Norwich Cancer Research Network

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NCRN Symposium

11th September 2024

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Welcome

We are delighted to have you here today to celebrate the wealth of cancer research taking place at Norwich Research Park. This symposium is an opportunity for scientists, clinicians and the public to come together and learn about the latest advances in cancer research.

During the day you will have the opportunity to:

- Find out about the breadth of cancer research taking place at Norwich Research Park.
- Gain an insight into the infrastructure support available to you.
- Find out about new developments in cancer research across the research spectrum.
- Get ideas for new collaborations with researchers from across the NRP.
- Present your research to a friendly and engaged audience.
- Learn from experienced researchers on how to set up research projects.

We encourage you to fully interact with the sessions and ask lots of questions. We particularly encourage you to attend the poster sessions, where you will have the opportunity to interact with researchers and learn more about their work. As this is an annual event, we would like to hear from you to help us make next year's symposium even better. You can provide feedback by completing the survey at the end of the day.

Thank you for coming. We hope you have a productive and enjoyable day at the Norwich Research Park Cancer Symposium.

We encourage you to use social media as much as possible citing **#NCRNSymposium2024** and **@CancerInNorwich**.

Your feedback would be valuable to us to help gauge how well the event went and how we can improve it for next year.
Please fill in the form here (https://forms.office.com/e/VYxUexBELZ)

Enjoy!

Daniel Brewer, Pinelopi Gkogkou, Emily Hobson, Maria O'Connell, Mark Williams, (NCRN Symposium organising group)

Sponsors

We'd like to thank our sponsors for their generous support of this event. Please visit their stands in the Atrium and attend their presentations in Session 2, where you can pick up tips on some of the latest technologies and resources for cancer research.

Agenda

10:00	Welcome – Julian Blow & Daniel Brewer

10.05am Session 1

Chair: Maria Marin

10:05	From bugs to drugs: the duocarmycins as potential antitumour agents – **Mark Searcey**
10:20	Adopting digital 3D technologies to improve breast cancer management – **Ken Tam**
10:35	Investigating the mechanism of action of acridine-4-carboxamides: from DNA intercalation to duplex to duplex crosslinking – **Victoria Kamperi**
10:50	Sponsor Talk - **Thermo Scientific**
11:00	Sponsor Talk - **Stratech**

11.10am Tea & Coffee Break

11.40am Session 2 - Lightning Talks

Chair: Emily Hobson

11:40	Sponsor Talk - **VectorBuilder**
11:50	**Lightning talks – part 1** Salonee Banerjee, Advait R Aithal, Owen Mullen, Rebecca Casson, Katherine Hampton
12:05	Sponsor Talk – **NanoString**
12:15	**Lightning talks – part 2** Amy Harden, Rachel Hurst, Carrie Wemyss, Alicia Nicklin, Angela Man
12:30	Sponsor Talk – **Oxford Nanopore**

12.40pm Lunch/Poster Session1

1.40pm Session 3

Chair: Maria O'Connell

13:40	From RCT to the 'real world': Implementing the Cessation of Smoking Trial in the Emergency Department (COSTED) into clinical practice **– Emma Ward**
13:55	Financial incentives for smoking cessation, including during pregnancy – A Cochrane review – **Caitlin Notley**
14:10	Natural products from Chinese medicinal plants that take the brakes off apoptosis – **Cathie Martin**
14:25	Dietary Isothiocyanates: Novel Insights into the Potential for Cancer Prevention and Therapy – **Yongping Bao**

2.40pm Tea & Coffee Break & Ice creams/Poster Session 2

3.30pm Session 4

Chair: Mark Williams

15:30	Reinvestigating microbial classifications and machine learning models in human cancer – **Abraham Gihawi**
15:45	Development of a non-invasive diagnostic tool for early detection of colorectal cancer (EDCC) study - **Dimitra Lamprinaki**
16:00	Repolarisation of M2 Macrophages Via cGAS-STING Activation Enhances Phagocytosis of Acute Myeloid Leukaemia – **Matthew Markham**

Keynote

Chair: Pinelopi Gkogkou

16:15	**Stuart Rushworth**

16:55	Close and Poster Prize Presentation – Daniel Brewer

5.00pm Drinks Reception & Poster Viewing

Talk Abstracts

Session 1
Mark Searcey

From bugs to drugs: the duocarmycins as potential antitumour agents

Mark Searcey

Natural products have formed the backbone of clinically used antitumour agents for many years, either through the direct use of the natural compound or as inspiration for purely synthetic analogues. Potent cytotoxics, prone to many side effects on their own, have found a new place in the cancer arsenal through the development of targeting agents, such as antibody drug conjugates (ADCs). The duocarmycins are a family of ultrapotent antitumour antibiotics that are under investigation as payloads for ADCs and other targeting moieties. In this talk, the progression of this intriguing family of natural products towards the clinic will be discussed, alongside efforts by the Searcey laboratory and collaborators to make and characterise new analogues.

Investigating the mechanism of action of acridine-4-carboxamides: from DNA intercalation to duplex to duplex crosslinking

Victoria Kamperi, Zoë Goddard, Maria O' Connell, Maria J. Marin, Andrew M. Beekman, Mark Searcey

DNA intercalating agents are well known as topoisomerase inhibitors, with agents such as doxorubicin or amsacrine clinically utilised for the treatment of cancer. Natural products such as nogalamycin or echinomycin are inhibitors of RNA transcription and consequently also have potent antitumour activity. In the latter case, that activity has been related to their long residence time (high Kd) on DNA and we and others have designed synthetic compounds to mimic this effect. In our case, we chose to focus on 9-aminoacridine-4-carboxamides as potential DNA interacting compounds, that bind to DNA through a threading mechanism, in which the 9-substituent is placed in the minor groove and the 4-carboxamide in the major groove[1].

Within the context of this project, we are investigating duplex-duplex DNA crosslinking using 9-aminoacridine-4-carboxamide dimers, which as monomers are well-known DNA intercalating agents. We have previously shown that flexibly linked 9-aminoacridine-4-carboxamides can form duplex to duplex crosslinks. A number of dimers have been synthesised, using click chemistry to generate dimers with varying linkers and also with the carboxamide either in the 3- or 4-position on the acridine chromophore. We previously developed a gold nanoparticle-based assay that allows for rapid screening of duplex-duplex DNA crosslinking ability, which has been utilised for initial studies of these compounds[2]. Interestingly, some of the dimers, particularly the 3-carboxamides, are clearly unable to form duplex to duplex crosslinks under the conditions of the assay. This has been compared to their ability to directly bind to linear CT-DNA through an ethidium bromide displacement assay. The dimers biological activity, particularly their ability to inhibit cell proliferation, has been assessed through MTS assays, showing an interesting structure activity relationship. Moving forward, we are investigating targeting secondary DNA structures, specifically three-way junctions through the synthesis of tri-acridine compounds.

1. L. P. G. Wakelin, X. Bu, A. Eleftheriou, A. Parmar, C. Hayek and B. W. Stewart, Journal of medicinal chemistry, 2003, 46, 5790–5802.
2. M. J. Marín, B. D. Rackham, A. N. Round, L. A. Howell, D. A. Russell and M. Searcey, Chem. Commun, 2013, 49, 9113.

Session 1
Ken Tam

Adopting digital 3D technologies to improve breast cancer management

Chak Hin Tam, Sheng Qi

This project explores innovative 3D design and printing applications to enhance breast cancer treatment, specifically in breast reconstruction surgery and the development of drug-eluting implants. Accurate assessment of breast volume is critical in the preoperative planning process for breast reconstruction after mastectomy. At the Norfolk and Norwich University Hospital (NNUH), 3D scanning was employed in this project to create customised 3D-printed moulds for the patient to improve breast volume measurement. Integrating 3D technologies into the current surgical workflow can 1) increase surgical precision and 2) reduce the number of surgical procedures, operational time in the theatre and potentially the need for post-operative correction.

Concurrently, we collaborated with James Paget University Hospital (JPUH) to develop a novel breast implant with the potential to elute anti-cancer drugs. The contour of this implant is customised based on MRI-derived dimensions of the excised tumour and adjacent healthy tissue from individual patients.

From RCT to the 'real world': Implementing the Cessation of Smoking Trial in the Emergency Department (COSTED) into clinical practice

Emma Ward, Pippa Belderson, Ian Pope, Caitlin Notley

Background: The Cessation of Smoking Trial in the Emergency Department (COSTED) was a multicentre, randomised controlled trial. Adults who smoked who attended one of six UK EDs were randomised to intervention (brief advice, e-cigarette starter kit and referral to stop smoking services) or control (written information on stop smoking services). The intervention was found to be twice as effective at supporting people to quit compared to control, prompting widespread interest from services looking to adopt the COSTED approach in their ED. This presentation aims to demonstrate how the research team: 1) capitalised on interest; 2) supported implementation; 5) measured implementation.

Methods: With the support of Action on Smoking and Health (ASH), a working group was set up including professionals from services at various stages of implementation offering peer support and shared learning. The research team developed a toolkit covering frequently asked questions for commissioners. The toolkit also provided a structured eLearning module aimed at advisors delivering the intervention, adapted from the original COSTED trial training, but also incorporating learning from the trial process evaluation. The research team continues to monitor implementation of the COSTED intervention by services via the working group.

Results: The COSTED intervention was well timed to coincide with national funding available to services to purchase e-cigarettes for smoking cessation support. The membership of the working group will be profiled, and an overview will be given of the function of the group including type of support offered and meetings to date. The development of the toolkit will be discussed with components presented. We will present a case study of one local authority who has successfully implemented the COSTED model into their practice.

Conclusion: Evidence based preventative interventions, like COSTED, are essential to reducing cancer rates. However, there is often a 'research-to-practice' gap and implementation of effective interventions into clinical practice can be delayed or even prevented due to services having limited access to resources, skills, or knowledge. We conclude that by combining access to resources via existing policy, with robust knowledge translation and peer support building practices, the COSTED intervention may be successfully implemented into practice by services.

Session 3
Caitlin Notley

Financial incentives for smoking cessation, including during pregnancy – A Cochrane review

C. Notley, S. Gentry, J. Livingstone-Banks, L. Bauld, R. Perera, M. Conde, J. Hartmann-Boyce

Background: Tobacco smoking is the leading preventable cause of cancer. Supporting people to quit smoking by incentivising abstinence has been tested in many trials worldwide. Previous reviews have found incentives to be effective for long-term tobacco smoking cessation in the general population, although evidence for pregnant people who smoke was less clear.
Methods: Cochrane systematic review of randomised controlled trials, allocating individuals or groups to smoking cessation incentives or control. Trials included mixed populations, those in substance misuse treatment, and pregnant people. The outcome was abstinence from smoking at longest follow-up (at least six months from intervention start).
Results: 47 mixed-population studies met inclusion criteria, including 21,964 participants in community settings, clinics, workplaces, and drug clinics. The pooled RR for quitting with incentives at longest follow-up (six months or more) compared with controls was 1.52 (95% CI 1.33 to 1.74; I2 = 23%; 39 RCTs, N = 18,303; high-certainty evidence). In a sub-group analysis of trials recruiting people who were pregnant, the 13 trials with usable data (eleven conducted in the USA, one in France and two in the UK), delivered a RR at longest follow-up (up to 48 weeks post-partum) of 2.13 (95% CI 1.58 to 2.86; I2 = 31%; N = 3942; high-certainty evidence), in favour of incentives.
Conclusions: There is high certainty evidence that incentives improve smoking cessation rates at long term follow-up in mixed population studies. There is also high certainty evidence (moderate certainty in the last review update), that incentive schemes conducted among pregnant people who smoke improve cessation rates, both at the end of pregnancy and post-partum.

This abstract is based on a draft and [pre-peer review/post-peer review] version of a [Protocol for a Cochrane Review/Cochrane Review]. Upon completion and approval, the final version is expected to be published in the Cochrane Database of Systematic Reviews (www.cochranelibrary.com).

Session 3
Cathie Martin

Natural products from Chinese medicinal plants that take the brakes off apoptosis

Cathie Martin

TBC

Session 3
Yongping Bao

Dietary Isothiocyanates: Novel Insights into the Potential for Cancer Prevention and Therapy

Q. Wang, G. Strusi, W. Wang, E. Sampson, Y. Bao

Epidemiological studies suggest that high intake of cruciferous vegetable can reduce the risk of cancer. Dietary isothiocyanates (ITCs) have been shown to possess cancer chemopreventive properties in many cellular and animal models. ITCs derived from glucosinolates in cruciferous vegetables can induce cell cycle arrest, apoptosis, autphagy and up-regulate several antioxidant enzymes via the nuclear factor E2-related factor 2 (Nrf2)-antioxidant responsive element (ARE) pathway. The induction of antioxidant enzymes protects cells against carcinogen-mediated DNA damage and free radical-mediated cell death. However, we have recently shown that isothiocyanates exhibit a hormetic effect on cell proliferation, migration and angiogenesis. Our recent study demonstrated that phenethyl isothiocyanate (PEITC) can enhance the anti-cancer activity of dasatinib (Sprycel, a tyrosine kinase inhibitor) in liver cancer models. Moreover, we have demonstrated that co-delivery of an isothiocyanate sulforaphane and anti-cancer drugs such as cisplatin (CDDP), and/or sorafenib in nanoparticles possess greater effects on cancer cell growth in animal models than treatment with the individual compounds alone. Ongoing work investigates interactions between ITCs and triterpenoids from medicinal plants. Therefore, we believe that isothiocyanates have the potential to be used as adjuvant drugs for cancer therapy.

Session 4
Abraham Gihawi

Reinvestigating microbial classifications and machine learning models in human cancer

Abraham Gihawi, Rachel Hurst, Colin, S. Cooper, Daniel S. Brewer

Bacteria and viruses are associated with numerous cancer types and have been attributed with 20% of cancers worldwide. Searching for additional associations between microbes and cancer is undoubtedly an important undertaking which could lead to significant clinical benefit. Cancer tissue is increasingly being sequenced, providing a great opportunity for a thorough investigation. Accurately identifying taxa within human tissue, however, is if rife with technical challenges. This makes finding translational associations all the moreproblematic. This talk will highlight and provide recommendations for some of the issues confronting cancer metagenomics as a field, which include misclassified host sequencing reads and issues with batch correction.

Session 4
Dimitra Lamprinkai

Development of a non-invasive diagnostic tool for early detection of colorectal cancer (EDCC) study

Dimitra Lamprinaki, Clare Ferns, Sarah Wilford, Teresa Kieloch, Sarah Phillips, Antonietta Hayhoe, Sarah Hughes, Gemma Beasy, Rachel Watt, Silas Triller, Roxanne Brunton-Sim, Anna Wordley, Nathalie Juge

Colorectal cancer (CRC) is one of the three most common cancers worldwide, with the majority of cases being sporadic and non-hereditary, primarily caused by environmental factors. Notably, in about 80% of CRC metastatic CRC cases, metastasis occurs before the primary tumor is detected, underscoring the critical need for early diagnosis to reduce disease severity and mortality.

In the UK, bowel cancer screening is offered every two years to asymptomatic individuals aged 58 to 74 using a faecal immunochemical test (FIT) home kit. This test detects blood in faecal samples, which could indicate early signs of colon cancer or polyps. If the test results are abnormal, individuals are offered a colonoscopy. However, there is an urgent need for novel approaches to enhance diagnostic accuracy and reduce the reliance on colonoscopy.

CRC is characterized by a distinct microbiota signature, with bacterial species such as Fusobacterium nucleatum being enriched in tumor tissues. F. nucleatum is associated with poor prognosis and chemoresistance, and at the molecular level, it contributes to CRC development and metastasis, primarily by modulating the innate immune response.

In a previous study (Lamprinaki et al. 2021), we identified Siglec-7, a member of the 'Sialic-acid-binding immunoglobulin-like lectins' family, as an immunomodulatory receptor mediating the interaction between F. nucleatum and immune cells. Both Siglec-7 and Fusobacterium spp. have significantly higher relative abundance in tumor tissues compared to non-tumor sites. In addition, our preliminary studies revealed an increased abundance of antibodies against F. nucleatum and soluble Siglec-7 in the blood of CRC patients, suggesting their potential as serum biomarkers.

This study, funded by Bowel Cancer UK, aims to evaluate the use of F. nucleatum and Siglec-7 as potential serum biomarkers for CRC by monitoring soluble Siglec-7 and antibodies against F. nucleatum in FIT abnormal cases. With a FIT screening positive predictive value of 7% at the current threshold, we plan to recruit 428 participants to obtain 30 participants per group (cancer-positive and cancer-negative). We are currently collecting serum samples from two groups of research participants with abnormal FIT results (≥120 µg Hb/g faeces) who have been invited for colonoscopy as part of the NHS Bowel Screening Programme at the NNUH endoscopy unit, hosted in QIB.

Repolarisation of M2 Macrophages Via cGAS-STING Activation Enhances Phagocytosis of Acute Myeloid Leukaemia

Matthew Markham, Rebecca S. Maynard, George Bell, Katherine Hampton, Dominic J. Fowler-Shorten, Alyssa Polski-Delve, Annalisa Altera, Rosie Taylor, Charlotte Hellmich, Kristian M. Bowles and Stuart A. Rushworth

The tumour microenvironment is composed of extracellular matrix and non-mutated cells supporting tumour growth and development. Tumour-associated macrophages are among the most abundant immune cells in the microenvironment. Studies of the innate immune compartment in the bone marrow of patients with acute myeloid leukaemia (AML) reveal a shift toward a tumour-supportive M2-polarised macrophage (Weinhäuser et al., 2023). Furthermore, previous work by our group has shown that AML drives activation of the stimulator of interferon genes (STING) pathway in macrophages in the AML bone marrow microenvironment (Moore et al., 2022). We found that AML-derived mitochondrial damage-associated molecular patterns were processed by bone marrow macrophages via LC3-associated phagocytosis. This activation of STING resulted in a suppression of AML growth.

To fully characterise the role of STING pathway in AML-associated macrophages we have combined RNA sequencing, flow cytometry and in vivo characterisation to reveal a shift of M2 tumour supportive macrophages towards an M1 phenotype in response to STING activation. We used various STING agonists including DMXAA, 2'3'-cGAMP, and CpG oligonucleotides to model STING activation of bone marrow derived macrophages (BMDM) in vitro. Functionally, STING activation of BMDM induced an anti-tumour response by increasing phagocytosis of AML blasts which was also established in an in vivo model of AML in C57Bl/6 mice. Using the well-established syngeneic AML murine model (MN1), STING activation in vivo slowed tumour progression and prolonged survival. RNA sequencing of STING activation in macrophages revealed a M1 proinflammatory polarisation phenotype. Moreover, AML-induced M2 macrophage polarisation was reversed in response to STING activation.

Additionally, we show that STING activation upregulates genes involved with cell-cell adhesion, and we used blocking antibodies to assess their involvement in the increased phagocytic clearance of AML blasts. We observed that inhibiting the ICAM1/LFA1 interaction blocked STING-induced phagocytosis of AML blasts, therefore demonstrating the role this interaction plays in mediating the anti-tumour response of STING activation. Finally, we show that AML cells have higher levels of LFA1 cell surface expression than non-AML cells within the bone marrow. This is further supported by the BloodSpot database which demonstrates a higher level of LFA1 expression in human AML subtypes compared to haematopoietic stem and progenitor cells (Bagger, F.O. et al., 2016).
Our study provides insight into mechanisms by which AML-associated macrophages can be reprogrammed through STING activation towards an anti-tumour, pro-phagocytic phenotype, thus providing an alternative strategy for targeting leukaemia progression.

Poster & Lightning Talk Abstracts

There will be a virtual poster session available throughout the day. Posters will be available for you to like and comment on. These sessions will be facilitated using the Padlet platform. This page works like a virtual bulletin board. If you would like to discuss one of the posters in more detail, please email the author.

Please follow this link: https://bit.ly/NCRN24posters

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3	Pippa Belderson	Digitalising A Specialist Treatment Programme For Smoking Cessation In Pregnancy
4	Emma Ward	Development of PrOactive primary care Smoking cessation Trial (POST): perspectives of patients and primary care professionals on a proposed smoking cessation intervention delivering NRT or e-cigarettes by post to people who smoke identified from primary care records
5	Jasenka Mazibrada	Dedifferentiated Mucinous Ovarian Carcinoma: A Case Report and Literature Review
6	Anbu Sellamuthu Kooduthurai	Alkaline Phosphatase-Responsive Mn-based MRI contrasts for Breast Cancer Diagnosis
7	Salonee Banerjee	Crosstalk between Hepatocyte Growth Factor and Epidermal Growth Factor signalling is involved in tunneling nanotube formation in A549 Lung Adenocarcinoma Cells
9	Advait R Aithal	Towards developing homogeneously labelled photoactive ADCs
10	Owen Mullen	Characterisation of Stapled DNA Binding Peptides to Inhibit Transcription Factors in Cancers
11	Emilia Szlosarek	The oxidative stress sensor DJ-1 as a therapeutic target in melanoma
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13	Enas Aljohani	Heterobimetallic Ruthenium-Ferrocene Complexes as Anticancer Agents
14	Pichamon Eiamampai	Evaluating the potential of VEGFA mRNA isoform measurement as a predictive biomarker for use of AvastinTM in ovarian cancer
15	Eduardo de la Vega	Single molecule DNA replication dynamics of the human genome.
16	Angela Man	Using the haploid cell line, HAP1, to investigate polyploidy in many ways.
17	Edyta Wojtowicz	Single-Cell Analysis Reveals Novel Insights into Megakaryocyte Heterogeneity and Microenvironment

18	Katherine Hampton	Acute Myeloid Leukemia induces an increased senescent phenotype in the large intestine which protects barrier function and reduces leakiness.
19	Alyssa Polski-Delve	Tumour Derived Interleukin-1 Drives AML Induced Muscle Atrophy
20	Amy Harden	Investigating the RNA Structurome of Ewing Sarcoma
21	Kate Manley	Prediction of Disease Progression for Patients on Active Surveillance using the Prostate Urine Risk (PUR) Score
22	Freddie Marlowe	Refining Prostate Cancer Subtyping Using a Breast Cancer Classification Framework
23	Calista Tink	Determining How Prostate Cancer Evolves by Analysing Molecular Subtypes in Single-Cell Sequencing Data
24	Tim Brendler-Spaeth	The Prognostic Potential of Methylation Patterns in Prostate Cancer: Insights from High-Resolution EPICseq Data
25	Seshadhri Subramanian	tRNA Charging and Methionine: Unleashing bacterial potency in prostate cancer
26	Jade Manning	Investigating the Mechanisms of Action of Intracellular Anaerobic Pathogens on Human Host Cancer Cell Metabolic Networks
27	Rachel Hurst	Multi-Step Mechanisms of Action of Novel Pathogens and Anaerobic Bacteria Associated with Cancer
28	Carrie Wemyss	Mining the Microbiota: Harnessing Bacterial Extracellular Vesicles for Improved Asparaginase Therapy
31	Alicia Nicklin	The use of novel microbial-based therapeutics in the treatment of cancer
32	Mark Williams	Cholinergic mobilisation of juxtaposed TPC1-InsP3R3 calcium stores triggers secretion of mucus and fluid to flush the human colonic stem cell niche
33	Mark Williams	Dysregulation of the calcium signalling pathway in colon cancer: identification of drug targets for chemotherapy
H30	Luke Mitchell	Exploring the immunomodulation pathways of Bifidobacterium in breast cancer through genetic modification

Tracey Brown
Poster Number 1

Electronic signposting in cancer prevention: a realist review protocol

Tracey J Brown, Felix Naughton, Natalie Tham, Zarnie Khadjesari

Background
Cancer is a leading cause of mortality worldwide, and deaths are growing annually, causing significant burden to healthcare and to individuals. Almost 40% of cancers can be prevented by changing health behaviours such as dietary intake, alcohol consumption, tobacco use and physical activity. Infections, including hepatitis B and human papillomavirus, account for approximately 15% of cancers; and cancer screening and early diagnosis significantly reduce cancer mortality. Importantly, there are pronounced cancer inequalities, with people of lower socioeconomic status being less likely to benefit from behavioural interventions and less likely to access healthcare services. Electronic signposting to behavioural interventions, and to vaccination and cancer screening appointments, has the potential to target at risk groups, and to help prevent cancer in a cost-effective manner.

Objectives
We will undertake a realist review to understand the contexts and mechanisms of action which influence the implementation of electronic signposting using electronic health records, for cancer prevention.

Methods
A realist review approach seeks to explain how and why an intervention works, rather than measuring effectiveness alone. We will develop an initial realist programme theory, and test this by reviewing the literature. Searches will be conducted in Medline (Ovid), EMBASE, CINAHL, Scopus, PsycINFO, ERIC and AMED databases. We will include studies which contribute relevant information on context and mechanisms of action for electronic health record signposting to cancer prevention interventions. Quality appraisal will be based on relevance, richness and rigour. We will extract context-mechanism-outcome configurations, and synthesise findings in order to refine our programme theory.

Implications for practice
A greater understanding of how best to implement electronic signposting to prevent cancer, offers the potential for substantial benefits to patients, services and to public health.

Lisa McDaid
Poster Number 2

Developing and testing a digital support package (eSupport) for smoking cessation in pregnancy

Lisa McDaid, Tim Coleman, Caitlin Notely, Joanne Emery, Pippa Belderson, Jo Leonardi-Bee, Sophie Orton, Sanjay Agrawal, Zarnie Khadjesari, Michael Ussher, Matthew Jones, Elizabeth Bailey, Matt Hammond, Carmen Glover, David Crane, Felix Naughton

Background
Smoking during pregnancy increases the risk of health problems for babies and drives health inequalities. In the UK, the NHS provides free interpersonal counselling and nicotine replacement therapy (NRT) to help pregnant people stop smoking. This is effective, but not widely used. Digital support - text messages, web, apps – could provide an alternative delivery model, and pregnant people and experts are enthusiastic about it. Few digital stop smoking tools are orientated towards pregnancy, which is the main reason most pregnant people try to quit, and none are available through the NHS. This study will design and test a comprehensive electronic support ('eSupport') package for smoking cessation in pregnancy. This would include help to use NRT, and possibly e-cigarettes.

Methods
1. Identify key delivery modalities, content and behaviour change techniques to promote engagement and smoking cessation by undertaking systematic reviews.
2. Co-develop and optimise an eSupport package (with a commercial partner) using collaborative workshops and iterative testing.
3. Test the effectiveness and cost-effectiveness of the eSupport package in a randomised control trial, with embedded process evaluation.
4. Develop and evaluate implementation strategies for promoting eSupport in real-world settings.

Expected results
The project will determine 'eSupport' (cost) effectiveness, and whether it can be made accessible in clinical and online settings such that pregnant people use and engage with it.

Discussion
We anticipate producing an effective eSupport package and implementation strategies to help more pregnant people stop smoking and, consequently, reduce infant morbidity and mortality.

Pippa Belderson
Poster Number 3

Digitalising A Specialist Treatment Programme For Smoking Cessation In Pregnancy

Pippa Belderson, Lisa McDaid, Joanne Emery, Felix Naughton

Background
Stopping smoking in pregnancy reduces the risk of many pregnancy complications and infant mortality/morbidity. The UK standard treatment programme for smoking cessation in pregnancy offers counselling and pharmacotherapy, but uptake is low. A digital support package could be offered as an alternative, but the evidence base is limited. This study aimed to investigate views on translating the UK standard treatment programme for cessation in pregnancy into a digital intervention.

Methods
Online group and individual interviews with 38 experts (11 focus groups, 3 interviews) and 25 pregnant smokers (all interviews) and analysed thematically.

Findings
Experts and pregnant people were supportive of a pregnancy-specific digital intervention. Most counselling content from the standard treatment programme was considered transferable. However, accountability to a human advisor, empathy and the ability to go 'off-script' were considered more difficult to replicate digitally. Suggestions to address this included personalisation, artificial intelligence tools, and the option to escalate support to a human advisor. While experts had mixed views on integrating remote carbon monoxide monitoring (for verifying smoking status) into a digital intervention, pregnant people were enthusiastic. Remote provision of free nicotine replacement therapy (NRT) without interpersonal support was considered feasible and pregnant people were receptive if the intervention felt trustworthy and provided tailored advice. However, experts had concerns about governance issues and exacerbating low NRT adherence.

Discussion
The standard treatment programme is largely transferable to a digital intervention and would potentially be helpful to pregnant smokers who are looking to quit, thus merits further development and evaluation.

Emma Ward
Poster Number 4

Development of PrOactive primary care Smoking cessation Trial (POST): perspectives of patients and primary care professionals on a proposed smoking cessation intervention delivering NRT or e-cigarettes by post to people who smoke identified from primary care records

Emma Ward, Caitlin Notley, Ian Pope

Background
In order to reach seldom heard populations who have the most to gain from quitting smoking, innovative proactive approaches are required. It is known that advice from primary care clinicians is effective in supporting people to quit and that combining advice with using a smoking cessation aid (such as NRT or an e-cigarette) is more likely to result in quitting. However, delivering advice remotely, combined with posting smoking cessation aids, has never been trialed in a proactive intervention targeted at undeserved populations identified via primary care records.
Aims: 1) To conduct Patient and Public involvement (PPI) work to assess the acceptability of a primary care based smoking cessation intervention incorporating remote brief advice delivered by a trained healthcare professional and smoking cessation aids posted to the patient; 2) to define the proposed intervention using the Template for Intervention Description Replication (TIDieR) framework.

Methods
GP surgery patients who smoke were recruited to undertake interviews to seek their views on the proposed intervention. To reflect the type of underserved groups which may be reached if the intervention is implemented, we specifically focused on patients resident in the 20% most deprived areas, patients working in routine and manual occupations, or patients accessing community organisations aimed at supporting mental health or financial issues. Primary care professionals including GPs, practice managers, nurses, and healthcare assistants were interviewed, as well as local authority public health staff. To ensure robust knowledge translation from PPI work to intervention development, interview data was analysed using the Template for Intervention Description and Replication (TIDieR) framework headings as qualitative themes to directly map onto the TIDieR framework to describe the proposed intervention.

Results
A ‘low pressure’ remotely delivered smoking cessation intervention offering advice and a choice of smoking cessation aids was found to be acceptable by both healthcare professionals and people who smoke. Both groups highlighted the need for patients to be reassured that the intervention was being delivered by or on behalf the practice (and was not a scam or sales cold call) and that an initial contact to warn patients about the intervention was necessary. Both groups agreed that remote follow up should be offered. Staff training and brief advice was reported as needed to reassure staff/patients about the effectiveness and safety of e-cigerettes in particular.

Conclusions
A remotely delivered smoking cessation intervention identifying smokers from primary care records is defined. Through knowledge translation of the presented PPI work and triangulation with the literature, it is expected that the intervention will be acceptable to the target population of primary care patients who smoke. This work will support a grant application for a feasibility trial to test the intervention.

Jasenka Mazibrada
Poster Number 5

Dedifferentiated Mucinous Ovarian Carcinoma: A Case Report and Literature Review

E. Owusu, N. Jayatunge, H. Turnbull, J. Mazibrada

Introduction
Dedifferentiated mucinous carcinoma of the ovary is a rare, highly aggressive and molecularly distinct ovarian malignancy, characterised by frequent inactivation of core SWI/SNF complex and typically a low response rate to platinum-based standard care for ovarian cancer.

Presentation of case
We here report a case of dedifferentiated mucinous carcinoma in a 50-year-old patient, who morphologically showed spectrum of well, moderate and undifferentiated carcinoma and a distinct immunohistochemical profile, as documented by application of wide range immunohistochemistry. It also underwent detailed molecular testing in two independent institutions. Results showed alterations in numerous genes such as BRCA2, SMARCA4, ARID1A, TP53, STK11, EP300, KRAS and MTAP. Tumour was MMR proficient. Moreover, distinct expression patterns in B-Catenin and E-Cadherin were noted in different areas of tumour, which warrant further studies, but raises the possibility of post-transcriptional alterations in Wnt signalling pathway.

Discussion
Morphological and immunohistochemical characteristics of tumour, differential diagnoses and review of literature have been discussed, as well as the utility of molecular profiling for precision cancer therapies.

Alkaline Phosphatase-Responsive Mn-based MRI contrasts for Breast Cancer Diagnosis

Anbu Sellamuthu, John Brooks, William Penny, Joseph Wright

Breast cancer is the most common cancer in women in the UK, with 55,500 new female cases and 370 new male cases every year[1]. It represents 15% of all new cancer cases and leads to about 11,600 deaths per year among women. Due to the increasing incidence, it is estimated that breast cancer-related deaths will globally increase by 43% from 2015 to 2030[2]. Of particular concern is that 60-70% of advanced breast cancer (metastases/stage-IV) patients develop bone metastases (BM), significantly affecting their quality of life[3]. Early detection of BM is crucial for better management and treatment options, highlighting the need for advanced diagnostics as global breast cancer incidences rise. MRI is a critical tool for diagnosing breast cancer, using contrast agents to enhance the visualisation of internal structures.

Unlike mammography and bone scanning, MRI offers superior specificity, detailed multi-azimuth imaging, and high spatial resolution, especially when detecting small tumours in dense tissues, making it highly effective for early and accurate breast cancer-induced BM diagnosis[4]. The research also advocates for a shift from traditional gadolinium-based contrast agents (GdCAs) to safer manganese(II)-based MRI contrast agents (MnCAs)[5]. Unlike GdCAs with limitations such as tumour non-specificity and potential kidney risks, MnCAs present a safer alternative for enhancing MRI imaging. Additionally, recent advancements in MnCAs have shown promising potential in improving the specificity and safety of MRI contrast agents, addressing the limitations associated with traditional contrast agents.

Recent research has identified a link between the overexpression of ALP and the rapid breast cancer progression to BM via calcium phosphate depositions[6]. Elevated serum ALP, indicating phosphatase activity and altered PPi/Pi levels, can signal potential BM in breast cancer[8], with higher ALP levels associated with a worse prognosis[8]. To address this unmet medical need, an advanced ALP-responsive MnCA has been developed by integrating the MRI probe (macrocyclic Mn(II)-chelate, MnLP) and ALP-responsive unit (4-nitrophenyl phosphate substrate). Preliminary MRI studies in vitro have shown that the MnLP exhibits enhanced relaxivity (r1) of 5.06 mM-1 s-1 at the clinical field 3 Tesla upon selective ALP recognition. Other preliminary relaxometry studies, including concentration, time, pH-dependent, and transmetalation studies of MnLP, indicate that it is a potential smart or ALP-responsive MnCA for early detection of hard-to-treat cancers, such as breast cancer-induced bone metastasis (BC-to-BM).

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Salonee Banerjee
Lightning Talk And Poster Number 7

Crosstalk between Hepatocyte Growth Factor and Epidermal Growth Factor signalling is involved in tunneling nanotube formation in A549 Lung Adenocarcinoma Cells

Salonee Banerjee, Aya Elmeligy, Griselda Awanis, Natalia Cicovacki, Stefan Bidula, Derek Warren, Anastasia Sobolewski

Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer cases and is associated with mutations in hepatocyte growth factor(HGF) and epidermal growth factor(EGF) expression. Tunneling nanotubes(TNTs) are thin cytoplasmic connections involved in long-distance cell-to-cell communication in cancer. In this study, we investigated the role of EGF/HGF signalling pathway crosstalk in TNT formation in A549 cells. A549 cells were cultured and treated with 0-700ng/ml EGF/HGF/EGF+HGF for 24h and phase-light image analysis was undertaken using ImageJ. Pharmacological inhibition of c-Met, EGFR, MEK, and PI3K was performed 24h before imaging to assess signalling pathways. Immunofluorescent labelling was performed to identify protein markers. LysoTracker, mitochondrial cytopainter and DiO(green) were used to visualise organelle/cargo trafficking. We found that EGF, HGF, and EGF+HGF treatment induced TNTs in a concentration-dependent manner, with maximal TNT induction at 100ng/ml. EGF+HGF induced TNTs to a similar extent as individual EGF and HGF treatment, and inhibition of both the c-Met+EGF receptors was required to abrogate EGF+HGF-induced TNTs, indicating crosstalk between EGF and HGF at the receptor level. We also found that the c-Met/HGF and EGFR/EGF pathways converge downstream to induce TNTs via the MAPK and PI3K pathways. SEM revealed vesicle-like structures within TNTs, which was later supported by the uni- and bidirectional trafficking of mitochondria, lysosomes, and lipid vesicles intercellularly via TNTs. The early endosomal proteins Rab5a and EEA-1 were also localised within the TNTs. Finally, EGFR and c-Met were identified as novel components of TNTs, however, these receptors did not exhibit colocalisation. Our findings highlight the role of EGFR/c-Met crosstalk, and their downstream pathways in TNT formation, and TNT-mediated organelle transfer as a potential means for chemoresistance. Clinically, combinatorial approaches to inhibit TNTs through simultaneous targeting of c-Met and EGFR hold promise to improve patient outcomes in NSCLC.

Advait R Aithal
Lightning Talk And Poster Number 9

Towards developing homogeneously labelled photoactive ADCs

Advait R Aithal, Amit Sachdeva.

Currently, several FDA approved antibody-drug conjugates (ADCs) are being used to treat a variety of cancers. They offer targeted drug delivery to cancer cells through the selective and precise interaction between the antibody and their target tumour antigens. However, traditional labelling techniques used to attach cytotoxic drugs to tumour targeting antibodies can result in a mixture of non-homogeneously labelled proteins, potentially affecting their functionality and efficacy. Furthermore, the very nature of the antigen-antibody interaction means that ADCs target any cell expressing that receptor, including non-cancerous healthy cells which leads to complications and adverse side effects termed “on-target, off-tumour” effects. These can range from nausea, alopecia, and pyrexia to life-threatening haemorrhages. Sachdeva and group have previously engineered a photoactive 7D12 nanobody by genetically encoding photocaged tyrosine site-specifically. The work described in this poster aims to extend the scope of these photoactive nanobodies by site-specifically labelling them with a cytotoxic drug to generate PhotoActive Nanobody-Drug conjugAtes (PANDAs). For this, we have employed two techniques: 1) a non-canonical amino acid containing a bio-orthogonal group is genetically encoded into the nanobody, and this bio-orthogonal group is then covalently attached to a cytotoxic drug, and 2) a site-specifically encoded unpaired cysteine is covalently attached to a maleimide functionalised cytotoxic drug, to create homogeneously labelled PANDAs.

Characterisation of Stapled DNA Binding Peptides to Inhibit Transcription Factors in Cancers

Owen Mullen, Maria O'Connell, Andrew Beekman

The Nrf2-MafG transcription factor complex forms in response to cellular oxidative stress, leading expression of cytoprotective genes for metabolism and cell survival[1]. Upregulation of this complex in cancers is associated with poor clinical prognosis, linked to increased cell growth, proliferation, survival, and chemoresistance[2]. In cancers, development of transcriptional modulation inhibitors offers great potential in therapeutics in overcoming resistance.

For transcriptional activity, Nrf2 requires MafG to bind to the antioxidant response element DNA sequences, found within MafG recognition elements (MARE)[3]. The crystal structure of this protein dimer in complex with ARE highlighted α-helical domains within both proteins that bind to the major groove of DNA. Furthermore, key residues for interaction with DNA were identified[4]. Previous work has utilised the Nrf2 DNA binding domain to generate peptide mimics with low µM affinity to ARE[5]. Herein, we used the MafG DNA binding domain to design peptide mimics to replicate MafG's role as a transcriptional modulator.

A core 18 amino acid region was used to design initial peptides that were found to bind to target DNA with low µM affinity. Peptide MafG52-73 showed the greatest affinity to ARE, KD = 1.295 µM, via fluorescence polarisation (FP), though lacked sequence selectivity with similar affinity to E-box DNA. As such, the peptide MafG52-73 sequence was used to design stapled peptide derivatives that should enhance the affinity to target DNA through α-helicity enhancements, potentially improving selectivity.

In this presentation, characterisation of five peptide MafG52-73 stapled peptide variants will be discussed. Peptide-DNA affinity to MARE and secondary DNA sequences were found by application of FP assays, supported by EMSA observations. Excitingly, a ~two-fold improvement was observed for i,i+4 stapled peptides compared to peptide MafG52-73. However, similar affinity for E-box DNA was observed, suggesting stapling may have enhanced the charged based interaction of the peptide to DNA. Consequently, peptide MafG52-73 will be used to generate a dimeric peptide with an Nrf2 peptide, assessing whether dual peptide recognition can enhance affinity and selectivity to target DNA by mimicking both proteins.

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Emilia Szlosarek
Poster Number 11

The oxidative stress sensor DJ-1 as a therapeutic target in melanoma

Emilia Szlosarek, Megan Williams, Sean Tattan, Emily Hobson, Maria O'Connell

Melanoma is the most lethal form of skin cancer. In the UK, approximately 16,700 people are diagnosed with melanoma each year, with the incidence rate of malignant melanoma rising rapidly[1]. Melanoma is treated successfully in its early stages with surgery; however, survival rates fall markedly after metastasis. Immunotherapies or targeted chemotherapy, for example BRAF inhibitors, are given to patients in later stages of the disease to slow the spread, yet in many cases the melanoma becomes chemoresistant. Novel pharmacological strategies are therefore urgently needed to combat chemoresistance. DJ-1 (also known as PARK7) is a Parkinson's disease-associated protein and oxidative stress sensor, which can activate the redox-sensitive Nrf2 pathway[2]. Similarly to Nrf2, it is overexpressed in certain cancers including melanoma. Recently, DJ-1 was reported to play a role in the progression of melanoma, suggesting that it could be a novel therapeutic target for this cancer[3]. Here, the role of DJ-1 in melanoma was investigated using siRNA knockdown in human A375 skin melanoma cells. DJ-1 protein was detected in A375 cells by confocal microscopy. Knockdown of DJ-1 mRNA and protein expression was validated by RT-qPCR and western blot. siRNA uptake was validated with siGlo. 100 nM DJ-1 siRNA inhibited DJ-1 mRNA by 80% but had no significant effect on Nrf2, heme oxygenase-1 or NADPH quinone oxidoreductase-1 mRNA expression, two downstream target genes of Nrf2. 100 nM DJ-1 siRNA decreased cell proliferation by 40%. Furthermore, a small molecule inhibitor of DJ-1, STK793590 (50 uM), in combination with the BRAF inhibitor, Vemurafenib (1 uM), reduced A375 cell proliferation by 60%. Studies are currently underway to determine if this inhibitor causes apoptosis and can reduce growth in a 3D tumoroid melanoma model.

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Rebecca Casson
Lightning Talk And Poster Number 12

Production of a suite of molecules through synthetic biology for comprehensive structure-activity relationship investigations

Rebecca Casson, Philip Spence, Adnane Aouidate, Emily Hobson, James Reed, Maria O'Connell, Anne Osbourn

Triterpenes are structurally diverse 30-carbon natural products with a long history of use in traditional remedies, which possess significant modern applications including as potential anti-cancer molecules. Triterpenes are often produced in plants in very low quantities, and their structural complexity makes chemical synthesis difficult. Transient expression is a technology that allows rapid expression of triterpene biosynthetic genes in a plant host. Previously, the "triterpene toolkit" suite of biosynthetic genes developed by the Osbourn lab was shown to produce both natural and novel triterpenes at a speed and scale which outcompetes chemical synthesis. In the current work, a comprehensive suite of triterpene molecules based on the β-amyrin scaffold was synthesised via transient expression in Nicotiana benthamiana, purified, and structurally verified by NMR. This suite includes modifications made by biosynthetic enzymes from plant, bacterial, and animal sources, and constitutes a significant level of structural diversity. Subjection of these molecules to anti-proliferative assays has revealed novel insights into triterpene structure-activity relationships, which have been mapped out through comparisons and evaluated using SAR. This work shows the possibility to unlock the therapeutic potential of these important molecules for development into the next generation of drugs.

Enas Aljohani
Poster Number 13

Heterobimetallic Ruthenium-Ferrocene Complexes as Anticancer Agents

E. Aljohani, T. Stringer, Y. H. Lee, R. M. Lord

Ruthenium-based compounds are emerging as alternatives to platinum-based chemotherapeutic drugs, due to the availability of the metal in a range of oxidation states under physiological conditions and the lower cytotoxicity against normal cells. Organometallic Ru(II) "piano stool" complexes have sparked a lot of interest, due to the simplicity of synthesis and the flexibility of altering their ligand environments. Several studies have been carried out to study the cytotoxic effects of changing the arene group and ligand environments.

Pichamon Eiamampai
Poster Number 14

Evaluating the potential of VEGFA mRNA isoform measurement as a predictive biomarker for use of AvastinTM in ovarian cancer

Pichamon Eiamampai, William English

Ovarian cancer is the 6th leading cause of cancer death in females, accounting for almost 4,100 deaths in the UK (United Kingdom) annually. Although clinical trials of chemotherapy in combination with Bevacizumab (AvastinTM) showed promise, the high rates of resistance and severe side effects remain an ongoing concern. This urgently calls for alternative treatment options. One possible treatment involves the Vascular Endothelial Growth Factor A (VEGFA), a potent activator of angiogenesis that plays an important role in the migration and proliferation of vascular endothelial cells in ovarian cancer. Nevertheless, although anti-VEGF therapies (e.g., Avastin from Genentech/Roche) indeed improve survival rates, its unreliable efficacy and high cost means that it cannot be routinely offered to patients. Therefore, the aim of our research is to find a predictive biomarker that can effectively identify patients who will likely benefit from anti-VEGF therapy and bring down the cost of treatment.

Various isoforms of VEGFA had been generated via mRNA splicing and proteolysis (Vempati et al.). In light of this background, we previously analysed VEGFA isoform expression in RNA sequencing data on ovarian cancer from the Cancer Genome Atlas (TCGA) and demonstrated that VEGFA-165 showed the highest expression (unpublished work). Further analysis of our unpublished work showed that women with High Grade Serous Ovarian Cancer (HGSOC) and higher expression of VEGFA-165 isoform had reduced overall survival. Retrospectively, they were also more likely to respond to anti-VEGF therapy. Altogether, this could suggest that VEGFA-165 does indeed have the potential to be a predictive biomarker for anti-VEGF therapy.

As a part of the ongoing research, this study specifically analysed 14 tissue microarrays (TMA) of ovarian cancer in situ using BaseScope, a tool used to identify short mRNA segments. Specifically, this allowed detection of short mRNA segments of VEGFA-165, VEGFA-121, and VEGFA-189 isoforms within the tissue sections. The programme QuPath is then used to quantify exact measurements and location of the mRNA.

Another existing potential biomarker is the mean vascular density (MVD), which is categorised into low or high density based on the expression levels of protein CD31 as described by Bais et al., 2017. From this, we endeavoured to investigate any correlation between areas of high MVD and high VEGFA-165 expression. Chalkey Point Array was laid over the images and 10 random areas were identified for each of the 14 TMAs. The corresponding image was opened on QuPath and the same 10 areas were identified in order to compare VEGFA-165 expression. We were able to conclude that there were no correlations between the two biomarkers.

Through this research, we hope to evaluate the effectiveness of VEGFA as the next predictive biomarker for ovarian cancer in anti-VEGF therapy. Its success could give a wider implication to treatment of not just ovarian cancer, but other forms of cancer as well. Although this is an ongoing research, we predict that it will point us in a new direction for more effective cancer treatment.

Eduardo de la Vega
Poster Number 15

Single molecule DNA replication dynamics of the human genome.

Jamie Carrington, Rose Wilson, Eduardo de la Vega, Sathish Thiyagarajan, Tom Barker, Leah Catchpole, Alex Durrant, Vanda Knitlhoffer, Chris Watkins, Karim Gharbi, Conrad A. Nieduszynski

Faithful DNA replication is fundamental to the survival of every organism. Errors during DNA replication are the major source of genetic variation that that can lead to disease. The location and distribution of DNA replication initiation sites have been implicated in genome instability and multiple human diseases, including early stages of cancer. However, there are currently no high-resolution or high-throughput genomic approaches for the reliable detection of replication initiation sites.

Here, we present DNAscent, an unbiased method for detectig replication initiation events on individual nanopore reads. DNAscent monitors replication fork dynamics across the human genome by detecting changes in BrdU incorporation during S phase, allowing to determine the direction of replication fork movement on individual nanopore sequence reads. This approach reveals that most initiation events are dispersed throughout the genome and are missed by traditional cell-population based methods. Unlike focused initiation sites, these dispersed sites do not correlate with transcription start sites or epigenetis signatures. Therefore, this single-molecule approach indicates that most initiation occurs at sites that, individually, are rarely used, yet cumulitative replicate the majority of the human genome.

Angela Man
Lightning Talk And Poster Number 16

Using the haploid cell line, HAP1, to investigate polyploidy in many ways.

Angela L. Man, Andrew J. Goldson, Wilfried Haerty, Conrad A. Nieduszynski

Somatic polyploidy is the state where cells, apart from the gametes, possess more than one set of chromosomes, and commonly arises from the process of whole genome duplication. Studies show that evolutionarily conserved mechanisms control the generation of polyploidy, and also that polyploidy plays important roles during normal development as well contributing to human disease. There is much known about molecular mechanisms that generate cellular polyploidy, but comparably little regarding other aspects of the polyploid state such as genomic, transcriptomic and epigenetic changes, and perturbations in DNA replication. We want to study such processes by looking at “polyploidy in many ways”, using as our model systems, the HAP1 cell line and natural examples of programmed polyploidy, such as the megakaryocyte. We hope that insights gained with our investigations into healthy cells could contribute to knowledge of how polyploidy maybe controlled in the disease states such as cancer.

Edyta Wojtowicz
Poster Number 17

Single-Cell Analysis Reveals Novel Insights into Megakaryocyte Heterogeneity and Microenvironment

Sonia Fonseca, Yuxuan Lan, Andrew Goldson, Stuart Rushworth, Edyta Wojtowicz

Megakaryocytes (MKs) are highly polyploid cells responsible for platelet production, exhibiting a unique model of physiological polyploidization. We observed dramatic ploidy shifts in bone marrow samples from acute myeloid leukemia (AML) models, prompting a comprehensive investigation of MK cellular and molecular features. We aim to elucidate the intrinsic and extrinsic factors influencing MK function through transcriptomic single-cell analysis.

We developed a robust MK isolation method combining manual sorting and imaging cell sorting, overcoming limitations of current protocols that fail to capture morphological information. This approach enabled discrimination between MKs containing/attached to neutrophils or platelets decorating other cells. High-quality single MK transcriptomes were obtained, providing unprecedented insights into mature MK transcriptional landscapes.

To investigate the influence of microenvironment on MKs, we employed spatial transcriptomics to study niches supporting polyploidization in vivo and cellular organization within tissues. This approach allows for the examination of MKs in different anatomical locations, considering variations in oxygen concentration, pathogen presence, and neighboring cells.

Our findings contribute to a deeper understanding of the correlation between ploidy, morphology, gene expression, and function in MKs. This research has significant implications for unraveling the complex interplay between MKs and the tumor microenvironment in AML, potentially leading to novel therapeutic strategies targeting megakaryopoiesis in hematological malignancies.

Katherine Hampton
Lightning Talk And Poster Number 18

Acute Myeloid Leukemia induces an increased senescent phenotype in the large intestine which protects barrier function and reduces leakiness.

Katherine Hampton, Alyssa Polski-Delve, Rebecca Maynard, Naiara Beraza, Kristian Bowles, Stuart Rushworth

The initiation and progression of multiple diseases and cancers has been associated with both intestinal dysfunction and microbiome changes, particularly those associated with aging, including acute myeloid leukemia (AML) (Wang et al., 2022). AML is a rapidly proliferating hematological malignancy, primarily affecting the elderly population, which interacts with and affects multiple organs in order to enhance its survival and ability to proliferate. One key hallmark of an aging phenotype is cellular senescence, defined as an irreversible state of cell cycle arrest in G1 phase, preventing cellular growth and division, as well as the secretion of a wide range of proinflammatory factors, creating a senescence-associated secretory phenotype (SASP). Our previous work has shown that AML can induce senescence in the bone marrow microenvironment, accelerating AML disease progression via the secretion of proinflammatory SASP-cytokines which support the survival and proliferation of AML blasts (Abdul-Aziz et al., 2019).

Multiple studies have linked AML to cellular and morphological alterations in the liver, spleen, central nervous system and kidneys. However, whether these changes are caused in the early stages of the disease to aid AML proliferation and survival or are just a consequence of latestage AML and the associated multi-organ failure is hard to define. Limited research has studied the impact of AML on the intestine and how these intestinal changes affect AML disease progression. Here, we investigate the role of AML on the intestine, and in particular the role of cellular senescence within the gut.

Intestines were harvested from mice engrafted with MN1 cells, a well established mouse model of AML, and intestinal epithelial cells were isolated from the colon. Analysis of the large intestine showed increased intestinal senescence, as evidenced by increased expression of p16 and p21 within intestinal epithelial cells, as well as positive Beta-galactosidase staining. This increase in senescence correlated with a significant decrease in proliferation by nearly 30% compared to control mice, evidenced by Ki67 immunohistochemistry. Increased senescence also correlated with increased tight junction RNA and protein expression. Immunofluorescent microscopy revealed there was a significant increase in zonula occludens-1 (ZO-1) and occludin expression in the intestinal epithelial cells at the tip of the colonic crypts, suggesting reduced overall gut leakiness within the colon of AML mice.

Further work is planned to investigate whether this increase of intestinal senescence was beneficial to or protective against the AML, by orally treating mice engrafted with AML with the senolytic, Navitoclax. This well help establish establish the impact of Navitoclax on the intestine, via elimination of senescent intestine cells, on AML progression and tumor burden.

Alyssa Polski-Delve
Poster Number 19

Tumour Derived Interleukin-1 Drives AML Induced Muscle Atrophy

Alyssa Polski-Delve, Rebecca Maynard, Katherine Hampton, Gwenaelle Le Gall, Timothy Pearson, Kristian M. Bowles, Stuart A. Rushworth.

Cachexic muscle wasting has been reported in multiple cancers including acute myeloid leukaemia (AML), though it is better defined in solid tumour types such as lung and gastrointestinal cancers than in haematological malignancies (Campelj D. et al 2022). With the average age of first diagnosis of AML being 69 (according to the American Cancer Society 2024), age related sarcopenia combined with AML associated skeletal muscle atrophy can lead to a severe and life threatening phenotype. This phenomenon is exacerbated by chemotherapy, limiting treatment options and efficacy. Though AML associated cachexia presents a poor prognosis, research is limited and the mechanisms behind it are inadequately understood. In this project, we aim to elucidate the mechanisms behind muscle atrophy during AML.

To identify the drivers of AML associated cachexia, the syngeneic AML mouse model was used in which C57/Bl6 mice were administered MN1 over-expressing cells via intravenous injection. Blood serum was collected, and muscles of the lower hind leg were harvested. Comparison of AML muscle weights to non-tumour bearing mice indicated a statistically significant decrease in the gastrocnemius muscles of AML mice, and a noticeable decrease in the anterior tibialis. Furthermore, RT-qPCR of gastrocnemius tissue revealed a significant upregulation of the atrophy associated genes TRIM63 and FBXO32, both of which are involved in the process of ubiquitin mediated protein degradation. Histological analysis by haematoxylin and eosin staining revealed areas of abnormal structure and reduced muscle fibre size within AML mouse muscles. These results indicate that muscle degeneration and atrophy occurred within our model.

To understand why atrophy occurs in muscle tissue in AML, we analysed the amino acid composition of serum from AML mice using nuclear magnetic resonance (NMR). Due to altered amino acid levels within the serum, namely large decreases in glutamine with increases in aspartate and serine, we investigated amino acid transport by analysing the expression of large neutral amino acid transporter 1 (LAT1) in gastrocnemius muscles. LAT1 was found to be significantly upregulated in the muscles of AML mice relative to non-tumour bearing mice.

To elucidate the factors involved in manifesting AML associated cachexia, we analysed serum from AML mice via proteome profiler cytokine array. Results indicated an increase in pro-inflammatory cytokines such as Interleukin (IL) 6, IL-1β and IL-1α, the latter two being implicated in the upregulation of TRIM63 and FBXO32 and subsequent myotube atrophy via the NF-κB pathway (Li W. et al 2009) (Huang N. et al 2017). IL-1 levels were also increased in MN1 cell conditioned media, indicating that the IL-1 seen within the serum of AML mice is produced by the cancer itself and not in response to it. The addition of IL-1β to C2C12 myotubes in culture produced a significant increase in TRIM63 expression, indicating that tumour derived IL-1 is a potential cause of muscle atrophy in our AML model.

We conclude that AML derived IL-1 can cause tumour associated muscle atrophy, this is likely due to the dependency of AML on amino acids released via muscle protein breakdown. Based on these results, therapeutic targeting of IL-1 could ameliorate AML associated cachexia.

Amy Harden
Lightning Talk And Poster Number 20

Investigating the RNA Structurome of Ewing Sarcoma

Amy M. Harden, Yiliang Ding, Darrell Green

Bone and soft tissue cancers (collectively termed 'sarcomas') account for just ~1% of all cancer diagnoses but are the third commonest cancer in the paediatric, adolescent and young adult (AYA) age group, making up 1 in 5 cases. Paediatric and AYA cancers are clinically and biologically highly distinct from older adult cancers. Sarcomas are characterised by abnormal terminal differentiation and genome rearrangements. Ewing sarcoma (EWS) is the second commonest bone sarcoma affecting ~2 per million individuals globally annually. The driver mutation for EWS is a fusion of the EWSR1 and FLI1 genes. The EWSR1::FLI1 fusion in a limb-derived mesenchymal progenitor produces an oncogenic transcription factor sufficient for tumorigenesis. A recent report showed that transient expression of IGF1 during puberty onset is required to cause full malignant transformation via YAP1 and TEAD DNA binding. It is unclear why EWS patients with the same driver mutation have significantly different outcomes to treatment. The answer to this long-standing question in the EWS field will add distinct value to basic biomedical science and will lead to improved therapies by converting 'poor responders' to 'good responders' to treatment. Most research into this question/problem has focused on the EWSR1::FLI protein and its role in regulating oncogenic transcription, however, cellular RNAs including those from EWSR1::FLI1 are heterogeneous with respect to their alternative processing and secondary (and hence tertiary) structures. The functional importance of this complexity at RNA level is poorly understood, for example, the ribosome binding site (RBS) can be made inaccessible or accessible by changing the RNA structure, through 'riboswitches', affecting translational output, and RNA bulges create unique recognition sites in RNA architecture both directly by acting as molecular handles (e.g. for RBPs and other proteins) in otherwise uniform helical regions and indirectly by distorting the RNA backbone and permitting access to base pairs. This PhD project will investigate RNA structure in vivo using chemical probing followed by Nanopore long-read sequencing in EWS cells, tissues (e.g. primary tumours and metastatic sites) and at single-cell resolution in circulating tumour cells. RNA structures will be linked to their clinical phenotype. RNA structure modifying drugs are in development; structures associated with increased sensitivity to treatment and better patient outcomes will be 'enforced' in patients who display poorer prognostic structures.

Kate Manley
Poster Number 21

Prediction of Disease Progression for Patients on Active Surveillance using the Prostate Urine Risk (PUR) Score

Kate Manley, Jeremy Clark, Daniel Brewer, Rob Mills, Omar Al Kadhi, Antonietta Hayhoe, Maria Traka, Richard Mithen, Colin Cooper

Background
The Prostate Urine Risk Score has been developed as a diagnostic tool in the diagnosis of prostate cancer (PCa). Through analysis of extracellular vesicle-derived RNA excreted in the urine, a risk score can be calculated which can determine the presence of prostate cancer prior to imaging or biopsy. In addition, the PUR score is able to predict the presence of low, intermediate or high grade disease thus avoiding unnecessary interventions for those patients with benign/low grade disease and facilitating choice of treatment for men with intermediate and high risk disease. For each patient, four PUR scores are returned which predict the probability of normal tissue (PUR-1), D'Amico Low risk (PUR-2), Intermediate risk (PUR-3) and High risk (PUR-4) prostate cancer. Prostate cancer is highly heterogenous and a mixture of all the above can exist in individual prostates. The sum of all four PUR signatures in each sample is always '1'.

PUR has also been demonstrated to predict outcomes for men on active surveillance (AS) at 5 years from diagnosis. The proportion of PUR-4 was found to have a significant association with clinical outcome (time to progression) up to five years following the collection of a single urine sample.

The ESCAPE study was a randomised double blinded intervention study examining the impact of dietary glucoraphanin on genetic expression in prostates of men on active surveillance. PUR scores were taken at diagnosis (T0) and at 12 months (T12) for this group of patients. Clinical follow up of the ESCAPE study cohort at 10 years was undertaken to further determine the predictive potential of the PUR score for men on AS.

Method
Clinical information was gathered for the 49 patients from the original ESCAPE study to determine disease progression and cancer specific mortality. Progression criteria included: significant volume or grade increase on biopsy histology, increase in radiological T or N stage on MRI, PSA progression leading to discontinuation of Active Surveillance. Clinical information on progression was correlated with PUR scores at T0 and T12. Patients were grouped on amount of PUR4 (> or < than 0.174) using the previously defined threshold

Results
Median length of follow up was 10.1 years (9-13.5), there was 1 mortality (non prostate cancer specific death), 44.8% progressed in 10 years (n=22), time to progression was median 4.3 years (1.3-8.9). Patients were grouped using the previously defined cut-off of >0.174 or <0.174 (Connell *et al.* 2019). Kaplan–Meier survival analyses were undertaken using progression as an endpoint for both groups. Patients with a T12 PUR4 >0.174 were found to have higher rates of disease progression than those with T12 PUR4<0.174 (log rank test P<0.05).

Discussion
This study provides further evidence of the ability of the PUR score to provide prognostic information for men on active surveillance, demonstrating its use over a much longer follow-up interval, supporting its use as a surveillance tool.

Freddie Marlowe
Poster Number 22

Refining Prostate Cancer Subtyping Using a Breast Cancer Classification Framework

Freddie Marlowe, Sergio Llaneza-Lago, Daniel S. Brewer

Prostate cancer (PCa) is the second most prevalent cancer in the UK, with approximately 50,000 new cases diagnosed annually. Its heterogeneous nature poses significant challenges for accurate diagnosis and treatment. To optimise patient outcomes, precise classification of PCa into distinct subtypes is essential. While current subtyping

methods remain inconsistent, recent research has applied Latent Process Decomposition (LPD), a Bayesian unsupervised approach, and successfully identified eight PCa subcategories with distinct clinical profiles. However, further research is imperative to fully characterise these subcategories. Given the shared molecular pathways between breast and prostate cancers, insights from breast cancer subtyping can be applied to prostate cancer. Therefore, we aim to apply the clinically validated breast cancer intrinsic subtype classification to refine PCa subtyping frameworks and develop more effective treatment strategies.

To achieve this, expression profiles from 370 PCa samples (Affymetrix Exon 1.0 ST) were analysed using five breast cancer subtyping algorithms (AIMS, PAM50, SCMGENE, SCMOD2, and SSP2006). A consensus classification was established by assigning the most frequent subtype across all algorithms to each sample. Differential expression analysis across the subtypes was conducted using the R package limma and survival analysis were performed using Kaplan–Meier (biochemical recurrence as clinical endpoint). Consensus subclassifications were compared to previously determined LPD-based molecular subtypes of the same samples.

Our results showed that the consensus breast cancer successfully classified the samples into four subtypes: Luminal A (n = 304), Luminal B (n = 18), Basal (n = 13), and HER-2 enriched (n = 35). Survival analysis revealed significant differences between subtypes ($p < 0.001$; Log-rank test), with Luminal B exhibiting the worse prognosis. We identified differentially expressed genes associated with each subtype. Comparative analysis with previous LPD classification, indicated significant enrichments of Luminal A and Basal samples with specific LPD groups ($p = 1.02e-16$; Chi_square test). Future research in this project will explore relationships between known breast cancer biomarkers and the LPD subtypes, and perform a comparative analysis of the differentially expressed genes between the two classifications to gain better insights into the LPD subtypes. This study will deepen our understanding of PCa heterogeneity and cross-cancer mechanisms, leading to improved classification frameworks and the development of new treatments to improve clinical outcomes.

Calista Tink
Poster Number 23

Determining How Prostate Cancer Evolves by Analysing Molecular Subtypes in Single-Cell Sequencing Data

Calista Tink, Sergio Llaneza Lago, Colin S. Cooper, Harveer Dev, Daniel S. Brewer

Prostate cancer is the most common cancer affecting males in the UK, with one in eight males being diagnosed in their lifetime. Prostate cancer is a highly heterogeneous disease, which makes it hard to decipher the underlying biological mechanism behind disease progression and the detection of clear molecular subtypes. Classification is important to accurately determine individual disease prognosis, effective patient treatment plans or identify novel treatment targets. There are currently no molecular subtypes used to diagnosis prostate cancer in the clinic. Previous work in our group focusing on determining prostate cancer molecular subtypes revealed an aggressive subtype named DESNT, using Latent Process Decomposition (LPD) to cluster bulk transcriptomic data into processes. Patients with DESNT showed poor outcomes and a higher risk of developing metastasis, highlighting its clinical utility. However, bulk sequencing omits rare subpopulations and averages gene expression across samples, which can obscure key molecular drivers of disease. Single cell sequencing offers a new perspective to accurately classify single cells into existing or novel molecular subtypes. In this project, we will analyse approximately 15,000 cells from 10 patients using scRNA-seq to identify differences between cell populations from the prostate cancer biopsy and matched normal tissue. This research will provide an opportunity to delve deeper into the underlying biology behind the existing molecular subtypes, such as DESNT, and identify potential novel subtypes at the single cell level. In the future, this could improve the accuracy of patient diagnosis through the implementation of an improved classification framework into the clinic. This could result in more tailored treatment strategies for patients and ultimately better patient outcomes.

Tim Brendler-Spaeth
Poster Number 24

The Prognostic Potential of Methylation Patterns in Prostate Cancer: Insights from High-Resolution EPICseq Data

Timothy Brendler-Spaeth, Abraham Gihawi, Colin S. Cooper, Daniel S. Brewer

Prostate cancer (PCa) is the second most prevalent cancer among men globally. The disease spectrum ranges from indolent cases that may remain harmless for many years to aggressive forms that rapidly progress. Disease stratification is a significant clinical challenge due to PCa's heterogeneity.

Accurate prognostication at the time of diagnosis is crucial for guiding treatment decisions. However, current clinical methods often fall short in accurately differentiating between aggressive and indolent forms of PCa. This can result in overtreatment with many patients undergoing radical treatments that may be unnecessary. There is a pressing need for improved prognostic biomarkers that can better stratify patients.

Our research uses data from the International Cancer Genome Consortium (ICGC). The ICGC multi-omic dataset, which includes genomic, methylomic, transcriptomic, and clinical information from PCa patients, provides a comprehensive resource for understanding disease aetiology through 'omic interactions and identifying potential biomarkers.

Notably, the ICGC dataset offers enhanced coverage of methylation sequencing using Illumina's TruSeq Methyl Capture EPIC Library Prep Kit (EPICseq). While other studies, such as those utilising The Cancer Genome Atlas methylation data, rely on the lower coverage 450k array, EPICseq offers a cost-effective, higher resolution of genome-wide methylation at over 3 million CpG sites, allowing for a more detailed and comprehensive analysis of methylation patterns.

Methylation changes are known to play a critical role in cancer biology, potentially revealing key disease drivers, novel therapeutic targets, and prognostic insights. By leveraging EPICseq's superior methylation information, our study aims to identify specific methylation changes that correlate with PCa progression and changes in other 'omics.

We are conducting an extensive analysis of the ICGC methylation data, focusing on its prognostic potential to differentiate between indolent and aggressive forms of PCa. The findings from this study could contribute to the development of more accurate prognostic tools, ultimately improving treatment stratification and enhancing the quality of life for patients with PCa.

Seshadhri Subramanian
Poster Number 25

tRNA Charging and Methionine: Exploring bacterial metabolic mechanisms in Prostate Cancer

Seshadhri Subramanian, Abraham Gihawi, Colin S. Cooper, Rachel Hurst, Daniel S. Brewer

Introduction
Prostate cancer is one of the most common cancers in the world (1 in 8 men will be diagnosed). The main risk factors are old age, ethnicity and familial history. Infection has also been established as a risk factor in many cancers. Prostate cancer is also influenced by many bacteria in the prostate microbiome. The identification of the Anaerobic Bacterial Biomarker Set (ABBS) bacteria could help identify patients at risk of developing aggressive prostate cancer. The five ABBS species so far are from the following genera – Fenollaria, Peptoniphilus, Anaerococcus, Porphyromonas, and Fusobacterium. Patients with the ABBS bacteria in them tend to have more aggressive cancers and lower long-term survival. What remains unclear are the metabolic mechanisms by which the ABBS bacteria act in these cancers.

Objectives
To use bioinformatics packages on The Pan Prostate Cancer Group whole genome sequencing dataset to determine the metabolic mechanisms which are enhanced in ABBS-positive versus ABBS-negative cancer patients.

Methods
867 tumour samples from the Pan-Prostate Cancer Group (PPCG) dataset were examined for this study. 164 were ABBS-positive tumours and 704 were ABBS-negative tumours. HUMAnN (HMP Unified Metabolic Analysis Network) were used to analyse metabolic profiles of these two sets of samples from sequencing data. Analysis was done using a Snakemake pipeline to run HUMAnN version 3.7 and carried out on the High Performance Computing Cluster supported by the Research and Specialist Computing Support Service at UEA. The three output files of HUMAnN denoting the abundance and names of Gene families (grouped by Enzyme Commission number to level 4 using the Uniref90 database), Path abundance and Path coverage are taken forward for further analysis together with a metadata file. This is done using the Maaslin2 – Microbiome Multivariable Associations with Linear Models - (version 1.12.0) package on R. Maaslin2 correlated the association between ABBS status (positivity or negativity) and the gene families that are significantly associated with this. A similar output was generated for path abundance

Results
667 gene families and 207 pathways were deemed to be significantly associated with ABBS positivity when the PPCG data was analysed. tRNA charging pathway genes – tRNA synthetases – for all the 20 amino acids are significantly associated with ABBS positivity with Glutamine, Valine and Aspartate among the most significant. ABBS positive tumours demonstrate a highly significant association with pathways that either synthesise or salvage methionine.

Discussion
tRNA charging which is undertaken by tRNA synthetases refers to the aminoacylation of tRNA through aminoacyl-tRNA synthetases (ARSs) to form an amino-acyl tRNA which can then allow for the formation of peptide bonds. Tumour suppression programmes are shown to be inactivated when leucyl-tRNA synthetase is downregulated.Cancer cells can be distinguished from normal cells due to their dependence on exogenous methionine – Hoffman effect. It has been shown that under methionine-restricted conditions, cancer cells can stop in the S/G2 phases of the cell cycle and altered methionine metabolism may be an underlying principle behind cancer development.Thus increased tRNA charging and methionine synthesis/salvage could be very important pathways in aggressive ABBS positive tumours.

Jade Manning
Poster Number 26

Investigating the Mechanisms of Action of Intracellular Anaerobic Pathogens on Human Host Cancer Cell Metabolic Networks

Jade Manning, Rachel Hurst, Abraham Gihawi, Dipali Singh, Rachel Dunn, Jonathan Tang, Gwenaelle Le Gall, John Wain, Colin S. Cooper, Daniel S. Brewer

Prostate cancer is the most common cancer in men in developed countries, and accounts for over 250,000 deaths per year worldwide. It is known that infectious agents, such as bacteria and viruses, are involved in the development of various cancers such as cervical, stomach, and bladder cancer. The causes of prostate cancer and the development of advanced disease remains obscure, but there is good evidence that infectious agents could be one cause. Hurst et al. found that the presence of a group of five anaerobic genera (called Anaerobic bacteria biomarkers set, ABBS), was associated with cancer progression: hazards ratio of 6.18 (OTU data, 95% CI:0.81-47.3, Cox regression) in urine sediment, 4.41 (RNAseq data, 95% CI:0.95-20.53) in urine extracellular vesicles, and 2.07 (whole genome sequencing (WGS) data, 95% CI:1.04-4.15) in cancer tissue. A critical question is how infectious bacterial agents are involved in the aetiology and evolution of prostate cancer, or whether their presence is opportunistic. To answer this question the global effect of ABBS infection on human host cell cancer metabolic networks will be investigated through experimental and computational analysis. Experimentally, nuclear magnetic resonance and mass spectroscopy will be used to determine the metabolomic profiles linked with prostate cancer in cells and human tissue explants. Additionally, RNAseq will be used to investigate changes in the expression profile of both bacteria and human tissue. Computationally, genome-scale metabolic models will be generated from the datasets to reveal potential mechanisms of interaction between bacteria and human cancer cells. Preliminary data will be presented on target metabolites produced by ABBS bacterial pathogens that may be linked to prostate cancer. This research has the potential to reveal how bacteria cause prostate cancer to develop and reveal new treatment possibilities to prevent or halt aggressive cancer.

Rachel Hurst
Lightning Talk And Poster Number 27

Multi-Step Mechanisms of Action of Novel Pathogens and Anaerobic Bacteria Associated with Cancer

Rachel Hurst, Daniel S. Brewer, Abraham Gihawi, John Wain, Colin S. Cooper

Several anaerobic bacteria genera and species have been associated with multiple cancer types, for example, bacteria belonging to genera including Peptoniphilus, Porphyromonas, Fusobacterium, Fenollaria, Anaerococcus, Prevotella, Sneathia and Veillonella[1]. The first five genera listed have also been associated with aggressive poor prognosis prostate cancer[2].

Bacteria associated with cancer have been described as intratumoural and have been detected intracellular inside cancer tissue and immune cells[3,4]. Several mechanisms of action of bacteria in cancer have been reported. We have analysed additional data from several novel species and will discuss the interlinked mechanisms and hypotheses of how multiple intracellular anaerobic bacteria may act together to cause host cell and tissue microenvironment changes associated with carcinogenesis and cancer cell invasion in a multi-step 7 step process. The seven steps include combined effects of multiple specific anaerobic bacteria on changes in the microenvironment, cell signalling pathways, DNA damage, cellular metabolism, hypoxia and immune evasion contributing to cancer growth, progression and invasion.

1. Hurst et al 2022 Eur Urol Oncol 5:412
2. Hurst et al 2024 J Med Microbiol 73(3):001817 doi: 10.1099/jmm.0.001817
3. Nejman et al 2020 Science 368(6494):973-80.
4. Geller et al 2017 Science 357(6356):1156-60.

Carrie Wemyss
Lightning Talk And Poster Number 28

Mining the Microbiota: Harnessing Bacterial Extracellular Vesicles for Improved Asparaginase Therapy

Carrie Wemyss, Regis Stentz, Emily Jones, Simon Carding

Acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL) are aggressive blood cancers with limited treatment options that often lead to severe side effects. Traditional therapies, including E.coli-derived L-asparaginase, are essential but can cause hypersensitivity and other adverse reactions due to their instability and high dosing regimens. Our project introduces a novel approach using bacterial extracellular vesicles (BEVs) generated by human commensal gut bacteria, which naturally encapsulate L-asparaginase. Our current research focuses on evaluating the efficacy of BEV-encapsulated asparaginase produced by different gut bacteria and benchmarking against clinical formulations of E. coli L-asparaginase. Results to date demonstrate promising cytotoxic effects of Bacteroides derived BEVs on the prototypical leukaemia cell lines HL-60 and THP-1 with potential for clinical application. This BEV based therapeutic has the potential to provide a safer and more effective treatment for AML and ALL by reducing the frequency and severity of side effects associated with current therapies.

Luke Mitchell
Poster Number H30

Exploring the immunomodulation pathways of Bifidobacterium in breast cancer through genetic modification

Luke Mitchell, Chris Price, Lindsay Hall, Stephen Robinson

Background
Increasing evidence has emerged highlighting the importance of gut microbiota in relation to cancers distant from the gastrointestinal tract. Specific members of the gut microbiota, such as strains belonging to the genus Bifidobacterium, have been shown to modulate host-immune responses and enhance cancer immune therapy. Further to this, the shift of the gut microbiota to dysbiosis through the use of antibiotics has shown to accelerate tumour growth in murine models. Research in the Robinson lab has focused on investigating the immunoregulatory and potential anticancer properties of Bifidobacterium.

Methods
Bifidobacterium underwent genetic manipulation through the use of electroporation inserting multiple selective markers. Following selection for successful transformation, WT (wildtype) strains were screened in vitro for immunoregulatory and potential anti-cancer properties by tumour cell killing assays (MTS), CD8 T cell activation assays, cytokine, and chemokine profiling.

Results
Using electroporation, we were able to generate GFP-positive and Chloramphenicol-resistant Bifidobacterium strains which will be crucial for downstream investigation. Moreover, we have also shown that potential immunoregulatory properties of Bifidobacterium are not only species dependent but also strain dependent, and that these properties vary between potential candidates.

Conclusions
Collectively, these studies demonstrate that Bifidobacterium have immunoregulatory properties that could be utilised to adapt the host-tumour immune response, offering a potential novel cancer treatment. Future work into murine cancer models is essential for progressing these candidates, and we believe that the mechanisms behind their properties can be confirmed through such genetic manipulation.

Alicia Nicklin
Lightning Talk And Poster Number 31

The use of novel microbial-based therapeutics in the treatment of cancer

Alicia Nicklin, Christopher Price, Anne Jordan, Luke Mitchell, Wesley Fowler, Mitch Rowe, Lindsay Hall, Stephen Robinson

Background
Accumulating evidence has emerged highlighting the importance of the gut microbiota in relation to cancers distant from the gastro-intestinal tract. Certain members of the gut microbiota, such as strains belonging to the genus Bifidobacterium and Lacticaseibacillus, have been shown to modulate host-immune responses as well as synergising with immune checkpoint inhibitor therapy. In addition, loss of these protective species through antibiotic-induced disturbances accelerated breast tumour growth in murine models. Research conducted in the Robinson lab has focused on manipulating the gut microbiota using a newly discovered live biotherapeutic product (LBP) to improve cancer outcomes.

Methods and Results
In various orthotopic mouse models of breast cancer (BRPKp110, PyMT-BO1, and 4T1), the administration of a specific microbial strain has been found to reduce primary tumour growth compared to control (PBS-treated) mice. Long-term administration of the microbial strain also significantly delayed tumour onset time and overall tumour burden in a genetically engineered spontaneous murine breast cancer model (MMTV-PyMT). Flow cytometric analysis of tumours has shown increased infiltration and activity of CD8+ T cells as well as increased polarisation to a CD44hiCD62Llo CD8+ memory T cell subset in the blood after LBP-supplementation, suggesting the induction of systemic immunological memory.

We have also shown that the therapeutic potential of this microbial strain is not limited to administration of the live bacteria. Previous work demonstrated that intravenous administration of purified bacterial extracellular vesicles (BEVs) significantly reduced melanoma tumour volumes in a B16-F10 mouse model. Observed immunomodulatory properties of BEVs include strong activation of toll-like receptor (TLR)-2 (as determined by TLR-2 reporter HEK293 cells) as well as increased infiltration of tumour-associated neutrophils in vivo.

Conclusion
Utilising specific members of the microbiota and microbial-derived products to adapt host-tumour immune responses could offer a novel approach to accompany conventional cancer treatments. Further work to assess efficacy in humans, who display large inter-individual microbiome variation, is integral for future clinical development.

Mark Williams
Poster Number 32

Cholinergic mobilisation of juxtaposed TPC1-InsP3R3 calcium stores triggers secretion of mucus and fluid to flush the human colonic stem cell niche

Nicolas Pelaez-Llaneza, Victoria Jones, Christy Kam, Alvin Lee, Alyson Parris, Sean Tattan, Martin Loader, Jordan Champion, George Russam, J. Benjamin Miller, Nathalie Juge, Iain Macaulay, Daniel S. Brewer, Ryan Cardenas, Richard Wharton, Christopher Speakman, Sandeep Kapur, James Hernon, Adam Stearns, Irshad Shaik, Anatu Pal, Alexia Tsigka, Diogenis Batsoulis, Mark Williams

Intestinal epithelial cells form a vital selective barrier between the mucosal immune system and a barrage of microorganisms, ligands and antigens derived from the hostile gut lumen. Not surprisingly, compromised barrier function is associated with an increased risk of inflammatory bowel disease and colorectal cancer. Preservation of barrier function is underpinned in part by calcium-dependent secretion of a protective mucus layer. The aims of the current study were to (i) unravel the mechanism of cholinergic calcium signals that initiate at the human colonic crypt base and (ii) determine the effects on secretion of mucus and fluid from crypt-base-goblet-cells (GCs) and neighbouring intestinal stem cells (ISCs). Methods: Human colonic crypts were isolated from colorectal tissue samples obtained at surgical resection (NREC approval) and cultured as crypts in the short-term or propagated as crypt-like organoids over the long-term (up to 5 years). The spatio-temporal characteristics of intracellular calcium was monitored by Fura-2/Fluo-4/Calbryte-630 imaging and the mechanism of receptor-mediated calcium mobilisation was characterised by pharmacological and knockdown gene approaches. Calcium signalling toolkit expression was visualised by fluorescence immunolabelling and super-resolution imaging. Mucus secretion was visualised by Muc2 immunofluorescence depletion assays and real-time imaging of fluorescently tagged mucin-2, using MUC2::mNEON crypt-like organoids generated by CRISPR-HOT. An organoid swelling assay was used as a proxy for fluid secretion. Results: A microdomain of juxtaposed InsP3R3 and TPC1 expression was present at the apico-lateral pole of ISCs and GCs at the crypt (or organoid crypt-like)-base and corresponded to the site of cholinergic calcium signal initiation (Carbachol, 1-100 μM; n>10). Calcium signals propagated across to the basal pole of initiation cells and laterally to neighbouring cells. Calcium signal amplitude was reduced >50% by TPC1 antagonists NED19 (250 μM; n>10; P<0.05) and tetrandrine (20 μM; n>10; P<0.05). Caffeine (10 mM), an inhibitor of InsP3Rs, and miRNA knockdown of InsP3R3s, also reduced calcium signal amplitude by >50% (n>5; P< 0.05). Carbachol (10 μM) stimulated both MUC2 depletion from GCs in colonic crypt bases (n>200; P<0.05) and luminal secretion of MUC2-mNEON in crypt-like organoids (n>5; P<0.05). Carbachol (10 μM) also stimulated an increase in organoid cross-sectional area in organoid swelling assays. Pharmacologic inhibition of TPC1 or InsP3Rs (see above) reduced cholinergic stimulation of: MUC2 immunofluorescence depletion from crypt GCs; luminal secretion of MUC2-mNEON from crypt-like organoids; cross-sectional area of organoids in swelling assays (n>5; P<0.05). Conclusion: Co-activation of juxtaposed TPC1 and InsP3R3 is required for generation of cholinergic calcium signals and downstream secretion of hydrated mucus, which culminates in the flushing of the human colonic stem cell niche. The implications for colon cancer risk will form a basis of future work.

Mark Williams
Poster Number 33

Dysregulation of the calcium signalling pathway in colon cancer: identification of drug targets for chemotherapy

Victoria Jones, Sean Tattan, Nicolas Pelaez-Llaneza, Alyson Parris, Alvin Lee, Jordan Champion, Ana Garcia Canadas, Ryan Cardenas, Daniel S. Brewer, Iain Macaulay, Simon Moxon, Marietta Xagorari, Alexia Tsigka, Dhanurjaya Thyagaraja, Richard Wharton, Chris Speakman, James Hernon, Sandeep Kapur, Irshad Shaikh, Diogenis Batsoulis, Anatu Pal, Adam Stearns, Mark Williams

Background
There are over 40,000 new cases of colorectal cancer and 16,000 related deaths per annum in the UK. The calcium signalling pathway regulates a host of (patho)physiological processes including cell proliferation, migration, and apoptosis. Genetic and epigenetic perturbation of the calcium signalling pathway has been implicated in carcinogenesis, and has attracted attention as a target for pharmacological intervention[1]. We have generated a living tissue biobank of patient-matched organoids and tumouroids to model the physiology of the human colonic epithelium in health and cancer *ex vivo*. The aim of the current study was to utilise a combination of in silico and in vitro approaches to investigate the status of the calcium signalling pathway in colon cancer. We hypothesise that bioinformatical and functional analyses will identify druggable calcium signalling components that can be screened for efficacy using the patient-matched organoid and tumouroid platform.

Methods
DNA/RNA was isolated from patient-matched human primary mucosa, isolated crypts, organoids and tumouroids, and subjected to WES/bulk RNA-seq (N=6). Analyses of WES identified a unique combination of cancer driver gene mutations for each patient. Transcriptomic data was examined by principal component analyses (PCA), Pearson correlation analyses, differential gene expression analyses (DGEA), and gene ontology (GO) analyses. Genes significantly upregulated in tumouroids (log2FC>2, padj<0.01) were queried against the drug gene interaction database (DGIdb https://www.dgidb.org/) to yield a drug gene interaction network. Spatial expression of calcium signalling toolkit components was investigated by immunocytochemistry, and calcium signalling status and mechanisms were investigated by fluorescence imaging and pharmacologic methods. Organoid/tumouroid viability was assessed by Cell Titer Glo and live/dead labelling.

Results
Each patient exhibited a distinct profile of mutations affecting cancer driver genes such as APC, K-RAS and TP53. A molecular function GO analysis on genes significantly upregulated in tumouroids (Log2FC>2, padj<0.001) showed an enrichment of genes pertaining to calcium signalling. Several calcium channels exhibited differential gene expression in patient-derived organoids versus tumouroids. Calcium channel protein immunofluorescence intensity analysis also revealed aberrant calcium channel expression in tumouroids v organoids. An in-silico drug library was generated against calcium channels significantly upregulated in tumouroids v organoids. Querying these targets against the DGIdb facilitated the generation of a network of drug gene interactions. Patient-matched organoid- and tumouroid viability exhibited differential sensitivity to intracellular calcium channel inhibitors.

Conclusions
The calcium signalling pathway is dysregulated in colon cancer and can be targeted to selectively kill cancer cells whilst sparing their healthy counterparts. Future work will determine the interactions between cancer-driver gene status, calcium signalling pathway function and cancer cell proliferation/survival.

Thanks

A huge amount of work has gone into this symposium and we would like to take this opportunity to thank everyone who contributed, including all the delegates who asked questions, those who gave talks, those who chaired sessions, poster judges and those who presented posters. Special thanks to Emily Hobson and Tim Brendler-Spaeth for organising and chairing the Early Career Researcher session, and for being on top of social media. Also, a huge thank you to Libby Brient from UEA RIN who provided excellent professional services support and manned the registration desk on the day. We are also indebted to Timothy Brendler-Spaeth, Sergio Llaneza-Lago, Freddie Marlowe, Calista Tink and Anna Russell for supporting all aspects of the day. We would also like to thank the Faculty of Science, the Faculty of Medicine and Health, Stratech, VectorBuilder, nanoString, Oxford Nanopore, Fisher/ThermoScientific for their financial support in making this event possible. Finally, we would like to thank you, the delegates, for your participation.

Daniel Brewer, Emily Hobson, Pinelopi Gkogkou, Maria O'Connell, Mark Williams (NCRN Symposium organising group)

Feedback

Thank you so much for taking part in this year's symposium. Your feedback would be valuable to us to help gauge how well the event went and how we can improve it for next year.

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