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Understanding Disease — Guiding Therapy

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Plenary Oral Abstracts

PL1

Histological Features of Neoadjuvant Endocrine Therapy Response and Effect on Tumour Phenotype in Luminal Breast Cancer

Ⓟ AM Shaaban[1]; N Badr[2]

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Introduction: Despite the increasing use of neoadjuvant endocrine therapy (NAET) in the treatment of oestrogen receptor positive (ER+) breast carcinoma (BC), there are no standard systems for histological assessment of response. The effect of NAET on the histological characteristics and hormone receptor status of BC is still unknown. This study aims to assess the histological features of response to NAET and its effect on tumour type, grade and molecular profile.
Methods: Patients who underwent NAET for primary and operable invasive breast carcinoma between 2008 and 2018 in a large UK tertiary referral centre were identified with detailed collection of clinical, histopathological and follow up data. A detailed histological review of residual tumour morphology including margin type, cellularity, scarring pattern and receptor status of paired pre and post tumour samples was undertaken.
Results: One hundred and twenty cases were included. Complete pathological response was achieved in 2 cases (1.7%). Following therapy, a highly significant change in the histological subtype was noted in 8.5 % of cases (p=0.003) with increase in tubular and mixed subtypes. Downgrading was observed in 15% of cases. A switch from Progesterone receptor (PR) positive to negative status occurred in 30% of cases (p=0.002). A total of 3.3% of cases changed to non-luminal subtypes especially in the Asian ethnicities (p=0.05). Response to therapy highly correlated with a reduction in tumour cellularity (p<0.001). An infiltrative margin of residual tumour was associated with poor overall survival (p=0.02).
Conclusion: Significant changes in tumour characteristics and PR status occur following NAET. We recommend assessment of residual tumour cellularity and margin status as predictors of clinical outcome.

PL2

Investigating Stromal-Epithelial Interactions Using a Novel 3D Breast Cancer Organoid Model: Regulation of the Basal Epithelial Phenotype by Cancer-Associated Fibroblasts

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Tumour organoids have provided novel insight into the intrinsic mechanisms regulating breast cancer invasion, highlighting the critical contribution of epithelial transdifferentiation to a conserved basal, keratin 14 (KRT14)-positive, phenotype. However, the mechanisms that regulate these alterations to tumour cell phenotype remain unknown. Weighted gene correlation network analysis of RNA-Seq data from the breast cancer genome atlas revealed a highly significant positive correlation between the basal epithelial phenotype and stromal genes involved in extra-cellular matrix (ECM) organisation. To investigate this correlation experimentally we developed a novel method to co-culture autologous tumour organoids and stromal cells in 3D -directly following isolation from primary tumours. In this system, stromal cells significantly increased tumour cell transdifferentiation to a KRT14-positive basal phenotype and invasion (MMTV-PyMT and C31-Tag ductal breast cancer models). Time-lapse and confocal microscopy showed stromal cells to be highly dynamic and motile within these 3D organotypic cultures, organising collagen fibres and interacting directly with tumour organoids. Comparison of organoids co-cultured with stromal cells or treated with stromal-conditioned media revealed that juxtacrine signalling was required for maximal impact on epithelial cell transdifferentiation and invasion. Inhibiting NOX4, an enzyme recently identified as a critical regulator of cancer associated fibroblast activation, prevented these stroma-mediated effects. Proteomic analysis of NOX4 inhibition's impact on fibroblast activation revealed a critical role of this enzyme on actin cytoskeleton remodelling, reducing stromal cell's contractility and ability to remodel the ECM.
In summary, we have developed a novel organotypic model to study tumour-stromal interactions and demonstrate the potential for targeting NOX4 to prevent stromal-mediated tumour cell invasion in ductal breast cancer.

PL3

Copy Number Signatures Elucidate Mechanisms Underlying Sarcoma Heterogeneity

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Background: Genomic instability is a recognised hallmark of cancer which can lead to numerous copy number driver alterations in a tumour. Sarcomas are a histologically and genetically heterogeneous group of cancers ranging from fusion-driven to karyotypically complex tumour subtypes. The genomic complexity of some of these tumour types has hindered the inference of their evolutionary histories.
Methods: Inspired by recent work on mutational signatures, we utilised a non-negative matrix factorization framework to generate copy number signatures from allele specific copy number profiles in a cohort of 320 sarcomas; 43 chondrosarcoma, 51 dedifferentiated liposarcoma, 17 myxofibrosarcoma, 6 malignant peripheral nerve sheath tumour, 112 osteosarcoma, 10 synovial sarcoma, 52 soft tissue leiomyosarcoma, 27 uterine leiomyosarcoma and 43 undifferentiated pleomorphic sarcoma SNP arrays, as well as 12 low-grade sarcoma whole-genomes. We extended the copy number signatures to a cohort of 53 undifferentiated sarcoma whole genomes to further elucidate some of the mechanisms underlying copy number signatures.
Results: Copy number signatures were identified that indicate chromothriptic events, early near-haploidisation, sequential genome doubling and hypodiploidy. The hypodiploid signature was found to associate with sarcoma subtypes such as synovial sarcoma (90% of samples clustered by largest exposure to this signature) or low-grade sarcomas (67%), whereas signatures of one or two genome doubling events associated with karyotypically complex sarcoma subtypes such as undifferentiated pleomorphic sarcoma (72% and 19% respectively).
Conclusion: Copy number signatures are a powerful tool to deconvolute complex copy number profiles, allowing inference about the life history of a tumour, and evolutionary processes that contribute to karyotypic complexity.

PL4

Potential Place of Risk-Reducing Salpingectomy in BRCA1/2 Carriers: Prevalence of Malignancies and/or In-Situ Lesions at Risk Reducing Salpingo-Oophorectomy Over a 16 Year Period

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Risk-reducing salpingo-oophorectomy (RRSO) is recommended to BRCA1 carriers before 40 years and BRCA2 carriers before 45 years, to prevent high grade serous carcinoma (HGSC). Clinical trials (TUBA study) are evaluating risk reducing salpingectomy (RRS) with delayed oophorectomy (DO) as an alternative procedure. This is in view of the tubal origin of majority of HGSC from serous tubal intraepithelial carcinoma (STIC), and to help avoid the adverse effects of surgical menopause. We assessed the potential of RRS for HGSC prevention by studying the prevalence and primary site of HGSC and/or STIC in BRCA1/2 carriers at RRSO. We also describe 2 BRCA1 cases with STIC at RRSO, who later developed peritoneal serous carcinoma (PSC). Retrospective study of consecutive BRCA1/2 carriers undergoing RRSO at Erasmus MC (2000—2016) was conducted. Histology was reviewed in cases with HGSC and/or STIC, with p53 and MIB-1 immunohistochemistry. In 2 cases with late PSC, Next Generation Sequencing (NGS) was used to establish clonality. We studied 528 BRCA1/2 carriers. Thirty two percent of BRCA1 carriers, and 47% of BRCA2 carriers, underwent RRSO at the recommended age; this group had a significantly lower rate of HGSC at RRSO (p=0.03), than those operated on later. Thirteen of the 528 patients (2.5%) had HGSC; of these 7 (54%) were of tubal origin, 4 (31%) were of ovarian origin, & 2 (15%) of probable ovarian origin. Ten (1.9%) patients had STIC; 6 with HGSC and 4 isolated STIC. Two BRCA1 carriers developed PSC 118 and 80 months post-RRSO respectively. Isolated STIC was identified retrospectively in both these cases, and showed identical p53 mutation and similar single nucleotide polymorphism pattern on NGS. The majority of HGSC in BRCA1/2 carriers is of tubal origin; this group may benefit from RRS. The advantage for women with HGSC of ovarian origin is less certain. Longer follow up of patients with STIC at RRS(O) should be considered, as STIC can give rise to PSC after long lag periods.

PL5

Frequent Homologous Recombination Deficiency in High-Grade Endometrial Carcinomas

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Purpose of the Study: High-grade endometrial cancers (EC) generally have a poor prognosis with limited treatment options. The high levels of genomic instability including high somatic copy number alterations (sCNA) in a subset of these EC are reminiscent of defects in pathways governing genome integrity. Here, we assessed the occurrence of homologous recombination deficiency (HRD) in EC in relationship to the underlying aetiology.
Methods: Fresh tumour tissue of 36 EC was prospectively collected between 2015-2017. The ability of replicating tumour cells to accumulate RAD51 protein at DNA double strand breaks (RAD51 foci) induced by ionizing radiation was used as a functional read out for HR capacity. Tumours were molecularly characterized by comprehensive genetic analysis (next generation sequencing and array comparative genomic hybridization). Additionally, we determined the prevalence of *BRCA*-like sCNA-profiles (surrogate marker HRD) in the TCGA-EC cohort.
Summary of results: HRD was observed for 24% of EC and was significantly associated with non-endometrioid morphologies (46% HRD, p=0.014). None of the low-grade endometrioid EC were HRD. HRD was exclusively found in TP53-mutant EC, and genetic characterization revealed either pathogenic variants in *BRCA1* or significant somatic copy number losses in HR-genes. TCGA data supported our finding, as *BRCA*-like profiles were present in 42% (81/192) of NEEC and 8% (33/404) of EEC.
Conclusions: Homologous recombination deficiency is a frequent event in EC, particularly in non-endometrioid, TP53-mutant EC. This finding supports treatment strategies for these patients that exploit HRD, like platinums and PARP inhibitors.

PL6

Upregulation of Aldo-Keto-Reductase 1C1 and 1C3 (AKR1C1, AKR1C3) is Associated with Poor Prognosis in Oropharyngeal Squamous Cell Carcinomas (OPSCC) Independent of Human Papillomavirus (HPV) Status

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HPV16-positive OPSCC can be subdivided based on integration status (integrated, episomal, mixed). Previously, we showed that integration did neither affect the levels of viral genes, nor those of virally disrupted human genes. Here we performed a genome-wide screen to identify human genes which expression is influenced by viral integration and to determine their clinical relevance. Total RNA from 33 fresh-frozen HPV-16 positive OPSCC (9 integrated, 4 mixed, 20 episomal) was analyzed by mRNA expression profiling (Agilent Whole Human Genome 4644K Microarrays). Non-hierarchical clustering and pathway analysis was carried out to identify genes of interest. AKR1C1 and AKR1C3 expression was confirmed by RT-qPCR and immunohistochemistry. Additionally, 141 OPSCC, including 50 HPV-positive cases, were used to validate gene expression by immunohistochemistry. Results were correlated with clinical and histopathological data. Non-hierarchical clustering resulted in two main groups of mRNA expression patterns corresponding to OPSCC with either exclusively integrated or episomal viral DNA. Several deregulated cellular pathways were identified, in which AKR1C1 and AKR1C3 were predominantly involved. Upregulation of gene expression was observed in OPSCC with exclusively integrated viral DNA. Survival analysis of 141 additionally immunostained OPSCC showed unfavourable survival rates for those patients with tumours exhibiting upregulation of AKR1C1 or AKR1C3 (both $p < 0.0001$) both in HPV-positive ($p \leq 0.001$) and -negative ($p \leq 0.017$) tumours. OPSCC with integrated HPV16 show upregulation of AKR1C1 and AKR1C3 expression, which correlates with worse survival rates. Also in HPV-negative tumours, upregulation of these proteins correlates with poor outcome. This is in agreement with data from other tumours, making these genes promising candidates as indicators of prognosis. In addition, the availability of inhibitors of these gene products may be utilized for drug treatment.

PL7

Predicting the Response to Pre-Operative Short Course Radiotherapy in Rectal Cancer: a Potential Role for Raman Spectroscopy?

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Colorectal cancer is common, affecting around 42,000 people each year in the UK. Currently, neoadjuvant radiotherapy (RT) is frequently given to high risk rectal cancer patients. However, a significant proportion of patients do not respond yet unnecessarily endure the side-effects of RT. Raman spectroscopy is a highly sensitive, non-destructive form of vibrational spectroscopy that can determine the chemical fingerprint of cells and tissues, prior to any morphological changes. Using Raman spectroscopy, we aimed to identify novel biomarkers capable of predicting the response to pre-operative RT in rectal cancer. 4 rectal cancer patients who underwent short course RT were identified. Response to RT was determined by calculating the percentage reduction in tumour cell density (TCD) following RT by comparing the biopsy and resection. Tumour rich and stromal rich regions of interest were annotated onto digitalised H&E stained sections and an inVia Raman confocal inverted microscope was used to collect the spectra of these areas. The good responders had a TCD reduction of 92% and 98%, and the bad responders a reduction of 6% and 30%. Preliminary results suggest that Principal Component Analysis, a form of non-supervised multi-variate analysis, was able to accurately classify the spectra obtained, showing clear differentiation between tumour and stroma, when correlated with the H&E. Further analysis to investigate the differences between good and poor responders is ongoing. There are currently no reliable predictors of response to radiotherapy in rectal cancer. Pilot work from our study has shown that Raman spectroscopy can be used to differentially identify tumour and stroma after short course radiotherapy. Further work is currently being performed to interrogate the spectra and identify novel predictors of response.

Oral Abstracts

O1

Raman Analysis of Breast Cancer: A Large Tissue Microarray Study

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Purpose of the study: The understanding of the morphological and molecular features of breast cancer of non-Caucasian women is insufficient affecting the optimal treatment of those patients. The aim of this work is analyse the chemical signature of a large series of invasive breast carcinoma in comparison to normal breast using Raman Spectroscopy (RS), a non-destructive technique with high molecular specificity.
Methods: African breast tumours from similar tribal origin in Nigeria were reviewed and assembled into tissue microarrays (TMAs). Breast cancer (n=440) and normal breast (n=80) cores were analysed with DXR™ Raman Microscope (Thermo Scientific, USA). Cancerous (CA) and normal breast (NB) epithelium within each TMA core was selected and analysed. For each area, 20 spectra were collected then averaged. A chemometric model was created using Principal component analysis (PCA) and validated with Linear discriminant analysis (LDA) using The Unscrambler (CAMO, Norway).
Results: Differentiation and biochemical characterisation were achieved with RS with a sensitivity of 90% and a specificity of 78%. Peaks at 544, 849, 1085, 1158 and 1448 cm^{-1} showed a higher intensity of cholesterol, lipids, and carotenoids and were associated with the NB classification. The peaks at 783, 1006, 1294, and 1667 cm^{-1} supported the CA grouping and indicated a higher content of DNA/RNA bases, phenylalanine, ceramides and structural proteins of tumours.
Conclusions: RS has proven to be a powerful technique in breast cancer research by identifying characteristic profiles for normal and cancerous tissue and allowing the chemical classification of invasive breast cancer. Further studies into its prognostic utility are warranted.

O2

Inter- and Intra-Laboratory Variation in the Histopathological Grading of Ductal Carcinoma *In Situ* of the Breast in a Nationwide Cohort in the Netherlands

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Purpose of the study: A considerable part of ductal carcinoma in situ (DCIS) lesions may never progress into invasive breast cancer. Yet, standard treatment consists of surgical excision. Trials aim to identify a subgroup of low-risk DCIS that can safely forgo surgical treatment based on histologic grade, which highlights the importance of accurate grading by pathologists. To improve standardization of DCIS grading, we aimed to gain insight into laboratory-specific variation of DCIS grading.
Methods: All synoptic pathology reports of pure DCIS resection specimens between 2013–2016 were retrieved from PALGA, the nationwide Dutch Pathology Registry. Absolute differences in proportions of grade I-III between laboratories were visualized using funnel plots. Multivariable analysis to correct for case mix was performed by logistic regression, providing odds ratios (ORs) and 95% confidence intervals (CI) for high-grade (III) versus low-grade (II-III) DCIS.
Summary of results: In total 4,952 DCIS cases from 36 laboratories were included, of which 12.5% were reported as grade I (range 6.1-24.4%), 39.5% as grade II (range 18.2-57.6%), and 48.0% as grade III (range 30.2-72.7%). After correction for case mix, 14 laboratories (38.9%) reported a significantly lower (n=4) or higher (n=10) proportion of high-grade DCIS than the reference laboratory. Adjusted ORs (95% CI) range from 0.52 (0.31-0.87) to 3.83 (1.42-10.39). Intra-laboratory analysis also showed significant differences between pathologists within 25% of participating laboratories.
Conclusions: We observed substantial inter-laboratory variation in the histologic grading of DCIS, not explained by differences in case mix. Similarly, significant variation was observed between pathologists. Therefore, there is an urgent need for nationwide standardization of grading practices, especially since the future management of DCIS may alter significantly depending on histologic grade.

O3

Prospective Verification of a Reverse Transcriptase qPCR Assay for Molecular Subtyping of Breast Cancers

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Breast cancer specimens can be subdivided in molecular subtypes, which guide systemic therapy. Subtyping may include ER, PR, Her2 and Ki67 immunohistochemistry (IHC) and/or Her2 FISH. Discrepancy rates for these biomarkers, however, are frequently debated due to intra- and interobserver variability. The MammaTyper® assay analyses mRNA for more precise, reproducible assessment of these markers. Here we prospectively evaluated the performance of MammaTyper with IHC/FISH analysis in breast cancer biopsies. IHC for ER, PR and Ki67 was performed on 74 FFPE breast cancer biopsies using a Dako autostainer (cut-off positivity: ER/PR ≥10%, Ki67≥20%). Her2 status was determined using dual-colour FISH (Abbott Molecular; cut-off amplification ASCO/CAP guidelines 2013). Extracted total RNA was analyzed using MammaTyper assay on a BioRad CFX96 2017 device (cut-off based on St Gallen consensus 2013). Initial correlation between IHC/FISH and qPCR results showed an overall concordance in 43/74 (58%) samples; high for PR (71/74;96%), but lower for HER2 (65/74;88%), ER (61/74;82%) and Ki67 (57/74;77%). Of 9 discordant Her2 FISH positive-MammaTyper negative samples, 4 were IHC negative (0, 1+), increasing the correlation to 93% (69/74). For ER, decreasing the cut-off for ESR1 mRNA positivity based on analysis of 20 additional ER-negative breast tumours cases and the mean +2 SD, correlation became 98% (92/94). 16/17 discordant Ki67 cases had high MKi67 mRNA content and IHC Ki67<20%, which could not be improved by varying cut-off levels. After optimization, our prospective data show excellent concordance for PR, ER and Her2 status using IHC/FISH and MammaTyper subtyping of breast cancer biopsies. Tumour heterogeneity and intra/interobserver variations in manual Ki67 scoring particularly around the cut-off value for positivity might explain the difficulty to improve concordance for Ki67. Correlation with clinical outcome will determine which approach shows best performance to guide therapy.

O4

Single-Cell Analysis of Cancer-Associated Fibroblast Heterogeneity in Non-Small Cell Lung Cancer: Mapping Molecular Phenotypes in Tumours

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This work aims to characterise the heterogeneity and spatial relationships of the cancer-associated fibroblast (CAF) population in non-small cell lung cancer.
Fresh primary lung tissue was dissociated to extract the highest possible fraction of CAF. Single-cell RNA sequencing was performed using a droplet-barcoded sequencing platform. Bioinformatic analysis was performed in R, with cell type identification and gene set enrichment analysis using the ToppFun tool and GSEA program. Spatial relationships between cell types were assessed using a multi-immunohistochemical (IHC) staining technique. Analysis of 11 non-small cell lung cancer (NSCLC) tumours and 5 matched non-involved lung samples revealed the presence of 5 discrete CAF subtypes. Three subgroups showed transcriptomic overlap with normal fibroblasts. Of the distinct CAF subtypes, one showed higher expression of genes normally associated with the 'myofibroblastic' CAF phenotype, including multiple collagens. The second CAF cluster showed higher expression of genes encoding growth factors (e.g. IGF1). Of the remaining three subtypes, one showed gene expression and enrichment in keeping with the previously-described 'inflammatory' fibroblast type. The remaining two clusters show significant prognostic impact, but their potential functions have yet to be elucidated. Multiplexed IHC using identified cluster markers demonstrated that these subtypes have different spatial distribution and relationships to other cell types. Despite their abundance in most solid cancers, CAF are a poorly characterised cell population. No single marker identifies all CAF; it is not yet clear whether different functionally or phenotypically distinct CAF types exist. We have identified five discrete CAF subtypes with differential gene set enrichment and prognostic impact. Identification of pro-malignant CAF subgroups may facilitate development of novel stromal targeting strategies.
This work is supported by a Pathological Society grant.

O5

Two Years Experience of *MET* Gene Exon 14 Skipping Analysis in Lung Adenocarcinoma: Identification of 32 Cases by NGS

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Introduction: In lung adenocarcinoma (LAC) mutations in *EGFR*, *HER2* and *BRAF*, specific translocations of *ALK*, *ROS1*, *RET* and amplification of *MET* all have standard diagnostic importance and lead to specific targeted treatment options. Recently, in 2-4% of LAC *MET* gene mutations leading to skipping of exon 14 were found. These mutations were described to occur more frequently in tumours with sarcomatoid histology. LAC with *MET* exon 14 skipping mutations showed impressive, although temporary, responses to *MET* tyrosine kinase inhibitors (TKI) crizotinib, cabozantinib and capmatinib. We will present our experience with routine molecular diagnostic detection of the most common *MET* exon 14 skipping mutations.
Methods: In January 2016 we included in our standard, DNA based, molecular diagnostics custom-made NGS analyses 4 amplicons for detection of *MET* skipping mutations. The analyses were performed on microdissected FFPE tissue sections or routine histology or cytology stained preparations. 25 different mutations were validated for their effect on splicing by RT-PCR on RNA isolated from the same samples.
Results: Between January 2016 and March 2018 1563 routine molecular diagnostic analyses on LAC were performed. In 32 (2%) cases *MET* mutations were detected possibly resulting in exon 14 skipping. Twenty out of 25 different mutations were tested on the RNA level by RT-PCR and all 20 different mutations were confirmed to result in *MET* exon 14 skipping.
Conclusion: *MET* exon 14 skipping mutations can reliably be detected in routine pathology tissue and cytology samples. These analyses can easily be included in routine molecular diagnostic NGS. When necessary, confirmation of the mutational effect on RNA splicing can be implemented as well. Routine identification of *MET* exon 14 skipping mutations (2% of cases) adds substantially to the personalized targeted treatment strategies for LAC patients.

O6

Searching for Diagnostic Criteria in Pre-Operative Biopsy Specimen for Large Cell Neuroendocrine Carcinoma (LCNEC)

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Pulmonary large cell neuroendocrine carcinoma (LCNEC) is diagnosed when neuroendocrine morphology and neuroendocrine immunohistochemical staining is present. The aim of this study was to establish if additional diagnostic features for diagnosing LCNEC on a biopsy specimen could be identified in a cohort of paired LCNEC biopsy samples. Using the Dutch pathology registry (PALGA) surgically resected LCNEC with matching pre-operative biopsy specimen were identified (n=110) and original Hematoxylin and immunohistochemical slides were requested. Subsequently, the WHO 2015 criteria and the cumulative size of all (biopsy) specimen were assessed by a panel of pathologists. For validation, a tissue micro array (TMA) of surgically resected non-small cell lung cancer (80) and LCNEC (18) was used. Original paired biopsy-resection slides were obtained in 60/110 cases; in 12/60 cases no diagnosis could be established. LCNEC was diagnosed in 32/48 resection specimen and LCNEC was also confirmed in 47% (n=15/32) of the paired biopsy specimen. Neuroendocrine morphology was absent in 53% (n=17/32) of the paired biopsy specimen, more often when a limited amount of tissue was available for evaluation ((29% <5mm (n=14) versus 67% ≥5mm (n=18) P=0.04). New insights showed that staining for ≥2 out of 3 positive neuroendocrine markers might be an argument for LCNEC in a biopsy specimen devoid of neuroendocrine morphology. Combined with the established WHO criteria this increased the sensitivity for LCNEC from 47% to 93%. Additionally, staining for ≥2 neuroendocrine markers occurred in 3/80 NSCLC and 16/18 LCNEC screened by TMA. LCNEC is difficult to diagnose because neuroendocrine morphology frequently is not present in the biopsy specimen. In NSCLC devoid of obvious morphological squamous or adenocarcinoma features, positive staining in ≥2 out of three neuroendocrine immunohistochemical stains may support a diagnosis of LCNEC requiring prospective evaluation.

O7

PD-L1 Staining in Histologic and Cytological Material: Validation and Results

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Purpose of study: To validate staining for PD-L1 with a the cheap 22C3 antibody.
Material and methods: The DAKO 22C3 kit and the anti-PD-L1 antibody (also clone 22C3), obtained from Dako were compared *Material* There were 64 clinical samples with requested PD-L1 staining, 49 histological and 15 cytological specimens *Staining protocol* Controlled antigen retrieval was performed. All further staining steps were done in the semi- automated Dako Autolink 48. Detection was done with Envision Flex-HRP. *Validation* All 64 specimens were stained simultaneously with both the kit and the 22C3Ab and were judged by 2 pathologists separately, blinded to the method used. The number of positive cells were expressed in the following categories: 0, <1%, 1-5%, 5-50% and >50%. Pit falls were recorded.
Results: 1) Comparison between kit and separate antibody After "unblinding" no systematic difference was detected in staining intensity or the number of positive cells. 2) Staining results The distribution over the categories for histology was: 0: 15, <1%: 8, 1-5%: 5, 6-50%: 4, >50%: 17 For cytology the distribution was: 0: 7, <1%: 0, 1-5%: 2, 6-50%: 2, >50%: 4 In 60/64 cases the results were identical. In 4 cases comparison would have resulted in a potential change of positive to negative or vice versa, 2 in the histological cases (4% of all studied cases), 2 in the cytological cases (13%) 3) Pit falls Staining of alveolar macrophages that occasionally made proper evaluation of the percentage of tumour cells difficult.
Conclusions: The results with the two methods were comparable and concordance was more than 90%, in the histology cases. For the cytology cases the number studied was a bit too low to be certain, but already gave a concordance percentage of 87%. The distribution over the categories of staining is similar. PD-L1 staining can be performed in clinical samples with the cheaper antibody.

O8

An Exquisitely Sensitive Method for Mutation Detection

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Purpose of Study: Knowledge of tumour mutations underpins precision medicine in the management of cancer patients. This knowledge can also support other care pathways such as tumour surveillance. Not infrequently, however, the proportion of mutant alleles in patient-derived DNA samples is very low and the presence of contaminating wild-type DNA may make mutation detection unreliable.
Methods: To circumvent this problem, we have developed a novel highly sensitive amplification-refractory mutation detection system (ARMS) PCR-based method. This method does not require special probes or wild-type blockers and can be performed on standard real-time PCR machines without the need for expensive equipment.
Results: We tested 12 different mutations (including single nucleotide variants and insertion-deletion mutations) in template derived from cell lines and formalin-fixed tissue. We showed robust mutation detection with a limit-of-detection as low as 0.004% mutant allele frequency (MAF) or 1 copy. The assay has a wide dynamic range and excellent precision even at low MAF (CV <1.5%). Each target tested required little or no optimisation and the tests could be multiplexed. Blind testing of 35 FFPE cases with known KRAS mutation and 32 cases with known BRAF mutation were correctly identified thus confirming the accuracy of the assay.
Conclusion: In summary, we have developed an extremely simple, robust and sensitive test. It is a single stage closed-tube test which could transform cancer patient management by widening access to genetic testing. The speed of the test means it could theoretically be established in the hospital out-patient and even the primary care setting. Future research will focus on mutation detection in solid and liquid biopsies.
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O9

Comparison of Mutational Status in the EGFR Pathway Across Four Different Platforms in the Fluoropyrimidine Oxaliplatin and Targeted Receptor Pre-Operative Therapy (FOxTROT) Trial

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The FOxTROT trial assessed the potential benefit of pre-operative chemotherapy in locally advanced colon cancer. RAS wild type patients were additionally randomised to receive an anti-EGFR antibody. The optimal method for RAS analysis is currently unclear. We aimed to look at the concordance in mutational calls across the EGFR pathway using four different platforms. Cases of complete/near complete response were not tested (n=8). DNA was extracted from resected tumour blocks in the phase II group (n=142). The mutational status of KRAS (codons 12/13/59/61/117/146), NRAS (12/13/59/61), BRAF (600) and PIK3CA (542/545/546/1047) were tested using next generation sequencing (NGS) of direct PCR products. This was compared to three alternative platforms, each covering a variable number of amplicons: pyrosequencing (no KRAS117/146, NRAS & PIK3CA); Fluidigm Access Array NGS (no KRAS117 & NRAS117); and Affymetrix DNA microarrays (no KRAS59/117 & NRAS 13/59/117). ***Part funded by PathSoc.*** Mutations were detected in 37% of samples for KRAS, 2% for NRAS, 16% for BRAF and 18% for PIK3CA using NGS of direct PCR products. Pyrosequencing showed excellent correlation for KRAS12 (97%), KRAS13 (99%), KRAS61 (100%) and BRAF (97%). Affymetrix chips showed excellent agreement for NRAS (100%) and KRAS 13 & 61 (99%), but slightly lower agreement for KRAS 12 (94%) and BRAF (95%). Fluidigm agreement ranged from 92% to 100%, however, due to technical difficulties the data were incomplete for a number of cases. NGS of direct PCR products is currently our gold standard test for determining EGFR pathway mutational status prior to treatment with anti-EGFR monoclonal antibodies. Pyrosequencing and Affymetrix chips produced very similar results with generally excellent agreement. Cases with discrepancies are currently undergoing further exploration. Due to technical difficulties when using FFPE, Fluidigm Access Arrays frequently failed to produce a result and are considered suboptimal for routine mutational detection

O10

The Impact of Short Course Pre-Operative Radiotherapy on Tumour Cell Density and Tumour Biology in Rectal Cancer: Analysis of the MRC CR07 Trial

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The MRC CR07 trial demonstrated that pre-operative short course radiotherapy (SCRT) is associated with reduced local recurrence following rectal cancer surgery. There are currently no reliable predictors of response to SCRT and the effect of SCRT on tumour biology is relatively unknown. We aimed to evaluate the impact of SCRT on the amount of residual tumour and tumour biology in rectal cancer. Affymetrix DNA microarrays were used to obtain genome-wide copy number variation (CNV) and point mutation data for 432 resected tumours in the MRC CR07 trial. 178 cases received pre-operative RT (5x5Gy) with a median delay time between RT and surgery of 4 days (IQR 3-6 days). The remaining cases received no pre-operative treatment. Tumour cell density (TCD) across the whole tumour area was assessed in a representative digitised H&E slide using point counting (300+/-15 points). Cases were classed as responders or non-responders after SCRT based on the median TCD value across all cases. TCD ranged from 0.35% to 69.31% and was significantly lower in the SCRT group (mean 26.73% vs. 39.95%, p<0.0001). 138 (77.55%) patients were classed as responders. Overall CNV was reduced by SCRT even when adjusting for residual TCD (mean 0.34 vs. 0.37, p=0.04). CNV was not significantly associated with the degree of response to radiotherapy. Point mutations in the Epidermal Growth Factor Receptor (EGFR) gene were associated with a lower TCD after SCRT: 19.11% of patients in quartile 1 had a mutation in EGFR compared to 5.00% of patients in quartiles 3 and 4. Pre-operative SCRT in rectal cancer significantly decreased the residual TCD and the proportion of the genome with CNV. Patients with a mutation in EGFR were found to have a lower TCD after SCRT, suggesting a possible positive predictive effect, however this needs confirming in a larger series.

O11

NDRG4 is a Specific Enteric Neuronal Protein Which Attenuates Intestinal Tumour Progression and Protects Against Colitis-Induced Injury

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Background: We have identified promotor CpG island methylation of *NDRG4* as a promising early detection marker for CRC (Melotte et al. JNCI, 2009), which is incorporated in the FDA-approved stool assay (Cologuard®) for CRC screening in the US. Surprisingly, we recently revealed that NDRG4 expression is restricted to the enteric nervous system (ENS), labelling cell bodies of the myenteric and submucosal plexus and nerve fibers (Vaes et al. NGM, 2017). Nevertheless, the role of NDRG4 during CRC remains to be elucidated.
Methods: We used *NDRG4* wild-type (*NDRG4WT*) and knockout (*NDRG4KO*) mice to explore the effect of *NDRG4* deletion on the normal gut. CRC was modelled in *NDRG4KO*, *NDRG4$^{fl/fl}$-Villin-Cre* (epithelial-specific *NDRG4KO*) and *NDRG4WT* mice by (A) crossing them with *APC$^{min/+}$* mice, (B) treatment with Azoxymethane (AOM) or (C) treatment with AOM and dextran sodium sulphate (DSS). Gastrointestinal tracts were excised post-mortem for histopathological analysis and assessment of polyp burden.
Results: We discovered that absence of *NDRG4* did not lead to increased numbers of polyps during CRC development. However, polyps of *NDRG4KO* mice tend to be enlarged (*APC$^{min/+}$*, P=0.0451; AOM, P=0.0810) and have a more aggressive phenotype, as shown by the higher nuclear β-catenin content (*APC$^{min/+}$*, P=0.0637; AOM, P=0.0919), compared to *NDRG4WT* mice. In the AOM/DSS model, significantly more *NDRG4KO* mice died before the end of the protocol (P=0.0488) and exhibited higher colonic inflammation compared to *NDRG4WT* mice (P=0.0328). Finally, the *NDRG4$^{fl/fl}$-Villin-Cre* model enabled us to demonstrate that potential undetectable levels of epithelial *NDRG4* did not influence colorectal carcinogenesis, as there was no difference in polyp development, size or nuclear β-catenin content (*NDRG4$^{fl/fl}$-Villin-Cre APC$^{min/+}$*, P=0.7174, 0.5533, 0.8822).
Conclusion: Our data suggest that NDRG4, via the ENS, tends to repress intestinal tumour progression and protects against inflammation-induced gut injury.

O12

Categorical Assessment of Stromal Inflammation in Ductal Carcinoma in Situ of the Breast Results in Substantial Interrater Reliability

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Purpose of the study: Ductal carcinoma in situ (DCIS) of the breast is considered to be a non-obligate pre-invasive precursor of invasive carcinoma of no special type. Adequate prognostic markers for DCIS require robustness of assessment, i.e. low interobserver variability and thus high reproducibility. This study compares the interrater concordance among pathologists between several histopathological features of DCIS. The aim of this study is to identify robust histopathological features with low interrater variability.
Methods: Haematoxylin/eosin stained tissue sections of 153 DCIS lesions were reviewed by one pathologist. One representative slide was selected for each case. Thirteen pathologists independently assessed the following characteristics according to predefined descriptions: nuclear grade, intraductal calcifications, necrosis, solid growth, stromal architecture, stromal inflammation and apocrine differentiation. All features were assessed as categorical variables. Krippendorff's alpha (KA) was calculated to assess interrater concordance among the thirteen observers. KA was selected as a reliability measure as it can be used regardless of the numbers of observers and sample size.
Summary of results: Highest KA's were observed for the presence of necrosis (0,6885), calcifications (0,6456) and stromal inflammation (0,6355). Lower KA's were observed for solid growth (0,5935), nuclear grade (0,5629) and stromal architecture (0,4463). Poor concordance was observed for apocrine differentiation (KA 0,2819).
Conclusions: This interobserver variability study shows that categorical assessment of stromal inflammation in DCIS results in a substantially higher degree of agreement than the assessment of well-known features such as nuclear grade. The degree of concordance for stromal inflammation was similar as for the presence of necrosis and intraductal calcifications. This study demonstrates the robustness of categorical assessment of stromal inflammation in DCIS.

O13

The Cooperative Expression of the Solute Carriers, SLC1A5, SLC7A5 and SLC3A2, Confers a Poor Prognosis in the Highly Proliferative ER+ Breast Cancer Subtype

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Purpose of the study: Breast cancer (BC) is a heterogeneous disease characterised by variant biology, metabolic activity, and patient outcome. Known clinic-pathological parameters are insufficient to unravelling the secrets of the BC molecular heterogeneity. This study aimed to evaluate the biological and prognostic value of the membrane solute carriers, SLC1A5, SLC7A5 and SLC3A2 in BC with emphasis on the intrinsic molecular subtypes.
Methods: SLC1A5, SLC7A5 and SLC3A2 were assessed at the proteomic level, using immunohistochemistry on tissue microarray sections constructed from a large well characterised BC cohort (n=2,784, n=2,664 and n=2,500) respectively. Clustering analysis was applied to stratify tumours into accredited clusters and correlated with clinicopathological parameters, molecular subtypes, and patient outcome.
Summary of results: Clustering of SLC1A5, SLC7A5 and SLC3A2 identified three groups, which had distinct correlations to known prognostic factors and patient outcome (p<0.001). When different BC subtypes were considered, the association with the poor outcome was only observed in the ER+ high proliferation/luminal B class (p=0.01). In multivariate analysis, SLC clusters were an independent risk factor for shorter breast cancer specific survival (p=0.02).
Conclusions: The cooperative expression of SLC1A5, SLC7A5 and SLC3A2 appears to play a role in the aggressive subclass of luminal BC and therefore they can act as potential therapeutic targets, particularly in synergism.

O14

Assessment of Tumour Microenvironment Immune Cell Profile in Inflammatory Breast Carcinoma Using Digital Multiplex Analysis

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Purpose of the study: Inflammatory breast cancer (IBC) is a rare and aggressive disease that shows different rates of response to neoadjuvant chemotherapy (NACT). This study aims to identify the profile of immune cells existing in IBC tumour microenvironment both within the tumour and stromal regions and correlate them with different clinical and histological parameters including response/resistance to therapy and patient overall survival.
Methods: A cohort of 67 IBC cases that received NACT was collected from both UK and international centres during the period from 1997 to 2015. Tumour core biopsy samples were stained using advanced multiplex immunofluorescence for CD20, CD4, CD8, CD68 and FOXP3 and scanned images were computationally analysed using the Inform 2.2.1 software.
Summary of results: 50.7% of cases were grade II tumours, 53.7% were ER negative and 61.2% HER2 negative. The most frequent immune phenotypes were CD68 followed by CD4 and CD8 positive cells. No significant correlation was found between CD68 expression and any of the clinical parameters. However, significant association was found between high grade tumour and CD20 positive cells total count (p=0.046) and density (p=0.020) in stroma. Intratumoural high CD20 density was associated with low grade tumours (p=0.039). High CD8 total stromal count trends to correlate with ER negativity (p=0.079) and high FOXP3 density in tumour showed a trend towards positive ER cases (p=0.079). High intratumoural CD8 total count and density were correlated with response to NACT therapy (p=0.05), (p=0.049) respectively. High intratumoural CD20 total count significantly correlated with partial pathological response (p=0.004). No significant association was found between immune cells recruitment and survival of these patients.
Conclusions: Profiling of immune cell subpopulations in IBC microenvironment is prognostic and can identify groups of patients with tumours that are likely to respond to neoadjuvant chemotherapy

O15

An Assessment of EGFR Pathway Mutations in Late Stage Colorectal Cancer by Next Generation Sequencing in a Regional NHS Genomic Testing Service

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Colorectal cancer (CRC) is the third most common cancer in the UK and those with distant metastatic disease have a 5-year survival of 5–10%. For metastatic CRC, treatment with monoclonal antibodies against EGFR may slow progression. Multiple studies have confirmed lack of efficacy in the presence of mutations in the genes that code for proteins downstream of EGFR; KRAS, NRAS and BRAF. We aimed to analyse the mutational profiles of all cases tested through a large NHS regional genomic testing service over a 2-year period and determine what constitutes an optimal sample. We analysed all molecular pathology reports following CRC mutation testing between January 2015 and December 2017. All cases underwent next generation sequencing (NGS) of PCR products for hotspot mutations in KRAS codons 12/13/59/61/117/146, NRAS 12/13/59/61 and BRAF 600 with an Illumina MiSeq. Additional clinicopathological data were also collected. 421 samples were included in the study, with mutations in KRAS, NRAS or BRAF in 59% of samples. There was a significantly higher BRAF mutation rate in female patients (17 vs. 6%, p=0.0085) and right-sided tumours (20 vs. 6%, p<0.001). There was also a larger proportion of BRAF mutations in the left colon compared to rectal tumours (9 vs 1%, p<0.01419). The number of blocks extracted from did not affect mutation yield (1 block 74%, 2 blocks 66%, 3 blocks 48%, 4 blocks 66%, >5 blocks 54%).Samples bearing a higher tumour percentage yielded more mutations, with samples with <20% tumour only having a 30% mutation rate. There was no significant difference in the mutation frequencies between biopsy and resection samples (57% vs. 62%, p=0.162). The study population had a similar mutation frequency to that reported in the literature. We demonstrated that testing biopsy material did not change the overall mutation frequencies seen. Also, we did not see any significant difference in the proportion of mutations and the number of blocks tested in resection specimens. Finally, it is important that samples have an adequate proportion of tumour with >20% likely to find the highest number of mutations.

O16

Histopathologist Features Predictive of Diagnostic Concordance at Expert Level Amongst a Large International Sample of Pathologists Diagnosing Barrett's Dysplasia

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Introduction: Histopathological diagnosis of dysplasia in Barrett's oesophagus (BO) is the gold standard for patient risk stratification, but is subject to significant interobserver variation. We investigated histopathologist features that predict diagnostic performance amongst a large international cohort of gastrointestinal (GI) pathologists.
Methods: An online scoring environment was developed for GI-pathologists (n=55) from over 20 countries to grade a case set of 55 digitalised BO biopsies encompassing the diagnostic spectrum from non-dysplastic Barrett's oesophagus (NDBO), indefinite, low and high-grade dysplasia (IND/LGD/HGD). Cases were assessed before and after revealing P53 immunohistochemistry. Detailed histopathologist demographic data (experience, centre volume, fellowship training etc.) was obtained, as well as a consensus gold standard diagnosis for the entire case set through a reference panel of four expert pathologists. Multivariate regression analyses was conducted to identify pathologist predictors of concordance.
Results: We recorded over 6,000 case diagnoses. Of 2,805 H&E diagnoses, we found excellent concordance for NDBE (643 of 816 diagnoses; 79%) and HGD (544 of 765 diagnoses; 71%) and intermediate concordance for LGD (382 of 918; 42%) and IND (70 of 306; 23%), replicating known glass slide test characteristics. Major over or under-interpretations were reported in 248 diagnoses (8.8%). Addition of p53 staining further improved consensus, but had limited impact on major over or under-interpretations. Predictors of diagnostic performance were; > 5 years of experience, working in a teaching hospital, viewing 5-20 BO cases/week, adherence to major guidelines, and an interest in digital pathology.
Conclusion: Using this rich dataset representing a heterogeneous group of gastrointestinal pathologists working globally, we have quantified diagnostic performance for BO dysplasia diagnosis using digital case review. Our results reveal predictors of diagnostic performance at expert level.

O17

Defining the Clinical Value of Pathological Lymph Node Status and Primary Tumour Regression Grading Following Neoadjuvant Therapy in Oesophageal Cancer: Results from the MRC OE02 Trial

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Purpose of study: The clinical value of tumour regression grading (TRG) in oesophageal cancer (OC) following neoadjuvant therapy (NAC) remains unclear. We analysed the prognostic value of TRG (by Mandard) and associated pathological factors in OC patients enrolled in the Medical Research Council OE02 trial.
Methods: Pathology was reviewed in n=497 resections from OE02 trial participants allocated to surgery (S group; n=244) or NAC followed by surgery (CS group; n=253). Associations between TRG grouping [responders (TRG1-3) versus non-responders (TRG4-5)], pathological lymph node (LN) status and overall survival (OS) was determined.
Summary of results: n=195/253(77%) of non-responder CS patients had a significantly higher mortality risk compared to responders, [hazard ratio (HR)=1.53, 95%CI=1.05-2.24, p=0.026]. OS was significantly longer in patients without LN metastases independent of TRG group allocation [non-responders HR=1.87, 95%CI=1.33-2.63, p<0.001 versus responders HR=2.21, 95%CI=1.11-4.10), p=0.024]. In multivariate models, LN status was the only independent factor predictive of OS in CS patients (HR=1.93, 95%CI=1.42-2.62, p<0.001). Exclusion of radiotherapy-exposed patients (n=48) in subgroup analyses showed similar prognostic outcomes.
Conclusion: LN status is the principal prognostic factor in OC post-NAC, irrespective of TRG. Potential clinical utility (e.g. adjuvant treatment / follow-up), highlight the importance of meticulous LN dissection for staging and prognostication.

O18

Mortalin Expression by Soft Tissue Sarcomas and Correlation with Grade

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Purpose of the study: Mortalin (mitochondrial heat-shock protein 70/ mtHsp 70) is a key regulator of p53 function involved in controlling a wide array of cellular functions with important roles in apoptosis and immortalisation. We investigated whether mortalin could play a role in the growth of different types of sarcoma.
Methods: Initially detected using differential expression proteomics, mortalin was separately identified in proteomic extractions of sarcoma samples using Liquid Chromatography Mass Spectrometry. Expression in large numbers of cases was investigated using a tissue microarray constructed from a well-defined series of 420 primary and secondary soft tissue sarcomas linked to a patient database immunostained for mortalin. Immunohistochemical expression levels were determined using a scoring system based on staining intensity and correlated with clinicopathological parameters.
Summary of results: Mortalin is expressed by a spectrum of sarcoma types including various small round blue cell sarcomas, synovial sarcomas, angiosarcomas, malignant peripheral nerve sheath tumours, lipoblasts in various liposarcoma types and de-differentiated components. In a series of 106 leiomyosarcomas, levels of mortalin expression correlated directly with FNCLCC grade (high expression in high grade tumours, p<0.0005) and sex (p<0.05) but not significantly with anatomical site or tumour size (p=0.283, p= 0.974, respectively). Metastases were more likely to highly express mortalin (p<0.0005). Sarcomas not typically graded, such as clear cell sarcoma and alveolar soft part sarcoma, displayed high expression levels.
Conclusions: Our findings suggest that mortalin may be fundamentally involved in sarcoma tumourigenesis. This is important because it is known that drug molecules specifically inhibiting mortalin/ p53 interaction induce tumour suppression and reduce metastatic signalling. Mortalin might provide a specific molecular therapeutic target for high grade sarcomas.

O19

Recurrent *FN1* and/or *ACVR2A* Fusion Genes are Implicated in the Pathogenesis of Synovial Chondromatosis, Chondrosarcoma Secondary to Synovial Chondromatosis, and Soft Tissue Chondroma

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Synovial chondromatosis (SC) is a rare benign cartilaginous tumour involving joints which rarely undergoes malignant transformation into synovial chondrosarcoma (SCS). In a previous chondrosarcoma targeted exome sequencing study we identified a *FN1-ACVR2A* rearrangement in a chondrosarcoma arising in a SC. This finding was confirmed by FISH using custom-designed *FN1* and *ACVR2A* break-apart probes. A similar alteration was previously reported in one other case of SCS. Herein we aimed to determine if *FN1-ACVR2A* fusion is a recurrent alteration in SC, SCS, and soft tissue chondroma (STC) using FISH.
Results: 81 informative cases including SC (n=53), SCS (n=9), STC (n=19) were analysed by FISH revealing both *FN1* and *ACVR2A* break-apart signals in 9/53 (17%) SC, and 1/9 (11%) SCS. Only *FN1* break-apart signals were identified in 5/53 (9%) SC and 3/9 (33%) SCS. A break-apart involving only *ACVR2A* occurred in 14/53 (26%) SC, but not in SCS. In view of the histological similarities between SC and STC we assessed 19 STC for the same alterations: *FN1* but no *ACVR2A* break-apart signals were detected in 6/19 (26%). In summary *FN1* and/or *ACVR2A* rearrangement were found in 52% (28/53) SC and 44% (4/9) SCS, whereas only a *FN1* rearrangement was detected in 26% (5/19) STC. The genetic alterations in the remaining cases remain to be identified. In conclusion, the biological consequence of the *FN1-AVCRA2* fusion is unclear but do not distinguish between benign and malignant disease. Both *ACVR2*, a member of the transforming growth factor beta receptor family, and *FN1* play important roles in bone and cartilage biology: understanding the mechanism by which this fusion gene induces disease could explain the pathological process giving rise to disease.

O20

Development of a Novel FISH Probe for Detection of 1p/19q Codeletion in Routine Glioma Diagnosis

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The World Health Organization Classification of Tumours of the Central Nervous System (2016) has introduced integrated phenotypic and genotypic criteria to define diffuse glioma entities. Diagnosis of oligodendroglioma requires demonstration of 1p/19q codeletion, i.e., simultaneous loss of one copy of the entire 1p and 19q caused by unbalanced translocation t(1;19)(q10;p10). The analysis is commonly carried out using fluorescence in situ hybridisation (FISH). However, pathologists need to be aware of potential methodological and interpretational pitfalls as some commercially available probes hybridise to loci at 1p36 or 19q13 and detect partial loss of these regions, which is a common feature of glioblastomas. This may lead to misdiagnosis, incorrectly attributing the tumour to codeleted status. To improve diagnostic accuracy of 1p/19q FISH, we have developed a novel FISH probe sets. They are designed to hybridise to 1p31 and 19q13.1, the loci rarely deleted in non-oligodendroglial tumours. Ten diffuse glioma formalin-fixed paraffin-embedded (FFPE) samples (two oligodendrogliomas, three anaplastic oligodendrogliomas, one diffuse astrocytoma, and four anaplastic astrocytomas) were examined. The 1p/19q status of all samples was validated by multiplex ligation-dependent probe amplification (MLPA) with corresponding frozen tissues. For FISH analysis, clear and bright target and control signals were detected in nine out of ten FFPE samples. Four oligodendroglial tumours lost a target signal yielded one red target signal and two green control signals. All astrocytic tumours showed balanced two red target signals and two green control signals. Our FISH results thus showed perfect concordance with the MLPA results and correctly identified 1p/19q codeleted tumours. Thus, we have successfully developed a novel FISH probe sets for detection of 1p/19q codeletion. A validation using a large number of various gliomas with known 1p/19q status determined by MLPA is underway.

O21

The Roles of Gap Junctional Intercellular Communication in Non-Alcoholic Steatohepatitis (NASH) and Hepatocarcinogenesis: Establishment a Model of NASH

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Purpose of the study: Non-alcoholic steatohepatitis (NASH) have the potential leading to develop cirrhosis and hepatocellular carcinoma. Connexin (Cx)32, a hepatocyte gap-junction protein, plays an important role in liver tissue homeostasis. We previously reported that Cx32 has protective roles in NASH by analysing methionine-choline deficient diet (MCDD) received Cx32 dominant negative transgenic (Tg) rat. Though MCDD is well-established tool for induction of NASH, this model system does not reflect metabolic syndrome, such as obesity and insulin resistance. In this study, we aimed to establish an improved NASH model.
Methods: Male Tg and wild-type (Wt) rats at 7 weeks of age were received high-fat diet (HFD) with dimethylnitrosamine (DMN) for 12 weeks. Liver tissues were collected from each animal for the histopathological, gene and protein expression analysis. The levels of hepatic enzymes (AST, ALT), lipid and insulin in the serum and blood glucose value were also analysed.
Summary of results: HFD gained body, liver and visceral fat weights in both genotypes. Serum insulin level and HOMA-IR (homeostasis model assessment of insulin resistance) score in Tg rats were higher than those in Wt rats. Elevation of AST, ALT, inflammatory cytokine expressions (Tnfα, Il6, Tgfβ, Timp2 and Col1a1), NF-κB activity, and progression of histological steatohepatitis, fibrosis were significantly severe in Tg rats as compared with Wt rats. Concerning NASH-related hepatocarcinogenesis, the number, area of GST-P positive foci (a marker for preneoplastic lesion for rat liver) and expression of brain expressed, X-linked 1 (Bex1), which was established as a maker for hepatocarcinogenesis with NASH, were significantly increased in Tg rats versus Wt rats.
Conclusion: Cx32 dysfunction promoted the development of steatohepatitis and carcinogenesis in NASH accompanied by metabolic syndrome. Therefore Tg-HFD-DMN model may be a useful tool for NASH study.

O22

Rapid Evaporative Ionization Mass Spectrometry to Differentiate Between Normal Liver and Tumour Tissues

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Resection margin status of liver resections is an important factor for successful surgical treatment of liver metastases and is currently assessed at the time of pathological specimen reporting. Thus, there is a clinical need to establish an alternative rapid intraoperative tissue classification to assist surgical decision making. Mass spectrometry tissue profiling using vapours produced by electrocautery (Rapid Evaporative Ionization Mass Spectrometry, REIMS) has been reported for several different cancer types. The aim of our ex vivo study was to assess the feasibility of accurately characterising liver resection tissue types using REIMS. Liver tissue slices from 10 patients undergoing liver surgery for colorectal metastases were collected instantly after surgery. Multiple samples were taken from each tissue piece using a 1mm diameter needle connected to an electrosurgical heat-generator (Covidien). The vapours were analysed by REIMS on a mobile mass spectrometer (Xevo-G2-XSQ-TOF, Waters). The remaining tissue was paraffin embedded, cut and stained by Haematoxylin and Eosin. REIMS sampling points were annotated based on the tissue histology surrounding each 'hole' as normal liver, viable tumour, tumour necrosis or fibrosis. REIMS tissue profiles were analysed using principal component analysis-linear discriminant analysis (PCA-LDA, AMX, Waters) and compared to the histology based tissue type to build a library of REIMS tissue profiles. In total, vapour from 312 tissue points was analysed and annotated as originating from tumour (colorectal metastasis) or normal liver. PCA showed that 96% of the REIMS generated profiles provide a clear separation between tumour versus normal liver samples. Generation of a single REIMS profile took 2 seconds. The ex vivo analysis using REIMS allows rapid distinction between tumour and normal liver tissues. Further studies are warranted to investigate the feasability of classifying complementary liver pathology changes.

O23

Mutation Screening Using Formalin-Fixed Paraffin-Embedded Tissues: A Stratified Approach According to DNA Quality

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DNA samples from FFPE tissues are highly degraded with variable quality, and this imposes a big challenge for mutation analysis due to false positives by PCR errors and cytosine deamination. To eliminate false positives, a common practice is to validate the detected variants by Sanger sequencing or perform targeted sequencing in duplicate. Technically, PCR errors could be removed by molecular barcoding of template DNA prior to amplification as in the HaloPlexHS design. Nonetheless, it is uncertain to what extent variants detected using this approach should be further validated. Here, we addressed this question by correlating variant reproducibility with DNA quality using HaloPlexHS target enrichment and Illumina HiSeq4000, together with an in-house validated variant calling algorithm. The overall sequencing coverage, as shown by analyses of 70 genes in 266 cases of large B-cell lymphoma, was excellent (98%) in DNA samples amenable for PCR of ≥400bp, but suboptimal (92%) and poor (80%) in those amenable for PCR of 300bp and 200bp respectively. By mutation analysis in duplicate in 93 cases, we demonstrated that 20 alternative allele depth (AAD) was an optimal cut-off value for separating reproducible from non-reproducible variants in DNA samples amenable for PCR of ≥300bp, with 97% sensitivity and 100% specificity. By cross validation with a previously established targeted sequencing protocol by Fluidigm-PCR and Illumina MiSeq, the HaloPlexHS protocol was shown to be highly sensitive and specific in mutation screening. To conclude, we proposed a stratified approach for mutation screening by HaloplexHS and Illumina HiSeq4000 according to DNA quality. DNA samples with good quality (≥400bp) are amenable for mutation analysis with a single replicate, with only variants at 15-20 AAD requiring for further validation, while those with suboptimal quality (300bp) are better analysed in duplicate with reproducible variants at >15 AAD regarded as true genetic changes.

O24

This abstract has been withdrawn

O25

Preclinical Assessment of 17β-Hydroxysteroid Dehydrogenase-1 in Endometrial Cancer

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In endometrial cancer (EC), 17β-hydroxysteroid dehydrogenase-1 (17βHSD1) converts low-active estrone (E1) to active 17β-estradiol (E2).
Purpose of the study: Assess the therapeutic value of 17βHSD1 inhibition.
Methods: EC Ishikawa cells modified to express 17βHSD1 similar to humans (Ishi-HSD1) were used for *in vitro* (colony formation assay) and *in vivo* studies (chick chorioallantoic membrane assay -CAM- and an E2-dependent orthotopic mouse xenograft model). 17βHSD1 inhibition (HPLC) and expression (RT-PCR) in paired primary/metastatic lesions were assessed in two EC patient cohorts.
Summary of results: In Ishi-HSD1, E1 elicited colony formation similar to E2, which was impaired by 17βHSD1 inhibitor FP4643 (Forendo Pharma). Ishi-HSD1 tumours grafted on the CAM showed that E1 upregulated the E2 responsive cyclin A expression similar to E2, which was impaired by FP4643. To assess the human relevance of the 17βHSD1 enzyme, its activity was demonstrated in all 52 analysed EC biopsies, and was inhibited by over 90% in more than 45% of ECs. In addition, the 17βHSD1 mRNA was detected in both primary EC and paired metastatic lesions (37 ECs). For future testing of the 17βHSD1 inhibitor (and other) drug(s), an E2-dependent orthotopic mouse xenograft model was developed based on ovariectomised mice with controlled E2 (or placebo) exposure by novel optimised subcutaneous E2-releasing implants (E2-MedRod). Tumours were E2 responsive as determined by bioluminescence imaging (BLI), contrast enhanced-computed tomography (CE-CT) and by weight of surgically resected tumours at sacrifice. Metastatic spread and lymph vessel invasion were observed thus mimicking the human disease.
Conclusions: Inhibition of 17βHSD1 blocks E2 generation, colony formation and tumour growth (CAM). 17βHSD1 was blocked in the majority of human EC biopsies and its expression was maintained in metastatic lesions. A highly relevant estrogen-dependent orthotopic EC model is ready to further explore endocrine drugs.

O26

Placental Maternal Vascular Malperfusion Lesions in Relation to Doppler Velocimetry in Pregnancies Complicated by Placenta Syndrome

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Purpose of the study: Placental insufficiency, also known as placenta syndrome (PS), leads to a high resistance utero-placental circulation and pathological maternal vascular malperfusion (MVM) lesions in the placenta. The aim of this study was to evaluate Doppler velocimetry findings in relation to the presence of MVM lesions in pregnancies complicated by PS.
Methods: A retrospective study was performed on placentas of singleton pregnancies complicated by PS, defined as the presence of pre-eclampsia and/or foetal growth restriction. MVM lesions were examined by one pathologist and classified according to the criteria of the Society for Pediatric Pathology. In order to adjust for gestational age, p-values of the pulsatility index of the uterine (UtPI), umbilical (UmbPI) and middle cerebral artery (MCAPI) Doppler measurements were collected within the last two weeks prior to delivery. Patients were divided into two groups according to the presence of MVM. Doppler velocimetry and pregnancy outcome were compared between groups, using independent T-test, Mann-Whitney U test and Fisher's exact test.
Summary of results: MVM lesions were present in 64% (n=32) of the collected placenta's (n=47) in pregnancies complicated by PS. This group showed a significantly higher UtPI (p=0.028) with more bilateral notching (p=0.009) and a higher UmbPI (p=0.007). Cerebro-placental ratio was significantly lower (p=0.016) and MCAPI (p=0.074) showed a trend to be lower. Moreover, significantly more placental lakes were observed (p=0.048) with ultrasound imaging. Clinical outcome did not differ significantly.
Conclusions: In pregnancies complicated by PS, two-thirds of the placenta's showed signs of MVM. These patients demonstrate abnormal Doppler velocimetry within the last two weeks prior to delivery, indicating that routine Doppler measurements can detect placental malperfusion.

O27

C4d Positive Renal Transplant Biopsies With No other Evidence of Rejection: Transcriptional Investigation of Biological Significance Using Nanostring nCounter Technology

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Immunohistochemical staining for C4d in peritubular capillaries has been part of the definition of antibody-mediated rejection (AbMR) in the Banff Classification for Allograft Pathology since 2003. However, it has limited sensitivity and specificity and the clinical significance of C4d-positive biopsies without other evidence of rejection (C4d WER) is unknown. Our aim was to investigate the molecular significance of C4d positivity in such biopsies from both ABO-compatible and ABO-incompatible renal transplant patients. RNA was extracted from formalin-fixed paraffin-embedded renal transplant biopsies (n=157) with a mix of diagnoses and gene expression analysis of 35 AbMR-associated transcripts was carried out using the NanoString nCounter system. Seventeen of the 35 AbMR-associated transcripts were significantly increased in samples with AbMR compared to C4d-negative samples without rejection. These 17 transcripts showed no differential expression between C4d negative and C4d positive biopsies WER from both ABO incompatible and ABO compatible transplants. The geometric mean of the 17 differentially expressed AbMR transcripts was used to generate a receiver operator characteristic (ROC) curve, comparing AbMR to non-rejection. Samples with a geometric mean greater than or equal to 58.7 were considered high risk for AbMR, maximising sensitivity (0.80) and specificity (0.92). C4d+ WER samples from both ABOi and ABOc patients were assigned a low or high risk score based on the 58.7 cut off. Kaplan-Meier survival plots were generated with subsequent biopsy proven AbMR used as the outcome measure. AbMR development was significantly higher in the high risk group (p=0.003). Gene expression analysis in samples showing C4d positivity without evidence of rejection provides evidence that most of these cases do not represent AbMR, and may be used to identify patients in this group that may represent incipient ABMR and are at risk of imminent AbMR.

O28

The Histopathological Spectrum of Monoclonal Gammopathies of Renal Significance: A Single Centre Experience

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Purpose of the study: Monoclonal Gammopathy of Renal Significance (MGRS) encompasses renal histopathological entities caused by Monoclonal Immunoglobulins (MIg) in patients who do not meet criteria of symptomatic lymphoma or myeloma (MM). We describe the spectrum of MGRS histopathological diagnoses in the context of the underlying haematological disease in our centre.
Methods: Native renal biopsies performed during 2006-2017 were reviewed. Those with evidence of MIg or light chain in glomeruli, tubules, vessels and/or interstitium were included. C3GN and TMA were excluded.
Summary of results: Out of 4,374 kidney biopsies, MIg-associated lesions were identified in 163 cases (3.7%). After exclusion of symptomatic MM and lymphomas 68 biopsies (1.5%) were consistent with MGRS. Renal histological diagnoses included: AIg amyloidosis (n=28, 41%), Proliferative GN with MIg Deposits(PGNMID) (n=13, 19%), MIg Deposition Disease(MIDD) (n=12, 18%), Light Chain Tubulopathy (n=5, 7%), Type-1 Cryoglobulinaemic GN (n=6, 9%), Intracapillary Monoclonocal IgM without Cryoglobulin (n=2, 3%), Crystal Cryoglobulinaemia (n=1, 1.5%) and Fibrillary Light Chain restricted GN (n=1, 1.5%). Only 24 out of 42 patients in whom Serum Free Light Chain assay was performed had an abnormal ratio. Overall, haematological diagnosis was possible in bone marrow histology (BMAT) in 53 patients. These included MGUS (n=30, 56.6%), smouldering myeloma (n=17, 32%), lymphoplasmacytic lymphoma/ Waldenstrom's macroglobulinaemia (n=2, 3.7%), chronic lymphocytic leukaemia (n=3, 5.6%) and marginal zone lymphoma (n=1, 1.8%). In 8/13 PGNMID and 3/12 MIDD cases a clonal B cell or plasma cell clone was undetectable in BMAT.
Conclusions: MGRS are increasingly recognised since the term introduction in 2012. Renal histopathology must be interpreted in conjunction with the haematological diagnosis to guide treatment decisions. However, current diagnostic methods underperform and consensus diagnostic approaches are lacking.

O29

Peritubular Capillary Loss is Induced in the First Month After Transplantation by Ischemic and Allo-Immune Injury

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Peritubular capillary (PTC) density is associated with renal function in animal models and human studies. We have shown previously that a decrease of PTC density occurs in the first three months after transplantation in association with delayed graft function (DGF) and rejection. We now extend these findings in a cohort of patients who underwent an indication biopsy in the first month after renal transplantation. Between August 2003 and December 2009, 102 patients underwent an indication biopsy in our transplant centre, either because of DGF (n=66) or rejection (n=36). In addition, protocol biopsies at baseline and after 3 and 12 months were analysed. PTC density was studied as described earlier (Steegh *et al.* JASN 2011), and histological scoring was performed according to Banff criteria. In the whole cohort, a significant decrease of PTC density was found in the indication biopsies, compared to baseline ($t=6.42$, $p<0.01$), which was due to a decrease in PTC number ($t=5.40$, $p<0.01$), but not in tubular number. PTC density decrease was more pronounced in patients with rejection than in patients with DGF. In the rejection group there was a stabilisation of PTC density between 1 and three months ($t=0.65$, $p=0.52$), concomitant with decreased inflammation ($t=2.12$, $p=0.04$), which may be treatment related. In contrast, in the DGF group decrease of PTC density is enhanced in the 3 month biopsy ($t=3.64$, $p<0.01$) in association with increased total inflammation ($t=2.85$, $p=0.006$). In the biopsy after 12 months there was more interstitial fibrosis and tubular atrophy (IF/TA) in the DGF group vs. rejection group ($chi^2=9.04$, $p=0.029$). Our results indicate that both ischemic and allo-immune injury are associated with decreased PTC density the first month after transplantation. In patients with DGF, decrease of PTC density aggravates over time in association with enhanced inflammation and more interstitial fibrosis after one year.

O30

Author requested abstract not to be published

O31

Early B Cell Gene Expression Pattern in Merkel Cell Carcinoma

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Merkel cell carcinoma (MCC) is a highly aggressive neuroendocrine skin carcinoma which is found in elderly and immunosuppressed patients. In 80% of MCCs the DNA of Merkel cell polyomavirus (MCPyV) was detected. MCC could be further characterized by its trilinear expression pattern of neuroendocrine, epithelial and pre-pro B-cell lymphocytic genes. The cellular origin of MCCs has remained elusive. Based on the early B-cell gene expression of the terminal deoxynucleotidyl transferase (TdT) and paired box gene 5 (PAX5) in combination with Ig expression and rearrangements, we hypothesized early B-cells might be the cellular origin of MCC. Here we are investigating the expression of more pre-pro and pro B-cell genes which are involved in the commitment of the B-cell differentation. Formalin-fixed and paraffin-embedded (FFPE) tissues of resection specimens of 21 MCCs were analysed for the early B cell genes AID, EBF-1, IKAROS, BTK, E2A, IRF-8, RAG-1 by immunohistochemistry. All 21 tested MCCs were stained positive for the early B-cell transcription factors E2A and EBF-1. A weak staining was observed of IKAROS in two MCCs. The Ig rearrangement recombinase complex molecules as RAG-1 and IRF-8 were 94.7% and 85% respectively positive in MCCs. The DNA repair enzyme Artemis was found in 11% tested tissues. The hypermutation enzyme AID was in an extent of 63% positive. In addition, all tissues were negative for BTK which is an important part of the pre-B-cell receptor. So, in total the early B-cell genes like E2A, EBF-1, RAG-1, IRF-8 and AID were expressed to an high extent in MCC. Taken these genes together with the previous published B-cell genes, the B-cell phenotype of MCC could be specified as a pre-pro B-cell phenotype. These similarities of pre-pro B-cells and MCC cells hints to a direct connection and hints to the possibility that MCC originates from pre-pro B-cells.

O32

The Use of PD1 and PDL1 as Prognostic Markers in Malignant Melanoma

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Introduction: Although it comprises less than 5% of skin cancer cases, melanoma accounts for the great majority of skin cancer-related deaths. Melanoma is a very immunogenic neoplasm, meaning it has a high mutation rate generating many neoantigens for a healthy immune system to recognize and target for destruction. Rather than simply avoiding the immune system, cancer cells can counterattack immune cells. A group of proteins known as immune checkpoints (ICPs) are known to inhibit the function and replication of active immune cells, and play a key role in immune homeostasis. Examples include programmed death 1 (PD1), programmed death ligand 1 (PDL1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). Many tumour cells, including melanoma, have been found to express these restricted ICPs, allowing them to suppress the function of any Tumour infiltrating lymphocytes (TIL) that migrate into the tumour microenvironment.
Aim: To assess PD1 and PDL1 in melanoma microenvironment (tumour and immune cells), and test the hypothesis of an association between ICP expression in primary melanoma and known clinicopathological factors and patient's prognosis that includes (overall survival, recurrent free survival or metastasis free survival). METHODS: Two hundred cases of malignant melanoma have been investigated for PD1, PDL1 and other inflammatory cell associated markers within the tumour and tumour microenvironment.
Result: PD1 is more prevalent in tumour infiltrating lymphocytes while PDL1 was more predominant in tumour cells. There is a positive correlation between PDL1 expression and the intensity of tumour infilterating lymphoctes and increased Breslow thickness. PDL1 expression in melanoma cells is associated with reduced overall survival.
Conclusion: PDL1 is a significant predictor of decreased overall survival. Further studies determining the relation between PDL1 expression and patient response to immunotherapy are warranted.

O33

Sequencing Older Archived FFPE DNA: First-Hand Experience and Potential Pitfalls

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Background: Previous studies have indicated archived formalin-fixed, paraffin-embedded (FFPE) tissue samples as a valuable source of genetic information. However, several factors might affect sequencing and reliable identification of somatic variants, including DNA fragmentation, formalin-fixation and tumour heterogeneity. We evaluated the usability of DNA isolated from FFPE material for somatic variant calling in a large nation-wide prospective cohort study in the Netherlands.
Methods: Routinely worked-up paraffin material from 266 clear-cell renal cell carcinoma cases (ccRCC), identified in a large prospective cohort study in the Netherlands during 20.3 years of follow-up, was collected from 51 pathology laboratories throughout the Netherlands. DNA was isolated directly from heterogeneous FFPE tumour blocks (GENRE I, N=119), or from macrodissected FFPE material (GENRE II, N=147). Coding regions of 48 frequently mutated genes in ccRCC, selected based on the COSMIC database, were sequenced following Single Primer Enrichment Technology (NuGEN).
Results: In total, 252 samples were successfully sequenced with a mean coverage of 39x and 46% of the target region was covered at least 20x. Decreased coverage of the exon was observed at increasing distance from the landing probes, likely due to FFPE-based DNA fragmentation. However, through careful selection of regions with sufficient coverage, poorly performing samples can still be used for somatic variant calling.
Conclusion: Taking into account the caveats of working with older archived FFPE material, FFPE material can be used for (targeted) genotyping. We will further elaborate on potential pitfalls of FFPE material and sources of potential differences in sequencing quality (a.o. formalin-fixation, storage time and DNA isolation).

O34

Sensitivity of Transperineal Template Prostate Biopsy: A Proposal to Estimate How Many Cores to Take Based on PSA Density

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Purpose of the study: Transperineal template prostate biopsy (TPTB) is considered a highly sensitive method to diagnose prostate cancer (PC). There are, however, limited studies into the factors associated with false negative TPTB and no consensus exists on the number of cores needed, with recommendations ranging between 24 cores per case to 1 core per each mL of prostate volume (PV). We wished to study the role of a number of histopathological, radiological and clinical parameters in the context of vanishing PC on TPTB after a diagnosis on TRUS biopsy and elucidate a way to reduce the incidence of false negative TPTB.
Methods: We identified all TPTB performed in our institution on men with a previous TRUS-based diagnosis of PC during 2013-2017 and separated into 2 cohorts: positive and negative for PC. Multiple variables including number of cores in TPTB, PC length in previous TRUS, PV, potential tumour on mpMRI, [number of cores in TPTB/PV], PSA density (PSAd) and [PSAd x number of cores in TPTB] were compared.
Summary of results: 100 cases were studied of which 73 had a positive TPTB and 27 had a negative TPTB. The following variables showed differences between both cohorts which were found to be statistically significant using Fisher exact test: PC length in previous TRUS, potential tumour on mpMRI, [number of cores in TPTB/PV], PSAd and [PSAd x number of cores in TPTB]. The last was found to be extremely statistically significant ($p<0.0001$). Furthermore, only 1 of the 62 (1.6%) cases which [PSAd x number of cores TPTB] value was ≥6 had a negative TPTB. Therefore, the sensitivity of TPTB to diagnose PC in our cohort is approximately 98% when [PSAd x number of cores TPTB]≥6.
Conclusions: The sensitivity of TPTB is strongly associated with the number of cores taken for a given PSAd and a simple formula is proposed to ensure a highly sensitive diagnostic test: number of cores=6/PSAd.

O35

Tumour Seeding in the Tract of Percutaneous Renal Tumour Biopsy: A Report of Six Cases from a UK Tertiary Referral Centre

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The role of the percutaneous renal tumour biopsy (RTB) in the management of radiological indeterminate renal masses is long-established. Patients with small renal masses who have biopsy-proven renal cell carcinoma (RCC) may be offered ablative therapy or active surveillance, and RTB can provide diagnostic tissue for patients with metastatic disease who may be candidates for systemic therapy. Current guidelines suggest that tumour seeding of the tract of RTB is anecdotal. From January 2014 to November 2017, we have performed 585 renal tumour resections within our tertiary-referral institution and 196 RTB have been performed. We report six patients in this series in whom RTB tract seeding by tumour has been identified on histological examination of the subsequent resection specimen; 5 papillary RCCs and 1 clear cell RCC. In five of these cases the presence of tumour within the fat upstaged the tumour, which would have otherwise been TNM 8th Ed stage pT1. Two of the patients have subsequently developed local recurrence of the tumour within the renal bed at a site consistent with the biopsy tract. In the modern literature the first published case of seeding of the tract of a RTB by RCC, which was histologically evident, was published in 2013. There have since been four further case reports of RTB tract seeding evident on histology, involving four patients, one of whom has had documented local recurrence, with three further reported cases of suspected RTB tract seeding and local recurrence. We hereby present the largest series of patients who have histological evidence of RTB tract seeding. The RTB site can be identified macroscopically in the majority of renal tumour resection specimens and should be targeted when taking blocks in order to exclude the presence of biopsy tract seeding.

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