CANCER RESEARCH UK | Oxford Centre

OXFORD CANCER

UNIVERSITY OF OXFORD

Annual Symposium

Wednesday 13th March 2024

Mathematical Institute, Woodstock Road, Oxford

Wednesday 13th March Programme

	8.30	**Registration Open**	
Session 1: Multidisciplinary Research Chair: Mark Middleton	**9.15**	**Symposium Welcome, Tim Elliott**	
	9.30	Is diet-induced weight loss feasible before colorectal cancer surgery and does it affect tumour characteristics?	Paired Talk **Simon Buczacki & Dimitrios Koutoukidis** NDM & NDPH
	9.55	Quantitative spatial characterisation of intestinal adenoma-carcinoma progression via multiplex imaging	Paired Talk **Eoghan Mulholland & Joshua Bull** CHG/NDM & MATHS
	10.20	Mechanistic modelling to improve CD8+ epitope immunogenicity prediction	Paired Talk **Tim Elliott & Miles Weatherseed** NDM & NDORMS
	10.45	Living human tumours outside the body: a normothermic perfusion model to accelerate the clinical translation of novel cancer therapeutics	Paired Talk **Kerry Fisher & Constantin Coussios** ONCOLOGY & IBME
	11.10	**Coffee Break and Poster Viewing (odd numbers)**	
Session 2: Paradigm Changing Biology Chair: Tim Elliott	**11.50**	IFNγ-dependent remodelling of the myeloid immune landscape underlies control of IFNγ-insensitive tumours	**Audrey Gérard** THE KENNEDY INSTITUTE
	12.02	CD4 T follicular helper cells, B cells, and tertiary lymphoid structures in cancer	**Isabela Pedroza-Pacheco** CAMS/NDM
	12.14	The elepHant in the room	**Pawel Swietach** DPAG
	12.26	APOBEC3B regulates R-loops and promotes transcription-associated mutagenesis in cancer	**Natalia Gromak** DUNN SCHOOL
	12.38	A computational pipeline for spatial mechano-transcriptomics	**Adrien Hallou** THE KENNEDY INSTITUTE
	12.50	Dicer dependent tRNA-derived small RNAs promote nascent RNA silencing	**Monika Gullerova** DUNN SCHOOL
	13.05	**Lunch, Poster Viewing (even numbers), Information Stalls**	
Session 3: PPI Chair: Catriona Gilmour Hamilton	**14.05**	Patient & Public Involvement: A Patient Perspective	**David Church & Tom Bartlett** NDM & PPI Representative
Session 4: Spatial Biology Chair: Helen Byrne	**14.25**	Quantitatively Modelling Short Peptide Vaccinations in Immuno-oncology	**Eamonn Gaffney** MATHS INSTITUTE
	14.50	Learning to Adapt: Rational Personalization of Cancer Treatment Schedules Using Deep Reinforcement Learning	**Kit Gallagher** MATHS INSTITUTE
	15.15	CD8+ T cells drive selection and segregation in the tumor microenvironment	**Anagha Krishnan** NDORMS
	15.40	Identifying phenotype-genotype-function coupling in live-cell imaging using SPOT	**Adam Norton-Steele** LUDWIG INSTITUTE
	16.05	**Coffee Break**	
Session 5: Keynote & Centre Announcements Chair: Tim Elliott	**16.35**	Exploring biological mysteries with condensate models	**Richard Young** Whitehead Institute for Biomedical Research Massachusetts **Keynote Speaker**
	17.15	Awards and Closing Remarks	**Mark Middleton** Co Director - Oxford Cancer
	17.25	**Networking Reception**	

Welcome from the Oxford Cancer Directors

Tim Elliott

Mark Middleton

It is our great pleasure to welcome you to the 11th Annual CRUK Oxford Centre Symposium, hosted by Oxford Cancer.
We are delighted to again present a programme of talks that demonstrate the cutting-edge research that is happening across Oxford. As well as showcasing some of your multidisciplinary research projects, we are highlighting those led by fundamental molecular biologists and spatial statisticians. We are particularly excited to welcome Professor Richard Young as our keynote speaker who will be discussing some of his seminal work in transcriptional condensates and epigenetic regulation, and how this can provide a better understanding of cancer.

It is with immense pride that we reflect on the steps we've taken in 2023. This year truly stands as a testament to Oxford's cancer researchers' collective dedication to advancing cancer research, detection, prevention and treatment.

Oxford received CRUK funding to become the UK's first clinical trials unit specialising in precision prevention cancer studies. This application, led by Professor Sarah Blagden as director of Oxford's Cancer Trials Office OCTO, builds on nearly 5 years of coordinating and nurturing Oxford's early cancer biology and early cancer detection community by Professor Xin Lu and the OxCODE network.

Oxford's Cancer Research UK Clinical Academic Training Programme was also renewed this year with over £10M to support 40 clinical trainee and medical undergraduate DPhil students over the next 5 years. This programme means that there are more opportunities than ever for Oxford cancer researchers to host students working on collaborative fundamental, translational, or clinical cancer research.

Professor Blagden also led Oxford's Experimental Cancer Medicine Centre (ECMC) application, which was awarded over £3 million from CRUK and The National Institute for Health and Care Research. The investment will enable Oxford to continue to lower the barriers to investigator-led early-phase clinical studies which provide a route to clinic for Oxford science.

Finally, we officially launched the Oxford Cancer Immuno-Oncology Network (OCION). The network is run by Professor Tim Elliott and aims to improve our understanding of the long-term benefits of immunotherapy by bringing together scientists from across the city. Through this network, we have already been able to bring a new generation of immunologists into the cancer field via a series of externally funded projects and programmes.

Without your continued support, advancements such as these could not happen; thank you.

About Oxford Cancer and the CRUK Oxford Centre

Oxford Cancer

Oxford Cancer is a city-wide network and partnership between Oxford University and Oxford University Hospitals NHS Trust, based on the University's Translational Biomedical Research Campus.

It currently comprises over 10000 members from around 90 different Departments, Units, and Institutes of the University, as well as the NHS Trust, which invests over £55m each year into translational cancer research.

Oxford Cancer is an inclusive network of organisations in Oxford for whom cancer research is a priority focus. We support and connect people working across a range of disciplines and aim to facilitate research collaboration on a local, national and international scale to speed up translation from scientific discovery to treatments in patients.

Any faculty member, student or employee of the University of Oxford interested in cancer research, and any clinician, nurse, or employee of the Oxford University Hospitals NHS Trust interested in cancer research can join our membership. To become a member, visit our website.

The CRUK Oxford Centre

The CRUK Oxford Centre harnesses Oxford's world-leading cancer research with the core aim of facilitating collaboration to ensure rapid translation from scientific discovery to treatments for patients. The Centre is run as part of the Oxford Cancer network, with a focus on funding Cancer Research UK strategic priorities.

Support and Services

Oxford Cancer is here to support your research by coordinating expertise, lowering barriers and helping you to access key research facilities.

We have access to a range of services that can help your research to reach its translational potential. These are available to any University of Oxford or Oxford University Hospitals staff member belonging our Oxford Cancer community. More details on the tools, teams and resources available can be found on our website.

Funding support

Translational histopathology lab

Clinical positioning network

Patient sample access

Clinical trial support

PPI

Molecular diagnostics centre

Comms & event support

Translational data platform

Get in touch

We are always interested to hear from our members!

Get in touch...

E: cancer@medsci.ox.ac.uk

T: +44 (0)1865 617043

: @OxfordCancer

W: cancer.ox.ac.uk

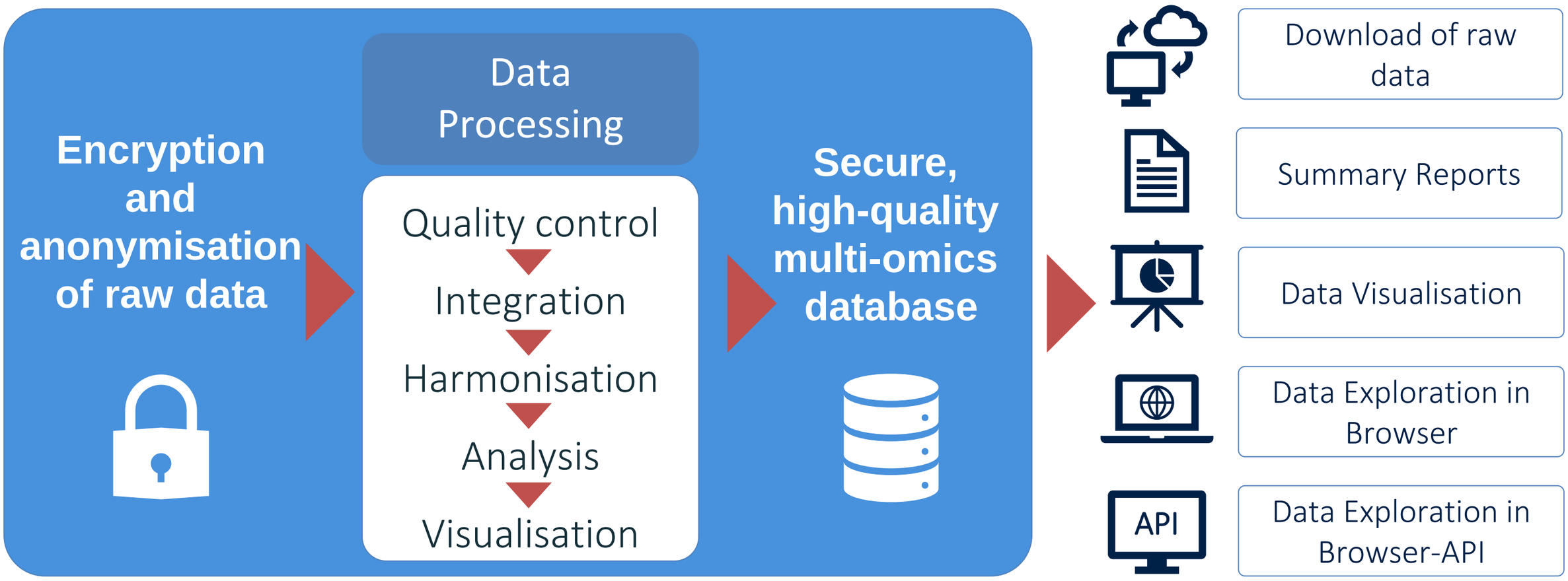
Translational Data Platform

“We enable Oxford researchers to fully capitalise on clinical, imaging and molecular data generated during clinical studies.”

We combine data from clinical trials and programs into a single, secure, user-friendly, multiomic data platform. Data includes:

- Single cell and bulk RNASeq expression data
- NGS mutations
- SNPs
- Copy number
- Methylation
- Proteomics
- IHC scores and images
- H&E images
- MRI and other scans
- Clinical data
- Outputs of machine learning image analysis algorithm models

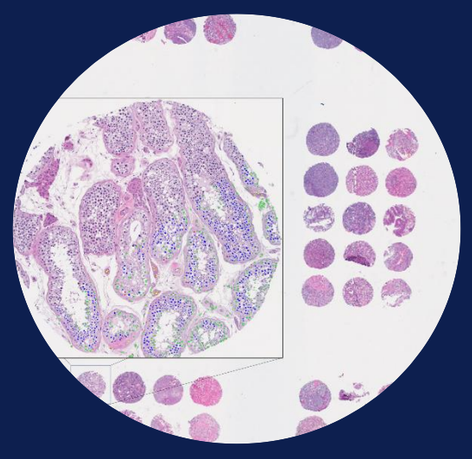
Translational Data Platform

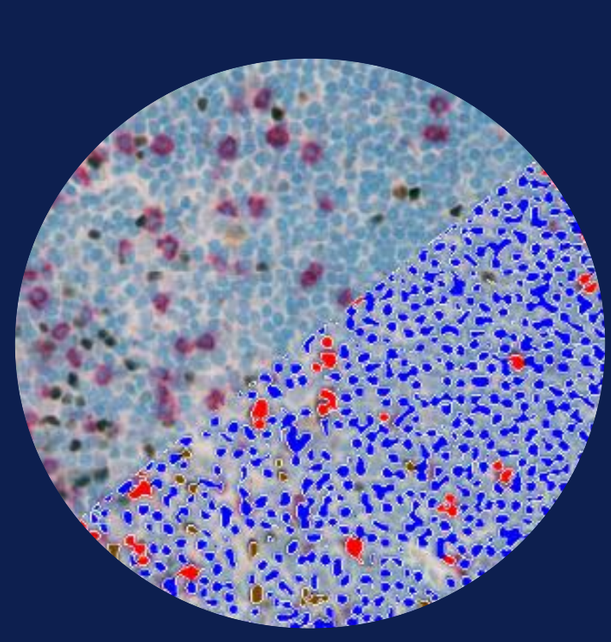


To request help with your clinical data management please email:
andrew.blake@oncology.ox.ac.uk or **cancer@medsci.ox.ac.uk**

Translational Histopathology Laboratory

thlenquiries@oncology.ox.ac.uk





Services

Tissue processing, embedding and sectioning

Tinctorial histochemical staining (H&E).

Multiplex immunofluorescence with panel optimisation and imaging

TMAs

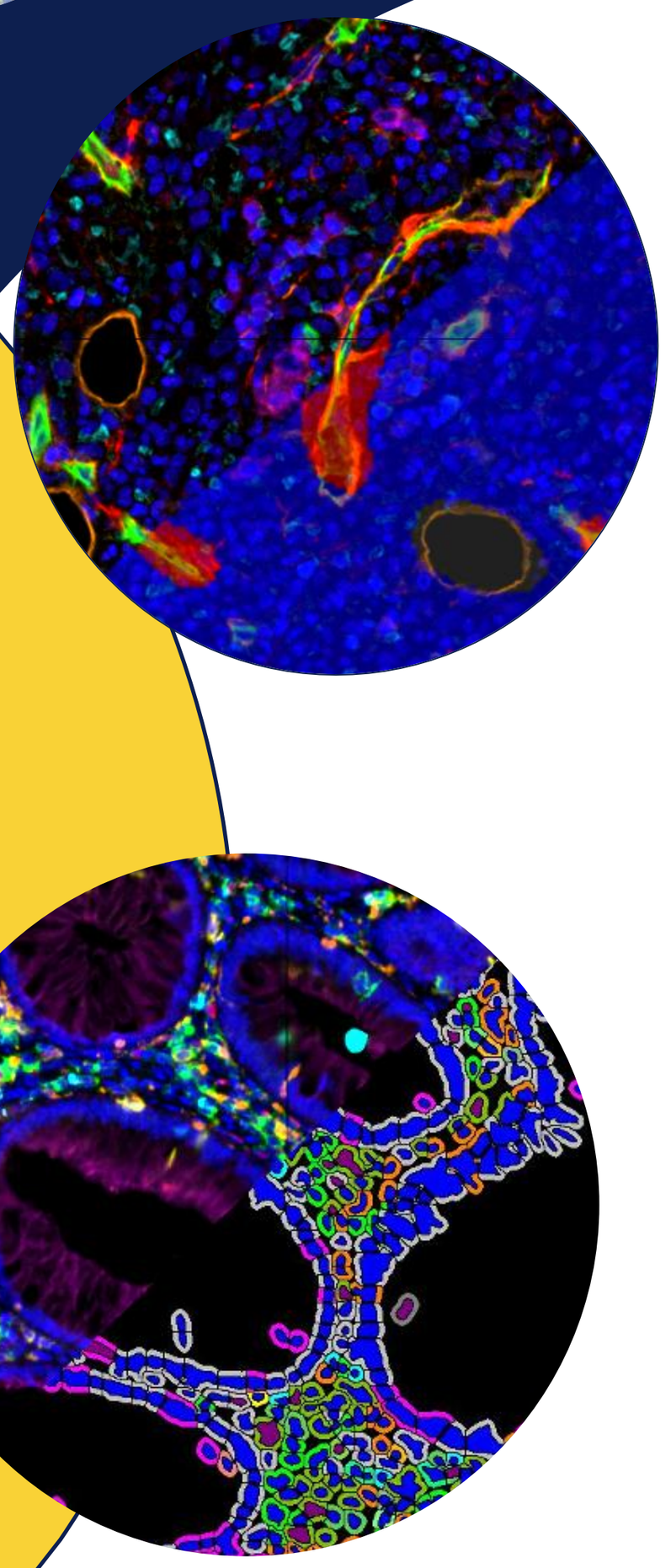
- **Oesophageal Cancer**
- **Ovarian Cancer**

Bespoke TMA creation

Indica's HALO image analysis software

Pathologist-led, computer-aided, analysis

Digital slide scanning



UNIVERSITY OF OXFORD

OXFORD CANCER

ecmc

CANCER RESEARCH UK | Oxford Centre

Halo® – Image Analysis Platform

- HALO® can separate **up to five stains simultaneously** within any cellular compartment.
- HALO® is capable of **automated tissue classification** thanks to machine learning.
- The software can perform **infiltration analysis, nearest neighbour/proximity analysis and density hotspot** analysis.
- HALO® allows automated batch analysis of whole slides and TMAs.

We offer software training, pathology and pathology-assisted analysis and unassisted use of HALO®.
To Join our **Pathology Clinics** please email thlenquiries@oncology.ox.ac.uk.

Bespoke Tissue Micro Arrays

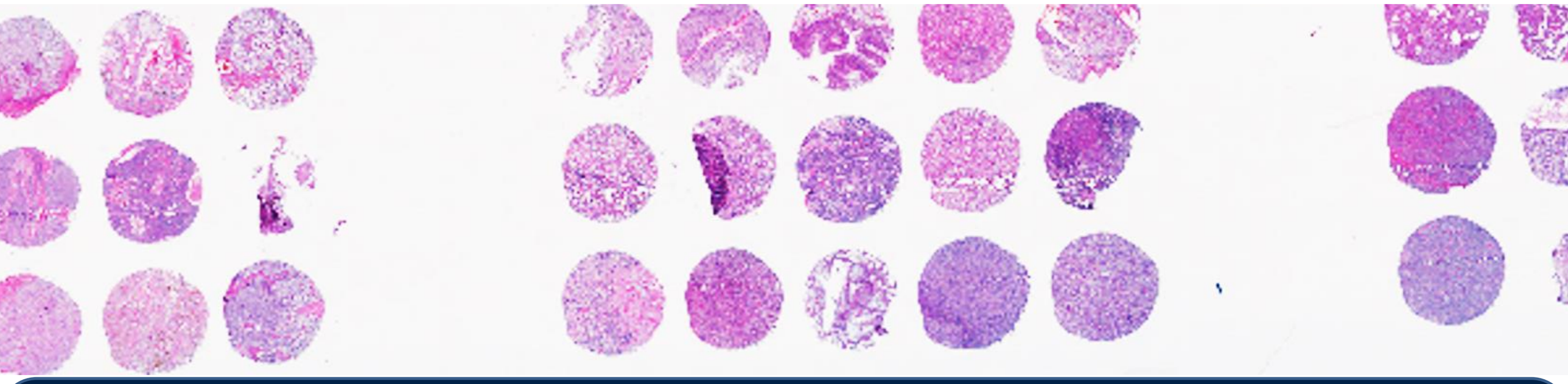
Tissue Micro Arrays (TMAs) are multi-tumour tissue blocks that allow standardised staining of up to **120 patient samples**.

Using TMAs reduces IHC cost and staining variations

The THL has access to diagnostic archive samples and **pathology expertise** to choose the most interesting regions to study

We offer:
- **pan-tumour and healthy tissue** TMAs for antibody optimisation
- **Oesophageal cancer** TMA
- **Ovarian Cancer** TMA (*in preparation*)

And **bespoke TMA** creation



We offer bespoke TMA creation for any type of cancer as well as access to existing ones. For enquires please email thlenquiries@oncology.ox.ac.uk.

Patient and Public Involvement

Successful cancer research can only happen in partnership with those affected by cancer: people who live with cancer; people who have recovered from it; friends and family members; and those who are at risk of getting cancer in future. At Oxford Cancer, we strive to ensure that our work learns from and responds to the experience of people like these.

What is PPI?

Patient and Public Involvement (PPI) refers to the many ways in which people with lived experience can work in partnership with cancer researchers as colleagues, advisors and collaborators. It is about ensuring that the culture of cancer research seeks out, cultivates and welcomes multiple voices to challenge and co-design.

Examples of PPI in action:

What matters most to the people who might benefit from your work?

Do you know if your research is important to patients? Do you know what questions patients want answers to? We work with groups of patients to identify priorities and shape our Centres of Excellence in response.

Building capacity in cancer researchers of the future.

We ensure that students on our DPhil programme have the opportunity to meet and learn from our PPI contributors from the outset of their studies.

Ensuring diversity in PPI contributors.

We are working with community groups locally and nationally to build greater diversity in our panel of consultees. This ensures that our work includes perspectives from the many different communities we serve.

Building relationships between the lab and "real world" experience.

Our 'buddy' programme allows people with cancer to see the science in action, and allows people working in labs to fully understand why their work really matters.

To find out more - visit our stand at the symposium, or contact us at: oxfordcancerppi@medsci.ox.ac.uk.

OCION

Oxford Cancer Immuno-Oncology Network

The Oxford Cancer Immuno-Oncology Network

OCION was established in June 2023 to bring together scientists from different disciplines, cutting-edge technologies and patient samples to progress our immuno-oncology understanding to the next level.

By applying Oxford's leading expertise in fundamental immunology, we hope to enable more patients, with a wide range of cancer types, to benefit safely from tailored immunotherapy use. The network is directed by Professor Tim Elliott and managed day-to-day by Beth Mann (elizabeth.mann@medsci.ox.ac.uk).

Alongside our annual symposium (pictured), our offering includes a seminar series with networking drinks, pump prime funding, a monthly newsletter, an immuno-oncology directory and support with grant writing, sample/data access and helping to foster industry relationships.

The 2024 OCION Annual Symposium will be hosted at the Mathematics Institute in Oxford on 7th June 2024 when we will launch our next round of pump prime applications. The day will provide ample opportunities for people from different disciplines to come together to share their research.

All University of Oxford or OUHFT researchers with an interest in immunology are welcome to join. If you wish become a member and to be added the mailing list to hear about future events and funding opportunities, please email: **cancer@medsci.ox.ac.uk**

OxCODE

The Oxford Centre for Early Cancer Detection

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, travel to key PPED conferences and grant writing support.

We will celebrate five years of OxCODE at our next symposium in December 2024.

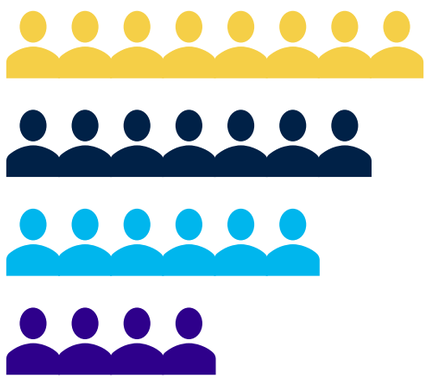
All University of Oxford or OUHFT researchers with an interest in PPED are welcome to join. If you wish to be added to the mailing list to hear about future events and funding opportunities, or have an idea for a new PPED research project, please email the OxCODE Scientific Coordinator (francoise.howe@ludwig.ox.ac.uk).

DPhil in Cancer Science

Launched in 2020, the DPhil in Cancer Science Programme provides research-based doctoral training for cancer researchers from clinical, biological, engineering, mathematics, and statistics backgrounds.

STUDENTS

The CRUK Oxford Centre funds around 12 full-time studentships each year, and currently has 43 on-course students spread across 17 Departments. We are looking forward to welcoming an additional 15 students in October 2024. Our current cohorts consist of:



31% Clinical trainees

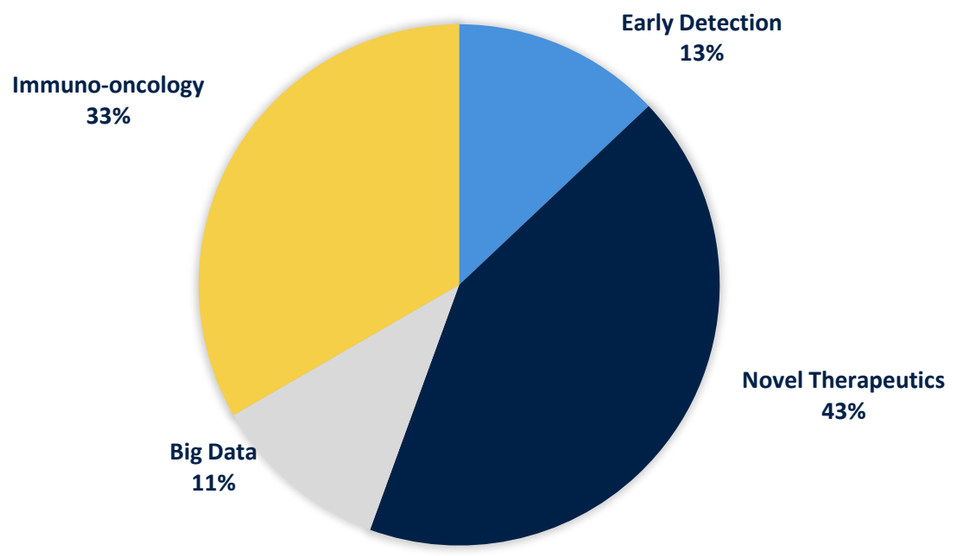
29% Medical Undergraduates

25% Fundamental scientists (biological/chemical science)

15% Fundamental scientists (engineering/mathematics/stats)

The programme attracts students on an international scale, with over 24 different nationalities currently enrolled.

Our students receive a world-leading research training experience that incorporates advanced level seminars; mentorship; and a subject-specific training programme tailored to individual research needs.



Main theme of current student projects

PROJECT THEMES

The programme is distinctive in offering integrated training across the following themes: Immuno-Oncology; Cancer Big Data; Novel Therapeutics; Early Cancer Detection.

It builds on Oxford's outstanding research record in these areas, spanning both the University and Hospital Trust.

NEW FOR 2025

Fundamental science students successfully enrolled onto our 2025 cohort will take on two 6-month rotations within their first year, before deciding on a final 3-year project for the remainder of their DPhil. If you are interested in submitting a DPhil project for 2025, we will be holding an information session in mid-April 2024, date to be announced in the Oxford Cancer Newsletter.

To find out more about the DPhil in Cancer Science programme, view our current student profiles, or join the mailing list, email cancer@medsci.ox.ac.uk or scan the QR code.

CRUK Oxford Centre Development Fund

Development Fund

Scheme Overview

The Development Fund is a scheme that was initiated at the very inception of the Centre back in 2010.

It aims to provide short-term, pump-priming funds to support innovative, high-risk, proof-of-concept cancer research.
Priority is given to projects with a clear translational trajectory, to early career researchers, for new collaborations between investigators, and those that map onto both CRUK Oxford Centre's & Oxford Cancer's Strategic Framework.

There are two types of awards, small - up to £7,500 and main - up to £15,000. Awards are for 12 months and usually start on 01st April and on 31st March.

Three scientific reviewers are assigned to each project and asked to score between 1-5, this is based on the scientific strengths and weaknesses of the application. Each application is asked to submit a 300-word lay summary, which is then reviewed by two Patient and Public Involvement (PPI) members and scored between 1-4 based on its context, patient impact and relevance along with presentation and content (readability).

Development Fund recipients from the past year are highlighted in the next few pages. We look forward to being able to share the successes of some of these projects at the Centre symposium in 2025.

CRUK Oxford Centre Development Fund

January 2023 Awards

Simeio Go & Eric O'Neil
Department of Oncology - £14,960.00
Title: Infiltrating NK cells as immunoregulators in pancreatic cancer?

Oni Chowdhury
Weatherall Institute of Molecular Medicine - £15,000.00
Title: Applying single cell genomics to clinical diagnostics - detection of TP53 mutant clones and collaborating genetic aberrations in myeloid neoplasms

Gemma Hancock
NDWRH and Wellcome Trust - £7,450.00
Title: Deep phenotyping of CD4+ T-cells in high-risk human papillomavirus (hrHPV) infection

Luis Alberto Baena Lopes
Sir William Dunn School of Pathology - £14,997.98
Title: Paired identification of gene targets and pharmacological drugs with anti-oncogenic potential

Ejung Moon
Department of Oncology – £7,500.00
Title: Characterisation of the role of MAFF in bone metastatic breast cancer

Matthew Bottomley
CAMS Oxford Institute, Nuffield Department of Medicine – £13,000.00
Title: Single-cell spatial transcriptomic profiling to delineate changes in tumour-associated macrophage behaviour in cutaneous squamous cell carcinoma with immunosuppression

Grigore-Aristide Gafencu
Dept of Oncology, Oxford Molecular Diagnostic Centre - £13,148.00
Title: Comprehensive multi-modal assessment of peripheral bloodfrom healthy elderly males: a key component in the interpretation of the data from the pilot collaboration between OXPLORED and CARTOGRAPHY

Nikolaos Kanellakis
China Oxford Institute, NDM - £7,500.00
Title: Phenotype and characterisation of the immunosuppressive mechanisms of malignant pleural effusion

Adam Cribbs & Jianfeng Sun
NDORMS, Botnar Research Centre - £7,500.00
Title: Single-cell spliceosome map establishment of immune cells

Jane McKeating
Nuffield Department of Medicine - £14,768.00
Title: Targeting the acidic microenvironment in hepatocellular carcinoma to overcome resistance to immunotherapy

Carol Leung
Ludwig Cancer Research - £15,000.00
Title: Developing T-cell engager bispecific antibodies targeting the Epstein-Barr virus latent membrane proteins

Chung Ying Chan
Department of Oncology - £15,000.00
Title: Stratifying treatment for MYC amplified prostate cancer

Eleni Adamopoulou & Isabela Pedroza-Pacheco
NDM Immuno-Oncology - £14,733.55
Title: Immune-profiling of malignant ascites from ovarian cancer patients as a route to sampling the tumour microenvironment

Margarida Rei
Ludwig Cancer Research - £7,500.00
Title: Discovery of T cell antigen targets in glioblastoma

Valentine Macaulay
Department of Oncology/ Nuffield Department of Surgical Sciences - £7,400.00
Title: Investigating immunosuppressive effects of IGF-1 as a growth factor for regulatory T-cells in prostate cancer

George Adigbli
Nuffield Dept of Surgical Sciences – £15,000.00
Title: Early Risk Stratification by Lymph Liquid Biopsy In Melanoma

Adam Cribbs & Jianfeng Sun
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Title: Investigating immunosuppressive effects of IGF-1 as a growth factor for regulatory T-cells in prostate cancer

George Adigbli
Nuffield Dept of Surgical Sciences – £15,000.00
Title: Early Risk Stratification by Lymph Liquid

CRUK Oxford Centre Development Fund

January 2022 Awards

Clive Wilson

Department of Physiology, Anatomy & Genetics

Title: Functional analysis of the sumoylation-dependent miRNA transcriptome in pro-tumorigenic Rab11a-exosomes.

Alex-Gordon-Weeks

Nuffield Department of Surgical Sciences

Title: Targeting LOXL2 Activity to Alter Extracellular Matrix Structure and Promote Immune Checkpoint Sensitivity in Colorectal Cancer.

John Parrington

Department of Pharmacology

Title: The roles and mechanisms of action of the two-pore channels TPC1 and TPC2 in melanoma tumourigenesis and metastasis'.

Diana Withrow

Department of Primary Health Care Sciences

Title: Exploring morbidity among long-term survivors of non-malignant meningioma.

Matthias Fredrich

Nuffield Department of Medicine (NDM), Translational Gastroenterology Unit- Experimental Medicine Division

Title: Validation of a core membrane marker set for reliable cell segmentation analysis across different multiplex imaging platforms. (Oxford Multiplex Imaging Users Group, OMIG).

Niamh Appleby

Dept of Oncology, Oxford Molecular Diagnostic Centre

Title: Comprehensive multi-modal assessment of early B-cell neoplasms: A pilot collaboration between OXPLORED and CARTOGRAPHY.

Benjamin Fairfax

Department of Oncology

Title: Examining the relationship between tertiary lymphoid structures within primary melanomas and the systemic response to Immune Checkpoint Blockade.

Eoghan Mulholland

Wellcome Centre for Human Genetics

Title: Development of a Bespoke Multiplex panel for the detection of CAF subtypes in Colorectal Cancer.

Doreen Lau

Nuffield Department of Medicine/Target Discovery Institute

Title: Molecular Imaging of Antigen-specific T cells in Cancer Immunotherapy.

Simon Buczacki

Nuffield Department of Surgical Sciences

Title: Linking Paneth cell differentiation and T-cell activation in colon cancer organoid cultures.

Karl Morten

The Nuffield Department of Women's and Reproductive Health

Title: Developing Raman micro-spectroscopy to determine the OXPHOS /glycolysis status of patient tumours.

Romuald Binet

Ludwig Cancer Research

Title: Deciphering the interaction between MITF and the MRE11-NBS1-RAD50 complex – a melanoma-specific regulation of the DNA Damage response.

The next Development Fund call for projects opens in the Autumn of 2024!

Clinical Trial Management Support from Concept to Reporting

OCTO is the first trials unit in the UK specialising in Cancer Precision Prevention (PP) and Early Detection (ED) Studies. Working in partnership with the Primary Care CTU our strategy is purposefully disease agnostic which allows us to conduct studies in areas of greatest unmet need.

Our approach aligns with Oxford Cancer's priorities of developing novel therapeutics including anti-cancer vaccines (immuno-oncology), supporting tumorigenesis research, 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 Precision Prevention and Early Detection studies over the next five years.

Sarah Blagden

Precision Prevention Lead and OCTO Director

Brian Nicholson

Early Detection Lead

Primary Care CTU

OCTO has two new immunotherapy trials in set up: VISTA and LungVAX

You can find out more about these trials from our poster.

From your initial approach to OCTO and throughout the lifetime of your trial you can expect a **proactive**, **pragmatic** and **proportionate** approach to trial development and management from our team.

Investigator approach to OCTO

↓

Initial discussion with OCTO's Senior Trial Development Manager

↓

Discussion in OCTO Clinical Trial Development Group

↓

Trial Development & Grant Applications

Patient and Public Involvement

Patients and the public are involved at every stage of our trials.

From helping us decide which trials to run, to designing those trials, through to publicising results, they are essential and valued collaborators in our work.

***SCAN HERE* to see our portfolio and for more information about how to work with us.**

FUNDRAISING WITH GRATEFUL PATIENTS

Many patients and their families and friends want to understand the research that affects them, and they often wish to philanthropically support it. The University of Oxford Medical Sciences Development Team can help you with these important conversations.*

•To learn more, visit the Development Stall at the Oxford Cancer Annual Symposium 2024 or contact us via the details below.

•Lucy Howe
•Acting Head of Development
•lucy.howe@devoff.ox.ac.uk

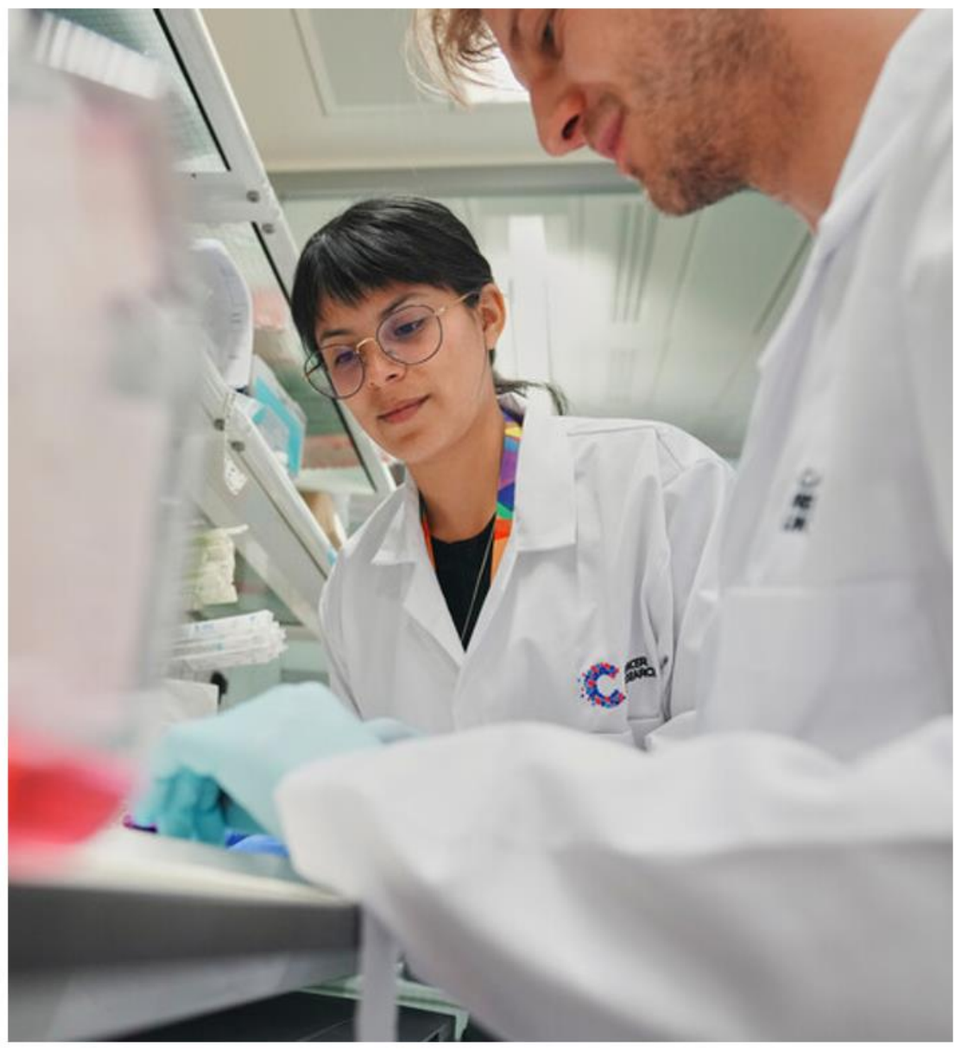
•Dr Sara Gordon
Development Executive
sara.gordon@devoff.ox.ac.uk

•*All fundraising conversations follow the ethical guidelines established by the GMC.

Postdoc futures: empowering postdocs to take the next step in their career

7 June 2024

Mercure Manchester Piccadilly Hotel



CANCER RESEARCH UK

CRUK Trusted Research Programme

We're unleashing the potential of data-driven research to improve our understanding of cancer and create better outcomes for patients. Our Trusted Research Programme offers support for researchers dealing with sensitive health and related data, including anyone harnessing the latest approaches in machine learning and AI, 'omics' and population scale data processing.

We also offer access to our own trusted research environment (TRE) for researchers across the UK who don't have a suitable, safe set-up to store and analyse patient data. Our TRE can provide access to HPC facilities for advanced analytics, machine learning, and AI development. We provide advice, such as technical support for research and information governance requirements.

Contact nathan.lea@cancer.org.uk for further information.

Cancer Research UK – Biology to Prevention Awards

To stimulate translational research that will build and use greater biological and mechanistic understanding of cancer aetiology, genesis and risk, in order to lead to precision prevention interventions.

Applicants are expected to have a clear justification of line of sight from the proposed work to the eventual impact on lowering cancer risk or incidence. These awards are for researchers at all post-PhD career stages across any research area. Scalable funding is available through biannual funding calls, from £100,000 for 1–2-year pilot studies to £600,000 for 3-5-year project awards.

Please contact the Cancer Research UK's office before applying at pprc@cancer.org.uk.

CANCER RESEARCH HORIZONS

FURTHER FASTER TOGETHER

HAVE AN IDEA THAT COULD HELP WITH THE DIAGNOSIS, MONITORING AND TREATMENT OF CANCER?

EXPLORE OPPORTUNITIES TO TRANSLATE YOUR RESEARCH WITH CANCER RESEARCH HORIZONS

Get in touch with Gill Shuttleworth, our Oxford representative

Senior Translation Executive

Gillian.Shuttleworth@cancer.org.uk

Scan to learn about how we can help you to translate your research(incl. funding opportunities and entrepreneurial programmes.

Our Symposium Sponsor

Thermo Fisher Scientific is a global leader in scientific solutions, serving customers in healthcare, research, and industrial sectors.

Our goal is to enable customers to make the world healthier, cleaner, and safer through innovative technologies and services.

We offer a comprehensive range of products catering to cancer research, including laboratory instruments, reagents, consumables, and software solutions.

Our cutting-edge technologies empower scientists and researchers to accelerate discoveries, enhance efficiency, and streamline workflows.

We provide exceptional customer support and technical expertise, assisting customers from instrument selection to troubleshooting and training.

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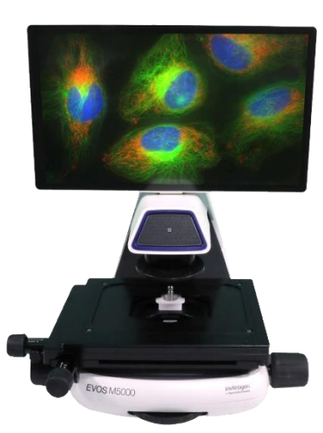

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Speaker Abstracts & Summaries

Speaker Abstracts & Summaries

Session1: Multidisciplinary Research

Is diet-induced weight loss feasible before colorectal cancer surgery and does it affect tumour characteristics?

Colorectal cancer (CRC) is the fourth most common cancer in the UK. Surgery is the standard treatment for ~70% of patients but leads to significant post-operative morbidity. Concomitant obesity affects a third of patients and independently doubles this post-operative morbidity risk. Through improvements in physical fitness, inflammation, and glucose regulation, weight loss in the period between diagnosis and surgery may improve morbidity.

We are currently running a randomised controlled trial to assess the feasibility of a low-energy, high-protein, and nutritionally replete total diet replacement programme (800kcal/day) with weekly remote behavioural support versus usual care in people with excess weight who are awaiting curative elective surgery for colorectal cancer. We aim to recruit 72 patients (n=36 per arm) from 9 sites across England. We are specifically testing whether recruitment, engagement, adherence, and retention are feasible. If promising, we will plan a definitive trial to assess the effectiveness of the intervention on post-operative morbidity.

Additionally, we plan to analyse currently collected samples from the diagnostic biopsy and surgically resected tumour. This analysis will aim to explore the potential mechanisms through which diet-induced weight loss (i.e., energy restriction) exerts its effects on the tumour microenvironment and colorectal cancer biology. We hypothesise that the intervention will reduce markers of tumour cell proliferation rate and insulin signalling pathway activation as well as alter tumour gene expression in favour of anti-tumour activity.

Dimitrios Koutoukidis

I am a dietitian and my research focuses on testing how behavioural science can change dietary intake and improve outcomes for patients with obesity-related diseases, as well as exploring the mechanisms through which weight loss could exerts its effects.

I am currently funded by an NIHR Advanced Fellowship leading a programme of work that examines the role of intentional weight loss before cancer surgery. This work focuses on assessing the feasibility of pre-operative diet-induced weight loss on post-operative complications in patients diagnosed with colorectal or endometrial cancer. This is complemented with qualitative interviews, health economic modelling, and mechanistic studies.

Prior to this, I built a programme of work on weight loss for the treatment of non-alcoholic fatty liver disease. This works used a variety of methodologies, including systematic reviews, meta-analyses, observational analyses, and clinical trials of low-energy diets. It showed that weight loss interventions meet the regulatory criteria for licencing pharmacotherapy for NAFLD and that low-energy diets are safe in an advanced form of NAFLD. It currently explores how the gut microbiome and the immune system might be implicated in these improvements and whether weight loss might be helpful in the treatment of liver cirrhosis.

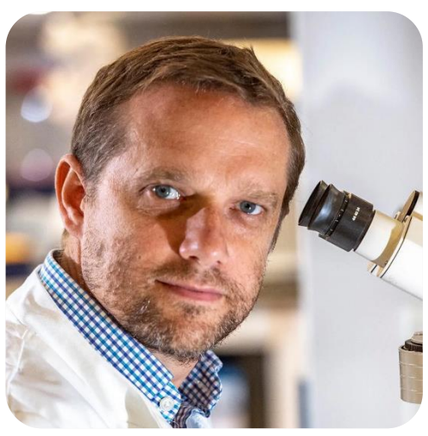
Simon Buczacki

My research focusses on understanding how colorectal (bowel) cancers evolve and how different mutations change the way cancer cells interact with each other and surrounding tissues.

The lifetime risk of being diagnosed with colorectal cancer is around 1 in 16 and in the UK approximately 45 people every day sadly die from the disease. Unlike some other cancers less progress has been made in identifying new drugs that improve survival rates for patients with colorectal cancer.

Recently, our understanding of colorectal cancer has radically changed. We now know that there are many types of bowel cancer and even different parts of a single patient's tumour may vary molecularly. My laboratory uses complex gene engineering techniques on surgical biopsies to better understand what drives this variance to help develop improved therapeutics.

My research aims to provide a mechanistic understanding of the implications of colorectal cancer heterogeneity. These experimental data may provide a clinical rationale for the trial of novel chemotherapy drugs or combinatorial treatments that ultimately could improve outcomes for patients with colorectal cancer.



09:30

Simon Buczacki & Dimitrios Koutoukidis

Professor of Colorectal Surgery, Senior Research Fellow

Nuffield Department of Surgical Sciences & Nuffield Department of Primary Care Health Sciences

Speaker Abstracts & Summaries

Session1: Multidisciplinary Research

Quantitative spatial characterisation of intestinal adenoma-carcinoma progression via multiplex imaging

The progression from adenoma to carcinoma in colorectal cancer is associated with substantial structural remodelling of the tissue cellular milieu. Using a set of 44 paired adenoma-carcinoma rectal biopsy samples, we have used multiplex-IHC technology (PhenoImager, Akoya) to identify immune, stromal, and epithelial cell populations to explore how cell-cell relationships differ between these evolutionary timepoints.

We use a suite of mathematical and statistical tools to characterise the “spatial rules of engagement” that govern cell-cell relationships. We show that different methods provide complementary ways of identifying different aspects cell-cell relationships, such as aggregation, co-localisation, exclusion, or more complex structural interactions. Importantly, by using many different interpretable methods to characterise the tissue, we increase our ability to detect key biological relationships. Using this comparative approach between methods, we show that, despite substantial spatial heterogeneity between patients, we can identify the key changes in spatial interactions associated with progression from adenoma to carcinoma on a per patient basis. Further, our work indicates that there are distinct subsets of patients whose progression is characterised by the same “rules of engagement”. These subsets are most notably mediated through interactions between innate immune cells and the stromal marker periostin. Previous studies have indicated that periostin-innate interactions promote macrophage PD-1 expression in CRC [1]. Furthermore, periostin deletion in pre-clinical breast cancer models decreases the number of tumour-associated neutrophils and reduces immunosuppressive pathway activation [2]. Together, identification of patients with periostin-innate cell interaction mediated progression suggests a potential biomarker for immune/combination therapy candidates.

1. Dorafshan, S., Razmi, M., Safaei, S., Gentilin, E., Madjd, Z., & Ghods, R. (2022). Periostin: biology and function in cancer. Cancer Cell International, 22(1), 315
2. Wang, Z., Xiong, S., Mao, Y., Chen, M., Ma, X., Zhou, X., Ma, Z., Liu, F., Huang, Z., Luo, Q. and Ouyang, G. (2016), Periostin promotes immunosuppressive premetastatic niche formation to facilitate breast tumour metastasis. J. Pathol., 239: 484-495.

Eoghan Mulholland

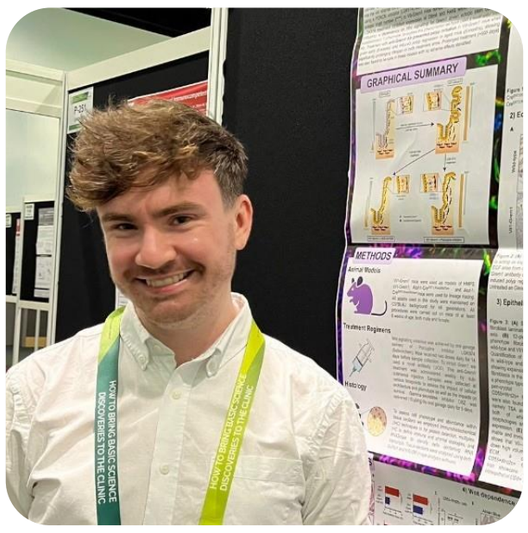
Eoghan is a Lee Placito Research Fellow in Gastrointestinal Cancers, exploring molecular and cellular mechanisms of colorectal cancer (CRC) initiation and progression. Eoghan is part of the ACRCelerate Consortium (CRUK) which focuses on accelerating translational research to pave the way for personalised bowel cancer treatments. His work brings together basic and clinical scientists at the forefront of CRC research to interrogate a suite of state-of-the art pre-clinical models.

Eoghan's research focus is understanding cell-cell interactions between tissue compartments and the genetic drivers behind them. This involves using spatial biology tools to elucidate the crosstalk between the stromal, epithelial and immune compartments, working with Human tissues, organoids and in vivo models (GEMMs). Finally, Eoghan is the Early Career Representative for the MRC’s National Mouse Genetics Network-Cancer Cluster.

Joshua Bull

Joshua is a senior postdoctoral fellow at the Mathematical Institute. His research focusses on the development and application of approaches to describe, quantify and understand spatial biology. He uses mathematical and statistical methods to characterise multiplex imaging data, and mechanistic agent-based modelling to investigate how tumour-immune interactions impact tumour progression and response to treatment.

His research is supported by CRUK through the Cancer Research UK Oxford Centre, and he is a Research Fellow at Reuben College.



09:55

Eoghan Mulholland & Joshua Bull

Lee Placito Fellow in Gastrointestinal Cancer, Postdoctoral Research Associate

Nuffield Department of Medicine & Mathematical Institute

Speaker Abstracts & Summaries

Session 1: Multidisciplinary Research

Mechanistic modelling to improve CD8+ epitope immunogenicity prediction

The development of cancer immunotherapies requires the identification of immunogenic neoantigen targets. Current approaches to this problem utilise modern machine learning techniques to try to implicitly learn the rules of antigen presentation and CD8+ T-cell recognition. However, the success of these is greatly limited by the limited availability of clinically validated immunogenicity data.

We propose a novel approach, at the heart of which is a validated mechanistic model of the Class I antigen processing pathway. Since this model is based on immunological understanding rather than being data-driven, we are confident in its ability to make accurate predictions of raw class I peptide-MHC presentation on an antigen presenting cell surface. The inclusion of these mechanistic outputs into a predictor of CD8+ immunogenicity appears to enhance predictive efficacy beyond what can be achieved through machine learning alone. The resulting predictor - an algorithm we call *POEM* - has displayed efficacy across a range of neoantigen and pathogenic immunogenicity prediction tasks that is competitive or superior to current state-of-the-art.

We believe that *POEM* and future improvements to the model should facilitate the development of personalised cancer vaccines.

Miles Weatherseed

Miles Weatherseed is a postdoctoral researcher in the Centre for Immuno-Oncology. He is a mathematician by background with a particular focus on mathematical biology. This led to the completion of a DPhil in computational immunology under the supervision of Mark Coles, Eamonn Gaffney and Tim Elliott.

Tim Elliott

Professor Tim Elliott is a world leader in the field of antigen presentation and T cell biology and has incorporated discoveries in the areas of antigen processing, T cell regulation and immunodominance into the development of new cancer immunotherapies and is the recipient of a Royal Society/Wolfson Research Merit Award. He is a Fellow of the Royal Society for Biology and Fellow of the Academy of Medical Sciences; founding Editor-in-Chief of the journal *Immunotherapy Advances* published by the British Society for Immunology; and chairs the Cancer Research UK Cancer Immunology expert review committee.

Professor Elliott was appointed to the Kidani Chair of Immuno-Oncology at the University of Oxford in 2020.

10:20

Tim Elliott & Miles Weatherseed

Kidani Professor of Immuno-oncology, Postdoctoral Researcher

Nuffield Department of Medicine & NDORMS

Speaker Abstracts & Summaries

Session 1: Multidisciplinary Research

Living human tumours outside the body: a normothermic perfusion model to accelerate the clinical translation of novel cancer therapeutics

The development and clinical translation of novel therapeutics and treatments is hindered by the paucity of clinically relevant pre-clinical tumour models. However, tumour-bearing liver lobes are commonly resected from cancer patients. We have developed a novel methodology that enables the connection and perfusion of these lobes to a normothermic machine perfusion device, making it possible to keep human solid tumours in a quasi-physiological metabolic, synthetic and haemodynamic state that preserves liver function and basic tumour biology for periods of several days. The validity of the model was first interrogated using non-tumour bearing livers, demonstrating that the pharmacokinetics of a range of therapeutic agents in the isolated perfusion model adequately predicted those observed clinically. The tumour-bearing model was then put to the test by intraarterial administration of oncolytic virus libraries, evidencing both its selectivity, infectivity and replication within the solid tumour. The tumour-bearing normothermically perfused liver lobe model therefore bridges a significant knowledge gap serving to make novel discoveries at the intersection between laboratory science and clinical practice through an enhanced understanding of basic tumour biology and therapeutic response, with the potential to substantially de-risk clinical trials.

Constantin Coussios

Professor Constantin Coussios is the Director of the Oxford Institute of Biomedical Engineering. He received his BA, MEng and PhD in Engineering from the University of Cambridge and was elected to the first statutory chair in Biomedical Engineering at the University of Oxford in 2011. He founded and heads the Biomedical Ultrasonics, Biotherapy and Biomaterials Laboratory (BUBBL) and in 2022, became the founding director of the £40m Podium Institute for Sports Medicine and Technology. The author of over 150 peer-reviewed publications and 30 patents, Prof. Coussios received the UK's Institute of Acoustics' Young Person's Award for Innovation in Acoustical Engineering in 2007, the Lizzi award of the International Society of Therapeutic Ultrasound in 2012 and was elected a fellow of the Acoustical Society of America in 2009, receiving the Lindsay award in 2012. In 2008, he co-founded OrganOx Ltd., which has developed a now CE-marked and FDA approved normothermic perfusion device for improved organ preservation prior to transplantation. In 2014, he co-founded OxSonics Ltd, commercializing ultrasound-based medical devices for cavitation-enhanced oncological drug delivery, and OrthoSon Ltd in 2018, which is translating a novel percutaneous biostructural hydrogel approach to treat lower back pain under an FDA breakthrough device designation. In 2017, he received the Silver Medal of the UK's Royal Academy of Engineering in recognition of his contributions to organ preservation and ultrasound-mediated drug delivery. In 2019, he was elected a Fellow of the Royal Academy of Engineering and in 2022 was awarded an OBE for services to biomedical engineering.

Kerry Fisher

My research is focused on the tumour microenvironment, notably addressing abnormal metabolism and dysfunctional immune activity. The structure and composition of tumour stroma can influence disease progression and, crucially, the performance of therapeutic drugs. Conventional laboratory models do not fully recapitulate the complexity of the tumour microenvironment and our research team makes extensive use of fresh biopsy material from patients. We have expanded this work to form a live tissue facility for the benefit of the department and our collaborators.

For the treatment of cancer, I am exploring the use of immunotherapy approaches that are designed to capitalise on and modulate the tumour phenotype. Oncolytic viruses are particularly interesting because they exploit both innate and adaptive immune dysfunction to replicate and spread while killing cells independently of drug resistance pathways.

Translating new therapies to the clinic often involves working with industry, either established companies or new spin-out biotechs from the University. Over the last 20 years I have been involved in many industrial collaborations in one form or another, helping to manage the development of new treatments from the initial innovation through to science-led clinical trials.

10:45
Kerry Fisher & Constantin Coussios
Research Lecturer
Director of the Oxford Institute of Biomedical Engineering
Department of Oncology & IBME

Speaker Abstracts & Summaries

Session 2: Paradigm Changing Biology

IFNγ-dependent remodelling of the myeloid immune landscape underlies control of IFNγ-insensitive tumours

I am an associate professor based at the Kennedy Trust of Rheumatology Research (KTRR). I completed my Ph.D. at the University of the Mediterranean, Marseille, France, focusing on the negative regulation of primary and leukemic T cell activation and undertook postdoctoral training at the Netherlands Cancer Institute, Amsterdam and UCSF, San Francisco, where I studied the molecular mechanism underlying T cell migration and differentiation in space and time. My group still has a broad interest in the spatio-temporal behaviour of T cells. We use multidisciplinary approaches to study how T cells directly communicate with each other and with their surroundings to control their fate in the context of infection and cancer. Cancer cells adapt to their environment to survive. This includes evading the immune system by inducing an immunosuppressive state. This is a dynamic process, whereby the immune system not only protects against cancer but also shapes emerging tumours. Tumour escape occurs following strong immune pressure, such as checkpoint blockade, during which many tumours acquire mutations to inhibit IFNγ signalling, thereby limiting cancer recognition by CD8 T-cells. While it was originally hypothesised that those types of mutations would lead to tumour escape and resistance to immunotherapies, new studies are challenging this dogma. In this talk, I'll present evidence that immune cells, in particular myeloid and CD8 T-cells, adapt to IFNγ-dependent tumour escape. Counter-intuitively, this adaptation results in enhanced immune fitness, explaining some of the discrepancies in the relevance of IFNγ signalling in cancer cells for anti-tumour immunity, broadening our understanding of the cancer/immune interface.

11:50

Audrey Gérard

Associate Professor

Kennedy Institute of Rheumatology

Speaker Abstracts & Summaries

Session 2: Paradigm Changing Biology

CD4 T follicular helper cells, B cells, and tertiary lymphoid structures in cancer

Recent evidence indicates the presence of ectopic lymphoid aggregates or hubs, often termed tertiary lymphoid structures (TLS) resembling GCs within tumour tissue, is associated with longer survival and better treatment response. However, the cellular mechanisms contributing to TLS formation remain incompletely understood due to their lifetime, scarcity, limited tissue availability, and infrequent occurrence in existing murine models of tumorigenesis. To this end, we aim to study paired blood and tissue samples from pre-treatment patients with colorectal cancer via the CRUK Oxford Centre. To characterise the cellular composition and spatial distribution of the cellular environment that promotes TLS and tumour-reactive antibodies, we have developed a multi-omics approach combining our recently developed 5-38-plex high-dimensional spectral flow cytometry panels for phenotypic/functional proteomic analysis of the tumour microenvironment, with single-cell RNA-TCR/BCR sequencing and spatial technologies. This work will provide a deeper mechanistic understanding of TLS formation, clarify the immunological mechanisms that underpin the protective effect of TLS in cancer, and leverage CD4 T cells and B cells to develop novel immunotherapies and cancer vaccines to recreate a microenvironment beneficial for TLS formation in refractive tumours.

Dr Pedroza-Pacheco is a Career Development Fellow at the Centre for Immuno-Oncology in the Nuffield Department of Medicine and the Chinese Academy of Medical Sciences Oxford Institute at the University of Oxford. She obtained her bioengineering and immunology training at the Monterrey Institute of Technology (ITESM, Mexico) and University College London (UCL, UK). She did her postdoctoral training at the University of Oxford, and in 2014 she joined the Consortium for HIV/AIDS Vaccine Development (CHAVD) as a young faculty member. Dr Pedroza-Pacheco's dual expertise in engineering and immunology gives her a unique skill set, enabling her to combine a systematic approach to understanding complex diseases in an integrative context. By developing techniques and technologies that comprehensively assess genetic and protein variations that shape cellular function, Dr Pedroza has made significant contributions in the field of HIV and vaccinology, from revealing cellular mechanisms that regulate Tfh and B cell responses to uncovering predictive cellular features needed in vaccination strategies to develop bnAbs. These techniques are currently being adapted to understand the role of tertiary lymphoid structures in the context of cancer.

12:02

Isabela Pedroza-Pacheco

Career Development Fellow

Nuffield Department of Medicine

Speaker Abstracts & Summaries

Session 2: Paradigm Changing Biology

The elepHant in the room

Hypoxia and acidosis are chemical signatures of the tumour microenvironment that act as selection pressures driving malignancy because they require cells to adapt in order to survive. Low oxygen and pH relate to inadequate vascular perfusion, but have distinct metabolic causes and signalling consequences. Since respiration is the principal O_2 consumer, survival under oxygen-depleted conditions is facilitated by switching to O_2-independent lactic acid fermentation, a process orchestrated by hypoxia-inducible factor (HIF). The metabolic strategy for surviving acidosis is less intuitive because both fermentation and respiration generate acidic end-products. Addressing this, our CRISPR/Cas9 screen of colorectal cancer (CRC) cells identified respiratory genes as essential for surviving low pH, which contrasts surviving hypoxia where these processes are redundant and is explained by the lower per-ATP yield of respiratory acid (CO_2), compared to lactic acid. Moreover, HIF signalling is inactivated at low pH even under persistent hypoxia, indicating that a switch from respiration to fermentation is permissive under alkaline hypoxia only. Indeed, the strong inhibition of fermentation by low pH protects tissues from excessive acidification, but also unveils respiration as a druggable vulnerability. Our phenotypic screen of ~70 CRC lines associated acid-resistant phenotypes with higher respiratory rates, stimulated further upon acid-adaptation. Furthermore, acid-resistant lines associated with the surface-expressed glycoprotein CEACAM6, revealing a strategy for targeting acid-resistant cells. We propose that eliminating acid-adaptation disarms a key survival strategy, without which CRC cells cannot exploit acid-resistance to gain a competitive advantage over normal cells and host defences.

Pawel Swietach is Professor of Physiology at DPAG. His group works on acid-base disorders and their interplay with hypoxia, ranging from oxygen transport by blood to inborn errors of metabolism. A special interest is the acidic tumour microenvironment. We aim to understand acid-adaptation, defining druggable vulnerabilities, and developing ways of exploiting this information therapeutically and diagnostically.

12:14
Pawel Swietach
Professor of Physiology
Department of Physiology, Anatomy & Genetics

Speaker Abstracts & Summaries

Session 2: Paradigm Changing Biology

APOBEC3B regulates R-loops and promotes transcription-associated mutagenesis in cancer

APOBEC3B (A3B) is a single-stranded DNA (ssDNA) cytosine deaminase, implicated in the innate immune response to viral infection. Despite this beneficial function, A3B activity is estimated to be the second most common mutator in cancer. Whilst this mutation signature is well-established, the underlying molecular mechanisms are unclear. Notably, the cellular substrates of A3B have not been fully delineated.

R-loops are three-stranded structures composed of an RNA-DNA hybrid and displaced ssDNA. These structures form naturally during transcription and involved in multiple biological functions. However, their mis-regulation is linked to human diseases. Our previous work has shown that R-loops accumulate in cancer and drive transcription-replication conflicts, promoting genome instability (*Kotsantis, Nature Comm 2016*). Using biochemical, genome-wide and cellular approaches, we demonstrate a previously unrecognised role for A3B in regulating R-loops. We show that A3B binds to R-loops and promotes mutations at these sites, through a genome-wide mapping of A3B-associated R-loops and A3B signature mutations in breast cancer. Hence, we unveil a distinct mechanism for transcription-associated mutagenesis in cancer (*McCann, Nature Genetics 2023*). This work also highlights the potential for targeting R-loops in new cancer therapies.

Natalia Gromak in an Associate Professor and an MRC Senior Research Fellow at the Sir William Dunn School of Pathology (University of Oxford). She is also a Tutorial Fellow in Medicine in Trinity College. Gromak lab works on biology of R-loops and their role in health and disease. The lab has identified the role of R-loops in transcriptional termination and established methods to map R-loops in human genome. Gromak lab has demonstrated that R-loops associate with human diseases, including cancer and neurodegeneration. Work in Gromak laboratory is funded by the MRC, Royal Society, Ataxia UK, Motor Neuron Disease Association, National Ataxia Foundation, EPA Trust, Rosetrees Foundation.

12:26

Natalia Gromak

Associate Professor

Sir William Dunn School of Pathology

Speaker Abstracts & Summaries

Session 2: Paradigm Changing Biology

A computational pipeline for spatial mechano-transcriptomics

Advances in spatial profiling technologies are providing insights into how molecular programs are influenced by local signalling and environmental cues. However, cell fate specification and tissue patterning involve the interplay of biochemical and mechanical feedback. Here, we propose a new computational framework that enables the joint statistical analysis of transcriptional and mechanical signals in the context of spatial transcriptomics. To illustrate the application and utility of the approach, we use spatial transcriptomics data from the developing mouse embryo to infer the forces acting on individual cells, and use these results to identify mechanical, morphometric, and gene expression signatures that are predictive of tissue compartment boundaries. In addition, we use geoadditive structural equation modelling to identify gene modules that predict the mechanical behaviour of cells in an unbiased manner. This computational framework is easily generalized to other spatial profiling contexts, providing a generic scheme for exploring the interplay of biomolecular and mechanical cues in tissues.

Dr. Adrien Hallou is a Group Leader in Tissue Biology at the Kennedy Institute of Rheumatology (University of Oxford). He leads an interdisciplinary research group that develops innovative experimental and computational approaches combining mechanobiology, spatial omics, advanced microscopy, machine learning and mathematical modelling to investigate the role of mechanical and biochemical signalling in cell fate decisions and tissue dynamics in development, homeostasis and inflammatory diseases.

12:38

Adrien Hallou

Group Leader in Tissue Biology

Kennedy Institute of Rheumatology

Speaker Abstracts & Summaries

Session 2: Paradigm Changing Biology

Dicer dependent tRNA-derived small RNAs promote nascent RNA silencing

Mammalian cells utilize small RNAs (sRNAs) molecules to regulate gene expression in a pathway known as RNA interference (RNAi). Transfer RNAs (tRNAs) play essential role in translation, but also generate tRNA-derived small RNAs (tsRNAs). We discovered that Dicer, an enzyme critical for canonical RNAi process, not only associates with actively transcribed tRNA genes, but also binds to tRNAs that are alternatively folded, converting them into tsRNAs. These Dicer-generated tsRNAs specifically target the introns of numerous protein-coding genes as well as long non-coding RNAs, leading to the degradation of their nascent transcripts. Notably, the genes targeted by tsRNAs are significantly linked to disease-related phenotypes, highlighting the clinical relevance of this pathway. We have outlined a novel mechanism by which nascent RNA silencing occurs, diverging from the established norms of post-transcriptional and transcriptional regulation of gene expression. Our approach includes the use of synthetic tsRNAs to diminish the aggressive cancer phenotype. Additionally, we demonstrate that tsRNAs can effectively silence long non-coding RNAs, such as NEAT1, under both normal and hypoxic conditions (Di Fazio et al., NAR, 2022; and international patent filed by Cancer Research UK).

The field of oligonucleotide therapeutics is experiencing rapid growth due to its potential to address a broad spectrum of diseases. The market for oligonucleotide synthesis is projected to grow, from USD 9.1 billion in 2023 to an anticipated USD 44.9 billion by 2033. Despite initial hurdles, the sector has achieved considerable progress, leading to the successful authorization and market launch of therapies such as Patisiran for hereditary amyloidosis. TsRNAs represent a novel class of therapeutic oligonucleotides, distinguished from siRNA by their nuclear activity and association with the protein Argonaute 2, conferring advantages in stability and stoichiometry over ASOs. As such, tsRNA-mediated silencing presents a promising new avenue for developing nuclear-targeted oligonucleotide therapies.

tRNA-derived small RNAs (tsRNAs) regulate gene expression via novel gene silencing mechanism. This innovative pathway, involving Dicer-generated tsRNAs, targets genes linked to diseases, offering a novel approach to gene therapy. With their distinct nuclear activity, tsRNAs represent a promising frontier in oligonucleotide therapeutics, potentially revolutionizing treatments for genetic disorders and cancer.

12:50

Monika Gullerova

Professor of Molecular Medicine

Sir William Dunn School of Pathology

Speaker Abstracts & Summaries

Session 3: Patient and Public Involvement

Come along to hear more about how patient and public involvement can benefit your research.

14:05

David Church & Tom Bartlett

Clinician Scientist Fellow, PPI Representative

Nuffield Department of Medicine

Session 4: Spatial Biology

Quantitatively Modelling Short Peptide Vaccinations in Immuno-oncology

A prospective immunologically-based cancer treatment is multi-peptide vaccination, targeting multiple tumour-associated peptides. However, a recent multiple short-peptide vaccination trial for renal cell carcinoma failed to show benefit, with many patients responding to only one of the administered peptides. An in-silico model is considered to enable an exploration of the determinants of the initial immunological response following multiple short peptide vaccination, suggesting mechanisms for the observed lack of benefit in the recent clinical trial. These insights may also used to suggest possible improvements to the trial design and more generally illustrate one means by which in silico studies can be used to test and improve the design of clinical trials.

Prof Eamonn Gaffney has over 25 years' experience in applying mechanism-based mathematical modelling to the life sciences. This has included the field of immunological modelling in the past seven years, working with Prof Mark Coles, an Oxford Systems Immunologist, to set up a Mathematical Immunology Research group that has considered diverse problems, especially but not exclusively related to immune cell dynamics.

14:25

Eamonn Gaffney

Professor of Applied Mathematics

Mathematical Institute

Speaker Abstracts & Summaries

Session 4: Spatial Biology

Learning to Adapt: Rational Personalization of Cancer Treatment Schedules Using Deep Reinforcement Learning

Abstract: Standard-of-care treatment regimens have long been designed for maximal cell kill, yet these strategies often fail when applied to metastatic cancers due to the emergence of drug resistance. Adaptive treatment strategies have been developed as an alternative approach, dynamically adjusting treatment to suppress the growth of treatment-resistant populations, delaying, or even preventing, tumor progression. Promising clinical results in prostate cancer indicate the potential to optimize adaptive treatment protocols. We propose the application of deep reinforcement learning (DRL) to guide adaptive drug scheduling, and demonstrate that these treatment schedules can outperform the current adaptive protocols in a mathematical model calibrated to prostate cancer dynamics, more than doubling the time to progression. The DRL framework also produces interpretable, adaptive strategies based on a single tumor burden threshold, replicating and informing optimal treatment strategies. Given the DRL framework has no knowledge of the underlying mathematical tumor model, this demonstrates the capability of DRL to help develop treatment strategies in novel or complex settings. Finally, we propose a five-step pathway combining mechanistic modeling with the DRL framework to translate this approach to the clinic with limited information for a given patient. Integrating conventional tools to ensure the interpretability of traditionally ``black-box'' DRL models, we generate personalized treatment schedules that consistently outperform clinical standard-of-care protocols.

Profile: Kit Gallagher is a DPhil student, co-supervised by Philip Maini in the Mathematical Institute, and Sandy Anderson at the Moffitt Cancer Center, Florida. His primary interests are centered around improving treatment scheduling and patient outcome prediction using tools from population and agent-based modelling, statistical inference and deep learning methods. His current work focuses on treatment-resistant prostate and ovarian cancers, but develops themes applicable to a range of disease and treatment settings.

14:45
Kit Gallagher
Postgraduate Student
Mathematical Institute

Speaker Abstracts & Summaries

Session 4: Spatial Biology

CD8+ T cells drive selection and segregation in the tumor microenvironment

While immunotherapies are promising cancer treatments, they remain unsuccessful in many patients. To understand how tumor heterogeneity affects the immune control of tumors, we studied how the immune system interacted with two different cancer cell lines: Interferon-gamma (IFNg) receptor-wild type (WT) and knockout (KO) B16F10 melanoma cells. In isolation, both WT and KO cell lines were equally controlled by the immune system; however, when admixed, WT tumors were selectively killed over the KO. By mathematically fitting our data and profiling tumor-infiltrating immune cells, we determined that while KO tumors were harder to kill than WT tumors, CD8+ T cells primed by KO tumor cells killed more efficiently than those primed by WT tumors. This polarization explained clonal selection within the tumor and demonstrated the importance of antigen priming in downstream tumor killing.

T cell infiltration also caused spatial segregation between WT and KO cells in admixed tumors. Using both an experimental spheroid culture system and an agent-based model, we determined that selective one-to-one combat between polarized CD8+ T cells and tumor cells could directly cause segregation within the tumor microenvironment. By repeating this work with other model systems, we have demonstrated a generalizable principle: immune-driven selective pressure can drive both numerical shifts and spatial reorganization within the tumor microenvironment. By quantifying these processes, we aim to better understand the way immunotherapies shape the tumor-immune microenvironment and, in turn, develop more successful cancer treatments.

Anagha Krishnan is a PhD candidate at the Kennedy Institute of Rheumatology and the National Cancer Institute, jointly supervised by Dr. Audrey Gerard and Dr. Gregoire Altan-Bonnet as part of the NIH Oxford-Cambridge Scholars Program. Previously, she earned a B.S. in biomedical engineering from the Georgia Institute of Technology. Anagha is broadly interested in applying systems biology approaches to understand cell-cell interactions in the tumor microenvironment.

15:15
Anagha Krishnan
Postdoctoral Student
NDORMS

Speaker Abstracts & Summaries

Session 4: Spatial Biology

Identifying phenotype-genotype-function coupling in live-cell imaging using SPOT

Live cells are highly plastic, phenotypically dynamic, and are affected by genetic and environmental perturbations. The perturbations that alter cell function manifest in phenotypical deviations, namely, changes to the shape, appearance and motion of a cell or tissue. Until now, much of this phenotypical information has remained untapped, simply because there has been no effective and holistic method of extracting, quantifying and analysing phenotypical data. For this reason, we developed SPOT – the Shape, Appearance and Motion Phenotype Observation Tool – which offers an unbiased and comprehensive description of phenotype, thereby enabling agnostic phenotype analysis.

SPOT can be applied to phenotypes that manifest across all levels of biological organisation, but is a particularly valuable tool for exploratory analysis in contexts such as phenotypical screening. We have validated the approach using simulation, computer vision, and single-cell tracking datasets; and applied it to intestinal organoid models subjected to genetic and environmental perturbations. SPOT is applicable to label-free live-cell imaging, and it advances biomedical discovery through its ability to quantitatively measure phenotypic heterogeneity over time, facilitating the identification of phenotype-genotype-function relationships. Further information will be presented at the conference.

I am a DPhil student in Xin Lu’s lab, working on developing phenotype analysis pipelines, and applying them to investigate intestinal cancers. My computational work involves using cutting-edge machine learning models in the context of biological data, including the data generated from high-throughput screening. In the lab, my experimental work is centred around mouse and human organoids derived from the gastrointestinal tract.

15:40
Adam Norton-Steele
Postdoctoral Student
Ludwig Institute

Speaker Abstracts & Summaries

Session 5: Keynote

Exploring biological mysteries with condensate models

Cellular processes such as transcription and signal transduction depend on the concerted action of many protein molecules. Many cellular processes are now understood to occur within large assemblies called biomolecular condensates, which compartmentalize the community of protein molecules involved in each process. I will describe new insights into the nuclear condensates that play prominent roles in regulation of cell identity and metabolism. I will also discuss the features of condensates that provide the cell with regulatory capabilities beyond canonical molecular regulatory mechanisms, note where these are dysregulated by pathological mutations, and explain how our new understanding of chemical partitioning is influencing the development of new therapeutics for cancer.

Richard Young is a Professor at the Whitehead Institute and MIT. Dr. Young studies gene regulation in health and disease. Therapeutic concepts from these studies have led to development of multiple anti-cancer drugs that are currently in clinical trials. Dr. Young has served as an advisor to the World Health Organization, the National Institutes of Health and numerous scientific societies and journals. His honors include Membership in the National Academy of Sciences and the National Academy of Medicine, and Scientific American has recognized him as one of the top 50 leaders in science, technology and business. Young has trained over 120 predoctoral and postdoctoral students, many of whom play prominent roles in academic and commercial settings. He has founded and advised companies in the biotechnology and pharmaceutical industry, and currently serves on the boards of multiple biopharmaceutical companies. Dr. Young is also an aviator and holds a commercial pilot license.

16:35

Richard Young

Professor Of Biology

Whitehead Institute for Biomedical Research and

Department of Biology, MIT

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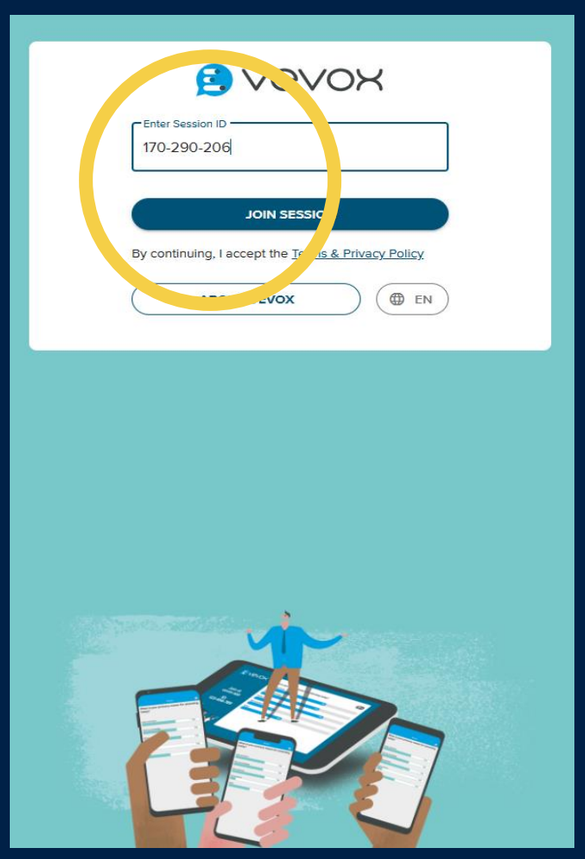

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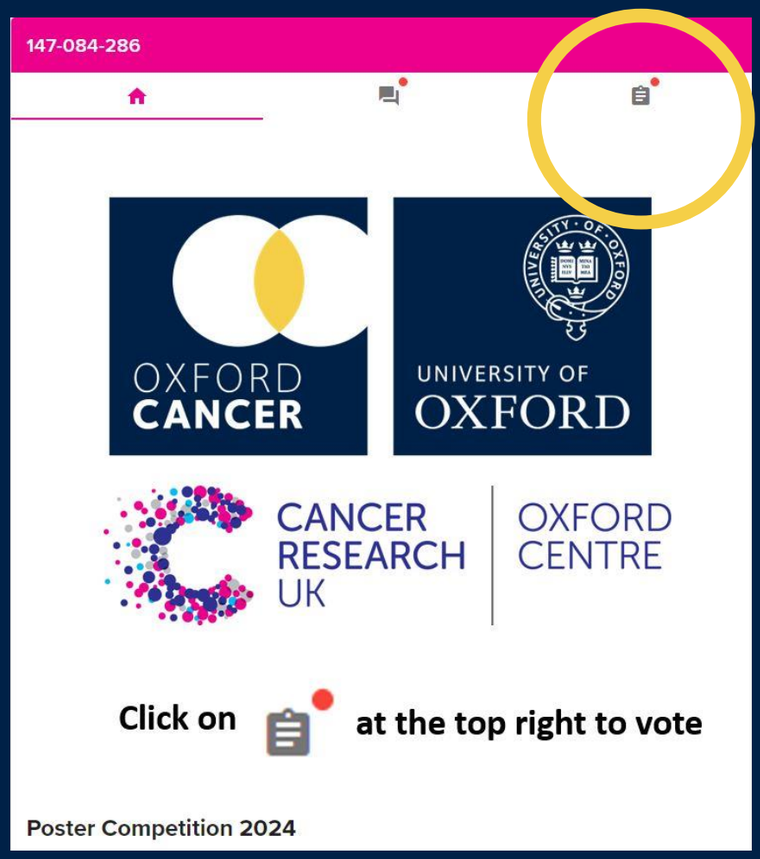

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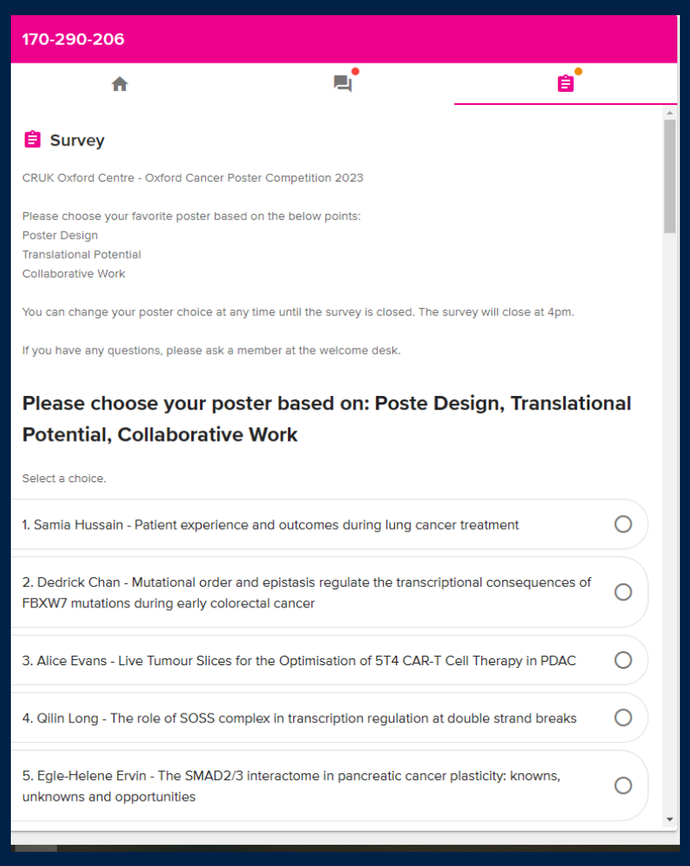

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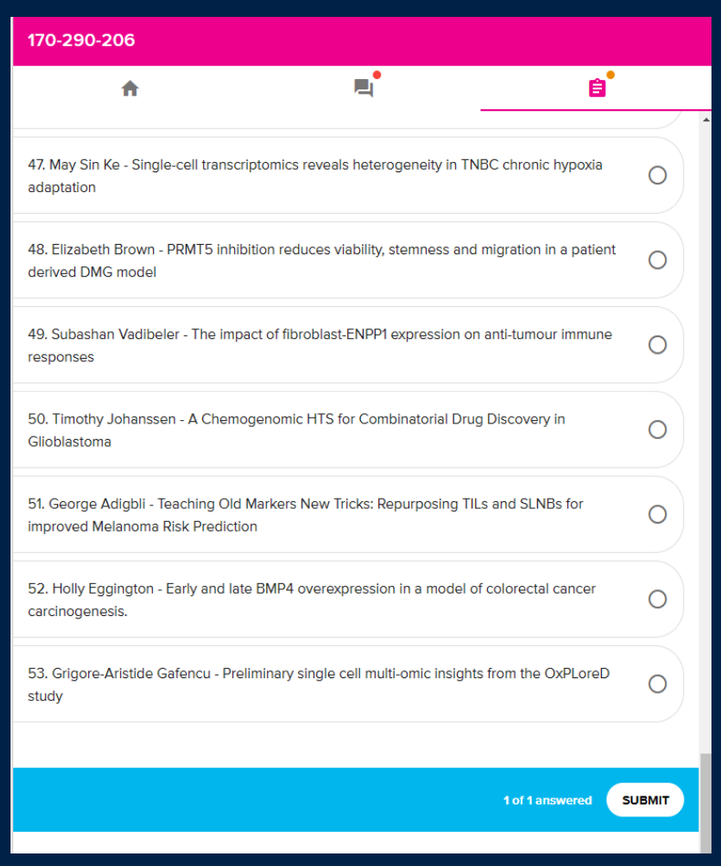

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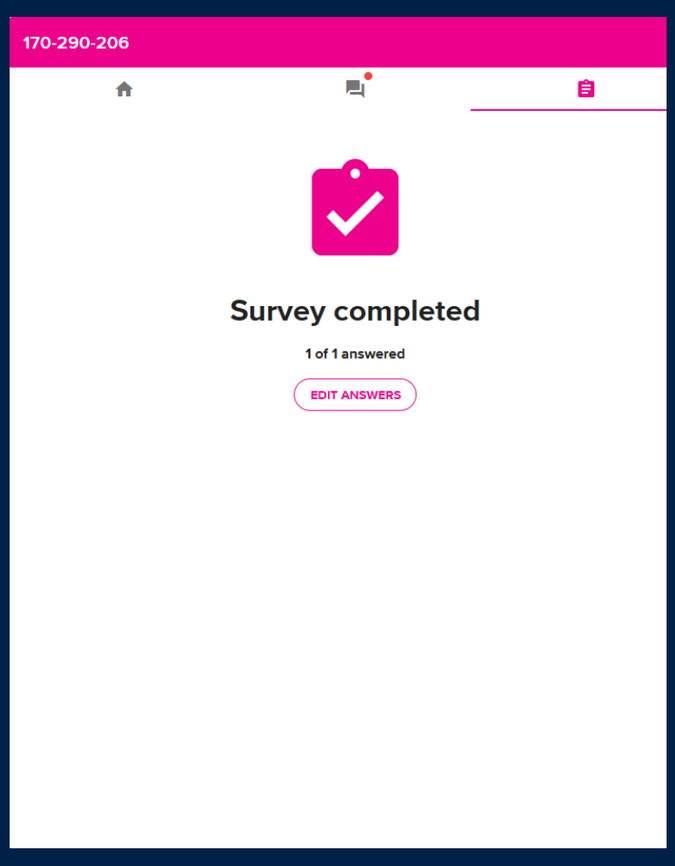

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Poster Abstracts

1

Targeting Complement System to Improve High-grade Glioma Response

Background: Radiotherapy is currently the standard treatment for glioblastoma (GBM), and the only treatment option available for paediatric diffuse intrinsic pontine glioma (DIPG). However, median survival for both brain cancers are only 14 months and 7 months respectively. Tumours invariably occur, making them incurable diseases. The complement receptor, C5aR1, is highly expressed in high grade gliomas compared to normal tissues, and as a G-protein coupled receptor, we aim to investigate how inhibiting C5aR1 could impede multiple pro-survival signalling pathways and improve treatment efficacy when combined with irradiation.

Method: Avacopan and PMX205 are both selective inhibitors of C5aR1 that have been approved for vasculitis, and currently undergoing clinical testing for ALS respectively, in which they are reportedly well-tolerated. We therefore investigate if these inhibitors could target C5aR1 signalling in DIPG and GBM cells. In addition to glioma cells, we also take into considerations of the C5aR1 inhibition impacts in microglia cells, which are resident macrophages in the brain that make up a large proportion of brain tumours.

Results: Following treatment with C5aR1 inhibitors and siC5aR1, we observed attenuated MAPK signalling, potentially mediating increased apoptosis following irradiation. Our results show that C5aR1 inhibition decreased glioma cell viability after irradiation, and also possibly reduced cell survival. Surprisingly, C5aR1 inhibition did not result in significant changes in microglia survival, however, it did induce changes in its activation, migration, and inflammatory profile.

Conclusions: C5aR1 inhibition impedes pro-survival signalling in glioma cells, and the effects are most observable when combined with irradiation. However, further investigation is needed to determine whether inhibiting C5aR1 signalling has an overall positive impact on microglia.

Contact: sze.lee@oncology.ox.ac.uk

2

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

Zinaida Dedeic, David Arcia Anaya, Christopher Pinder, Caroline Dive, Phil Crosbie, James Reading, Sergio Quezada, Catherine Green, Charles Swanton, Carol Leung, Benoit Van den Eynde, Mariam Jamal-Hanjani, Benjamin Simpson, Kevin Litchfield, Tim Elliott & Sarah Blagden.

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 developed a cancer vaccine, called LungVax, using the ChAdOx platform. LungVax vaccine is based on shared clonal neoantigens that are present throughout lung tumorigenesis. Preliminary pre-clinical evaluation of the LungVax vaccine demonstrate the successful expression of the vaccine insert in cell lines. The administration of the vaccine in BALB/C mice induces a promising immunogenic response. We provide an overview of the prospective clinical development pathway for LungVax.

3

Epigenetic mechanisms governing interactions between cancer and microenvironmental cells in melanoma

Background: Despite improvements in survival with the advent of immunotherapies, melanoma still represents an area of clear clinical need, with the 1-year survival rate for those diagnosed at stage 4 being just 57.5% in the UK. Contributing to the development, progression, and recurrence of melanoma is the tumour microenvironment (TME) which comprises a variety of immune and stromal cell types. Whilst the immune TME has been an area of intense study, less work has been carried out on alternative TME cell types. Previous data from our lab has demonstrated that melanoma-keratinocyte interactions could be of particular interest, due to nuclear size and epigenetic dysregulation occurring in the latter population upon interaction with cancer cells. This raises the hypothesis that epigenetic alterations in the TME affect growth of the tumour.

Methods: We developed a fate mapping system to track melanoma-keratinocyte interactions. We engineered human A375 melanoma cells to express a secreted form of Cre recombinase, which is taken up into nearby HaCaT keratinocytes with a floxed reporter (CMV-loxP-dsRed-loxP-eGFP). An epigenetic library containing 157 small molecule compounds was applied to this system in a high-throughput assay to identify those able to enhance or diminish melanoma-keratinocyte interactions (as indicated by the number of eGFP positive HaCaT cells per well). Candidate hits were validated via dose-response experiments and subsequent assays involving pre-treating the cells with compounds 48 hours prior to co-culture were used to determine which cell type the inhibitors were affecting.

Results: In vitro co-cultures containing A375-Cre donor cells and HaCaT recipient cells revealed frequent interactions between the two populations, which could be enhanced by the application of C6 ceramide (a compound identified in previous work with the same system). Application of small molecule epigenetic inhibitors to this co-culture system revealed 59 compounds able to increase the number of eGFP positive HaCaT recipient cells per well (an indicator of inter-cellular interactions) and 80 compounds which decreased this readout. Follow up dose-response experiments highlighted the PRMT1 inhibitor TC-E 5003 and the HDAC6 inhibitor CAY10603 as particularly promising compounds able to increase the number of interactions between the two cell types. Pre-treatment of HaCaT recipient cells, but not A375-Cre donor cells, with either of these two compounds prior to co-culture significantly increased the number of eGFP positive HaCaT recipient cells.

Conclusions: PRMT1 and HDAC6 have been identified as promising epigenetic candidates regulating interactions between melanoma cells and keratinocytes. This regulation appears to arise within the HaCaT recipient cell population as opposed to melanoma cells. Ongoing experiments are aimed at delineating the mechanism and in vivo consequences of these interactions.

Contact: hannah.pook@sjc.ox.ac.uk

4

CD8+ T cells drive selection and segregation in the tumor microenvironment

Authors: Anagha Krishnan, Vivian Lau, Gracie Jennah Mead, Hannah Dada, Davide Randazzo, Gregoire Altan-Bonnet, and Audrey Gerard

Background: While immunotherapies are promising cancer treatments, they remain unsuccessful in many patients. Intratumor heterogeneity, the genetic and non-genetic diversity between individual cells in a tumor, is strongly implicated in this variable response. To understand how cellular heterogeneity affects the immune control of tumors, we studied how the immune system interacted with two different cancer cell lines: Interferon-gamma (IFNg) receptor-wild type (WT) and IFNg receptor-knockout (KO) B16F10 melanoma cells. Tumor sensing of IFNg drives positive feedback loops within the immune system and plays an important role in both immunity and tolerance; therefore, loss of the IFNg receptor on tumor cells is a common mechanism of immune escape.

Methods: We utilized a variety of experimental and computational techniques: syngeneic mouse models, intravital imaging, spectral flow cytometry, 2D and 3D microscopy, in vitro timeseries assays, machine learning methods, differential equation modeling, and agent-based modeling.

Results: In isolation, both WT and KO cell lines were equally controlled by the immune system; however, when admixed, WT tumors were selectively killed over the KO. By mathematically fitting our data and using spectral flow cytometry to profile surface proteins on infiltrating immune cells, we determined that differences in T cell receptor binding to tumor surface proteins distinguished the immune responses to WT and KO tumors. While KO tumors were more difficult to kill than WT tumors, CD8+ T cells primed by KO tumor cells killed more efficiently than T cells primed by WT tumors. This polarization explained clonal selection within the tumor and demonstrated the importance of antigen priming in downstream tumor killing.

T cell infiltration also caused spatial segregation between the WT and KO cells in admixed tumors. Using both an experimental spheroid culture system and an agent-based model, we determined that selective one-to-one combat between polarized CD8+ T cells and tumor cells could directly cause segregation within the tumor microenvironment.

Conclusions: By repeating this work with other model systems, we have demonstrated a generalizable principle: immune-driven selective pressure can drive both numerical shifts and spatial reorganization within the tumor microenvironment. By quantifying these processes, we aim to better understand the way immunotherapies shape the tumor-immune microenvironment and, in turn, develop more successful cancer treatments.

Contact: Anagha.krishnan@gtc.ox.ac.uk

5

A novel IRE1 activity prognostic score is a proxy of Prostate Cancer Evolution

Introduction & Objectives: Prostate Cancer (PCa) evolution depends on transcriptional events, forming an adaptive response to intrinsic/extrinsic stresses, culminating in lineage reprogramming and treatment resistance. PCa results in ~360000 deaths annually due to metastatic progression, supported by the emergence of stress and treatment-resistant cancer cells which experience high proliferative rates and androgen receptor (AR)-driven high metabolic demand. These events require high protein turnover, leading to misfolded-protein accumulation, causing Endoplasmic Reticulum (ER) stress. The unfolded protein response (UPR) resolves this stress through its transducer Inositol Requiring Enzyme (IRE1) and downstream effector XBP1 (X-box binding protein 1).

We have previously shown that IRE1/XBP1 may be determining responses to AR deprivation therapy (ADT). Consequently, we proposed to generate an IRE1 activity prognostic/response biomarker signature as a proxy of lineage plasticity and treatment resistance, applicable to individual tumours, thus informing preclinical/ clinical development of IRE1-targeting strategies in PCa.

Results: We have generated an IRE1 activity score that is a proxy of multiple driving biologies (AR, Inflammation, DNA repair, Cell Cycle, EMT), multiple histological features (stroma, varying histological tumour grades, varying benign structures), and can prognosticate large clinical cohorts.

Conclusions: We have employed bulk and spatial transcriptomics to refine our understanding of the impact of IRE1 activity on PCa and its microenvironment and to better define the crosstalk with the AR. By comparing our outputs to clinical transcriptomic datasets, we have developed a refined prognostication score which may be used as a tool alongside imaging AI and other multiomic modalities to inform dynamic disease progression.

Contact: Dr Dimitrios Doultsinos

6

Circulating tumour cell isolation and enrichment methodologies for investigation of prostate cancer metastasis

Sophia M. Abusamra, Robert Barber, Daniele Cotton, Todd M. Morgan, Ian Mills, Jason J. Davis, Alastair D. Lamb, Claire M. Edwards

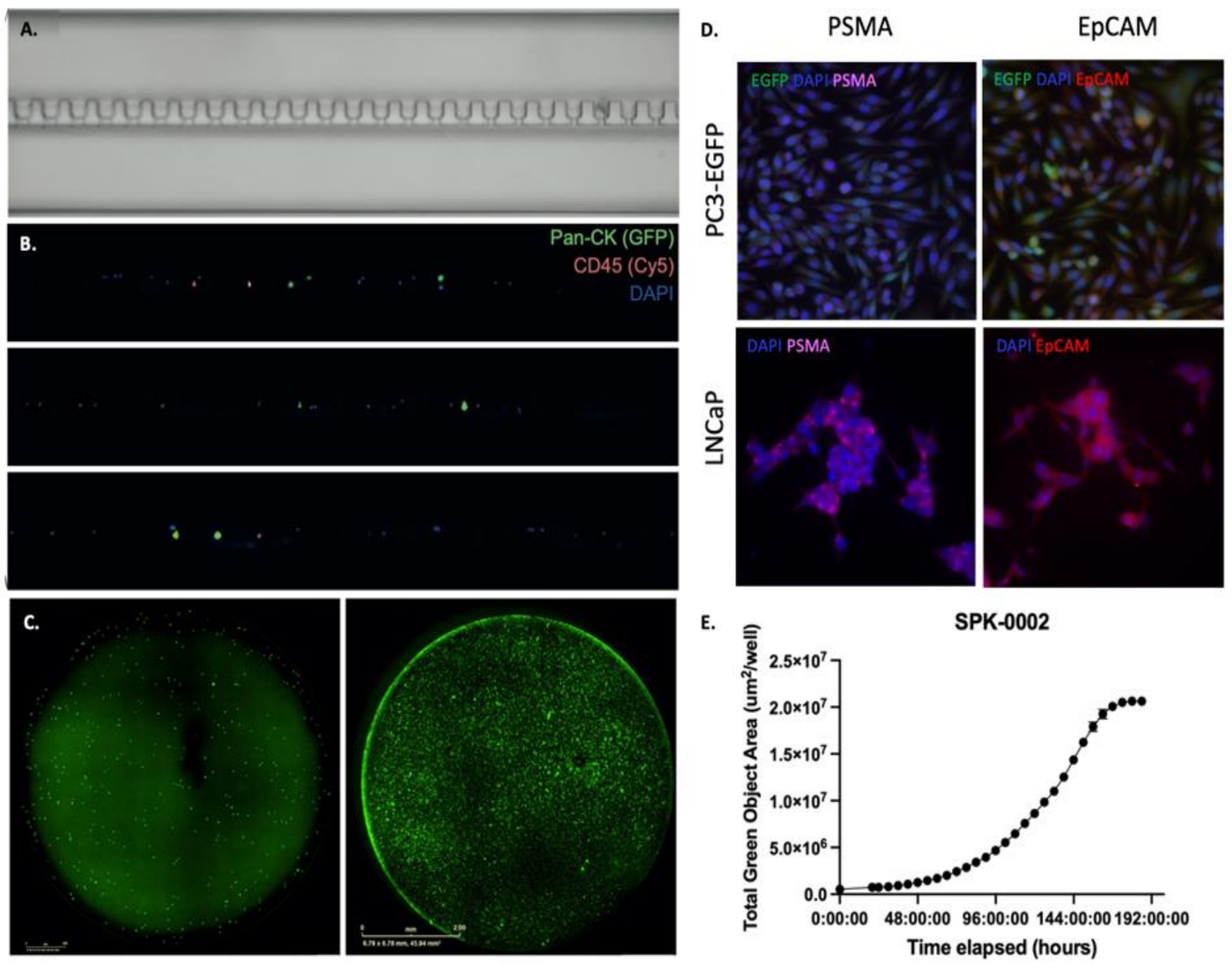
Background: Circulating tumour cell (CTC) burden is an established marker of poor prognosis and worse overall survival for patients with metastatic prostate cancer, offering insight into disease progression. Methods to enrich for CTCs often rely on immune-affinity techniques targeting EpCAM. Such methods may fail to capture CTCs that have downregulated EpCAM expression, for example as a result of epithelial-to-mesenchymal transition (EMT). Label-free methods of enrichment are able to capture CTCs in all stages of the EMT. This study aimed to assess a novel antigen-independent circulating tumour cell enrichment method via metrics including capture efficiency and purity.

Materials and Methods: Either 1) PC3 or LNCaP cells spiked into 4 mLs whole blood drawn from a healthy male donor, or 2) CTCs from blood of a metastatic prostate cancer patient were enriched for using the Genesis System with Celselect slides [BioRad] according to manufacturer's instructions. Captured cells were either fixed and stained via immunofluorescence for DAPI, PanCK, and CD45 on enumeration slides for imaging and quantification for assessment of capture efficiency based on optical identification, or placed into culture for assessment of capture efficiency using FIJI image analysis. LNCaP and PC3-EGFP cells were analysed with immunofluorescence (IF) staining of EpCAM and PSMA.

Results: We quantified captured LNCaP cells on enumeration slides, and estimated capture efficiency at 70.5%. PC3–GFP cells spiked into and enriched from whole blood proliferated in culture for 8 days following normal growth kinetics, and with a 69.1% capture efficiency. LNCaP cells displayed an $EpCAM^{positive}/PSMA^{positive}$ signature, whereas PC3-EGFP cells were $EpCAM^{positive}/PSMA^{negative}$.

Figure 1: A. Brightfield view of chambers after capture (20X). B. Fluorescent view of captured cells showing successful isolation of tumour cells (GFP) alongside WBCs (Cy5) at a ratio of 1:5, confirming >99.9% background reduction for removal of white cells. C. PC3-EGFP cells in culture at 0 and 8 days following spiking into and enrichment from whole blood. D. Fluorescent images of PC3-EGFP and LNCaP cells stained for EpCAM (Texas Red) and PSMA (Cy5). E. Growth curve for PC3-EGFP cells.

Conclusions: This label – free method of CTC enrichment was able to successfully enrich for PC3-EGFP and LNCaP cells from whole blood with high capture efficiencies (69.1% and 70.5%, respectively). Furthermore, we were able to elute cells spiked-in PC3-EGFP cells for successful proliferation in culture. We plan to apply this method to blood samples from patients with metastatic prostate cancer to isolate CTCs for downstream analyses including RNA sequencing and *ex vivo* expansion.



Contact*:* sophia.abusamra@nds.ox.ac.uk

7

Microenvironmental Induction of a Cancer Stem Cell-like State in Medulloblastoma

Background: The malignant brain tumour, sonic hedgehog medulloblastoma (SHH-MB) originates in cerebellar granule cells. The properties of cancer cells are influenced by their microenvironment, but the nature of those effects and the phenotypic consequences for the tumour are poorly understood. The aim of this study was to identify phenotypic properties of malignant granule cells that are driven by the tumour microenvironment.

Methods: Human iPSC were differentiated to cerebellar organoids to simulate the non-malignant microenvironment. Tumour spheroids were generated from two well-characterised SHH-MB cell lines which were co-cultured with cerebellar organoids. We profiled the cellular transcriptomes of malignant and non-malignant cells by droplet-based scRNA-seq (Fig. 1A). The transcriptional profiles of tumour cells in co-culture were compared with those of malignant control cells cultured as monolayers or tumour spheroids and with public SHH-MB datasets of patient medulloblastoma.1

Results: In malignant cells in co-culture, advanced differentiation markers and neural stem cell (NSC) markers were upregulated (Fig. 1B). NSC markers were associated with cancer stem cell (CSC)-like states centred on a SOX2 regulatory network (Fig. 2A) that were not observed in controls.2 An equivalent signature was found in scRNA-seq of patient SHH-MB (n = 7/8), but not in other MB subtypes (Fig. 2B, C).

Conclusions: For SHH-MB cell lines grown in co-culture with human cerebellar organoids to simulate a tumour microenvironment, malignant cell states converged towards in vivo cell states. In particular, the microenvironment non-cell autonomously induces a CSC-like state in SHH-MB cells.

Contact: john.jacob@ndcn.ox.ac.uk

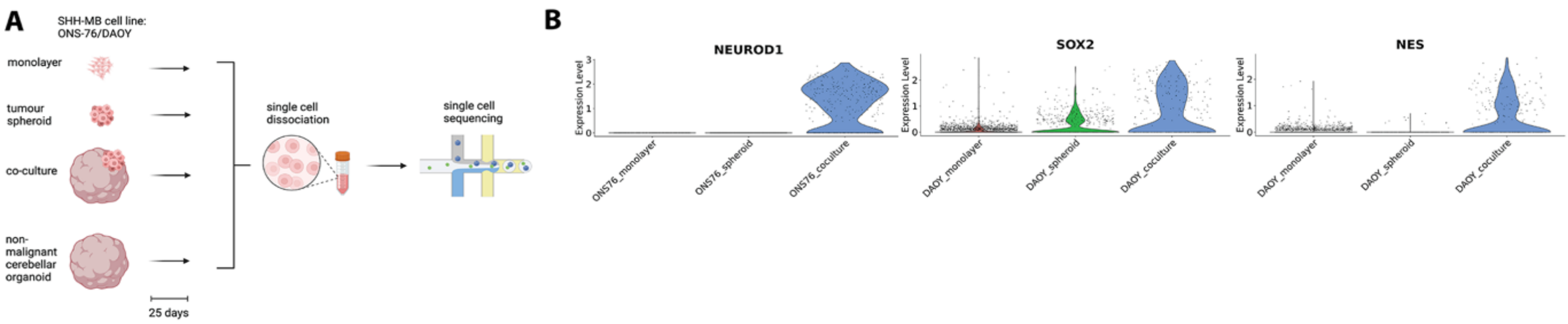


Figure 1 (A) Experimental workflow. (B) Violin plots of the granule cell post-mitotic marker, *NEUROD1* and NSC marker upregulation in malignant cells in co-culture.

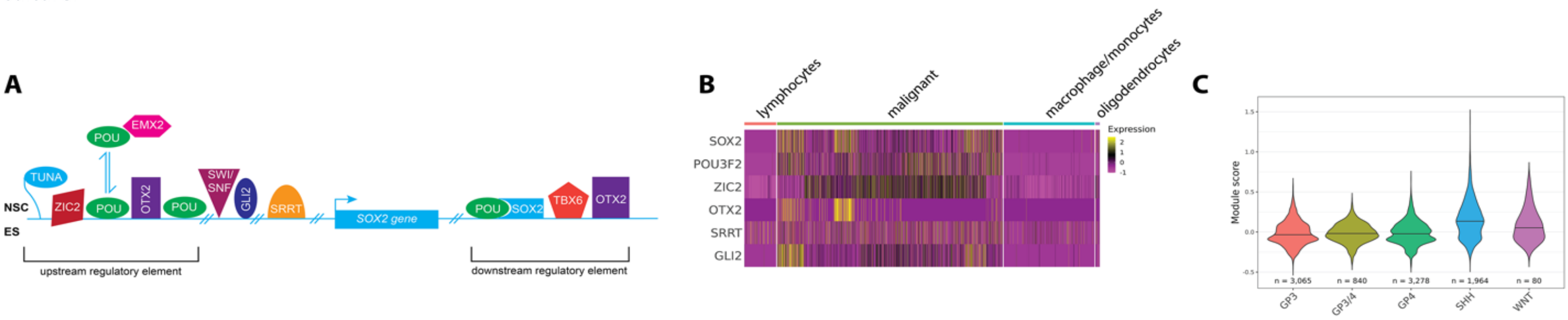


Figure 2 (A) SOX2 regulatory network in pluripotent cells.2 (B) Heatmap of SOX2 network in patient SHH-MB (dataset from 1). (C) SOX2 network signature score in patient MB.1

References: 1 Riemondy, K. A. et al. Neoplastic and immune single-cell transcriptomics define subgroup-specific intra-tumoral heterogeneity of childhood medulloblastoma. Neuro Oncol 24, 273-286 (2022). https://doi.org:10.1093/neuonc/noab135
2 Bertolini, J. et al. in Sox2: Biology and Role in Development and Disease (eds Hisato Kondoh & Robin Lovell-Badge) Ch. 11, 187-216 (Academic Press, 2016).

8

Targeting Bone Metastasis in Prostate Cancer: Isolation and Characterisation of Highly Migratory Subclones with Microfluidic Technology.

Daniele T Cotton, Joseph A E Morgan, Sophia M Abusamra, James R Edwards, Srinivasa R Rao, Edmond J Walsh, Claire M Edwards.

Background: Prostate cancer (PCa) is the most common male malignancy in the UK. Bone is a frequent and clinically important site of PCa metastasis, indicating progression to incurable disease. Though tumours are clonally derived, somatic mutations drive tumour evolution, resulting in metastasis-competent subclones that drive progression to advanced disease. Identifying these cells and their unique characteristics offers an opportunity to develop targeted therapeutics to combat the inevitably fatal outcome of PCa bone metastasis.

Methods: We deployed fluid-walled microfluidic technology to design miniaturised multiplexed assays to (1) identify rare but functionally significant subclonal populations distinguished by elevated migratory capacity and (2) study the impact of cellular crosstalk between microenvironment and tumour cells in PCa migration. Each multiplexed unit is a medium-filled circuit in a dumbbell configuration (two chambers connected by a narrow conduit), overlain with a bio-inert fluorocarbon. The fluid-walled system possesses a major advantage in that it permits live-cell retrieval at any moment for targeted study. Experiments used the bone-metastasis-derived cell line PC3.

Results: Initial validation experiments demonstrated clear preferential migration of PC3 cells towards chemoattractant (FBS) relative to controls (reversed FBS gradients and serum-free circuits). Introducing co-culture into the circuits revealed preferential migration of PCa cells to bone stromal cells when PCa cells are themselves co-cultured with bone stromal cells (HS-5 & HS-27A). Further experiments identified a unique subpopulation of highly migratory PC3 ‘super spreaders’ with morphological characteristics resembling the polyaneuploid cancer cell state – a cellular state implicated in cancer treatment resistance and relapse. We further demonstrated a reproducible workflow to isolate individual highly migratory PCa cells, harvest them with a robotic cell picker system (IotaSciences), and deposit cells into microplates for downstream analysis. Though initially non-proliferative, we demonstrated induced proliferation in highly migratory picked cells after a period of quiescence using a feeder cell bed.

Conclusion: This study leveraged cutting-edge microfluidic assays to characterise and isolate highly migratory PCa subclonal populations in monoculture and co-culture conditions. The highly migratory ‘super-spreader’ cells appear to undergo a period of quiescence followed by proliferation, reminiscent of the steps of the metastatic cascade and providing a promising avenue for targeted therapies against bone metastasis.

Contact: daniele.cotton@seh.ox.ac.uk

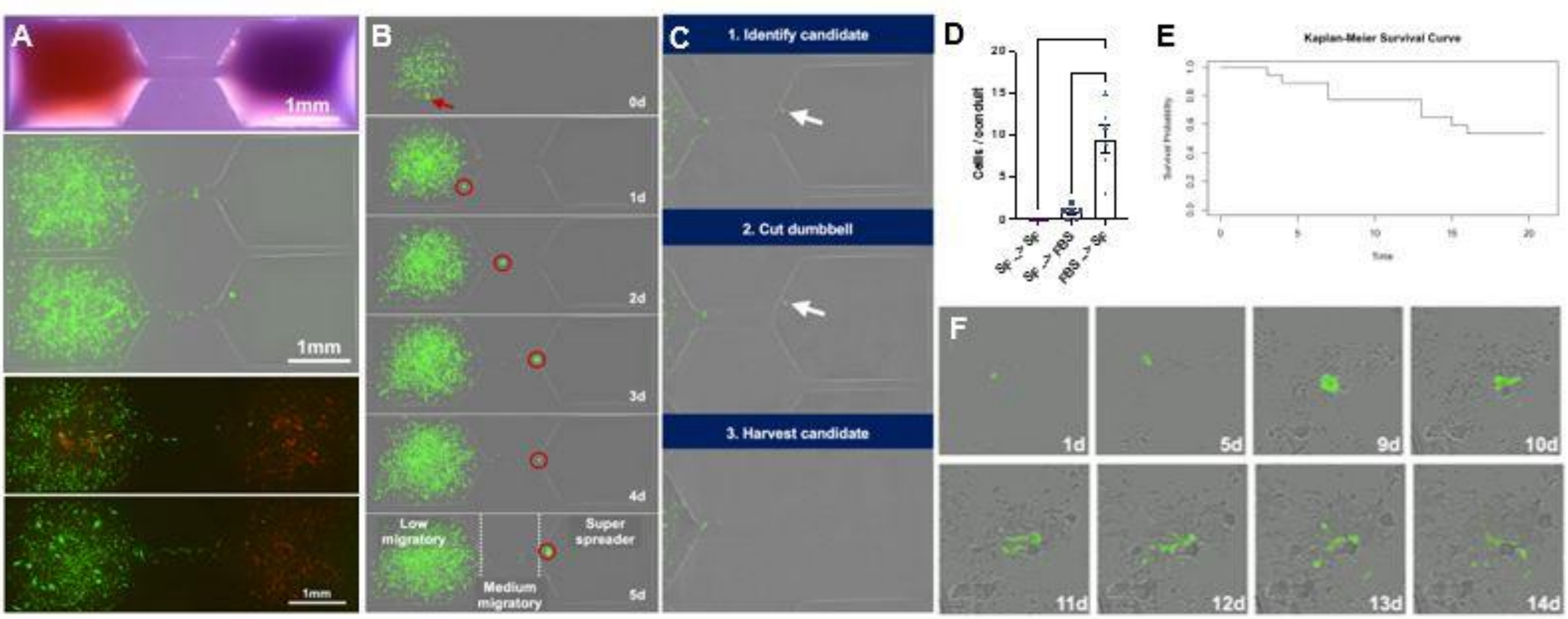


Figure 1. *A) Circuit filled with (i) dyes (ii-iii) GFP-PC3 (iv-v) varied PC3-HS5 co-cultures. B) Sample super-spreader PC3 cell trajectory. C) Migrated single-cell isolation and picking retrieval workflow. D). Quantitation of migration validation. E) Survival data for harvested super-spreaders. F) Restored super-spreader proliferation.*

9

OGT: A Regulator of Transcriptional and Oxidative Stress with Implications for Treatment Selection

Background: Our study explores the potential of an enzyme of O-GlcNAc Transferase (OGT) in prostate cancer cells. OGT is often overexpressed in prostate cancer and correlates with disease progression and poor prognosis. OGT is an enzyme that glycosylates serine/threonine residues on oncogenes and tumour suppressors thus impacting their phosphorylation, stability or activity including androgen receptor (AR) and MYC, oncogenes associated with prostate cancer (PCa) progression.

Method: We have undertaken a drug screen on prostate cancer cells to identify compounds that synergize with OGT inhibition. In addition to identifying factors that are regulated by OGT activity, we have performed whole-cell proteomics with O-GlcNAc-specific antibody enrichment of proteins and RNA-seq analysis on cells with knocked-down OGT expression.

Results: Our drug screen has identified CDK9 inhibitors as synergistic with OGT inhibition. CDK9 regulates RNA polymerase II processivity. Whole-cell proteomics with OGlcNAc enrichment identified proteins associated with the Nonspecific lethal (NSL), Nucleosome remodelliing and deacetylase (NURD) and polycomb repressive complex 2 (PRC2) complexes as OGlcNAc-modified and dependent on OGT for their stability/abundance. These complexes are responsible for generating chromatin modifications (principally H4K8Ac/H4K16Ac and H3K27me3) which regulate transcription and DNA repair. Co-treating cells with OGT siRNA and a histone deacetylase (HDAC) inhibitor, entinostat, led to the restoration of histone acetylation patterns resembling cells treated with scrambled siRNA. RNA-seq analysis revealed that genes associated with antioxidant biosynthesis, oxidative phosphorylation, autophagy, ferroptosis, and lipid metabolism were most significantly differentially expressed when OGT expression was suppressed.

Conclusion: Collectively our data implicate OGT (O-GlcNAc transferase) as a regulator of transcriptional processivity, chromatin modification and oxidative stress/lipid metabolism. Collectively these biologies are permissive for the activity of oncogenic transcription factors, principally c-Myc and the androgen receptor (AR) in prostate cancer progression. Each of these biologies is targetable and assessing OGT expression and/or activity in tumour samples may support the selection of patients most likely to benefit from these treatments. Future work will assess this and further characterise the response of pre-clinical models to drugs targeting these biologies alongside OGT.

Contact: Reema Singh1 Harri Itkonen2, Ninu Poulose1 and Ian G Mills1

1University of Oxford, Nuffield Department of Surgical Sciences, Oxford, United Kingdom 2University of Finland, Department of Biochemistry and Development Biology, Finland.

10

Leukaemic stem cells hack into epigenetic innate immune memory programs to drive myelomonocytic differentiation in TET2 mutant chronic myelomonocytic leukaemia

Richard A Salisbury[1], Sean Wen[1,2], Christina Simoglou-Karali[1] and Adam J Mead[1]

[1]MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, UK

[2]Centre for Genomics Research, Discovery Sciences, BioPharmaceuticals R&D, AstraZeneca, Cambridge, UK

Background: Chronic myelomonocytic leukaemia (CMML) is a blood cancer characterised by the proliferation of myelomonocytic immune cells within the bone marrow and peripheral blood. CMML is caused by somatic mutations in epigenetic controllers (TET2 and ASXL1), spliceosome machinery (SRSF2) and inflammatory signalling (RAS/KRAS) pathways originating in multipotent haematopoietic stem cells (HSC/MPP). Causative links between these mutations and myelomonocytic proliferation have remained elusive, although multiple studies have identified increased inflammatory cytokine signalling as a potential driver of CMML. Using single cell multiomic profiling of nuclear RNA (snRNA) and chromatin accessibility (scATAC), we found that multipotent haematopoietic progenitors are epigenetically primed for myelomonocytic differentiation. In particular through the CEBPβ mediated innate immune memory pathway that is utilised in emergency myelopoiesis. We also identify CSF1R, a downstream target of CEBPβ, as a putative therapeutic target in CMML.

Methods: 10X Multiome was performed on bone marrow mononuclear cells from 6 CMML patients and 3 Healthy donors. Cytotoxicity of CSF1R inhibition was assessed by culturing monocytes with emactuzumab, a CSF1R inhibitor, for 72hrs. Viability was assessed by CellTitre Glo. CSF1R expression was quantified by flow cytometry.

Results: Paired snRNA and scATAC data from 64423 cells were integrated to identify 10478 multipotent haematopoietic progenitor (HSC/MPP) cells. HSC/MPP subgroup analysis found enrichment of MYC-targets and MTORC1-signalling gene sets in genes upregulated in CMML. GREAT analysis of differentially accessible peaks found enrichment of Myeloid cell differentiation and activation gene sets in CMML HSC/MPPs. These gene sets were previously found to be enriched in murine HSCs that had been trained to display innate immune memory by LPS treatment[1]. Transcription factor motif enrichment analysis of differentially accessible peaks found that CEBP family motifs were highly enriched in CMML, and CEBPB had increased expression over myeloid differentiation pseudotime. CSF1R is a downstream target of CEBPβ and increased expression of CSF1R was found on CMML monocytes compared to healthy donors by flow cytometry. Inhibiting CSF1R using emactuzumab in monocytes, cultured in the presence of M-CSF (CSF1), led to a dose dependent reduction in viability.

Conclusions: HSC/MPP cells in CMML are epigenetically primed for myelomonocytic differentiation via a CEBPβ orchestrated homologous innate immune memory pathway. Targeting cytokine signalling that feeds into this pathway, such as the CSF1-CSF1R axis, may identify new therapeutic vulnerabilities in CMML.

Reference: 1. Laval et al. Cell Stem Cell(2020). https://doi.org/10.1016/j.stem.2020.01.017

Contact: Richard.salisbury@hertford.ox.ac.uk

11

IdentifyinG Radiotherapy and ImmunE ResistanCe Mechanisms In Anal CaNcer (GRECIAN)

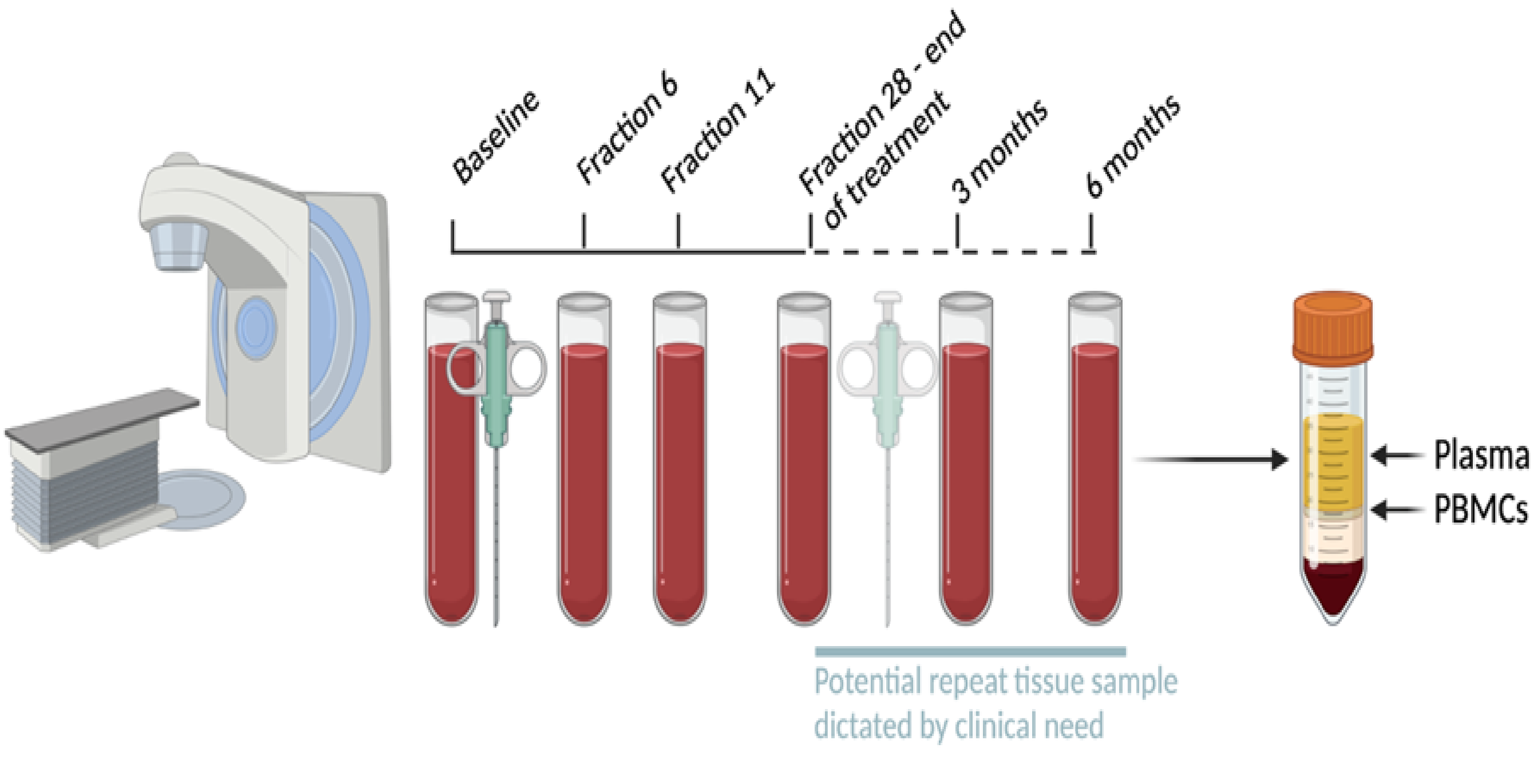
Anal squamous cell cancer (ASCC) is the most common type of anal cancer, accounting for approximately 90% of all cases. Although it is a relatively rare disease, with 1500 new diagnoses in the UK each year, incidence rates have been rapidly increasing. While chemoradiotherapy (CRT) is the standard of care for most localised anal cancers, alternative treatment strategies, including immunotherapy, are needed to decrease the risk of locoregional failure.

This project aims to use expertise across 3 RadNet Centres (Oxford, Manchester, Leeds) to identify candidate immune resistance mechanisms and potential biomarkers for locally advanced anal cancer. Serial peripheral blood and tumour samples from ASCC patients
(n = 40) were collected before, during and after CRT, alongside 6-month clinical and radiological outcomes (*Figure 1.*). Experiments studying immune resistance and potential biomarkers conducted across the centres include immunophenotyping, cytokine expression, HPV DNA expression, tumour microenvironment profiling and complement system assays.

The Olcina group (University of Oxford) are using a novel multiplex ELISA-based technology (MicroVue Complement Multiplex assays from Quidel) to simultaneously monitor multiple complement proteins per plate. A total of 15 complement proteins will be assessed per patient at baseline, during CRT, and up to 6 months post-CRT, and compared to healthy controls. This will be the first detailed characterisation of complement protein activity in CRT-treated ASCC patients, and may highlight potential prognostic and therapeutic biomarkers. Combined with the wider study data, results from GRECIAN will improve our understanding of anal cancer biology and predictors of treatment failure and clinical outcome in ASCC, aiding the establishment of treatment and clinical trial stratification methods.

Contact: dominika.majorova@oncology.ox.ac.uk

Figure 1 Blood and tissue collection schedule for locally advanced anal cancer undergoing CRT.



12

Severity of COVID-19 is associated with cancer and high expression of the RNA binding protein LARP1

James Chettle[1], Zinaida Dedeic[1], Sarah Blagden[1]

[1] Department of Oncology, University of Oxford

Background

The relationship between cancer and the risk of severe COVID-19 has been a topic of significant concern within the medical community. Individuals with cancer are known to be more prone to critical COVID-19 complications, independent of receiving chemotherapy or radiotherapy. Patients with cancer or other chronic diseases such as diabetes or hypertension, are associated with higher cellular endoplasmic reticulum (ER) stress, linked to enhanced viral burden following SARS-CoV-2 infection. We have previously shown that the RNA-binding protein LARP1, known to be aberrantly overexpressed in breast, ovarian and other cancers, drives a post-transcriptional programme of gene expression activated by ER stress. Here, we show that LARP1 drives increased SARS-CoV-2 replication *in vitro,* providing a putative link between cancer and COVID-19 susceptibility.

Method

We measured the infection and replication of several different viral variants from the pandemic (B.1.1.7, B.1.351, P1 and Omicron) in cells expressing or lacking LARP1, using both plaque assays and viral PCR. We also measured the effect of LARP1 depletion on ER stress and oxidative stress during infection, as well as the effect of the CK2 inhibitor CX-4945 (silmitasertib) , which has been tested in various cancer clinical trials and is known to inhibit the phosphorylation of LARP1.

Results

We show that LARP1 promotes increased viral replication. We demonstrate that it directly binds the SARS-CoV-2 nucleocapsid protein and viral RNA and localises to foci of replication in infected Calu3 and Vero cells. Furthermore, we show that treatment with the CK2 inhibitor CX-4945, which prevents LARP1 phospho-activation, inhibits SARS-CoV-2 replication.

Conclusions

We show here that LARP1 directly binds SARS-CoV-2 and drives its replication *in vitro*. Inhibition of LARP1 phosphorylation, using the CK-2 inhibitor CX-4945, reduces SARS-CoV-2 replication in all cell lines tested. As LARP1 is over-expressed in cancers and immune cells, this could provide a mechanistic link between cancer, ER stress, and SARS-CoV-2 susceptibility. This provides a mechanism by which cancer patients (and those with other chronic ER stress-associated conditions) are more susceptible to SARS-CoV-2 and possibly other mRNA viruses.

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Detection and characterisation of whole genome duplication using a novel spatial whole genome sequencing method

Srinivasa Rao[1], Clare Verrill[1], Willem Bonnaffe[1], Jasmine Reese[2], Olaf Ansorge[2], Simon Davis[3], Roman Fischer[3], Claire Edwards[1], Ian Tomlinson[4], Jens Rittscher[5], Ian Mills[1], Dan Woodcock[1], Freddie Hamdy[1]

Presenting author: Dr. Srinivasa Rao, Srinivasa.rao@nds.ox.ac.uk

1 Nuffield Dept. of Surgical Sciences, 2 Nuffield Dept. of Clinical Neurosciences, 3 Nuffield Dept. of Medicine, 4 Dept. of Oncology, 5 Dept. of Engineering Science, University of Oxford

Copy number profiling is performed in cancer samples using whole genome or exome sequencing. However, bulk sequencing results in loss of histological context. We developed a novel method called Adaptive Resolution Multiscale Spatial Whole Genome Sequencing (ARMS WGS) to characterise the whole genome in formalin fixed paraffin embedded tissue sections. A 4 µm section from a prostate cancer patient sample consisting of high grade, low grade, and benign regions was divided into an unbiased grid of 96 x 1 mm^2 tiles. Uniquely barcoded and extraction-free whole genome libraries from each of these tiles were pooled and sequenced on the Illumina platform.

We successfully called whole genome copy number profiles in each 1 mm^2 tile. The copy number calls were confirmed by comparison with bulk whole genome sequencing data from matching regions. Furthermore, copy number calls from each tile were included in Principal Component Analysis (PCA), showing clear clustering of the high grade region tiles distinct from the low grade and benign region tiles. The copy number profiles in the high grade region were consistent with whole genome doubling (ploidy > 3), whereas the low grade region was diploid. Nuclei in the high grade region were also more intensely stained (Haematoxylin OD mean and OD sum) ($p < 0.000$) and exhibited differences in glandular architecture compared to the low grade region.

This method has the potential to call single nucleotide variants in addition to copy number alterations, greatly adding to the information that can be obtained from other spatial methods.

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Prostate Cancer – spatial phenotyping in mice and men

Prostate cancers are frequently multifocal, with individual tumours in various stages of aggressiveness within the organ. Analysis of large-scale genomic studies on prostate cancer cells concluded that genetic instability such as losses and gains in copy numbers relates to poor-prognosis for the patient. At the same time, genetic instabilities of prostate cancer cells alone do not fully account for the biology of these tumours, as the tumour micro-environment rapidly adapts to genetic changes. However, we can learn from the cell-interaction dynamics in prostate cancer by drawing a spatial map of the tumour micro-environment. We believe that in the future such a map will assist in the design of combination therapy. We applied *PhenoCycler* multiplex imaging platform that uses antibodies conjugated to a unique oligonucleotide sequence. A panel of antibodies targeting the tumour microenvironment and key immune cell components was composed and samples from mouse model of prostate cancer and human tissue from radical prostatectomy were imaged. *PhenoCycler* multiplex imaging has identified at least five groups of prostate epithelial cells that display phenotype and show behaviour associated with migration and invasion in the human samples such as vascular co-option and perineural invasion. These events were observed within the same cross section taken from a prostatic disk. Detailed proteomic and single cell spatial transcriptomic evaluation and analysis is ongoing, while we are exploring alternative data analysis through machine learning algorithms.

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Predictive accuracy of standard versus flexible parametric survival models for long-term cancer survival

Background: Estimates of long-term cancer survival are important for funding recommendations of new cancer treatments, as they can play a key role in impacting cost-effectiveness estimates used in health technology assessment (HTA). Due to the relatively limited follow-up observed in clinical trials, standard parametric models are frequently used to extrapolate long term survival outcomes beyond the trial period. There is a growing awareness that standard models may not be sufficiently flexible to capture complex hazard patterns and may be limited in accurately predicting long term survival. The appropriateness of flexible parametric models for predicting long term cancer survival is unclear. The aim of this study is to investigate if flexible parametric models perform better than standard parametric models in predicting long term survival for eight different cancers using primary care records from the United Kingdom.

Methods: Adults with a primary incident diagnosis of breast, colorectal, pancreatic, liver, stomach, prostate lung, or head and neck cancer were selected from CPRD GOLD between 2000 to 2019 with a potential follow-up time of 20 years. Follow-up time for each cancer cohort was right censored at two years. Standard parametric models (exponential, Weibull, Gompertz, log-logistic, log-normal, generalised gamma) and flexible parametric spline models were fitted to each right-censored dataset. Goodness-of-fit (GOF) was assessed using Akaike Information Criterion (AIC). Prediction accuracy was assessed by comparing predicted 10-year restricted mean survival time (RMST) and percentage surviving at 10 years with observed values from Kaplan Meier estimates.

Results: Flexible parametric spline models generally performed best in terms of GOF and prediction accuracy across all cancers with absolute errors ranging from 0.05 to 7.34. However, most models underpredicted 10-year survival compared to Kaplan Meier estimates (80%). The poorest performing models, in terms of GOF and prediction accuracy, were the exponential and Weibull standard parametric models with absolute errors ranging between 4 and 25 across all cancers.

Conclusions: The findings emphasise the enhanced performance of flexible parametric spline models in predicting long-term cancer survival compared to standard parametric models. Flexible parametric models should be routinely assessed alongside standard parametric models when extrapolating cancer survival data for HTA submissions. Further research is required to determine applicability of findings to trials.

Contact: danielle.newby@ndorms.ox.ac.uk

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The role of C5aR1 under hypoxia in cancer

Background: Cancer cells acquire treatment resistance due to the stress in the tumour microenvironment (TME). Immune evasion is one of the major reasons, however, targeting the immune system often increases adverse events. The complement system is an innate immunity that plays different roles in tumours and normal tissues. The complement system is supposed to induce inflammation post treatment in normal tissue, while complement proteins are locally produced in TME to create a disordered complement system, leading to tumour malignancies. Through *in silico* screening, we previously identified a complement receptor, C5AR1, as a potential target to increase anti-tumour effects without causing adverse events, and reported the effects of C5aR1 inhibitors in a colorectal cancer mouse model. However, the role of C5aR1 in TME remains unclear.

Method & Results: In this study, we found that intracellular C5aR1 is upregulated in a UPR-dependent manner under severe hypoxia in various types of cancer, contributing to cancer cell survival in TME. IHC analysis using *in vivo* and clinical samples suggested that C5aR1 highly expresses in tumour, particularly in hypoxic regions, compared with normal tissue, and that higher C5aR1 expression correlated with poor prognosis in various kinds of cancer patients.
In vitro experiments showed that C5aR1 mRNA and protein levels increased in a UPR-dependent manner under severe hypoxia. The increased C5aR1 was intracellular but not on the cell membrane. We found that the increased C5aR1 reduced apoptotic cell death and increased cell survival under hypoxia; RNA-seq showed that C5aR1 knockdown suppressed gene biosynthesis pathways, particularly at translation level, under hypoxia.

Conclusions: Taken together, these results show the molecular mechanisms by which C5aR1 induces cancer cell survival in TME. Further studies will be performed to obtain POC for targeting intracellular C5aR1.

Contact: tatsuya.suwa@oncology.ox.ac.uk

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Spatial transcriptomic clonal deconvolution identifies the ‘lethal clone’ in prostate cancer as defined by ability to metastasize to lymph nodes

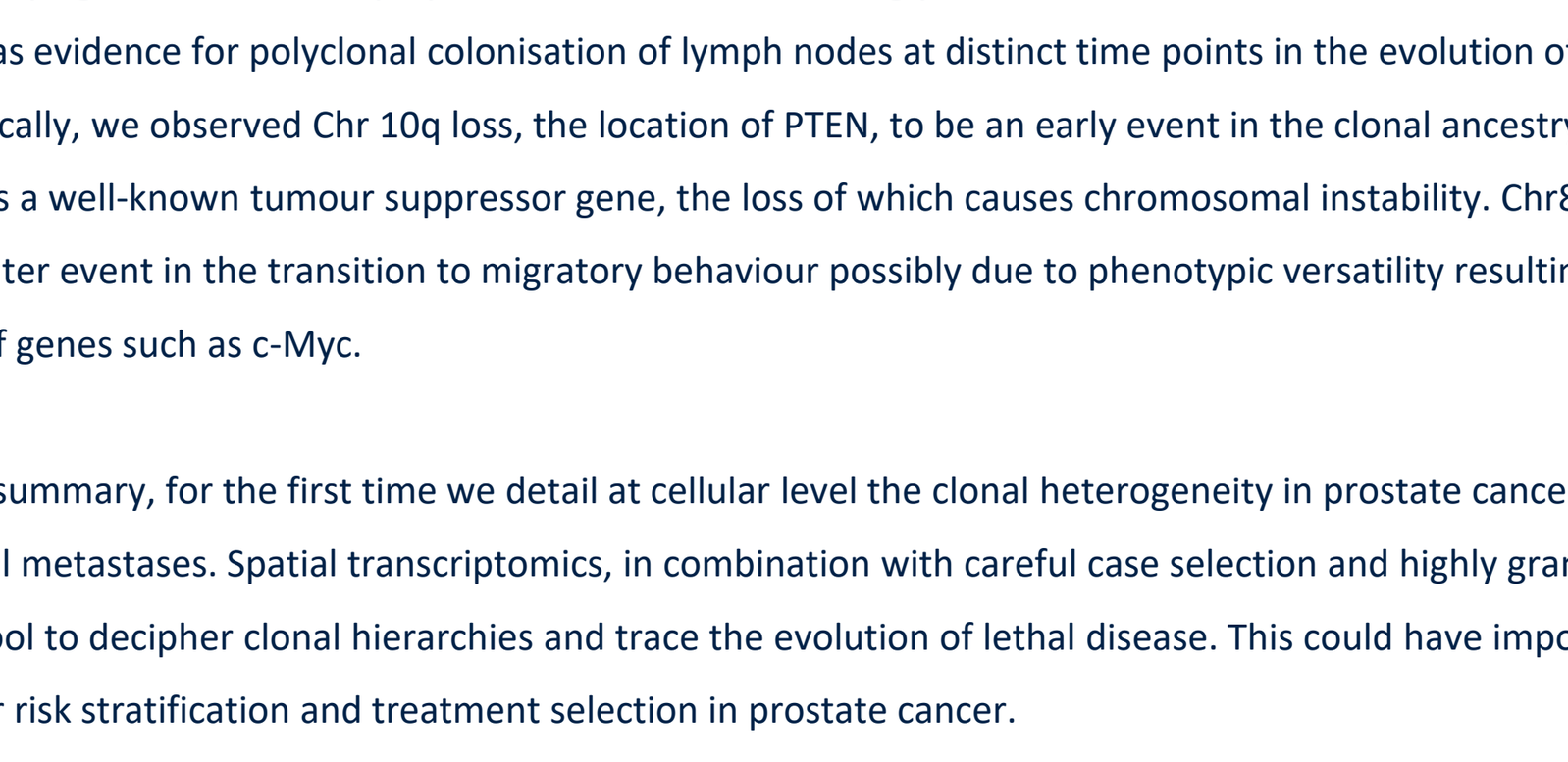
Introduction: Prostate cancer epitomises intratumoural heterogeneity, a phenomenon that has hindered accurate risk stratification and treatment. A distinctive feature of this heterogeneity is the frequent occurrence of copy number alterations acquired by dividing cells, which can be used to define clonal heritage. The precise contribution of local cancer clones to lethal metastatic prostate cancer has been unclear. This study leveraged cutting-edge spatial transcriptomics technology to infer clonal genomic identity in prostate cancer to identify regions of primary tumour that can metastasize to lymph nodes.

Methods: We collated FFPE samples from primary and nodal metastatic tissue for four patients recruited to a local trial (ISRCTN10046036). We performed organwide spatial transcriptomics (Visium™ v2, 10x Genomics), processed fastq files using SpaceRanger™ and analysed according to our published protocol (https://github.com/aerickso/SpatialInferCNV). Each section contains approximately 5,000 (6.5mm sq) or 14,000 (11 mm sq) spots of 55 µm diameter – each spot underwent meticulous histological annotation by a certified pathologist. We show the clonal relationships, location and distribution throughout the prostate and nodal environment.

Results: Focussing on epithelial tissue alone, we identified 11 clonal subtypes. We show the distinct features of specific clones which exist in both in the primary prostate and lymph nodes (“X clones”) as well as their clonal ancestry (figure 1). We constructed a phylogenetic tree to display clone evolution. Interestingly, we also observed subclonal events within the lymph nodes, as well as evidence for polyclonal colonisation of lymph nodes at distinct time points in the evolution of primary disease. Specifically, we observed Chr 10q loss, the location of PTEN, to be an early event in the clonal ancestry of lethal disease. PTEN is a well-known tumour suppressor gene, the loss of which causes chromosomal instability. Chr8 gain/loss was an important later event in the transition to migratory behaviour possibly due to phenotypic versatility resulting from amplification of genes such as c-Myc.

Conclusion: In summary, for the first time we detail at cellular level the clonal heterogeneity in prostate cancer, with direct linkage to nodal metastases. Spatial transcriptomics, in combination with careful case selection and highly granular pathology, is a powerful tool to decipher clonal hierarchies and trace the evolution of lethal disease. This could have important implications for risk stratification and treatment selection in prostate cancer.

Contact: Wencheng.yin@nds.ox.ac.uk



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Investigating tissue/tumour immune microenvironment after FLASH radiotherapy

Authors: Jia-Ling Ruan[1], Carl Lee[2], Shihong Wu[3], Doreen Lau[4], Iain DC Tullis[1], Dylan Windell[2], Christian Cooper [1], Kristoffer Petersson[1]

[1]Department of Oncology, [2]Kennedy Institute of Rheumatology, [3]Department of Biochemistry, [4]Centre for Immuno-Oncology, University of Oxford

Radiotherapy delivered at ultra-high dose rates (≥ 40 Gy/s, FLASH IR) has shown promise in reducing normal tissue toxicity, like radiation-induced neurotoxicity, while maintaining cancer management efficacy comparable to conventional dose rates (< 0.1 Gy/s, CONV IR). Despite these promising outcomes, the precise mechanisms underlying the modulation of the local tumor or tissue microenvironment by FLASH IR remain poorly understood.

In this comprehensive study, we seek to elucidate the impact of FLASH IR and CONV IR on both brain and clinically relevant muscle-invasive bladder tumors using a variety of advanced imaging and analysis techniques. Alterations in protein expression profiles of different cell types within the tumour and brain tissue were assessed by multiplex immunofluorescent imaging multiplexed ion beam imaging. Additionally, three-dimensional visualization of tissue morphology was conducted through light sheet microscopy. Immune cell populations in the tumour microenvironment were characterized by multicolour flow cytometry, and the systemic immune response evaluated by whole-body immune tracking.

By integrating these methods, we uncovered novel insights into the complex interplay between radiation dose rate and the tumour/tissue microenvironment. This knowledge could inform the development of more effective cancer radiotherapy strategies.

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Is risk of immune escape inversely proportional to T-cell production?

The risk of cancer and infectious disease rises with age, often exponentially at 4.5% per year. This means risk doubles every 16 years and is inversely proportional to T-cell production and thymus volume, which both halve every 16 years (starting in adolescence) [1]. Diseases with this property include cancers such as brain cancers and chronic myeloid leukemia, as well as infectious diseases such as MRSA and COVID-19 [2]. I will discuss these trends as well as a mechanistic mathematical model which involves T-cell exhaustion and an immune escape threshold (around the order of 106 cells) [1]. Supporting evidence for this model includes inverse gender biases in disease risk and thymic output, approximately constant risk of T-lymphoblastic leukemia with age, and experimental results involving cancer inoculations in mice.

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Genetically engineering T cells to reduce the risk of autoimmune cross-reactivities in T cell therapies

Background: T cell receptor-engineered T cell (TCR-T) therapies are limited by the potential risk of cross-reactivity against healthy tissues. For example, T cells engineered with the MAGE-A3 specific a3a TCR caused a lethal autoimmune toxicity due to cross-reactivity to a lower affinity peptide derived from the muscle protein titin. Therefore, there is an urgent need to identify methods that reduce T cell activation to lower-affinity self-peptides, whilst maintaining a potent response to higher-affinity target peptides.

Methods: We have selected the well-studied NY-ESO-1 specific c259 TCR to study ligand discrimination. Firstly, we have measured the affinity of a panel of 8 peptides to the c259 TCR by Surface Plasmon Resonance. Using this panel, we performed a targeted CRISPR-Cas9 knock-out screen in human primary T cells to identify genetic modifications that can modulate ligand discrimination.

Results: We have generated a panel of 8 peptides spanning affinities from 1-100 µM to the c259 TCR. We then identified genetic modifications that can selectively abrogate T cell activation against lower affinity peptides, whilst maintaining a potent response to higher affinity peptides. Finally, we have demonstrated that these genetic modifications can abolish a3a TCR T cell cross-reactivity to titin, without reducing activation against MAGE-A3, its target tumour peptide.

Conclusions: Our findings suggest that T cells can be engineered to exhibit different degrees of enhanced ligand discrimination. This approach could be applied to other clinically relevant TCRs for the development of T cell therapies with high efficacy and reduced risk of lethal autoimmune toxicities.

Contact: jose.cabezascaballero@wadham.ox.ac.uk

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Defining er-associated degradation factors required for cancer cell survival

Background: Constitutively engaging the pro-survival branches of the unfolded protein response (UPR) permits cancer cells experiencing lethal proteotoxic stress levels in the endoplasmic reticulum (ER) to remain viable. Disabling UPR-mediated adaptation represents a potential strategy for cancer therapies to break this form of non-oncogenic addiction. The Hrd1 ubiquitin ligase complex is the principal executor of ER-Associated Degradation (ERAD), clearing proteotoxic burden and mitigating ER stress. ERAD capacity through Hrd1 is a key aspect supplemented by the UPR's pro-survival Ire1-XBP1 and ATF6 branches through transcriptional upregulation of key genes in the Hrd1 complex.

Method/results: To assess whether attenuating ERAD could compromise viability of stressed cancer cells, individual Hrd1 complex components were knocked down by shRNA in multiple myeloma (AMO-1/L363) cell lines. Using both 2D growth and 3D spheroid assays, Hrd1 and important cofactors were found to be required for cell growth and viability. We next modelled ER stress in cancer cells using a HeLa cell line expressing a mifepristone-induced IgM heavy chain (μS). Hrd1 and its cofactors were dispensable for unstressed cells while ER stress elicited by μS induction revealed dependence on the Hrd1 complex and its ubiquitylating activity. To establish the contribution made by ERAD to ER stress resolution and viability made by the Hrd1 complex added during the UPR, we generated μS-expressing HeLa cell lines in which canonical XBP1/ATF6^{p50} transcription factor (TF)-binding ERSE sequences of Hrd1, Herp and Derl3 were mutated by CRISPR/Cas9. Disrupting TF binding sites in Hrd1, Herp and Derl3 was sufficient to decouple each factor from UPR regulation, indicated by blunted responsiveness of genes to ER stress induced by μS. Decoupling UPR regulation for Hrd1, Herp and Derl3 attenuated growth upon μS induction, but neither were as severe as complete knockouts.

Conclusions: These data indicate the key roles played by the Hrd1 complexes constitutively expressed and those supplementing ERAD activity during the UPR. Together with ongoing studies of composition changes in Hrd1 complexes, these studies will provide insight toward identifying the key targets for small-molecule inhibitors aimed specifically at selectively disrupting the Hrd1 complex, as new avenues for therapeutic benefit in cancer.

Contact: giorgia.gaeta@st-hughs.xo.ac.uk

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Histological types of invasive breast cancer in 830,000 women diagnosed in England during 1988-2016

Kezia Gaitskell*,[1-3] Jake Probert,[1] John Broggio,[4] Sarah C. Darby, [1] Paul McGale, [1] David Dodwell, [1,3] Carolyn Taylor [1,3]

[1]Nuffield Department of Population Health, University of Oxford, Oxford, UK

[2]Radcliffe Department of Medicine, University of Oxford, Oxford, UK

[3]Oxford University Hospitals, Oxford, UK;

[4]National Disease Registration Service, NHS England, Birmingham, UK

Background: Breast cancer can be categorised into different histological types, based on microscopic appearances. There is some evidence that these histological types are associated with different patient and tumour characteristics, but few previous studies have been large enough to investigate this systematically, including rare histological types.

Methods: National cancer registration data were used to describe trends in incidence of specific histological types of invasive breast cancer in women diagnosed when aged 18-89 years in England from January 1988 to December 2016, and to investigate associations between breast cancer histological types and patient and other tumour characteristics.

Results: There were 838,776 women diagnosed with a first primary invasive breast cancer in this 29-year period, including 614,698 (73%) cases of ductal carcinoma NST (no special type), 90,028 (11%) lobular carcinomas, and more than 16,000 (2%) each of tubular and mucinous carcinomas. Rarer histological types included medullary, metaplastic, papillary and cribriform carcinomas, with >1000 cases of each. Data quality and completeness improved substantially during the study period. The different breast cancer histological types showed different patterns in incidence by age at diagnosis, calendar period of diagnosis, and breast screening status. In the 246,477 women diagnosed during 2010-2016, the breast cancer histological types also showed different associations with tumour characteristics such as grade, stage at diagnosis, and molecular subtype.

Conclusions: This large national study provides an overview of incident invasive breast cancer in England over almost 30 years and gives an opportunity to investigate the characteristics of rare histological types, which smaller studies have been unable to explore. The results also reveal the continuing value of histological types defined by microscopic morphology, alongside newer molecular classifications.

Contact: Kezia.gaitskell@ndph.ox.ac.uk

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Effects of C5aR1 Inhibition in Tumour on Macrophage Immunogenic Cell Death

Background: Despite being one of the crucial components of the innate immune system, the role of the complement system within the tumour microenvironment (TME) remains poorly understood. Previous studies have shown that targeting C5aR1, a G protein-coupled receptor for the most potent complement anaphylatoxin C5a, can improve radiotherapy effectiveness. Within the immune population, macrophages were found to increase post-radiation and were associated with high C5aR1 expression. Radiation-induced immunogenicity within the TME is facilitated by immunogenic cell death and macrophages play a major role in this process. This project investigates the effects of C5aR1 on immunogenic cell death in macrophages, triggered by Damage-Associated Molecular Patterns released by tumour cells following radiation treatments.

Methods: RT-qPCR and Western Blots were used to establish genetic and protein expression of necroptosis and pyroptosis gene. LDH assay was carried to determine necrosis cell death of macrophages. Finally, cytokine array was carried to determine cytokine release by tumour cells following C5aR1 inhibition.

Results: We found that C5aR1 inhibition in tumour cells may prime macrophages for necroptosis, potentially via an altered cytokine release profile. However, when combined with radiotherapy, C5aR1 inhibition appears to have the opposite effect and instead rescues macrophage cytotoxicity.

Conclusion: Preliminary findings indicate contrasting effects of C5aR1 inhibition on necroptotic behaviours in macrophages, contingent upon the presence of radiotherapy. Future works will focus on identifying DAMPs released by tumour cells following radiation and investigate how the DAMPs profile changes with C5aR1 signal inhibition.

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Tetrameric INTS6-SOSS1 complex facilitates DNA:RNA hybrid autoregulation at double-strand breaks

Qilin Long[1], Kamal Ajit[1], Katerina Sedova[2], Vojtech Haluza[2], Richard Stefl[2,3], Sadat Dokaneheifard[4], Felipe Beckedorff[4], Monica G Valencia[4], Marek Sebesta[2], Ramin Shiekhattar[4] and Monika Gullerova[1,*]

[1] Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE, United Kingdom

[2] CEITEC-Central European Institute of Technology, Masaryk University, Brno CZ-62500, Czech Republic

[3] National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno CZ-62500, Czech Republic

[4] Department of Human Genetics, University of Miami, Miller School of Medicine, Sylvester Comprehensive Cancer Center, 1501 NW 10th Avenue, Miami, FL 33136, USA

* Correspondence to: monika.gullerova@path.ox.ac.uk

Background: DNA double strand breaks (DSBs) represent a lethal form of DNA damage that can trigger cell death and initiate oncogenesis. The activity of RNA polymerase II (RNAPII) at the break site is required for efficient DSB repair. However, the regulatory mechanisms governing the transcription cycle at DSBs are not well understood.

Method: In this study, we conduct both *in vitro* and *in vivo* methods, including electrophoretic mobility shift assay (EMSA), Proximity ligation assay (PLA), Co-immunoprecipitation (CoIP), Chromatin immunoprecipitation (ChIP), Chrome RNA sequencing (ChrRNA -seq) *etc.*

Result: Here, we show that Integrator complex subunit 6 (INTS6) associates with the trimeric SOSS1 (comprising INTS3, INIP, and hSSB1) to form a tetrameric SOSS1 complex following DNA damage. INTS6 binds to DNA:RNA hybrids and plays a crucial role in Protein Phosphatase 2 (PP2A) recruitment to DSBs, facilitating the dephosphorylation of RNAPII. Furthermore, INTS6 prevents the accumulation of damage-induced RNA transcripts (DARTs) and the stabilization of DNA:RNA hybrids at DSB sites. INTS6 interacts with, and promotes the recruitment of Senataxin (SETX) to DSBs, facilitating the resolution of DNA:RNA hybrids/R-loops.

Conclusion: Our results underscore the significance of the SOSS1 complex in the autoregulation of DNA:RNA dynamics and the promotion of efficient DNA repair.

Contact*:* Qilin Long, qilin.long@path.ox.ac.uk

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Utilising Mass Spectrometry to Spatially Interrogate the Immunopeptidome of Colorectal Cancer

Colorectal cancer (CRC) is the third most prevalent malignancy and second leading cause of cancer-related mortality worldwide. Despite recent advances in screening, predictive high throughput sequencing technologies, and emerging targeted therapies, CRC incidence is projected to increase by close to 20% by 2030. Excitingly, several large-scale prognostic assessments have highlighted a meaningful association between the density and location of tumour infiltrating lymphocytes (TILs) and the increased overall survival of CRC patients[2]. The significant disease burden of the cancer, paired with the presence of biologically relevant and immunogenic antigens in CRC tumours are therefore indicative of a pressing need for novel immunotherapeutic strategies for the disease. However, early-stage clinical trials involving immune checkpoint inhibitors (ICIs) and neoantigen-based vaccines have yielded positive results for only a small proportion of CRC tumours, the majority of which were characterised by high mutational burden and microsatellite instability[2]. Since approximately 80% of CRC tumours are microsatellite stable[2], it is vital to identify novel, actionable, and tumour-specific antigens, which could be effectively exploited for immunotherapeutic gain. In the present study, we employed immunopeptidomics – the most effective method for detection of targetable antigens for T cell recognition – to spatially characterise the inter- and intra-tumoral antigenic landscape of patient biopsies, whilst using normal adjacent tissue samples as controls. Peptides presented on class I human leukocyte antigens (HLA-I) on the surface of cancer cells were immunoaffinity-purified and subjected to data-dependent acquisition (DDA) using liquid chromatography with tandem mass spectrometry (LC-MS/MS) on LCUltiMate™ 3000 RSLC nano-HPLC and TimsTOF SCP. Subsequent spectral matching against all reviewed human SwissProt protein entries and filtration using a peptide-level false discovery rate (FDR) of 1% were performed using the MSFragger (v4.0) proteomics software (Nesvizhskii). A total of 51,217 unique class-I restricted peptides were identified exclusively in the cancer compartment across 12 tumour biopsies from 4 CRC patients. Of those 25,027 8-14 amino acid-long peptides were discovered in more than one patient, while 2,429 antigens were shared between all 4 patients. Validation against the Human Protein Atlas highlighted 23 peptides originating from 17 proteins (16 of which are known testis proteins) as potential tumour-associated (cancer/testis) antigens with immunotherapeutic potential. A number of identified peptides were derived from source proteins encoded by oncogenes known to be involved in CRC progression, which is indicative of their possible therapeutic utility. These findings could aid in understanding the spatial presentation of candidate targets in patients, which could benefit the development of specific T cell-based vaccines with translatable therapeutic potential.

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Prediction of recurrence free survival of head and neck cancer using PET/CT radiomics and clinical information

The 5-year survival rate of Head and Neck Cancer (HNC) has not improved over the past decade and one common cause of treatment failure is recurrence. For this research, we built Cox proportional hazard (CoxPH) models that predict the recurrence free survival (RFS) of oropharyngeal HNC patients. Our models utilise both clinical information and multimodal radiomics features extracted from tumour regions in Computed Tomography (CT) and Positron Emission Tomography (PET). Furthermore, we were one of the first studies to explore the impact of segmentation accuracy on the predictive power of the extracted radiomics features, through under- and over-segmentation study. Our models were trained using the HEad and neCK TumOR (HECKTOR) challenge data, and the best performing model achieved a concordance index (C- index) of 0.74 for the model utilising clinical information and multimodal CT and PET radiomics features, which compares favourably with the model that only used clinical information (C-index of 0.67). Our under- and over-segmentation study confirms that segmentation accuracy affects radiomics extraction, however, it affects PET and CT differently.

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Live PDAC tumour slices for the interrogation of CAR-T cell efficacy in pancreatic cancer

'Chimeric antigen receptor (CAR) T cell therapy has improved the prognosis of haematological malignancies and increasing efforts are being made to translate this success to solid tumours. Often candidates show success in preclinical testing which fails to be replicated at clinical trial due to the lack of patient relevance of many *in vitro* models. Patient-derived organotypic tumour slices preserve the structural integrity, cytokine landscape and cellular heterogeneity of the native tumour. This information provides valuable insight into barriers preventing clinical efficacy which cannot be assessed in other model systems.

We are using live tumour slices to assess the efficacy of a novel CAR-T cell therapy in pancreatic ductal adenocarcinoma (PDAC). The preservation of physicochemical properties is particularly important in this disease as PDAC is characterised by a rich desmoplastic stroma – presenting a physical barrier to infiltrating immune cells.

Exploration of the platform in the assessment of novel therapies has involved investigation of target antigen expression, development of imaging techniques and generation of CAR-T cells from patient PBMCs. We have determined CAR-T cell efficacy in vitro and identified the ability of activated patient T cells to infiltrate the tumour microenvironment (TME) ex vivo. We are analysing matched patient CAR-T cell distribution within the tumour slice and progress towards live cell imaging to visualise activation in situ. This patient specific model will determine major barriers hindering efficacy and allow identification of potential treatment combinations'

Contact: alice.evans@keble.ox.ac.uk

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Causal attribution of smoking and BMI to disease

Observational studies typically report “hazard ratios” (for longitudinal cohort studies) and “odds ratios” (for case-control studies), for associations between diseases and potential risk factors. Although hazard and odds ratios can be rigorously calculated from data, they cannot quantify the absolute burden of disease in a population, or allow extrapolation of disease risk to ages outside of the population studied. As others have pointed out [1], causal assumptions are necessary for the statistical analysis of most observational studies [2], but this is rarely acknowledged [3]. Consequences include the “Table 2 fallacy” [1], where estimates for causal quantities are presented equivalently to estimates of parameters that adjust for confounding factors, whose interpretation are often unclear. When stated explicitly, causal assumptions in the form of a directed acyclic graph (DAG), can allow a much broader range of quantities to be considered [2,4]. As an example, circumstances are described where proportional hazard estimates can be used to estimate attributable fractions, that quantify the proportion of disease caused by an exposure [4]. The approach is illustrated by estimating the influence of smoking and BMI on a range of diseases in UK Biobank [4].

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Quantifying cellular microenvironments in multiplex images

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.

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T cells are physics gurus: let’s put them to the test

Hannah Dada, Kun Do, Huw Colin-York, Alexander Cartagena-Rivera, Marco Fritzsche[3] Michael L. Dustin, Grégoire Altan-Bonnet

Background: Improving upon T cell therapies is critical in the fight against cancer. Current immunotherapies such as T cell-based therapies mostly take advantage of the biochemical features of T cell activation and function, such as strengthening the antigenic responses of T cells. Although these immunotherapies have shown impressive responses in patients, the overall response rate is only about 15-20% and the benefits are largely specific to tumor type. In addition to the variability that exists the in response based on tumor type, there is also stark differences in the amount of T cell-based therapies required to realize a treatment benefit. Treating a liquid tumor such as lymphoma requires 3x10^5 CAR-T cells per kg while treating a solid tumor such as melanoma requires 10^7 tumor infiltrating T cell lymphocytes per kg. These variabilities present in tumor types regarding therapy efficacy and amount of T cells needed to confer response indicates that there are other factors outside of biochemical features that govern T cell immune response. Since the differences seen are in tumors of varying physical attributes, the biophysical properties and the physical environment of T cells could also be relevant to T cell function. Teasing out the key mechanical properties essential for maximal T cell capacity is important in improving treatment outcomes for patients regardless of their tumor type.

Methods: Polyacrylamide (PAA) and other hydrogels (i.e. alginate and polydimethylsiloxane [PDMS]) are used to vary the mechanical environment experienced by T cells. The PAA gels consist of different concentrations of acrylamide and the cross-linker bis-acrylamide to introduce T cells to different mechanical landscapes. Alginate and PDMS gels feature different stiffnesses by using different calcium concentrations and modifying the cross-linker respectively. Mouse and engineered human killer T cells co-cultured with either tumors of varying antigenic strengths or activated PMA+ionomycin are plated on the PAA gels. A robotic platform that enables the automatic collection of cytokines from T cells is used to assess the function of T cells on the hydrogels. Rheology and atomic force microscopy are used to measure biophysical properties including as viscoelasticity, porosity, and stiffness that might mediate T cell function.

Results: We show that the mechanical environment of T cells dictates their function. From cytokine measurements, our results suggest that not only does antigenic quality influence T-cell function, but altering the crosslinking of the PAA gel, and thus the physical environment, results in varied T-cell immune response. Low PAA gel crosslinking leads to a dramatic hindrance of T cell function, while increasing the crosslinking results in a gradual increase in T cell response. Alginate and PDMS hydrogels allowed us to rule out substrate stiffness as a mechanical mediator of T cell response.

Conclusions: Biochemical features of T cells are not the only cues that dictate T cell function. From immunotherapies, we know that the success of T cell based therapies largely depend the overall physical properties of the tumor (i.e. liquid vs solid, soft vs hard). Using a hydrogel system to vary the mechanical environment T cells find themselves in, we observe that the T cell immune response is significantly governed by the physical landscape experienced by the T cells. Measuring biophysical properties will help uncover the specific physical characteristic (i.e. viscoelasticity, porosity, etc) that dictates improved or impaired responses in T cells housed in these environments. Modeling these properties will be essential in identifying the mechanical landscape responsible for immune response, and predicting this response based on the biophysical environment of the tumor. These findings will introduce a novel approach to treating patients with tumors by assessing the mechanical properties of the tumor and changing the physical environment to one that allows for a more favorable immune response.

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KBTBD4 mutations elicit neomorphic ubiquitylation activity to promote stemness in medulloblastoma

Zhuoyao Chen [1], Rafael Ioris [2], Stacey Richardson [3], Ava Van Ess [2], Iolanda Vendrell [1, 2, 4], Benedikt Kessler [1, 4], Francesca Buffa [2], Luca Busino [5], Steven Clifford [3], Alex Bullock [1], Vincenzo D'Angiolella [2]

[1] Centre for Medicines Discovery, University of Oxford, UK

[2] Department of Oncology, University of Oxford, UK

[3] Wolfson Childhood Cancer Research Centre, Newcastle University Centre for Cancer, UK

[4] Target Discovery Institute, University of Oxford, UK

[5] Department of Cancer Biology and Abramson Family Cancer Research Institute, University of Pennsylvania, USA

Background: Drivers of non-WNT/non-SHH medulloblastomas are poorly understood, presenting challenges for targeted drug discovery. Genetic analyses have identified a subset of group3/4 medulloblastomas harbouring mutations in KBTBD4, a previously uncharacterised E3 ligase. These tumours lack other typical cancer pathogenesis events such as high MYC amplification, indicating that the KBTBD4 mutations are the cancer drivers. However, the molecular mechanisms were not clear.

Methods: We performed Affinity Purified Mass Spectrometry Proteomics to look for cancer mutant specific interactors, followed by cellular interaction validation in both HEK293 and Group3 Medulloblastoma D283 cell lines harbouring KBTBD4 mutations. We also performed ubiquitylation, degradation and proteasomal inhibition assays to confirm the E3 ligase activity. The changes in epigenetic programmes were assessed via RNA seq and the stemness via tumorsphere assay and ALDH activities.

Results: Unlike common loss-of-function mutations, the indel mutations in KBTBD4 cause unique binding to CoREST complex which result in ubiquitylation and degradation of CoREST complex as neosubstrates. Our epigenetic analyses show that the degradation of CoREST complex upregulates genes that favour proliferation and increased stemness in D283 medulloblastoma cells.

Conclusions: Here we establish a new paradigm for tumorigenesis through neomorphic mutations in an E3 ubiquitin ligase KBTBD4. We propose KBTBD4 a novel drug target for cancer specific treatment for previously understudied Medulloblastoma subtypes.

Contact: zhuoyao.chen@cmd.ox.ac.uk

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Pipeline For Biomarker Discovery By RNA Mutation Detection Using RNA-seq And Machine Learning Of Blood Extracellular Vesicles from Pancreatic Cancer Patients

Background: Identification of biomarkers is critical for early detection of cancers such as pancreatic ductal adenocarcinoma (PDAC), which is often diagnosed in later stages leading to poor survival. An important part of carcinogenesis is the accumulation of DNA mutations, which can be reflected in the downstream expression of the RNA. Extracellular vesicles (EVs) released into the blood from the parent tumour cells contain all types of RNA providing a good source of biomarkers for cancer detection. These have advantages over other blood diagnostics as the EVs protect their nucleotide cargo from the harsh environment, can be enriched and are usually quickly cleared from the blood providing a real-time diagnosis.

Method: In a novel approach, blood based EV mRNA mutations were identified and analysed from patient RNAseq data from 100 PDAC patient sample against 100 “healthy” patient samples. For this we developed a pipeline to calculate the variants compared to human genome version 38 across the EV RNA for each patient sample across all genes.

Results: This showed the detection of mutations in actionable genes like KRAS And TP53. Using mutations and expression levels with machine learning methods showed a panel of genes offering an AUC of 0.998. Analysis of associated pathways were used to offer clinical insight.

Conclusions: Overall a novel pipeline and methodology for cancer biomarker discovery has been built, providing new combinations of candidate PDAC biomarkers with clinical relevance that could be expanded out to multiple cancers and clinical testing.

Contact: rpink@brookes.ac.uk

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ZFHX4 as a Potential Regulator of Immunogenicity in Colon Cancer

Background: Immunotherapy has revolutionised metastatic colon cancer (CC) treatment, with immune checkpoint modulators approved as first-line treatment in certain settings. Efficacy unfortunately appears mostly restricted to mismatch repair deficient (MMRd) or microsatellite unstable (MSI) immunogenic tumours which only represent <5% of metastatic cases[1,2]. However, MMRd and MSI aren't exclusive routes to tumour immunogenicity, a phenomenon inconsistent within literature with contributions aside from tumour mutational burden (TMB) rarely investigated. For example, clinical trials have demonstrated varied immunotherapeutic responses irrespective of MMR/MSI status, with around 30-50% MMRd/MSI patients lacking response[3-5] and some microsatellite stable (MSS) patients responding[6,7]. Solutions to refine immunotherapy targeting and maximise its benefit to all CC patients are here proposed: (1) ascertaining precise biomarkers delineating immunogenic tumours from non-immunogenic tumours and (2) a novel approach termed "immunosensitisation" employing targeted agents during immunotherapy to generate a pro-immunogenic environment independent of TMB. To explore these therapeutic avenues, research into genes and pathways altering immunogenicity can be translated from studying somatic mutations and their respective immune-phenotypic impact in CC patients.

Methods: TCGA-COAD data[1] were interrogated with R-programming. CC samples were classified into distinct immune subtypes based on unsupervised clustering of curated determinants of immunogenicity (DOI) including antigenicity and immune infiltration/signalling derived from genomic, transcriptomic and clinical data. Subtypes were analysed for associations with somatic mutations and other molecular correlates.

Results: Conventionally classed as "immune-cold", MSS samples interestingly spanned >1 subtype and were selected for further investigation because they eliminate confounding of high TMB in mutation analysis and permit conclusions from the immune-phenotypic impact of mutations by quality rather than quantity. ZFHX4 mutation incidence in MSS-CC was associated with multiple DOI including abundant anti-tumorigenic immune infiltrate and potentially enhanced tumour immunogenicity.

Conclusions: These preliminary bioinformatic data exhibit three-fold biological and clinical impact: classification of CC into robust subtypes defined by immunogenicity, identification of genes such as ZFHX4 potentially involved in routes to immunogenicity and provision for combinatorial therapy sensitising CC tumours to immunotherapy.

Future Directions: ZFHX4 appears on attractive research focus as ZFHX4 and related ZFHX3 mutations are also shown to correlate with immunotherapy response[8,9], yet knowledge regarding their impact on the translated transcription factor is limited. Thus alongside investigation into currently elusive ZFHX4 biology, I will employ CRISPR-mediated ZFHX4 knock-outs in normal-tissue-derived CC organoids before performing co-cultures with primary autologous human T-cells to measure T-cell activation, function and migration as readouts for immunogenicity. Additionally, I will aim to externally validate my bioinformatic analyses of ZFHX4 by exploring non-TCGA datasets such as immunotherapy-treated cohorts accompanying DNA/RNA/sc-RNAseq to elucidate associations between ZFHX4 and immunotherapy response.

Contact: jack.flower@linacre.ox.ac.uk)

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Oxford Cancer

New Richards Building
Old Rd,
Headington,
Oxford
OX3 7LA
Email: cancer@medsci.ox.ac.uk