VIRTUAL WINTER MEETING, 25-26 JANUARY 2022

4TH JOINT MEETING OF THE PATHOLOGICAL SOCIETY AND THE ROYAL SOCIETY OF MEDICINE

Embracing complexity in modern pathology

PLENARY ORAL PRIZE WINNER

PL3

A Genetic Model for Central Chondrosarcoma Evolution that Associates Patient Outcome

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Background Central conventional chondrosarcoma (CS) is the most common subtype of primary malignant bone tumour in adults. While surgery is curative in low grade disease, higher grades have variable outcomes, making prognoses challenging. Dedifferentiated disease is often fatal. Here, leveraging a large patient cohort of genomic and clinical data, we present the first genetic model of CS and suggest its clinical utility. Methods Whole genome sequencing, targeted sequencing, and methylation arrays were compiled from the Genomics England 100,000 Genomes Project, and from the RNOH (n = 350). Previously identified driver mutations in IDH1, IDH2, TERT, and other genomic features such as genome doubling and haploidisation, were examined against tumour grade and outcome. Results Mutations in the TERT promoter, previously associated with poor outcome, are more frequent in IDH2 mutant tumours (%TERT, IDH1: {tilde}20%, IDH2:{tilde} 50%) yet only effect outcome in tumours driven by IDH1 mutations. IDH2 mutations pertain to an increased likelihood of dedifferentiated disease. Tumours wild type for IDH1 and IDH2 are driven by a separate mutational pathway involving genome doubling and prior haploidisation. These tumours acquire TERT mutations rarely and have a reduced tendency to evolve to dedifferentiated disease. Conclusion We suggest that diagnostic testing for IDH1, IDH2 and TERT mutations could help clinical management and prognostication.

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RAPID FIRE ORAL PRESENTATION PRIZE WINNERS

JOINT 1ST RAPID FIRE ORAL/SIR ALASTAIR CURRIE POSTER PRIZE

RFO2

Circulating Tumour DNA is a Promising Biomarker for Risk Stratification in Chondrosarcoma

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Central conventional chondrosarcoma (CS) is the most common primary malignant bone tumour in adults. The prognosis is currently based on tumour grade, imaging and anatomical site, however, the inter-observer variation in grading CS by pathologists and radiologists demonstrates that these criteria are unreliable and consequently more objective biomarkers are needed. In this study, we aimed to determine if measuring circulating tumour DNA (ctDNA) could be used to predict outcome for CS patients. In this multi-institutional study, 145 patients with cartilaginous tumours were recruited, 41 of whom were excluded, mainly due to lack of sufficient tumour DNA. ctDNA levels for the 83/104 patients, whose tumours harboured a hotspot mutation in IDH1/2 or GNAS, were assessed using digital droplet PCR. ctDNA was detected pre-operatively in 31/83 (37%), and in 12/31 (39%) of these patients post-operatively. The detection of ctDNA was more accurate than pathology for identification of high grade tumours and was associated with a poor prognosis ($p<0.0001$). Moreover, ctDNA was never associated with CS grade 1/ atypical cartilaginous tumours, neoplasms sited in the small bones of the hands and feet or in tumours measuring less than 80 mm. Implementation of this assay into clinical practice is the major challenge now faced. This assay should be introduced into clinical practice as a complementary assay as this would allow patients with CS to receive more personalised care.

RFO5

Phenotypic Plasticity Limits Subclonal Selection in Colorectal Cancer

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Clonal expansions in cancers occur via genetic variation which alters selected phenotypes. Meanwhile, there is evidence that phenotypic plasticity, the ability to switch phenotype without a change in genotype, is common in cancer. For instance, we have previously found that the majority of colorectal cancers (CRCs) evolve without stringent subclonal selection, and have speculated that these neutral tumours are formed by a plastic clone. Furthermore, many candidate genetic drivers have been identified in CRC but the selective advantages and phenotypic effects of these candidates need to be assessed. We produced a dataset of 30 multi-region multi-omic CRCs involving 1,373 samples in order to detect phenotypic heterogeneity and any evidence of underlying genetic causes.

This included 504 whole genome sequences, 297 whole transcriptomes and 1,185 ATAC-seq measuring chromatin accessibility. At the phenotypic level, we observed extensive variation in gene expression programs across a tumour. This intra-tumour phenotypic heterogeneity was often not a reflection of evolutionary history and showed only infrequent evidence of genetic control. We employed a novel mathematical inference framework that can handle spatial-genomic data to sensitively detect selection and, consistent with the phenotypic data, we inferred that only a minority of CRCs undergo stringent subclonal selection, and that most putative driver mutations had no discernible effect on subclonal evolution. Together, the data showed that phenotypic plasticity is established early in the majority of CRCs, and defines evolutionary trajectories. Subclonal genetic alterations, even in candidate driver genes, can alter gene expression but frequently have limited impact on tumour evolution. In conclusion, every genetic alteration does not necessarily alter the phenotype and phenotypic variation may not always be under selection.

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3RD RAPID FIRE ORAL PRESENTATION PRIZE / POSTER PRIZE

RFO1

Development of an Artificial Intelligence Solution for the Analysis of Histopathological Images as an Improved Clinical Test for Coeliac Disease

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Purpose of the study: Around 1% of the population of the UK and North America have a diagnosis of Coeliac Disease (CD), due to a damaging immune response to the small intestine. Assessing whether a patient has CD relies primarily on the examination of a duodenal biopsy using the Marsh-Oberhuber classification system. This is an unavoidably subjective process with poor inter-observer concordance. There is a clear unmet need for a more sensitive, objective and reproducible test. Methods: A neural network has been built to accurately predict the presence of CD in whole slide images of duodenal biopsies. The Marsh-Oberhuber classification system was used to guide the development of the neural network architecture. Stain normalization, data selection, group equivariances and local-global information trade-off techniques were selected to mimic the way pathologists diagnose CD. Summary of Results: The neural network was trained on a dataset of 500 carefully annotated, haematoxylin and eosin stained duodenal biopsies. It correctly classified 93% of unseen data. Conclusions: Designing a classification neural network by modelling the way pathologists examine duodenal biopsies when considering the diagnosis of CD resulted in a powerful, comprehensive, data-driven CD diagnostic tool with greater reproducibility than that seen in some studies of concordance between pathologists. This work was funded by a Pathological Society of Great Britain and Ireland PhD studentship.

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PRIZE WINNING ABSTRACTS

CAMBRIDGE UNIVERSITY PRESS POSTER PRIZE WINNERS

P02

Pre-clinical Fast Field Cycling Nuclear Magnetic Resonance: New Applications in Breast Cancer

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Fast Field Cycling-Nuclear Magnetic Resonance (FFC-NMR) is emerging as a new tool that can provide unique insights into molecular dynamics of biological samples. This study aimed to explore its potential in breast cancer. We obtained NMR dispersion curves (0.001-8MHz) from 148 fixed breast cancer samples using a bench-top FFC-NMR relaxometer. Tissues were acquired, with ethical approval, from the tumour, peritumoral zone and distant normal areas (non-adipose and adipose). Dispersion curves were fitted using standard models. Numerical parameters, corresponding to the shape of the curves, including its overall vertical offset (A); slope at different fields ({alpha-x}low, {alpha-x}med and {alpha-x}high) and frequency at discontinuity ({nu-x}low and {nu-x}high), were derived from the models and investigated in the context of tumour proximity and prognosis. Tumour and non-tumour breast tissue could be distinguished, based on A and {alpha-x}low, {alpha-x}med and {alpha-x}high ($p<0.05$). We then stratified the numerical parameters to known indicators of prognosis: Nottingham Prognostic Index, lymph node and estrogen receptor status. The numerical parameters that could significantly ($p<0.05$) distinguish the different prognostic categories were A and {alpha-x}low, but only when sampling the distant normal breast tissue. This suggests that the earliest biophysical changes related to disease progression occur in the periphery rather than the tumour itself. This finding appears to be consistent with the idea that the tumour microenvironment plays a critical role in cancer progression and metastasis. Our findings may have translational potential, when combined with whole-body Field-Cycling Imaging, to assist in patient stratification and predicting clinical outcome in breast cancer.

P26

RFWD3 - a Potential Biomarker for Platinum Response in High Grade Serous Ovarian Cancer

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High grade serous ovarian carcinoma (HGSOC) is the most common type of ovarian cancer, demonstrating a 5-year survival of just 40%. This relatively poor outcome is attributed to the development of resistance to platinum chemotherapy. Alterations in DNA damage repair pathways - such as the Fanconi Anaemia pathway, which repairs DNA interstrand crosslinks induced by platinum - are often involved in development of chemoresistance. We investigated Fanconi Anaemia pathway components and found that RFWD3 (FANCW) expression alterations appear to play a role in chemoresistance of HGSOC. The expression profiles of these components were assessed in 5 HGSOC cell lines via Western blot, and RFWD3 was found to have a wide dynamic range of expression across the panel. Interestingly, COV318 - one of the most carboplatin-sensitive cell lines, had significantly lower levels of RFWD3. Knockdown of RFWD3 in HGSOC cell lines significantly increased cellular sensitivity to carboplatin. RFWD3 expression was assessed in 274 HGSOC cases using immunohistochemistry of tumour tissue microarrays; these data were quantified using QuPath and

demonstrated differential expression of RFWD3 across cases. HGSOCs which demonstrated complete response to platinum-based chemotherapy were associated with significantly lower levels of RFWD3 than those which progressed ($p<0.01$). CRISPR cell lines with modified RFWD3 expression were generated to further characterise the cellular roles of RFWD3 in drug response, proliferation, and migration. We have demonstrated that the DNA damage repair protein RFWD3 can mediate cellular response to carboplatin, and differences in protein levels are linked to platinum chemotherapy outcomes in HGSOC patients. RFWD3 may also have further functions beyond its classical role in DNA damage response, which will be further explored.

P36

Development of a Semi-automated Method for Tumour Budding Assessment in Colorectal Cancer and Comparison with Manual Methods

Fisher, N.C.[1]; Loughrey, M.B.[2]; Coleman, H.G.[1]; Gelbard, M.D.[3]; Bankhead, P.[3]; Dunne, P.D.[1]

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Tumour budding (TB) is the histological manifestation of local tumour cell dissemination, usually most evident at the invasive front region of a tumour mass. Despite TB being an established prognostic feature in multiple cancers, inconsistent qualitative criteria, definitions and non-standardised reporting have proven an obstacle to routine implementation in pathology practice. Efforts to standardise and automate assessment have shifted from haematoxylin and eosin (H&E)-stained images towards cytokeratin (CK) immunohistochemistry. In this study, we compare manual H&E and CK assessment methods with a new, semi-automated approach built within QuPath open-source software. Budding was assessed in cores from the advancing tumour edge in a cohort of stage II/III colon cancers (n=186). More than four times the number of buds were detected manually using CK compared to H&E. 1734 individual buds were identified on both manual and semi-automated assessments applied to CK images, representing 75.7% of the buds identified manually (n=2290) and 33.7% of the buds detected using the semi-automated method (n=5138). Higher semi-automated bud scores were due to any discrete area of CK immunopositivity within an accepted area range being identified as a bud, regardless of shape or crispness of definition, and to inclusion of tumour cell clusters within glandular lumina ("luminal pseudobuds"). Although absolute numbers differed, semi-automated and manual bud counts were strongly correlated across cores ($\rho=0.81$, $p<0.0001$). All methods of budding assessment demonstrated poorer survival associated with higher budding scores. We present a new QuPath-based approach to tumour budding assessment, which compares favorably to established methods. More importantly, it offers a freely-available, rapid and transparent tool for TB assessment, which can be used in translational research as a standalone method or as an aid in developing future approaches suitable for clinical implementation.