologists, our data indicates that a large proportion of pathologists are undertaking academic research out-with their job plan. In addition, most pathologists are using or beginning to use molecular pathology in their diagnostic work without formal training. This demonstrates the need to enable time and opportunity for training in molecular and academic pathology, to support both NHS diagnostic work and research.

## PL4

**Impact of Postmortem CT on Coronial Autopsies: Review of a Digital Autopsy Service**

© AMS Shavick<sup>1</sup>; JH Hamblin<sup>1</sup>; REB Benamore<sup>2</sup>; ZCT Traill<sup>2</sup>; ISR Roberts<sup>3</sup>

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The use of postmortem CT (PMCT) can reduce the requirement for traditional invasive Coroner's autopsy and demonstrate injuries not detected by dissection. Here we review the first 300 cases in our digital autopsy service in order to determine the impact of PM imaging on autopsy practice. Deaths were referred from 24 Coroners. All cases had PMCT, followed by angiography and invasive autopsy as required. 272 were at the request of families who objected to traditional invasive Coroner's autopsy, and 28 for police to supplement PM dissection in forensic investigation, including 4 paediatric autopsies. Excluding these forensic/trauma cases, a cause of death was issued in 222 (82%) on the basis of PMCT imaging  $\pm$  angiography without invasive autopsy. 104 (38%) required CT alone and 118 (43%) CT + angiography. 11% of deaths occurred in hospital and there was a significantly higher requirement for invasive examination in this group when compared to deaths in the community (38% vs 16%,  $p=0.05$ ). There was also a greater requirement for invasive autopsy in younger adults. In the age groups  $<50$ , 50-70 and  $>70$  years, an invasive procedure was carried out in 31%, 24% and 13% respectively,  $p=0.01$ . The commonest causes of death were cardiac disease (49%), followed by non-coronary arterial disease (15%) and respiratory disease (12%). The use of PMCT with angiography results in an 82% reduction in the number of invasive Coroner's autopsies. Hospital deaths and deaths in young adults have a higher requirement for an invasive procedure, reflecting a different spectrum of causes of death.



## PL5

### Evaluating Sudden Cardiac Death: Insights from the Largest Pathology Database for Sudden Cardiac Death in the World

© JD Westaby; CJ Miles; MN Sheppard

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Most sudden cardiac deaths are due to atherosclerotic coronary artery disease. In younger individuals, cardiac genetic disorders are more common. Causes of death from 1998 to 2016 were reviewed and classified based on pathological findings and circumstances. All cases had undergone expert cardiac pathological review. 13 cardiac sections are taken as standard. Cases were classified as sudden arrhythmic death syndrome (SADS) based upon negative autopsy, negative expert cardiac evaluation and negative toxicology. A total of 5340 cases were analysed. Average age was 36(0-101). M:F ratio was 1.86:1. The most frequent classification was SADS(43.8%). Cardiomyopathies(CM) were diagnosed in 1229 cases(23.0%). The cause of death was hypertrophic cardiomyopathy(HCM) in 220 cases(4.1%), arrhythmogenic cardiomyopathy(ACM) in 195 cases(3.7%) and dilated cardiomyopathy in 148 cases(2.8%). The other CMs made up 665 cases(12.5%). In 2015, we received 13 referrals of HCM and 23 referrals of ACM. Ischaemic heart disease(IHD) accounted for 912 deaths(17.1%). Myocarditis or endocarditis were reported in 145(2.7%) cases. Other cardiovascular causes included valvular disease(138 cases/2.6%), congenital heart disease(88 cases/1.6%) and aortic related disease(77 cases/1.4%). This is the largest pathology series available on SCD. It highlights that SADS and CM make up 66.8% of referrals. These have possible genetic aetiology and with the increasing availability of molecular material combined with cardiological screening of relatives we should be able to increase our diagnostic yield. We now almost have as many referrals of ACM as of HCM, the previously thought predominant cause of sudden cardiac death in cardiomyopathy. We postulate that ACM is, and has been, under recognised by the general autopsy practitioner. It will be interesting to see the future trends of these two important causes of sudden death. IHD is less common in the younger population and may not be referred.

## PL7

### Genetic and Epigenetic Landscape of Chordoma with a Focus on Studying Inactivation of *CDKN2A/p16*

© L Cottone<sup>1</sup>; P Lombard<sup>1</sup>; N Eden<sup>1</sup>; C Unwin<sup>2</sup>; H Ye<sup>2</sup>; R Tirabosco<sup>2</sup>; S Behjati<sup>3</sup>; N Pillay<sup>1</sup>; AM Flanagan<sup>1</sup>

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The authors have requested that this abstract is not published.

## PL6

### Examining the Genomic Changes Which Occur in the Development of Oral Cancer from a Pre-Cancerous Background

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**Purpose of study:** It is generally assumed that oral pre-cancer contains a limited subset of the genomic changes seen in invasive disease. Driver events are thought to occur early, but it not known to what extent they are present in pre-invasive disease. These assumptions need to be tested as clinical focus moves increasingly towards both early detection and personalised medicine. The purpose of this study was to examine the suspected driver events in a number of oral cancers, and to assess how many of these events were present in the associated pre-cancerous samples from the same patients.

**Methods:** Genomic copy number was examined from 256 pre-invasive and invasive lesions taken from 69 oral cancer patients using low pass genome sequencing. A more detailed examination of 48 samples from 16 patients was carried out using exome sequencing.

**Summary of results:** One third of dysplasias contained copy number events absent in the associated carcinoma, demonstrating independent evolution. The remainder exhibited a copy number profile with a subset of the events seen in the carcinoma. All dysplasias examined had somatic point mutations absent in the matched carcinoma. Copy number events commonly seen in oral cancer, and point mutations in TP53 were frequently shared, indicating the early development of key driver events. The timing of other putative driver mutations was more sporadic.

Analysis of the mutation rates of the samples indicated that most samples develop neutrally, with no sudden expansion of an invasive sub-clone.

**Conclusions:** These findings suggest that the genomic changes driving oral cancer develop gradually during the pre-cancerous stage by random accumulation, rather than one dramatic event dividing the pre-invasive and invasive states.

## PL8

### Development of a Semi-Automated Analysis Pipeline for Next-Generation Sequencing Based Targeted Mutation Data in the FOCUS4 Trial

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**Purpose of study:** As next-generation sequencing (NGS) becomes more routine in both research and clinical settings, the development of robust analysis pipelines is increasingly important. As such, the task of processing, analysis and interpretation of the raw data needs to move away from that of a specialist bioinformatician and become more accessible to workers with a general background. The purpose of this work was to collate all the bioinformatic tools used into a user-friendly pipeline, minimising the specialised skills required to perform routine analysis.

**Methods:** By a series of benchmarking studies and analysis of best practice protocols, the tasks of adapter trimming, alignment, indel realignment, mutation calling, and functional effect prediction were collated into an integrated workflow, which can be operated in full with a single command. All calls were visually checked before being reported. Validation of the pipeline was carried out by comparing the results with those previously obtained by pyrosequencing on patients recruited into the FOCUS4 trial.

**Summary of results:** All pyrosequencing calls were also detected using the NGS pipeline, although one call was rejected due to being below a pre-agreed 5% mutation cutoff. Two low level calls in mutation hotspots were called using NGS that were not called using pyrosequencing, but could be seen as low level events in the pyrograms. An additional benefit to using NGS is that mutations can be called outside of hotspots, and in genes with multiple pathogenic loci, which are difficult to fully screen by pyrosequencing.

**Conclusions:** A well planned NGS analysis pipeline can process samples with no need for the user to develop advanced informatics skills. We have shown that this pipeline compares favourably to pyrosequencing in the task of mutation detection. Currently the pipeline is being used for the limited analysis of five genes for the FOCUS4 trial, but could be used for any number of genes with little modification.

## O1

**How Robust is the Expression of CK20, CK7 and CDX2 in Diagnosing Colorectal Cancer?**© MM Davie<sup>1</sup>; A Alnabulsi<sup>2</sup>; GI Murray<sup>1</sup><sup>1</sup>Aberdeen Royal Infirmary, Aberdeen, UK; <sup>2</sup>University of Aberdeen, Aberdeen, UK

The classic immunophenotype of colorectal cancer (CRC) is CK20+/CK7-/CDX2+. This immunohistochemistry (IHC) panel can be used to accurately classify primary and metastatic CRC. IHC can be particularly helpful in the diagnosis of poorly differentiated tumours and metastatic deposits. However, not all CRCs express the classic immunophenotype. Aberrant IHC expression could lead to incorrect diagnosis. The purpose of this study was to analyse the expression of CK20, CK7, and CDX2 by a large cohort of colorectal cancers. IHC was performed on a colorectal cancer microarray, using antibodies to CK20, CK7, and CDX2. The microarray was constructed from blocks of formalin fixed, paraffin embedded tissue, from 650 primary CRCs. All patients had undergone elective surgery for CRC at a regional cancer centre between 1994 and 2009. A selection of clinical and pathological data was available for each case. Staining for each antibody was scored and recorded as either negative or positive. 650 cases were studied, with a mean patient age of 69 years. Most tumours were staged as Dukes B (37.5%) or Dukes C (44%). Overall, only 63% of cases expressed the classical phenotype of CK20+/CK7-/CDX2+. 10% of cases were CK20+/CK7-/CDX2-, and 9% of cases displayed the phenotype CK20-/CK7-/CDX2+. 9% of cases were triple negative, and 6% of cases were triple positive. Only 2% of cases were CK20-/CK7+/CDX2-. Looking at markers individually, 79% of cases were CK20 positive and 78% were CDX2 positive, whilst 91% of cases were CK7 negative. Less than two thirds of CRCs in this cohort express the classic immunophenotype of CK20+/CK7-/CDX2+, with a significant proportion of tumours (37%) showing aberrant staining of at least one of the three markers. However, only 2% of cases showed the opposing immunophenotype of CK20-/CK7+/CDX2-. Using the panel of three markers together is still a useful tool in diagnosing colorectal cancer, as long as results are interpreted with a degree of caution.

## O3

**Retrospective Study of the Correlation Between Radiological and Histopathological Staging in Advanced Rectal Cancers Requiring Multi-Visceral Resection**

© AC Huskinson; A Quyn; P Sagar; N West

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**Purpose of study:** Colorectal cancer affects around 41,000 people a year in the UK. A significant minority present with locally advanced rectal cancer requiring multi-visceral resection preceded by chemoradiotherapy. Increasingly, tumours are restaged by MRI prior to surgery to determine the degree of regression and confirm the optimal operative planes. We aimed to correlate the post-treatment MRI report with the final histopathological staging.

**Methods:** We identified 16 patients following multi-visceral resection for locally advanced rectal cancer between January 2015 and September 2016 within a large teaching hospital specialist advanced colorectal cancer practice. Of these, 4 were excluded: 3 didn't receive pre-operative therapy and 1 had no MRI imaging. The post-treatment MRI report and histopathology reports were obtained and compared.

**Summary of results:** Of the 12 patients, 9 had adenocarcinoma and 3 squamous cell carcinoma. Correlation between radiology and histopathology reports was challenging due to differences in reporting style. In 8 cases T staging was identical; in the other 4, the final T stage was lower as adjacent structures were not involved as predicted on MRI. In the 6 cases where vascular invasion was not reported by MRI, these were assumed to be V0; with this assumption, there was agreement in over half of cases (7 cases). Resection margin status was predicted accurately in 10 cases with the remainder having clear margins despite the MRI predicting involvement. Assessment of neoadjuvant treatment response was difficult to compare due to differences in the grading systems used, but only 3 cases showed a marked difference in response.

**Conclusion:** In the majority of cases, post-neoadjuvant treatment MRI accurately predicted T stage and resection margin status in advanced rectal cancers requiring multivisceral resection. Restructuring the synoptic reporting systems used in radiology and histopathology could be explored to facilitate comparative studies.

## O2

**Improving the Management of Early Colorectal Cancer: Assessment of Quantitative Markers to Predict the Need for Major Resection**© SF Brockmoeller<sup>1</sup>; E Toh<sup>1</sup>; S Fleming<sup>2</sup>; E Morris<sup>2</sup>; P Quirke<sup>1</sup><sup>1</sup>Leeds Institute for Cancer and Pathology, Leeds, UK; <sup>2</sup>University Leeds, Leeds, UK

Detection of early colorectal cancer lesions through implementation of the National Health Service Bowel Cancer Screening programme has increased by a factor of three to about 17%, but how these lesions are managed is unclear. Therefore, new guidelines for predicting cancer spread to determine treatment are urgently needed. We build a multivariate model of quantitative reproducible markers to predict lymph node metastases (LNM). Our cohort consisted of 206 symptomatic patients with pT1 colorectal cancer (19 with LNM). Qualitative factors were obtained from routine pathological reports. Quantitative markers of tumour stroma content, area of submucosal invasion of the tumour, tumour budding, and width of cancer were measured, and cut-off values were assessed as previously described. Associations between categorical data and LNM were performed with the x2 test and Fisher's exact test. Multivariate modelling was carried out with logistic regression to estimate predicted probabilities for LNM. Youden's rule was used to define the probability cut-off point for LNM. All significant parameters in the univariate model were included in a multivariate model: tumour stroma content (odds ratio 9.7, 95% CI 2.3 - 41.0; p=0.002), area of submucosal invasion (24.5, 2.1 - 284.6; p=0.011), poor grade of differentiation (26.7, 2.0 - 358.3; p=0.003), and lymphatic invasion (16.1, 1.3 - 192.6; p=0.028) were predictive of LNM, but tumour budding and vascular invasion were not significant. Youden's rule gave a cut-off value of p>0.05 capturing 18(95%) of 19 LNM cases, leading to resection in 70(34%) of 206 cases. When the model only included quantitative factors all quantitative factors were significant. Youden's rule gave an optimum cut-off point at p>0.05 capturing 17(90%) of 19 LNM cases and leading to resection of 72(35%) of 206 cases. In this small retrospective pilot study, we show that we could reduce the resection rate in pT1 colorectal cancer to 34% while detecting up to 95% of all LNM cases.

## O4

**Deriving Prognostic Information from the Tumour Microenvironment in Stage II/III Colonic Adenocarcinoma: Reinvigorating the Soil and Seed Hypothesis**© SO Hynes<sup>1</sup>; HG Coleman<sup>2</sup>; PJ Kelly<sup>3</sup>; S Irwin<sup>3</sup>; RF O'Neill<sup>2</sup>; RT Gray<sup>2</sup>; C McGready<sup>1</sup>; PD Dunne<sup>1</sup>; S McQuaid<sup>1</sup>; JA James<sup>1</sup>; M Salto-Tellez<sup>1</sup>; MB Loughrey<sup>3</sup><sup>1</sup>Northern Ireland Molecular Pathology Laboratory, Belfast, UK; <sup>2</sup>Centre for Public Health, Queen's University Belfast, Belfast, UK; <sup>3</sup>Royal Victoria Hospital, Belfast, UK

**Purpose of the study:** Modern classification of colorectal adenocarcinoma by molecular subtypes has reinvigorated interest in the "soil and seed" hypothesis of cancer, including inflammatory and stromal signatures in an attempt to better prognosticate and predict response to treatment. Despite this, microscopic assessment of tumour microenvironment has not been adopted into routine histopathology reporting practice.

**Methods:** From existing literature, we selected specific morphological features relating to peritumoral inflammatory and stromal responses, agreed criteria for scoring, and assessed these in representative H&E-stained whole tumour sections, from a population-based cohort of 445 stage II/III colon cancer cases.

**Summary of results:** Moderate/severe peritumoral diffuse lymphoid inflammation and Crohn's disease-like reaction were associated with significantly reduced risks of CRC-specific death (adjusted HR 0.48, 95% CI 0.31-0.76; and HR 0.60, 95% CI 0.42-0.84, respectively). The presence of >50% tumour stromal percentage, assessed by global evaluation of tumour area, was associated with a significantly increased risk of CRC-specific death (HR 1.60 95% CI 1.06-2.41). A composite, "fibroinflammatory score" (0-3), combining dichotomised scores of these three features, showed a highly significant dose-response association in relation to survival outcomes. Those with a score ≥2 had an almost 2.5 fold increased risk of CRC-specific death (HR 2.44, 95% CI: 1.56-3.81), compared with those scoring zero. These associations were stronger in MSI-high tumours, potentially identifying a subset of MSI-high colon cancers which lack characteristic morphological features and have an associated worse prognosis.

**Conclusions:** Selected microenvironmental microscopic features readily identified on H&E sections, without additional staining, can provide valuable prognostic information in stage II/III colon cancer, potentially identifying higher risk disease and guiding management.

## 05

**The Development of a Semi-Quantitative Assessment Tool for Analysing Regression Patterns Following Chemoradiotherapy in Colorectal Cancer**

© EL Clarke; NP West

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**Purpose of the study:** Following neoadjuvant chemoradiotherapy (CRT) in colorectal cancer (CRC), pathologists evaluate the degree of regression in the resection specimen. Patterns of regression and their significance are poorly understood and the availability of diverse scoring systems has led to widespread confusion. Moreover, a wide range of morphological patterns of regression following CRT have been observed, and consequently, the current scoring systems lack the required flexibility to document these patterns in detail. We sought to analyse patterns of regression in CRC through the development of a semi-quantitative assessment tool.

**Methods:** A literature search reviewed current methods of regression grading in CRC and other cancers, and where applicable, applied previously validated definitions for observed features. This enabled the construction of a semi-quantitative assessment tool for analysis of regression patterns. The assessment tool was developed using an iterative process through its application to a pilot colorectal case series (n=10) treated with CRT. Each case was reviewed by a consultant gastrointestinal pathologist.

**Summary of results:** Features selected for inclusion comprise: Mandard regression grade; deepest layer with viable cancer cells; pattern of response across the whole tumour; mucosal ulceration; other damage to the mucosa; residual mucosal adenoma; regression towards the lumen; mucoid change; fragmentation; fibrosis; calcific deposits; inflammatory cell infiltrate; cancer cell necrosis; blood vessel damage; regressed tumour in lymph nodes. All of these features were assessable across the pilot series leading to a detailed description of the pattern of response.

**Conclusions:** We have developed a robust, semi-quantitative method of analysing regression patterns in CRC following CRT, tested against a small case series. Further work involving a larger case series will be conducted to thoroughly evaluate patterns of response.

## 07

**RAS Screening in Colorectal Cancer: Comprehensive Analysis of the Results from the UK NEQAS Colorectal Cancer External Quality Assurance Schemes (2009–2016)**© SD Richman<sup>1</sup>; J Fairley<sup>2</sup>; R Butler<sup>3</sup>; ZC Deans<sup>2</sup>*<sup>1</sup>Leeds Institute of Cancer and Pathology, Leeds, UK; <sup>2</sup>UK NEQAS for Molecular Genetics, Edinburgh, UK; <sup>3</sup>University Hospital of Wales, Cardiff, UK*

Extended RAS testing should be undertaken in mCRC patients, prior to prescribing anti-EGFR therapies. With more laboratories implementing testing, the requirement for External Quality Assurance schemes increases, thus ensuring high standards of molecular analysis. Data was analyzed from 15 UK NEQAS for Molecular Genetics Colorectal cancer EQA schemes, delivered between 2009 and 2016. Participating laboratories were provided annually with nine colorectal tumour samples for genotyping. Information on methodology and extent of testing coverage was requested, and scores given for genotyping, interpretation of results and clerical accuracy. Laboratory participation has increased six-fold (18 in 2009 to 108 in 2016). For RAS genotyping, fewer laboratories now use Roche cobas, pyrosequencing and Sanger sequencing, with more moving to next generation sequencing (NGS). NGS is the most commonly employed technology for BRAF and PIK3CA mutation screening. KRAS genotyping errors were seen in <10% laboratories, until the 2014-15 scheme, when there was an increase to 16.7%, corresponding to a large increase in scheme participants. NRAS genotyping errors peaked at 25.6% in the first 2015-16 scheme, but subsequently dropped to below 5%. Interpretation and clerical accuracy scores have been consistently good throughout. Within this EQA scheme, we have observed that the quality of molecular analysis for colorectal cancer samples has continued to improve, despite changes in the required targets, the volume of testing and the technologies employed. It is reassuring to know that laboratories clearly recognize the importance of participating in EQA schemes.

## 06

**FOCUS4: MAMS Trial Design in Action. Early Closure of FOCUS4-D (Pan-HER 1, 2 and 3 Inhibitor Versus Placebo) in Advanced Colorectal Cancer (aCRC) Patients, with Tumours Wildtype (WT) for KRAS, NRAS, BRAF and PIK3CA**© SD Richman<sup>1</sup>; R Adams<sup>2</sup>; E Brown<sup>3</sup>; L Brown<sup>4</sup>; R Butler<sup>5</sup>; S Falk<sup>6</sup>; D Fisher<sup>7</sup>; R Kaplan<sup>8</sup>; G Middleton<sup>7</sup>; P Quirke<sup>1</sup>; L Samuel<sup>9</sup>; J Seligmann<sup>1</sup>; MT Seymour<sup>1</sup>; KK Shiu<sup>4</sup>; H Wasan<sup>9</sup>; R Wilson<sup>10</sup>; T Maughan<sup>11</sup>*<sup>1</sup>Leeds Institute of Cancer and Pathology, Leeds, UK; <sup>2</sup>Cardiff University and Velindre Cancer Centre, Cardiff, UK; <sup>3</sup>Edinburgh Cancer Centre NHS Lothian, Edinburgh, UK; <sup>4</sup>MRC Clinical Trials Unit at UCL, London, UK; <sup>5</sup>All Wales Genetics Laboratory, University Hospital of Wales, Cardiff, UK; <sup>6</sup>University Hospital Bristol NHS Foundation Trust, Bristol, UK; <sup>7</sup>University of Birmingham, Birmingham, UK; <sup>8</sup>Aberdeen Royal Infirmary, Aberdeen, UK; <sup>9</sup>Imperial College London, London, UK; <sup>10</sup>Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, UK; <sup>11</sup>CRUK/MRC Oxford Institute for Radiation Oncology, University of Oxford, Oxford, UK*

Clinical trial designs are becoming ever more complex, providing the ability to evaluate multiple novel treatments, in parallel with multiple biomarker analyses. FOCUS4 is a phase II/III trial programme, assessing targeted agents in aCRC patients, within molecularly stratified cohorts. Pan-HER inhibition (blocking EGFR, HER2 & HER3 signalling) was hypothesised to be superior to blocking only EGFR signalling in WT tumours, by reducing *de novo* resistance, and slowing acquired resistance. In FOCUS4-D, the orally active AZD8931 was compared to a placebo in WT patients. Tumours underwent biomarker analyses in Leeds or Cardiff; pyrosequencing of KRAS, NRAS, PIK3CA & BRAF, and immunohistochemical analysis of MLH1, MSH2, MSH6, PMS2 & pTEN. A 1st-line treatment break was planned for after 16 weeks chemo, at which point, patients with WT tumours were randomised to FOCUS4-D, receiving either AZD8931 or placebo. The primary outcome measure was progression-free survival. The multi-arm, multi-stage (MAMS) trial design allowed pre-planned interim analyses to ascertain whether to continue the randomisation. Thirty two patients were randomised to FOCUS4-D between Jan 2014 and Mar 2016; 16 in each arm, with both arms balanced for baseline characteristics. Following the results of the first pre-planned interim analysis, the Independent Data Monitoring Committee recommended trial closure, on the basis of a lack of activity of the drug AZD8931. The observed hazard ratio did not fall below the critical pre-defined threshold value of 1.0. Median PFS was 3.7 months and 3.5 months in the placebo and AZD8931 cohorts respectively. A skin rash was the most common toxicity (20% of AZD8931-treated patients). AZD8931 failed to demonstrate improvements in PFS in WT aCRC patients. The novel MAMS trial design did however allow the early closure of this molecular cohort, without affecting the remaining trial comparisons. Furthermore, novel biomarkers and agents can be introduced, as and when identified.

## 08

**Introducing a New Panel of Markers for Microsatellite Instability (MSI) Detection in Colorectal Cancer by High Resolution Melting Analysis**© W Fadhil<sup>1</sup>; S Susanti<sup>1</sup>; HO Ebili<sup>1</sup>; J Field<sup>2</sup>; K Stafford<sup>2</sup>; H Hadjimichael<sup>1</sup>; M Ilyas<sup>1</sup>*<sup>1</sup>School of Medicine/ University of Nottingham, Nottingham, UK; <sup>2</sup>Nottingham University Hospitals NHS Trust, Nottingham, UK*

The microsatellite instability status in CRC can be inferred by immunostaining of the mismatch repair (MMR) proteins; MLH1, MSH2, MSH6 or PMS2. Direct MSI testing mostly involve the use of capillary electrophoresis (CE) or by denaturing high performance liquid chromatography (DHPLC). Recently, determination of microsatellite instability in CRC clinical samples showed that High Resolution Melting (HRM) exhibits more than 90 % sensitivity and specificity in separating the MSI from MSS group. This paper describes a new panel of microsatellite markers, consists of 3 novel markers (ESWR1, MYB and TP53) and 3 previously reported markers (BCAT25, BAT25 and BAT 26) for a low cost, rapid and highly sensitive MSI detection using HRM analysis for archival clinical samples. A total of 53 clinical samples which had been tested for MSI status using CE and for MMR protein expression by immunostaining. The HRM analysis was performed using 2 systems, ABI HRM software v2.0.1 (Applied Biosystem) and the Light-Scanner (LS) (Idaho Technology). Limit of detection (LOD) of the MSI markers by HRM was also compared to the CE platform. Overall, for the novel MSI markers, sufficient difference of melting pattern could be observed in HRM when as few as 6.25% mutant alleles were present and this is comparable to the currently used CE analysis. There was some difference between the HRM platforms and the auto grouping feature of the LS software could identify samples with very low percentage of mutant allele (1.56%). Among 53 case, 20 cases were confirmed as MSI by HRM analysis, showing 100% sensitivity, specificity and concordance to both CE and immunohistochemistry (IHC) data. Based on HRM analysis on LS, the MSI samples showed instability on least 5 microsatellite markers. In summary, this study revealed the potential clinical use of HRM-based MSI screening using a novel panel of mononucleotide microsatellite markers on archival pathology specimen that is low-cost, robust and highly sensitive.

**O9**

**Sudden Unexplained Death in Alcohol Misuse Patients have Different Characteristics to Those Who Died from Sudden Arrhythmic Death Syndrome**

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**Purpose:** Sudden arrhythmic death syndrome (SADS) is a diagnosis of exclusion with no obvious cause of death and a morphologically normal heart. Sudden unexplained death in alcohol misuse (SUDAM) is also a diagnosis of exclusion affecting heavy alcohol users. As in SADS, there is no obvious cause of death and acute alcohol toxicity and alcoholic ketoacidosis are specifically excluded. It is important to differentiate between SADS and SUDAM because SADS is often associated with heritable channelopathies that may affect surviving family members. Such family members can benefit from screening for genetic mutations associated with channelopathies, therefore preventing further sudden deaths. This study describes the characteristics of a cohort with SUDAM from a tertiary cardiovascular referral centre and compares the findings with those of individuals who died from SADS.

**Method:** Cases in this retrospective cross-sectional study were identified from a database of referrals to our centre spanning approximately 40 years. Cases with recorded heavy use of alcohol and non-alcohol users were selected, then limited to those with SUDAM or SADS aged 16 to 64 years of age.

**Results:** 62 cases of SUDAM and 41 cases of SADS were identified. The SUDAM group were older than the SADS group; mean age 35.8 years and 27.7 years respectively (P=0.0002). There was also a higher incidence of significant psychiatric illness in SUDAM (19.7%) than SADS (2.4%). Post mortem liver examination was more likely to reveal heavy livers in SUDAM than SADS (2196.1g and 1572.4g respectively; P=0.0033) and more fatty liver change (24.2% and 2.4%).

**Conclusion:** SUDAM tends to occur in individuals who are older and have heavier livers than those with SADS. Psychiatric illness is also more common. These different characteristics can help identify families most likely to benefit from screening for channelopathies, thus aiding in preventing future deaths from SADS.

**O11**

**The Effects of Cold Ischaemia on Tumour Morphology and DNA Quality for Whole Genome Sequencing: A Systematic Comparative Study of a Series of Renal Tumours in a Single Institution**

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The requirement for tumour tissue for molecular pathology, including whole genome sequencing (WGS), is increasingly important within clinical and research settings, with preference for fresh frozen (FF) tissue over formalin-fixed paraffin embedded (FFPE) tissue. However the provision of FF tissue is labour intensive, influenced by many factors including the delivery of a fresh surgical specimen to a pathologist who may be off site or 'out of hours'. Validated alternative storage methods for fresh specimens until FF tissue can be sampled are much needed. We carried out a systematic study of ten renal tumours, to compare the quality of DNA extracted from FF samples, from half of the tumour formalin-fixed upon receipt on day 0 with that from the half of the tumour fixed on day 1 following a further period of up to 24 hours at 4°C without formalin. We also compared the morphology of the tumour from both halves of the specimen. The DNA extracted from FF samples taken on day 1 was of acceptable quality for WGS in all ten cases. The morphology of the tumour formalin-fixed on day 0 and day 1 was considered to be comparable and suitable for diagnostic purposes in all cases following blinded review of representative H&E sections by eight histopathologists. We conclude that prolonged cold ischaemia for up to 24 hours prior to formalin-fixation of a renal tumour specimen does not impact significantly on the morphology, and that the DNA extracted from these samples is of suitable quality for WGS.

**O10**

**With a Heavy Heart: Sudden Cardiac Death in Hypertensive Heart Disease**

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**Introduction:** The prevalence of mortality due to hypertensive heart disease (HHD) is 1.2% in the UK. HHD is a well-established cause of sudden cardiac death (SCD). This study reports the characteristics of a cohort of SCD in HHD from a tertiary referral centre for cardiac pathology.

**Method:** We performed a retrospective cross sectional study, identifying cases from a national database recorded between 1994 and 2016. Cases were selected based on the diagnosis of HHD after expert cardiac review. Hypertension had to be diagnosed pre-mortem. Diagnostic criteria included increased heart weight, left ventricular thickness of greater than 15mm, myocyte hypertrophy and fine interstitial fibrosis. Cases with significant coronary artery disease were excluded.

**Results:** 63 cases of sudden death in hypertensive heart disease were identified. The average age was 53±15 years; 32 were male and 31 were female. Females tended to be older than males. Average BMI was 33±9 and 60% were obese. Average weight of heart was 580±165 (males 577±161, females 556±169). Female hearts showed a greater increase above normal weight than male hearts (p=0.027). Mean maximal left ventricular thickness was 15.9mm (range 11-20). All hearts showed concentric hypertrophy. Left ventricular fibrosis was present in 46 cases (73%). The presence of fibrosis was not associated with sex (p=0.55), BMI (p=0.09) or heart weight (p=0.20).

**Discussion:** SCD in HHD occurs with similar frequency in both sexes. Females show a greater increase in heart weight and may be protected by oestrogen pre-menopause. Hypertension and obesity may act synergistically to increase the risk of SCD. Fibrosis was present in most cases and appears to occur independently of sex, BMI and heart weight. SCD appears to occur in those with concentric hypertrophy rather than eccentric hypertrophy. These findings highlight the importance of early identification and treatment of hypertension to prevent progression to HHD.

**O12**

*This abstract has been withdrawn*



## O13

**A Genomic Classification Model Enables Risk Stratification of Paediatric Endemic Burkitt Lymphoma in Malawi**

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**Purpose:** Endemic Burkitt lymphoma (eBL) is the most common childhood cancer in sub-Saharan Africa. Treatment of sporadic BL (sBL) with intensive chemo-immunotherapy results in excellent cure rates in high-income settings but delivery of similar therapy in low-income countries is prevented by high costs and insufficient supportive care facilities. Consequently, patient outcome remains unsatisfactory. We aimed to develop a genetic risk model to stratify patients and predict response to protocols currently used in Malawi.

**Methods:** High-resolution genomic copy number array profiling of 72 eBL cases identified regions of recurrent copy number alteration (CNA). CNAs previously associated with outcome in sBL were examined by FISH. Univariate and multivariate analyses examined the association of CNAs with risk of relapse (RR), event-free (EFS), and overall survival (OS). A two-group risk model was defined for eBL and applied to 50 paediatric sBL patients treated using European protocols.

**Results:** Gain of either or both of two loci on 1q and 13q, involving known cancer-related genes, identified one third of eBL patients as having a high-risk of relapse (77%) compared to the low-risk group (21%) (hazard ratio 5.36,  $p=0.005$ ). Despite differences in treatment intensity, there was no difference in outcome for the low-risk eBL and sBL groups. However, the outcome for high-risk patients was significantly different (RR 77% versus 20%,  $p=0.002$ ; EFS 23% versus 75%,  $p=0.005$ , OS 44% versus 88%,  $p=0.03$  for eBL and sBL, respectively).

**Conclusions:** We report a genomic risk classifier for eBL which identified patients at high-risk of relapse. Importantly, most high-risk children in Malawi who relapsed remained curable. These patients may benefit from moderate intensification of up-front therapy, reducing the overall cost and burden of treatment by reducing the need for salvage chemotherapy. In contrast, low-risk patients may be candidates for treatment de-intensification in some healthcare settings.

## O14

**shRNA Mediated PPARalpha Knockdown in Glioma Stem Cells Reduces *In Vitro* Proliferation and Decreases Tumourigenicity in an Orthotopic Nude Mouse Xenograft Model**

© HR Haynes<sup>1</sup>; H Scott<sup>1</sup>; G Shaw<sup>2</sup>; T Brend<sup>2</sup>; CL Killick-Cole<sup>1</sup>; H Bulstrode<sup>3</sup>; K Hares<sup>1</sup>; J Redondo<sup>1</sup>; KC Kemp<sup>1</sup>; WG Singleton<sup>1</sup>; SC Short<sup>2</sup>; J Uney<sup>1</sup>; A Wilkins<sup>1</sup>; KM Kurian<sup>1</sup>

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**Purpose of study:** PPAR $\alpha$  is a transcription factor governing fatty acid and carbohydrate metabolism. Drugs that target PPAR $\alpha$  are in clinical use for hyperlipidaemia. Our previous work has shown that PPAR $\alpha$  expression is increased in glioblastoma and has independent prognostic significance in multivariate analyses. In this current work we analysed the expression of PPAR $\alpha$  in glioma stem cell (GSC) models and investigated the effects of PPAR $\alpha$  knockdown (KD).

**Methods:** We investigated the expression of PPAR $\alpha$  *in vitro* and using accessioned microarray data. Using *de novo* PPAR $\alpha$  shRNA design we transduced GSC using HIV based lentiviral vectors with active (KD) or control scrambled (SCR) shRNA constructs. Population doubling, proliferation indexes, colony formation, apoptosis and senescence rates were investigated. We analysed the downstream gene and protein expression of stem cell and mitogenic pathways. Stably transduced KD and SCR cells were luciferase tagged and stereotactically xenografted into the striatum of NOD-SCID mice ( $n=10$  per group). Tumour formation was monitored with bioluminescent imaging and MRI.

**Summary of results:** PPAR $\alpha$  is overexpressed in GSC compared to fetal and adult neural stem cells. Stable (long-term) KD of PPAR $\alpha$  in GSC results in significantly decreased growth rates and mitotic activity with an increase in cellular senescence. This is accompanied by a downregulation of cMYC, CyclinD, EGFR, Nestin and SOX2. 4 months after xenograft initiation, PPAR $\alpha$  KD GSC mediate a decreased intracranial tumour load compared to SCR controls.

**Conclusions:** GSC are considered to be responsible for glioblastoma initiation, recurrence and therapy resistance. GSC overexpress PPAR $\alpha$  and PPAR $\alpha$  KD reduces proliferation *in vitro* and tumour formation *in vivo*. Targeting PPAR $\alpha$  in GSC may therefore be a therapeutically beneficial strategy with translational potential in an adjuvant setting.

*Work supported by The Pathological Society and Jean Shanks Foundation Research Training Fellowship.*

## O15

**Inflammatory Cell Infiltrates in Advanced Metastatic Uveal Melanoma**

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**Purpose of study:** Current treatments for metastatic uveal melanoma (mUM) are limited and rarely prolong patient survival. Immunotherapy trials for mUM are few, and to-date have demonstrated only marginal success. High densities of tumour-associated-macrophages (TAMs) and infiltrating T-lymphocytes (TILs) in primary UM are associated with poor prognosis. Little is known about the immune microenvironment of mUM. Our aim was to examine the presence and distribution of TAMs and TILs in mUM within the liver.

**Methods:** Whole tissue-sections of liver mUM ( $n=16$ ) were examined by immunohistochemistry. For TAMs, monoclonal antibodies against CD68 and CD163 were used. Macrophage density and morphology were scored using previous established systems. Density and spatial distribution of TILs were highlighted using antibodies against CD3 (pan-lymphocyte marker), CD4 (T-helper cells) and CD8 (T-cytotoxic cells).

**Summary of results:** CD68+ and CD163+ TAMs were seen within the tumour in all 16 specimens; their density was 'few' in 66%, 'moderate' in 34% of cases and the majority showed an 'indeterminate' phenotype. CD3+ TILs were noted both within mUMs and surrounding the tumour. Of these CD8+ TILs were 'few' in number within mUM but were predominantly seen peri-tumourally at the tumour/normal liver interface, whilst CD4+ TILs showed a high perivascular density within mUM.

**Conclusions:** In this study we present novel data for inflammatory cell infiltrates within hepatic mUM. CD68+ and CD163+ TAMs of 'indeterminate' morphology were observed in mUM, suggesting a tendency towards the pro-tumourigenic M2-phenotype. CD4+ TILs were seen mainly infiltrating mUM, whereas CD8+ TILs tended to be peri-tumoural. The biological role of inflammatory cells in mUM requires further investigation, to determine if they represent potential targets for future therapies in mUM.

## O16

**Novel Molecular Events in Phosphaturic Mesenchymal Tumour**

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**Background:** Phosphaturic mesenchymal tumours (PMT) are rare neoplasms of bone and soft tissue associated with tumour-induced osteomalacia and characterised by high levels of FGF23 gene expression. *FN1-FGFR1* and *FN1-FGF1* fusion genes are recurrent events in PMT, although in over half of cases specific molecular events have not been identified.

**Methods:** We analysed 7 PMTs by FGF23 RNA in situ hybridisation, whole genome sequencing (WGS; 4/7), methylation profiling (4/7) and RNA sequencing (2/7). Our findings were validated with fluorescent in situ hybridisation (FISH), reverse transcription polymerase chain reaction (RT-PCR) and quantitative PCR (qPCR).

**Results:** We showed that one case harboured the previously reported *FN1-FGFR1* fusion transcript. We then identified a novel *BRAF* fusion by WGS, corroborated by FISH and RT-PCR. Using RNA Seq and qPCR we identified a third case with striking high levels of  $\alpha$ Klotho expression, providing a potentially novel mechanism for initiating FGFR signalling in this tumour. Copy number loss at 9p21.1-9p23, encompassing the locus for *CDKN2A*, was found in two PMTs, one of which showed complete deletion. Finally, methylation profiling identified *FGF1* promoter hypomethylation across all cases tested (4/4), regardless of fusion status, and this was associated with significantly higher levels of FGF1 expression compared with other bone tumours ( $n=14$ ) by qPCR ( $p=0.003$ ).

**Conclusion:** By utilising high-throughput sequencing methods we have expanded the range and understanding of genetic alterations in PMT. In future work it will be important to determine in particular whether the *BRAF* fusion gene is a recurrent event in this tumour, since PMT, although rarely, can behave in a malignant fashion, and this represents a potential therapeutic target.



## O17

**The Chemokine CXCL10 is a Potential Regulator of Cancer Stem Cells in High Grade Serous Carcinoma**

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High grade serous carcinoma (HGSC) is the most aggressive type of epithelial ovarian cancer. Patients initially respond well to chemotherapy but they almost invariably relapse. An explanation for this may lie in a population of cells that are chemo-resistant allowing the tumour to metastasise. This type of cell has been designated a cancer stem cell (CSC). We have shown that the chemokine CXCL10 is associated with HGSC disease progression. CXCL10 may be important for the function of CSC in HGSC.

**Methods:** 1. Cell lines: Four HGSC cell lines were studied.

2. CSC isolation: Flow cytometry sorted using an Aldefluor assay.

3. Sphere and clonogenic assays: Spheres were counted after 9-12 days and colonies were counted after 21 days.

4. Stem cell gene expression: Nanog, OCT3/4 and Sox 2 quantified by qRT-PCR

5. Chemokine and receptor expression: Analysed by RT profiler PCR array

6. CXCL10 ELISA: Supernatants taken after 12 days.

7. CSC and non-CSC were cultured either with recombinant CXCL10 or with CXCR3 blocking antibodies for 12 days.

**Results:** 1. Each cell line had a population of CSC. Carboplatin-resistant cells had a larger CSC population.

2. CSC are more proliferative or form more spheres and colonies than non-CSC

3. HGSC cell lines expressed high levels of CXCR3, the receptor for CXCL10

4. CSC express higher levels of CXCL10 than non-CSC

5. CXCL10 treated CSC express high levels of CXCL12.

6. Blocking CXCR3 reduces the expression of CCL3

**Conclusions:** Carboplatin resistance enhances the CSC population, suggesting that CSC may increase when patients become disease-resistant. CSC express high levels of CXCL10 and this may have an autocrine effect, since the cells also express its receptor, CXCR3. CXCL10 appears to have a regulatory effect on CSC by 1) increasing CXCL12 expression, a chemokine strongly implicated in metastases in many cancers and by 2) maintaining expression of the macrophage chemoattractant CCL3. CXCL10 could be a possible therapeutic target.

## O19

**Myoepithelial Cell Phenotype in DCIS Progression: Functional Significance of Integrin  $\alpha\beta6$  and Fibronectin**

© M Hayward; MD Allen; JJ Gomm; L Haywood; JF Marshall; LJ Jones

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**Introduction:** Progression to invasive breast cancer follows transition through a pre-invasive stage, ductal carcinoma *in-situ* (DCIS). DCIS represents disease limited to the ductal system and it is clear that not all DCIS will progress to invasion leading to concerns of overtreatment. There is a need to identify robust prognostic markers to better direct therapeutic intervention, and focus has turned to 'normal' host cells to identify such markers. Normal myoepithelial cells (MECs), which form the interface between the epithelium and stroma, exert a tumour suppressor function. In some DCIS, MECs are altered, with de-novo expression of integrin  $\alpha\beta6$  and up-regulation of Fibronectin (FN). This study aims to evaluate the functional relevance of these changes.

**Methods:** Established MEC lines with ( $\beta6$ -1089) and without (N-1089)  $\alpha\beta6$  expression were used to model DCIS and normal MECs, respectively. Together, with primary normal MECs, these cells were used to assess the mechanisms regulating their expression, and the role of FN in  $\alpha\beta6$ -mediated TGF $\beta$  activation and signalling.

**Results:**  $\beta6$ -1089 exhibit significant up-regulation of FN and FN isoforms; EDA and EDB, at both the protein and mRNA level, compared to N-1089. Knockdown of either  $\alpha\beta6$  or FN in  $\beta6$ -1089 significantly reduced TGF $\beta$  activation and signalling, as well as breast cancer cell invasion. In turn, TGF $\beta$  up-regulates the expression of  $\alpha\beta6$  and FN in MECs at the protein and mRNA level. Moreover, immunohistochemical analysis of pure DCIS and DCIS with co-existing invasion indicates that both  $\alpha\beta6$  and FN expression are significantly associated with the progression of DCIS.

**Conclusion:** Expression of both  $\alpha\beta6$  and FN by MECs is required to generate enhanced TGF $\beta$  activation and signalling, which promotes breast cancer cell invasion. These changes may be used to determine which DCIS lesions will and will not progress, allowing for more robust patient stratification.

*This work was funded by the Pathological Society PhD Studentship.*

## O18

**Uterine Tumour Resembling Ovarian Sex Cord Tumour (UTROSCT): Follow-Up of a Case Series Confirming a Neoplasm of Low Malignant Potential**

© M Moore; WG McCluggage

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**Purpose of the study:** Uterine tumour resembling ovarian sex cord tumour (UTROSCT) is an uncommon mesenchymal neoplasm of unknown histogenesis which exhibits immunohistochemical evidence of sex cord differentiation. It is considered a neoplasm of uncertain but low malignant potential but there is limited evidence for this since there are no large studies with follow-up.

**Methods:** From a series of 34 cases of UTROSCT, mainly from the consultation files of one of the authors, we report follow-up information. Follow-up was obtained by contacting referring pathologists and clinicians.

**Summary of results:** Eight of 34 patients (23.5%) developed extrauterine metastasis to a variety of sites, including pelvis and abdomen, ovary, lymph nodes, bone, liver and lung and 3 patients died of tumour.

**Conclusions:** While our figure of 23.5% of cases exhibiting malignant behaviour may reflect some bias related to consultation practice, our results show that these neoplasms not uncommonly exhibit malignant behaviour with extrauterine metastasis. Clinicopathological features associated with the development of metastasis are not well described but will be investigated in this series.

## O20

**Glutamine Transporters SLC1A5 and SLC7A5 are Key Therapeutic Targets in Luminal B Breast Cancer**© R El-Ansari<sup>1</sup>; MA Aleskandarany<sup>1</sup>; KW Cheng<sup>1</sup>; M Diez-Rodriguez<sup>2</sup>; CC Nolan<sup>1</sup>; C Caldas<sup>2</sup>; IO Ellis<sup>1</sup>; EA Rakha<sup>1</sup>; AR Green<sup>1</sup><sup>1</sup>University of Nottingham, Nottingham, UK; <sup>2</sup>Cancer Research UK Cambridge Institute, University of Cambridge, Cambridge, UK

**Purpose of the study:** SLC1A5 and SLC7A5 are amino acid transporters involved in glutamine transport and play an important role in cancer cell survival/growth. Both transporters are co-expressed in many cancers suggesting a functional coupling to support tumour progression. Breast cancer (BC) is a heterogenous disease characterised by variant biology and patient outcome. This study aimed to determine whether SLC1A5 and SLC7A5 are co-ordinately expressed and co-operate to support BC proliferation, particularly in the highly proliferative more aggressive subtypes which tend to have 'glutamine addiction'.

**Methods:** SLC1A5 and SLC7A5 were assessed at the genomic (n=1,980 using the METABRIC data) and proteomic (n=950 assessed in TMA) levels in large and well-characterised BC cohorts and were correlated with clinicopathological parameters, molecular subtypes, and patient outcome.

**Summary of results:** Strong correlation between mRNA and protein expression was observed for each of the transporters, where high expression was observed in triple negative (TN), HER2+, and luminal B subtypes. Both transporters were associated with larger tumour size, higher grade, and positive lymph node metastasis. High expression of SLC1A5 and SLC7A5 mRNA and protein was associated with poor breast cancer specific survival (BCSS) in the highly proliferative, luminal B, subclass, but not in other subtypes (p<0.001 and p=0.003 respectively). Multivariate analysis showed that co-expression of both transporters was independent of grade, size, lymph node stage, ER, HER2 and adjuvant therapy and predictive of shorter BCSS (p=0.009).

**Conclusions:** SLC1A5 and SLC7A5 appear to play a role in the aggressive luminal B subtype of BC and could act as potential therapeutic targets particularly in synergism. Functional assessment is necessary to reveal the specific role played by these glutamine transporters in luminal B BC.

## O21

**Elevated Expression of STK3 mRNA and Protein is Associated with Poor Outcome in Invasive Breast Cancer**

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**Purpose of the study:** The mammalian sterile 20-like kinase (MST2/STK3) and its close homologue MST1 (STK4) are members of the germinal centre kinase group II (GCK II) family of mitogen-activated protein kinases (MAPK). High STK3 expression is known to be correlated with poor prognosis in various cancers playing a role in cell migration and invasion. This study aimed to determine correlations of STK3 expression with clinicopathological variables in BCs.

**Methods:** STK3 mRNA expression was investigated in the METABRIC BC cohort (n=1980) and externally validated using online BC expression datasets [bc-GenExMiner v4.0]. STK3 protein expression was studied in a well characterised series of primary invasive BCs (n=1024) using immunohistochemistry including correlations with clinicopathological parameters, other biomarkers and patient outcome.

**Results:** Copy number (CN) gain of STK3 was correlated with adverse prognostic features: higher grade and poor NPI (p<0.0001). High STK3 expression was also associated with poor prognostic factors, including high grade, younger age, larger tumour size, poorer NPI and negative ER/PR status (p<0.001). In PAM50 subtypes, high STK3 expression was associated with Luminal B/basal like tumours. Cytoplasmic STK3 (c-STK3) protein expression was associated with increased mitotic index, poorer NPI (p<0.001) and basal-like markers CK5/6 and EGFR (p<0.05). In univariate analysis, high c-STK3 expression showed poorer outcome in the whole cohort and ER+ subgroups (p<0.05). Pooled STK3 gene expression data in the external validation cohort confirmed association with poor outcome (p<0.0001, HR = 1.60, 95% CI 1.28–2.01).

**Conclusions:** Results suggest c-STK3 as a poor prognostic marker in invasive BC including ER+ subgroups warranting further functional studies.

*Project supported by a CDF from the Pathological Society.*

## O23

**The Heterogeneity of Tumour Infiltrating Lymphocytes and its Effect on Patient Outcome**

© M Althobiti; MA Aleskandarany; AR Green; C Joseph; M Diez Rodriguez; CC Nolan; IO Ellis; EA Rakha

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**Background:** Assessment of tumour-infiltrating lymphocytes (TILs) in breast cancer (BC) confers prognostic and predictive information. This study aims to assess the degree of TILs intra-tumour heterogeneity and its impact on outcome.

**Method:** Full face sections from 247 BC cases were examined for TILs heterogeneity including intraslide, interslides (52 cases with 3–4 slides/cases) and between primary and recurrent tumours. In addition, association of TILs with lymphocyte subtypes (CD3, CD8, CD20, CD68 and FOXP3) and immune check point (PDL-1 and PD1) assessed using IHC was evaluated.

**Result:** Friedman test showed intraslide variation between the average (ATILs) and hotspots (HSTILs) scores (p=<0.001) in primary BC while showed less variation in recurrent tumours (P=0.303). Interslide AVTILs scores were less heterogeneous than intraslide differences (p=0.418). Average TIL score in primary BC was significantly associated with features of aggressive behaviour including, high grade, advanced nodal stage and LVI positive. Moreover, higher AVTILs in primary BC was associated with longer BC – specific survival (long rank, LR=11.2, P=0.004) and distant metastasis (LR=12.3, P=0.002). Higher AVTILs and HSTILs in recurrence cases were significantly associated with shorter BC – specific survival (LR=8.513, P=0.004, LR=3.578 P=0.059, respectively). The HSTILs score in primary BC cases were positively association with CD3, CD20 and FOXP3 (P=0.05, P=0.028 and p=0.014, respectively). Both AVTILs and HSTILs scores were positively association with PDL-1 and PD1 expression (P<0.05).

**Conclusion:** This observational study demonstrates that TILs assessment on one tumour slide can reliably represent whole tumour TILs. TILs has prognosis significance in primary and recurrent BC. The association of Hotspot TILs with CD3, CD20 and FOXP3 emphasised the heterogeneity of TILs and could prompt to consider the hotspot when assessing TILs.

## O22

**Cell Division Cycle 25C (CDC25C) Expression Confers Poor Prognosis in Invasive Breast Cancer**

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**Background:** CDC25C, belonging to the Cdc25 phosphatase family, plays a major role in cell cycle control, impacting on DNA repair and apoptosis. It has been shown that poor prognosis/copy number high Luminal A breast cancers (BCs) are enriched for the Aurora kinase pathway including CDC25C leading to CDK1 activation (Ciriello et al, Breast Cancer Research Treatment, 2013:409). This study examined the associations of CDC25C with clinicopathological and molecular features in BCs including the low grade ER positive cohort.

**Methodology:** CDC25C mRNA expression was studied in the METABRIC BC cohort (n=1980) and externally validated using online expression datasets [bc-GenExMiner v4.0]. CDC25C protein expression level was assessed immunohistochemically on a large annotated series of BC (n= 1330) and correlations made with clinicopathological parameters and patient outcome.

**Results:** High CDC25C expression was significantly associated with poor prognostic factors including high grade, large tumour size, medullary like tumours, poorer NPI, ER-/PR- Her2+ status (p<0.001) and was differentially expressed in poor prognosis integrative clusters 5 and 10 (p<0.001). Cytoplasmic CDC25C (c-CDC25C) protein showed positive association with non-NST and non-medullary tumour subtypes while nuclear CDC25C (n-CDC25C) negatively associated with tumour stage (p<0.05). There was no association with ER, PR status, NPI and lymph nodes. However, high c-CDC25C resulted in poor survival at 20 years in the Grade 1 ER+ cohort (p=0.007), while high n-CDC25C showed better long term survival (p<0.001). Pooled CDC25C expression data in the external validation cohort showed an association with poor outcome (p<0.0001, HR = 1.45, 95 % CI 1.28–1.64).

**Conclusion:** CDC25C appears to be associated with poor prognosis in BC including the Grade 1 ER+ cohort, indicating the importance of further functional analyses.

*Project supported by a CDF from the Pathological Society.*

## O24

**Loss of MED23 Leads to Poor Prognosis in Invasive Breast Cancer**

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**Purpose of the study:** The molecular mechanism of lymphovascular invasion (LVI) which determines the early metastatic phenotype in breast cancer is still not fully understood. Lead from the METABRIC study revealed that MED23 correlated with negative LVI status (p=0.00005). Hence MED23 expression was studied at the protein level for correlations with LVI and other clinical-pathological parameters.

**Methods:** The METABRIC BC cohort (n=1980) was evaluated for MED23 mRNA expression and prognostic impact externally validated using the online bc-GenExminer 4.0. Correlation between MED23 protein expression with clinicopathological parameters, patient outcome and other biomarkers were explored (Nottingham Tenovus series; n=1255) using immunohistochemistry (IHC).

**Results:** High MED23 mRNA expression was negatively associated with tumour stage and was differentially expressed in good prognosis integrative clusters 7 and 8 (p<0.001). MED23 IHC revealed nuclear expression (n-MED23). Although no association was found with LVI, higher n-MED23 expression correlated with low NPI, low grade, older age, ER+ status, low Ki67 index and low N-cadherin expression (p<0.05). Positive correlations with PTEN, GATA3, STAT3 and CDC42 (p<0.001), indicate possible interacting pathways. In univariate analysis, high n-MED23 expression showed better long-term patient outcome in the whole cohort and ER+ subgroups (p<0.05). Pooled MED23 expression in an external validation cohort (ER+LN-) also showed association with better patient outcome (p<0.02, HR=0.82, 95% CI 0.69-0.98).

**Conclusion:** Results of the study suggest that loss of n-MED23 is a marker of poor prognosis in invasive BC, results re-enforced by expression data. The difference in correlation with LVI at gene and protein level highlights the importance of IHC validation and indicates MED23 as a probable bystander in the LVI cascade.

*Project supported by Academy of Medical Sciences and the Pathological Society.*

## O25

**FOXP1 Expression Correlates with Better Prognosis in Invasive Breast Cancer Including the ER-Positive Luminal Subtype**

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**Background:** Fork head box P1 (FOXP1) is a FOX family transcription factor influencing ER $\alpha$ ; regulated transcription by interaction with the ER $\alpha$ ; related pioneer factor FOXA1. Altered FOXP1 expression is seen in breast and prostate cancers. This study investigated FOXP1 at the protein level in breast cancer (BC) and examined associations with clinicopathological and molecular features.

**Methods:** FOXP1 mRNA expression was investigated in the METABRIC BC cohort (n=1980) and validated using online expression datasets [bc-GenExMiner v4.0]. Protein expression was studied in a well characterised BC primary series (n=621) using immunohistochemistry and correlations made with clinicopathological parameters and outcome.

**Results:** High FOXP1 mRNA and protein expression was significantly associated with low grade, low NPI, positive ER/PR status, lobular BCs, low Ki67 and negative Her2 status (p<0.001). Within PAM50 subtypes, high FOXP1 expression was associated with Luminal A BCs and good prognosis integrative clusters (IC3 and IC8). Nuclear FOXP1 (n-FOXP1) protein positively associated with luminal markers: CARM1, RERG and FOXA1 (p<0.05). Negative association with PIK3 (p=0.023) indicates a possible dual role whereby n-FOXP1 reduces EGFR-mediated ligand-independent ER activation, but enhances AKT-mediated activation. Positive correlations with GATA3, STAT3 and CDC42 (p<0.001), suggest interacting pathways. On univariate analysis, n-FOXP1 overexpression showed better long term outcome in the whole cohort and ER+ subgroups (p<0.05). Pooled FOXP1 gene expression data in the ER+ external validation cohort showed similar association with better outcome even when adjusted for NPI/proliferation (p<0.001).

**Conclusions:** Higher n-FOXP1 correlated with low grade ER positive BCs and spelt better prognosis with increased long-term survival. The marker may be helpful to distinguish between good versus poor prognosis luminal A tumours.

Supported by CDF from the Pathological Society.

## O27

**Enterobius Vermicularis Ova are a Normal Finding in the Gastric Mucosa of Children Undergoing Endoscopy in Birmingham**

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The threadworm *Enterobius vermicularis* is a small nematode frequently seen at colonoscopy and is probably the commonest helminth to infect humans. Its life cycle takes place within the gastrointestinal tract, with ova being shed from adult worms in the colon. Ingested eggs hatch in the stomach. Threadworms are often seen in appendectomy specimens, but ova in the stomach are not regularly recognised by histopathologists. We describe the histopathological appearances of the embryonated ova, which lie in the surface epithelium of the stomach. We found ova in almost all gastric biopsies over a four month period from over 100 children undergoing diagnostic endoscopy for a wide range of clinical indications. It is not clear that the ova cause any local reaction or disease state in the stomach. Threadworms were seen at colonoscopy in the right colon, but not the left colon, and were not seen at sigmoidoscopy. No threadworms were seen in the colon in children with a diagnosis of inflammatory bowel disease. The significance of these findings is unclear. Ova are so common a finding in the stomachs of children undergoing gastroscopy that they could be regarded as a normal finding. The absence of threadworms in the colons of children with inflammatory bowel disease is at this state of knowledge just an observation, with further studies required to elucidate the significance of the finding of embryonated ova in gastric biopsies and adult threadworms in the colon, whether or not they are also found in adults, and the part they play in the normal and the diseased colon.

## O26

**The Importance of Resting CD8+ T Cells and Proliferating Tumour Cells in Gastric Cancer**

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**Purpose of the study:** Discovery of prognostic factors based on cell density distributions and co-occurrence of proliferating CD8+ T cells and cancer cells in gastric cancer (GC) patients as potential biomarker for immunotherapies.

**Methods:** Consecutive sections of tissue microarrays (TMAs) from 213 GC patients were stained for cytotoxic T cells (CD8), proliferating cells (Ki67), and epithelial cells (CK). Using automatic image analysis algorithms, marker-positive cells were detected in the virtually multiplexed TMAs. Individual TMA cores were divided into tiles of size 64  $\mu\text{m}^2$ , and the average ratio of CD8+ to Ki67+ cells per tile/ per patient was computed. The relationship with pT, pN and histological tumour type was assessed using non-parametric Kruskal-Wallis test and Dunn's test. Prognostic features were determined by univariate stratification which optimizes Kaplan-Meier p-value using a 50x3-fold pre-validation and ranked by the median pre-validation p-values. P-values < 0.05 were considered significant.

**Summary of results:** Median (range) number of tiles analysed per patient was 291 (81-345), the median CD8+/Ki67+ ratio was 0.39 (0.01-0.92). Manual inspection of selected image tiles showed that CD8+ cells are rarely Ki67+. Median (range) percentage of tiles per patient where CD8+Ki67- cells co-occurred with Ki67+ tumour cells was 17% (0%-93%). Significant difference of CD8+/Ki67+ ratio was observed between diffuse and intestinal (p=0.015) or mucinous (p=0.026) histological subtypes. A high CD8+/Ki67+ ratio was related to better survival (p=0.012).

**Conclusions:** This is the first study to suggest that the majority of CD8+ T cells in GC appear to be resting Ki67- T cells. The co-occurrence of CD8+ T cells and Ki67+ tumour cells seems to be clinically relevant and characterise certain histological phenotypes in GC.

## O28

**Gastric Cancer with Primitive Enterocyte Phenotype: An Aggressive Subgroup of Intestinal-Type Adenocarcinoma**

© T Ushiku; S Yamazawa; A Shinozaki-Ushiku; M Fukayama

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**Purpose:** A primitive cell-like gene expression signature is associated with aggressive phenotypes of various cancers. We aimed to characterize features of gastric cancer with primitive phenotype.

**Methods:** Immunohistochemical analysis of a panel of primitive phenotypic markers, including embryonic stem cell markers (OCT4, NANOG, SALL4, CLDN6, and LIN28) and known oncofetal proteins (AFP and GPC3), was performed using tissue microarray on 386 gastric cancers.

**Results:** Based on the expression profiles, the 386 tumours were clustered into three groups: group 1 (primitive phenotype, n = 93): AFP, CLDN6, GPC3, or diffuse SALL4 positive; group 2 (SALL4-dispersed, n = 56): only focal SALL4 positive; and group 3 (negative, n = 237): all markers negative. Groups 1 and 2 predominantly consisted of intestinal-type adenocarcinoma, including 13 fetal gut-like adenocarcinomas exclusively in group 1. Group 1 was significantly associated with higher T-stage, presence of vascular invasion and nodal metastasis when compared to groups 2 and 3. Group 1 was associated with patients' poor prognosis and was an independent risk factor for disease-free survival. Group 1 showed frequent TP53 overexpression and little association with EBV or mismatch repair deficiency. Further analysis of The Cancer Genome Atlas (TCGA) dataset validated our observations and revealed that tumours with primitive phenotypes were mostly classified as "chromosomal instability" (CIN) in TCGA's molecular classification.

**Conclusions:** We identified gastric cancer with primitive enterocyte phenotypes as an aggressive subgroup of intestinal-type/CIN gastric cancer. Therapeutic strategies targeting primitive markers, such as GPC3, CLDN6, and SALL4, are highly promising.



## O29

**Epstein-Barr Virus-Associated Gastric Carcinoma: The Roles of Cellular and Viral MicroRNAs in its Carcinogenesis**

© A Shinozaki-Ushiku

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**Purpose of the study:** To clarify the roles of cellular and viral microRNAs in the carcinogenesis of Epstein-Barr virus-associated gastric carcinoma (EBVaGC).

**Methods:** The expressions of cellular and viral microRNAs were investigated by real time RT-PCR using formalin-fixed, paraffin-embedded tissues of surgically resected gastric carcinoma. Several microRNAs were specifically upregulated or downregulated in EBVaGC and the biological roles of these microRNAs were investigated using cell line models of EBVaGC.

**Summary of results:** The expression levels of two cellular microRNAs, hsa-miR-200a and 200b, were significantly lower in EBVaGC compared with those in EBV-negative gastric carcinoma. In vitro experiments demonstrated that suppression of these microRNAs induced epithelial-to-mesenchymal transition through the upregulation of ZEB1 and ZEB2 which are transcriptional suppressors of E-cadherin. Comprehensive analysis of viral microRNA expression in EBVaGC identified several highly expressed viral microRNAs. One of these microRNAs, ebv-miR-BART4-5p, showed the anti-apoptotic effect by suppressing BID, an apoptosis activator.

**Conclusion:** Both cellular and viral microRNAs play important roles in the carcinogenesis of EBVaGC through the regulation of epithelial-to-mesenchymal transition and apoptosis.

## O31

**Non-HPV Related Squamous Cell Carcinoma of the Anal Canal and Perianal Region**© JM Trainor<sup>1</sup>; J Jamison<sup>2</sup>; H McBride<sup>1</sup>; L Travers<sup>1</sup>; WG McCluggage<sup>1</sup>; PJ Kelly<sup>1</sup><sup>1</sup>Royal Victoria Hospital, Belfast, UK; <sup>2</sup>Antrim Area Hospital, Antrim, UK

It is assumed that virtually all squamous cell carcinomas (SCC) of the anal/perianal region are associated with human papillomavirus (HPV). We have encountered SCCs in this region with a morphology (non-basaloid and keratinising) raising the possibility of a non-HPV-related neoplasm. To explore the possibility of a non-HPV-related pathway, we reviewed anal/perianal SCCs diagnosed in our institution over a 13-year period. All cases considered to possibly represent non-HPV-related neoplasms by morphology were stained for p16 and if negative (non-block staining) underwent molecular testing for HPV DNA. Cases were considered HPV-related if there was typical morphology by consensus diagnosis (basaloid/non-keratinising, adjacent classical AIN, koilocytosis); p16 immunohistochemistry was not performed in all such cases. Cases without typical morphology required positive block-type p16 staining and a positive molecular test to be classified as HPV-related. Cases with negative p16 staining and a negative HPV molecular test were classified as non-HPV related. All material was available in 56 cases (M=22; F=34; 36-85y). 39 cases (70%) were classified as HPV-related based on morphology. p16 was available for 13/39 of these cases (all positive). 12 cases were considered to be non-HPV related. In 5 cases the results were indeterminate (block p16 positivity but HPV negative). Our results suggest a significant percentage of SCCs of the anal and perianal region are non-HPV-related. As in the vulva, a subset of SCCs with morphology suggesting a non-HPV-related neoplasm is HPV related. Conversely a small number of basaloid SCCs are HPV negative. While currently there is no difference in management between HPV positive and negative tumours in the anal / perianal region, improved recognition of non-HPV-related neoplasms will help determine if the better prognosis of HPV-related SCCs in the vulva also applies to the anal/perianal region

## O30

**Functional Analysis of GLI1 and ZIC2, Hedgehog-Related Transcription Factors in Pancreatic Cancer Cells**

© SI Inaguma; KK Kasai; HI Ito; TT Tsunoda; HI Ikeda

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**Purpose of the study:** Hedgehog (Hh) signaling is indispensable for the development of vertebrates and its disturbance causes severe developmental anomalies including Holoprosencephaly. To date, Krüppel-like zinc-finger transcription factors GLI1 and ZIC2, the key molecules of the Hh signaling pathway have been shown to regulate the expression of several genes crucial for a variety of cancer cell properties in several types of cancers. Recently, we uncovered GLI1 and ZIC2 over-expression during pancreatic carcinogenesis by immunohistochemical analyses of pancreatic ductal adenocarcinoma (PDAC) tissues. Based on these observations, we hypothesized that GLI1 and ZIC2 might play crucial roles in pancreatic carcinogenesis.

**Methods:** At first, GLI1- and ZIC2-inducible PDAC cells were established from PANC-1 cell line. To identify transcriptional target genes of GLI1 and ZIC2 in PDAC cells, comprehensive cDNA expression analyses were performed using Agilent 44x4 cDNA microarray (Agilent Technologies). After the selection of candidates, we tried to uncover the functions of GLI1 and ZIC2 axes by using molecular biological techniques.

**Summary of results:** From cDNA microarray analyses, we identified 31 and 43 up-regulated genes upon GLI1 and ZIC2 induction, respectively. Among the candidate target genes of GLI1, we focused on *BHLHE41*. From ZIC2 candidates, *FGFR3* and *ANXA8* were chosen for further analyses. The methylnitrosourea (MNU) tolerance assay revealed that GLI1-BHLHE41 axis functionally depressed the activity of the mismatch repair system through MHL1 down-regulation cooperatively with the low copy number status of *MLH1* gene. On the other hand, ZIC2 was proved to regulate apoptosis of PDAC cells in *FGFR3* and *ANXA8* dependent manner.

**Conclusions:** These lines of evidence highlight the pivotal roles of GLI1 and ZIC2 in pancreatic carcinogenesis. It was also suggested that GLI1, ZIC2 and the members of these signaling axes might be potential therapeutic targets for PDAC.

## O32

**Acetone Clearance of Pericorectal Fat Increases the Detection of Positive Lymph Nodes in Junior and Senior Pathologists**

© CAW Leung; D Rennspiess; GE Fazzi; HI Grabsch

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**Purpose of the study:** Accurate lymph node (LN) staging is critical in patients with colorectal cancer (CRC) since it determines prognosis and need for adjuvant therapy. It has been suggested that acetone clearance (AC) of pericorectal fat increases LN yield. We hypothesised that AC increases positive LN yield by enhancing detection of small LNs, irrespective of the experience of the dissecting pathologist. The objective of this retrospective study was to explore this hypothesis and to identify factors that influence LN yield with and without AC.

**Methods:** In 80 consecutive CRC resections LNs were dissected by 9 junior and senior pathologists before AC. Second dissection was performed by an experienced technician after one night incubation in acetone. Positive (LNpos) and negative LNs (LNneg) before and after AC were counted. LN width was measured using Leica Qwin.

**Summary of results:** In total, 1548 (94%) LNneg and 96 (6%) LNpos were found. Median (range) total LN yield per specimen was 12 (3-41) before AC and 18 (4-48) including LNs after AC. In 75 (94%) specimens, a total of 534 (33% of all) LNs were found after AC: 34% (n=524) of all LNneg and 10.4% (n=10) of all LNpos. The LNpos found in 6 (7.5%) specimens after AC did not change pN. Median (range) of LNpos width before AC (5.0 (1.1-17) mm) was similar to LNpos width after AC (4.0 (2.1-6.3) mm), p=0.064. In 5 specimens, the biggest LNpos after AC was larger than the smallest LNpos before AC. 2 LNpos were missed by senior pathologists compared to 8 by junior pathologists. Neoadjuvant treatment showed no influence on LN yield before or after AC.

**Conclusions:** Our study confirms that AC increases LN yield substantially. In contrast to previous studies, this study shows LN yield before and after AC is not related to LN size. As LNpos were also missed by senior pathologists, we would currently recommend to perform AC for all CRC resections despite its costs to ensure high quality LN staging.

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