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Norwich Cancer Research Network

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NCRN Symposium

5th June 2023

Sponsored By

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Oxford NANOPORE Technologies

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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.

Our programme includes a variety of sessions, including:

- Poster sessions: This is your chance to hear about the latest research from our scientists.
- Lightning talks: These short talks are a great way to learn about new research in a fast and engaging way.
- Longer talks: These talks will give you a deeper understanding of specific areas of cancer research.

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 tweet as much as possible citing **#NCRNSymposium2023** and **@CancerInNorwich**.

Enjoy!

Daniel Brewer, Emily Hobson, Pinelopi Gkogkou, Maria O'Connell, Mark Williams, Rianne Lord (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 and Introduction to NCRN – Daniel Brewer

10.05am Session 1 – Drug Discovery

Chair: Maria O'Connell

10:05	The design of heterobimetallic compounds for the treatment of cancer – **Rianne Lord**
10:20	Nanoparticles in cancer research: from early detection to targeted treatments – **María J. Marín**
10:35	Using Light to Control the Immune System - **Dee Hayward**
10:50	Development of a personalised chemotherapy pipeline for colorectal cancer - **Sean Tattan**
11:05	Dasatinib and Phenethyl isothiocyanate synergystically reduces hepatocellular carcinoma growth via oxeiptosis and cell cycle arrest - **Gabriele Strusi**

11.20am Tea/Coffee Break

11.45am Session 2 - Lightning Talks and Sponsors

Chair: Rianne Lord

11:45	Epidemiology of skin cancers using national registry data – **Zoe Venables**
12:00	Sponsor Talk 1 – **CancerTools**
12:10	**Lightning talks – part 1** Salonee Banerjee, Emma Ward, Vicky Kamperi, Silvia Ogbeide, Suzanne van Wier
12:25	Sponsor Talk 2 – **Oxford Nanopore**
12:35	**Lightning talks – part 2** Conrad Nieduszynski, Rebecca Casson, William R English, Stephanie Smith, Bethany Hood

12.50pm Lunch/Poster Session

1.50pm Session 3 - Biomedicine and bacteria

Chair: Mark Williams

13:50	Big roles for small RNAs: Fighting childhood and TYA cancer **- Darrell Green**
14:10	Acute myeloid leukemia reprograms lipid metabolism by downregulating CD36 expression in hepatocytes – **Rebecca Maynard**
14:25	Role of senescence during chronic liver disease – **Naiara Beraza**
14:45	Anaerococcus Species Associated With Increasing Prostate Cancer Risk - **Anastasios Bampalis**

3pm Tea/Coffee Break & Ice creams

3.25pm Session 4 – Clinical and Infrastructure Services

Chair: Pinelopi Gkogkou

15:25	The UEA BioAnalytical Facility: Your Sample Analysis Partner - **Isabelle Piec**
15:40	Turning an idea into a successful clinical trial - **Eleanor Mishra**
15:55	Insights into the potential collaborations with the MRI UEA department and the possibilities for research projects – **William Penny**
16:10	NRP biorepository - **Louise Jones and Rachael Stanley**

Keynote

16:25	Setting up clinical trials in Oncology: The story of PORTICO studies - **Dr Thankamma Ajithkumar**

17:05	Close and Poster Prize Presentation

5.10pm Drinks Reception & Poster Viewing

Talk Abstracts

Session 1
Rianne Lord

The design of heterobimetallic compounds for the treatment of cancer

R. M. Lord, E. Aljohani, M. Allison, B. J. Hofmann, T. Stringer, Y.-H. lee, and P. C. McGowan

Ruthenium compounds are promising alternatives to clinical platinum chemotherapeutics, due to their lower cytotoxicity against normal cells. Ruthenium research was excelled in the early 2000s, by the excellent cancer cell selectivity and different modes of action that NAMI-A ([$RuCl_4$(DMSO)(Im)][ImH], where Im = imidazole, DMSO = dimethylsulfoxide) displayed against solid tumours[1]. The main mode of action of these complexes is the intracellular reduction to an active ruthenium(II) species. Due to their ease of synthesis and characerisation, the understanding of such ruthenium(II) complexes have dominated the field of inorganic anticancer drug alternatives.

It is well documented that modifications to the organic ligands that surround the metal centre and the overall charge of the complex, can effect their biological activities. Our group have focused on b-diketonate ligands, in particular those that contain ferrocene [2-4], as this molecule is known to increase reactive oxygen species (ROS) in cells, and we have reported their complexation to several transition metals. This talk will give an update on both ruthenium(II) ferrocenyl β-diketonate coordination complexes (Figure 1A) [5], and both neutral (Figure 1B) and charged (Figure 1C) ruthenium(II) ferrocenyl β-diketonate arene complexes [6-8]. Reporting on their activities, cancer cell selectivity and intracellular modes of action.

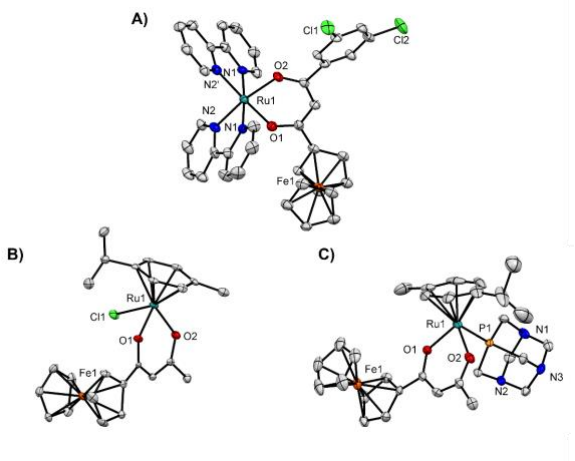

Figure 1 *Molecular structures of* ***A)*** *a [Ru(bpy)$_2$(Fc-diketonato][PF_6] (where X = 2,2'-bipyridine) and [p-cymRu(Fc-diketonato)X]$^{0/+}$ complexes where* ***B)*** *X = chloro and* ***C)*** *X = 1,3,5-Triaza-7-phosphaadamantane (PTA). Hydrogen atoms and counterions are omitted for clarity.*

References: 1. G. Sava et al. *Clin. Cancer Res.* 2003, **9**(5),1898-905; 2. R. M. Lord et al. *J. Med. Chem.* 2015, **58**(12), 4940; 3. R. M. Lord et al. *ChemEurJ*, 2019, 25(2), 495; 4. M. Allison et al, *ChemBioChem*, 2020, **21**(14), 1988; 5. M. Allison et al. *ChemEurJ*, 2021, **27**(11), 3737; 6. S. Gadre et al, *J. Med. Chem.* 2022, **65**(24), 16353; 7. M. Allison et al. Organometallics, 2023, just accepted; 8. unpublished work

Session 1
María J. Marín

Nanoparticles in cancer research: from early detection to targeted treatments

Carla Arnau del Valle, Alexandra Schroter, Paul Thomas, M. Paz Muñoz-Herranz, Francisco Galindo, Thomas Hirsch, and María J. Marín

The application of nanotechnology to diagnose, prevent and treat diseases has attracted increase attention in the past decades. For example, in photodynamic therapy (PDT) of cancer, nanoformulation-based delivery systems can be used to overcome some of the drawbacks of most photosensitiser drugs including their low water-solubility and non-specific biodistribution that leads to severe side effects [1]. Nanoparticles can be designed to be hydrophilic and their targeted delivery to the tumour side can be achieved passively via the enhanced permeability and retention (EPR) effect and actively following further functionalisation with targeting that selectively recognise specific cell surface receptors overexpressed in cancer cells [2-6]. Furthermore, nanoparticles such as upconverting nanoparticles (UCNPs) can be used to shift the excitation wavelength of commonly used photosensitisers to near-infrared (NIR) light, that penetrates deeply in the biological tissues producing minimal photodamage and no autofluorescence of the tissue upon long-term irradiation [2]. In addition, nanoparticles can be used to developed theranostic agents, in which diagnostic tools are combined with therapeutic agents in a single nanoplatform.

This presentation will show how nanoparticles (gold nanoparticles and UCNPs) have been used for the development of new nanoparticle-based photosensitiser-delivery systems that are water soluble, tumour specific and can be excitable with NIR light to overcome some of the drawbacks of current PDT of cancer. This work will also show advances on the development of NIR excitable nanoplatforms that can be used for the detection and quantification of nitric oxide (NO), which has been shown to have a double effect in cancer, being involved in tumour-promotion and tumour-suppression effects, with the cytotoxic effect of NO being exploited in the treatment of cancer.

1. A. B. Ormond et al, Materials, 2013, 6, 817
2. C. Arnau del Valle et al., Methods Appl. Fluoresc., 2022, 10, 034003
3. Z. R. Goddard et al., Chem. Soc. Rev., 2020, 49, 8774
4. J. Fang et al., Adv. Drug Delivery Rev., 2011, 63, 136
5. A. K. Iyer et al., Drug Discov. Today, 2006, 11, 812
6. S. Tran et al., Clin. Transl. Med., 2017, 6, 44

Session 1
Dee Hayward

Using Light to Control the Immune System

Dee Hayward, Mark Searcey, Andrew Beekman

Immunotherapy provided a turning point in oncology, transforming treatment efficacy with a more targeted approach than conventional treatments. By blocking the interaction between the immune checkpoint molecule and its ligand, T cells can be activated to eliminate tumour cells. Programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1) are well described checkpoint proteins that have been successfully targeted with monoclonal antibodies. Recent research has identified cyclic and turn-motif peptides capable of inhibiting the PD-1/PD-L1 interaction, offering cheaper antibody alternatives with less adverse immune effects.

Incorporation of a photo-switchable compound into a peptide can provide a light controllable inhibitor of the PD-1/PD-L1 interaction. Azobenzenes have been extensively studied and are typically found in the trans-form to isomerize to the cis-form upon ultraviolent (UV) irradiation at 350 nm. In the cis-form, the azo-peptide has the ability to mimic the conformational turn of cyclic and turn-motif peptides to inhibit the interaction by binding to PD-L1.

The design and synthesis of light activatable azo-peptides that show the capability to inhibit the PD-1/PD-L1 interaction will be reported. In addition, modifications to the azobenzene structure to tune wavelength sensitivity will be disclosed.

Session 1
Sean Tattan

Development of a personalised chemotherapy pipeline for colorectal cancer

Victoria Jones, Sean Tattan (presenting), Ryan Cardenas, Daniel S Brewer, Jordan Champion. Nicolas Pelaez-Llaneza, Alvin Lee, Alyson Parris, Thomas Marshall, Tom Palmer, Iain Macaulay, Simon Moxon, Alexia Tsigka, Dhanurjaya Thyagaraja, Gaurav Kapur, Richard Wharton, Chris Speakman, James Hernon, Sandeep Kapur, Irshad Shaikh, Diogenis Batsoulis, Anatu Pal, Adam Stearns, and Mark Williams

Colorectal cancer (CRC) is the second leading cause of cancer-related death in the Western world. High-throughput DNA sequencing has demonstrated that each tumour harbours a unique mutational profile. This patient-patient heterogeneity likely underpins the differential responses to 'one-size-fits-all' chemotherapy and the need for personalised medicine. Personalised pharmacogenomic pipelines utilising patient-matched healthy and tumour tissue are under development [1]. Proof-of-principle studies have identified cancer-driver genes from genome sequencing data and tested the efficacy of candidate pharmacologic agents [2]. This approach is challenged by the potential for multiple mutations in cancer-driver pathways to interact and thereby affect drug responsiveness. This sends out a call to develop pharmacogenomic pipelines that incorporate documented interactions between mutated cancer-driver gene pathways, and which more accurately model the efficacy of pharmacologic agents.

In this study, whole exome sequencing and KEGG pathway data sets were integrated into an in silico chemotherapy pipeline to predict personalised treatments for colorectal cancer. A living tissue biobank of patient-matched organoids and tumouroids was generated to model the physiology of the human colonic epithelium in health and cancer ex vivo and serve as a platform to validate the efficacy of the predicted personalised treatments. High content image analysis was applied to patient-matched organoid and tumouroid data sets to validate the efficacy of drug targets predicted by the personalised chemotherapy pipeline. Combined in silico and in vitro analysis of CRC organoid data sets is a significant advance towards personalised chemotherapy.

References
1. Van De Wetering et al., 2015, doi:10.1016/j.cell.2015.03.053.
2. Tamborero et al., 2018, doi:10.1186/s13073-018-0531-8

Dasatinib and Phenethyl isothiocyanate synergystically reduces hepatocellular carcinoma growth via oxeiptosis and cell cycle arrest

Gabriele Strusi, Caterina M. Suelzu, Nicole J. Horwood, Andrea Münsterberg, and Yongping Bao

Hepatocellular carcinoma (HCC) is the 4th most lethal tumour with a five years survival rate of 13%. Combination therapy exploits multiple drugs to target synergistically key pathways to reduce tumour growth. Isothiocyanates have shown anticancer potential and to complement the anticancer activity when combined with other compounds. This study assessed the potential of phenethyl-isothiocyanate to synergistically improve the activity of dasatinib in HCC. MTT and cell clonogenic assay were used to assess the combination anti-tumour effect in vitro, and a murine syngeneic subcutaneous model to assess the combination efficacy in vivo. Flow cytometry and western blot were used to elucidate the mechanism of action. Phenethyl-isothiocyanate and dasatinib showed a synergistic effect both in vitro and in vivo. By inducing oxidative stress, through the production of reactive oxygen species (ROS), PEITC and dasatinib combination promoted the formation of a premature CDK1-Cyclin B1 complex that leads to mitosis catastrophe. Furthermore, ROS activated oxeiptosis, a novel programmed cell death. Phenethyl-isothiocyanate can complement dasatinib's action in treating HCC with ROS-induced cell cycle arrest and induction of oxeiptosis, highlighting the role that ITCs may have in cancer therapy as a complement of clinically approved chemotherapeutic drugs.

Session 2
Zoe Venables

Epidemiology of skin cancers using national registry data

Zoe Venables

Skin cancer is the commonest cancer in the UK and incidence and mortality rates are increasing. We present data from national cancer registries to highlight the need for research development for skin cancers and in particular non-melanoma skin cancers (NMSC) which are more than ten times more common than melanoma. A paradigm shift in melanoma care has occurred over the last decade including the NICE recommendation for 12 targeted and immunotherapies for advanced melanoma. NMSC therapeutic development lags behind with only one immunotherapy NICE recommended for cutaneous squamous cell carcinoma (cSCC) and one for basal cell carcinoma (BCC). cSCC and BCC research has been long neglected as a result of poor epidemiological data and a belief that these cancers are not of significance in terms of survival outcomes.
In England 2013-2019, melanoma crude incidence rates increased by 2% per annum, BCC by 2.9% per annum and cSCC by 4.7% per annum. Crude mortality rates in England from 2010-2019 have reduced by 1% per annum, whereas NMSC mortality has increased by 4% per annum. As a result, we expect NMSC deaths to outpace melanoma deaths by 2035. Furthermore, cSCC 5 year net survival from 2014 is 90% which is the same as melanoma, breast and prostate cancer.
Additionally, we present data on a predictive modelling calculator developed from National Disease Registration Service data to identify high risk cSCC to inform clinical care and discuss projects in development including accessing CPRD data to better understand associations between skin cancers and prescribed medications.

Session 3
Darrell Green

Big roles for small RNAs: Fighting childhood and TYA cancer.

Darrell Green, Lee Jeys, Vaiyapuri Sumathi, Tamas Dalmay, and William D. Fraser

Sarcomas are heterogeneous and clinically challenging bone and soft tissue cancers. Sarcomas disproportionately affect children, teenagers and young adults (TYA) 10-fold when compared to adults. According to Public Health England's children and TYA UK cancer statistics report 2021, sarcoma is the third commonest childhood cancer. Almost half of all sarcomas are primary bone cancer (PBC), which affects ~6 per million individuals annually worldwide. Primary bone cancer (PBC) comprises several subtypes each underpinned by distinctive genetic drivers. This driver diversity produces novel morphological features and clinical behaviour that serendipitously makes PBC an excellent metastasis model. We report that some transfer RNA-derived small RNAs termed tRNA fragments (tRFs) perform as a constitutive tumour suppressor mechanism by blunting a potential pro-metastatic protein-RNA interaction. This mechanism is reduced in PBC progression with a gradual loss of tRNAGly^{TCC} cleavage into 5′ end tRF-Gly^{TCC} when comparing low-grade, intermediate-grade and high-grade patient tumours. We detected recurrent activation of miR-140 leading to upregulated RUNX2 expression in high-grade patient tumours. Both tRF-Gly^{TCC} and RUNX2 share a sequence motif in their 3′ ends that matches the YBX1 recognition site known to stabilise pro-metastatic mRNAs. Investigating some aspects of this interaction network, gain- and loss-of-function experiments using small RNA mimics and antisense LNAs, respectively, showed that ectopic tRF-Gly^{TCC} reduced RUNX2 expression and dispersed 3D micromass architecture *in vitro*. iCLIP sequencing revealed YBX1 physical binding to the 3′ UTR of RUNX2. The interaction between YBX1, tRF-Gly^{TCC} and RUNX2 led to the development of the RUNX2 inhibitor CADD522 as a PBC treatment. CADD522 assessment *in vitro* revealed significant effects on PBC cell behaviour. In xenograft mouse models, CADD522 as a single agent without surgery significantly reduced tumour volume, increased overall and metastasis-free survival and reduced cancer-induced bone disease. Our results provide insight into PBC molecular abnormalities that have led to the identification of new targets and a new therapeutic.

Acute myeloid leukemia reprograms lipid metabolism by downregulating CD36 expression in hepatocytes.

Rebecca Maynard, Katherine Hampton, Charlotte Hellmich, Dominic Fowler-Shorten, Martha Ehikioya, Niara Beraza, Kristian Bowles, and Stuart Rushworth.

Acute myeloid leukemia (AML) is a highly proliferative disease, requiring a high metabolic turnover. We and others have previously shown that primary human AML blasts rely on fatty acid (FA)-oxidation for survival. The liver plays a key role in fatty acid (FA) metabolism, producing energy in the form of ATP or ketone bodies. FFA oxidation in the liver is induced by the hormone-mediated release of FFA from adipose stores and subsequent uptake into hepatocytes, providing an excess of substrates for metabolism. This project aimed to determine if AML can alter FA metabolism in the liver to support disease progression and by what mechanism.

C57Bl/6 mice were engrafted with HOXA9/Meis or MN1 AML cells. This was confirmed by flow cytometry of the blood and bone marrow. The effect of the disease progression was seen in the body weight of the mice, which was stable over the initial period of AML progression but reduced during later stages. Epididymal and inguinal fat deposits were smaller in AML mice. These data support the notion that AML induces lipolysis in adipose tissue. qRT-PCR analysis of liver cells from the animals showed reduced expression of genes associated with FA metabolism, in AML mice. In vitro analysis of primary hepatocytes cultured with MN1 or HOXA9/Meis1 on transwells, showed downregulation of CD36 and CPT1 indicating that AML directly regulates expression of liver genes associated with FA metabolism. Cells were also cultured with BODIPY conjugated long-chain FA and representative images showed reduced uptake in AML-treated hepatocytes. These data suggest that AML is able to redirect FFA away from the liver by down-regulating CD36 which mediates FFA uptake in the liver.

To summarise, AML alters the expression of FA metabolism genes in the liver. Our in vivo and in vitro studies indicate that AML secretes cytokines which interact with hepatocytes, altering gene expression and thus FA metabolism in the liver. Increasing our understanding of how AML accesses more metabolites, allowing the disease to successfully infiltrate and expand throughout healthy BM, may aid in developing novel therapeutic targets.

Role of senescence during chronic liver disease

Mar Moreno-Gonzalez, Katherine Hampton, Caitlin Bone, Ane Alava-Arteaga, Meha Patel, Paula Ruiz, Aimee Parker, Gemma Beasy, Charlotte Hellmich, Pablo Luri-Martin, David Baker, Simon M Rushbrook, Falk Hildebrand, Stuart Rushworth, and Naiara Beraza

Senescence is present in liver cells during human and murine cholestatic disease. Liver disease associates with loss of intestinal barrier function and changes in the microbiome. Here, we found increased senescence in intestinal epithelial cells during cholestatic liver disease in patients and in preclinical mouse models. In vitro studies pointed to reduced bile acids and increased bacterial products (lipopolysaccharide) as contributors to intestinal senescence. Elimination of senescent cells during cholestasis in vivo had a detrimental impact on the intestine, decreasing the proliferative response while increasing apoptosis and intestinal permeability that overall associated with exacerbated liver injury and fibrosis. Overall, our results support that the elimination of senescent cells during cholestasis has a detrimental impact on the gut-liver axis.

Anaerococcus Species Associated With Increasing Prostate Cancer Risk

Anastasios Bampalis, Colin Cooper, Jeremy Clark, Mark Winterbone, Robert Mills, Daniel Brewer, Rachel Hurst

INTRODUCTION
There are continuous interactions between the microbiome and the human body. Infectious microbial agents can cause and influence the severity of diseases, including cancer. The inflammation that results from such infections can contribute to the development and progression of prostate cancer (PCa). In addition, unique microbial compositions have been isolated from the urogenital system, which differed between benign and PCa patients.

Five bacteria genera were associated with prostate cancer risk and progression, called the Anaerobic Bacteria Biomarker Set (ABBS).

Our research aimed to investigate the presence of bacterial species belonging to ABBS, particularly the Anaerococcus genera, in urine samples from participants with PCa compared with control groups.

MATERIALS AND METHODS
After a digital rectal examination, urine samples were collected from study participants (n=290). Following collection, DNA was extracted from the urine cell sediment. The DNA samples were tested for ABBS bacterial presence using qPCR assays which targeted specific ribosomal-protein gene sequences that offered high taxonomic distinction. PCR amplicons were examined and analysed using melt curve analysis, agarose gel electrophoresis and sequencing techniques.

RESULTS AND DISCUSSION
Six bacteria species belonging to ABBS were detected across clinical classifications ranging from clinically benign to advanced cancer. Anaerococcus lactolyticus and Anaerococcus sp.i were significantly associated with increased prostate cancer aggressiveness ($\chi 2$ test for trend, p<0.006 and p<0.003 respectively). These two species were also flagged as important features with the Boruta feature selection algorithm.

Research is now focussed on investigating the potential of ABBS bacterial detection as a prognostic tool for different risk groups and cancer aggressiveness.

CONCLUSION
We have linked the presence of specific bacteria species in urine with PCa aggressiveness. Research is underway for the development of a prognostic bacterial test, that in combination with the patient's clinical information, could improve the accuracy of predicting disease outcomes and potential treatment strategies.

Session 4
Isabelle Piec

The UEA BioAnalytical Facility: Your Sample Analysis Partner

Isabelle Piec, Rachel Dunn, Jonathan Tang & William D. Fraser

Located within the Bob Champion Research and Education Building at the University of East Anglia, the BioAnalytical Facility (BAF) provides analytical services of the very highest quality. Using our advanced equipment and expertise, we support medical, biological, molecular, and macromolecular research within Norwich Research Park and beyond. We offer our services to other schools and institutions within the university sector, NHS trusts, and external commercial organisations. Our facility has a wide range of state-of-the-art analytical equipment, operated by highly skilled and experienced personnel who are supported by academic experts. Analytical methods can be developed or adapted to suit individual requirements.

We offer a wide range of analytical services:
- Analysis of many types of samples
- Sample storage
- Quality assurance
- Rapid, reliable, and confidential results
- Interpretation

As part of the Musculoskeletal Science Group within the Faculty of Medicine and Health Sciences, we have research expertise in biochemical investigations of metabolic bone diseases and cancers, calcium disorders, fracture treatment and outcomes, endocrinology, inflammation, and kidney diseases. Our 30+ years' expertise in clinical biochemistry enables us to support analytical analysis in many other fields (e.g., COVID-19, asthma, sleep, and exercise science).

We welcome enquiries at an exploratory level, where we can offer advice on the best methods of analyses or on the use of a specific technique. All enquiries are treated in strict confidence.

Session 4
Eleanor Mishra

Turning an idea into a successful clinical trial

Eleanor Mishra

Translating a great idea from the lab to use in patients requires a clinical trial. However, the process is challenging, often in unexpected ways. In this talk, I will draw on my 15 year experience of delivering practice-changing clinical trials to talk you through the route to success. I will cover building a team, developing your ideas, what resources are available for support and running a trial, using examples drawn from studies I have worked on.

Session 4
William Penny

Insights into the potential collaborations with the MRI UEA department and the possibilities for research projects

William Penny

Session 4
Louise Jones and Rachael Stanley

NRP biorepository

Louise Jones, Rachael Stanley

Keynote
Dr Thankamma Ajithkumar

Setting up clinical trials in Oncology: The story of PORTICO studies

Dr Thankamma Ajithkumar

Setting up a new clinic trial in Oncology is challenging. In this talk, I would like to narrate the journey of establishing PORTICO studies, which is an ongoing co-clinical platform study evaluating the role of preoperative radiotherapy in combination with immunotherapy and novel agents in operable pancreatic cancer. I will highlight the clinical significance of PORTICO-studies and share tips on how to utilities different national and regional resources to design and conduct a new clinical trial in Oncology.

Poster & Lightning Talk Abstracts

POSTER NUMBER	FULL NAME	TITLE OF RESEARCH
1	Jake Rigby	Synthesis and evaluation of ubiquitin ligase inhibitors as anticancer leads
2	Ellie Hyde	Controlling Coiled Coils: Harnessing Transcription Factors With Protein-protein Interactions
3	Suzanne van Wier	Removing cancer's immortality: the design and synthesis of peptides and small molecules targeting the dyskerin-dyskerin PPI in telomerase
4	Jack Connor	Targeting the Protein-Protein Interactions of Telomerase in Cancers
5	Rebecca Casson	Biosynthesised Triterpenes Provide Novel Insights into Structure-Activity Relationships
6	Megan Williams	DJ-1 as a potential therapeutic target in cancer
7	Vicky Kamperi	Investigating duplex-duplex DNA crosslinking with acridine bis-intercalators
8	Lily Cassidy	The synthesis of DSA analogues for the treatment of cancer
9	Marco M D Cominetti	Novel approaches to the synthesis of Duocarmycin derivatives via C-H activation
10	Bethany Hood	Distamycin-Duocarmycin conjugates for use as DNA sequence selective antitumor agents
11	Zoe Goddard	The smallest active pharmacophore of duocarmycin SA for use on the solid phase
12	Aya Elmeligy	Regulation of EGF/HGF pathway crosstalk is associated with TNT formation.
13	The Dung Nguyen	Hydrogen peroxide induces tunnelling nanotube formation in the A549 lung adenocarcinoma cells
14	Tameryn Stringer	Repurposing metallo-quinolines as therapeutics for cancer
15	James Mccoll	To the diffraction limit and beyond
16	Seshadhri Subramanian	Specific Bacteria in Urine Can Indicate Higher Risk for Aggressive Prostate Cancer
17	Stephanie Smith	A urine test for prostate cancer: Patient and public involvement work
18	Abraham Gihawi	Investigating Microbe Associated Prostate Cancer from Short Read Whole Genome Sequences
19	Valeriia Haberland	A comprehensive comparison of proposed prostate cancer molecular subtypes
20	Sergio Llaneza-Lago	Detecting Novel Subtypes of Osteosarcoma Using Bayesian Unsupervised Clustering
21	Emma Bull	Investigating Novel RNA Transcripts in Primary Bone Cancer-derived Circulating Tumour Cells
22	Polly Drewczynska	An mRNA-based approach to restore TP53 expression in TP53-null osteosarcoma
23	Calista Tink	Unravelling the Driver Landscape of Chondrosarcoma

24	Mark Williams	Cholinergic mobilisation of juxtaposed TPC1-InsP3R3 calcium stores triggers secretion of mucus and fluid to flush the human colonic stem cell niche
25	Mark Williams	Dysregulation of the calcium signalling pathway in colon cancer: identification of drug targets for chemotherapy
26	William R English	Alternative mRNA splicing is associated with an immunosuppressive tumour microenvironment in soft tissue sarcoma
27	Luke Mitchell	Therapeutic potential of microbial modulation in pancreatic cancer
28	Alicia Nicklin	The use of *Bifidobacterium* in the treatment of cancer
29	Dominic Fowler-Shorten	Acute myeloid leukaemia drives BCL-2 upregulation in normal HSPCs which is targeted by Venetoclax and causes cytopenia
30	Martha Egheose Ehikioya	Multiple Myeloma Derived Il-6 Reduces Fatty Acid Metabolism In The Liver By Downregulating CD36 And CPT1A In Hepatocytes
31	Silvia Ogbeide	Single-cell multiomics profiling in the study of colorectal cancer evolution
32	Edyta Wojtowicz	Cellular barocoding method unravels bone marrow changes upon acute platelet depletion
33	Iain Macaulay	Single-cell sequencing at EI – platforms and applications in cancer biology
34	Andrew Goldson	Spatial Transcriptomics using the Vizgen Merscope
35	Conrad Nieduszynski	Single molecule DNA replication dynamics of the human genome
36	Anna Varley	A qualitative exploration of homeless smokers and the services they receive: Contextual factors
37	Emma Ward	Developing a community-based intervention to support deprived coastal communities to stop smoking
H2	Lucy Clark	Cessation Of Smoking Trial In The Emergency Department (COSTED)
H3	Derrick Tsang	Non-visible haematuria: an audit of referral to diagnosis of bladder cancer
H4	Helen Walker	Efficacy of MRI and targeted biopsy in detecting clinically significant prostate cancer
H5	Salonee Banerjee	Hepatocyte Growth Factor and Epidermal Growth Factor signalling crosstalk is involved in tunneling nanotube formation in A549 human lung adenocarcinoma cells

Abraham Gihawi
Poster Number 18

Investigating Microbe Associated Prostate Cancer from Short Read Whole Genome Sequences

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

Prostate cancer is the most common cancer in men in the UK with more than 52,000 new diagnoses annually. We recently discovered that a subset of anaerobic bacteria appears to be associated with aggressive prostate cancer – the ABBS genera [1]. In this study, we explored whether these findings could be validated in the Pan-Prostate Cancer Group (PPCG, *N*=2,176), using the taxonomic classification of short non-human whole genome sequencing reads.

As in our initial report we found that the detection of at least one ABBS genera in the primary tumour samples was associated with a poorer outcome in the patient (log-rank *p*=0.023, relapse free survival). We also confirmed that ABBS positive samples tend to originate from older participants (*p*=5.5x10-11).

Investigating pks, an experimentally validated genotoxic bacterial pathogenicity island, alongside evidence for Escherichia reveals four distinct groupings of samples: 41 samples contained evidence for the pks gene without Escherichia, suggesting that the gene may exist in genera other than Escherichia in samples from patients with prostate cancer.

In this study we have provided further evidence for the important role that anaerobic bacteria may play in the development of prostate cancer and revealed that a bacterial gene driven approach may be more reflective of impact on cellular biology than a purely taxonomic driven approach.

[1] Hurst R, Meader E, Gihawi A, Rallapalli G, Clark J, Kay GL, et al. Microbiomes of Urine and the Prostate Are Linked to Human Prostate Cancer Risk Groups. Eur Urol Oncol. 2022.

Alicia Nicklin
Poster Number 28

The use of Bifidobacterium in the treatment of cancer

Alicia Nicklin, Christopher Price, Luke Mitchell, Wesley Fowler, Anne Jordan, Nancy Teng, Lindsay Hall, and Stephen Robinson

Bifidobacterium (Bif) species have long been considered beneficial microbes for health, and recent studies suggest they may enhance the efficacy of cancer therapeutics. The Robinson lab conducted experiments using newly discovered strains of Bifidobacterium in murine breast cancer models, which resulted in reduced tumor volumes compared to the control group. Understanding the immune modulations and optimising the administration of Bifidobacterium has been crucial in unraveling its mechanism of action. Furthermore, exploring the anti-tumorigenic potential of Bifidobacterium could indicate its applicability as a chemopreventive agent. Additionally, the lab demonstrated that Bifidobacterium-based cancer therapeutics are not limited to the administration of live bacteria. For instance, intravenous administration of bacterial extracellular vesicles (BEVs) isolated from one of the Bifidobacterium strains significantly reduced melanoma tumor volumes and modulated tumor-associated immune populations.

Andrew Goldson
Poster Number 34

Spatial Transcriptomics using the Vizgen Merscope

Iain Macaulay and Andrew Goldson

Single-cell transcriptomics has revolutionised how we analyse gene expression in heterogeneous tissues. However, these approaches lose the spatial context in which cells exist, and thus important information about tissue organisation and cell-cell interactions is lost. We are implementing Vizgen Merscope technology, which uses the Multiplexed Error-Robust Fluorescence in situ Hybridization (MERFISH) approach, to resolve sub-cellular transcript localisation in regions of up to 1 cm2. Here, we present an overview of the approach and our preliminary data from a mouse brain section in which the expression of 140 genes was measured in parallel, enabling resolution of cell types and their position within the tissue.

Anna Varley
Poster Number 36

A qualitative exploration of homeless smokers and the services they receive: Contextual factors

Lead Author: Anna Varley (UEA), Co-Authors: Lauren McMillan (Stirling), Allison Ford (Stirling), Caitlin Notley (UEA), Emma Ward (UEA). PIs: Lynne Dawkins (LSBU), Sharon Cox (UCL)

Background: In the UK, people experiencing homelessness have a smoking prevalence rate of up to four times the average. There are marked health inequalities, with this population likely to live 30 years less than the general population. Most of this excess mortality may be due to tobacco smoking. Despite complex circumstances, the feasibility study demonstrated that it is possible to recruit this population, that participants were willing to attempt smoking cessation, and that the e cigarette-based intervention was feasible to deliver. The wider trial aims to ascertain effectiveness. An embedded process evaluation aims to explore fidelity of intervention delivery; mechanisms of change; contextual influences and sustainability.

Methods: Cluster RCT, comparing the effectiveness and cost-effectiveness of providing free Electronic Cigarette (EC) starter kits to people who smoke accessing homeless centres compared with usual care. Recruiting centres (*n*=32) across Scotland, Wales and England. The embedded mixed methods process evaluation purposely sampled eight centres in the EC arm for in-depth interviews with staff (*n*=2 per centre) and participants (*n*=4 per centre). Interviews were thematically analysed.

Results: Initial findings from interviews suggest that organisational, contextual and geographical contexts influence implementation. The mechanisms through which smoking behaviour may have changed are influenced by the culture of the centre and the context where social support is provided, or not.

Conclusions: A finding of the feasibility study was that the building of trust, communication and relationships between participants (and researchers) and centre staff was key. The current findings support this, although the impact of contextual factors on the primary outcome remains to be seen. Initial findings suggest that strong and positive relationships with researchers and centre staff are central to successful delivery of the EC intervention.

Aya Elmeligy
Poster Number 12

Regulation of EGF/HGF pathway crosstalk is associated with TNT formation.

Aya Elmeligy, Dung Nguyen The, Salonee Banerjee, and Anastasia Sobolewski

Regulated intracellular communication between cancer cells is pivotal for tumour progression and is associated with the known hallmarks of cancer. Tunnelling nanotubes (TNTs) are a subtype of direct contact between cancer cells that allows for transfer of cell cargo including proteins, vesicles, and important organelles (mitochondrion). This allows for transfer of phenotypes associated with cancer cell survival and tumour progression. These have been identified in many different cancers including non-small cell lung cancer (NSCLC) both in vivo and in vitro.

Epidermal Growth Factor (EGF) and Hepatocyte Growth Factor (HGF) crosstalk mediates the formation TNTs, influencing their growth, abundance and length. A concentration-response experiment was set up to determine the optimal concentration of growth factor that would induce the most TNTs. Cells were seeded in 12 well plates at a density of 5000 cells/ml and treated with the following concentrations in ng/mL: 0.3, 1, 3, 10, 30, 100, 300, 700. The plates were then incubated for 72 hours, and white light images taken for analysis. A concentration of 100µg/ml for both EGF and HGF was therefore used for all subsequent experiments. A549 cells were treated with 100ng/ml of each growth factor separately and in combination and incubated for 72 hours after which white light images were taken and analysed. Three parameters were measured: percentage of TNTs, TNTs per cell and the length of the TNTs with appropriate analysis performed. TNT appearance under EGF/HGF treatment was observed using scanning electron microscopy, vesicles could be seen being transported between the A549 cells. Images collated showed both adherent and non-adherent TNTs.

Immunofluorescence labeling and confocal microscopy will be used to stain TNTs and observe the transfer of cell cargo such as mitochondria. EGFR and C-met receptor signaling via the Rac1 and Arp2/3 complex will be investigated to identify components that induce TNT formation.

Bethany Hood
Lightning Talk And Poster Number 10

Distamycin-Duocarmycin conjugates for use as DNA sequence selective antitumor agents

Bethany Hood, Zoë Goddard, Marco Cominetti, and Mark Searcey

The ability to target DNA in a sequence specific manner could prove revolutionary in terms of cancer treatments, with targeted treatments potentially increasing cytotoxicity to tumour cells while limiting damage to healthy cells. Targeting of specific DNA sequences is already seen in both SiRNA - RNA based gene silencing - and CRISPR - protein based gene editing -, with clinical trials involving CRISPR gene editing for targeted cancer immunotherapy already underway.

DNA binding can be achieved through non-covalent minor groove binding small molecules, with distamycin, a polyamide consisting primarily of N-methyl pyrrole units, being of particular interest to this work. This is perhaps one of the most well-known of the DNA binding small molecules, with its curved planar shape and base pair specific hydrogen bonding interactions leading to good binding in the minor groove of AT heavy sequences. Through the addition of a flexible linker to encourage a hairpin conformation, and the inclusion of imidazole units in the pyrrole-based chain for selective CG over AT binding, studies suggest Py-Im distamycin analogues have a potential for DNA sequence specific binding.

In this work, we focus on combining the sequence selective minor groove binding distamycin analogues with duocarmycin SA, a highly potent antitumor antibiotic with high activity (IC50 = 10 pM) but limited selectivity, by solid phase peptide synthesis. This results in binding of the distamycin to the active DNA alkylating subunit of duocarmycin SA in place of the natural binding unit. It is hoped that these duocarmycin-distamycin conjugates combine the ultrapotent cytotoxicity of the DNA alkylating duocarmycins with the sequence specific binding of the distamycin chains to deliver a small molecule with ultrapotent, DNA targeted toxicity.

Calista Tink
Poster Number 23

Unravelling the Driver Landscape of Chondrosarcoma

Calista Tink, Sergio Llaneza Lago, Vaiyapuri Sumathi, Lee Jeys, William D. Fraser, and Darrell Green

Chondrosarcoma (CS) is the most common primary bone cancer and comprises a group of heterogeneous cartilage matrix-forming bone tumours. The five-year survival rate for conventional low-grade CS is 75% but conventional high-grade CS and dedifferentiated CS is significantly worse at 38% and 25% respectively. Grading and sub-classification of CS relies on histological interpretation. This analysis can be subjective and cause interobserver variability. Inaccurate grading can lead to insufficient treatment and result in a worse prognosis, which could be made avoidable. Molecular biomarkers determined to be specific to the grade would reduce diagnostic variability. Here, we have identified significant differential gene expression in patient-derived CS samples in comparison to controls. Our patient series included control cartilage tissue from the femoral neck (n=2), conventional CS grade 2 (n=1), conventional CS grade 3 (n=3), clear-cell CS (n=2), dedifferentiated CS (n=2). We performed mRNA-seq using the Novaseq 600 (Illumina). Differential expression (DE) analysis was conducted on the gene counts per sample using the R package DESeq2 to identify significant DE of genes between CS tumour and control samples. Our early data shows distinct hierarchal clustering of CS samples and controls, illustrating expected heterogeneity between the two populations. DE analysis found a total of 2,141 genes that were significantly differentially expressed in CS when compared to non-transformed controls. Of these genes, 736 were significantly downregulated and 1,405 were significantly upregulated ($\log_2$ Fold change > |1|, p-adj <0.05). This data suggests that further downstream functional analysis could identify significant DE of genes involved in CS molecular pathology. We have since collected an additional 36 CS samples, but these were not included in the present analysis. In the future, we will compare between different CS histological grades to identify potential novel biomarkers that could more robustly support diagnosis.

Conrad Nieduszynski
Lightning Talk And Poster Number 35

Single molecule DNA replication dynamics of the human genome

Jamie Carrington, Rose Wilson, Tom Barker, Leah Catchpole, Alex Durrant, Vanda Knitlhoffer, Chris Watkins, Karim Gharbi, and Conrad A. Nieduszynski

Faithful DNA replication is fundamental to the survival of every organism. Mistakes during DNA replication are the major source of genetic variation that underlies 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 presently no high-resolution or high-throughput genomic approaches for the reliable detection of replication initiation sites. Here we present our findings using nanopore sequencing (DNAscent) to detect replication fork dynamics across the human genome. DNAscent detects the changes in BrdU incorporation during S phase to determine the direction of replication fork movement on individual nanopore sequence reads. This identifies the sites of replication fork initiation, pausing and termination across the genome, at near base resolution and on single molecules.

Derrick Tsang
Poster Number H3

Non-visible haematuria: an audit of referral to diagnosis of bladder cancer

Derrick Tsang, Johanna Penfolds, Omar Ali, Nikita Bhatt, Charlotte Dunford, and Sarah Wood

Introduction
NICE guidance (2021) recommends patients aged 60 years and older with unexplained non-visible haematuria (NVH) are referred under the two week wait rule (2 WW). There is no formal guidance on patients who fall outside these criteria. We aimed to audit GP referrals with NVH, evaluating our current pathway and patient outcomes.
Methods
Audit standards were set according to NICE guidance. The audit was registered locally (URO_TC _23-24_A01). Retrospective analysis of all GP referrals with NVH 1/9/2022- 30/11/22 was performed. All patients were triaged by Consultant Urologists. Data was collected on patient demographics, referral/triage priority and management outcomes.
Results
114 patients were referred, and the majority were aged <60. 2% as 2WW, 38% as 'urgent and 60% as 'routine. After triage 29% referrals were upgraded and 4% downgraded. 3 patients were diagnosed with malignancy (2/3 upgraded at triage). Average time to be seen in one stop clinic for 2WW, urgent and routine referral was 1.7, 2 and 15.8 weeks respectively. No malignancy was identified in those patients referred/triaged as 'routine'.
Conclusion
Audit standards were met in patients referred with NVH aged 260. The majority of patients referred were younger, sometimes as 'urgent and no malignancy was diagnosed. We propose seeing all patients aged <60 with NVH routinely (in line with GIFT recommendations). We plan to educate our department and GPs and re-audit late 2023.

Dominic Fowler-Shorten
Poster Number 29

Acute myeloid leukaemia drives BCL-2 upregulation in normal HSPCs which is targeted by Venetoclax and causes cytopenia

Dominic Fowler-Shorten, Charlotte Hellmich, Rebecca Maynard, Katherine Hampton, Martha Ehikioya, Ravindu De Silva, Edyta Wojtowicz, Kristian Bowles, and Stuart Rushworth

Introduction: The BH3-mimetic Venetoclax, in combination with low-dose chemotherapy has recently shown clinical efficacy in newly diagnosed acute myeloid leukemia (AML) patients. Venetoclax selectively inhibits the BCL-2 protein which is overexpressed in AML to reactivate intrinsic apoptosis. However, this treatment regime is associated with cytopenias including neutropenia which leaves patients immunocompromised. The underlying cause of cytopenia in the context of Venetoclax-treated AML remains unknown.

Aims: Here, we hypothesise that Venetoclax depletes normal haematopoietic stem and progenitor cells (HSPCs) in AML leading to cytopenia. Using in vitro and in vivo modelling, we aim to investigate the mechanism of cytopenia in Venetoclax-treated AML.

Methods: Two established AML models, HOXA9/Meis1 and MN1, were co-cultured with isolated Lineage negative Sca+ and cKit+ (LSKs) and BCL-2 gene expression was assessed in LSKs by qPCR. MN1 cells were next injected into C56Bl/6 mice followed by Venetoclax treatment by oral gavage. Flow cytometry assessed isolated bone marrow and bloods before and after treatment. LSKs were also FACS purified and qPCR assessed BCL-2 expression post-treatment. Mechanisms for increased BCL-2 transcription in HSPCs were later explored using the co-culture system and targeted pathway inhibitors.

Results: We identified BCL-2 overexpression in LSKs co-cultured with either HOXA9/Meis1 or MN1 compared to LSK-only controls. In vivo analysis of BCL-2 in LSKs from AML engrafted mice showed increased BCL-2 expression confirming the in vitro data. Next, we confirmed that Venetoclax causes cytopenia in AML engrafted mice allowing us to explore the mechanism. We found that interleukin-3 induces upregulation of BCL-2 transcription in HSPCs.

Conclusions: Here, we demonstrate in vitro and in vivo that BCL-2 is overexpressed in HSPCs during AML progression, and that Venetoclax depletes HSPCs to cause cytopenia. Finally, we identify interleukin-3 as a potential target to explore further to determine if inhibitors of interleukin-3 can reverse cytopenia in Venetoclax-treated AML.

Edyta Wojtowicz
Poster Number 32

Cellular barocoding method unravels bone marrow changes upon acute platelet depletion

Edyta E. Wojtowicz,, Jayna J. Mistry, Vladimir Uzun, Charlotte Hellmich, Anita Scoones, Desmond W. Chin, Laura M. Kettyle, Francesca Grasso, Allegra M. Lord, David J. Wright, Graham J. Etherington, Petter S. Woll, Mirjam E. Belderbos, Kristian M. Bowles, Claus Nerlov, Wilfried Haerty, Leonid V. Bystrykh, Sten Eirik W. Jacobsen, Stuart A. Rushworth, and Iain C. Macaulay

Platelets and erythrocytes constitute over 95% of all hematopoietic stem cell output. However, the clonal dynamics of HSC contribution to these clinically relevant lineages remains largely unexplored.

We use lentiviral genetic labeling of hematopoietic stem cells to quantify output from all lineages, nucleate and anucleate, simultaneously linking these with stem and progenitor cell transcriptomic phenotypes using single-cell RNA-sequencing. We observe dynamic shifts of clonal behaviors through time in same-animal peripheral blood and, demonstrate that acute platelet depletion shifts output of multipotent hematopoietic stem cells to exclusive production of platelets. Additionally, we observe emergence of new myeloid-biased clones, which support short and long-term production of blood cells.

Our approach enables kinetic studies of multi-lineage output in peripheral blood and transcriptional heterogeneity of individual hematopoietic stem cells. Our results give unique insight into hematopoietic stem cell reactivation upon platelet depletion and of clonal dynamics in both steady state and under stress.

Ellie Hyde
Poster Number 2

Controlling Coiled Coils: Harnessing Transcription Factors With Protein-protein Interactions

Ellie Hyde, Emily Hobson, Maria O'Connell, Mark Searcey, and Andrew Beekman

The Nrf2-MafG protein-protein interaction (PPI) activates transcription of cytoprotective genes in the body [1]. Cancer can overexpress Nrf2 to create resistance to cytotoxic chemotherapeutic drugs [2]. Designing inhibitors to the Nrf2 pathway would allow us to control the Nrf2-MafG PPI for resensitising cancer cells to chemotherapeutic drugs.

Nrf2-MafG bind through dimerisation at the alpha helical, leucine zipper region featured on both BZIP proteins which follows the coiled coil binding theory [3]. Armed with this information, we can identify key amino acids for Nrf2 binding to design MafG mimicking peptides that disrupt the PPI. Using an AlphaFold homology model [4], a section of the MafG leucine zipper region $_{76}$KEELEKQKAELQQEVEKLASENASMKLE$_{104}$ has been identified to synthesise peptides using solid phase peptide synthesis.

This presentation will describe the synthesis and design of MafG mimicking peptides and explores their ability to bind Nrf2 and inhibit the formation of the Nrf2-MafG heterodimer.

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Emma Bull
Poster Number 21

Investigating Novel RNA Transcripts in Primary Bone Cancer-derived Circulating Tumour Cells

Emma Bull, Sergio Llaneza-Lago, William Fraser, and Darrell Green

Primary bone cancers (PBCs) are a heterogeneous group of cancers. PBCs primarily arise in children and adolescents. The five-year survival rate for localised disease is ~50% but ~20% for metastatic disease. Standard of care and survival rates have not improved since the 1960s. Better understanding of PBC biology leading to more effective and less toxic treatments is urgent. Metastasis is the leading cause of cancer-related death, but metastatic samples are difficult to obtain because surgery is scantly used at late-stage disease. We recently developed protocols to isolate circulating tumour cells (CTCs) from PBC patient blood to investigate the “seeds” of metastasis at single cell resolution. We recently identified novel RNA transcripts in CTCs that were not observed in normal or tumour tissue. This data suggests that (i) some novel RNAs are unique to metastasis and/or (ii) pro-metastatic cancer cells are capable of producing their own disease-promoting gene networks. This project aims to validate and characterise the mechanism of action of these novel RNAs. Whole blood samples (7.5mL) were obtained from ten patients with osteosarcoma or Ewing sarcoma. The ClearCell FX (Biolidics) was used to isolate live and viable CTCs. Single CTCs were then manually picked from the output using brightfield microscopy and pipetting. The SMART-Seq® mRNA Single Cell LP kit was used to generate libraries for single cell RNA-seq (scRNA-seq). scRNA-seq revealed 347 significantly differentially expressed genes between CTCs and control cell lines. A completely uncharacterised 2498 base pair transcript that we term LNC441 was expressed in CTCs but not observed in controls or tumours. Early bioinformatics analysis suggest that this transcript is a long non-coding RNA housing multiple new and unknown microRNA hairpins. Characterising LNC441 will reveal previously unknown networks promoting PBC metastasis. Our study may identify new therapeutic targets for reducing or halting PBC dissemination.

Emma Ward
Lightning Talk And Poster Number 37

Developing a community-based intervention to support deprived coastal communities to stop smoking

Emma Ward, Anna Varley, Ian Pope, and Caitlin Notley

Background: People living in coastal communities have some of the worst cancer incidence rates in the UK, driven in part by high smoking rates. Coastal communities include socially disadvantaged groups that struggle to access traditional stop smoking services. Research shows that the most effective smoking cessation support includes e-cigarettes, intensive behavioural support, and financial incentives. However, these evidence-based approaches have only been tested individually, mostly within motivated populations.

Aims: To conduct Patient and Public involvement (PPI) work to assess the acceptability of a combined evidenced-based smoking intervention within coastal communities. Specifically, it aimed to explore the community context, the best location for intervention delivery, the acceptability of the provision of e-cigarettes/NRT, behavioural support and financial incentives.
Methods: 25 people who smoke were recruited to participate in qualitative interviews from a range of community locations including social supermarkets, community cafes, a community college, a Portuguese Café, drop-in centres for the elderly, women, migrant workers and men with mental health issues. A thematic analysis of the interview data was undertaken and the Template for Intervention Description Replication (TIDieR) framework was used to describe a proposed intervention.

Results: Barriers to quitting smoking in the targeted population include low motivation, high anxiety/boredom, normalisation of smoking and widespread illicit tobacco use. There was broad support for all three proposed interventions. There was a strong preference for the intervention to be delivered opportunistically and locally within (non-healthcare) community settings, meeting people where they are, in a non-pressurising manner, by a community worker specially trained to give stop smoking support.

Conclusion: Interventions to target smoking cessation within costal communities are acceptable to the targeted population. It is recommended that smoking cessation interventions are developed and implemented locally in coastal communities. These interventions should be thoroughly evaluated, and feasibility testing may be required prior to a full-scale trial.

Helen Walker
Poster Number H4

Efficacy of MRI and targeted biopsy in detecting clinically significant prostate cancer

Kate Manley, Helen Walker, Omar Al Kadhi, and Krishna Sethia

Introduction: Multiparametric MRI MP-MRI) is widely accepted as the key imaging modality for detection, facilitation of image-guided biopsy, and staging of prostate cancer with a high sensitivity for clinically significant disease.

The introduction of the Prostate Imaging Reporting and Data System (PIRADS) classification for MRI reporting has further standardised MRI reporting. Whilst MP-MRI improves the diagnostic accuracy of targeted biopsy for prostate cancer, it is not without its limitations.

Much of the published literature on this topic comes from specialised centres - we report a snapshot of clinical experience at a single centre routinely using pre-biopsy MP-MRI.

Methods: 112 consecutive cases over 16 months at a single centre were analysed.

Results: PIRADS 4/5 lesions had a positive predictive value for high grade prostate cancer (98.7%). 14 (12.5%) clinically significant lesions did not have a target identified on pre-biopsy MP-MRI.
The lesions with no target had a mean PSA density of 0.21 (0.07-0.4). MRI staging was correct in 40% of cases (under-staged in 32% and over-staged in 8%), with 11 cases (9.8%) of T3b disease not identified on initial imaging.

Overall positive biopsy concordance with MRI target was 91%, as was location of disease on MRI when compared with final RP histology (79%). Upstaging on final RP histology compared with image-guided biopsy histology occurred in 26%.

Conclusion: MP-MRI has a high positive predictive value for clinically significant disease.

Biopsy/MRI concordance rate is high for PIRADS 4/5 lesions. Caution should be taken if considering the use of MR-MRI to rule out the need for biopsy, as clinically significant lesions did not have a target identified on MRI in 12.5%.

Even when employing the gold-standard approach of target biopsy plus systematic sampling on biopsy, upstaging from biopsy histology to final RP histology specimen still occurs in over a quarter of patients.

Iain Macaulay
Poster Number 33

Single-cell sequencing at EI – platforms and applications in cancer biology

Iain Macaulay and Andrew Goldson

This poster will give a brief overview of the single-cell technologies established at EI, outlining how they can be applied to the study of cellular identity in normal and cancer cells.

Jack Connor
Poster Number 4

Targeting the Protein-Protein Interactions of Telomerase in Cancers

Jack Connor, Mark Searcey, and Andrew Beekman

In 2020, there were an estimated 19.3 million diagnosed cases of cancer, with an estimated 10 million deaths attributed to various cancers. Current chemotherapeutics afford a number of drawbacks, with the most glaring being off-target effects, which can often be as severe as the disease itself.

Telomerase is an enzyme complex involved in maintaining or lengthening telomeres, the protective caps at the terminus of DNA. In normal adult human cells, telomerase has little to no activity, hence why we age. However, in 90% of cancer cells, telomerase is over-expressed and is thought to be means for the immortality of cancer cells. Theoretically, this provides us with a target with a very high degree of specificity.

This research attempts to develop small-molecule inhibitors derived from a disordered peptide sequence of dyskerin, a subunit of the human telomerase complex. The disordered peptide sequence is derived from a protein-protein interaction commonly mutated in diseases which exhibit poor telomerase activity.

Jake Rigby
Poster Number 1

Synthesis and evaluation of ubiquitin ligase inhibitors as anticancer leads

Jake Rigby, Ashley Dudey, Tom Storr, Andrew Hemmings, Andrew Chantry, and Richard Stephenson

The ubiquitin proteasome system (UPS) is essential for the regulation of cellular protein quality and quantity within cells [1]. Within the UPS, E3 ligases are selective for certain proteins, tagging them with ubiquitin for eventual protein degradation. WWP2 is a HECT E3 ligase overexpressed in several forms of cancers, such as oral and prostate cancers, suggesting WWP2 and its isoforms could be promising disease targets [2,3,4]. Within the research group, several molecules have been investigated for inhibitory activity against WWP2 by utilising STD and DEEP-STD NMR [5]. Currently we are exploring the synthesis and functionalisation of a number of hits, including imidazo[4,5-b]pyrazine, indole, alloxazine, isoquinoline as well as biaryl and benzidine cores. Molecular docking is being utilised to aid in the design of analogues for structure-activity studies.

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James Mccoll
Poster Number 15

To the diffraction limit and beyond

James Mccoll

Housing a range of optical microscopes, the Bio imaging facility based at UEA can image a wide range of samples. From fast widefield microscopes to super-resolution Airyscan technology we can image almost any sample. All systems having full environmental control so that all samples are happy and backed up by cutting edge AI analysis software. Many look but very few can really see.

Lily Cassidy
Poster Number 8

The synthesis of DSA analogues for the treatment of cancer

Lily Cassidy, Andrew Beekman, and Mark Searcey

Cancer is the second leading cause of death in the world, with more than one in two people being diagnosed within their lifetime. Cancer can be treated through different means including chemotherapy, the choice of drug depends on the type of cancer and the extent of its spread.
The duocarmycins are natural compounds that are highly potent DNA alkylating agents. They bind to the minor groove in AT rich regions of DNA and are among the most potent cytotoxic compounds known, offering a great potential for cancer treatment. However, high toxicity and low selectivity mean there has been limited progress of duocarmycins as chemotherapeutic agents. One approach to achieve selective cytotoxicity is with antibody drug conjugates, or ADCs. Here the cytotoxic drug is bound by a linker to the antibody, in doing this the payload can be targeted directly to the tumours. A duocarmycin based ADC called SYD985 is in phase III clinical trials for the treatment of HER-2 Metastatic Breast Cancer. This shows an exciting new field to be explored, this project will look at preparing a dimeric payload that could potentially be used as a warhead for an ADC.

The duocarmycin family consists of different alkylating subunits, including the duocarmycin SA unit, DSA, one of the most potent subunits and of great interest in the design of analogues. This project follows work previously done by Searcey where DSA was synthesised with an appropriate protection strategy. In doing this, analogues can be made using solution phase synthesis and allows a library of analogues to be made and studied, with focus on making dimers. Duocarmycin payloads in ADCs have shown promise as dimers with dimeric seco-CBI payloads and PBD dimers both in clinical trials. Using our approach we will be able to “tune” the physicochemical characteristics of the dimers by varying amino acid based linker structures.

Lucy Clark
Poster Number H2

Cessation Of Smoking Trial In The Emergency Department (COSTED)

Emma Ward, Pippa Belderson, Lucy Clark, Caitlin Notley, and Ian Pope

Background: Tobacco smoking is a leading cause of cancer, and negatively impacts significantly on recovery outcomes. To date there have been no randomised controlled trials of smoking cessation interventions in the Emergency Department (ED) using e-cigarettes. This approach may offer a valuable opportunity to reach unmotivated quitters and provide them an acceptable alternative to tobacco, with the ultimate aim of encouraging complete tobacco cessation. The aim of this study is to definitively test real-world effectiveness of an ED based smoking cessation intervention (including provision of an e-cigarette) with usual care.

Methods: Two-group, multi-centre, pragmatic, individually randomised, controlled trial (ClinicalTrial.gov: NCT04854616). We randomly assigned people who smoked tobacco and were attending one of six UK EDs to either control (in which case they were given written information about local stop smoking services) or intervention (in which case they received a brief smoking cessation intervention, provision of an e-cigarette starter kit, and referral to stop smoking services). Both groups were followed up 1, 3 and 6 months after randomisation and asked if they had smoked tobacco in the past 7 days. Smoking cessation is biochemically verified at 6 months.

Results: A total of 972 participants have undergone randomisation and data collection is now complete. In this presentation we report overall (intervention and control group combined) smoking status at 1, 3 and 6 months, demographic data of recruits (gender, age, ethnicity, level of deprivation, employment status) as well as changes in e-cigarette usage (percentage who had used an e-cigarette at least twice a week at baseline and frequency of use at 6 months).

Conclusion: It is feasible to implement a smoking cessation intervention in EDs with dedicated staff to deliver the intervention. Recruitment was above target indicating that EDs may represent an excellent opportunity to engage hard to reach smokers.

Luke Mitchell
Poster Number 27

Therapeutic potential of microbial modulation in pancreatic cancer

Luke Mitchell, Christopher Price, Alicia Nicklin, Lindsay Hall, and Stephen Robinson

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of exocrine pancreatic neoplasm and accounts for around 95% of all pancreatic cancers. Globally PDAC is the 10th most common cancer in the UK but has the lowest survival rate of all the 20 common cancers with a five-year survival of less than 6%.

Imbalances in the gut microbial community (dysbiosis) are found to be associated with a range of pathologies including cancer. Live biotherapeutic product (LBP), which is often used as a probiotic, is a member of the human gut microbiota and is associated with improved cancer outcomes. However, its use as a therapeutic strategy against PDAC progression has yet to be determined.
By orally administering different LBP strains in murine PDAC models, we demonstrate a strain-specific response in inducing tumour reduction. In addition, we observe differences in tumour infiltrating immune populations, most notably a significant increase in natural killer cells following LBP administration. These results provide vital insights into the development of LBP-based novel anti-cancer therapies which could be utilised to improve prognosis in PDAC patients.

Marco M D Cominetti
Poster Number 9

Novel approaches to the synthesis of Duocarmycin derivatives via C-H activation

Marco M. D. Cominetti and Mark Searcey

Duocarmycins are a class of natural compounds discovered in the 70's. They are selective minor groove binders with a peculiar DNA alkylation mechanism. Their high potency and stability in absence of DNA has attracted great interest, but striking the correct balance between efficacy and side effects has proven very difficult, with clinical trials failing early on. A sapient use of cleavable linkers, modulation of biophysical properties and antibody drug ratio has led to fast-track advancement of a first ADC candidate (SYD985) to Phase 3 clinical trial.

The synthesis of these complex molecules has been approached by different groups with different strategies. The available synthesis methods are still fairly long and low yielding. Our previous approaches to the problem have focussed on the synthesis of the key alkylating unit with suitable protections for solid phase synthesis and synthetic plans which avoid DNA alkylating intermediates, thus increasing the safety and scalability of the approach.

Following in these steps, we will disclose a new approach which exploits rapid and high yielding access to a key intermediate and its chiral resolution. The major novelty in our approach is a C-H activation reaction to generate a borylated derivative as a flexible building block, which can be converted into its active counterpart on demand.

Mark Williams
Poster Number 24

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, and Mark Williams

Introduction: 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 mM; $n>10$). 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 mM; $n>10$; $P<0.05$) and tetrandrine (20 mM; $n>10$; $P<0.05$). 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 mM) 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 mM) 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 25

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, and 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 intervention1. 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 ($log_2FC>2$, p-adj<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 ($log_2FC>2$, p-adj<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.

Martha Egheose Ehikioya
Poster Number 30

Multiple Myeloma Derived Il-6 Reduces Fatty Acid Metabolism In The Liver By Downregulating CD36 And CPT1A In Hepatocytes

Martha Egheose Ehikioya, Rebecca Maynard, Katherine Hampton, Charlotte Hellmich, Kat Ravindu De Silva, Dominic Fowler-Shorten, Naiara Beraza, Edyta Wojtowicz, Kristian Bowles, and Stuart Rushworth

Background: Normally, the liver processes free fatty acids (FFA) and stores small amounts as triglycerides. In multiple myeloma (MM), cancer cells accumulate in the bone marrow and produce paraproteins. MM cells use fatty acid (FA) oxidation to obtain energy in the form of adenosine triphosphate, for proliferation and survival.

Objectives: To investigate FFA metabolism in response to MM progression and determine the role of the liver in redirecting FFA to the bone marrow.

Methods: We injected 5TGM1(GFP) cells into KaLwRij mice to model MM. We took blood samples to measure levels of serum paraprotein (to confirm engraftment), IL-6 and FFA. Bone marrow was analysed for engraftment using anti-CD138 antibodies and GFP via flow cytometry. Real-time PCR was performed on liver samples to determine expression of genes associated with FA metabolism.
For in-vitro modelling, primary mouse hepatocytes were co-cultured with conditioned media from 5TGM1 cells or IL-6 cytokine. RNA was extracted and real-time PCR was performed. Seahorse was performed on primary hepatocytes to determine reliance on FFA metabolism.

Results: Levels of FFA in serum of 5TGM1(GFP)-engrafted mice was higher than in control. Expression of CD36 and CPT1A in liver samples of 5TGM1(GFP)-engrafted mice was reduced compared to controls, and in hepatocytes cultured in 5TGM1 conditioned media.
We and others have previously shown that IL-6 is upregulated in MM and others have shown that IL-6 can regulate liver FA metabolism. Serum IL-6 was increased in 5TGM1(GFP)-engrafted mice. Seahorse analysis showed that primary hepatocytes had decreased reliance on FA oxidation when treated with IL-6.

Conclusion: MM changes FA metabolism in the liver by upregulating IL-6. Expression of FA genes shows that CD36 and CPT1A are downregulated in hepatocytes in response to IL-6. This suggests that MM can redirect FFA away from the liver by downregulating CD36, which mediates FFA uptake in the liver.

Megan Williams
Poster Number 6

DJ-1 as a potential therapeutic target in cancer

Megan Williams, Emily Hobson, Marco Cominetti, Zoë Goddard, Mark Searcey, and Maria A O'Connell

DJ-1 is a multi-functional protein which acts as a redox sensor and regulator of apoptosis and metabolism. DJ-1 is a homodimer, which can interact with several signalling pathways, including the NF-κB, PTEN, MAPK and Nrf2 signalling pathways [1] to regulate the expression of cytoprotective genes, such as NQO1, HO-1 and Trx1 [2]. In cancer, DJ-1 is upregulated in many patient tumours, such as in melanoma, lung and pancreatic cancers, and is associated with increased cancer cell survival, proliferation, migration and chemoresistance [3]. Disruption of the DJ-1 homodimer may therefore be a useful target in cancer chemotherapy. Here, two novel peptides (TAT14DJ1C1 and TAT14DJ1C2) were designed to disrupt the DJ-1 homodimer and were studied alongside the small molecule DJ-1 inhibitor STK793590, for their effects on the DJ-1 signalling pathway in several cancer cell lines.

The highest DJ-1 mRNA and protein expression was observed in SKMEL-28 melanoma and LNCAP prostate cancer cell lines; hence the SKMEL-28 cells were selected for further experiments. STK793590, a small molecule inhibitor of DJ-1, had greater inhibitory effects on SKMEL-28 cell proliferation compared to the TAT14DJ1 peptides, with an IC50 value of 62.71 µM, compared to that of >100 µM for the TAT14DJ1 peptides. Stimulation of the SKMEL-28 cells with the TAT14DJ1 peptides reduced Nrf2 protein expression, whilst DJ-1 and PTEN expression remained unaffected via western blot.

High DJ-1 expression was observed in melanoma, therefore DJ-1 may serve as a useful therapeutic target in melanoma. Methods to target DJ-1 were explored using both a small molecule inhibitor, STK793590, and novel peptides. While STK793590 displayed some effects on melanoma cell proliferation, the TAT14DJ1 peptides reduced expression of Nrf2, a DJ-1-related and tumour promoting protein.

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Polly Drewczynska
Poster Number 22

An mRNA-based approach to restore TP53 expression in TP53-null osteosarcoma

Polly Drewczynska, Eloise Whittle, Willam D. Fraser, and Darrell Green

Primary bone cancer (PBC) affects ~6 per million individuals annually worldwide. Osteosarcoma (OS) is the most common PBC subtype in children, teenagers and young adults. The five-year survival rate is ~50%. Treatment for OS comprises non-specific combination chemotherapy and surgery. This treatment protocol was established in the late 1970s and has remained unchanged since. TP53 is a major tumour suppressor protein that functions to detect DNA damage and then trigger a cellular response involving either repair or apoptosis. The TP53 gene is altered in half of all human cancers, but is the major driver mutation in most OS cases (>90%). Here, we are restoring TP53 function not by classical gene therapy, but by delivering TP53 mRNA directly into OS cells and then measuring the cell response. We produced TP53 mRNA in vitro and packaged into cationic lipids before transfecting into normal bone (hFOB) and OS (143B and SAOS-2) cell lines. Cells were incubated with TP53 mRNA for 24 h. Restored TP53 protein was quantified using a BCA assay and visualised by western blot. We have demonstrated that TP53 mRNA can be produced in vitro and that this mRNA can be transfected into null OS cells, which then produce TP53 protein.

Biosynthesised Triterpenes Provide Novel Insights into Structure-Activity Relationships

Rebecca Casson, Maya Valmiki, James Reed, Maria O'Connell, and Anne Osbourn

Triterpenes are 30-carbon natural products built from isoprenoid units and are an important class of plant specialised metabolites, where they have roles including plant defence. These natural products have a long history of use in traditional remedies and have significant modern applications in a number of therapies, including anti-cancer and anti-inflammatory applications and are very structurally diverse, with over 20,000 triterpene structures known to science. However, many triterpenes are produced in plants in very low quantities, and their structural complexity makes chemical synthesis difficult. Transient expression, a technology that allows rapid expression of triterpene biosynthetic genes in a plant host, allows for the production of significant quantities of triterpenes in 3-5 days. Previously, the “triterpene toolkit” suite of triterpene biosynthetic genes developed by the Osbourn lab at the John Innes Centre was shown to produce both natural and novel triterpenes biosynthetically at sufficient quantities for biological activity assays, at a speed and scale which outcompetes chemical synthesis. In this talk I will present key findings from my PhD project, in which specific triterpenes were produced and tested for their bioactivity. Anti-proliferative activity was discovered in a number of cancer cell lines of different cell types, representing newly discovered active positions on the β-amyrin triterpene scaffold. As a result, fresh insights have been gained into the structure-activity relationships of β-amyrin derived triterpenes. I will also detail the design of novel triterpenes generated through the insights gained during this project, optimised for improved bioactivity and pharmacokinetic properties. This work shows the considerable possibility to unlock the biological potential of these important molecules and develop them further into new sources of therapeutics.

Salonee Banerjee
Lightning Talk And Poster Number H5

Hepatocyte Growth Factor and Epidermal Growth Factor signalling crosstalk is involved in tunneling nanotube formation in A549 human lung adenocarcinoma cells

Salonee Banerjee, Griselda Awanis, Robert Johnson, Derek Warren, and Anastasia Sobolewski

Non-small cell lung cancer accounts for 85% of all lung cancer cases and is often associated with overexpression of hepatocyte growth factor (HGF) and epidermal growth factor (EGF). Tunneling nanotubes (TNTs) are thin cytoplasmic protrusions involved in long-distance intercellular communication via cargo transfer and can encourage cancer development and metastasis. We investigated the role of EGF/HGF crosstalk in TNT formation and the markers and signalling pathways involved in TNT formation in A549 cells. The A549 cell line was cultured and treated with 100ng/mL EGF/HGF for 24h. Phase light microscopy was used to capture cell images and TNT analysis was undertaken using Fiji ImageJ. Pharmacological inhibition of c-Met, EGFR, MEK, PI3K, Rac1, Cdc42, and the Arp2/3 complex and siRNA-mediated knockdown of Paxillin was used to assess signalling pathways. Immunofluorescent labelling was performed to visualise TNT markers. EGF, HGF and EGF+HGF induced TNTs in 42%, 39% and 46% of cells respectively, with TNT lengths ranging up to 300µm. Combined treatment of EGF and HGF yielded effects consistent with individual EGF and HGF treatments, suggesting convergence of EGF and HGF signalling pathways. We also found the Ras/MAPK/MEK and PI3K/Akt pathways and the Arp2/3 complex regulated EGF and HGF-induced TNTs. While singular inhibition of MEK or PI3K diminished TNTs induced by HGF/EGF individually, this was not sufficient to inhibit EGF+HGF-induced TNTs, and simultaneous inhibition of both pathways was required for suppression of TNTs to basal levels. This provides insight into how the MAPK/MEK and PI3K/Akt pathways, traditionally thought to be independent, may participate in compensatory signalling to overcome TNT inhibition in A549 cells. Furthermore, the knockdown of Paxillin inhibited EGF/HGF-induced TNTs, marking it as an important scaffolding protein in TNT formation. The observed TNTs showed co-localisation of the novel markers c-Met and β1 integrin along the TNT length, in addition to expressing the classical TNT markers F-actin, α-tubulin and M-sec. Further characterisation of TNTs using scanning electron microscopy showed presence of vesicles within TNTs and revealed the position of the TNTs to be above the substratum, demonstrating non-adherence of the TNTs to the substrate. Future work will investigate the functional consequences of mitochondria/organelle transfer and chemoresistance via TNTs.

Sergio Llaneza-Lago
Poster Number 20

Detecting Novel Subtypes of Osteosarcoma Using Bayesian Unsupervised Clustering

Sergio Llaneza-Lago, William D. Fraser, and Darrell Green

Osteosarcoma (OS) is a rare and aggressive bone cancer that primarily affects children and young adults. OS has a poor prognosis due to the difficulty in eliminating all tumour cells using currently available treatments, leading to relapse and metastasis. Accurate classification of osteosarcoma into subtypes is essential to optimize treatment pathways and develop targeted drugs. The heterogeneity of OS patients poses a significant challenge to traditional classification methods. One better-suited approach is the Bayesian soft clustering method termed latent process decomposition (LPD). LPD has recently identified a clinically useful aggressive subtype of prostate cancer.

The objective of this study is to apply LPD to RNA-seq data from OS patients to detect novel stratifications and characterise them by studying differentially expressed genes and clinical associations.

Total RNA expression data plus clinical information of OS patients were downloaded from five publicly available cohorts in the GEO database. Non-biotechnical batch effect was corrected with the R package ComBat. Cohorts were merged and LPD was performed to detect stratifications. OS patients were distributed into groups according to their most representative stratification. Differential expression (DE) analysis was performed using the R package DESeq2 to characterise the identified stratifications. KEGG pathway and GO enrichment analyses were conducted using the R package ClusterProfiler on DE genes. Clinical associations were analysed by assessing relationships between the stratifications and age, gender, survival time, survival status and metastatic occurrence.

LPD detected five distinct OS subtypes to which DE genes were identified. These were characterised as a typical cancer-progression subtype, a primary cilium promoter subtype, an indolent subtype associated with muscle development, a bone mineralisation-enriched subtype and a low-prognosis subtype with downregulation of muscle development. Our data highlight the importance of LPD for the in-depth examination of OS subtypes. Further validation and study of these subtypes in other sample cohorts is still required.

This preliminary data provides comprehensive insights into the complex genomic processes occurring in OS and a better understanding of its subtypes. This approach will contribute to improving patient care and trial outcomes through better prognosis stratification and facilitate the development of new treatment strategies.

Seshadhri Subramanian
Poster Number 16

Specific Bacteria in Urine Can Indicate Higher Risk for Aggressive Prostate Cancer

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

Background: Prostate cancer is one of the most common cancers in the world. Infectious causes for cancer are well documented. A group of five bacteria genera called the Anaerobic Bacteria Biomarker Set (ABBS), may help to identify the risk of developing aggressive prostate cancer.

Objectives: To detect bacteria in urine using 16S bacterial sequencing data, and to compare bacteria profiles across samples from participants with different grades of prostate cancer.

Methods: Urine samples from a total of *n*=46 participants were subjected to 16S rRNA Illumina sequencing and the results were analysed through the DADA2 pipeline to give an output of number of reads for each amplicon sequence variant (ASV).

Analysis: The analysis on the family level taxonomic results was completed using the Phyloseq package in R. A heatmap of abundances was produced. K-Means clustering was done with Manhattan distances on a Principal Co-Ordinate Analysis Plot to reveal clusters of participant samples based on bacterial families.

Results: The participant urine samples clustered into three groups in terms of their bacterial communities. One group demonstrated a higher proportion of participants that developed metastases, *n*=5 out of 6 samples from patients with prostate cancer metastasis were in one cluster while there was only 1 sample in the other two clusters..

Conclusions/Recommendations: The results show that participants with metastatic prostate cancer tend to group in one cluster when they are clustered on bacteria taxa at the family level. The data analysed indicates that specific bacteria that contribute to the urine microbiome may indicate a higher chance of aggressive prostate cancer.

Impact: The outcome of this research may help detect patients at risk of aggressive prostate cancer early and lead to potential treatment options in the future.

Silvia Ogbeide
Lightning Talk And Poster Number 31

Single-cell multiomics profiling in the study of colorectal cancer evolution

Silvia Ogbeide; Erika Yara, Erica Oliveira, Javier Fernandez Mateos, Andrea Sottoriva, and Iain Macaulay

The heterogenic nature of cancers limits the efficacy of targeted therapies and poses a significant challenge for precision medicine. This is mainly due to the introduction of artificial selection by chemotherapy and radiation, which inadvertently exert a selective pressure that eliminates sensitive cancer phenotypes while promoting the proliferation of variant cells with higher Darwinian fitness and malignant potential. Looking at evolutionary cues in cancer will help us understand the disease and provide insights that can help design novel therapeutic strategies that account for its adaptive nature, promote treatment responsiveness, and delay therapeutic resistance.

Current knowledge of genomic abnormalities in cancer is mostly derived from high-throughput sequencing analyses performed on bulk tumour specimens. However, bulk-sequencing methods are not sensible enough to detect genomic changes present in minority subclones. For this reason, single-cell sequencing methods are preferable to understand cell-to-cell variability.

We have applied single-cell genome and transcriptome sequencing (G&T-seq) on patient-derived organoids (PDOs) of metastatic colorectal cancer samples to characterise the cellular diversity of organoids, follow the evolution of clones under two AKT-inhibitors (MK-2206 and AZD5363) and explore the mechanisms by which chemoresistance is achieved. We have first analysed the transcriptomes of 316 cells of organoid-derived single cells from control and treated organoids. We observe a shift in gene expression in cells isolated from drug resistant PDOs, with one cluster expanding and another decreasing in the resistant PDOs compared to the untreated organoid. These resistant cell clusters express genes that may have a role in the development of resistance to chemotherapy in colorectal cancer, including ODC1 and DKK4.

Intratumor heterogeneity is closely related to cancer progression. The combined examination of a single cell's genome and transcriptome in PDOs offers scientists the opportunity to model tumour progression in vitro, focusing on understanding the clinical relevance of resistant subclones to drug therapies.

Stephanie Smith
Lightning Talk And Poster Number 17

A urine test for prostate cancer: Patient and public involvement work

Stephanie Smith

Background: PUR (Prostate Urine Risk) is a prostate cancer biomarker developed at the University of East Anglia, based on a 38-gene expression readout of prostatic microvesicular RNA extracted from urine. PUR is predictive of D'Amico risk status at diagnosis and PUR is correlated to the amount of Gleason pattern 4 cancer within the prostate.

To avoid side effects some men with prostate cancer are offered active surveillance (AS) rather than immediate radical treatment. AS entails regular blood tests, imaging and biopsies. In a pilot study of men on AS, PUR predicted cancer progression up to 5 years later (HR 8.2). Patient and public involvement work has been undertaken as part of planning for proposed research evaluating PUR in the context of active surveillance.

Methodology: Volunteers were recruited from local and national prostate cancer support groups. One-to-one interviews were performed via Microsoft Teams to explore research priorities. Written and verbal feedback was sought on the Plain English Summaries of research proposals.

Two educational events have been held (Norfolk and Waveney Prostate Cancer Support Group, East Suffolk Prostate Cancer Support Group). At these events, an introduction to the field of research and the project was presented with an interactive "question and answer" session.

Patient feedback forms were collated from urine collections sent previously by the department comprised of three closed questions (Likert scale 1-5) as well as a free text box for additional comments/feedback.

Results: Patient education events were valuable in recruiting volunteers to a PPI panel.

1335 patient feedback forms were collated. Using a five-point Likert scale, clarity of instructions and patient information sheet was deemed very clear (median score 5, mean 4.62 and median score 5, mean 4.66, respectively), kit use very easy (median score 5, mean 4.66). Free text feedback highlighted issues such as the definition of "first urine".

Removing cancer's immortality: the design and synthesis of peptides and small molecules targeting the dyskerin-dyskerin PPI in telomerase

Suzanne van Wier, Mark Searcey, and Andrew Beekman

One of the hallmarks of cancers is their ability to replicate limitlessly making them immortal. In 80-90% of cancer cells this is due to the reactivation of telomerase, a protein complex which elongates telomeres at the end of chromosomes, protecting the chromosomes from degradation and preventing cell senescence.

The Cryo-EM structure of telomerase published in 2021 provided an opportunity to identify new ways to target telomerase [1]. In patients with Dyskeratosis Congenita, a disease characterised by shortened telomeres, the structure showed that genetic mutations are transcribed to the dyskerin-dyskerin protein-protein interaction (PPI) in telomerase. In this project we aim to target this PPI, inhibiting the telomerase activity of cancer cells and thus removing cancer's immortality.

We will describe the design and synthesis of a peptide derived from the dyskerin sequence at this PPI. This initial peptide showed low α-helicity and low proteolytic stability as well as not showing any effect on cell viability. To improve these properties hydrocarbon stapling was applied, which increased the α-helicity by 31-48% with the best peptide showing an 83% decrease in cell viability at 200 μM as well as showing an increase in proteolytic stability. Current work is focused on assay development to evaluate the binding of these peptides to dyskerin and their effect on telomerase activity.

When compared to small molecule drugs, peptide therapeutics can have drawbacks such as their limited stability and cell permeability. Therefore, peptide-directed binding was used to go from peptide to small molecule inhibitor by computationally identifying fragments able to replace parts of the peptide. This method has previously been applied successfully to quickly identify hit compounds for PPIs whilst minimising the organic synthesis and biological screening needed [2].

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Tameryn Stringer
Poster Number 14

Repurposing metallo-quinolines as therapeutics for cancer

Tameryn Stringer; Aran E. Boakye-Smith

Quinolines have been used extensively to prevent and treat malaria. Chloroquine, specifically, is the most successful of these quinoline antimalarials, but has been rendered less effective due to the development of drug-resistant strains. There has been a large amount of research dedicated to finding suitable alternatives that overcome resistance in malaria, specifically with the development of metal-based quinolines, however not many has been as effective as ferroquine in treating chloroquine-resistant infections. Chloroquine and ferroquine have also been investigated for other diseases such as cancer and SARS-CoV-2. Chloroquine has also been shown to sensitise cancer cells to radiation and other treatments, suggesting that it may change the current cancer therapeutic strategies [1]. This paves the way forward to repurpose metal-based antimalarials [2,3] as potential anticancer agents. Metal complexes are increasingly popular anticancer agents, especially if biologically active metals, such as platinum or ruthenium are incorporated as part of a pharmacophore. This often leads to an increase in activity, due to a collaborative effect. Another effect of the metal incorporation is a change in the shape and binding of the pharmacophore to biomolecules (DNA and proteins). The mechanisms of many metal-based compounds are still not yet fully understood, but this study aims to shed light on the mechanism of action of quinoline-based piano-stool complexes containing ruthenium.

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The Dung Nguyen
Poster Number 13

Hydrogen peroxide induces tunnelling nanotube formation in the A549 lung adenocarcinoma cells

The Dung Nguyen, Aya Elmeligy, Salonee Banerjee, and Anastasia Sobolewski

Tunnelling nanotubes (TNTs) are long and thin actin-based protrusions that extend from the plasma membrane and enable contact between different animal cells over a wide range of distances (up to several hundred microns). TNTs act as a conduit for the trafficking of intracellular organelles such as mitochondria, viruses and even oncogenes from a donor cell to an acceptor cell that can have consequences to acceptor cell functions such as migration, grow and survival. TNTs are known to play an important role in cancer cell metastasis and resistance to chemotherapy, radiotherapy, and surgery and can be induced by different cell stresses such as reactive oxygen species that are present in the tumour microenvironment. TNTs have been observed in vivo in different cancers, including lung adenocarcinoma cells that cause non-small cell lung cancer (NSLC). NSCLC has a poor prognosis with low survival rates. However, there have not been many studies on the mechanisms of TNTs formation in non-small cell lung cancer cells. The aim of the present research was to investigate the effect of hydrogen peroxide, an agent capable of causing cellular stress, on TNT formation in A549 lung adenocarcinoma cells. Methods: A549 cells were seeded at 5000 cells per well in a 12-well plate and cultured for 72 hours in 10% FBS, pen/strep DMEM after which hydrogen peroxide was added at different concentrations (0.0001-100µM) for 24-72 hours. Images were taken with a white like microscope and the number of TNTs per cell, the average number of TNTs per cell and the average length of TNTs was determined using image J software. Cell viability was determined using an alamar blue assay. Scanning electron microscopy was also used to visualize TNTs at high resolution. Results: hydrogen peroxide at 100µM (n=3) caused cell death. At 10-4 µM (n=3), it induced a significant increase in the average number of TNTs per cell, average number of cells with TNTs and the average length of TNTs (n=3, p<0.05) after 24 hours incubation, with no effect at 48 or 72 hours (n=3). Scanning electron microscopy showed that hydrogen peroxide induced TNTs were non adherent in places and showed exosomes or vesicle structures in the TNT, which was different compared to growth factor induced TNTs. Conclusions: Hydrogen peroxide at high concentrations is the main cause of cell death, but at low concentrations it induces the formation of TNTs in A549 lung adenocarcinoma cells. Not only that, the cargo transported by hydrogen peroxide-induced TNTs formed by was different from that of growth factor-induced TNTs, thus indicating the pathway of TNTs formation under the influence of cellular stressors may be different from the normal TNT-forming. The study contributes to a better understanding of the formation mechanism, signaling pathway as well as the molecules involved in the formation of TNTs. Thereby may pave the way for future pharmacological studies to increase the effectiveness of treatment for patients with non-small cell lung cancer.

Valeriia Haberland
Poster Number 19

A comprehensive comparison of proposed prostate cancer molecular subtypes

Valeriia Haberland, Dan Woodcock, David C. Wedge, and Daniel S. Brewer

Motivation: Prostate cancer is the most common type of cancer among men. It is highly heterogeneous and may progress slowly requiring just regular surveillance or progress fast requiring radical treatment. No molecular subtype classification framework for prostate cancer is used routinely in the clinical practice, despite its need. Many studies have attempted to classify prostate cancers using unsupervised machine learning algorithms, but no comprehensive and standardised comparison has been presented in the literature. Our goal is to replicate and compare these studies using the large multi-omics dataset of 2000 patients provided by the Pan Prostate Cancer Group consortium, and potentially combine them to create an optimal disease progression predictor.

Methods: Our multi-omics dataset consists of RNA-Seq count data, copy number alterations, single nucleotide variants, indels and methylation data. We have reviewed the literature to identify the subtype classification studies suitable for inclusion into our framework based on these criteria: primary cancer, unsupervised machine learning algorithms and possibility to replicate the results. A semi-automated pipeline to characterise and compare these subtypes has been developed, and incorporates a survival analysis, gene differential expression analysis as well as investigating associations between the subtypes and known driver genes, CNA regions, mutational signatures and types of mutations.

Results: As a result of our literature review, 15 studies were deemed to be suitable for inclusion into our comparison framework. Five subtype classification frameworks have been incorporated, and our results have demonstrated interesting overlaps between some subtypes. We have identified potentially two aggressive subtypes, which may be driven by different biological processes.

Vicky Kamperi
Lightning Talk And Poster Number 7

Investigating duplex-duplex DNA crosslinking with acridine bis-intercalators

Victoria Kamperi, Zoë Goddard, Marco M. D. Cominetti, Maria J. Marin, Andrew M. Beekman, and Mark Searcey

Damaging the DNA of cancer cells is a well-known strategy for cancer therapy. One way this can be accomplished is through DNA crosslinking, which can occur when crosslinking agents covalently bind to two nucleotides in the same or opposite strand of a dsDNA molecule [1]. An interstrand crosslink (ICL) can form when a compound binds to two nucleotides located in opposite strands of the dsDNA molecule and is very toxic to the cell. The ICL prevents essential DNA processes from being carried out such as DNA replication and transcription, ultimately blocking cell growth and proliferation and leading to cell death. Based on this principle, a stronger binding effect can be reached through DNA bis-intercalation, where a compound (bis-intercalator) can bind to a DNA molecule through two intercalating groups, connected by a linker, or have each intercalating moiety symmetrically bind to a different dsDNA molecule.

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 inhibiting topoisomerase activity. Here we will discuss the synthesis of a variety of acridine dimers and a gold nanoparticle-assay that was carried out to test their ability to crosslink two duplex DNA strands [2].

[1] Huang, Y.; Li, L. DNA Crosslinking Damage and Cancer - a Tale of Friend and Foe. Transl Cancer Res 2013, 2 (3), 144. https://doi.org/10.3978/J.ISSN.2218-676X.2013.03.01.
[2] Marín, M. J.; Rackham, B. D.; Round, A. N.; Howell, L. A.; Russell, D. A.; Searcey, M. A Rapid Screen for Molecules That Form Duplex to Duplex Crosslinks in DNA †. Chem. Commun 2013, 49, 9113. https://doi.org/10.1039/c3cc45600e.

Alternative mRNA splicing is associated with an immunosuppressive tumour microenvironment in soft tissue sarcoma

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Soft tissue sarcomas (STS) are the most common form of sarcoma seen in adults. 5-year survival for patients with STS with complex karyotypes (CK STS) and recurrent or metastatic disease is less than 20%. Identification of effective therapies is urgently needed.

mRNA splicing is a common post-transcriptional event that leads to the generation of alternative transcripts, often with altered biological function. mRNA splicing is aberrantly regulated in cancer and is considered an emerging therapeutic target. How alternative mRNA splicing regulates STS biology and its potential as a therapeutic target is unknown.

Using an immunocompromised mouse model of STS, we have shown differential expression of splice variants of VEGFA impacts on metastasis and response to therapy (English et al, Cancer Res, 2017). RNAseq of whole tumour extracts has since identified that expression of VEGFA120 versus VEGFA188 leads to increased expression of PD-1 immune-checkpoint pathway genes. To confirm its clinical relevance, we combined TCGA SARC and VORTEX phase III trial mRNA splice variant data to double the number of CK STS patients available for analysis. Patients with CK STS fall into three groups of VEGFA alternate splicing, two of which are comparable to our pre-clinical model. GESA and cell subset prediction showed patients with high VEGFA121 versus VEGFA189 were enriched in pathways associated with immunosuppression including PD-1 signalling and oxidative phosphorylation, as well as high levels of MDSCs and CD8 cells, with no change in TReg. By expressing VEGFA120 or VEGFA188 in T241 sarcomas in C57Bl6/J mice, we were able to show VEGFA188 expression led to decreased tumour take, increased levels of CD8 cells and no change in TReg. Together our data shows alternative mRNA splicing can alter the tumour immune microenvironment in CK STS and has potential for therapeutic intervention or augmentation of immune checkpoint therapy.

Zoe Goddard
Poster Number 11

The smallest active pharmacophore of duocarmycin SA for use on the solid phase

Zoë R. Goddard, James Williamson, and Mark Searcey

Duocarmycin SA is a potent DNA alkylator. To date, however, it has not been clinically approved for use as a chemotherapeutic. Much work has been done on harnessing the power of duocarmycin, and analogues of the alkylating unit show varied potency and toxicity. The structure of duocarmycin SA consists of two DNA binding units, surrounding a core alkylating unit. The alkylating unit of duocarmycin, also known as CI, has been shown to alkylate DNA, but its potency is decreased due to the loss of the binding units. Here, we hypothesise that we can isolate the alkylating unit and encourage DNA binding through addition to peptides and small molecules. To do this, we have pioneered the use of solid phase synthesis to produce analogues of duocarmycin through the synthesis of Fmoc-protected analogues. Here, we will present the synthesis of CI for use on the solid phase and discuss its versatility for the synthesis of duocarmycin analogues for the design of novel DNA alkylating agents.

How to join virtually

Talks

We will be using Microsoft Teams for the symposium. The package works like a lecture theatre, with a large audience watching and able to ask questions or chat. They will be able to see the slides and the speaker.

Please follow this link: https://bit.ly/NCRNsymp23 or Click here to join the meeting

Poster Session

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/NCRN23posters

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 for organising and chairing the Early Career Researcher session, and for being on top of social media. Also, a huge thank you to Dani Lefeuvre from UEA RIN who provided excellent professional services support and manned the registration desk on the day. We are also indebted to Sean Tattan, Paul Jackson, Timothy Brendler-Spaeth, Sergio Llaneza-Lago and Anastasios Bampalis for supporting all aspects of the day. We would also like to thank the Faculty of Science, the Faculty of Medicine and Health, CancerTools and Oxford Nanopore 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, Rianne Lord (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.

Please fill in the form here:
https://forms.microsoft.com/r/E8ggaDKFKp

NCRN Virtual Seminar Series

We are continuing the NCRN virtual seminar series. If you would like to speak at this or have suggestions for an external speaker, please email Daniel Brewer (d.brewer@uea.ac.uk).