Young Cancer Researchers Networking (YCRN)

Translational Cancer Research Conference

21-22 June 2018, University College Cork, Ireland

Inspire Connect Interact

12 Royal College of Surgeons Ireland CPD Credits

PROGRAMME

Day1

Thursday, 21st June 2018

Western Gateway Building, University College Cork, Western Road, Cork, T12 XF62

From 9 am	Registration and Poster Hanging
10.45 am	Opening Ceremony
11 am	Cancer Research Overview – Prof Mary Cahill
11.15 am	Opening Keynote Talk

Prof Stephen R Pennington, President Elect HUPO, UCD Conway Institute, Ireland

"Discovery, Development and Delivery of Protein Biomarkers for Cancer Patient Benefit"

12 noon – 1.20 pm	**SESSION 1 Cancer Cell Death and Survival**; Chairs: Dr Tracey O'Donovan (UCC) and Dr Kheshwant Gill (UCC)

12 pm – 12.20 pm **Rebecca Amet**, Trinity College Dublin, Ireland

"Novel Anti-Cancer Therapeutics for Multiple Myeloma which target the STAT3 signalling pathway"

12.20 pm – 12.40 pm **Sarah Riis**, University College Cork, Ireland

"Insulin-like Growth Factor-1 signalling is essential for mitochondrial protection in cancer cells"

12.40 pm – 1.00 pm **Jennifer Quinn**, University College Cork, Ireland

"Investigating a role for autophagy in ovarian cancer chemoresistance"

1.00 pm – 1.30 pm **Dr Eva Szegezdi**, Director of Blood Cancer Biobank Ireland

"The differential effects of decitabine and 5-azacytidine on AML cells – implications for NK cell-based immunotherapy"

1.30 pm – 2.30 pm	LUNCH + POSTER VIEWING
2.30 pm – 3.50 pm	**SESSION 2 Cancer Cell Signalling and Trafficking**; Chairs: Dr Andrew Lindsay (UCC), Joanne Nolan (UL)

2.30 pm – 2.50 pm **Máté Nászai**, University of Glasgow, UK

"The Ras effector RalA promotes Wnt pathway activation and controls intestinal stem cell dynamics"

2.50 pm – 3.10 pm **Zhi Liu**, University College Dublin, Ireland

"Functional analysis of the MAPK scaffold KSR1 in malignant melanoma"

3.10 pm – 3.30 pm **Amira Mahdi**, University of Limerick, Ireland

"Elucidating the role of newly synthesised proteins in the progression of breast cancer"

3.30 pm – 4.00 pm **Dr Patrick Caswell**, University of Manchester, UK

"Controlling tumour-stroma interactions through endocytic trafficking"

4.00 pm – 4.30 pm	COFFEE BREAK + POSTER VIEWING
4.30 pm – 5 pm	R&D IN INDUSTRY – **CAREER FORUM**

Dr Giuliano Bensi, GSK Vaccines S.r.l. Siena, Italy

Dr Emiliano Chiarot, GSK Vaccines S.r.l. Siena, Italy

"R&D in vaccine industry: contribution of internal and external activities to innovation"

5.00 pm – 6.30 pm	**SESSION 3 Cancer Biomarker Discovery – translation towards personalised medicine** Chairs: Dr Mark Tangney (UCC), Shona Cronin and Yensi Flores
5.00 pm – 5.20 pm	**Dr Elizabeth Foxall**, King's College London/The Francis Crick Institute, UK "Rare cancer helper genes disrupt related biological processes in oesophageal adenocarcinoma"
5.20 pm – 5.40 pm	**Aisling Heeran**, Trinity College Dublin, Ireland "Profiling inflammatory protein secretions from the ex vivo rectal cancer microenvironment compared to normal tissue"
5.40 pm – 6.00 pm	**Natalia Koerich Laureano**, German Cancer Research Center (DKFZ), Germany “Integrative multiscale omics analysis identified epigenetic regulation of SOX2 expression by DNA methylation in disseminating HNSCC tumour cells"
6.00 pm – 6.30 pm	**Dr Sudipto Das**, Honorary lecturer at Royal College of Surgeons in Ireland "Dissecting genetic and epigenetic alterations mediated metastatic colorectal cancer progression"
6.30 pm – 7.30 pm	POSTER SESSION
8.30 pm – 11 pm	DINNER at CORNSTORE (http://cornstore.ie/) and CORK REBEL PUB CRAWL

Day2

Friday, 22nd June 2018

Western Gateway Building, University College Cork, Western Road, Cork, T12 XF62

8.45 am	EACR-sponsored Keynote Talk **Prof Julie Gehl**, Center for Experimental Drug and Gene Electrotransfer (C*EDGE), Zealand University Hospital, University of Copenhagen, Denmark "Electrochemotherapy and calcium electroporation; development of new treatments for cancer”
9.30 am – 10.50 am	**SESSION 4 Cancer Immunology and Immunotherapy**; Chairs: Dr Patrick Forde (UCC), Vamsi Bharadwaj Yallapragada (UCC)
9.30 am – 9.50 am	**Maria Davern**, Trinity College Dublin, Ireland "The effect of clinically relevant chemotherapies on the expression of immune checkpoints in oesophageal adenocarcinoma"
9.50 am – 10.10 am	**Liam Tremble**, University College Cork, Ireland "Development of a preclinical model for the development of macrophage targeting therapies in melanoma"
10.10 am – 10.30 am	**Dr Lisa Loughney** and **Mairéad Cantwell**, Dublin City University, Ireland "Exercise Training throughout the Cancer Journey: The MedEx Experience"
10.30 am – 11.00 am	**Dr Urska Kamensek**, Institute of Oncology Ljubljana, Slovenia “Gene electrotransfer for cancer immunotherapy”
11.00 am – 11.30 am	COFFEE BREAK

11.30 am – 1.00 pm **SESSION 5 Novel Drugs and Drug Delivery**;

Chairs: Dr Órla Barry (UCC), Keiran Logan (Ulster University)

11.30 am – 11.50 am **James Mc Keown**, Trinity College Dublin, Ireland

"Discovery of therapies for lymphomas and leukaemia: Synthesis and antiproliferative action of novel ethanoanthracenes"

11.50 am – 12.10 pm **Amy Buckley**, Trinity College Dublin, Ireland

"The action of a novel radiosensitiser within the Oesophageal Adenocarcinoma tumour microenvironment"

12.10 pm – 12.30 pm **Lauren Gutgesell**, University of Illinois at Chicago, USA

"Estrogen receptor agonists, antagonists, and beyond for characterizing and overcoming resistance in ER+ breast cancer alone and in combination therapy"

12.30 pm – 1.00 pm **Prof John Callan**, The Norbrook Chair in Pharmaceutical Science, Ulster University, UK

"Ultrasound Targeted Microbubble Destruction for the targeted Chemo-Sonodynamic Therapy of Pancreatic Cancer"

1 pm – 2 pm LUNCH

2 pm – 4 pm **SESSION 6 Translational Research**;

Chairs: Prof Caitriona O'Driscoll (UCC) and Glenn Hogan

2.00 pm – 2.40 pm **Dr Verena Murphy**, Operations Lead for CNS and Paediatric Studies, Cancer Trials Ireland

"Translational Research in Cancer Trials Ireland"

2.40 pm – 3.20 pm **Prof Mark Corrigan**, Director of Core Surgical Training at Cork University Hospital, Ireland

“We need to stop translating”

3.20 pm – 4.00 pm **Prof Jacintha O'Sullivan**, Trinity Translational Medicine Institute, Ireland

"Is Angiogenesis still an attractive target in Colorectal Cancer drug discovery?"

4 pm – 4.30 pm **Prof Anita Magurie** - Closing Ceremony and Best Speakers/Best Posters Awards

Invited Speaker Abstracts:

Opening Keynote Talk

Prof Stephen R Pennington, President-Elect HUPO, UCD Conway Institute, Ireland

Discovery, Development and Delivery of Protein Biomarkers for Cancer Patient Benefit

Despite a few decades of proteomics research and the apparent discovery of hundreds if not thousands of new protein biomarkers, the number of that have been developed to the stage of being used routinely in clinical practice is disappointingly low. So, whilst there's huge interest in the development of new biomarker and major unmet clinical needs as yet biomarker development and delivery is proving very challenging. Many aspects of these challenges have been described and reviewed.

In this presentation, early-stage efforts to develop multiplexed protein signatures will be described. The process we adopt begins with identifying the unmet clinical need and using it to define the context and intended use of a new diagnostic test. Then having established potential biomarkers we exploit the flexibility, sensitivity and specificity of targeted mass spectrometry approaches to develop assays to measure multiplexed protein signatures for biomarker evaluation. Drawing on examples in prostate cancer our on-going efforts to develop and validate multiplexed protein assays that are sufficiently robust, reproducible and operationally routine for use as advanced diagnostic tests will be presented.

EACR-sponsored Keynote Talk

Prof Julie Gehl, Clinical Professor, Center for Experimental Drug and Gene Electrotransfer (C*EDGE), Zealand University Hospital, University of Copenhagen, Denmark

Electrochemotherapy and calcium electroporation; development of new treatments for cancer

Brief electric pulses may be used to transiently permeabilize cell membranes (electroporation), and this can be used to increase uptake of drugs in cancer cells. Of note the increase in cytotoxicity of the chemotherapeutic agent bleomycin is increased several hundred folds, enabling highly efficient once-only treatment of tumours that can be accessed with an electric field. Also, calcium may be used as a cytotoxic drug as supraphysiological doses of calcium are poorly tolerated by tumour cells. Novel electrodes are being developed, enabling treatment of not only cutaneous tumours but also tumours in internal organs. This talk will explain the scientific basis of electroporation-based drug delivery, will describe present clinical results and expand thoughts on future therapies using electroporation-based drug delivery.

SESSION 1 Cancer Cell Death and Survival

Dr Eva Szegezdi, Director of Blood Cancer Biobank Ireland

The differential effects of decitabine and 5-azacytidine on AML cells – implications for NK cell-based immunotherapy

Delphine Ohayon, Andrea Tirincsi, Hojjat Alizadeh Zeinabad, Eva Szegezdi

Apoptosis Research Centre and Blood Cancer Network Ireland, National Universiy of Ireland, Galway

Decitabine and 5-azacytidine are DNA hypomethylating agents (HMA). They inhibit DNA methyltransferases (DNMT) by getting incorporated into the DNA where they form an irreversible complex with DNMTs. 5-azacytidine and 5-aza-2-deoxycytidine (decitabine) are nucleoside analogues. They differ from each other in that decitabine is a DNA nucleoside (contain deoxy-ribose), while 5-azacytidine is an RNA nucleoside.

Decitabine and 5-azacytidine are widely used for the treatment of elderly acute myeloid leukaemia (AML) patients due to their relatively mild side effects.

AML is characterised with aggressive growth of immature myeloid cells derived from malignant hematopoietic stem or progenitor cells (HSPC), termed leukemic stem cells (LSCs). DNMTs are central for HSPC differentiation and proliferation but the effect of HMAs on LSCs is poorly characterised.

In this study, we show that decitabine and 5-azacytidine have an opposing effect on the expression of the interleukin-3 receptor (IL3Ra, AKA CD123), a characteristic receptor of malignant LSCs associated with IL-3-driven proliferation. While decitabine enhanced the expression of CD123 on the cell surface, 5-azacytidine had a tendency of reducing it. By measuring protein expression rate we found that 5-azacytidine robustly blocks general protein synthesis and decitabine has a much milder effect.

As CD123 is a selective marker of LSCs, it is a good target for immunotherapy. Here we show that AML cells treated with decitabine can be opsonised more effectively with an anti-CD123 antibody than 5-azacytidine treated cells and these cells can be recognised and killed by natural killer (NK) cells, thus offering a treatment strategy to effectively target LSCs, the recognised cause of drug resistance and disease relapse in AML.

SESSION 2 Cancer Cell Signalling and Trafficking

Dr Patrick Caswell, Wellcome Trust Centre for Cell-Matrix Research, University of Manchester, UK

Controlling tumour-stroma interactions through endocytic trafficking

The endocytosis of cell surface receptors, and subsequent trafficking through the endocytic system, regulates their ability to switch on downstream signalling modules. It is now clear that endocytosis controls signalling during proliferation, differentiation and migration of cells, and plays an important role in cancer progression. Our work has focused on how the endolysosomal system controls the master regulators of the cytoskeleton, RhoGTPases, in cells migrating in 3D-matrix.

We have shown that Rab11-driven recycling of integrins and co-cargo receptors controls cancer cell migration in 3D-matrix. Rab11 and its effector Rab-coupling protein (RCP) coordinate signals that control the architecture of F-actin protrusions, by switching the balance of RhoGTPase signalling to favour RhoA activation and formin-dependent filopodia formation to generate actin-spike protrusions (rather than lamellipodia). We are now focusing on the functions of Rab11 trafficking pathways in metastasis of high grade serous ovarian cancer to the omentum, a fatty peritoneal fold that is the major colonisation site in this form of cancer.

SESSION 3 Cancer Biomarker Discovery – translation towards personalised medicine

Dr Sudipto Das, Honorary Lecturer at Royal College of Surgeons in Ireland

Dissecting genetic and epigenetic alterations mediated metastatic colorectal cancer progression

Sudipto Das1,2*, Kirsha Naicker1*, Rut Klinger1, Bruce Moran1, Elton Rexhepaj3 Yue Fan1, Rory Casey4, Fredrick Ponten3, Karen Jirstrom5, Jacintha O' Sullivan4, William M Gallagher2, Donal J Brennan6§, Darran P O'Connor1,2§

1Conway Institute, University College Dublin,2 Department of Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin, 3Uppsala University, Uppsala Sweden4 Trinity College Dublin,5Department of Clinical Sciences, Lund University, Sweden,6 University of Queensland, Australia *Equal Contribution, § Shared Senior Authorship

Despite continual efforts in developing more effective diagnostic and therapeutic options, metastatic colorectal cancer (mCRC) associated survival remains significantly low compared to non-metastatic CRC. This talk focuses on a targeted DNA methylation approach, which has allowed us to identify tumour specific differentially methylated enhancer regions which control disease-associated genes such as SATB2, a member of a family of Special AT-rich Binding proteins and a novel transcription factor that orchestrates gene expression. Previously, we described SATB2 as colorectal cancer (CRC) diagnostic marker, here, we assess the precise functional role of SATB2 in the development and progression of colorectal cancer, initiating from the pro-inflammatory phenotype. Differential expression of SATB2 was observed in colorectal cancer with the early pre-neoplastic tissue demonstrating highest SATB2 levels, which diminishes in adenomas followed by complete loss of SATB2 expression in metastatic disease. Functionally, we demonstrate that siRNA-mediated knock down of SATB2 in SW480 cells was associated with the acquisition of an aggressive phenotype. Gene set enrichment analysis of gene expression data derived from two independent CRC cohorts (n=776), revealed

that loss of SATB2 mRNA expression was associated with a TH2 cytokine response and checkpoint genes like CTLA4 and PD1. Intriguingly, SATB2 expression was associated with chromosomal stability as knock-down of SATB2 resulted in an increase of anaphase bridging. Given the relationship between SATB2, chromosomal instability and local inflammatory response, SATB2 protein expression was assessed in a large cohort of patients with Ulcerative Colitis (UC) some of whom developed UC related carcinoma. SATB2 protein expression decreased across the disease spectrum from normal to UC to dysplasia to carcinoma, suggesting that SATB2 expression can be used to monitor UC patients at risk of developing CRC. Taken together, these findings for the first time demonstrate the role of SATB2 as potential master immune-regulator in colon cancer.

SESSION 4 Cancer Immunology and Immunotherapy

Dr Urska Kamensek, Institute of Oncology Ljubljana, Department of Experimental Oncology, Zaloska cesta 2, 1000 Ljubljana, Slovenia

Gene electrotransfer for cancer immunotherapy

Gene electrotransfer (GET) is one of the most efficient non-viral gene therapy approaches for localized gene transfer into tumors in vivo. Therefore, it is especially promising for delivering different cytokines that are toxic if administered systemically. Currently, electrotransfer of plasmids encoding the cytokine interleukin 12 (IL-12) is approaching clinical use for treatment of various superficial solid tumors. Plasmids used in ongoing IL-12 GET clinical trials in the USA, contain antibiotic resistance genes and are thus, according to safety recommendations of the European Medicines Agency, not suitable for clinical trials in the EU. Hence, in our group we are striving to prepare plasmids without antibiotic resistance genes. Likewise, we are investigating different immunological capacities of GET: from using GET as an adjuvant to standard local ablative therapies or to induce in situ vaccination, to investigating the adjuvant effect of plasmid DNA itself.

In one of the immunological GET based approaches, we utilize concomitant intratumoral GET of two plasmids: a plasmid encoding a potent cytotoxic cytokine tumour necrosis factor alpha (TNFα) to induce in situ vaccination and a plasmid encoding an immunostimulatory cytokine IL-12 to boost the primed local immune response into a systemic one. The results of our recent study in a mouse melanoma tumour model confirmed the feasibility and effectiveness of the proposed approach in eliciting a potent and durable antitumor response. Furthermore, the ability of the approach to induce in situ vaccination was indicated by the expansion of effector immune cells in lymph nodes, and vitiligo-like depigmentation of the treated area. However, further studies are needed to directly prove the systemic effectiveness, such as the abscopal effect, of the approach and to push the local effectiveness from 80 to 100%. In addition, the approach needs to be tested in other tumour models, especially since one of the advantages of in situ vaccination is its ability to harness the patient's own immune system and tumour's own tumour-associated antigens, meaning it has the potential to be effective in different cancer types.

SESSION 5 Novel Drugs and Drug Delivery

Prof John Callan, The Norbrook Chair in Pharmaceutical Science, Ulster University, UK

Ultrasound Targeted Microbubble Destruction for the targeted Chemo-Sonodynamic Therapy of Pancreatic Cancer

Abstract: Pancreatic cancer has the lowest survival rate among the 21 most common forms of cancer with only 5% of patients surviving 5 years following diagnosis. Surgery remains the only cure for pancreatic cancer but surgery with curative intent is only possible in approximately 20% of patients. The remaining 80% of patients present with either metastatic disease (40%) or locally advanced/borderline resectable disease (LAPC / BRPC) (40%). In recent years, significant effort has focussed on treating LAPC / BRPC patients with neo-adjuvant chemo- or chemo/radiotherapy in an attempt to downstage their tumours and increase the proportion of patients eligible for surgery. Unfortunately, the chemotherapy used in these treatment regimes is extremely toxic and results in significant off-target side effects. We have developed a microbubble based platform capable of carrying drug payloads on their surface and oxygen gas in their core. We have demonstrated the ability to target delivery of the oxygen gas and drug payloads to murine pancreatic cancer tumours using externally applied ultrasound to disrupt (burst) the microbubbles in the tumour vasculature. We have also demonstrated that combining conventional cancer chemotherapeutics with sonodynamic therapy (SDT) produces a significantly improved tumour response compared to chemotherapy or radiotherapy alone. In this talk, we present our pre-clinical results to date and outline the next steps toward translation of this technology to the clinic.

SESSION 6 Translational Research

Dr Verena Murphy, Translational Research Leader Operations Lead for CNS and Paediatric Studies, Cancer Trials Ireland

Translational Research in Cancer Trials Ireland

Cancer Trials Ireland is a not for profit clinical trials organisation, which was founded in 1996 (as All Ireland Cooperative Research Group, ICORG). The aim was to create more research opportunities for patients by putting a formal structure in place to make Ireland more attractive as a location to international cancer research groups and the pharmaceutical industry. Today it counts more than 95% of the island's cancer treating professionals and a large amount of academic cancer researchers among its membership ensuring that research into cancer develops at a national level across all localities.

Cancer Trials Ireland collaborates with both national cancer research groups (e.g. BREASTPREDICT and the Blood Cancer Network), as well as leading international groups such as ECOG, NSABP, TRIO, ANZUP, UNC Cancer Network and CRUK. Currently, the Cancer Trials Ireland portfolio consists of 154 trials in various different stages (in development, open to accrual, in follow-up), of which 42 are translational/registry, 25 are radiotherapy and 87 are clinical studies addressing all major cancer types.

Resulting from a strategic decision in 2009, a resource for translational research and a requirement for all in-house studies to include a biological sample collection has been put in place. Since then, the majority of Cancer Trials Ireland sponsored studies include translational sub-studies, which together with related clinical data present an invaluable source for cancer research projects. In addition, Standard Operating Procedures (SOPs) for sample collection and processing were put in place to ensure consistently high quality of the samples, which are a prerequisite for good and meaningful research.

Prof Mark Corrigan, Director of Cork Breast Research Centre (CBRC) UCC, Director of Core Surgical training & Consultant Surgeon Cork University Hospital

We need to stop translating

Language is important. Our goal is to improve the outcomes of patients with cancer. The term 'translating' infers the use of separate languages by at least two participants. Different languages create the need to translate, therefore creating barriers. Barriers are impediments to accomplishing goals. Our aim to date has been to improve translation, however, we now need to look at developing an organisational vernacular that facilities the incorporation of clinical, basic biomedical and theory directed research into delivering improved outcomes for patients with cancer. Accepting the significant talent and experience available to us, we need to construct a model of medical research that brings us beyond needing to translate and instead one that immediately harmonises the available skillset towards our stated objectives. The evolution of patient, hospital, university and commercial industry conglomerates in targeted areas will help facilitate this.

Prof Jacintha O'Sullivan, Professor in Translational Oncology, Director of MSc. in Translational Oncology, Trinity Translational Medicine Institute, Department of Surgery, Trinity College Dublin,

Is Angiogenesis still an attractive target in Colorectal Cancer drug discovery?

Colorectal cancer (CRC) is the third most frequent cause of cancer death worldwide, responsible for over six hundred thousand deaths per year. 25%-30% of CRC patients present with metastases at diagnosis. Despite the introduction of molecularly targeted therapies, the five-year survival rate for patients with metastatic CRC remains at a dismal rate of 12%. Targeted therapies for these patients include cetuximab (Erbitux) which targets the epithelial growth factor (EGF) receptor, and bevacizumab (Avastin) which is a humanized monoclonal antibody that targets vascular endothelial growth factor (VEGF). However, response rates to bevacizumab are only about 40% or less. In contrast to cetuximab where assessing KRAS mutation status does predict survival, there is very limited or inconclusive data on biomarkers for predicting response to bevacizumab treatment.

Selection of patients for bevacizumab therapy based on molecular predictors of individual tumours is regarded as an important treatment strategy for these patients, however, there are currently no biomarkers used clinically to predict benefit to Bevacizumab targeted treatment, to monitor treatment response or to assess whether and when to discontinue treatment. This is a major clinical challenge. Without appropriate tools, clinicians are now using bevacizumab in unselected patients without awareness of pre-existing or emerging resistance. Therefore, the identification of robust

biomarker panels with demonstrated biological relevance to VEGF inhibition in order to predict or monitor the efficacy of Bevacimuab treatment is a challenging field.

In my talk, I will discuss the work we have done on biomarker screening in this field. We have also taken a theranostic approach linking this diagnostic work with developing novel small molecule drugs that may have utility in those tumours non-responsive to bevacizumab using human ex vivo models and in vivo mouse studies. The output of these studies is focused on delivering a more personalised medicine approach in treating metastatic CRC patients.

Early-Career Researchers - Talk abstracts:

SESSION 1 Cancer Cell Death and Survival

YCRN18-1-12 Ms Rebecca Amet, School of Biochemistry and Immunology, Trinity College Dublin, Ireland

NOVEL ANTI-CANCER THERAPEUTICS FOR MULTIPLE MYELOMA WHICH TARGET THE STAT3 SIGNALING PATHWAY

Amet R.1,2,3, Previtali V.3, Browne P.V.4, Rozas I.3, McElligott A.M.2 & Zisterer D.M.1

1 School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, 152-160 Pearse St., Dublin 2. 2 John Durkan Leukaemia Laboratories, Trinity Translational Medicine Institute, Trinity College Dublin. 3 School of Chemistry, Trinity Biomedical Sciences Institute, Trinity College Dublin, 152-160 Pearse St., Dublin 2. 4 Department of Haematology, St James's Hospital, Dublin.

Introduction: Multiple myeloma (MM) is a plasma cell malignancy characterized by the secretion of monoclonal immunoglobulins which are detectable in the serum or urine. A better understanding of the pathophysiology of myeloma and advancements in the development of novel therapeutics have allowed the median survival of patients to increase from 3 to 6 years in the past decade. However, despite the effectiveness of the first-line treatments, patients invariably relapse and become drug refractory; therefore, novel therapeutics which target this incurable disease are still required.

The RAS/RAF/MEK/ERK signal transduction pathway also significantly contributes to MM cell growth and survival, as well as to angiogenesis and to the development of drug resistance. Targeting this pathway to prevent aberrant oncogenic signaling is therefore a promising anti-MM strategy and, indeed, the multi-tyrosine kinase inhibitor sorafenib has been shown to exhibit anti-tumour activity in MM. Signal transducer and activator of transcription 3 (STAT3) is activated by the MM growth and survival factor IL-6 secreted by the bone marrow stromal cells within the tumour microenvironment. STAT3 upregulates expression of genes involved in apoptosis, proliferation and angiogenesis. Consequently, STAT3 has emerged as a therapeutic target in various cancers including MM (Hua et al., 2014).

We have previously rationally designed and synthesized guanidinium based novel compounds to target the RAS/RAF/MEK/ERK signaling pathway, which demonstrated anti-tumour activity in leukaemia cells (Diez-Cecilia et al., 2014). In the present study, we evaluated the anti-cancer activity of an optimised series of these compounds in MM cells and examined their ability to target both the ERK and STAT3 signaling pathways.

Materials and methods: The effect of seven guanidinium based compounds on the viability of MM cell lines, NCI-H929 and U266B1, was evaluated by AlamarBlue assay. IC50 values were determined and a lead compound VP79s was identified. Apoptosis was determined through flow cytometric analysis of Annexin V/PI stained cells and by immunoblotting of caspase-3 cleavage products. Targeting of the ERK and STAT3 signalling pathways was examined through immunoblotting using antibodies directed against phosphorylated and, thus, activated forms of ERK and STAT3. The effect of VP79s on expression of STAT3 mediated gene products including anti-apoptotic Mcl-1 and survivin was investigated by immunoblotting.

Results: The novel lead compound VP79s reduced the viability of NCI-H929 and U266B1 cells with IC50 values of 3.2 ± 0.4 μM and 4.9 ± 0.9 μM respectively. VP79s induced apoptosis in a dose and time-dependent manner resulting in caspase-3 activation. Interestingly, VP79s induced a sustained ERK activation in MM cells. VP79s rapidly inhibited both constitutive and IL-6 induced STAT3 activation, and was found to decrease expression of STAT3 mediated anti-apoptotic gene products, Mcl-1 and survivin, and the cell cycle protein cyclin D1.

Conclusions: The novel compound VP79s can target dysregulated STAT3 activation and induce apoptosis in myeloma cells, suggesting its potential as a novel anti-cancer therapeutic. Identification and development of novel drugs that can target STAT3 remains an important scientific and clinical challenge.

YCRN18-1-13 Ms Sarah Riis, Biosciences Institute, University College Cork, Ireland

Insulin-Like Growth Factor 1 Signalling is Essential for Mitochondrial Protection in Cancer Cells

Sarah Riis1, Amy Lyons1, Michael Coleman1, 2, Cedric Favre1, Ciara H. O'Flanagan2, Stephen D. Hursting2, and Rosemary O'Connor1

1. School of Biochemistry and Cell Biology, University College Cork, Ireland, 2. Division of Nutritional Biochemistry, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, USA

Mitochondrial activity and cellular metabolic reprogramming may influence the phenotype of cancer cells and mediate resistance to targeted therapy. Previously, we reported that an IGF-1-inducible mitochondrial carrier protein for nucleotides (PNC1/SLC25A33) is essential for maintenance of mitochondria and cell growth(1, 2). This suggested an underlying role for IGF-1 signalling in mitochondrial homeostasis. To explore this further we investigated the signalling pathways by which IGF-1 promotes mitochondrial maintenance.

We found that activation of the IGF-1 pathway promotes mitochondrial biogenesis through induction of the transcriptional co-activators PGC-1β and PRC in a wide range of cell lines(3). This response requires both PGC-1β and PRC, and their suppression with siRNA is sufficient to reduce mitochondrial mass, and to disrupt mitochondrial morphology and membrane potential. Moreover, IGF-1 induces expression and mitochondrial accumulation of the mitophagy receptor BNIP3, as well as the Nrf2/NFE2L2 gene, which has been implicated in both mitochondrial biogenesis and anti-oxidant responses. Interestingly, suppression of Nrf2 with siRNA caused reduced induction of BNIP3 in response to IGF-1, suggesting a role for Nrf2 in integrating IGF-1-mediated regulation of mitophagy with survival and metabolic reprogramming in cancer cells.

Overall, we conclude that IGF-1 signalling has an essential function in mitochondria function, stimulating biogenesis and turnover. This mitochondria protective signal is likely to strongly influence responses to therapy and the phenotypic evolution of cancer.

YCRN18-1-16 Ms Jennifer Quinn, Cancer Research at UCC, University College Cork, Ireland

Autophagy is induced in ovarian cancer cells and promotes recovery following treatment with chemotherapeutic agents

Quinn J, O'Donovan TR, McKenna SL. ; Cancer Research at UCC, University College Cork, Ireland

Ovarian cancer (OC) is the seventh most common gynaecological malignancy among women worldwide. In Ireland, ovarian cancer was the fourth most commonly diagnosed cancer among women between 1994 and 20101. A major challenge in the clinical management of OC is the high rate of disease recurrence. Approximately 80% of women whom exhibit an excellent response to first line therapy present with recurrent disease. Autophagy is a highly conserved catabolic process, which enables cells to cope with stressful conditions. Our hypothesis is that ovarian cancer cells upregulate and utilise autophagy as a repair mechanism following treatment with chemotherapeutics. We have demonstrated that ovarian cancer cells elevate autophagy following 24-hour treatment with Paclitaxel, a drug commonly used in the clinical management of OC. Flow cytometry and western blot analysis of the autophagy specific marker LC3II revealed an increase in autophagic vesicle accumulation following treatment with paclitaxel. Analysis of Beclin1 and ATG7, key autophagy genes, by qPCR analysis revealed an increase in their expression by 1.5 and 1.3-fold respectively following 24hr paclitaxel treatment. Immunofluorescence has demonstrated co-localisation of autophagic vesicles and lysosomes following treatment with paclitaxel. Additionally, clonogenic assays revealed an ability of these cells to recover and regrow following treatment. Cell cycle analysis demonstrated the ability of OC cells to retain a 'normal' DNA profile following a 7-day recovery period. Pharmacological inhibition of autophagy with chloroquine significantly reduced the ability of ID8 cancer cells to recover from 5uM Paclitaxel ($p=0.025$). Chloroquine treatment also impaired the ability of OVCAR-5 and OVCAR-8 cells to recover following 5nM paclitaxel treatment ($p=0.04$, $p=0.01$ respectively). This data indicates an important role for autophagy in the ability of OC cells to recover. siRNA knockdown of key autophagy regulators will also be performed to further evaluate the impact of autophagy on recovery. This work aims to identify a new therapeutic target that could be used to combat recurrence in ovarian cancer.

1National Cancer Registry Ireland

SESSION 2 Cancer Cell Signalling and Trafficking

YCRN18-2-21 Mr Máté Nászai, University of Glasgow, Wolfson Wohl Cancer Research Centre, UK

The Ras effector RalA promotes Wnt pathway activation and controls intestinal stem cell dynamics

Mate Naszai[1], Joel Johansson[2], Pascal Peschard[3], Owen J Sansom[1,2] and Julia B Cordero[1,2]

[1] University of Glasgow, Glasgow G61 1B, United Kingdom; [2] Cancer Research UK Beatson Institute, Glasgow 61 1B, United Kingdom; [3] McGill University, Montréal, Canada

BACKGROUND: Wnt signalling is the evolutionarily conserved central orchestrator of intestinal stem cell behaviour in intestinal homeostasis and regeneration. Internalisation of frizzled receptors is crucial for proper activation of canonical Wnt-signalling, and is also relevant to cancer, where hyper activation of the WNT pathway is often a triggering event that drives tumour progression. Ral small GTPases, effectors of Ras signalling, have been indirectly linked to endocytosis, where it is known that the Ral effector protein RalBP1 facilitates both clathrin dependent and independent endocytosis.

HYPOTHESIS: We hypothesise that Ral Small GTPase signalling promotes internalisation of the Wnt signalosome through the actions of RalBP1.

METHODS: We performed knock-down and knock-out experiments in vivo, in Drosophila and mouse to understand the role of Ral proteins in stem cell behaviour and their requirement for the Wnt signalling pathway. In vitro experiments provided further insight into the molecular mechanism of Ral action.

RESULTS: Ral was dispensable for steady-state tissue maintenance, but stem cell autonomously required for tissue regeneration, with reduced Wnt pathway activation observed when Ral levels are reduced. Wnt ligand overexpression induced hyperproliferation was found to be rescued by Ral or RalBP1 knock-down, while reduced Ral had no effect in the context of the loss of the downstream negative regulator of the Wnt pathway Apc. Ral knock-out HEK293T cells were found to have reduced internalisation of the Wnt receptors, Frizzled 5 and 7. Ral dependent reduced Frizzled internalisation correlated with reduced activation of the Wnt pathway.

CONCLUSIONS: Our findings suggest an important link between Ras and Wnt-signalling through regulation of internalisation of Frizzled receptors mediated by Ral GTPases.

YCRN18-2-22 Mr Zhi Liu, System Biology Ireland, University College Dublin, Ireland

Functional analysis of the MAPK scaffold KSR1 in malignant melanoma

Zhi Liu1, Brendan McCann1, Alfonso Blanco2, Walter Kolch1,2, Jens Rauch1

1 Systems Biology Ireland, University College Dublin, Ireland, 2 Conway Institute, University College Dublin, Ireland

The RAS-RAF-MAPK pathway is considered one of the central signalling highways of the cell and, due to a high number of mutations, is directly involved in 30% of all human cancers. Fine tuning and spatial regulation of the pathway output is mediated by a number of scaffold proteins by bringing together MAPK components and meditating crosstalk to other pathways. The scaffold protein Kinase Suppressor of RAS 1 (KSR1) is considered as a major facilitator of oncogenic RAS signalling by binding the RAF, MEK, and ERK. Surprisingly, KSR1 knockout mice, when treated with oncogenic retrovirus expressing RAS, do not develop cancer, suggesting that KSR1-mediated signalling plays a crucial role in tumorigenesis. However, this cancer protective function seems to be regulated through signalling crosstalk and not the canonical MAPK pathway. With the exact functions of KSR1 in cancer remaining enigmatic, we analysed the function of KSR1 in a malignant melanoma model.

After establishing the workflow of CRISPR/Cas9 mediated gene editing in the HEK293 cell model, we successfully generated KSR1 knockout homozygous and heterozygous of SK-MEL-239 melanoma single cell clones. Gene editing was confirmed by sequencing and immunoblotting. In line with the current understanding, we can show that depletion of KSR1 did not impact bulk MAPK signalling or signalling dynamics. However, in melanoma cells lacking KSR1, we observed impaired proliferation and increased cellular senescence. In addition, we investigated KSR1â€™s role in MAPK substrate specificity using a combination of ERK substrate specific immunoprecipitation and label free mass spectrometry. In order to isolate KSR1 signaling complexes under physiological conditions and analyze the dynamic signaling complexes, we are currently establishing CRISPR/Cas9 mediated knock-ins of V5 tag in a number of

melanoma cell lines. We are also going to check the effect of the depletion of KSR1 on the transcription level by performing RNA-seq.

In conclusion, our data suggest that the signaling crosstalk of KSR1 is crucial for RAF and RAS-mediated oncogenesis in malignant melanoma cells.

YCRN18-2-24 Ms Amira Mahdi, University of Limerick, Ireland

Elucidating the role of newly synthesised proteins in the progression of breast cancer

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In Ireland, almost three thousand women are diagnosed with invasive breast cancer every year. This makes it the most common form of malignant tumour found in Irish females and accounts for 17% of all female cancer deaths. As a result, a large effort is being made to improve detection and survival rates for women affected by this disease. In order to achieve this, there is an urgent need to identify novel biomarkers and molecular targets so that we can more accurately and quickly diagnose cancer and understand cancer progression.
Our study aims to investigate exactly how the progression of breast cancer is influenced by specific micro-environmental cues and ascertain the “main players” involved in the growth and migration of tumour cells. We used Click-iT chemistry and Mass Spectrometry analysis to identify proteins that are synthesised by the cancer cells as they are stimulated to migrate towards an epidermal growth factor (EGF) chemoattractant, in both 2D and 3D in vitro models of cancer metastasis. We have identified a list of 97 proteins spanning different functions such as metabolism, intracellular calcium sensing, anti-oxidation and proteins that regulate the cell structure. We hypothesise that these newly synthesised proteins play a vital role in cancer cell migration and metastasis.

We are currently investigating the role of calcium binding proteins which interact with both the ribosomal machinery, cell membrane and the structural skeleton of the cell. We believe these proteins are influencing cell proliferation, migration and invasion in response to EGF. Directing pharmacological interventions towards newly synthesised proteins has the potential to pinpoint invasive cancer cells and provide a highly specific and effective anti-cancer therapy.

SESSION 3 Cancer Biomarker Discovery – translation towards personalised medicine

YCRN18-3-31 Dr Elizabeth Foxall, King's College London/The Francis Crick Institute, London, UK

Rare cancer helper genes disrupt related biological processes in oesophageal adenocarcinoma

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Oesophageal cancer is the eighth most frequent cancer worldwide, with a five-year survival rate below 20%. Oesophageal adenocarcinoma (OAC) is highly heterogeneous with frequent copy number alterations. As a result, recurrence of cancer driver gene alterations is rare, making OAC difficult to treat using therapies that target driver genes. We developed a novel method based on machine learning - sysSVM - that, unlike other recurrence-based methods, uses the properties of known cancer driver genes to identify all genes relevant to cancer in individual patients. We applied sysSVM to 261 OACs from the International Cancer Genome Consortium (ICGC), identifying 908 genes relevant to cancer. While these genes are mostly rare or sample-specific, clustering them based on their associated pathways revealed a convergence towards the perturbation of biological processes such as cell cycle regulation, DNA replication and intracellular signalling. We propose that these genes help to promote cancer through the disruption of key, cancer-related biological processes. By experimentally perturbing five cancer helper genes in OAC cells we validated their role, observing various cancer-promoting phenotypes. sysSVM is therefore a valuable tool for the detection of rare cancer helper genes in heterogeneous and unstable cancers. Our study demonstrates that helpers can provide insight into the biological processes underlying such diseases and allow patient stratification for targeted therapy.

YCRN18-3-33 Ms Aisling Heeran, Trinity College Dublin, Department of Surgery, Ireland

Profiling inflammatory protein secretions from the ex vivo rectal cancer microenvironment compared to normal tissue

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INTRODUCTION: Neoadjuvant-radiotherapy is the standard of care for rectal cancer, however 80% of patients either achieve a partial or no response to neoadjuvant treatment. Upregulation of inflammation is usually associated with a poor response to this treatment, however a comprehensive profile of inflammatory mediators released from rectal cancer tissue and normal tissue has not previously been performed, nor do we know the effect of irradiation on altering inflammatory protein secretions from this microenvironment and if this correlates with clinical parameters.

METHODS: Following patient consent, fresh ex vivo human rectal cancer and normal rectal tissue was cultured and irradiated with either 0Gy or 1.8Gy radiation. Following 24 hours in cell culture, the tissue conditioned media was collected and screened for the expression of 54 inflammatory mediators using MSD-multiplex system.

RESULTS: Several alterations in inflammatory cytokines and immune markers were observed. Preliminary analysis indicated an upregulation of many pro-inflammatory mediators including IL-17A, GM-CSF, MDC, MIP-1Î±, MIP-3Î±, IL-5 and IL-1RA in rectal cancer tissue compared to normal rectal tissue taken from patients that do not have cancer.

CONCLUSION: We have identified inflammatory pathway components that show alterations in secretion patterns between normal tissue and rectal cancer tissue. This may potentially reveal novel therapeutic targets for radio-sensitising agents.

YCRN18-3-34 Ms Natalia Koerich Laureano, German Cancer Research Center (DKFZ) Heidelberg

Integrative multiscale omics analysis identified epigenetic regulation of SOX2 expression by DNA methylation in disseminating HNSCC tumor cells

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Background: Despite advances in interdisciplinary therapy, treatment failure in head and neck squamous cell carcinoma (HNSCC) is a common event and mainly attributed to the plasticity and aggressiveness of invasive cancer cells leading to locoregional relapse or distant metastasis. Recently, we identified the transcription factor SOX2 as a novel key regulator of tumor cell plasticity and tumor cell motility in vitro, and demonstrated that low SOX2 expression serves as a risk factor for unfavorable prognosis of HNSCC patients. However, the molecular mechanisms involved in the regulation of SOX2 expression are not well understood.

Material & Methods: Integrative data analysis of multiscale omics data (TCGA and HIPO/POP) was conducted to unravel complex interactions between copy number variations and DNA methylation on SOX2 transcription in HNSCC. In addition, 2D and 3D-spheroid cell culture models have been established to investigate SOX2-related gene regulatory networks during tumor cell dissemination and to proof the concept, whether restoration of SOX2 expression by inhibitors of DNA methyltransferases (DNMT) exhibit therapeutic activity. Finally, immunohistochemical (IHC) staining of HNSCC samples analyzed SOX2 expression in disseminated tumor cells in the adjacent tumor microenvironment.

Results: Integrative data analysis revealed that DNA methylation at the SOX2 gene locus is a common event in HNSCC and is correlated with low SOX2 expression despite copy number gain. Treatment with the DNMT inhibitor Decitabine (DAC) restored SOX2 expression and inhibited tumor cell migration and invasion. IHC staining of tissue sections demonstrated low SOX2 in disseminated tumor cells and confirmed an inverse correlation between SOX2 expression and the presence of tumor budding in primary HNSCC.

Conclusion: Silencing of SOX2 expression by DNA methylation is a common event in HNSCC. Detection of low SOX2 expressing tumor cells in primary HNSCC might stratify a patient subgroup with a high risk for loco-regional treatment failure, who might benefit from treatment with DNMT inhibitors.

SESSION 4 Cancer Immunology and Immunotherapy

YCRN18-4-41 Ms Maria Davern, Trinity College Dublin, St James's Trinity Translational Medical Institute, Ireland

The effect of clinically relevant chemotherapies on the expression of immune checkpoints in oesophageal adenocarcinoma

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The current treatments for oesophageal adenocarcinoma (OAC) patients include chemotherapy, radiotherapy and surgery. Unfortunately, greater than 70% of patients fail to respond to these treatments and five-year survival rates remain low at <20%.Therefore, there is an urgent unmet clinical need to identify new treatments options for OAC patients. In recent years use of immunotherapeutics in cancer therapy has sparked huge excitement and has been described as a paradigm-shift for the treatment of several cancer-types and life-changing for patients. Targeting immune checkpoints (ICs) using immune checkpoint inhibitors (ICIs) to keep tumour killing T-cells active, has proved among the most beneficial approaches in the clinic. Currently no ICI has been approved for use in OAC patients but clinical trials are ongoing. One of the biggest clinical challenges is how to effectively combine ICIs with current standards of care such as chemotherapy. As focus is now shifting to identify the best scheduling regimens for combination chemotherapy with immunotherapy, no study has focused on the direct effect of individual chemotherapy agents on IC expression in OAC. Therefore, this novel study examines the expression of a range of newly identified ICs in OAC in order to identify potential ICs that could be targeted in OAC patients and importantly determine how clinically relevant chemotherapies affect their expression. Preliminary data demonstrates that combination chemoradiotherapy significantly decreases the expression of PD-1 in OAC patients. Additionally, in vitro experiments show that a range of chemotherapies including 5-fluorouracil, cisplatin and capecitabine, substantially alter the expression levels of a range of ICs and their ligands on the surface of OAC cells as determined by flow cytometry. This research has incremental value in providing key insights into identifying the most appropriate ICIs to combine with specific chemotherapies for the treatment of OAC patients and potentially many other cancer types.

YCRN18-4-43 Mr Liam Tremble, Cancer Research at UCC, University College Cork, Ireland

Development of a preclinical model for the development of macrophage targeting therapies in melanoma.

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Background: Immunotherapy for cancer patients has been widely hailed as the fourth pillar of cancer care along with the traditional treatments of chemotherapy, radiotherapy and surgery. However, the majority of successful therapies rely on activating T cells, either by checkpoint inhibitors or more recently by designer T cells. While the immune system works as a complex network of signals and cells, each playing a critical role, the development of anti-cancer therapies targeting other cell types has lagged behind anti-T cell therapies.

Recent evidence across many cell types has shown that immune-related side effects of anti-CTLA4 and anti-PD-1 checkpoint inhibitors are severe in many patients and that the side effects increase with the dose given, number of cycles received and are cumulative with each other. In a recent study examining Nivolumab plus Ipilimumab in metastatic melanoma, only 39% of patients were able to receive the full dose and 72% of patients required systemic steroids due to irAEs.

Thus, in order to continue to improve patient outcome it is vital we develop new generations of immunotherapies or immunotherapy complimenting therapies which are tolerable. There has been significant research into the viability of macrophage orientated therapies to improve anti-cancer therapies. Macrophages can contribute up to 50% of the tumour mass in melanoma, and under homeostatic conditions macrophages, and their precursors, monocytes, are orchestraters of the immune response and wound repair.

While we have begun to see clinical benefit from macrophage targeting therapies in other cancer types, progress has been slow due to our poor understanding of these cells and heterogeneity of nomenclature and protocols used to prepare and study these cells. A seminal paper by world leaders (Wynn et al. 2014) has improved research efforts but much remains undone.

Using bone marrow-derived monocytes (BMDMs) and genetically matched B16F10 cells we are developing a preclinical model which will allow the translation of novel anti-cancer therapeutics to clinical trials. We have successfully designed an in vitro protocol that allows the development and identification of CD11b+, Ly6C+, Ly6G- inflammatory monocytes, which mirror circulating monocytes isolated from C57BL/6J mice bearing subcutaneous B16F10 tumours.

Current research efforts are being made to define the effect of B16F10 conditioned medium on BMDMs and their role in anti-cancer immunity. Conditioned medium can effectively skew monocyte polarization towards a phenotype similar to alternatively activated macrophages (activated by IL-4 and IL-13), but with greatly reduced arginase activity, while it can inhibit LPS and IFNγ induced iNOS activity.

YCRN18-6-63 Dr Lisa Dr Loughney and **Mairéad Cantwell**, Dublin City University, Ireland

Exercise Training throughout the Cancer Journey: The MedEx Experience

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Acknowledgement: Mairéad Cantwell is funded by the Irish Cancer Society.

Introduction: MedEx is a community-based chronic illness rehabilitation programme based at Dublin City University. MedEx delivers Cancer Prepare (CP), which is a pre-operative exercise training programme for people with newly diagnosed cancer, and Move On (MO), which is an exercise programme for people who have completed adjunctive cancer treatment. These programmes represent a more scalable and sustainable alternative to hospital-based programmes. The results of two studies investigating the feasibility and effectiveness of CP and MO are presented which, to our knowledge, are the first to investigate such community-based exercise programmes for people with cancer in Ireland.

Methods: All participants were referred from local cancer centres in Dublin. All participants completed baseline assessments: lower limb strength (10 repetition sit-to-stand test), cardiorespiratory fitness (6-minute time trial) and HRQoL (EQ-5D or FACT-G questionnaire). In addition, CP participants completed assessments of upper limb strength (handgrip test) and flexibility (sit-and-reach test) whilst MO participants completed 7-day accelerometry and assessments of fatigue (FACIT-F questionnaire). CP participants undertook either a supervised or home-based exercise programme for the time window available and completed re-assessment within 1 week before surgery. MO participants undertook a 12-week programme (60 minutes twice-weekly) and completed re-assessment at the end of week 12.

Results: 37 CP participants (prostate, colorectal cancer) were recruited, of which 81% (n=30) completed the programme. 86 MO participants (incl. breast, colorectal, prostate, lung cancer) were recruited, of which 64% (n=55) completed the programme. Outcome measures for physical fitness variables, HRQoL, fatigue and physical activity are reported in the table below.

Table 1. Pre- and post-exercise intervention results

Outcome	CP (Prostate) Baseline (n=16)	CP (Prostate) Re-assessment (n=16)	p Value	CP (Colorectal) Baseline (n=14)	CP (Colorectal) Re-assessment (n=14)	p Value	MO - Baseline	MO – Re-Assessment	p Value
Cardiorespiratory fitness: 6-min Time Trial (metres)	705 (156)	734 (149)	>0.05	785 (228)	816 (249)	>0.05	555 (87)	659 (132)	<0.01*
Lower body strength: Sit-to-Stand Test (secs)	16.4 (6.4)	14.4 (5.6)	<0.05*	15.5 (5.7)	14.1 (4.4)	>0.05	20 (5)	17 (5)	<0.01*
Health related quality of life	71 (18) % (EQ-5D)	77 (14) % (EQ-5D)	<0.05*	77 (15) % (EQ-5D)	87 (6.7) % (EQ-5D)	>0.05	82 (15) (FACT-G Score)	89 (14) (FACT-G Score)	<0.01*
Upper body strength: Handgrip Test (kg)	33(10)	34 (9)	>0.05	33 (9)	35 (9)	>0.05	-	-	-
Flexibility: Sit-and-reach Test (cm)	4 (13)	6 (10)	>0.05	7 (7)	11 (7)	<0.05*	-	-	-

Fatigue	-	-	-	-	-	-	36 (10) (FACIT-F Score)	40 (11) (FACIT-F Score)	<0.05*
PA - Step Counts	-	-	-	-	-	-	7537 (2797)	8409 (3151)	<0.01*
PA - Light Intensity (Hours)	-	-	-	-	-	-	1.2 (.35)	1.3 (.34)	<0.05*

Statistical significance is denoted by * and was taken as p<0.05 following paired sample t-tests. Abbreviations: CP (Cancer Prepare programme); MO (Move On programme); PA (physical activity) Note: where there are no data outcome measures were not tested. EQ-5D (overall health status, scale 0 – 100, higher is better); FACT (quality of life, scale 0-108, the higher the score, the greater the HRQoL); FACIT-F (fatigue scale, 0-52, the higher the score, the greater the HRQoL)

Conclusion:. Community-based exercise training programmes are feasible pre-surgery and post-cancer treatment. Exercise training has beneficial effects on physical and psychological well-being as well as fatigue levels throughout the cancer journey.

SESSION 5 Novel Drugs, Drug Delivery and Synthetic Biology

YCRN18-5-51 Mr James Mc Keown, School of Pharmacy & Pharmaceutical Sciences, Trinity College Dublin, Ireland

Discovery of therapies for lymphomas and leukaemia: Synthesis and antiproliferative action of novel ethanoanthracenes

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CLL (Chronic Lymphocytic Leukaemia) is the most common leukaemia in developed countries, primarily affecting the elderly. CLL is classed as a clonal disorder of mature B-lymphocytes with its clinical patient prognoses being affected mainly by the mutational status of the Immunoglobulin G Heavy Chain Variable region (IGHV) (with mutated IGHV holding a better patient prognosis than the wild type variant) [1].

Structures related to tricyclic and tetracyclic anti-depressants (fluoxetine and maprotiline respectively) have been previously shown to express potent, selective antiproliferative and pro-apoptotic effects in-vitro and were met with marked success in related B cell malignancy cell lines, namely Burkitt's Lymphoma (BL) cell lines DG-75 and MUTU-1 [2].Based on these preliminary studies, libraries of structurally related compounds were designed, based on the proven effectiveness of nitrostyrene core moiety derivatives and evidence of effectiveness of chalcone moieties in leukaemic cell lines [3].

These compounds were synthesised using Henry-Knoevenagel condensation, Claisen-Schmidt condensation and Diels Alder cycloaddition reactions, with each compound subsequently characterised by 1H NMR,13C NMR, IR spectroscopy and high resolution mass spectrometry (HRMS).

The antiproliferative activity of each compound was determined using the Alamar Blue assay on the two types of CLL cell lines: HG-3 and PGA-1, representative of bad and good prognosis respectively. The antiproliferative effects and structure-activity compound relationships, together with preliminary effects on the induction of apoptosis will be discussed, leading to the identification of potent proapoptotic compounds for future evaluation.

YCRN18-5-52 Ms Amy Buckley, Trinity College Dublin, Department Of Surgery, Ireland

The action of a novel radiosensitiser within the Oesophageal Adenocarcinoma tumour microenvironment

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Background: Oesophageal Cancer (OAC) is an aggressive disease with survival rates of ~15-20%. Current therapeutic regimes focus on neo-adjuvant chemo-radiation prior to surgery. Unfortunately, only 20-30% of patients show a beneficial response. This major clinical challenge of treatment resistance reinforces the need for the identification of novel radio-sensitisers.

Methods: Through a drug screening approach in-vivo, we have identified a novel anti-angiogenic and anti-metabolic compound, 11B_CC8 with radiosensitising activity. The ability of 11B_CC8 to act as an anti-metabolic in-vitro and ex-vivo under normoxia and hypoxia was assessed using Seahorse technology and the Don Whitley i2 workstation. The effect of 11B_CC8 on radiosensitivity our isogenic OAC cells was assessed by clonogenic assay. The effect of 11B_CC8 on inflammatory protein secretions from OAC treatment-naÃ¯ve biopsies was evaluated by multiplex ELISA. The elucidation of a mechanism of action of 11B_CC8 was evaluated by Mass-Spectrometry.

Results: 11B_CC8 can enhance radiosensitivity in our isogenic model of OAC under both normoxic and hypoxic (0.5% O2) conditions. 11B_CC8 significantly reduces oxygen consumption rate (OCR) under normoxic but not hypoxic conditions. Ex-vivo, 11B_CC8 significantly reduced IL1Î² secretion (p=0.0117). 11B_CC8 does not affect dendritic cell maturation when assessed by flow cytometry. Real-time ex-vivo metabolic rate analysis of our treatment naÃ¯ve OAC biopsies showed significantly elevated OCR, when compared to a measure of glycolysis (p=0.0059) where treatment with 11B_CC8 produced a reduction in OCR (p=0.0039).

Conclusion: Our novel anti-angiogenic and anti-metabolic agent can enhance radiosensitivity in-vitro under both normoxic and hypoxic conditions. Ex-vivo, 11B_CC8 can significantly reduce the secretion of IL1Î² and altered metabolic programming, specifically oxidative phosphorylation in human explants.

YCRN18-5-53 Ms Lauren Gutgesell, University of Illinois at Chicago, USA

Estrogen receptor agonists, antagonists, and beyond for characterizing and overcoming resistance in ER+ breast cancer alone and in combination therapy

Lauren Gutgesell (1), Rui Xiong (1), Jiong Zhao (1), Huiping Zhao (2), Debra A Tonetti (2), Gregory RJ Thatcher (1)

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Endocrine therapy is the standard of care for breast cancer expressing estrogen receptor (ER), which occurs in 70% of patients. Unfortunately, acquired or de novo resistance to endocrine therapy is observed in up to 50% of patients, leaving a significant portion of patients with insufficient treatment options. A vast majority of these tumors remain ER+ and ultimately remain responsive to other classes of endocrine therapy. Our group has developed a library of ER ligands that do not adhere to the canonical binary agonist/antagonist model of ER-ligand interactions. Our library includes novel orally-bioavailable SERDs, selective estrogen mimics (SEMs), selective human ER partial agonists (ShERPAs), and compounds that do not fit into a category. Importantly, the compounds in this library vary only slightly in structure and are based on the scaffold of raloxifene, a selective ER modulator (SERM), known for its safety profile and similar efficacy to the standard SERM, tamoxifen. These ligands stabilize a variety of ER conformations, thereby resulting in activity not previously categorized. To test these ligands in monotherapy and combination therapy, it was imperative to model multiple resistance mechanisms in vitro. We have developed 5 stable, endocrine-resistant cell lines from a parent MCF-7 cell line, which all retain ER. One of these cell lines, MCF-7:CFR is resistant to both the SERD, fulvestrant, and tamoxifen but showed sensitivity to the ShERPA, TTC-352, developed in our laboratory. This antiproliferative activity is amplified in combination with non-endocrine targeted therapies, such as the PI3K inhibitor, alpelisib. Paradoxically, all endocrine-resistant cell lines responded to at least one of the endocrine therapies tested, demonstrating that if ER is not lost in the metastatic state, it remains a vulnerability suitable for therapeutic targeting.

Poster Abstracts

YCRN18-1-10 Mr Felipe Rodrigues University of Sao Paulo / CRUK Beatson Institute

Exploring IKKbeta kinase as a therapeutic target for KRAS-driven lung tumour-initiating cells

Felipe S. Rodrigues (1,2); Tatiana C. Carneiro-Lobo (1); Luiza C. Scalabrini (1); Daniel J. Murphy (2,3); Daniela S. Bassères (1)

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INTRODUCTION: Lung cancer induced by oncogenic mutations of the KRAS GTPase is a very frequent disease and RAS downstream effectors are directly involved in the acquisition of important malignant properties, such as the cancer stem-like phenotype. Since directly targeting of oncogenic RAS has previously failed, it is necessary to identify the downstream pathways activated by KRAS so that better therapeutic targets can become available.

OBJECTIVES: We have previously shown that IKKbeta kinase plays an important role in KRAS-induced lung tumorigenesis, and here our goal was to explore whether IKKbeta could be therapeutically explored in the context of KRAS-driven lung tumor-initiating cells (TICs), which are, in turn, self-renewing tumor cells thought to be responsible for tumor recurrence and metastatic spread.

MATERIAL AND METHODS: We targeted IKKbeta kinase in KRAS-positive lung cancer A549 and H358 cells by RNA interference (RNAi) or with a highly specific IKKbeta inhibitor (Compound A) and analysed expression of stem cell surface markers by flow cytometry, expression of other stem cell factors by qPCR, tumorsphere formation and self-renewal and clonogenic growth of tumorsphere-forming cells.

RESULTS AND DISCUSSION: When compared to parental cells grown in adherent cultures, tumorsphere cells displayed increased expression of stem cell factors, a hallmark of TICs. In addition, when compared to parental cells in adherent cultures, A549 and H358 tumorsphere-derived cells displayed increased activity of IKKbeta kinase, increased clonogenic growth, and are more sensitive to Compound A treatment. Targeting IKKbeta kinase by RNAi or with Compound A decreased the expression of stem cell markers, reduced primary and secondary tumorsphere formation and decreased clonogenic growth of tumorsphere-forming cells.

CONCLUSIONS: Our results suggest that IKKbeta kinase inhibition therapy can reduce KRAS-positive lung TIC function and is, therefore, a promising therapeutic target for KRAS-induced lung cancer.

YCRN18-1-11 Dr Adam Sharp Institute of Cancer Research and The Royal Marsden Hospital, Sutton, London, UK

Targeting the bromodomain and extra-terminal (BET) family proteins in metastatic castration resistant prostate cancer (mCRPC): Overcoming aberrant androgen receptor (AR) signaling.

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Hypotheses: The constitutively active androgen receptor splice variant-7 (AR-V7) drives persistent AR signalling and resistance to AR targeting therapies in mCRPC. BET family proteins are critical regulators of AR-V7 generation and provide a novel strategy to overcome aberrant AR signalling in mCRPC.

Methods: RNA sequencing (RNAseq) and immunohistochemistry (IHC) were used to determine the relationship between BET family proteins and AR signalling, and the clinical significance of BRD4 expression in mCRPC. Next, we investigated the effect and mechanism of chemical BET inhibition (BETi) and BET family protein knock down on AR-V7 generation and AR signalling in mCRPC models. Following which we developed patient derived models from resistant to AR targeting therapies with known AR aberrations and compared the effect of BETi and AR targeting therapy.

Results: We demonstrate that BRD4 protein expression increases significantly (p=<0.01) from castration sensitive prostate cancer (median H-score; interquartile range: 100; 100-170) to CRPC (150; 110-200), and higher BRD4 expression at diagnosis associates with worse outcome (HR 3.25, 95% CI 1.50-7.01; p=<0.001). Our data shows that BRD2, BRD3 and BRD4 RNA expression correlates with AR driven transcription (all p=<0.001) in CRPC biopsies demonstrating a role of BET family proteins in regulating AR activity. Consistent with this, chemical BETi, and BET

family protein knockdown, reduced AR-V7 expression and AR signalling in prostate cancer models. In addition, we describe a novel role of BETi in regulating RNA processing thereby reducing alternative splicing and AR-V7 expression. Furthermore, BETi reduce growth of prostate cancer cells and patient derived organoids with known AR mutations, AR amplification and AR-V7 expression. Finally, BETi, unlike enzalutamide, decreases persistent AR signalling and growth ($p=<0.001$) of a patient derived xenograft with AR amplification and AR-V7 expression.

Conclusion: BETi merit clinical evaluation as inhibitors of AR splicing and persistent AR signalling in treatment resistant mCRPC.

YCRN18-1-12 **Ms Stephanie Mc Kenna**, University College Dublin

Development of an In-silico Model of Intrinsic Apoptosis

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Apoptosis is a major form of programmed cell death which is essential in development and homeostasis. Caspases are the key drivers of intrinsic and extrinsic apoptotic cell death. Tight regulation of these cleaving proteins which are integral to the disassembly of the dying cell is important for normal physiological function. Caspases are classified as initiator (-2,-8,-9,-10) or effector (-3,-6,-7) caspases based on where they lie within the apoptotic cascade. Upon cleavage by initiator caspases, effector caspases act directly on cell dismantling substrates. Dysregulation of caspase activity can lead to aberrant cell death and severe pathological alterations leading to autoimmunity, neurodegeneration and cancer. Although we have an understanding of the signalling pathways involved in apoptosis, we lack an in-depth, mechanistic characterisation of the underlying network. Here we use a systems biology approach in order to gain an insight into the underlying intrinsic apoptotic network. We have generated an in-silico model of caspase activation within the intrinsic apoptotic cascade built by combining experimental data and previous literature findings. To further refine our model, we have calibrated it with clinical data from metastatic melanoma patients. Results to date demonstrate bistability of the apoptotic switch and how perturbations to the initiation of apoptosis can affect the bistability of the system. Moreover, our model indicates the important role of caspase deregulation in subgroups of melanoma patients which could lead to the development of personalise treatments for these patients.

YCRN18-1-13 **Ms Viktorija Juric**; Royal College of Surgeons in Ireland

Validation of the APOPTO-CELL modelling environment as a superior predictor of treatment responsiveness in newly diagnosed and recurrent GBM patients

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Glioblastoma (GBM) is the most common and aggressive primary brain tumour in adults. Median survival for newly diagnosed patients is 15 months and currently no cure exists. A major contributor to the poor survival rates of GBM patients is the extreme resistance to death-inducing treatment stimuli, e.g. temozolomide (TMZ) which GBM cells display. Such resistance to TMZ therapy greatly limits chemotherapeutic effectiveness in GBM as TMZ is the front-line therapy used for GBM patients. We are particularly interested in the ability of GBMs to resist the programmed cell death pathways of apoptosis that TMZ attempts to induce. Our group has published that a systems modelling approach (APOPTO-CELL), examining the expression of key players within the intrinsic mitochondrial apoptotic pathways can be exploited to predict patient response to TMZ. The aim of this project is to enhance the clinical relevance of our novel systems modelling approach by quantitatively analysing a large cohort of GBM patient samples for the expression of apoptosis regulators. By integrating this analysis with our systems model, we aim to examine the competency of the patient samples to execute apoptosis and to correlate such responses with patient progression free survival times. We further aim to analyse the ability of the cyclin dependent kinase inhibitors to enhance treatment responses to TMZ in both primary and recurrent patient-derived GBM cell lines and investigate how our system model can predict such responses.

YCRN18-1-14 Ms Marie Claire Fitzgerald, Royal College of Surgeons in Ireland

Improving and Personalising Chemotherapy Treatment Options for Paediatric Brain Tumour Patients

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Cancer is the most common cause of childhood death, and brain tumours are the most commonly occurring cancer. Of these, medulloblastoma (MB) is most prevalent, comprising 20% of all childhood brain tumours. Aggressive multimodal treatment consisting of surgery, chemotherapy and craniospinal radiation therapy (RT) is associated with an overall survival rate of 70-80%, though the side effects associated with aggressive treatment and subsequent radiation-induced brain injury are considerable, with survivors experiencing developmental, neurological, neuroendocrine and psychosocial deficits. Children < 3yrs experience greatest risk for development of long-term sequelae.

This highlights that the current 'one size fits all' approach to treatment is not valid, and a more personalised approach is needed to select the most appropriate drug for each patient to (1) reduce/eliminate the need for RT and (2) reduce side effects associated with inefficient therapy. Mathematical modelling holds considerable potential to personalise and optimise treatment strategies, and provide new patient stratification tools. Our group has previously demonstrated how an innovative systems-based strategy (SYS-ACT) can be exploited to predict the response of adult brain tumour cell lines to existing drugs and novel drug combinations. The aim of this project is to explore the applicability of SYS-ACT in the paediatric setting.

We now highlight the biological heterogeneity of a panel of medulloblastoma cell lines with respect to their expression of apoptosis regulators. Additionally, we will utilise these results as input to our model which is able to correlate the unique apoptotic signature of each cell lines with its subsequent therapeutic response. In the long term, this work has the potential to create a clinical decision-making tool to facilitate the de-escalation of therapy and elimination/reduction of RT in specific patient cohorts, thus reducing the long-term sequelae.

YCRN18-1-15 Dr Dalyia Benjamin, University College Cork

VALPROIC ACID AND ATRA CAN PROMOTE DIFFERENTIATION IN MYELOID LEUKAEMIA CELLS BY A MECHANISM DEPENDENT ON THE AUTOPHAGY REGULATOR TFEB

D Benjamin[1,2], TR O'Donovan[1], MR Cahill[1,2], NP Mongan[3], LI Gudas[3], SL McKenna[1]

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Acute myeloid leukemia" (AML) -a heterogenous group of clonal disorders of hematopoietic progenitor cells is characterized by aggressive proliferation and defective differentiation. Differentiation therapy has been achieved with the use of all-trans-retinoic acid (ATRA) for the treatment of one type of AML -acute promyelocytic leukemia (APL)1. This has not been replicated in other forms of AML.

Autophagy is a conserved protein degradation pathway that is induced in response to cellular stress or nutrient deprivation. Previously research from our group has shown that autophagy is required during ATRA induced differentiation of APL cells (1). We hypothesize that pharmacological induction of autophagy may promote ATRA mediated differentiation in cells that are normally unresponsive to differentiation therapy.

We assessed differentiation and autophagy in AML cell lines that are resistant to ATRA (NB4R, THP-1 and HL60 Diff-R). Differentiation was assessed by RTPCR analysis of differentiation markers (CD11B, GCSFR, CEBPe, ID2) and FACS analysis of CD11b. Autophagy was assessed by RT PCR analysis of known autophagy markers ATG16, GABARAP, TFEB and CTSD and western blot analysis of LC3II. Cells were treated with clinically relevant compounds that have been previously reported to induce autophagy: Valproic acid(VPA), Lithium, Arsenic Trioxide, m TOR inhibitors (Rapamycin, AZD8055) and PI3K/AKT/MTOR inhibitor(PI103) in combination with ATRA. Valproic acid and ATRA was the leading combination inducing differentiation in all resistant AML cell lines. In NB4R cells, the Mean fluorescent intensities(MFI) of CD11b was 1929 for ATRA alone, 5324 for VPA alone and 10994 for the combination treatment ($P<0.05$ comparing single and combination treatments (one-way Anova)). In NB4R and HL60DiffR cells, the combination treatment also increased differentiation by more than 50% ($p<0.05$ comparing single and combination treatments (one-way Anova).

TFEB transcriptionally regulates the expression of many autophagy and lysosomal genes. In order to assess whether autophagy is required for the differentiation induced by VPA and ATRA, we knockdown-TFEB, with shRNA in NB4 cells.

Cells with depleted TFEB were significantly impaired in their ability to differentiate, as measured by FACS and Real Time PCR analysis of myeloid differentiation markers CD11b and GCSFR, which were both reduced by more than 50%. These DATA have identified a combination treatment that can promote myeloid differentiation, by a mechanism dependent on autophagy regulator TFEB.

YCRN18-1-16 Ms Jennifer Quinn, Cancer Research at UCC, University College Cork, Ireland

Autophagy is induced in ovarian cancer cells and promotes recovery following treatment with chemotherapeutic agents

Quinn J, O'Donovan TR, McKenna SL. ; Cancer Research at UCC, University College Cork, Ireland

Ovarian cancer (OC) is the seventh most common gynaecological malignancy among women worldwide. In Ireland, ovarian cancer was the fourth most commonly diagnosed cancer among women between 1994 and 20101. A major challenge in the clinical management of OC is the high rate of disease recurrence. Approximately 80% of women whom exhibit an excellent response to first line therapy present with recurrent disease. Autophagy is a highly conserved catabolic process, which enables cells to cope with stressful conditions. Our hypothesis is that ovarian cancer cells upregulate and utilise autophagy as a repair mechanism following treatment with chemotherapeutics. We have demonstrated that ovarian cancer cells elevate autophagy following 24-hour treatment with Paclitaxel, a drug commonly used in the clinical management of OC. Flow cytometry and western blot analysis of the autophagy specific marker LC3II revealed an increase in autophagic vesicle accumulation following treatment with paclitaxel. Analysis of Beclin1 and ATG7, key autophagy genes, by qPCR analysis revealed an increase in their expression by 1.5 and 1.3-fold respectively following 24hr paclitaxel treatment. Immunofluorescence has demonstrated co-localisation of autophagic vesicles and lysosomes following treatment with paclitaxel. Additionally, clonogenic assays revealed an ability of these cells to recover and regrow following treatment. Cell cycle analysis demonstrated the ability of OC cells to retain a 'normal' DNA profile following a 7-day recovery period. Pharmacological inhibition of autophagy with chloroquine significantly reduced the ability of ID8 cancer cells to recover from 5uM Paclitaxel ($p=0.025$). Chloroquine treatment also impaired the ability of OVCAR-5 and OVCAR-8 cells to recover following 5nM paclitaxel treatment ($p=0.04$, $p=0.01$ respectively). This data indicates an important role for autophagy in the ability of OC cells to recover. siRNA knockdown of key autophagy regulators will also be performed to further evaluate the impact of autophagy on recovery. This work aims to identify a new therapeutic target that could be used to combat recurrence in ovarian cancer.

1National Cancer Registry Ireland

YCRN18-2-11 Ms Leanne O'Sullivan, Cork University Hospital, Cork, Ireland

Assessment of activation status in platelets of Multiple Myeloma and MGUS patients and study of paraprotein influence on this process.

Leanne O'Sullivan (School of Biochemistry & Cell Biology, University College Cork)
Dr. Gerardene Meade-Murphy (Department of Pharmacology & Therapeutics, University College Cork)
Prof. Mary Cahill (Haematology Department, Cork University Hospital)
Dr. Paul Young (School of Biochemistry & Cell Biology, University College Cork)

Thrombotic events are a major cause of death and morbidity in haematological cancer patients, being reported in up to 10% of patients with Multiple Myeloma (MM) and the premalignant disorder, monoclonal gammopathy of undetermined significance (MGUS). Platelet dysfunction, arising from the disease state or associated treatment regimes, is a key modulation of thrombotic risk reported in these patients which is incompletely characterised. Platelet hyperactivity is assessed in this study by determining platelet activation status in MM, MGUS and healthy controls.

Fibrinogen receptor activation (PAC-1) and platelet activation (CD62P, CD63) were assessed by flow cytometry at baseline and following treatment. In addition, changes in platelet activation markers in response to ADP were assessed by flow cytometry. Platelet-Leucocyte Aggregates (CD61/CD45), an emerging marker of platelet activation and thrombotic risk, were also assessed for the first time in MM patients. Identification of activated pathways allowed further study of pathway components and assessment of paraprotein influence using a novel fluid shear assay simulating haemodynamic flow on thrombogenic surfaces,

Although platelet dysregulation in terms of aggregation, adhesion, production and lifespan have been described in MM, the involvement of protein activation in this dysregulation remains inconclusive. This study of platelet activation addresses this deficit in our knowledge. Identifying activated pathways in these patients will also allow further study of the role of key regulators in these pathways, including cytoskeletal proteins, and may highlight alternative therapeutic targets in the prevention of myeloma associated thrombosis.

YCRN18-2-23 Ms Natasha Malik, Paul O'Gorman Leukaemia Research Centre, University of Glasgow

ROLE OF mTORC1 IN NORMAL HAEMOPOIESIS AND LEUKAEMOGENESIS

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Mammalian target of rapamycin (mTOR) is a protein kinase that mediates phosphoinositide-3-kinase (PI3K)/Akt signalling. This pathway is involved in a plethora of cellular functions including protein and lipid synthesis, cell migration, and apoptosis. Our laboratory has shown that mTOR substrates are activated in samples isolated from chronic lymphocytic leukaemia (CLL) patients, and in our kinase dead PKCα mouse model (PKCαKR) which exhibits a CLL-like disease.

We proposed to delineate the role of mTOR complex 1 (mTORC1) using conditional knock out (cKO) mouse models to determine its role in normal haemopoiesis and to determine whether it plays a role in CLL initiation and/or development.

We exploit the cre-loxP system in murine models where the gene of interest (raptor) is excised either, in a time-controlled manner (Mx1) or at the haemopoietic stem cell stage (HSC, Vav) to assess molecular events through q-PCR, western blotting and cell viability assays. We also retrovirally (RV) transduce BM cells with either a PKCα-KR (CLL-like) or MIEV construct for transplantation into host mice.

Upon cKO of raptor (mTORC1; Mx1-cre+raptorfl/fl) in adult mice, we observe splenomegaly, a disruption in normal haemopoiesis, along with a significant reduction in the B cell lineage in the BM. A block in B cell lineage commitment was indicated by the significant increase in Lin-Sca-1+CD117+ (LSKs) in the spleen, and a decrease in proB cells in the BM.

While assessing the role of mTORC1 during early developmental stages in vav-cre+raptorfl/fl mice, we established that the embryos do not survive due to a disruption in erythropoiesis. A significant decrease in Ter119+ cells was noted in E15 foetal livers (FL) in KO mice, with aberrations at megakaryocyte-erythrocyte progenitor (MEP) stage. Thus, we analysed the ability of mTOR inhibitors to block the differentiation of K562 cells towards red blood cells (RBCs) in galactose-rich cultures in vitro. Treatment of K562 cells in galactose-rich cultures with either mTORC1 inhibitor rapamycin or the pan-mTOR inhibitor AZD8055 significantly reduces key molecular events of RBC differentiation: generation of CD71+GlyA+ cells; expression of HBB (β-globin) and GATA2, thereby establishing the importance of mTORC1 signalling/function in the promotion of RBC development.

To address the role of raptor in CLL initiation/maintenance in vitro, we RV-transduced Mx1-cre- or Mx1-cre+raptorfl/fl BM (post cKO) with a PKCα-KR or MIEV construct. RV-transduction from raptor cKO mice with PKCα-KR failed to rescue the B cell lineage block or generate a CLL-like disease. To test mTORC1 requirement for maintenance of disease in vivo, Mx1-cre- or Mx1-cre+raptorfl/fl BM cells were RV-transduced with PKCα-KR-and transplanted into host mice. Once disease was established, as detected by blood sampling and analysis of GFP+CD19+CD5+ cells, mice were inoculated with polyI:C to induce cKO. Our results indicate that cKO of raptor with established CLL-like disease results in increase in survival time and decrease in disease load.

Taken together, our data supports the work of others demonstrating a block in B cell lineage commitment. Elevation in the MEP population observed in the vav-cre+-raptorfl/fl mice leads to neonatal lethality due to a block in erythropoiesis. Importantly, our results suggest a critical role for mTORC1 in CLL initiation and maintenance in vivo. These studies will pave the way towards testing the efficacy of clinically-relevant mTOR inhibitors in blocking/reversing CLL in pre-clinical mouse models.

YCRN18-2-25 Ms Joanne Nolan, Graduate Entry Medical School, University of Limerick

External substrates alter gene expression in breast cancer

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Metastasis is the number one cause of death in breast cancer patients and remains a scientific enigma, with no early detection methods or targeted therapies available. Therefore, understanding how and why cancer cells migrate is of significant biological and clinical interest. Accumulating evidence suggests the extracellular matrix (ECM) influences the spread of breast cancer. The ECM is the non-cellular component surrounding the tumour composed of fibrous proteins

and proteoglycans, providing an essential physical framework and facilitating biochemical cues to influence tumour structure and behaviour.

We are interested in examining genes that code for proteins involved in regulating the composition of the ECM. The set of genes we are interested in code for proteins involved in signal transduction from the cell membrane or code for proteins secreted into the ECM. These include fibrous proteins, glycoproteins, transmembrane proteins, growth factors and proteases. We refined our gene panel using STRING1, an online database, which allowed us to examine the relationships between the proteins coded by our genes based on experimental determination of protein co-expression and protein homology.

Using qRT-PCR, we examined the expression of our gene panel in three cell lines, (representing major sub-categories of breast cancer). Cells were cultured on collagen, fibronectin, laminin and stimulated with various growth factors. We extended our study to examine the expression pattern in cells maintained in 3-Dimensional culture over a period of 10 days to more closely mimic the physiological process. Our results indicate that there is a change in the expression of most of the genes in response to specific factors, with a strong shift towards differential expression of the genes regulating the epithelial to mesenchymal transition.

We are refining the signature set by identifying those genes whose expression is dysregulated in response to environmental changes. We then aim to determine whether dysregulation of our gene panel correlates with disease progression by examining the expression in matched normal and diseased patient tissue. This could provide the opportunity to generate a novel gene signature that may be used as a predictive prognostic tool of invasive cancers and may provide information on design of novel treatment therapies for patients with metastatic cancers.

YCRN18-3-10 Ms Paula Azorin Pardo, Institut Curie Paris, France

KINDLINS IN BREAST CANCER: DIFFERENTIAL FUNCTIONS, EXPRESSION PROFILES AND PROGNOSTIC VALUES

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Kindlin-1, -2, and -3 are members of the Kindlin family of focal adhesion proteins best known as regulators of integrin functions. They bind to the cytoplasmic tail of β-integrins contributing to fundamental biological processes including cell survival, cell adhesion, migration and differentiation. Deregulations of these proteins have been associated with diverse pathologies including a broad range of cancers.

There is not yet a clear picture of the role of the three Kindlins in breast cancer; whether they have redundant and/or complementary roles in mammary tumors remains largely unknown. To better characterize the role of Kindlins in breast cancer we have performed in vitro experiments that put in evidence that only Kindlin-1 and Kindlin-2 are expressed by breast cancer cells, participating in cell motility. However, only Kindlin-1 drives cell invasion.

Furthermore, the integrated expression study of the three Kindlin members in a large series of human breast tumors, both at RNA and protein levels, demonstrates that each Kindlin has a different expression profile. In line with our in vitro experiments, only Kindlin-1, but not Kindlin-2, is highly expressed in subsets of aggressive breast tumors and stands out as a potential biomarker of bad prognosis. Surprisingly, although Kindlin-3 is only expressed by immune cells infiltrating the tumors, higher levels of Kindlin-3 expression are associated with a worse patients' outcome.

These results suggest that both Kindlin-1 and Kindlin-3 might be associated with breast cancer progression. The up-regulation of Kindlin-1 in breast tumors is specifically associated with a higher propensity to develop lung metastases. However, whether Kindlin-3 expression may contribute to tumor progression via a potential role in a dysfunctional immune response is worthy of further investigations.

YCRN18-3-11 Ms Nicola Cosgrove, Royal College of Surgeons in Ireland

Differential Co-expression Network Analysis in Metastatic Breast Cancer

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Breast cancer is a heterogeneous disease affecting 1 in 9 Irish women with 2800 new cases diagnosed every year with approximately 675 of those progressing to metastatic disease. Metastatic breast cancer (MBC) is difficult to treat and is the main cause of cancer-related death in patients. In MBC, it has been observed that a primary breast tumour will preferentially metastasise to certain distant organs first, a phenomenon called "metastatic organ tropism". An intrinsic molecular subtype association with certain distant organ sites has also been observed. Transcriptomics has been critical for our understanding of tumour biology and for advancing the classification, management and diagnosis of breast cancer. However, the molecular mechanisms underpinning MBC and metastatic organ tropism still remain poorly understood. There is an urgent need for accelerated innovation in the discovery and development of effective biomarkers and molecular techniques for the diagnosis and treatment of MBC. A differential network-based approach for investigating the complexity of expression, regulatory and functional networks implicated in MBC using transcriptomic data has been underexplored and in particular for metastatic organ tropism. Although both single markers and multi-gene expression signatures associated with disease recurrence have been identified using microarrays based on primary breast tumour, added value may be gained from the re-analysis of several such transcriptomic datasets with the inclusion of metastatic tumour tissue using a network-based approach. A differential co-expression network analysis of publicly available non-malignant breast, good and bad prognosis primary breast tumours and distant metastatic tumour tissue gene expression profiled on microarray was used to find rewired gene networks in breast metastatic disease. Molecular subtype specific co-expression networks were also identified.

YCRN18-3-12 **Ms Karen Crowley**, Royal College of Surgeons in Ireland

Epcam, CD49f and CD24 identify functionally-relevant populations in endocrine resistant breast cancer

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Background: Breast cancer is a complex and heterogeneous disease. Gene expression profiling has provided a remarkable insight into the heterogeneity of breast cancer and has characterized at least four intrinsic subtypes: luminal A, luminal B, basal-like and HER2-enriched. Despite advances, heterogeneity within these subtypes remains poorly understood. Endocrine treatment is the gold standard for luminal breast cancer. However, almost 30% of patients develop endocrine resistance with metastatic progression. This underscores the need to better characterize intra-tumour heterogeneity to tailor treatment options.

Methods: EpCAM, CD49f, CD24, CD44 and HER2 were used to define tumour heterogeneity in breast cancer by flow cytometry.

Results: EpCAM, CD49f, CD24, CD44 and HER2 identified inter-tumour heterogeneity across luminal, basal-like and HER2-enriched subtypes. EpCAM, CD49f and CD24 together identified heterogeneity within luminal breast cancer. Endocrine sensitive breast cancer was most enriched in mature luminal (EpCAM+CD49f-) cells and endocrine resistant breast cancer in luminal progenitor (EpCAM+CD49f+CD24+) cells. Luminal progenitor cells exhibited traits associated with a more aggressive and metastatic biology in endocrine resistant breast cancer. Luminal progenitor cells were less differentiated with a higher stem activity, were more migratory and had an increased anchorage independent growth compared to mature luminal cells in endocrine resistance.

Conclusion: EpCAM, CD49f and CD24 identified heterogeneity across breast cancer subtypes and within luminal breast cancer. These markers identified functionally-relevant populations in endocrine resistant breast cancer which differ in their metastatic potential in vitro. Future work will involve transcriptional profiling and lineage tracing to elucidate the metastatic properties of these populations in vivo. Ultimately, an increased understanding of heterogeneity in luminal breast cancer will help stratify patients at an increased risk of disease progression and redefine current treatment options.

YCRN18-3-13 **Ms Croí Buckley**, Trinity Translational Medicine Institute (TTMI), Dublin, Ireland

MERiT: Targeting MEtabolism for individualised Rectal Cancer Treatment

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Introduction: The resistance of rectal cancer to neoadjuvant chemoradiation therapy (nCRT) is a significant clinical problem. Currently, there are no routinely used clinical biomarkers predicting patient response to nCRT. Emerging evidence supports an association between altered tumour metabolism and radioresistance in a variety of cancer types,

including gastrointestinal cancers. However, the role of altered metabolism in the radioresistance of rectal cancer is largely unknown. This project aims to investigate the potential role of altered tumour metabolism in radioresistant rectal cancer.

Methods: Radiosensitivity was assessed by the gold-standard clonogenic assay. Basal metabolism was assessed using the Seahorse live-cell metabolic assay. Metabolic rates were normalised to cell number using a crystal violet assay.

Results: To identify in vitro model of inherent radioresistant colorectal cancer, the basal radiosensitivity of a panel of colorectal cancer cell-lines was investigated. SW837, a rectal cancer cell-line was demonstrated to be significantly more radioresistant than the radiosensitive HCT116 colon cell-line at 2 Gy, 4 Gy and 6 Gy X-ray irradiation. The basal metabolic profile of the panel of colorectal cell-lines was characterised, with SW837, SW1463 and HCT116 each utilizing both glycolysis and oxidative phosphorylation, but with a trend towards increased glycolytic metabolism in the radiosensitive HCT116 cell-line.

Conclusions and Future Work: We have identified an in vitro model of inherent radioresistant colorectal cancer, and assessed the basal metabolic phenotype. We are currently investigating mitochondrial function and the potential radiosensitizing and anti-metabolic effect of a novel drug, CC8, in this colorectal cancer cell-line panel. We are also collecting pre-treatment tumour and blood samples from consenting rectal cancer patients for future metabolomic and transcriptomic analysis.

YCRN18-3-32 Ms Julia Samson, University College Cork, Ireland

Investigating the association between long non-coding RNAs and ductal carcinoma in situ (DCIS)

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Breast cancer is the most common cancer in women worldwide with incidence rates increasing and survival rates varying widely depending on early detection and access to treatment. In Ireland, the number of diagnoses is increasing with 89% of new cases being invasive breast cancer. To reduce the number of individuals with invasive cancer, there is an urgent need for specific and sensitive diagnostic biomarkers for the earliest stages of breast cancer. Ductal carcinoma in situ (DCIS) is a non-obligate precursor to invasive ductal carcinoma. With an increasing number of studies linking long, non-coding RNAs (lncRNAs) to various cancers, we have specifically selected to examine lncRNAs as novel DCIS biomarkers and to characterise their biological roles.

Here, we present RNA sequencing (RNAseq) results from two DCIS patient-derived cell lines and one DCIS cell line model identifying several lncRNAs with altered expression. In parallel, we are investigating the role of BC200, a lncRNA associated with high, nuclear-grade DCIS. Ultimately our work aims to identify and characterise lncRNA biomarkers in DCIS in an effort to improve patient outcomes.

YCRN18-4-10 Dr Pablo Moreno-Ruiz, Karolinska Institutet, Cancer Center Karolinska, Stockholm, Sweden

Analysis of stroma FAP+ fibroblasts as survival marker in lung cancer.

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Tumour biology studies have highlighted the role of tumour microenvironment (TME) in the regulation of cancer progression. Emerging studies are also highlighting crosstalk between different TME cell types, including fibroblasts and immune cells, as important regulatory pathways. These findings motivate detailed analyses of multiple cell types in well-annotated clinical cohorts for analyses of clinically relevant interactions.

This study presents data from immunohistochemistry (IHC) analyses of fibroblast active protein (FAP)+ fibroblasts andCD8+ T-cells in a series of clinically well-annotated non-small cell lung cancer (NSCLC) collection.

A tissue microarray(TMA) set was constructed of 354 NSCLC patients surgically treated at the Uppsala University from 2006 until 2011. IHC with FAP and CD8 anti-bodies was performed. The TMAs were scanned and analysed. The intensity of FAP staining was scored 0,1,2 or 3. Scores <2 were considered as low FAP intensity and scores ≥2 were

noted as high. CD8 annotations included separate scoring of CD8 cell density in stromal and tumor epithelial areas. Patients were divided in CD8 low and high “density” using the median as cut-off. Overall survival curves for different subgroups were calculated using Kaplan-Meier with log-rank test. Furthermore, hazard ratios (HR) were calculated using Cox Regression proportional hazard models.

Notably, high intensity of FAP-1 expressionin the cohort was related with shorter overall survival compared to low intensity of FAP (p=0.017). Subset analyses showed that the association between FAP+ cells and survival was retained in the “low density” stromal CD8+ cells group (p=0.012). In contrast, there was no significant difference in the “high density” stromal CD8+ group between the high and low FAP intensity groups (p= 0.397).

These observations confirm earlier findings linking FAP+ fibroblasts to poor prognosis. The restriction of the survival association to the “CD8 low stromal density” groups merits further analyses.

YCRN18-4-11 Ms Niamh Leonard, National University of Ireland Galway, Ireland

Colon cancer cells alter stromal cell PD-L1 and CD47 expression which can modulate the anti-tumour immune response

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The colon tumour microenvironment is highly stromal in composition and a greater stromal cell density correlates with a poor patient prognosis. Stromal cells of mesenchymal origin reside below the epithelial compartment and provide structural support in the intestine and interact with both the epithelial cell compartments as well as infiltrating hematopoietic immune cells. The importance of these cells in regulating immune homeostasis during inflammation is well recognised, however, little is known about their function and phenotype in the inflammatory tumour microenvironment. Using a syngeneic immunogenic model of colorectal cancer, we show that CT26 colorectal cancer cells, alters the secretome and induces up-regulated PD-L1 and CD47 expression in bone marrow derived mesenchymal stromal cells (BM-MSCs). This effect was significantly enhanced by TNF-Î± mediated tumour cell inflammation. Multiplex analysis of the tumour conditioned media showed that macrophage and T cell selective chemo-attractants, MCP-1 and RANTES were present at high levels and were further increased in the TNF-Î± treated group. We could show that tumour conditioned MSCs have a potent ability to suppress syngeneic CD8 T-cells proliferation and alter macrophage phenotype and polarisation, when compared to control BM-MSCs. Further experiments will allow us to determine the functional relevance of the expression of both PD-L1 and CD47 on tumour associated stromal cells in modulation of the anti-tumour immune response. Together, this data shows the ability of stromal in the tumour microenvironment to alter their immunomodulatory phenotype in response to the inflammatory tumour secretome and the potential to modulate the anti-tumour immune response.

YCRN18-4-12 Mr Venkata Vamsi Bharadwaj Yallapragada, University College Cork, Ireland

In situ bacterial production of therapeutic antibody fragments

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The therapeutic potential of antibodies has undergone unprecedented scientific and commercial development in the last 20 years. Due to the rise of engineered antibody fragments such as ScFVs, diabodies, Bi-specific T-cell Engagers (BiTE), tri-specific fragments, etc., therapeutic antibodies have gained wide attention in recent times. Designing such semi-customized antibody fragments against one or more epitopes needs an interdisciplinary approach to translate the in silico-aided design into a potential therapeutic. Today, with the availability of protein engineering and synthetic biology tools, the ‘design, model, build and test’ of these fragments is rapidly accelerating and reducing the cost and time of research. However, some properties such as pharmacokinetics, bioavailability, half-life and antigenicity are also dependent on the production and delivery platform used.

Bacteria have been a widely used production platforms. Given the natural ability of bacteria to selectively grow within tumours, they could conceivably form the final ‘drug’ product for administration, to provide in situ production of the therapeutic. In silico-aided, in situ delivery of bacterial-produced synthetic antibody fragments has exciting potential to deliver therapeutics locally, thus solving the problems associated with antibodies such as bioavailability, and reducing side effects.

In the present work, we demonstrate in silico aided design and in vitro testing of monovalent ScFVs, mono-specific bi-valent diabodies and bi-valent BiTEs against hHER2, hMucin1 and mCD3 antigens.

YCRN18-4-13 Mr Venkata Vamsi Bharadwaj Yallapragada, University College Cork, Ireland

In silico design of synthetic antibody fragments

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Recent advances in protein engineering has equipped us with the tools to design, modify and build various proteins for therapeutic purposes. In the last 30 years, the design of such complex and sophisticated proteins has been complimented by a parallel increase in the availability of computational resources and biological databases. However, the potential of in silico design is yet to be fully exploited. Antibody fragments are one such example of complex proteins. Several types of antibody fragments are currently being exploited by researchers for their therapeutic potential. Today, with predefined design requirement sets, a semi customized antibody could be designed with existing computational tools.

In the present work, we demonstrate the concept of in silico-aided design and how it could inform and guide wet lab experiments. Over 50 different models of various constructs of mono-valent ScFVs and bi-valent mono-specific diabodies against hMUC1 and hHer2 have been modelled and studied for their hydrophobicity, net surface charge, active site exposure and 3D structure. Appropriate solubility tags were added to the antibody fragments to reduce surface hydrophobicity. Linker types and sizes were optimized to find the right 3D conformation of the chains. Distances between the different affinity tags were adjusted to enable proper exposure of the active sites. Furthermore, to examine the binding of these constructs, the selected constructs were docked in silico onto their corresponding antigen to screen for the constructs with optimal functionality. We believe that such an in silico aided design would be very useful and efficient approach to reduce wet lab efforts, cost and the time taken to conduct research.

YCRN18-4-43 Mr Liam Tremble, Cancer Research at UCC, University College Cork, Ireland

Development of a preclinical model for the development of macrophage targeting therapies in melanoma.

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Background: Immunotherapy for cancer patients has been widely hailed as the fourth pillar of cancer care along with the traditional treatments of chemotherapy, radiotherapy and surgery. However, the majority of successful therapies rely on activating T cells, either by checkpoint inhibitors or more recently by designer T cells. While the immune system works as a complex network of signals and cells, each playing a critical role, the development of anti-cancer therapies targeting other cell types has lagged behind anti-T cell therapies.

Recent evidence across many cell types has shown that immune-related side effects of anti-CTLA4 and anti-PD-1 checkpoint inhibitors are severe in many patients and that the side effects increase with the dose given, number of cycles received and are cumulative with each other. In a recent study examining Nivolumab plus Ipilimumab in metastatic melanoma, only 39% of patients were able to receive the full dose and 72% of patients required systemic steroids due to irAEs.

Thus, in order to continue to improve patient outcome it is vital we develop new generations of immunotherapies or immunotherapy complimenting therapies which are tolerable. There has been significant research into the viability of macrophage orientated therapies to improve anti-cancer therapies. Macrophages can contribute up to 50% of the tumour mass in melanoma, and under homeostatic conditions macrophages, and their precursors, monocytes, are orchestraters of the immune response and wound repair.

While we have begun to see clinical benefit from macrophage targeting therapies in other cancer types, progress has been slow due to our poor understanding of these cells and heterogeneity of nomenclature and protocols used to prepare and study these cells. A seminal paper by world leaders (Wynn et al. 2014) has improved research efforts but much remains undone.

Using bone marrow-derived monocytes (BMDMs) and genetically matched B16F10 cells we are developing a preclinical model which will allow the translation of novel anti-cancer therapeutics to clinical trials. We have successfully designed an in vitro protocol that allows the development and identification of CD11b+, Ly6C+, Ly6G- inflammatory monocytes, which mirror circulating monocytes isolated from C57BL/6J mice bearing subcutaneous B16F10 tumours.

Current research efforts are being made to define the effect of B16F10 conditioned medium on BMDMs and their role in anti-cancer immunity. Conditioned medium can effectively skew monocyte polarization towards a phenotype similar to alternatively activated macrophages (activated by IL-4 and IL-13), but with greatly reduced arginase activity, while it can inhibit LPS and IFNγ induced iNOS activity.

YCRN18-5-09 Ms Shona Cronin, School of Biochemistry and Cell Biology, UCC, Ireland

Bacteria tumour targeting and in situ delivery of therapeutics

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Aim: The aim of this study is to investigate the potential of intra-tumoural bacteria to express proteins capable of entering tumour cells.

The tumour environment offers a unique set of conditions favouring the selective growth of certain bacteria species. Several strategies exploiting them as therapeutic agents have been examined. As with all therapeutic strategies, the key to success lies in achieving a suitable balance between efficacy and safety.

In situ production of therapeutics in tumours allows for bioaccumulation (higher-doses) with reduced off-target effects. Non-invasive bacteria (lacking the ability to enter host cells) offer a safer alternative to invasive (pathogenic) bacteria. However, these bacteria are incapable of directly depositing therapeutic agents within target tumour cells.

Cell penetrating peptides (CPPs), are short peptides enabling the introduction of cargo through the membrane of mammalian cells. The successful protein transduction of cancer cells has been validated in vitro and in vivo following administration of purified recombinant protein. We hypothesise that regulated in situ bacterial production of CPPs-tagged proteins might facilitate selective intracellular delivery of proteins to tumour cells.

Preliminary Results: The production and secretion of a CPP-GFP was demonstrated. The transduction of cancer cells in vitro and in vivo was demonstrated via FACS analysis.

These novel preliminary data indicate that bacteria can produce self-transducing proteins within tumours, suggesting a valid role for non-invasive bacteria in tumour therapeutic strategies.

YCRN18-5-10 Ms Shona Cronin, School of Biochemistry and Cell Biology, UCC, Ireland

Biochemical Characterisation of lncRNA, BC200

Shona Cronin, Kellie Dean; School of Biochemistry and Cell Biology, University College Cork

Breast cancer is the most commonly diagnosed cancer in Irish women and the second most common cause of cancer-related death. Treatment includes surgery, radiotherapy, chemotherapy, hormone therapy and/or biological therapy. Understanding the various cellular mechanisms underlying the vast array of breast cancers is key to developing new therapies specifically targeting cancer cells.

Long, noncoding RNAs (lncRNAs) are a recently discovered class of RNAs greater than 200-nucleotides that are transcribed, but not translated into proteins. LncRNAs have emerged as regulators of normal cellular events, with links to various disease states, including breast cancer. One lncRNA associated specifically to cancer is brain cytoplasmic 200 (BC200). BC200 was first found in the nervous system; however, it is associated with breast cancers and other cancers of the oesophagus, lung, ovary and cervix.

Recent work on BC200 lncRNA in breast cancer cells suggests that it interferes with splicing, leading to aberrant splicing of a messenger RNA encoding a pro-apoptotic protein. Importantly, this describes only one nuclear role of BC200; it does not represent the cytoplasmic roles/interactions of BC200, where the bulk of the RNA is located.

Throughout their lifetimes, all RNAs are bound by dynamic sets of RNA-binding proteins, forming ribonucleoprotein complexes (RNPs), that can profoundly affect their function. To uncover the biological roles of BC200, it is crucial to identify binding proteins and analyse their interactions.

To identify BC200-binding proteins, we have pioneered a biochemical approach that utilises streptavidin-aptamer tagged BC200 sense, anti-sense and scrambled control lncRNAs synthesised in vitro. By creating an RNA-affinity matrix, BC200-binding proteins were isolated from invasive and pre-invasive breast cancer cell line lysates. Using quantitative mass spectrometry in combination with microscale thermophoresis and gel shift assays, progress on identifying and confirming BC200:protein interactions will be presented.

The determination of the protein binding partners of BC200 will contribute immensely to our knowledge of the biological roles of this lncRNA in breast cancer. Given that BC200 is essential for cancer cell survival and proliferation, BC200 represents a promising therapeutic target. Thus, a detailed understanding of its cellular interactions is of great clinical importance

YCRN18-5-11 Dr Yingjie Sheng, Ulster University, Coleraine, Northern Ireland, UK

Oxygen Generating Nanoparticles for Improved Photodynamic Therapy of Hypoxic Tumours

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Photodynamic therapy (PDT) is a clinically approved anti-cancer treatment that involves the activation of an otherwise inactive sensitisier drug with light, which in the presence of molecular oxygen, generates cytotoxic reactive oxygen species (ROS). As oxygen is a key requirement for the generation of ROS in PDT and given the fact that hypoxia is a characteristic of most solid cancerous tumours, treating hypoxic tumours using PDT can be a challenge. In this manuscript, we have prepared a CaO2 nanoparticle (NP) formulation coated with a pH-sensitive polymer to enable the controlled generation of molecular oxygen as a function of pH. The polymer coat was designed to protect the particles from decomposition while in circulation but enable their activation at lower pH values in hypoxic regions of solid tumours. The oxygen generating capability of the polymer coated NPs was demonstrated in aqueous solution with minimal oxygen produced at pH 7.4, whereas it increased significantly when the pH was reduced to 6.2. The polymer coated CaO2 NPs were also observed to significantly increase tumour pO2 levels ($p<0.05$) in mice bearing ectopic human xenograft MIA PaCa-2 pancreatic tumours with an average increase in tumour pO2 of 6.5 mmHg in the period 10-30 min following administration. A statistically significant improvement in PDT mediated efficacy ($p<0.001$) was also observed when the particles were administered to mice bearing the same tumours 20 min prior to PDT treatment. These results suggest that the polymer coated CaO2 NP formulation offers significant potential as an in situ method for oxygen generation to enhance the efficacy of treatments that depend on the presence of oxygen to elicit a cytotoxic effect.

YCRN18-5-12 Dr Federica Foglietta, Ulster University, Coleraine, Northern Ireland, UK

Anticancer activity of ultrasound-responsive drug-loaded microbubbles in a three-dimensional and in vivo breast cancer models "

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Taxanes and antracyclines have emerged as critically important drugs in the treatment of patients with breast cancer. Among them doxorubicin (DOX) and paclitaxel (PTX) are the most active agents used in solid tumour. Although response rates of combination studies with DOX and PTX were often very high, several concerns are observed according to the toxicity in particular with cardiotoxicity [1]. Recently, ultrasound-responsive drug-loaded microbubbles (MB) offer the possibility of targeting chemotherapy delivery to the tumour and reducing off-target side effects [2]. In the present work, we show the anticancer activity of DOX functionalized MB-PTX in combination with Rose Bengal (RB) functionalized MB-PTX (PTX-MB-DOX/PTX-MB-RB) exposed to US treatment on an in vitro three-dimensional (3D) model of human breast cancer (MCF-7) cells and on an in vivo breast cancer model.

In vitro data showed a significant reduction of spheroid volume when MCF-7 cell were treated for 48 h with a combination of PTX-MB-DOX [DOX = 1 ÂµM] and PTX-MB-RB [RB = 10 ÂµM] before exposing them to US (frequency of 1 MHz, a power density of 3.0 W/cm2 and 50% duty cycle) as compared to the treatment with free drugs. At the same time point, a significant reduction of spheroid viability was observed ($p < 0.001$) along with a significant increase of Propidium Iodide signal ($p < 0.001$).

In vivo data showed a significant reduction in tumour volume for animals treated with the mixed MB suspension, compared to the free drugs treatment.

In conclusion, these results suggest that combination of US treatment with MB as a delivery vehicle for chemotherapeutics has significant potential as an efficacious treatment for breast cancer.

YCRN18-5-13 Dr Simanpreet Kaur Dhillon, Ulster University, Coleraine, Northern Ireland, UK

Photodynamic Therapy of Malignant Melanoma using a Peptide Modified Sensitiser

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Maligant melanoma is an aggressive skin cancer with current standard treatment regimes involving surgical excision, immunotherapy, targeted therapy and radiotherapy.1 In metastatic disease, systemic therapy using expensive immunotherapy drugs such as Pembrolizumab, Ipilimumab or targeted molecular therapeutic agents such as Vemurafenib or Dabrafenib are the most common treatments. Although these drugs have significantly improved melanoma prognosis, they are costly, have associated toxicity issues and resistance can develop quickly.2 We have discovered a novel and inexpensive treatment for malignant melanoma which employs photodynamic therapy (PDT) together with a Rose Bengal (RB)-C(KLAKLAK)2 conjugate to provide a highly efficacious therapy. The viability of luciferase expressing B16 melanoma cells (B16-F10 Luc-2) that were treated with the Rose Bengal-C(KLAKLAK)2 conjugate and exposed to white light reduced significantly while only a minor effect was observed for the conjugate alone or Rose Bengal mediated PDT at any of the concentrations tested. Furthermore, when B16-F10 Luc-2 tumours were established in SCID mice and treated with either (i) PDT using Rose Bengal alone (ii) PDT using the Rose Bengal-C(KLAKLAK)2 conjugate or (iii) the Rose Bengal-C(KLAKLAK)2 conjugate alone (i.e. no light), a significant reduction in tumour growth was observed for PDT using the Rose Bengal-C(KLAKLAK)2 conjugate compared to the other groups. Indeed, 12 days after treatment commenced, tumours in the group treated with PDT using Rose Bengal-C(KLAKLAK)2 conjugate barely grew at all, while tumours in the other groups grew in excess of 600% from their pre-treatment value.

Collectively, these results highlight a synergy between Rose Bengal and C(KLAKLAK)2 when combined as a conjugate that makes PDT treatment of malignant melanoma significantly more effective than conventional PDT treatment using Rose Bengal alone.

YCRN18-5-14 Mr Ryan Kruschel, University College Cork, Ireland

A structured approach to CDC25 inhibition as potential anticancer therapeutics

Ryan D. Kruschel, Dr Florence McCarthy

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A common characteristic in most cancers is that of a disordered cell cycle. A family of proteins known collectively as cell division cycle 25 (cdc25) are highly conserved phosphatases which play a key role in the regulation of cell division checkpoint pathways. Overexpression of cdc25 phosphatases is observed in many cancer types including thyroid, breast, lymphoma and gastric resulting in a loss of checkpoint control and uncontrolled proliferation. The inhibition of cdc25 has been shown to selectively inhibit various cancer cell lines. The pharmacophore of the quinolinequinone molecular structure is a privileged framework in many known inhibitors of cdc25, some revealing cdc25 isoform enzyme inhibition in the nano-molar range. Herein, we will describe the generation of a number of quinolinequinone and isoquinolinequinone analogues with micro-molar cytotoxic activity and high cancer cell line selectivity tested using the National Cancer Institute 60 cell screen. Further in silico correlation studies have been conducted on the most active analogues to known anti-cancer agents to discover a plausible mode of action.

YCRN18-5-15 Dr Heather Nesbitt¸ Ulster University, Coleraine, Northern Ireland, UK

Gemcitabine loaded Microbubbles for Targeted Chemo-Sonodynamic Therapy of Pancreatic Cancer

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Pancreatic cancer remains one of the most lethal forms of cancer with a 10-year survival of < 1%. With little improvement in survival rates observed in the past 40 years, there is a significant need for new treatments or more effective strategies to deliver existing treatments. The antimetabolite gemcitabine (Gem) is the most widely used form of chemotherapy for pancreatic cancer treatment, but is known to produce significant side effects when administered systemically. We have previously demonstrated the benefit of combined chemo-sonodynamic therapy (SDT), delivered using oxygen carrying microbubbles (O2MB), as a targeted treatment for pancreatic cancer in a murine model of the disease. In this manuscript, we report the preparation of a biotin functionalised Gem ligand for attachment to O2MBs (O2MB-Gem). We

demonstrate the effectiveness of chemo-sonodynamic therapy following ultrasound-targeted-microbubble-destruction (UTMD) of the O2MB-Gem and a Rose Bengal loaded O2MB (O2MB-RB) as a targeted treatment for pancreatic cancer. Specifically, UTMD using the O2MB-Gem and O2MB-RB conjugates reduced the viability of MIA PaCa-2, PANC-1, BxPC3 and T110299 pancreatic cancer cells by greater than 60% ($p<0.001$) and provided significant tumour growth delay (>80%, $p<0.001$) compared to untreated animals when human xenograft MIA PaCa-2 tumours were treated in SCID mice. The toxicity of the O2MB-Gem conjugate was also determined in healthy non-tumour bearing MF1 mice and revealed no evidence of renal or hepatic damage. Therefore, the results presented in this manuscript suggest that chemo-sonodynamic therapy using the O2MB-Gem and O2MB-RB conjugates, is potentially an effective targeted and safe treatment modality for pancreatic cancer.

YCRN18-5-16 **Mr Shehzad Ali**, University of Turin, Italy

Expressional changes in stemness markers post electrochemotherapy in pancreatic cancer cells.

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Pancreatic cancer is one of the most lethal cancers with high metastatic potential and strong chemoresistance. The capability of a tumor to grow and propagate is dependent on a small subset of cells within a tumor, termed cancer stem cells. Cancer stem cells exhibit great tumorigenicity and are closely correlated with drug resistance and tumor recurrence. The aim of our study was to illustrate electrochemotherapy as an effective treatment for pancreatic cancer along with the expression change in stemness genes (Nanog, Sox2 and Oct3/4) in pancreatic cancer cells post electrochemotherapy with bleomycin, cisplatin and oxaliplatin. Our results showed the enhanced expression of Nanog and decreased expression level of Oct3/4 after electrochemotherpy. We thus propose that these stemness marker may have important roles in the initiation and/or recurrence of pancreatic cancer, and consequently may serve as important molecular diagnostics and/or therapeutic targets for the development of novel treatment strategies in pancreatic cancer patients. In conclusion, targeting these stemness factors could potentially improve electrochemotherapy as a treatment and preventing recurrence.

YCRN18-5-17 **Ms Yensi Flores Bueso**, University College Cork, Ireland

The Tumour Microbiota

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The human body is home to a large and diverse population of bacteria with properties that are both harmful and beneficial to our health. The human microbiome differs from one individual to another, with the majority of available information confined to 'tract' related body regions. However, sites once thought of as sterile, such as the stomach, bladder, lungs and placenta, are recently being shown to harbour an endogenous microbiota.

Tumour-inhabiting bacteria have been linked with various cancers in a number of ways. For decades, bacteria of different types have been isolated from patient tumours of various organs. Recently, we and others have characterised the bacterial populations naturally present within malignant and nonmalignant tissue of the breast, and reported the presence of a wide range of bacteria that varies between individuals and tumours.

While bacterial presence in certain tumours is associated with tumorigenesis, in many cases bacterial presence may reflect selective replication of bacteria within tumours. Since it is becoming apparent that bacteria exist in some tumours, this study seeks to establish what tumours have what bacteria, and what influence on tumour initiation, progression or treatment they may have.

YCRN18-5-18 **Ms Yensi Flores Bueso**, University College Cork, Ireland

Synthetic Biology in the driving seat of the Bioeconomy

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Synthetic Biology (SB) has evolved as an umbrella term, representing a methodological framework adopted by a variety of research streams aligned in the quest to create synthetic life and downstream products. Its unprecedented pace of growth is evidence of the support provided by governments, the efforts made in developing a thriving community, and programmes facilitating routes to market.

The SB community seeks to annul the primary pitfalls of traditional biotech in terms of cost, reliability and productivity by discontinuing *ad hoc* practices often characteristic of traditional biotechnology by implementing systematic methods. Also, it is perceived that the reduced skill set, community repositories etc. will lead to a different breed of idea generators than traditional biotech, thus leading to previously unthought-of products and markets. Further to that, the SB community from the outset, placed much attention on bioeconomy aspects (product development needs, routes to market etc.) in order to avoid commercial failures observed with biotech. As a consequence, the SB is revolutionising the biotech industry and its applications are poised to conquer previously unthought-of markets.

We discuss the importance of the SB industry in the bioeconomy, and how it was forged by two key factors; i) the SB approach to R&D distinguishing it from the traditional, and ii) the unique nature of the field's carefully designed, all stakeholder inclusive, 'community-directed evolution'.

YCRN18-5-19 Mr Joseph Murphy, University College Cork, Ireland

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Utilising electrochemotherapy in the treatment of pancreatic ductal adenocarcinoma

Cell culture has been a vital tool in the field of cancer research since the isolation of HeLa cells in 1951. There are currently thousands of cell lines representing various cancer subtypes and genetic modifications available commercially. These are an invaluable tool in research but are mostly used in 2 dimensional culture models that do not adequately represent the tumour microenvironment. This can be overcome to some degree, through the use of 3D cell culture. This involves growing adherent cells in a spheroid shape. This introduces a layer of heterogeneity into the cell population with regard to their access to drugs, oxygen and metabolites. This creates a varied response to candidate treatments and may better bridge the gap in the massive drop off between in vitro and in vivo drug development.

While this allows the reduction in the use of in vivo models, it does not completely recapitulate the tumour microenvironment. Another focus of our work is to apply an orthotopic pancreas cancer model in vivo to our research interests to better understand the immune component of this disease.

Our work focuses on combining physical therapies with various candidate modulators in order to treat pancreatic ductal adenocarcinoma (PDAC) which has the highest ratio of mortality to morbidity of any cancer subtype, the 5 year survival for this disease currently stands at less than 10%. The focus of this research is to enhance tumour cell killing through the activation of the immune system via electroporation and coupling this physical therapy with candidate chemo and immunotherapies.

YCRN18-6-11 Ms Shauna Malone, Irish Cancer Society Research Scholar, Dublin City University, Ireland

Effect of preoperative and postoperative exercise on physical, clinical and psychological outcomes in lung cancer patients: Protocol for a randomised controlled trial

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Introduction: Lung cancer surgery offers a curative intent but is associated with post-operative complications and prolonged recovery time. Surgery is also associated with a significant reduction in physical fitness. Physical fitness is in fact an independent predictor of post-operative outcome and perioperative exercise training may improve physical fitness and ultimately post-operative recovery. However, the optimal model of delivery for perioperative exercise merits investigation.

Aim: To investigate the effect of preoperative and postoperative exercise compared to usual care on physical, clinical and psychological outcomes in lung cancer patients.

Methods: A randomised controlled trial will assign 60 participants with lung cancer scheduled for surgery to 4 groups; Group 1: pre-surgical and post-surgical exercise; Group 2: pre-surgical exercise and post-surgical usual care; Group 3: pre-surgical usual care and post-surgical exercise; Group 4: usual care. The exercise intervention will involve attendance at MedEx, a community-based chronic illness rehabilitation programme at Dublin City University. Participants assigned to exercise will complete 60 minutes of moderate to vigorous intensity exercise involving a combination of aerobic and resistance training on a minimum of 3 days per week.

Assessments will be completed at baseline, following the pre-surgical intervention, 2 weeks post-surgery, and following the post-surgical intervention. Assessments will include physical fitness, muscular strength, body composition, lung function, immune function, smoking status, physical activity and quality of life. Post-operative morbidity, cancer stage, hospital readmissions, surgery completed, post-surgical pain and length of hospital stay will also be recorded following surgery.

A group x time repeated measures ANOVA will be used to compare means.

Conclusion: Evidence of the relation between physical fitness and surgical outcomes may improve the accuracy of risk estimation of patients undergoing surgery and thus inform and improve patient care. Improving physical fitness may translate into better patient outcomes, including reduced morbidity and mortality and enhanced quality of life.

This work is supported by Irish Cancer Society Research Studentship CRS17MAL

YCRN18-6-61 Dr Lisa Loughney, Dublin City University, Ireland

A pre-operative community-based exercise programme for people with prostate cancer: feasibility and preliminary effectiveness study

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Introduction: Low physical fitness predicts poor surgical outcome. Pre-operative exercise training optimises fitness. To our knowledge, this is the first time to investigate the feasibility and preliminary effectiveness of delivering a pre-operative community-based programme in Ireland.

Methods: Prostate cancer patients scheduled for surgery referred from the Mater hospital to MedEx commenced the study within 2 - 7 days following diagnosis. Participants underwent baseline assessment to measure physical measures for lower limb strength (sit-to-stand test); upper limb strength (handgrip test); cardiorespiratory fitness (6-minute time trial); flexibility (sit-and-reach test) and health related quality of life (HRQoL) (EQ-5D questionnaire). Exercise training was delivered in the time window available (either at MedEx or at home) and included aerobic and resistance training using a range of exercise modalities, in 60 minute sessions, 3-5 days per week. Repeat assessments were conducted before surgery.

Results: Seventeen prostate participants were recruited. Baseline mean (SD) age was 63 (7) years with a body mass index of 28.9 (3.6) kg/m2. Sixteen completed the study (13 undertook the supervised programme at MedEx and 3 the home programme) and 1 was lost to follow up due to change in treatment pathway. The average number of exercise training sessions was 9 (5) over a duration of 3.5 weeks. There were no serious adverse events. Pre-operative exercise training resulted in improvements in physical fitness and HRQoL (although not all were statistically significant) (Table 1).

Conclusion: Community-based pre-operative exercise training is feasible. Physical fitness levels and HRQoL can be increased within short pre-operative time-windows.

Table 1. Outcome measures at baseline and post-intervention

Outcome measure	Baseline	Post-intervention	p-value
Lower limb strength (sec)*	16.4 (6.4)	14.4 (5.6)	<0.05
Upper limb strength (kg)	33 (10)	34 (9)	>0.05
Cardiorespiratory fitness (metres)	705 (156)	734 (149)	>0.05
Flexibility (cm)	4 (13)	6 (10)	>0.05
HRQoL (%)*	71 (18)	77 (14)	<0.05

*P<0.05 was taken as statistically significant following paired sample t-test.

YCRN18-6-62 Mrs Mairead Cantwell, Irish Cancer Society Postgraduate Research Scholar, Dublin City University, Ireland

"Move On" from Cancer: An Evaluation of a Community-based Exercise Model for Cancer Rehabilitation

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Introduction: MedEx Wellness is a community-based chronic illness rehabilitation programme located at Dublin City University. It offers exercise classes in a medically supervised environment to patients with a range of chronic illnesses. MedEx “Move On” is the oncology rehabilitation programme that caters for cancer survivors. This study aimed to determine the effect of “Move On” on cancer survivors’ physical activity levels, physical function and quality of life (QOL).

Methods: A single arm pre-test, post-test design was used. Adults with an established diagnosis of cancer, who had completed their adjunctive therapy, were referred to “Move On” by members of their oncology team and recruited to this study. Participants attended two 60 minute supervised exercise classes per week for 12 weeks. At baseline and week 12, assessments of cardiorespiratory fitness (6 minute time trial), strength (timed sit-to-stand test), body composition (BMI and waist:hip ratio), QOL (FACT-G Questionnaire), fatigue (FACIT-F Questionnaire) and physical activity levels (7 day accelerometry) were performed. Paired sample t-tests were used to compare means.

Results: 86 cancer survivors were referred to the programme during a four month period. The programme completion rate was 64% (n=55). Accelerometry data (n=40), physical test results (n=55) and questionnaire data (n=41) are presented. There was a statistically significant increase in 6 minute time trial (p<.01) and timed sit-to-stand performance (p<.01). Statistically significant increases were observed for participantsâ€™ daily steps counts (p<.01), hours spent in light intensity physical activity (p<.05) and QOL (p<.01). A statistically significant reduction in fatigue was also observed (p<.05).

Discussion: MedEx “Move On” significantly improved cancer survivors’ physical function, physical activity levels and QOL. Exercise can play a key role in the management of long-term treatment related side effects and community-based exercise programmes are well placed to support cancer survivors to increase their physical and psychological well-being.

YCRN18-6-63 **Mr Glenn Hogan**, University College Cork

Living Microbes Within Tumours

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The recent discovery by our group of a bacterial presence within healthy and malignant human breast tissue has pointed towards the existence of a tumour microbiome. This microbiome has been described via deep sequencing techniques. However, while high-throughput sequencing can help to define the composition of a bacterial community, it is disadvantageous in that it cannot confirm the viability of the microbes it detects. Further work is therefore required to create a more comprehensive portrait of the breast microbiome, and to potentially obtain tumour-adapted bacteria from human tumours to serve as drug delivery vehicles.

Our aim in this study was to develop a culture-dependent system, capable of capturing as wide an array of bacteria as possible from murine and human tissues. A procedure was developed and optimised using murine tumour models, before applying to fresh patient samples. Multiple bacterial genera and species were cultured from patient breast tissue, unlike control samples for skin or enviornmental ‘background’. There was significant divergence in the profiles of murine and human tissues in terms of their bacterial make-up. To our knowledge, this is the first study in which viable bacteria have been recovered from human tumour tissue.

YCRN18-6-64 **Mr Glenn Hogan**, University College Cork, Ireland

The Who, What, and Why of Drug Discovery and Development

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The healthcare industry is experiencing a significant decline in R&D productivity, casting doubt on its sustainability, and provoking considerable investigation into the prevailing forces at the root of the issue. In the literature, we noticed much attention being paid to ‘what’ could be improved to correct the downward trend in R&D efficiency (e.g. inadequacies of high-throughput screening). However, we found another facet of R&D efficiency dangerously less attended, namely, ‘who’ in R&D could be improved.

There is a need to examine the people-centric complexities of the health technology industry, because change within this industry can be institutionalised only by the people who influence triage from idea through to market. This topic is of increasing importance to scholars who attempt to provide an evidence-based rationale for advancing public and private scientific policy (e.g. The Science of Science and Innovation Policy).

We present a portrait of the actors who play a major role in discovering and developing investigational medicinal products (IMP) and show that the discovery of “historically novel” therapeutics, like gene therapies, is avoided in larger companies. We propose that a central problem in R&D concerns incompatibilities in risk tolerance and gatekeeper familiarity between early-stage and late-stage actors. By illustrating this, we identify a zone of compromise in which R&D efficiency could be improved.

Here, we present a framework for medical technology development in the context of actors and their preferences. We argue that practices which enable all actors in the idea to product chain to have a more holistic knowledge of the behaviour and incentives of each other, can optimise the process significantly. We ultimately provide a case for why the involvement of all actors from the earliest stages of R&D can enhance R&D productivity and restore the industry’s viability, reasoning that if the intellectual ideas of humans serve as the raw material for fully-formed medicines, these ideas will be optimised if the people from whom they come are likewise optimised.

YCRN18-6-65 **Dr Kheshwant Gill**, University College Cork, Ireland

The effects of metabolic modulation and immunogenicity in Osteosarcoma

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Osteosarcoma is the most common (75%) high-grade malignant tumour of the skeletal system in paediatric patients. In 2014, there were 579 new cases of osteosarcoma in the United Kingdom.

The mortality rates in osteosarcoma are due to both intrinsic and acquired resistance (60%) to currently used chemotherapies that lead to multidrug resistant phenotypes and the occurrence of ‘second malignancies’. Attempts to improve therapy efficacy by dose escalation, alterations in combinations of chemotherapy and irradiation therapy have not improved survival outcomes.

The tumour microenvironment poses as a major barrier to treatments due to immunosupression by chronic inflammation, tumour survival and angiogenesis. A low presence of immune cells within the tumour microenvionment correlates with a poorer prognosis in cancer.

Electroporation is a non-thermal, cell permeabilising technology that renders the treated cell membranes permeable to poorly permeant anti-cancer drugs thus facilitating a potent local cytotoxic effect from the improved cell membrane porosity. Metabolic modulators in combination with chemotherapy has been shown to be effective in cancer treatment, due to increase sensitivity of cancer cells from depletion of intracellular ATP levels from glycolytic modulation.

The aims of this study were to examine the cytotoxic effects of electroporation delivered metabolic modulators and low dose chemotherapy that subvert therapy resistance by increasing osteosarcoma sensitivity to existing treatments. Recruiting an immune response by modulating the tumour microenvironment – cell cycle modulation combined with low-dose chemotherapy, promotes an anti-tumour response and thus improving treatment efficacy and outcome.

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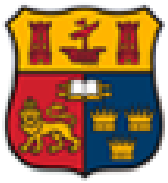

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