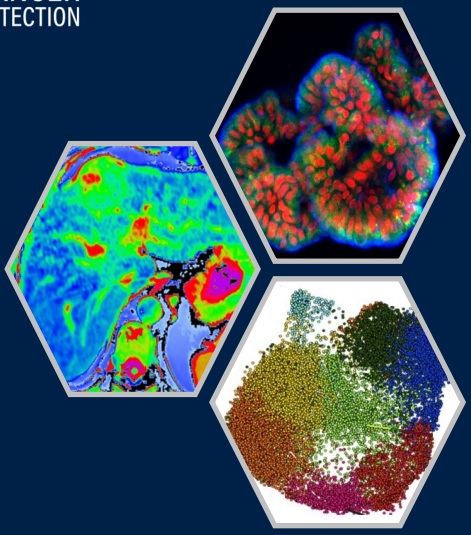
OXFORD CENTRE FOR EARLY CANCER DETECTION

UNIVERSITY OF OXFORD



Oxford Centre for Early Cancer Detection Annual Symposium

Tuesday 5th December 2023

Mathematical Institute, Oxford

Welcome

I am delighted to welcome you to this third in-person Annual Symposium for the Oxford Centre for Early Cancer Detection (OxCODE). We've had another productive year, including the Oncology Clinical Trials Office, in partnership with the Primary Care Clinical Trials Unit, becoming a CRUK trials unit dedicated to conducting precision prevention and early detection studies – an outstanding achievement! In parallel, OxCODE has expanded its remit to include the full breadth of early cancer biology research that is applied to precision prevention and early detection.

Following a fantastic Precision Prevention Symposium a few weeks ago, today we focus on early cancer and early detection. We have three excellent keynote speakers: Caroline Dive, Samra Turajlic and David Hunter, the latter of whom we are dedicating the first session on epidemiology to mark his appointment as Companion of the Order of Australia earlier this year. Many congratulations David! We are also grateful to Steph Phillips, one of our patient and public representatives, who will share how Li Fraumeni Syndrome is affecting her family and how she is buddying up with DPhil student Miriam Dixon-Zegeye for ongoing input into research.

As we look forward to our 5-year anniversary next year, our community is continuing to grow and with that growth comes new opportunities for collaborations. We have included ample networking breaks, so please take the time to talk to someone new and learn about an area of early detection research that is different from yours. You never know where these chance conversations may lead!

Finally, I would like to thank all the speakers, poster presenters and chairs at today's event, and CRUK, the Medical Sciences Division, the John Fell Fund, the CRUK Oxford Centre and the NIHR Oxford BRC for their support of OxCODE and this event.

Xin Lu

OxCODE Director

Schedule

09.30-09.40 Welcome

Xin Lu, Oxford Centre for Early Cancer Detection Director

09.40-11.00 Session 1: Cancer epidemiology

Chair: Clare Bankhead

09.40 **Julia Hippisley-Cox** - Personalised approaches to the early detection of cancer

10.00 **Ruth Travis** – Proteins and cancer: aetiology, early detection and prognosis

10.20 **Keynote talk: David Hunter** – Some thoughts on Polygenic Risk Scores and Our Future Health

11.00 *Coffee*

11.30-12.00 Session 2: Working with patients

Chair: Catriona Gilmour Hamilton

11.30 **Steph Phillips & Miriam Dixon-Zegeye** – The Researcher-PPI Buddy scheme: integrating patient and public involvement into basic research and clinical trials

12.00-12.25 Session 3: Lightning talks

Chair: Dan Woodcock

12.00 **Eoghan Mulholland** – Epithelial Grem1 drives ectopic stem cell niche formation through stromal remodelling and tissue co-evolution in intestinal dysplasia initiation

12.05 **James Chettle** – The RNA binding protein LARP1 drives tumorigenesis by promoting metabolic plasticity and resistance to oxidative stress

12.10 **Jingfei Cheng** – A human tissue atlas of DNA methylation and hydroxymethylation

12.15 **Talisia Quallo** – Opportunities from Cancer Research UK

12.25 *Lunch & posters*

Schedule

13.40-15.00 Session 4: Cancer detection using liquid biopsy

Chair: Chunxiao Song

13.40 **Brian Nicholson** – Why we need early detection studies in primary care

14.00 **Anna Schuh** – Liquid biopsies: from depth to breadth

14.20 **Keynote talk: Caroline Dive** – Our approach to earlier lung cancer detection using liquid biopsies

15.00 *Coffee*

15.30-16.50 Session 5: Early cancer biology

Chair: Beth Psaila

15.30 **Adam Mead** – Single-cell multi-omics identifies chronic inflammation as a driver of TP53-mutant leukaemic evolution

15.50 **Simon Buczacki** – Does mutational order matter in early colorectal cancer evolution?

16.10 **Keynote talk: Samra Turajlic** – Exploring early stages of cancer evolution by rewinding the tape of malignant transformation

16.50-17.00 Closing remarks

Simon Leedham, Oxford Centre for Early Cancer Detection Associate Director

17.00 *Drinks reception and canapés*

About OxCODE

The Oxford Centre for Early Cancer Detection (OxCODE) launched in June 2019 to build on the existing momentum and galvanise early cancer detection research in Oxford. We have recently expanded OxCODE's remit to also include cancer precision prevention research.

The formation of OxCODE consolidated our significant expertise to realise the full potential of cross-disciplinary discourse and collaboration for advancing early cancer research for patient benefit. The Centre comprises >300 members from >40 Departments, Units and Institutes from the University of Oxford and the Oxford University Hospitals NHS Foundation Trust (OUHFT). OxCODE aims to stimulate more cancer precision prevention and early detection (PPED) activity in Oxford. We are expanding our multidisciplinary research community by hosting a series of events and increasing the scale and scope of PPED research at Oxford by providing infrastructure funding for new projects and grant writing support.

All University of Oxford or OUHFT researchers with an interest in PPED are welcome to join. If you wish to be added to the mailing list to hear about future events and funding opportunities, or have an idea for a new PPED research project, please email the OxCODE Scientific Coordinator (francoise.howe@ludwig.ox.ac.uk).

OxCODE is supported by:

NIHR | Oxford Biomedical Research Centre

CANCER RESEARCH UK | OXFORD CENTRE

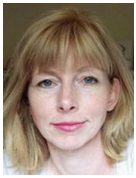
OxCODE Committees

OxCODE Management Committee

The OxCODE Management Committee determines the overarching vision and strategy for OxCODE.

Xin Lu - Director

Ellie Barnes



Sarah Blagden

David Hunter

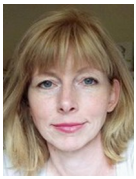
Simon Leedham

Paresh Vyas

OxCODE Operational Group

The OxCODE Operational Group reviews, supports and guides the activities of OxCODE, within the overarching strategy set by the OxCODE Management Committee.

Simon Leedham



Sarah Blagden

Emma Culver

James McCullagh

Eva Morris

Brian Nicholson

Beth Psaila

Jens Rittscher

Chunxiao Song

OxCODE Funding Scheme

The OxCODE Funding Scheme aims to advance innovative Oxford-based research that can be applied to preventing and/or detecting cancer earlier. OxCODE aims to pump-prime this research by providing short-term awards that will enable the development of projects to a stage at which more long-term external funding can be sought.

Since 2020, funds have been awarded to:

- **Sarah Blagden** "Defining the immunopeptidomic landscape of ovarian cancer"
- **Alistair Easton** "Multiplex immunofluorescence: Developing a pipeline for early cancer phenotyping, diagnosis and research"
- **Skirmantas Kriaucionis** "A pilot study to investigate biomarkers predicting recurrence in patients with non-muscle invasive bladder cancer treated with intravesical BCG therapy"
- **James McCullagh** "Towards early detection of altered cancer metabolism"
- **Brian Nicholson** "Enhancing non-invasive patient stratification for endoscopic detection of colorectal cancer"
- **Smita Patel** "Methylation, malignancy and primary immunodeficiency: early detection of pre-malignant changes and sub-clinical disease"
- **Siim Pauklin** "Early detection of pancreatic cancer by identifying exosome marker signatures in blood".
- **Bethan Psaila** "Basophils and mast cells as non-canonical drivers of inflammation and cancer progression in patients with myeloproliferative neoplasms''
- **Monica Olcina** "Developing probes for ovarian cancer early detection".
- **Susie Shapiro** "Unprovoked venous thromboembolism and early detection of increased blood cancer risk"
- **Nicola Sibson** "Biofluid metabolomics for detection of cancer in patients with non-specific signs"

OxCODE Travel Award

The mission of the OxCODE Travel Award is to raise the profile of Oxford's precision prevention and early detection research by competitively funding the attendance of early career researchers (students and post-doctoral researchers) at the Early Detection of Cancer conference. Applications from researchers working on fundamental scientific and/or clinical early detection research are welcome. Priority is given to applications with an abstract of high scientific quality with a clearly articulated relevance to the early detection of cancer, and applications with a strong justification for how the applicant will benefit by attending this conference.

This year's OxCODE Travel Award recipients were:

- **James Chettle** (Blagden group, Department of Oncology) "The RNA binding protein LARP1 drives tumorigenesis and can be exploited for the early detection of breast and ovarian cancer"
- **Masato Inoue** (Song group, Ludwig Institute for Cancer Research) "A human tissue atlas of DNA methylation and hydroxymethylation"
- **Eoghan Mulholland** (Leedham group, Wellcome Centre for Human Genetics) "Epithelial Grem1 drives ectopic stem cell niche formation through stromal remodelling and tissue co-evolution in intestinal dysplasia initiation"

Look out for announcements via the OxCODE mailing list about future opportunities.

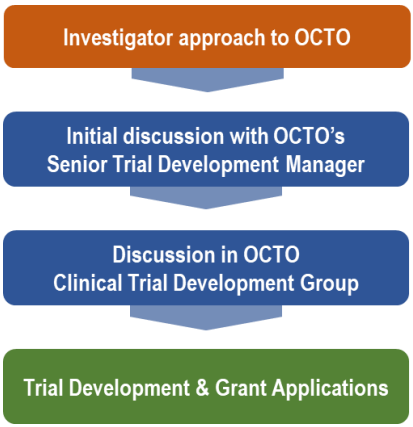
About OCTO

The Oncology Clinical Trials Office (OCTO) provides clinical trial management support to investigators across Oxford Cancer to deliver trials from concept to reporting.

OCTO joined the CRUK Clinical Trial Unit (CTU) network in October 2023. We are the UK's first CRUK trials unit specialising in cancer precision prevention (PP) and early detection (ED) studies. In this, we are working in partnership with the Primary Care CTU in Oxford. OCTO's strategy is purposefully disease-agnostic, enabling studies in areas of greatest unmet need.

The inclusion of PPED studies aligns with Oxford Cancer's priorities of supporting tumorigenesis research, developing novel therapeutics including anti-cancer vaccines (immuno-oncology), developing MCED tests (early detection) and building partnerships with biotech and pharma. Work on trials in these areas is supported by funding from CRUK to facilitate the launch of a series of national PPED studies over the next five years.

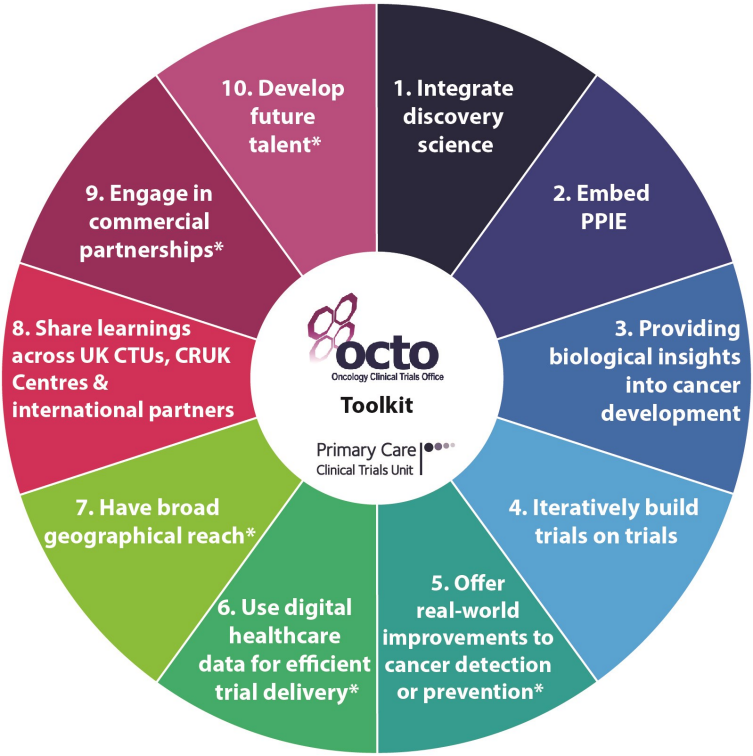
Our trial management expertise enables investigators to design and deliver multi-centre studies from concept to final reporting.



Our approach is collaborative and communicative; from your initial approach to us and through the lifetime of your trial, you can expect a proactive, pragmatic and proportionate approach to trial development and trial management from our team of experienced trial management professionals.

About OCTO *cont*

The OCTO toolkit comprises 10 components elemental to our precision prevention and early detection trials:



** developed by the Primary Care CTU and in partnership with OCTO to deliver the SYMPLIFY study*

Graphic: Dr Françoise Howe

SCAN HERE **to see OCTO's portfolio** ***and*** **for more information about how to work with us:**

Session 1 Abstracts

Personalised approaches to the early detection of cancer

Julia Hippisley-Cox

Nuffield Department of Primary Care Health Sciences

We now have unprecedented opportunities to use large scale multi-modal NHS patient datasets to drive improvements in the early detection of cancer though the development, validation and implementation of personalised cancer risk assessment tools. In this talk, we will focus on two new such tools (the 'CanPredict' tools) funded by INNOVATE UK and Cancer Research UK. They have been designed to improve early detection of lung cancer (via the new lung cancer screening programme) and oesophageal cancer (in conjunction with the CytoSponge) to better target those at highest risk of developing cancer for early detection and targeted interventions.

Session 1 Abstracts

Proteins and cancer: aetiology, early detection and prognosis

Ruth Travis

Cancer Epidemiology Unit, Nuffield Department of Population Health

Proteins are integral to most biological processes including those known to lead to carcinogenesis, such as tissue growth and proliferation. Proteomics can highlight novel opportunities for the therapeutic prevention of disease given that proteins are the targets of the majority of drugs. Previous prospective studies of individual or small panels of blood proteins have identified aetiological cancer proteins, such as insulin-like growth factor-I, which is a risk factor for breast, colorectal, and prostate cancers, and microseminoprotein-beta, which is associated with a lower prostate cancer risk. Recent advances in multiplexed and high throughput platforms now allow us to measure the levels of thousands of circulating proteins. The availability of large-scale proteomics in mature prospective cohorts presents key opportunities to advance our understanding of the role of proteins in cancer development and progression, and to identify novel strategies for cancer prevention and control. This presentation will summarise recent results from studies of proteins and cancer risk within the UK Biobank and EPIC cohorts, as well findings from corresponding genetic analyses (Mendelian randomisation and exome burden studies), and will illustrate the importance of functional and experimental analyses to triangulate the role of risk proteins. We will also highlight some notable findings for proteomics markers for early detection and cancer prognosis.

Session 1 Keynote

Some thoughts on Polygenic Risk Scores and Our Future Health

David J Hunter

Nuffield Department of Population Health

Polygenic risk scores (PRS) have recently been criticised as insufficiently predictive when evaluated by screening test criteria. These are the wrong criteria by which they should be evaluated. PRS are risk factors, not stand-alone screening tools. The question is whether PRS are useful when added to standard "clinical" cancer risk predictors when incorporated into Integrated Risk Scores. The answer is "yes" for at least some of the major cancer types. This moves the conversation to whether it is possible to calculate these for individuals, how to do this, how to communicate the information, what the effects on vertical screening programmes would be, and what are the costs and potential adverse effects of doing so. Our Future Health is a new charitable company set up by the UK Government to explore these questions, among many others. The data from Our Future Health will be available to registered researchers from December 2023 in a Trusted Research Environment, and will include a genome array that has been designed to include common cancer-associated genetic variants.

Session 2 Abstract

The Researcher-PPI Buddy scheme: integrating patient and public involvement into basic research and clinical trials

Steph Phillips and Miriam Dixon-Zegeye

George Pantziarka TP53 Trust/Department of Oncology

Integrating Patient and Public Involvement (PPI) into basic science is often perceived as challenging. Researchers may feel their science lacks enough clinical line-of-sight to appeal to PPI members, whilst PPI members feel they do not have a sufficient understanding of basic science to support a researcher.

Oxford Cancer is trialling a method for integrating PPI into basic science through “researcher-PPI” buddying. Miriam is a clinical research fellow undertaking a DPhil investigating the role of RNA binding proteins and mutant p53 in tumourigenesis. Steph has two daughters with Li Fraumeni Syndrome (LFS), a rare autosomal dominant cancer predisposition disease caused by a mutation to TP53. Steph is also a trustee of the George Pantziarka TP53 Trust which co-developed with Oxford University the Metformin in Li Fraumeni (MILI) Trial: A Phase II Randomised open-label cancer prevention study of metformin in adults with LFS.

The buddying scheme involves an initial face-to-face and then quarterly virtually catch-up meetings. Here we will discuss the mutual benefits of buddying, the ease of implementation and how this scheme is being expanded to involve other researchers.

Session 3 Abstracts

Epithelial Grem1 drives ectopic stem cell niche formation through stromal remodelling and tissue co-evolution in intestinal dysplasia initiation

Eoghan Mulholland

Wellcome Centre for Human Genetics

We investigate how the protein Grem1 influences the development of abnormal intestinal growths known as polyps with ectopic crypt foci (ECF). These are associated with the disease Hereditary Mixed Polyposis syndrome (HMPS) and have the potential to turn cancerous. Our focus is on understanding how Grem1 affects the cells lining the intestine (epithelial cells) and the surrounding tissue. We conducted experiments using mice with aberrant Grem1 expression in their intestinal lining. Excess Grem1 disrupted normal intestinal signalling, leading to certain cells exiting the stem cell area and forming ECF. Furthermore, Grem1 brought about alterations in the supportive tissue of the intestine (stroma), promoting the expansion of specific fibroblast cells that produce a protein called Wnt2b. By inhibiting Wnt signalling, a reduction in polyps was observed. Furthermore, testing a new anti-Grem1 antibody showed potential to prevent the early development of these growths and even shrink them in older mice, thereby extending their lifespan without causing any adverse effects. To summarise, Grem1 disrupts the balance in the intestine, leading to polyp formation. Blocking Wnt signalling or using an anti-Grem1 antibody holds promise as potential treatments for these conditions and may offer new approaches to treating colorectal cancer.

Session 3 Abstracts

The RNA binding protein LARP1 drives tumorigenesis by promoting metabolic plasticity and resistance to oxidative stress

James Chettle

Department of Oncology

It is now known that most epithelial cancers transition from precancerous dysplastic lesions to invasive, detectable cancers over several years. Examples of this progression are in high grade serous ovarian cancer which progresses from serous tubal intraepithelial carcinomas (STICs), and in invasive breast cancer which often progresses from ductal carcinoma in situ (DCIS). This 5–10 year latency provides a window of opportunity for both early detection and intervention. However, as little is known about the molecular behaviour of precancerous lesions, there are no reliable biomarkers and hence they are rarely detected early enough to intervene.

The mRNA-binding protein LARP1 post-transcriptionally regulates thousands of mRNAs encoding metabolic and antioxidant proteins and is elevated in tissues with high levels of oxidative stress. We have discovered that LARP1 is strongly upregulated in precancers such as STICs and DCIS as well as in invasive breast and ovarian cancer. In mouse models of ovarian cancer tumorigenesis, LARP1 upregulation is an early event in STIC development where it colocalises with TP53 mutated cells. Our findings suggest that precancers have high levels of oxidative stress and that staining tissue for LARP1 is useful for detecting nascent precancer lesions as well as providing insights into precancer biology.

Session 3 Abstracts

A human tissue atlas of DNA methylation and hydroxymethylation

Jingfei Cheng (on behalf of Masato Inoue)

Ludwig Institute for Cancer Research

Cell-free DNA (cfDNA), derived from cell death in various healthy and diseased tissues, holds significant promise for non-invasive cancer diagnosis. Genetic alternations in cfDNA are weakly indicative of tissue-of-origin and challenging to detect in early-stage cancer. In contrast, epigenetic modifications, such as 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) exhibit tissue/cancer-specific patterns, enabling precise estimation of tissue origin. Current tissue atlases cannot differentiate 5mC and 5hmC due to the limitation of bisulphite sequencing technology, and lack cancer signatures essential for distinguishing cancer and other tissue degenerative diseases. To address these limitations, we introduce the first tissue atlas featuring distinct 5mC and 5hmC modifications. Utilising advanced TET-assisted pyridine borane sequencing (TAPSβ) and chemical-assisted pyridine borane sequencing (CAPS) techniques, we conducted deep whole-genome epigenetic sequencing on 13 normal tissue types, 10 tumour types, and 9 blood cell types. Harnessing the rich sequencing data, we identified genomic regions that are uniquely 5mC/5hmC-modified in a tissue/cancer-specific manner. These regions are expected to improve the sensitivity and specificity of cancer detection based on cell-free DNA deconvolution. Our atlas will also serve as a valuable resource for various biological studies on tissue-specific epigenetic regulation.

Session 3 Abstracts

Opportunities from Cancer Research UK

Talisia Quallo

Cancer Research UK

Cancer Research UK is the world's leading cancer charity dedicated to saving lives through research, influence and information. Our ambition is to accelerate progress so that 3 in 4 patients survive cancer by 2034.

Through our Early Detection and Diagnosis Research Committee (EDDRC), we provide funding for early detection and diagnosis (ED&D) research. This includes the discovery and validation of signatures of early cancer, the development of the technologies to enable this, non-confirmatory clinical trials of ED&D technologies and approaches, ED&D health systems research, research to understand and optimise clinician and public behaviour to enhance ED&D and research into the health economics of ED&D research.

EDDRC supports ED&D research via three funding streams:

- Primer Awards - support researchers at all stages to develop early, novel and outside-the-box ideas and collaborations to build and make progress in the early detection field.
  Next deadline: 21 March 24
- Project Awards – support specific research projects that aim to have a significant impact on how and when cancer is detected.
  Next deadline: 14 December 23
- Programme Awards - support established researchers to perform large, integrated and renewable research programmes which have the potential to transform early cancer detection.
  Next outline deadline: 4 April 24

To discuss an application, please contact early.detection@cancer.org.uk

Session 4 Abstracts

Why we need early detection studies in primary care

Brian Nicholson

Nuffield Department of Primary Care Health Sciences

GPs request simple tests after consulting with patients with cancer symptoms to identify who should be sent to hospital for cancer investigation. Sounds simple, but there are very few tests that accurately select the highest risk patients from the rest. In this talk, the case will be made for the need to develop early detection technologies for use in primary care. It will draw on current practice guidelines, the existing literature, and recent technological advances including the potential role for Multi-Cancer Early Detection testing.

Session 4 Abstracts

Liquid biopsies: from depth to breadth

Anna Schuh

Department of Oncology

Technologies to detect circulating tumour DNA (ctDNA) in liquid biopsies must first be tailored to specific clinical use cases and then validated and evaluated using appropriate controls. Applying ctDNA detection has so far mainly focused on either screening asymptomatic individuals with multicancer early detection tests or on tracking minimal residual disease post treatment. However, there are other potential clinical applications and we have developed and evaluated artificial intelligence (AI) to derive cancer signals from liquid biopsies for different clinical scenarios and DNA sequencing platforms. First, we employ deep whole genome sequencing (WGS) of TET-assisted pyridine borane sequencing (TAPS) modified DNA to triage patients with cancer from a high-risk patient group with non-specific symptoms presenting to primary care. We show that multi-modal analysis of deep WGS data allows sensitive and specific detection of cancer signals from plasma including in patients with clinical stage 1 or 2 disease. Second, we prospectively show that applying our AI-approach to plasma samples of individuals with pre-malignant myeloma (low and high-risk MGUS) allows detection of the same genomic aberrations seen in CD138 selected bone marrow plasma cells, at least two years before clinical progression. Finally, we have developed, validated and implemented liquid biopsy testing as a diagnostic aid for EBV-driven lymphomas occurring in children living in sub-Saharan Africa. While additional analyses are still on-going, our aim is to make these AI tools accessible to low-middle-income countries for earlier cancer diagnosis. With this in mind, we have spun-out SerenOx, a social enterprise that supports AI driven diagnostics in sub-Saharan Africa.

Session 4 Keynote

Our approach to earlier lung cancer detection using liquid biopsies

Caroline Dive

CRUK Manchester Institute

I am Professor of Pharmacology at The University of Manchester and a Senior Group Leader at the CRUK Manchester Institute, where I direct the Cancer Biomarker Centre. My research team is developing liquid biopsies for earlier cancer detection and treatment monitoring, with a focus on lung cancer. Early detection of lung cancer is key to improving patient outcomes and we are working with the Lung Health Checks programme that targets lung screening towards people at higher risk of developing cancer. We employ methods that detect circulating biomarkers such as circulating tumour cells and cell-free DNA. In this talk, I will summarise our recent work and plans for future testing.

Session 5 Abstracts

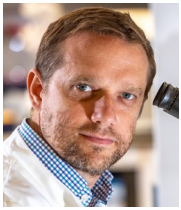
Single-cell multi-omics identifies chronic inflammation as a driver of TP53-mutant leukaemic evolution

Adam Mead

MRC Weatherall Institute of Molecular Medicine

Understanding the genetic and nongenetic determinants of tumour protein 53 (TP53)-mutation-driven clonal evolution and subsequent transformation is a crucial step toward the design of rational therapeutic strategies. Here we carry out allelic resolution single-cell multi-omic analysis of hematopoietic stem/progenitor cells (HSPCs) from patients with a myeloproliferative neoplasm who transform to TP53-mutant secondary acute myeloid leukaemia (sAML). All patients showed dominant TP53 'multihit' HSPC clones at transformation, with a leukaemia stem cell transcriptional signature strongly predictive of adverse outcomes in independent cohorts, across both TP53-mutant and wild-type (WT) AML. Through analysis of serial samples, antecedent TP53-heterozygous clones and in vivo perturbations, we demonstrate a hitherto unrecognised effect of chronic inflammation, which suppressed TP53 WT HSPCs while enhancing the fitness advantage of TP53-mutant cells and promoted genetic evolution. Our findings will facilitate the development of risk-stratification, early detection and treatment strategies for TP53-mutant leukaemia, and are of broad relevance to other cancer types.

Session 5 Abstracts



Does mutational order matter in early colorectal cancer evolution?

Simon Buczacki

Nuffield Department of Surgical Sciences

Somatic driver mutations have been discovered in phenotypically normal colonic tissue, however their role in cancer initiation remains elusive. Here, using patient-derived human colon organoids as models of early tumour evolution, we investigate the consequences of FBXW7 mutations in normal and gene-edited organoids. We observe that FBXW7 mutations exert an epistatic effect where the transcriptional consequences of the mutation are dependent on the mutational background. Specifically, we find the timing of acquiring an FBXW7 mutation relative to APC mutation, leads to profound differences. When FBXW7 is mutated before APC, repression of the APC transcriptional response and maintenance of near-normal cell state is seen. However, when APC is mutated before FBXW7, cells acquire classic cancer-stem cell features. Single-cell RNA-sequencing reveals that FBXW7 mutations in normal tissue also function by reordering stem cell hierarchies and priming a foetal/regenerative phenotype through upregulation of YAP/TAZ signalling. Further analysis using ATAC-seq finds this cellular plasticity driven by changes in chromatin accessibility associated with TEAD1/TEAD2 motifs, which in turn upregulate YAP. Taken together, we find a critical role of FBXW7 mutations in preventing the initiation of colorectal cancer, and provide exemplar evidence for the importance of epistasis and mutational order in cancer biology.

Session 5 Keynote

Exploring early stages of cancer evolution by rewinding the tape of malignant transformation

Samra Turajlic

Royal Marsden Hospital & The Francis Crick Institute

Cancer evolution is a function of the combined forces of mutation, selection and drift. The relative weighting of factors within the selective component, such as germline variation, environmental exposure, cell and tissue context remains unclear. This uncertainty arises as patients with sporadic cancer develop tumours from a singular clonal origin. Patients with von Hippel-Lindau (VHL) disease have a pathogenic germline mutation in *VHL* gene and develop multiple clonally independent tumours in a tissue-specific manner, typically following biallelic *VHL* loss. Therefore, clonal evolution proceeds in a setting where the initiating event, patient germline and environment are controlled, offering a unique opportunity to evaluate the factors that shape clonal evolution and the repeatability of cancer evolution.

Here we assemble the largest ever cohort of patients (n=132) with VHL disease, profiling tumours from a range of tissue sites (n=1010). Challenging the notion that all tumours are clonally independent, we detect instances of persistent clones that frequently precede widespread metastasis. We observe that the cell-of-origin acts as a powerful constraint even when the founding event is fixed and that somatic profiles of multiple RCCs from the same patient proceed through a range of possible paths across the cohort, highlighting the dominant role of chance in evolution. There are several exceptions with evidence of convergence, including a patient with a co-deletion of VHL and BRK1 in the germline and a bias towards somatic mutation of VHL as the second hit. Furthermore, we identify novel routes to biallelic inactivation of VHL, and a subset of tumours without VHL loss suggesting alternative evolutionary paths that may represent evolutionary ‘deadends’.

Poster Presentations

	Presenter	Title
1	Jingfei Cheng	A human tissue atlas of DNA methylation and hydroxymethylation
2	James Chettle	The RNA binding protein LARP1 drives tumorigenesis by promoting metabolic plasticity and resistance to oxidative stress
3	Zinaida Dedeic	LungVax: A Precision Prevention approach to vaccinate against lung cancer in an at-risk population
4	Holly Eggington	Interrogating the adenoma to carcinoma transformation of human rectal cancer
5	Zoe Grenville	Perturbations in the blood metabolome up to a decade before prostate cancer diagnosis in 4,387 matched case-control sets from the European Prospective Investigation into Cancer and Nutrition
6	Seham Helmi	Programmable DNA-origami based platform for label-free protein profiling
7	Maira Khan	Sex differences in cancer risk in the UK Biobank
8	Joshua Moore	Quantifying cellular microenvironments in multiplex images
9	Eoghan Mulholland	Epithelial Grem1 drives ectopic stem cell niche formation through stromal remodelling and tissue co-evolution in intestinal dysplasia initiation
10	Lauren Murphy	Platelets sequester extracellular DNA, capturing tumour-derived and free fetal DNA

Poster Presentations

	Presenter	Title
11	Defne Saatci	Derivation and validation of a risk prediction model for childhood, teenage and young adult cancers
12	Alaina Shreves	Amount and intensity of daily physical activity and step count measured by accelerometers in relation to incident cancer in the UK Biobank
13	Dimitris Vavoulis	Multimodal cell-free DNA whole-genome analysis combined with TAPS reveals cancer signals in patients presenting with non-specific symptoms

Poster Abstract – 1

A human tissue atlas of DNA methylation and hydroxymethylation

Jingfei Cheng, Masato Inoue, Chunxiao Song

Ludwig Institute for Cancer Research

Cell-free DNA (cfDNA), derived from cell death in various healthy and diseased tissues, holds significant promise for non-invasive cancer diagnosis. Genetic alternations in cfDNA are weakly indicative of tissue-of-origin and challenging to detect in early-stage cancer. In contrast, epigenetic modifications, such as 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) exhibit tissue/cancer-specific patterns, enabling precise estimation of tissue origin. Current tissue atlases cannot differentiate 5mC and 5hmC due to the limitation of bisulphite sequencing technology, and lack cancer signatures essential for distinguishing cancer and other tissue degenerative diseases. To address these limitations, we introduce the first tissue atlas featuring distinct 5mC and 5hmC modifications. Utilising advanced TET-assisted pyridine borane sequencing (TAPSβ) and chemical-assisted pyridine borane sequencing (CAPS) techniques, we conducted deep whole-genome epigenetic sequencing on 13 normal tissue types, 10 tumour types, and 9 blood cell types. Harnessing the rich sequencing data, we identified genomic regions that are uniquely 5mC/5hmC-modified in a tissue/cancer-specific manner. These regions are expected to improve the sensitivity and specificity of cancer detection based on cell-free DNA deconvolution. Our atlas will also serve as a valuable resource for various biological studies on tissue-specific epigenetic regulation.

Poster Abstract – 2

The RNA binding protein LARP1 drives tumorigenesis by promoting metabolic plasticity and resistance to oxidative stress

James Chettle[1], Eleni Adamopoulou, Nicola Ternette, Sarah Blagden[1]

[1]Department of Oncology

It is now known that most epithelial cancers transition from precancerous dysplastic lesions to invasive, detectable cancers over several years. Examples of this progression are in high grade serous ovarian cancer which progresses from serous tubal intraepithelial carcinomas (STICs), and in invasive breast cancer which often progresses from ductal carcinoma in situ (DCIS). This 5–10 year latency provides a window of opportunity for both early detection and intervention. However, as little is known about the molecular behaviour of precancerous lesions, there are no reliable biomarkers and hence they are rarely detected early enough to intervene.

The mRNA-binding protein LARP1 post-transcriptionally regulates thousands of mRNAs encoding metabolic and antioxidant proteins and is elevated in tissues with high levels of oxidative stress. We have discovered that LARP1 is strongly upregulated in precancers such as STICs and DCIS as well as in invasive breast and ovarian cancer. In mouse models of ovarian cancer tumorigenesis, LARP1 upregulation is an early event in STIC development where it colocalises with TP53 mutated cells. Our findings suggest that precancers have high levels of oxidative stress and that staining tissue for LARP1 is useful for detecting nascent precancer lesions as well as providing insights into precancer biology.

Poster Abstract – 3

LungVax: A Precision Prevention approach to vaccinate against lung cancer in an at-risk population

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Lung cancer is the most common cause of cancer-related death, alone accounting for up to 25% of all deaths from cancer. Although one of the most lethal cancers, lung cancer takes 8-10 years to develop and offers many opportunities for early intervention and prevention as the population at risk is easily identifiable. Smoking cessation and regular screening are proving effective in disease mitigation and earlier diagnosis. However, even those with the earliest stage (stage 1) of lung cancer have a high chance of recurrence or developing new primary cancers following surgical resection, estimated at 40% within 2 years. For this target population, the TracerX team (Crick), the CRUK Lung Cancer Centre of Excellence (UCL/Manchester) and the University of Oxford teams are developing a cancer vaccine, called LungVax, using the ChAdOx platform. This is based on finding of shared clonal neoantigens that are present throughout lung tumorigenesis. Here we describe the development of the LungVax vaccine and its future clinical development pathway.

Poster Abstract – 4

Interrogating the adenoma to carcinoma transformation of human rectal cancer

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Wellcome Centre for Human Genetics

While the genetic acquisition of malignancy within colorectal cancer is well studied, assessing non-mutational changes in transition from the pre-cancerous adenoma to malignant carcinoma is often neglected, and is further limited by variation in patient origin.

Through use of a paired human TEM Adenocarcinoma Rectal Adenoma with Carcinoma (TARMAC) dataset, where adenoma and carcinoma are present within a single patient resection, parallel interrogation of 'on-slide' imaging alongside gene expression data was carried out. The 'on-slide' phenotype was inclusive of immune and stromal cellular populations quantified via multiplex imaging, whereas gene expression analysis was performed via interrogation of paired bulk RNA-seq data.

Neoplastic status acted as a stronger differentiator than patient origin, and supervised analysis via GSEA and GSVA interrogation unveiled alterations in key signalling pathways as part of this transition.

Most notably, WGCNA (Weighted Gene Co-expression Network Analysis) revealed the presence of a novel adenoma enriched module with B-cell associated ontology, which was lost in carcinoma. This enrichment was further reflected in cell-type deconvolution methods. Further work aims to characterise the key B-cell associated populations via multiplex imaging, and to assess the potential of B-cell disruption as a key stage in the transition from pre-cancer to carcinoma.

Poster Abstract – 5

Perturbations in the blood metabolome up to a decade before prostate cancer diagnosis in 4,387 matched case-control sets from the European Prospective Investigation into Cancer and Nutrition (EPIC)

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Measuring pre-diagnostic blood metabolites may help identify novel risk factors for prostate cancer. Using data from 4,387 matched case-control pairs from the European Prospective Investigation into Cancer and Nutrition (EPIC), we investigated the associations of 148 individual metabolites and three previously defined metabolite patterns with prostate cancer risk. Metabolites were measured by mass spectrometry (AbsoluteIDQ p180 Kit, Biocrates Life Sciences AG). Multivariable-adjusted conditional logistic regression was used to estimate the odds ratio per standard deviation increase in metabolite concentration and metabolite patterns (OR1SD) for prostate cancer overall, and for advanced (T3–4 and/or N1–3 and/or M1, or coded as advanced), high grade (Gleason score 8+ or coded as undifferentiated tumors), aggressive (advanced, and/or high grade, and/or preoperative PSA > 20ng/ml, and/or death from prostate cancer as the underlying cause of death during follow-up). We corrected for multiple-testing using the Benjamini-Hochberg method. (*continued next page)*

Poster Abstract – 5 *cont*

Zoe S. Grenville *et al*

There were no significant associations between metabolites or metabolite patterns and overall, aggressive, or high grade prostate cancer. Six phosphatidylcholines (PCs) were inversely associated with advanced prostate cancer diagnosed within 10 years of blood collection. Metabolite patterns 1 (64 PCs and three hydroxysphingomyelins) and 2 (two acylcarnitines, glutamate, ornithine, and taurine) were also inversely associated with advanced prostate cancer; when stratified by follow-up time, these associations were observed for diagnoses within 10 years of recruitment (OR1SD 0.80, 95%CI 0.66-0.96 and 0.76, 0.59-0.97, respectively) but not after longer follow-up (0.95, 0.82-1.10 and 0.85, 0.67-1.06). Pattern 3 (8 lyso PCs) was associated with prostate cancer death (0.82, 0.68-0.98). Our results suggest that the plasma metabolite profile changes in response to prostate cancer up to a decade before detection of advanced stage disease.

Poster Abstract – 6

Programmable DNA-origami based platform for label-free protein profiling

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Early cancer detection critically depends on the precise identification of biomarkers, an endeavour challenged by the intrinsic heterogeneity of tumours, the masking effects of biological noise, and the imperative to concurrently detect multiple biomarkers. To address these diagnostic challenges, we have developed a novel DNA-origami-based platform that utilizes mass photometry (MP) for the label-free detection of protein biomarkers. This platform comprises an assortment of DNA origami nanostructures, each selectively functionalised with a specific antibody through DNA-sequence hybridization to target key cancer-associated proteins, including CA-125, HER2, AFP, EGFR, and Rb. These nanostructures are diffusing on a supported lipid bilayer, a setup that allows for their individual masses and diffusion properties to be quantified on a single-molecule level, generating a unidirectional mapping between the mass and the diffusion of the DNA origami nanostructures and their biomarker binding specificity. We demonstrate the simultaneous detection of biomarkers within complex biological matrices, such as cellular and plasma matrices in a densely multiplexed format, while negating concerns of cross-reactivity. The programmable nature of our DNA-origami platform lends itself to broad adaptability, facilitating the incorporation of various biomarkers, enhancing the platform's utility in personalised diagnostic applications, and holding a promise for refining early cancer detection.

Poster Abstract – 7

Sex differences in cancer risk in the UK Biobank

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There are sex differences in the incidence of cancers at shared anatomic sites, and previous research has hypothesised that the generally greater risk in men may be explained by anthropometric, lifestyle, and environmental risk factors. We investigated the associations between sex and the risk of cancer endpoints by systematically adjusting for established risk factors that may explain disparities and provide insights into cancer aetiology. Prospective analyses were performed with UK Biobank data (470,771 participants) to examine associations between biological sex and 15 cancers (and 13 subtypes) using multivariable-adjusted Cox proportional hazards models. With 10.5 (SD 2.2) years of follow-up and 50,065 cancers diagnosed (51.8% in women), we observed that while some differences in cancer risk between the sexes were attenuated in the cancer site-specific multivariable-adjusted models, men were at greater risk of cancers at more sites than women. Eight cancer sites had a higher risk in men compared to women; these were cancers of the oesophagus (adenocarcinoma; hazard ratio [HR] 5.45; 95% CI, 4.17-7.12), gastric cardia (HR 3.65; 95% CI, 2.48-5.38), bladder (HR 3.47; 95% CI, 2.85-4.24), oral cavity (HR 2.06; 95% CI, 1.69-2.51), liver (HR 1.91; 95% CI, 1.48-2.47), kidney (HR 1.77; 95% CI, 1.51-2.09), rectum (HR 1.7; 95% CI, 1.47-1.96), and leukaemia (HR 1.43; 95% CI,1.21-1.69). Five cancers had a higher risk in women compared to men; these were cancers of the thyroid (HR 0.36; 95% CI, 0.26-0.49), anus (HR 0.41; 95% CI, 0.26-0.64), gallbladder (HR 0.56; 95% CI 0.31-0.99), and lung adenocarcinoma (HR 0.72; 95% CI 0.62-0.84). Future studies of unexplained sex differences may provide insights into cancer aetiology.

Poster Abstract – 8

Quantifying cellular microenvironments in multiplex images

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Advances in multiplex imaging technologies have provided unprecedented access to the rich spatial information contained in biological samples, enabling a deeper exploration of the intricate cellular interactions in development and disease. Consequently, there is a growing demand for quantitative techniques capable of interpreting these high-dimensional data, specifically in the context of detecting cellular patterns across multiple spatial resolutions. While machine learning methods have displayed potential in discerning variations in tissue morphology and composition using multiplex images, challenges in interpreting these findings and scaling image resolution have limited their capacity to investigate the underlying biological mechanisms that govern cancer progression and treatment responses.

Subsequently, we present novel mathematical metrics for quantifying cellular microenvironments, specifically designed to simultaneously measure the cellular composition and spatial distribution of local regions of cell types of interest. Using spatial networks, we respect the underlying tissue morphology, whilst exploiting the computational efficiency of network kernels which describe the interactivity of all cellular subpopulations present in a microenvironment. Critically, these methods are not limited by the number of markers or tissue size, whilst providing biologically interpretable microenvironment descriptors. Fundamentally, we show how these metrics can be used in combination with other established spatial statistics to identify 'spatial biomarkers' for cancer detection and treatment.

Poster Abstract – 9

Epithelial Grem1 drives ectopic stem cell niche formation through stromal remodelling and tissue co-evolution in intestinal dysplasia initiation

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Wellcome Centre for Human Genetics

We investigate how the protein Grem1 influences the development of abnormal intestinal growths known as polyps with ectopic crypt foci (ECF). These are associated with the disease Hereditary Mixed Polyposis syndrome (HMPS) and have the potential to turn cancerous. Our focus is on understanding how Grem1 affects the cells lining the intestine (epithelial cells) and the surrounding tissue. We conducted experiments using mice with aberrant Grem1 expression in their intestinal lining. Excess Grem1 disrupted normal intestinal signalling, leading to certain cells exiting the stem cell area and forming ECF. Furthermore, Grem1 brought about alterations in the supportive tissue of the intestine (stroma), promoting the expansion of specific fibroblast cells that produce a protein called Wnt2b. By inhibiting Wnt signalling, a reduction in polyps was observed. Furthermore, testing a new anti-Grem1 antibody showed potential to prevent the early development of these growths and even shrink them in older mice, thereby extending their lifespan without causing any adverse effects. To summarise, Grem1 disrupts the balance in the intestine, leading to polyp formation. Blocking Wnt signalling or using an anti-Grem1 antibody holds promise as potential treatments for these conditions and may offer new approaches to treating colorectal cancer.

Poster Abstract – 10

Platelets sequester extracellular DNA, capturing tumour-derived and free fetal DNA

Lauren Murphy[1], Jeanne Inchauspe[1], Nikolaos Sousos[1], Natalie Jooss[1], Hayley L Belnoue-Davis, Pamela Holland, Rong Li, Giampiero Valenzano, Fenella Roseman, Sujata Biswas, Sally-Ann Clark, Jennifer O'Sullivan, Michael Rimmer, Abdullah O. Khan, Christina Simoglou Karali, Eric O'Neill, Nadia Nasreddin, Ian S. Hitchcock, Milka Koupenova, Manu Vatish, Paul Rees, Simon Leedham, Adam J. Mead[1], Benjamin Schuster-Boeckler, Christopher D. Gregory, Bethan Psaila[1]

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Platelets are small, multi-functional cells that lack a nucleus but contain RNA and translational machinery for protein synthesis. Platelet RNA derives from megakaryocytes, but they also sense and sequester endogenous and pathogen-derived nucleic acids during circulation. Nucleated cells release DNA after cell death or aberrant mitosis, resulting in 'cell-free' DNA in plasma (cfDNA). Given their role in sensing nucleic acids, we hypothesised that platelets may clear cfDNA from plasma, and that clinically-relevant insights may be derived from the analysis of DNA fragments contained in platelets.

Using live cell imaging and flow cytometry, significant DNA content (Draq5+) was detected in ~8% of platelets from healthy donors. FISH and ddPCR of platelets from pregnant women carrying male foetuses detected Y-chromosome fragments (n=10), confirming that platelet DNA is not solely derived from parent megakaryocytes but also sequestered during circulation. Platelet uptake in vitro was rapid, visible using live cell microscopy within 2 minutes of co-culture with cancer cells labelled with a probe that irreversibly intercalates to nuclear DNA. ddPCR of platelet DNA following co-culture of healthy donor platelets with cancer cell lines detected a range of canonical cancer driver mutations.

(*continued next page)*

Poster Abstract – 10 *cont*

Lauren Murphy *et al*

Whole genome sequencing revealed that platelets contain a repertoire of DNA fragments that map across the nuclear genome, similar to cfDNA. To explore utility in cancer screening, we analysed patients with high-risk, pre-malignant colonic lesions (serrated polyps). Remarkably, the driver mutation BRAFV600E was detected in platelets in 16% patients despite the small-size lesions (5/30).

Overall this study establishes a role for platelets in sequestration of cfDNA, an aspect of platelet biology that has not previously been highlighted but is of substantial clinical relevance.

Poster Abstract – 11

Derivation and validation of a risk prediction model for childhood, teenage and young adult cancers

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Background: Childhood, teenage and young adult (CTYA) cancers are rare and diverse, making timely diagnosis challenging. Use of cancer risk stratification tools in primary care has been shown to aid earlier detection, yet, they have not been explored for CTYA cancers.

Aims: To develop and validate a risk prediction model to identify CTYA at increased risk of cancer in primary care.

Methods: Using QResearch Database, we generated a cohort of CTYA (0 -24 years) between 1st January 1998-31st December 2018. The primary outcome was a blood cancer diagnosis (leukaemia or lymphoma) at 6 months following first symptom onset. The model was derived using logistic regression. An internal-external cross-validation framework based on geographical region was used to assess model performance and transportability.

Results: Out of 5,024, 892 CTYA, there were 306 blood cancer diagnoses. The derived model included symptoms, blood test results and demographic factors (age and sex). The model had good discriminative ability (Harrell's C-statistic 0.85 [95%CI 0.82-0.88]) and was well-calibrated (slope 0.99 [95%CI 0.95-1.03]).

Conclusions: This is the first model developed to estimate cancer risk in CTYA presenting to primary care and shows good performance. Further external validation is required prior to its integration as a risk stratification tool across primary care settings.

Poster Abstract – 12

Amount and intensity of daily physical activity and step count measured by accelerometers in relation to incident cancer in the UK Biobank

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We investigated the associations between accelerometer-measured activity, step counts, and incident cancer using data from UK Biobank participants.

Machine learning models derived total activity and step counts. The primary outcome was a composite of 13 cancers previously associated with low activity. Hazard ratios and 95% confidence intervals were calculated using Cox proportional hazard regression models and compositional data analyses, with age as the timescale and adjustments for sex, ethnicity, smoking, alcohol, education, deprivation, and reproductive factors.

Among 86,556 participants, 2,669 physical-activity-related cancers occurred. Higher total activity was associated with a lower risk of the 13 cancers (HR1SD=0.85, [95%CI 0.81-0.89]). For an average individual, reallocating one hour per day from sedentary behaviour to moderate-to-vigorous activity was associated with a lower risk (HR=0.93, [0.90-0.96]), as was reallocating one hour to light-intensity activity (HR=0.95, [0.93-0.97]). Compared to 5,000 daily steps, individuals taking 9,000 steps had a lower risk (HR=0.82, [0.74-0.90]). There was no association with peak 30-minute cadence after adjusting for total steps.

Higher total activity and substituting sedentary time for activity time were associated with a lower risk of certain cancers. For less active adults, increasing step counts by 4,000 may be a practical intervention for lowering the risk of some cancers.

Poster Abstract – 13

Multimodal cell-free DNA whole-genome analysis combined with TAPS reveals cancer signals in patients presenting with non-specific symptoms

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Liquid biopsies promise to revolutionise Clinical Oncology by simplifying tumour sampling for early cancer detection and disease monitoring. We developed an integrated bioinformatics pipeline for ctDNA detection based on whole genome TET-Assisted Pyridine Borane Sequencing (TAPS) of plasma samples sequenced at 80x or higher and applied it in a cohort of patients presenting to the UK National Health Service (NHS) primary care pathway with non-specific symptoms, who either did not have cancer or who were subsequently diagnosed with cancer and referred to surgery with curative intent. The proposed methodology combines copy number aberrations and single nucleotide variants with methylation calls from TAPS-treated plasma from 61 patients with Stage 1-4 cancer, while plasma from 30 age-matched non-cancer controls were used for data denoising and for establishing the minimum level of detection. Matched tumour samples were used for validation only, not for guiding the analysis, imitating an early detection scenario. At a fixed specificity of 100%, sensitivity was 85.2%. Experiments on synthetic data suggest excellent discriminatory capacity (AUC > 80%) at ctDNA fractions 0.7% or higher. Furthermore, we demonstrate successful tracking of tumour burden post-treatment and ctDNA shedding in precancerous adenomas in patients with colorectal cancer without matched biopsies.