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IACR COUNCIL MEMBERS 2014-2015

President, William Watson
Jacintha O’Sullivan, Honorary Secretary
Ann Hopkins, Honorary Treasurer
Emer Bourke
Niamh Buckley
Tara Dalton
William Gallagher
Patrick Kiely
Simon McDade
Ken Nally
Darran O’Connor
Norma O’Donovan
Graham Pidgeon
Olga Piskareva
Afshin Samali
Mark Tangney

Disclaimer

Although the information contained in this programme and abstract booklet is believed to be true and accurate, the Irish Association for Cancer Research (IACR) cannot accept any legal responsibility for any errors or omissions which have been made. The opinion and conclusions expressed in the abstracts are those of the authors, and not the IACR.
February 2015

Dear Colleagues

On behalf of the IACR council it is our great pleasure to welcome you to the 2015 IACR Annual Meeting in the Castletroy Hotel in Limerick. This annual cancer meeting is a major event for our association allowing us to showcase both national and international cancer research. We would like to extend a warm welcome to all our invited speakers and thank them for their time and participation at this cancer conference. We look forward to the many scientific discussions throughout the conference with you.

We sincerely thank the Irish Cancer Society (ICS) for their continuous support and financial help for this conference. We would like to congratulate Dr. Robert O’Connor on his recent appointment of Director of Research at the ICS. The IACR council looks forward to working with Rob over the coming years. Sadly, since our last annual conference, we lost a dear colleague, Professor John Fitzpatrick. John was always so engaging with our young scientists in the oral poster session every year. In his honour we will be giving an award for the best oral poster presentation. We are delighted that John’s family will join us for this presentation.

As always, this conference would not be possible without the generous financial support from our donors and sponsors. We would like to sincerely thank you for your continued support of this annual meeting. We encourage all delegates to visit the trade stands during the breaks.

The council would also like to thank Mr. Rob Kenney and his colleagues at the European Association for Cancer Research (EACR) for their working relationship with the IACR. We are grateful to the EACR for sponsoring the EACR young scientist awards at this conference. Congratulations to Dr. James Brown and Dr. Aideen Ryan who will be presented with these awards at the conference.

Since our last conference, we held an election for an IACR junior council which allows our postdoctoral fellows to be more involved in the running of the association. The ratification of this junior council will be done during the Annual General Meeting which we encourage all of you to attend. This is an opportunity for you to bring new ideas to the council.

At this conference, Jacintha and Ann’s 3 year term as secretary and treasurer respectively is ending. We would like to thank all council members we have worked with over the years. Congratulations to Pat Kiely (secretary elect) and Olga Piskareva (treasurer elect) on their new roles and we wish them every success.

We hope you enjoy both the science and social aspects of the conference and please remember this is your association so we encourage you to attend the AGM and participate in the session.

With best wishes

Sincerely

Bill Watson
President

Jacintha O’Sullivan
Honorary Secretary

Ann Hopkins
Honorary Treasurer
### DAY ONE – THURSDAY 26 FEBRUARY 2015

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<td>07.45 - 08.30</td>
<td>REGISTRATION – TEA/COFFEE AND MEET THE SPONSORS</td>
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<td>08.30 – 08.40</td>
<td>Welcome Address: William Watson, President</td>
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| 08.40 – 10.10 | Proffered Papers – Session 1  
Chairs: Dr Simon McDade (QUB) and Dr Mark Tangney (UCC) |
| 08.40 – 08.55 | PRE-CLINICAL EFFICACY OF A NOVEL ANTI-INVASION NANOPARTICLE THERAPEUTIC IN COMBINATION WITH THE ANGIgenesIS INHIBITOR BEvacizumab FOR THE TREATMENT OF Glioblastoma  
David Murray¹, P O’Halloran¹,², M Jarzabek¹, B MacCarthy¹, J.N. Sarkaria³, R.M. Schiffelers⁴, M Symons⁵, A.T. Byrne¹,¹¹Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin 2, ²National Neurosurgical Department, Beaumont Hospital, Dublin 9, ³Department of Radiation Oncology, Mayo Clinic, Rochester, MN 55905, USA, ⁴Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht, The Netherlands, ⁵Department of Oncology and Cell Biology, Feinstein Institute for Medical Research at North Shore-LI, Manhasset, NY 11030, USA |
| 08.55 – 09.10 | THE DEVELOPMENT OF ADAM22 AS A PREDICTIVE MARKER FOR ENDOCRINE RESISTANT BREAST CANCER AND AN LGI1 MIMETIC AS A COMPANION THERAPEUTIC  
Ben Doherty¹, J Bolger¹, D McCartan¹, C Byrne¹, D Vareslija¹, S Cocchiglia¹, A Hill¹, L Young¹  
¹Endocrine Oncology Lab, RCSI, Dublin, ²Surgery, Beaumont Hospital, Dublin |
| 09.10 – 09.25 | CDK7: A MARKER OF POOR PROGNOSIS AND TRACTABLE THERAPEUTIC TARGET IN TRIPLE-NEGATIVE BREAST CANCER  
Bo Li¹, T Ni Chonghaile¹, Y Fan¹, R Kinger¹, AE O’Connor¹, DP O’Connor¹, WM Gallagher¹  
¹Cancer Biology and Therapeutics Laboratory, Conway Institute, University College Dublin, Dublin |
| 09.25 – 09.40 | SYNERGISTIC COMBINATION OF ONCOlytic REovirus Type 3 (DEARING) AND RADIOTHERAPY IN MELANOMA IS MEDIATED THROUGH ENHANCED VIRAL REPLICATION AND MITOCHONDRIAL APOPTOTIC SIGNALLING  
Grainne McEntee², J Kyula², V Roulstone², C Gregory², M Coffey², KJ Harrington¹  
¹Targeted Therapy Team, Institute Cancer Research, London UK, ²Oncolytics Biotech. Inc, Calgary Canada |
| 09.40 - 09.55 | EphA2 is an essential driver of invasion and associated with the poor prognostic stem-like subgroup in early stage colorectal cancer.  
Philip Dunne¹, D McCarty¹, J Blayney¹, M Salto-Tellez¹, P Johnston¹, S Van Schaeybroeck¹  
¹CCRCB, Queens University Belfast, Belfast |
| 09.55 – 10.10 | ERYTHROPOIETIN RECEPTOR PROMOTES C-Myc DRIVEN BREAST TUMOURIGENESIS  
Kyle B Matchett¹, KK Chan¹, JA Coulter¹, MA Davidson¹, HF Yuen², CM McCruden¹, P Maxwell¹, M El-Tanani¹, TR Lappin¹  
¹Centre for Cancer Research and Cell Biology, Queen’s University Belfast, Belfast, ²Institute of Molecular and Cell Biology, A*STAR, Singapore |
| 10.10 - 10.30 | Gold Sponsor Platform – Fannin - Dr. Stephen Hague, European Droplet Digital PCR Applications Specialist for Biorad |
| 10.30 – 11.00 | TEA/COFFEE/POSTERS AND MEET THE SPONSORS |
| 11.00 – 12.30 | Plenary Session 1: Epigenetics and Disease States  
Chairs: Dr Olga Piskareva (RCSI) and Dr Ken Nally (UCC) |
| 11.00 – 11.30 | “Epigenetic Drugs: Exploring New Pathways For Manipulating Tumour Cell Death”  
Nick LaThangue (University of Oxford) |
| 11.30 – 12.00 | “Decoding Long non-coding RNAs in Cancer”  
Jo Vandesompele, Ghent University, Belgium |
| 12.00 – 12.30 | “Polycomb Group Proteins in Cancer”  
Adrian Bracken, Trinity College Dublin |
<p>| 12.30 – 12.50 | Gold Sponsor Presentation – Kevin Francis, Perkin Elmer |
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<td>Plenary Session 2: From Mouse To Man; Imaging And Cancer Chairs: Prof. William Gallagher (UCD) and Dr Norma O’Donovan (DCU)</td>
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<td>14.00 – 14.30</td>
<td>“Advances in Cancer Imaging Using Multispectral Optoacoustic Tomography” Dr Christoph Hinzen (Technische Universität München, Germany)</td>
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<td>14.30 – 15.00</td>
<td>“Improving the Predictive Value of Preclinical Models Through the Co-Clinical Trial Paradigm” Andrew L. Kung M.D., Ph.D., Chief, Division of Pediatric Hematology/Oncology/Stem Cell Transplantation, Robert and Ellen Kapito Professor of Pediatrics, Columbia University Medical Center, New York, USA</td>
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<td>15.00 – 15.30</td>
<td>“Exploring New Frontiers in the Diagnostic and Therapeutic Applications of Microwaves” Martin O’Halloran, National University of Ireland, Galway</td>
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<td>15.30 – 16.00</td>
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<td>16.00 – 17.00</td>
<td>ORAL POSTER PRESENTATIONS (Best Oral Poster Presentation will receive the Prof. John Fitzpatrick Award) Chairs: Dr Robert O’Connor (ICS) and Dr Emer Bourke (NUIG)</td>
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<td>16.00 – 16.06</td>
<td>INFLUENCE OF BACTERIA ON CHEMOTHERAPEUTIC DRUGS</td>
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Panos Lehouritis¹, J Cummins¹, M Stanton¹, C Murphy¹, F McCarthy¹, G Reid², C Urbaniak², W Byrne³, M Tangney¹-²Cork Cancer Research Centre, University College Cork, Cork Ireland
²Lawson Health Research Institute, Lawson Health Research Institute, London Ontario

16.06 – 16.12 | PERSONALISED MODELLING OF THE JNK ACTIVATION DYNAMICS CAN PREDICT NEUROBLASTOMA SURVIVAL
Dirk Fey¹, M Halas¹, D Drediax², SP Kennedy¹, J Hastings³, N Rauch¹, AG Munoz¹, R Pilkinson¹, M Fischer⁴, F Westermann², W Kolch³, BN Khodorolok⁴, DR Croucher³-⁴
¹Systems Biology Ireland, University College Dublin, Dublin, ²Department of Neuroblastoma Genomics (B087), German Cancer Research Center (DKFZ), Heidelberg, ³The Kinghorn Cancer Centre, Garvan Institute of Medical Research, Sydney, ⁴Department of Pediatric Hematology and Oncology, University Hospital Cologne, Cologne

16.12 – 16.18 | THE ROLE OF C-MET/HGF SIGNALLING AS A CRITICAL MEDIATOR OF AN INVASIVE AND RESISTANT PHENOTYPE IN COLORECTAL CANCER
Conor A Bradley¹, P Dunne, D McArt, S McQuaid, M Salto-Tellez, PG Johnston, S Van Schaeybroeck, ¹CCRCB, QUB, Belfast

16.18 – 16.24 | TOPOISOMERASE 2-α IS A NOVEL CDC7/DBF4 INTERACTING PROTEIN
Kevin ZL Wu¹,², G O’Brien¹,², MD Rainey¹,², C Santocanale¹,²
¹Centre for Chromosome Biology, NUI Galway, Galway,
²National Centre for Biomedical Engineering Science, NUI Galway, Galway

16.24 – 16.30 | BRCA1 DEFICIENCY EXACERBATES ESTROGEN-INDUCED DNA DAMAGE AND GENOMIC INSTABILITY
Kyle B Matchett¹, KI Savage¹, EM Barros¹, KM Cooper², GW Irwin³, JJ Gorski¹, KS Orr¹, J Vohhodina¹, JN Kavanagh¹, AF Madden¹, A Powell¹,², L Manti³, SS McIade¹, BH Park⁴, KM Prise¹, SA McIntosh¹, M Salto-Tellez¹, DJ Richard⁵, CT Elliott⁶, DP Harkin¹
¹Centre for Cancer Research and Cell Biology, Queen’s University Belfast, Belfast, ²Institute for Global Food Security, Queen’s University Belfast, Belfast, ³Department of Physics, University of Naples Federico II, Naples, ⁴The Sidney Kimmel Comprehensive Cancer Centre, The Johns Hopkins University School of Medicine, Baltimore, ⁵Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane

16.30 – 16.36 | RATIONAL TARGETING OF INHIBITOR OF APOPTOSIS PROTEINS (IAPs) FOR EFFECTIVE THERAPY OF COLORECTAL CANCER
Nyree T Crawford, C McCann, DJJ Waugh, PG Johnston, DB Longley
¹Centre for Cancer Research Biology, Queen’s University, Belfast
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<td>16.36 – 16.42</td>
<td><strong>HIGH-THROUGHPUT SCREEN IDENTIFIES A NOVEL SPECIFIC HISTONE DEACETYLASE 6 INHIBITOR THAT KILLS CHEMORESISTANT BREAST CANCER CELLS.</strong>&lt;br&gt;Triona Ni Chonghaile¹, B Smith², M Hemann³, JE Bradner⁴, W Gallagher¹, A Letai³&lt;br&gt;¹The Conway Institute of Biomolecular and Biomedical Science, University College Dublin, Dublin&lt;br&gt;²The Department of Hematology and Oncology, Dana Farber Cancer Institute, Boston&lt;br&gt;³The Department of Biology, Massachusetts Institute of Technology, Boston</td>
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<td>16.42 – 16.48</td>
<td><strong>THE EDUCATION OF MSCs BY PROSTATE CANCER CELL LINE CONDITIONED MEDIA</strong>&lt;br&gt;Sarah Ridge¹, Ms. Shaw², Prof. Sullivan³, Prof. Giles¹, Dr. Glynn¹&lt;br&gt;¹Prostate Cancer Institute (PCI), NUI Galway, Galway, ²Regenerative Medicine Institute (REMED), NUI Galway, Galway, ³Radiation Oncology, Galway University Hospital, Galway</td>
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<td>17.00 – 18.00</td>
<td><strong>IRISH CANCER SOCIETY SCHOLAR AND FELLOW PRESENTATIONS</strong>&lt;br&gt;Chairs: Dr Aisling O’Connor (ICS) and Dr Jacintha O’Sullivan (TCD)</td>
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<td>17.00 – 17.15</td>
<td><strong>TAILORING APPROACHES FOR GLOBAL EPIGENOME ANALYSIS FROM ARCHIVAL FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUE SAMPLES</strong>&lt;br&gt;Sudipto Das¹, B Moran¹, R Klinger¹, B Fender², G Peutman³, D Smeets³, A Byrne⁴, M Ebert⁵, D Lambrechts³, WM Gallagher³, DP O’Connor¹, ¹Cancer Biology &amp; therapeutics, Conway Institute, University College Dublin, Dublin, ²Oncomark Ltd., Nova UCD, Dublin, ³Department of Translational genetics, VIB, K.U., Leuven, Belgium, ⁴Department of Physiology, Royal College of Surgeons in Ireland, Dublin, ⁵University of Heidelberg, University of Heidelberg, Mannheim, Germany</td>
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<td>17.15 – 17.30</td>
<td><strong>MicroRNA-10a IS SIGNIFICANTLY DOWN-REGULATED IN BREAST CANCER AND REGULATED AT LEAST IN PART THROUGH RETINOIC ACID</strong>&lt;br&gt;Sonja Khan, D Wall, C Curran, J Newell, MJ Kerin, RM Dwyer, ¹Surgery, School of Medicine, National University of Ireland, Galway, ²Clinical Research Facility and School of Mathematics, Statistics and Applied Mathematics, National University of Ireland, Galway, ³Surgery, School of Medicine, National University of Ireland, Galway, ⁴Clinical Research Facility and School of Mathematics, Statistics and Applied Mathematics, National University of Ireland, Galway, ⁵Surgery, School of Medicine, National University of Ireland, Galway, ⁶Surgery, School of Medicine, National University of Ireland, Galway</td>
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<td><strong>Investigation of the role of STAT3 signalling in obesity-associated adenocarcinoma of the oesophagus</strong>&lt;br&gt;Katie O’ Sullivan¹, MC Cathcart¹, E O’ Regan¹, J Michaelson¹, N Gilmartin¹, A Cannon¹, G Moore¹, W Gao², U Fearon², J Lysaght¹, J O’ Sullivan¹, JV Reynolds¹&lt;br&gt;¹Department of Surgery, Institute of Molecular Medicine, Dublin&lt;br&gt;²Education and Research Centre, Conway Institute of Bimolecular and Biomedical Research, Elm Park, Dublin</td>
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<td>17.45 – 18.00</td>
<td><strong>FUNCTIONAL GENOMIC SCREENING IDENTIFIES USP11 AS A NOVEL THERAPEUTIC TARGET IN BREAST CANCER</strong>&lt;br&gt;Lisa Dwane¹, AE O’Connor¹, L Mulrane¹, AM Dirac², K Jirstrom³, JP Crown⁴, R Bernards², WM Gallagher¹, DP O’Connor¹, ¹UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Dublin, ²Division of Molecular Carcinogenesis, Netherlands Cancer Institute, Amsterdam, Netherlands, ³Department of Laboratory Medicine, Malmö University Hospital, Lund University, Malmö, Sweden</td>
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<td><strong>IRISH CANCER SOCIETY LECTURE – Introduced by: John McCormack</strong>&lt;br&gt;“GENETIC PREDISPOSITION TO PROSTATE CANCER AND ITS CLINICAL APPLICATIONS”&lt;br&gt;Prof. Ros Eeles, The Royal Marsden &amp; The Institute of Cancer Research, London</td>
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<td>19.30 – 22.30</td>
<td><strong>FORMAL POSTER SESSION, TRADE EXHIBITION/WINE RECEPTION AND BUFFET DINNER</strong></td>
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<td>08.40 – 08.55</td>
<td>IDENTIFICATION OF A NOVEL UBIQUITIN SPECIFIC PEPTIDASE INVOLVED IN MAINTAINING GENOMIC STABILITY</td>
<td>Edel McGarry¹, David Gaboriau¹, Umberto Restuccia², Michael D. Rainey¹, Angela Bachi² and Corrado Santocanale¹.¹ Centre for Chromosome Biology and National Centre for Biomedical Engineering Science, National University of Ireland, Galway, ²Functional Proteomics Unit, IFOM, FIRC Institute of Molecular Oncology, Milan, Italy</td>
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<td>08.55 – 09.10</td>
<td>INVESTIGATING THE ROLE OF POLYMORPHISMS IN mir146a AND mir196a2 IN MODIFYING BREAST CANCER RISK AND PHENOTYPE</td>
<td>Robert J Mulligan¹, TP McVeigh¹, KJ Sweeney¹, JB Weidhaas³, N Miller¹, MJ Kerin¹</td>
<td>¹Discipline of Surgery, NUI Galway, Galway, ³School of Medicine, Yale University, New Haven</td>
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<td>09.10 – 09.25</td>
<td>ELUCIDATING THE IMMUNOTHERAPEUTIC POTENTIAL OF GAMMA DELTA T CELL SUBSETS AND MAIT CELLS</td>
<td>Margaret Dunne¹, R Dunne¹, N Clarke¹, N Gilmartin¹, D O'Toole², F MacCarthy², N Ravi², JV Reynolds¹, JN O'Sullivan¹.¹Department of Surgery, Trinity College Dublin, Dublin, ²Department of Clinical Medicine, St James's Hospital, Dublin</td>
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<td>09.25 – 09.40</td>
<td>DEVELOPMENT OF AN EX-VIVO PRIMARY ACUTE MYELOID LEUKEMIA (AML) ASSAY TO PREDICT EFFICACY OF CHEMOTHERAPY</td>
<td>Denis Baev¹, J Krawczyk², M O'Dwyer¹, E Szegedi¹, ¹Apoptosis Research Center, National University of Ireland, Galway, ²Haematology, Galway University Hospital, Galway</td>
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<td>09.40 – 09.55</td>
<td>THE ROLE OF ZEB1/ZEβ2 AND βIII-TUBULIN IN MEDIATING DOCETAXEL-RESISTANT PROSTATE CANCER</td>
<td>Karen Hanrahan¹, M Prencipe¹, J Bugler¹, L Murphy¹, A O'Neill¹, RW Watson¹</td>
<td>¹UCD School of Medicine and Medical Science, Conway Institute of Biomolecular and Biomedical Research, University College Dublin</td>
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<td>09.55 – 10.10</td>
<td>DELINEATING AN EPIGENOMIC ROADMAP OF PROSTATE TUMORIGENESIS</td>
<td>Cj O'Rourke¹, AL Walsh¹,², B Hayes¹, J Hansen¹, S O'Meachair⁵, D Golden⁶, R Anney¹, S Madden⁷, C Morrissey⁸, R Vessella⁸, M Emmert-Buck⁴, SP Finn³, D Hollywood¹, T Lynch², RWG Watson⁹, Antoinette Perry¹.¹Institute of Molecular Medicine, Trinity College Dublin, Dublin 8, ²Department of Urology, St. James's Hospital, Dublin 8, ³Department of Histopathology, St. James's Hospital, Dublin 8, ⁴Pathogenetics Unit, National Cancer Institute, NIH, Bethesda, MD, USA, ⁵Dublin Centre for Clinical Research and Centre for Health Decision Science, Trinity College Dublin, Dublin 2, ⁶Trinity Centre for High Performance Computing, Trinity College Dublin, Dublin 2, ⁷National Institute for Cellular Biotechnology, Dublin City University, Dublin 9, ⁸Department of Urology, University of Washington, Seattle, USA, ⁹Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin 4</td>
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<td>10.55 – 12.25</td>
<td>Plenary Session 3: Growth Factor &amp; Adhesion Signalling</td>
<td>Chairs: Dr Pat Kiely (UL) and Dr Ann Hopkins (RCSI)</td>
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<td>10.55 – 11.25</td>
<td>“Rna Screen Identifies New Regulators of Epithelial Cell-Cell Adhesion”</td>
<td>Vania Braga, Faculty of Medicine – Imperial College, London</td>
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<td>11.25 – 11.55</td>
<td>“Forcing Tumor Progression and Metastasis”</td>
<td>Valerie Weaver, University of California, San Francisco</td>
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<td>11.55 – 12.25</td>
<td>“IGF and Adhesion Signalling in Cancer Cell Stemness, EMT and Therapy Resistance”</td>
<td>Rosemary O'Connor, University College Cork</td>
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<td>Gold Sponsor Platform – Kile Green, NanoString Technologies</td>
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<td>14.00 – 15.30</td>
<td>Plenary Session 4: Longitudinal Monitoring of Acquired Resistance to Therapy</td>
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<td>Chairs: Darran O’Connor, UCD and Prof. Afshin Samali, NUI, Galway</td>
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<td>14.00 – 14.30</td>
<td>“Therapy Resistance in Human Prostate Cancer”</td>
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<td>Norman Maitland, University of York</td>
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<td>14.30 – 15.00</td>
<td>“Non-Invasive Cancer Genomics”</td>
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<td>Dr Dana Tsui, University of Cambridge</td>
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<td>15.50 – 17.20</td>
<td>Plenary Session 5: Lessons from Research Centres: Translating Academic</td>
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<td>Research into Innovative Tools</td>
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<td>Chairs: William Watson, UCD and Dr Tara Dalton, UL</td>
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<td>15.50 – 16.20</td>
<td>“CRC-Associated Microbiota Profiles Are Linked With Distinct Mucosal Gene</td>
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<td>Expression Profiles”</td>
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<td>Burkhardt Flemer, PhD, Alimentary Pharmabiotic Centre &amp; Dept., Microbiology,</td>
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<td>University College Cork</td>
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<td>16.20 – 16.50</td>
<td>Professor Brian Caulfield (INSIGHT)</td>
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<td>Lead Investigator ARCH Centre, Dean of Physiotherapy, University College</td>
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<td>16.50 – 17.20</td>
<td>BREAST-PREDICT, the Irish Cancer Society’s First Collaborative Cancer</td>
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<td>William Gallagher (BREAST-PREDICT)</td>
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<td>17.20 – 18.20</td>
<td>EACR Young Scientist Awards</td>
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<td>Chairs: Dr Ann Hopkins and Dr Jacintha O’Sullivan</td>
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<td>17.20 – 17.50</td>
<td>Presented by: James Brown</td>
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<td>RATIONAL DESIGN AND VALIDATION OF A TIP60 HISTONE ACETYLTRANSFERASE INHIBITOR</td>
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<td>FOR THE TREATMENT OF BREAST CANCER SUBTYPES</td>
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<td>C Gao¹,², E Bourke³, M Scobie⁴, MC Casey⁶, A McGuire⁶, A Shalaby³, M Webber³,</td>
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<td>C Curran⁶, MA Famme⁴, T Koolmeister⁴, M Kerin⁶, T Helleday⁴, LA Eriksson¹,²,</td>
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<td>NF Lowndes⁵, James Brown⁵,⁶</td>
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<td>¹Department of Chemistry and Molecular Biology, Gothenburg, Gothenburg,</td>
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<td>Department of Medi, Karolinska Institute, Stockholm, Sweden, ⁴Centre for</td>
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<td>Chromosome Biology, School of Natural Sciences, National University of</td>
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<td>Ireland Galway, Galway, ⁵Department of Surgery, School of Medicine,</td>
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<td>17.50 – 18.20</td>
<td>TARGETING COLON CANCER CELL NF-kappaB PROMOTES AN ANTI-TUMOR M1-LIKE</td>
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<td>MACROPHAGE PHENOTYPE AND INHIBITS PERITONEAL METASTASIS</td>
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<td>AE Ryan¹,², A Colleran¹, A O’Gorman¹, L O’Flynn², J Pindjakova², P Lohan²,</td>
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<td>G O’Malley¹,², M Nosov², C Mureau¹, LJ Egan¹</td>
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<td>¹Discipline of Pharmacology and Therapeutics, NUIG, Galway, ²Regenerative</td>
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<td>Medicine Institute, NUIG, Galway</td>
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<td>AWARDING OF PRIZES</td>
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<td>GALA DINNER BUNRATTY CASTLE WITH ENTERTAINMENT</td>
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<td>TRANSPORT TO AND FROM VENUE PROVIDED – BUS DEPARTS AT 19.30!!!!</td>
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PLENARY SPEAKER BIOGRAPHICAL SKETCHES
(Alphabetical)

Dr. Adrian Bracken is an Assistant Professor of Cancer Epigenetics, at Trinity College Dublin, Ireland. During his post-doctorate years he gained an international reputation for using Cancer Epigenetics to explore cancer pathways and identify novel therapeutic targets. He is a PhD graduate of Trinity College Dublin (2000) and has held fellowships at The European Institute of Oncology, Milan, Italy and The Biotechnology and Research Innovation Centre at The University of Copenhagen, Denmark. Currently he is consolidating his own independent research group. The lab’s most recent two papers include a study on the role of Polycomb group proteins in embryonic stem cells (Brien et al., 2012 Nature structural & Molecular Biology), and the role of the tumour suppressor, CHD5, during neurogenesis (Egan et al., Developmental Cell. 2013). Recently, his lab developed and patented a novel method called “OncoMasTR”, which can categorise early stage breast cancer patients into groups based on their risk of recurrence, and thus it is designed to aid both clinicians and patients in making informed treatment decisions.

Vania Braga is currently a Reader in Cell-cell Adhesion Signalling. She started her own lab in 1995 at the MRC Laboratory for Molecular and Cellular Biology, University College London. In 2001, she was awarded the prestigious MRC Non-Clinical Senior Fellowship and moved to the Faculty of Medicine, Imperial College London. In 2012, she was appointed Deputy-Head of the Molecular Medicine Section, NHLI and South Kensington Campus Director for the Faculty of Medicine (2012-2015).

Her research interests include understanding the signalling pathways required for epithelial specification and how these processes are subverted during diseases and tumour progression. In particular, her lab focuses on signalling triggered by E-cadherin-dependent cell-cell contacts and small GTPases of the Rho and Rab subfamilies. The lab investigates the mechanisms via which E-cadherin contacts co-regulates different cellular and signalling processes to enable assembly and maintenance of tight adhesion, epithelial function and differentiation. Dr. Braga participates in a number of editorial boards of scientific journals, review panels and grant agencies in Europe and the UK and is regularly invited to present talks in international conferences in Europe, USA and Asia such as Gordon conferences, Keystone Meeting and FASEB.

Work in the lab is currently funded by Cancer Research UK (C1282/A11980); Medical Research Council (MR/J007668/1), The Wellcome Trust (GR081357MA) and Brunei Government Studentship (JPLL/A/4:A[2010]/J1(703)).
Professor Brian Caulfield leads University College Dublin’s Connected Health Programme. He is the Lead Investigator in Ireland’s industry led Connected Health Technology Centre, ARCH (www.arch.ie) and is a Director of Ireland’s largest research centre, the INSIGHT centre for Data Analytics (www.insight-centre.org), where he leads the Connected Health research programme. A physiotherapist by training, Brian is also Dean of Physiotherapy at UCD.

Brian is a champion for a Connected Health ecosystem in Ireland and has forged strong relationships with key clinicians, industry leaders, policy makers and research groups to drive advances in the field. Previously, he was a PI in the CLARITY Centre for Sensor Web Technologies (www.clarity-centre.org) and Director of the TRIL Centre (www.trilcentre.org), a research centre focused on technologies for independent living, which was funded by GE and Intel. As well as these companies, he has worked with a range of multinational and start-up companies such as BioMedical Research Ltd, Fujitsu Laboratories, and Shimmer to deliver innovative technology enabled solutions for areas such as rehabilitation medicine, COPD, and gerontology. His research has led to significant commercial opportunities in terms of exploitation of intellectual property by industry partners and spin out activity.

Professor Rosalind Eeles, FMedSci MA FCRP FRCR PhD
Professor of Oncogenetics and Honorary Consultant in Clinical Oncology and Cancer Genetics, The Institute of Cancer Research (ICR) and The Royal Marsden NHS Foundation Trust

Professor Ros Eeles is a leading expert in the field of cancer genetics and currently runs a research programme at The ICR on genetic predisposition to prostate cancer and management of individuals with BRCA mutations. She leads several large international consortia conducting research in genetic predisposition to prostate cancer and has authored over 313 papers. She is Co-PI on the ICGC – prostate cancer initiative in the UK.

She studied at the University of Cambridge and St Thomas’ Hospital Medical School, and went on to train in Clinical Oncology at The Royal Marsden. Her research training was in genetic predisposition to cancer at The ICR and The University of Utah, USA, and she has a PhD in Cancer Genetics from the University of London.

Professor Eeles is accredited in Clinical Oncology with a sub-specialism in cancer genetics – cancer risk assessment, genetic testing for predisposition to cancer, targeted screening advice based on cancer genetic risk assessment and the application of cancer genetics to cancer care. She has sat on several genetics advisory committees, including the Department of Health Genetics Advisory Committee of England, has given evidence to the House of Lords’ Select Committee on genomic medicine and edited a special edition of the Familial Cancer journal on delivery of cancer genetics services.

Professor Eeles is a Fellow of the Royal College of Physicians of London, a Fellow of the Royal College of Radiologists (Clinical Oncology Faculty), and Fellow of the Academy of Medical Sciences, UK.
Burkhardt Flemer graduated from Beuth-Hochschule, Berlin, Germany with a Major in Biotechnology. His PhD thesis, carried out in Cork under the supervision of Prof. Alan Dobson, encompassed studying the diverse microbial ecology of marine sponges and the discovery of novel, sponge-derived bioactive substances. After his PhD he joined Prof. Paul O’Toole’s laboratory in the Alimentary Pharmabiotic Centre, University College Cork to study the human gut microbiota. In his current role Burkhardt is analysing the microbiota profiles associated with cancers of the colon and rectum and the potential impact of the microbiota on tumour development.

Professor William M. Gallagher BSc PhD CBIol MSB
Director, BREASt-PREDICT (Irish Cancer Society Collaborative Cancer Research Centre), Professor of Cancer Biology, UCD School of Biomolecular & Biomedical Science, Conway Fellow, UCD Conway Institute, Co-Founder/Chief Scientific Officer, OncoMark Limited

Prof. Gallagher is Professor of Cancer Biology at the UCD Conway Institute, where he leads the Cancer Biology and Therapeutics Lab (www.cbtlab.ie). He is also the Chief Scientific Officer (CSO) at OncoMark Ltd., a private company he co-founded in 2007, which is centred on the development and application of biomarker panels and associated technologies (www.oncomark.com). A major focus of Prof. Gallagher’s research work is the identification and validation of candidate biomarkers of breast cancer and melanoma, with particular emphasis on translation of transcriptomic and proteomic datasets into clinically relevant assays.

Prof. Gallagher is currently Director of BREASt-PREDICT, which is the first Irish Cancer Society Collaborative Cancer Research Centre (CCRC) to be funded. This country-wide CCRC, which is supported to the level of €7.5 million by the Irish Cancer Society, runs from 2013 to 2018 and involves 6 academic institutions (UCD, TCD, RCSI, DCU, NUIG and UCC), as well as the not-for-profit clinical trials organisation, the All-Ireland Co-Operative Oncology Research Group (ICORG). In addition, he is co-PI and Deputy Co-ordinator of a major Science Foundation Ireland-funded Strategic Research Cluster, Molecular Therapeutics of Cancer (2009-2015) (www.mtci.ie); which involves comprehensive interactions with a range of major pharmaceutical, biotechnology and imaging companies. Prof. Gallagher also co-ordinates a number of EU programmes, including RATHER (www.ratherproject.com), which is focused on providing new rationalised therapy options for difficult-to-treat breast cancer subtypes (2011-2015); FAST-PATH (www.fastpathproject.com) which is focused on applying high-performance computing and automated image analysis to fast-track pathological assessment in prostate cancer (2011-2015); and SYS-MEL (www.sysmel.com), which is focused on applying systems medicine approaches to development of new diagnostic solutions in melanoma (2013-2017). His research group is also involved in several other FP7 research programmes as partners, including AngioTox (www.angiotox.com) and AngioPredict (www.angiopredict.com). Prof. Gallagher has received a number of awards based on his research work to date, including the BACR/AstraZeneca Young Scientist Frank Rose Award in 2004, the St. Luke’s Silver Medal Award in 2008 and the NovaUCD 2011 Innovation Award. Prof. Gallagher has had productive collaborative interactions with a variety of other industrial partners throughout his research, and has filed/been awarded multiple patents.
Dr. Christoph Hinzen received a diploma in biotechnology from the Westfälische Wilhelms-Universität Münster in 2008 studying intracellular transport processes in *S. cerevisiae*. In 2012 he earned his PhD degree at the department of biology of the Ludwig-Maximilians Universität München for his work on the function of Early B Cell Factor 2 in normal and malignant hematopoiesis of mouse. Since 2013 he works as a Postdoc at the Institute for Biological and Medical Imaging (IBMI), Helmholtz Center Munich, applying optoacoustic imaging technology to drive biomedical discovery in the fields of stem cells, immunology and oncology.

Dr. Andrew L. Kung, the Robert and Ellen Kapito Professor of Pediatrics, is Chief of the Division of Pediatric Hematology Oncology and Stem Cell Transplantation at the Columbia University Medical Center and Morgan Stanley Children’s Hospital. In this role, he oversees the clinical and research programs of the Division with the dual goal of providing outstanding care for patients today, and developing the next generation of therapies for children with cancer and blood disorders.

Dr. Andrew Kung received his M.D. and Ph.D. at Stanford University School of Medicine and completed a residency in Pediatrics at Children’s Hospital Boston. He served as a fellow in Pediatric Hematology/Oncology at the Dana-Farber Cancer Institute, where he also worked with Dr. David Livingston as a postdoctoral fellow. After completion of his training in 1999, Dr. Kung joined the faculty of the Harvard Medical School. He is the recipient of numerous honors and awards, including the Howard Temin Award from the National Cancer Institute, and the Eugene O’Kelly Award from the American Cancer Society. Dr. Kung is an elected member of the Society for Pediatric Research and the American Society for Clinical Investigation.

Informed by a multidisciplinary approach, Dr. Kung’s translational oncology research integrates molecular biology, genomics, bioinformatics, cell biology, preclinical models, drug development and molecular imaging. The research in his lab is focused on identification of novel cancer targets, cancer genomics, and development of targeted therapies. Dr. Kung has published over 160 scientific papers, and his research has been funded by the National Institutes of Health, American Cancer Society, St. Baldrick Foundation, and the Leukemia Lymphoma Society.

At Columbia, Dr. Kung has led the development of a comprehensive personalized medicine program for children with cancer and blood disorders. Through the Precision in Pediatric Sequencing (PiPseq) program, the power of comprehensive genome sequencing is made available to every child treated in the Division. Coupled with the Developmental Therapeutics Program and a robust clinical trials portfolio, the Division uses the latest technologies to formulate a personalized treatment and care plan for every patient that they treat.
Nicholas La Thangue, MA BSc PhD FRSE FMed Sci is Professor of Cancer Biology at the University of Oxford, and was previously Cathcart Professor of Biochemistry at the University of Glasgow, and before that a scientist at the Medical Research Council. He is a Fellow of the Royal Society of Edinburgh, a Member of the European Molecular Biology Organisation (EMBO), a Fellow of the Academy of Medical Sciences, a Fellow of the European Academy of Cancer Sciences, a Fellow of the Lister Institute and Professorial Fellow at Linacre College Oxford. He has authored over 200 publications and is an inventor on an extensive number of patents. His academic research is focussed on the molecular mechanisms that give rise to the abnormal proliferation of tumour cells, and translating novel mechanistic insights into new therapeutic opportunities. He has founded several biotech companies, most recently Oxford Cancer Biomarkers.

Professor Norman J Maitland, Professor of Molecular Biology  
Director of the YCR Cancer Research Unit

Professor Norman J Maitland graduated with First Class Honours in Biochemistry from the University of Glasgow, and holds a PhD in Cancer Studies from the University of Birmingham. Part of his Postgraduate research was carried out as a Robertson Research Fellow in Cold Spring Harbor laboratory (NY, USA). After postdoctoral research at the University of Edinburgh, he was appointed as the first UK Lecturer in Molecular Pathology at the University of Bristol in 1983, to apply basic molecular biology in the diagnosis and treatment of clinical problems, including childhood, cervical and oral cancers. He remained in Bristol until 1991, until his appointment to his current post as Yorkshire Cancer Research Professor of Molecular Biology at the University of York, where he is also Director of the YCR Cancer Research Unit.

His current research, with funding from Yorkshire Cancer Research and Prostate Cancer UK, is focused on studies of gene expression control and therapy for human prostate cancer, and also on exploitation of protein structure information. The Cancer Research Unit has particular expertise in the analysis of tissue stem cells from prostate in vivo and in vitro.

He is currently the Chairman of the Scientific Advisory Board for Orchid Trust, Bart’s Hospital, London.
Rosemary O'Connor joined the Department of Biochemistry at University College Cork in 1997. She was appointed as Professor of Cell Biology in 2007 and was Head of Department from 2008 to 2010. She holds a BSc. from University College Galway (NUIG) and a Ph.D. from NUI Maynooth. Research experience was gained in the USA and Germany in academia (at the Institute of Pathology of the University of Wuerzburg, Germany and the Wistar Institute at U PENN, Philadelphia); and in Industry (at Immunogen, Inc., Cambridge, Massachusetts).

Her research is focused on Insulin like Growth Factor actions particularly in cancer, neurodegeneration, and ageing. The current Science Foundation Ireland-funded PI programme explores how the IGF-1 Receptor Tyrosine Kinase is regulated by its own C terminal tail and by other interacting proteins; and also investigates the activity of newly discovered proteins in cancer progression. Prof O’Connor is a PI in the Irish Cancer Society Collaborative Research Centre, Breast Predict and is Director of the structured PhD training program in Cancer Biology and the MSc Programme in Molecular Cell Biology with Bioinnovation at UCC.

Dr Martin O’Halloran is an SFI-funded researcher in Electrical and Electronic Engineering at NUIG. He is currently developing a range of new electromagnetic-based imaging technologies. These technologies range from low-frequency Electrical Impedance Tomography to high-frequency Microwave Imaging. Dr O’Halloran is particularly focused on the translation of these technologies from "research bench to patient bedside" and is leading a large European COST Action in medical device translation, involving over 180 researchers from 24 countries.

In the last three years, he has secured 1.38 million euro in medical device funding. During that time, he has been awarded over 18 national and international research and commercialisation awards, including the Ireland-Canada University Foundation Dobbins Scholarship and the International Union of Radio Science Young Scientist Award (twice in 2011 & 2013). This year, he was awarded NUIG’s inaugural Early-Stage Researcher of the Year, and Engineers Ireland Chartered Engineer of the Year.
Dana Tsui - Dana did her PhD with Prof Dennis Lo from the Chinese University of Hong Kong and developed non-invasive approaches to diagnose genetic diseases of the foetus using cell-free DNA that shed from the foetus in to the blood circulation of pregnant women. She then moved to the UK and joined Dr Nitzan Rosenfeld in Cancer Research UK Cambridge Institute, University of Cambridge, to develop non-invasive cancer diagnostics tool using similar strategy: using cell-free DNA that shed from the tumours in to the blood circulation of cancer patients. Dana and Dr Rosenfeld’s team have established robust methodology for accurate measurement of circulating tumour DNA from plasma samples, and demonstrated its potential to monitor the evolving genetic information of cancer in a safe and non-invasive way.

Jo Vandesompele has a Master of Science in Bioscience Engineering (1997), a PhD in Medical Genetics (2002) and is professor in Functional Cancer Genomics and Applied Bioinformatics at Ghent University, Belgium since 2007. He is author of more than 180 scientific articles in international journals, including some pioneering publications in the domain of quantitative PCR. His H-index is 57, with 22,748 citations (Google Scholar, January 2015).

The main themes of his research are the study of non-coding RNAs in cancer, the study of the genetic basis and identification of prognostic gene signatures in the childhood tumor neuroblastoma, and the development of nucleic acid quantification strategies. His lab uses a combination of high-throughput genomics technologies and bio-informatics tools to answer the various research questions. The Vandesompele lab comprises 7 PhD students, 4 postdocs, and 5 research technicians and resulted in 8 PhD theses since 2006.

Jo is also the co-founder and CSO of Biogazelle, a Ghent University spin-off company. Biogazelle’s mission is to understand the transcriptome for next generation diagnostics and therapeutics through excellence in science and technology.
Valerie M. Weaver, Ph.D., is the Director of the Center for Bioengineering and Tissue Regeneration in the Department of Surgery. She earned her Bachelor of Science degree at the University of Waterloo in Biochemistry/Chemistry in 1985, continuing her education for an Honors Bachelor of Science in Biochemistry at the University of Ottawa (graduating Summa cum Laude). Dr. Weaver earned her doctorate degree, Ph.D, from the University of Ottawa in Biochemistry in 1992.

After receiving her doctorate, Dr. Weaver was awarded the Molecular Cell Biology and Apoptosis Research Group and the Institute for Biological Sciences of Canada Postdoctoral Fellow from 1992-1994. From 1994-1998, she was distinguished as the Postdoctoral Fellow at the Lawrence Berkeley National Laboratory at the University of California, Berkeley, in the Cancer and Cell Biology Group, Life Sciences Division.

Following her fellowships, Dr. Weaver has held principle positions within academia. Prior to her appointment at UCSF, she was an assistant professor in the Department of Pathology at the University of Pennsylvania she was an Adjunct Associate Professor with the Department of Bioengineering at the University of Pennsylvania from 2006 to 2009. In her current position, she is a Professor with the Department of Surgery at the University of California, San Francisco.

Additionally, Dr. Weaver is the Director for the Center for Bioengineering & Tissue Regeneration with the Department of Surgery and UCSF, an Associate Professor in the Department of Anatomy at UCSF, and Adjunct Associate Professor for the department of Bioengineering at UPenn. From 1999 to 2006 she was a member at the Institute for Medicine and Engineering at UPenn. She joined the department of Surgery at UCSF in late 2006 as Associate Professor and was appointed dirctor of the Center for Bioengineering and Tissue Regeneration. I became full Professor in 2010. I also hold cross appointments in the Department of Anatomy and the Department of Bioengineering and Therapeutic Sciences. She is also Co Directing the Bay area Physical Sciences and Oncology center and the UCSF brain Tumor Microenvironment Center.

Dr. Weaver’s extensive honors and awards include: the Breast Cancer Scholar Award with the Department of Defense, the International Society of Differentiation Young Investigator Travel Award, the Alberta Heritable Foundation for Medical Research Lecturer Award, and the University of California Breast Cancer Research Program Postdoctoral Grant.


*(Please note that the above Biography not in final form when going to print and therefore may contain some inaccuracies)*
PLENARY SPEAKER ABSTRACTS

(Alphabetical)

A PRC2 independent role of Polycomb-like 1 (PCL1/PHF1) to stabilise p53 and maintain cellular quiescence
Gerard L. Brien¹, Andrei V. Krivtov², Emilia Jerman¹, Conor J. Kearney¹, Ariane Watson³, Gerard Cagney³, Séamus J. Martin¹, Scott A. Armstrong², Adrian P. Bracken¹
1. Smurfit Institute, Trinity College Dublin, Dublin 2, Ireland.
2. Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY 10065, US.
3. Conway Institute, University College Dublin, Dublin 4, Ireland.

The Polycomb-like proteins (PCL1-3) function to recruit and maintain the PRC2 complex on target genes. However, it is unclear why three Polycomb-like proteins with apparent functional redundancy exist in mammals and how they contribute to proliferation control and cancer. Here we show that PCL1-3 play divergent roles in cell growth control. Ectopic expression of PCL1-3 recruit the PRC2 complex to repress the INK4A gene. However, only PCL2 and PCL3 confer an INK4A dependent growth advantage in 3T3 assays. Instead, PCL1 mediates a p53 dependent proliferative block by directly binding to and stabilizing p53 leading to the activation of p53 target genes, including CDKN1A. We characterize the PCL1-p53 interaction and show that it occurs off chromatin and is independent of the PRC2 complex. Tellingly, unlike PCL2 and PCL3, PCL1 is highly expressed in quiescent hematopoietic stem cells and is a direct transcriptional target of p53 suggesting the existence of a feed forward regulatory loop between PCL1 and p53. Finally, we provide functional evidence for this by demonstrating that the depletion of PCL1 phenocopies the defects in maintaining cellular quiescence associated with p53 loss. Thus, our study not only reveals a PCL1-p53 regulatory axis required to stabilize cellular quiescence, but also demonstrates a critical PRC2 independent function of a Polycomb-like protein.

RNAi screen identifies new regulators of epithelial cell-cell adhesion
Vania Braga, Faculty of Medicine – Imperial College London

Dynamic remodelling of junctions supports the maintenance of tissue integrity, morphogenesis and homeostasis. Conversely, tumour de-differentiation in epithelial tissues is accompanied by disruption of cell-cell contacts and re-writing of signalling to drive uncontrolled proliferation and migration. In epithelia, stabilization of E-cadherin contacts relies on the reorganization of the cortical actin cytoskeleton to maintain clustered receptors. Actin cytoskeleton regulators that drive specific F-actin remodelling at adhesive complexes have been a major focus of recent research because of their key role during malignant progression.

Although actin polymerization and contraction play a key role in the preservation of cell-cell contacts, the contribution of additional actin functions and specific cytoskeletal proteins involved are not well understood. I will discuss a novel RNAi screen to identify new actin regulators of E-cadherin complex stability at cell-cell contacts. Optimized phenotypic clustering and custom-made enrichment analysis highlight the combinatorial actions of distinct proteins and their actin
remodelling properties at junctions. Our computational analyses allow interrogation of novel signalling pathways relevant for epithelial differentiation and functionality.

Brian Caulfield, Lead Investigator ARCH Centre, Dean of Physiotherapy, University College Dublin

It is well known that our healthcare systems are under significant stress. Thankfully, we now have the capability to leverage a range of technology supports that could help to underpin a much more efficient and proactive approach to management of health as opposed to the current reactive system that is based on management of illness. One of the key enabling technology developments that we have seen in recent years is the massive expansion in our capability to generate data relating to human performance and health using mobile, wearable, and ambient sensors. These sensors are becoming more accessible and common with every passing day and we have entered the age where multimodal sensing and quantification of human behavior and performance on a 24-7 basis is a reality. If we combine this sensing capability with other sources of data, like genetic or clinical data, we can easily see the potential for generation of new knowledge leading to a truly connected, integrated approach to management of health where faster, more informed decisions about health can be made by relevant stakeholders and the end user can be fully empowered and engaged in management of their own health. However, getting from here to there is not straightforward. And we have a way to go in terms of understanding how we can optimally acquire and leverage all the data that we can generate for best effect. This talk will outline how we are addressing this challenge in the Insight Centre for Data Analytics, by bringing researchers from a variety of disciplines and sectors together with data scientists to better understand how we can develop new insights, knowledge and applications using personal sensed data.

Genetic Predisposition to Prostate Cancer and Its Clinical Applications
Prof Ros Eeles, Professor of Oncogenetics, The Institute of Cancer Research and Royal Marsden NHS Foundation Trust, London, UK

There is evidence from epidemiological and twin data that there is genetic predisposition to the development of prostate cancer. We now know from large consortia studies in over 100 000 samples that there are 100 common variants in the genome which are associated with prostate cancer in populations. We are now at the stage where populations can be risk stratified based on the typing of such variants and the levels of risk for the population at the top 10% of the risk profile are such that targeted screening studies should be conducted to assess if these advances will be helpful for the application of genomics to public health. There are also rarer variants, most in the DNA repair pathway, which are particularly associated with more aggressive prostate cancer which already have implications for the management of such men and targeted screening of men harbouring mutations in these genes. To date, most of the data have been obtained from men with germline mutations in the breast/ovarian predisposition genes, \( BRCA1 \) and \( BRCA2 \), and results from an international consortium will be presented which will alter international screening guidelines.
“CRC-Associated Microbiota Profiles Are Linked With Distinct Mucosal Gene Expression Profiles”
Burkhardt Flemer, PhD, Alimentary Pharmabiotic Centre & Dept., Microbiology, University College Cork

Although several bacteria such as *Fusobacterium nucleatum* have been linked with colorectal cancer (CRC), a unifying microbiota signature across multiple studies has not been identified. In addition to methodological variance, heterogeneity may be due to both microbial and host response differences, which were addressed in this study. We conducted a prospective study of the colonic microbiota and the expression of specific host response genes using paired faecal and mucosal samples (‘on’ and ‘off’ the tumor, proximal and distal) from 36 patients undergoing surgery for colorectal cancer and 37 controls. The microbiota profiles were analysed using 16S-rRNA amplicon sequencing, and expression of host genes involved in CRC progression and immune responses was quantified by RT-qPCR.

The microbiota associated with distal and proximal cancers was significantly different. Six bacterial co-abundance groups (CAGs) associated with the gut mucosa were defined, at least one of which was of increased abundance in any CRC patient. Furthermore, the CAGs interacted in such a way that cancer individuals could be grouped into three clusters based on the abundance of each CAG in each individual. The different CAGs were also associated with different mucosal gene-expression profiles. Two of the CAGs defined (named after their most abundant member, Bacteroides fragilis and Prevotella copri, respectively) were both significantly more abundant in cancer patients, and were differentially correlated with the expression of host response genes for TLRs, CCL20 and STAT3. In conclusion, CRC-associated microbiota profiles are linked with distinct mucosal gene-expression profiles. Compositional changes in the microbiota are not restricted to cancerous tissue and differ between right- and left-sided cancers.

BREAST-PREDICT: LEVERAGING THE POWER OF SYSTEMS MEDICINE TOWARDS PERSONALISED CANCER THERAPY
William Gallagher, Breast-Predict

Although the incidence of breast cancer is steadily increasing, mortality rates are decreasing. This means that the majority of women with breast cancer now survive, making it even more important to tailor therapy appropriately, reducing toxicity and long-term side-effects. Conversely, despite early intervention, some patients still succumb to this disease, and improved stratification approaches and more diverse treatment options are required.

This presentation will outline how the Irish Cancer Society Collaborative Cancer Research Centre, BREAST-PREDICT (www.breastpredict.com), is addressing these unmet needs for breast cancer patients by bringing together researchers from multiple disciplines to solve key problems in breast cancer research. The BREAST-PREDICT model incorporates the diverse skill sets of several disciplines, including biological, mathematical, computational, pharmaco-epidemiological and systems medicine approaches, in order to determine how to treat individual breast cancer patients according to the particular characteristics of their own cancer.

One of the central goals of BREAST-PREDICT is to consolidate existing breast cancer biobank and data resources, and make these available for research projects. Pharmaco-epidemiology approaches are also being employed to determine the effects of previous drug exposures on outcome, and multi-omic analyses are being performed on longitudinal samples from primary and metastatic tumours, in order to map the molecular evolution of breast cancer. The group is also applying systems medicine approaches in order to identify new therapeutic targets and combinatorial treatment strategies for specific breast cancer subtypes, and is developing new tools for improved prediction of patient outcome and response to treatment. The presentation will outline the benefit of this approach and how it can be used to fast-track progress towards personalised therapy in breast cancer.
“Advances in Cancer Imaging Using Multispectral Optoacoustic Tomography”
Dr Christoph Hinzen (Technische Universität München, Germany)

Optical imaging is unequivocally the most versatile and widely used visualization modality in the life sciences. Yet it is significantly limited by photon scattering, which complicates imaging beyond a few hundred microns. For the past few years however there has been an emergence of powerful new optical imaging methods that can offer high resolution imaging beyond the penetration limits of microscopic methods. These methods can prove essential in cancer research. Of particular importance is the development of multi-spectral opto-acoustic tomography (MSOT) that brings unprecedented optical imaging performance in visualizing anatomical, physiological and molecular imaging biomarkers. Some of the attractive features of the method are the ability to offer 10-100 microns resolution through several millimetres to centimetres of tissue and real-time imaging. In parallel we have now achieved the clinical translation of targeted fluorescent probes, which opens new ways in the interventional detection of cancer in surgical and endoscopy optical molecular imaging. This talk describes current progress with methods and applications for in-vivo optical and opto-acoustic imaging in cancer and outline how new opto-acoustic and fluorescence imaging concepts are necessary for accurate and quantitative molecular investigations in tissues.

IMPROVING THE PREDICTIVE VALUE OF PRECLINICAL MODELS THROUGH THE CO-CLINICAL TRIAL PARADIGM
Andrew L. Kung, MD, PhD, Professor of Pediatrics, Columbia University Medical Center

Studies in preclinical models play a critical role linking basic discovery to clinical trials. In the cancer disease area, however, there have been longstanding concerns about the predictive value of preclinical cancer models. Nearly 90% of new chemical entities that enter into clinical testing for cancer eventually fail, the majority due to lack of efficacy. The co-clinical trial paradigm seeks to improve the predictive value of preclinical models by emulating the design of human clinical studies. First, models that more closely emulate human cancer are utilized including genetically-engineered mouse models and patient-derived xenograft models. Second, study endpoints are aligned with human clinical endpoints, including the use of non-invasive imaging. Finally, the measures of success in preclinical studies are aligned with desired objective response criteria in human studies. Co-clinical studies mirror preclinical and clinical studies with the goal of better predicting the outcome of human clinical trials. The rapidity of preclinical studies also allows for the identification of biomarker strategies that predict response and resistance, and the validation of rapid response measures that can be incorporated into human clinical studies.

Epigenetic drugs: exploring new pathways for manipulating tumour cell death
Professor Nick La Thangue, University of Oxford, Department of Oncology, Old Road Campus Research Building, Roosevelt Drive, Oxford OX3 7DQ

Aberrant epigenetic control has been strongly linked to tumourigenesis. We have used a genome-wide loss-of-function screening platform, to identify the critical pathways and targets through which inhibitors of histone deacetylase (HDAC) kill tumour cells. Several novel and unexpected pathways were identified that impact for example on proteostasis and signal transduction. In turn, this approach has allowed us to identify new proteins which are targets for epigenetic modification, and address the role of the modification in the control of protein activity and drug sensitivity. The information has enabled us to deliver a personalised medicine approach to studying new epigenetically acting drugs in clinical trials.
THERAPY RESISTANCE IN HUMAN PROSTATE CANCER
Norman J Maitland, PhD, YCR Professor of Molecular Biology and Director, YCR Cancer Research Unit, University of York, UK

Current treatments for prostate cancer provide an improved response compared to those available before the millennium. There are now targeted next generation inhibitors, with increasing potency, but all have one common property: they are programmed to fail. Once a tumour is resistant, median survival is around 2 years, despite improved chemotherapies and secondary use of endocrine inhibitors.

In 2005, my laboratory first described a phenotype for the tumour initiating cells in prostate cancers, proposing that these cells also represented a therapy resistant fraction, capable of reinitiating tumour growth both within the prostate and at extraprostatic (lymph node and bone) sites. We recently directly tested this hypothesis for radiotherapy (see Frame et al, Br J Cancer, 2013), seeking to determine the mechanism by which the tumour initiating cells were able to both resist and rapidly repair DNA lesions.

As part of an EU-funded programme (PRONEST), we have also mapped both genome methylation and histone code in the different cell types, which comprise a human prostate acinus, and in high grade prostate cancers. Whilst genomic methylation patterns were distinctive, they offered few clues to the nature of the resistant phenotype. The more readily reversible histone code, and its effects on chromatin condensation were clearly implicated, as the resistant cells adopted a unique chromatin conformation after non-lethal irradiation, which could be unwound using low doses of histone deacetylase inhibitors, thereby restoring 40-50% of radiosensitivity.

However, more potent mechanisms of resistance were uncovered by mapping the expression patterns of non-coding microRNA in the individual cell types. We have devised an algorithm which allowed an unbiased correlation of the expression of microRNA to cellular functions (Rane et al, European Urol. 2014 and submitted for publication) instead of single mRNAs. The strategy was designed to overcome one of the weaknesses of many studies, which select familiar or significant genes from the many controlled by a single miRNA for more detailed study. The major conclusions from our analysis were (i) Most miRNA in prostate are concerned with maintaining specific cellular subtypes i.e. in cellular differentiation as opposed to carcinogenesis (ii) The miRNA expression patterns in tumour initiating cells are most closely aligned with those in not only embryonic stem cells but also the total cell miRNA from therapy resistant prostate cancers and (iii) The miRNA -associated function most associated with tumour initiating cells, and normal epithelial stem cells, was resistance to ionising radiation. The two top miRNA candidates, showing highly significant changes, are miR548c-3p and miR99a/100, which are respectively up and down regulated in the SC compartments. Manipulation of both miRNA and target mRNA levels were able to directly confer and abrogate radiation sensitivity.

Such epigenetic mechanisms offer cells a rapid, multivalent and reversible response to therapeutic insults, which preserve stem cell integrity in normal tissues, and when present in the cancer stem cells/tumour initiating cells, result in resistance to therapies. A further consequence of treatments was revealed when whole tumour population dynamics were studied in vitro. When a drug effectively reduced tumour volume by cytotoxicity, a loss of stem cell quiescence was observed, which ‘repairs’ the tumour in much the same way as stem cell expansion drives repair of normal tissues after insult or injury. The outcome in cancer patients is an expansion of the most metastatic cells, resulting in a catastrophic relapse with multiple metastatic lesions derived from the original tumour: precisely what is seen in prostate cancers, even after primary endocrine treatment, and in glioblastomas after radiotherapy.
IGF and Adhesion Signalling in Cancer Cell Stemness, EMT and Therapy Resistance
Dr Rosemary O’Connor, UCC Cork

The Insulin-like Growth Factor 1 system is strongly associated with cancer susceptibility and progression. However, drugs that target this system at the level of the Receptor (IGF-1R) or downstream kinases have not been generally effective. Many reasons have been suggested to account for this unexpected failure. These include the complex biological regulation of this essential signaling system. IGF-1R expression levels and activation levels generally cannot be correlated in cancer cells. This suggests that IGF-1R activity and susceptibility to inhibition is modulated by other cellular and extracellular proteins. Strong candidates for such modulation are found in cell-matrix signaling complexes where activated IGF-1R is located with integrins, focal adhesions kinase (FAK), RACK1 PDLIM2 and other cytoskeleton proteins. This talk will discuss how adhesion complex proteins modulate IGF-1R signaling during cancer progression and adaptive responses to therapy. Identifying key modulators of IGF-1 signalling and biomarkers of activity will be important for developing effective therapeutic strategies to inhibit this pathway cancer.

Exploring New Frontiers in the Diagnostic and Therapeutic Applications of Microwaves
Martin O’Halloran, B.E., M.A. PhD., CEng.

The current de-facto method for detecting non-palpable early stage breast cancer is X-ray mammography. Despite the fact that X-ray mammography provide high-resolution images at low radiation doses, its limitations in terms of sensitivity and specificity are well documented. One of the most promising alternative imaging modalities is Microwave Imaging. The rationale for using Microwave Imaging to detect breast cancer is the dielectric contrast that exists between normal and cancerous breast tissue at microwave frequencies. Furthermore, the dielectric properties of tumours show no significant variation with age, suggesting that the dielectric contrast exists at the earliest stage of tumour development. Therefore, when a microwave pulse illuminates the breast, the dielectric contrast between healthy and cancerous tissue in the breast tissue will ensure that a reflected wave is generated, which can be used to detect, localise and classify tumours. Microwave imaging is non-ionising, non-invasive, does not require uncomfortable breast compression, and is potentially very low cost.

From a therapeutic perspective, microwave hyperthermia can be used as a treatment for chronic cancer, particularly when used in combination with radiotherapy or chemotherapy. The feasibility of microwave hyperthermia for cancer treatment has been clearly shown in the medical literature, revealing the close relation between microwave hyperthermia treatment quality and cancer treatment outcome. However, bringing these technologies from "research bench to patient bedside" will require transdisciplinary experience and expertise, with particular support from the medical community.
All cells experience force and possess mechanosensory mechanisms that enable them to detect physical stimuli and to transduce these cues into biochemical signals that modulate cell and tissue behavior. Tumors consistently exhibit abnormally high cell and tissue level forces and transformed cells demonstrate perturbed mechanosensing. We have been studying how cells sense and transduce mechanical cues to regulate their behavior and the physiological relevance of altered mechanical force in cells and tissues to tumor formation and metastasis. Using an array of in vitro and in vivo models we find that the ECM progressively stiffens in various solid tumors including breast, pancreas, glioblastoma and skin, and we have been able to show that ECM tension modulates transition to invasion and metastasis. ECM stiffness compromises tissue morphogenesis and destabilizes tissue architecture by promoting integrin focal adhesions which potentiate growth factor receptor signaling and induce cytoskeletal remodeling and actomyosin contractility. By enhancing integrin signaling to ERK and PI3 kinase, ECM stiffness potentiates the tumorigenic effects of oncogenes and promotes tumor progression and aggression. Recently, we could show that a stiff ECM promotes tumor aggression by compromising the tissue vasculature to induce hypoxia and activate HIF1a and by directly activating HIF1a. High tumor HIF1a in turn alters tissue metabolism, stimulates inflammation and induces an epithelial to mesenchymal transition. We also find that many oncogenes such as Ras or ErbB2 or the loss of signaling in tumor cells such as the absence of TGF beta increase tumor cell contractility. We find that high tumor cell tension also induces malignancy and contributes to tumor aggression by inducing ECM remodeling and stiffening to drive focal adhesion assembly and enhance integrin signaling in a vicious feed forward circuit. The relevance of these findings to tumor prevention and therapy will be discussed. (Supported by DOD BCRP W81XWH-05-1-0330 and NIH U54CA163155-01, 1U01CA151925-01, RO1CA138818-01A1, 2RO1CA085492-11A1, and R01CA140663-01A2 to VMW, NIH 1U01ES019458-01 to ZW and U54CA143836-01 to J.L., NCI P50CA058207 to VMW, CP, LC & SH and KG110560 to S.H. & L.C.).
PRE-CLINICAL EFFICACY OF A NOVEL ANTI-INVASION NANOPARTICLE THERAPEUTIC IN COMBINATION WITH THE ANGIOGENESIS INHIBITOR BEVACIZUMAB FOR THE TREATMENT OF Glioblastoma

D.W. Murray1, P O’Halloran1,2, M Jarzabek 1, B MacCarthy 1, J.N. Sarkaria 3, R.M. Schifferlers 4, M Symons 5, A.T. Byrne 1,1, Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin 2, National Neurosurgical Department, Beaumont Hospital, Dublin 9, 3 Department of Radiation Oncology, Mayo Clinic, Rochester, MN 55905, USA. 4 Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht, The Netherlands, 5 Department of Oncology and Cell Biology, Feinstein Institute for Medical Research at North Shore-LIJ, Manhasset, NY 11030, USA

Glioblastoma (GBM), a highly invasive primary brain tumour, remains an incurable disease (1). Angiogenesis, the formation of new blood vessels, is a defining feature of GBM (2). Targeting GBM angiogenesis with Bevacizumab (Bev) is associated with improved progression free survival, but may also significantly enhance local tumour invasion (3). RhoGTPases and their activators, guanine nucleotide exchange factors (GEFs), play central roles in the invasive process (4). Herein, we sought to identify and target GEFs of importance in mediating GBM invasion with a view to improving Bev response. We report a novel strategy by which GBM tumours invade and proliferate via overexpression of the GEF β-PiX which was shown to be increased at the invasive edge in 74% of GBM tumours assessed (n=19) compared with tumour core (5). We have further shown that siRNA-mediated knockdown of β-PiX in GBM patient-derived xenograft cell cultures and cell lines resulted in decreased cell invasion in 3D, cell proliferation and survival assays in vitro. Furthermore, we have uncovered a role for β-PiX expression in endothelial cell function, as knockdown of β-PiX inhibits HUVEC cell migration in vitro. To interrogate in vivo effects, we established an imagable orthotopic model of invasive GBM in mice, by stereotactically implanting GBM cells stably overexpressing firefly luciferase (U87R-GFP-luc2). β-PiX-(human and mouse) specific siRNA or scrambled control siRNA (2 x 10 µg i.c.) were delivered to tumours using an αVβ3 integrin targeting-(RGD peptide conjugated)-nanoparticle (InVivoPlex Aparna Bio Corp, Rockville, MD). Animals were treated with- or without- Bev (6 x 10mg/kg i.p. over 12 days). Initial data has shown that animals treated with β-PiX siRNA nanoparticles in combination with Bev had an improved median survival of 100 days compared with Bev- alone treated mice (median: 88 days). Our data may support the future clinical use of a β-PiX-targeting nanoparticle therapeutic, to improve Bev outcomes in GBM.

THE DEVELOPMENT OF ADAM22 AS A PREDICTIVE MARKER FOR ENDOCRINE RESISTANT BREAST CANCER AND AN LGI1 MIMETIC AS A COMPANION THERAPEUTIC

B Doherty1, J Bolger1, D McCartan1, C Byrne1, D Vareslija1, S Cocchiglia1, A Hill2, L Young1
1Endocrine Oncology Lab, RCSI, Dublin, 2Surgery, Beaumont Hospital, Dublin

Endocrine therapy is the first line treatment strategy for oestrogen receptor positive (ER+) breast cancer. Despite improved survival rates for the majority of these patients, approximately one third will develop resistance to endocrine based therapy. Our lab has previously shown that high expression of the nuclear receptor co-activator SRC-1 is significantly associated with endocrine resistance and the development of metastasis. Recently, our lab identified ADAM22 as a downstream target of SRC-1 in endocrine resistant breast cancer. Knockdown studies showed ADAM22 to play a key role in migration, poor differentiation and anchorage independent growth. Furthermore, in a large patient cohort, ADAM22 was shown to be a strong independent predictor of poor disease free survival. To further elucidate the mechanisms through which ADAM22 functions in endocrine resistant breast cancer, the ADAM22-dependent signalling network was examined using a reverse phase protein array. The results suggest ADAM22 may play an anti-apoptotic role in endocrine resistant breast cancer via interactions with pro-apoptotic proteins. The endogenous ligand to ADAM22, LGI1, has been shown to have tumour suppressing functions in several cancer types. Treatment of endocrine resistant cells with recombinant LGI1 showed comparable results to those seen in ADAM22 knockdown studies, providing the rationale to use LGI1 as a basis for a targeted therapy. Consequently, an LGI1 mimetic was synthesised, based on the binding domain with ADAM22. The peptide mimetic has shown good efficacy in vitro, inhibiting migration and anchorage independent growth, while promoting differentiation in endocrine resistant cell lines. Preliminary results from in vivo studies suggest the mimetic is effective in inhibiting the development of metastases in an LY2 xenograft model. Taken together, these results further support ADAM22 as a target for endocrine resistant breast cancer.
CDK7: A MARKER OF POOR PROGNOSIS AND TRACTABLE THERAPEUTIC TARGET IN TRIPLE-NEGATIVE BREAST CANCER

B Li¹, T Ni Chonghaile¹, Y Fan¹, R Kinger¹, AE O'Connor¹, DP O'Connor¹, WM Gallagher¹
¹Cancer Biology and Therapeutics Laboratory, Conway Institute, University College Dublin, Dublin

Triple-negative breast cancer (TNBC) is defined by absent expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), and accounts for 15% - 20% of all breast cancer. TNBC patients commonly exhibit poor prognosis. Currently, there is a lack of targeted therapies for this difficult-to-treat subset of breast cancer patients. During tumour development and progression, alterations in cellular behaviour are frequently linked with kinase expression and activity. Here, we aimed to identify novel kinase targets that may play a pivotal role in the progression of TNBC and, thus, offer new therapeutic vantage points. We re-analysed a publicly available TNBC microarray dataset (n=579) and identified 9 kinases that were associated with survival. From this screen, CDK7 (cyclin-dependent kinase 7) was found to be correlated with poor relapse-free survival (p=0.00879, HR=1.989, CI=1.457-2.717). The value of CDK7 as a marker of poor prognosis was further validated via assessment of mRNA expression data (extracted from transcriptomic studies) within another TNBC cohort (n=116), as well as via tissue microarray analysis within the same TNBC cohort (n=109). CDK7 protein expression was associated with reduced breast cancer-specific survival (p=0.017, HR=2.516, CI=1.189-5.324), recurrence-free survival (p=0.019, HR=2.208, CI=1.123-4.344) and distance recurrence-free survival (p=0.013, HR=2.506, CI=1.185-5.299). A similar, albeit not significant, trend was seen within the same cohort in respect to link between CDK7 mRNA expression and poor outcome, across these 3 survival indices. To test the strategy of therapeutically targeting CDK7, two TNBC breast cancer cell lines (BT549 and MDA-MB-231) were selected for functional studies to evaluate phenotypic alterations post shRNA-mediated CDK7 knockdown. CDK7 silencing led to decreased cell proliferation, colony formation and migration in vitro. CDK7 down-regulation also increased sensitivity to two chemotherapeutic agents, namely doxorubicin and carboplatin. BS-181 and THZ1, two highly specific CDK7 inhibitors, induced G2/M cell cycle arrest and caused apoptosis in both TNBC cell lines evaluated. Moreover, THZ1 demonstrated 1000-fold higher potency than BS-181. Downstream signalling studies revealed that BS-181 and THZ1 attenuate TNBC tumour growth by inhibiting global RNA transcription and cell cycle progression, as indicated by decreased levels of phospho-RNA polymerase II, phospho-CDK9, phospho-CDK1 and phospho-CDK2. These results demonstrate that CDK7 is a promising marker of poor prognosis in TNBC. Moreover, our data also suggest that targeting CDK7 may be a useful therapeutic strategy for TNBC.
Synergistic combination of oncolytic reovirus type 3 (Dearing) and radiotherapy in melanoma is mediated through enhanced viral replication and mitochondrial apoptotic signalling.

G McEntee¹, J Kyula¹, V Roulstone¹, C Gregory¹, M Coffey², KJ Harrington¹
¹Targeted Therapy Team, Institute Cancer Research, London UK, ², Oncolytics Biotech. Inc, Calgary Canada

Oncolytic viruses selectively target and replicate in cancer cells, providing us with a unique tool with which to target and kill tumour cells. These viruses come from a diverse range of viral families including reovirus type 3 Dearing (RT3D), a non-pathogenic human double-stranded RNA oncolytic virus, which has been shown to be an effective therapeutic agent, both as a mono-therapy and in combination with traditional chemotherapeutic drugs. Here we show that, in melanoma, the effect of RT3D and ionizing radiation (RT) combination therapy is to enhance both viral replication and mitochondrial apoptotic signalling. An in vitro study of RT3D and RT in multiple melanoma cell lines, of varying genetic backgrounds (BRAF/Ras mutant and wild type), indicates that the combination therapy leads to an increase in viral replication through the up-regulation of CUG2 (cancer up-regulated gene 2) and the subsequent down-regulation of phospho-PKR and pEl2Falpha allowing for the translation of viral proteins. Activation of intrinsic apoptotic signalling also plays a role in the enhanced cytotoxicity observed in the combination group. Pro-apoptotic BCL2 proteins, including Bax and Bak show an increase in expression with a corresponding decrease in pro-survival proteins such as phospho-BCL2(S70). The downstream decrease in the expression of the IAP (inhibitor of apoptosis) family of proteins and the activation of both caspase 9 and caspase 3 is a further indication of mitochondrial apoptotic signalling. Preliminary in vivo data, using a BRAF mutant melanoma xenograft model, correlates with the in vitro data with the RT3D and RT combination cohort displaying a reduced tumor volume when compared to both control and monotherapy cohorts.

EphA2 is an essential driver of invasion and associated with the poor prognostic stem-like subgroup in early stage colorectal cancer.

P Dunne¹, D McArt¹, J Blayney¹, M Salto-Tellez¹, P Johnston¹, S Van Schaeybroeck¹
¹CCRCB, Queens University Belfast, Belfast

Background: Colorectal cancer (CRC) is the third most common cancer worldwide. Among patients with early stage CRC, there are subsets of patients who will not benefit from adjuvant 5-FU based treatment, highlighting the need for improved therapy options for early stage disease. Receptor tyrosine kinase analysis of our previously published pre-clinical adjuvant invasive cell line models identified the Ephrin receptor EphA2 as a key driver of an invasive phenotype in CRC.

Results: Our comprehensive approach identified overexpression of Ephrin receptor EphA2 in each of our invasive isogenic models. EphA2 silencing inhibited migration and invasion in a panel of CRC cells. Regulation of EphA2 expression was shown to be tightly control by both transcription and receptor degradation. EphA2 has previously been reported as an essential factor for tumour formation in vivo and in-line with this our EphA2 overexpressing invasive subpopulations displayed an increased ability to form colonies. Importantly, high EphA2 mRNA and protein expression were found to be associated with poor overall survival in early stage CRC tissues and correlated with the stem-like subgroup. Targeting of EphA2 was sufficient to inhibit invasion and remove the CD44 positive stem like cells from the overall population. Conclusion Using our novel pre-clinical adjuvant models we have identified EphA2 as a key driver of invasion and migration in CRC. We have also revealed novel transcriptional and proteasomal regulators of EphA2. In addition, we show that EphA2 is associated with the stem-like subgroup and a poor prognostic marker in the adjuvant disease setting. Taken together these findings highlight EphA2 as an important novel target for the most aggressive subgroup of CRC tumours.
Erythropoietin receptor promotes c-Myc driven breast tumourigenesis
KB Matchett¹, KK Chan¹, JA Coulter¹, MA Davidson¹, HF Yuen², CM McCrudden¹, P Maxwell¹, M El-Tanani¹, TR Lappin¹
¹Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast
²Institute of Molecular and Cell Biology, A*STAR, Singapore

Erythropoietin (Epo) regulates the survival and differentiation of erythroid progenitor cells through activation of its cell surface receptor, EpoR. In haematopoietic cells, EPO stimulates EpoR homodimerisation, leading to JAK2 autophosphorylation, STAT5 activation and triggering of the MEK/ERK and PI3K/Akt signalling pathways. EPO was once thought to act solely on erythroid compartment, but is now recognised to be pleiotropic cytokine. Functional erythropoietin receptor (EpoR) has been identified on a variety of normal and tumour cell lineages, such as normal brain, breast carcinoma, melanoma, and cervical cancer. Recent studies have reported that recombinant human EPO (rHuEpo) antagonises tamoxifen and trastuzumab treatment in estrogen receptor-α (ERα) positive and epidermal growth factor receptor-2 (HER2) positive breast cancer patients, respectively, leading to reduced patient survival. Indeed, elevated EpoR expression correlates with higher local cancer recurrence. However, the role of EpoR in mediating breast tumourigenesis is unknown. Here, we show that EpoR mediates epithelial to mesenchymal transition (EMT) in breast cells by regulating Snail, β-catenin, Slug, E-cadherin and fibronectin. EpoR knockdown impaired PI3K/AKT and MAPK signal transduction, inhibited cell growth, reduced cell migration and invasion, and triggered apoptosis through the induction of Bim expression in breast cancer cells. We identified c-Myc as an underlying mechanism of EPO/EpoR-induced breast cancer growth. Indeed, analysis of datasets of 1,283 breast cancer patients revealed a significant association between EPO, EpoR and c-Myc expression. Furthermore, examination of EpoR protein expression in >100 breast tumour samples by immunohistochemistry revealed that EpoR expression was associated with reduced patient survival. Using a tetracycline-inducible shEpoR xenograph model, our preliminary data suggests that EpoR knockdown delays tumour development and decreased tumour volume. Our findings provide new evidence for how the EPO/EpoR axis drives breast tumourigenesis and highlights EpoR inhibition as a potential novel therapeutic strategy.
INFLUENCE OF BACTERIA ON CHEMOTHERAPEUTIC DRUGS
P Lehouritis¹, J Cummins¹, M Stanton¹, C Murphy¹, F McCarthy¹, G Reid², C Urbaniak², W Byrne¹, M Tangney¹ ¹Cork Cancer Research Centre, University College Cork, , Cork Ireland
²Lawson Health Research Institute, Lawson Health Research Institute, London Ontario

In this study, the potential effects of tumour-associated bacteria on the efficacy of frequently used chemotherapies was examined. The bacterial content of patient breast tumours was analysed by sequencing, and the most frequently occurring species identified. Various bacteria and cancer cell lines were examined in vitro for changes in the efficacy of cancer cell killing mediated by chemotherapeutic agents. Of 30 drugs examined, the efficacy of 10 was found to be significantly inhibited by certain bacteria, while the same bacteria improved the efficacy of six others. HPLC and mass spectrometry analyses of sample drugs (gemcitabine, fludarabine, cladribine, CB1954) demonstrated modification of drug chemical structure. The chemoresistance or increased cytotoxicity observed in vitro with sample drugs (gemcitabine and CB1954) was replicated in in vivo murine subcutaneous tumour models. These findings suggest that the presence and types of bacteria in tumours and other body locations may influence tumour responses to certain chemotherapies in a subset of pertinent clinical cases. This work was funded by the Irish Health Research Board (HRA_POR 2012/99), Breakthrough Cancer Research and University College Cork School of Medicine TRAP.
Personalised modelling of the JNK activation dynamics can predict Neuroblastoma survival
D Fey¹, M Halasz¹, D Dreidax², SP Kennedy³, J Hastings³, N Rauch¹, AG Munoz¹, R Pilkington¹, M Fischer⁴, F Westermann², W Kolch¹, BN Kolodenko¹, DR Croucher¹,³
¹Systems Biology Ireland, University College Dublin, Dublin, ²Department of Neuroblastoma Genomics (B087), German Cancer Research Center (DKFZ), Heidelberg, ³The Kinghorn Cancer Centre, Garvan Institute of Medical Research, Sydney, ⁴Department of Pediatric Hematology and Oncology, University Hospital Cologne, Cologne

Signalling pathways exert finely tuned control over cell fate decisions that ultimately determine the behaviour of cancer cells. It could therefore be expected that the dynamics of key pathway activation may contain prognostic information over and above that which is contained in the static nature of traditional biomarkers. To investigate this hypothesis we focused on the JNK signalling pathway in Neuroblastoma.

JNK signalling plays a prominent role in stress induced apoptosis, but the precise network architecture that determines the apoptotic JNK response and its dysregulation in Neuroblastoma remained mostly uncharacterised.

Here, we have examined physiologically and therapeutically induced JNK stress signalling in neuroblastoma cells, built a mathematical model of this network and generated patient-specific simulations by populating this model with measured parameters and gene expression levels from tumour samples.

Our combined computational-experimental analysis revealed a network structure whose dynamic behaviour is determined by feedback and crosstalk. A feedback from JNK to its upstream kinase MKK7 promoted switch-like JNK activation, which is constrained by inhibitory crosstalk from Akt to both MKK4 and MKK7. A mathematical model based on this network structure recapitulated the dynamic behaviour of JNK observed under all experimental conditions and correctly predicted the apoptotic JNK response across different neuroblastoma cell lines. Survival analysis based upon the dynamics of the patient-specific simulations revealed that an inability to initiate switch-like JNK signalling was significantly associated with poor overall survival for both MYCN amplified and non-amplified neuroblastoma patients. Furthermore, our robustness analyses demonstrated considering the dynamics and extant network structure is critical for extracting prognostic information.

Summarising, as a proof of principle, we have demonstrated that personalised models of JNK-induced apoptotic switches can stratify Neuroblastoma cancer patients into groups with different survival probabilities.
THE ROLE OF C-MET/HGF SIGNALLING AS A CRITICAL MEDIATOR OF AN INVASIVE AND RESISTANT PHENOTYPE IN COLORECTAL CANCER
CA Bradley1, P Dunne, D McArt, S McQuaid, M Salto-Tellez, PG Johnston, S Van Schaeybroeck, 1CCRCB, QUB, Belfast

The c-MET proto-oncogene is frequently overexpressed (50-60%), amplified (5-10%), and mutated (5%) in colorectal cancer (CRC). Hepatocyte growth factor (HGF)-induced c-MET activation has been linked with enhanced proliferation, migration, survival and invasion, and has been suggested as a possible prognostic biomarker for CRC. In order to model CRC tumour cell invasion and metastasis, we have generated invasive subpopulations from CRC cells using Boyden Invasion chambers. Invasive cell lines were characterised for protein expression/activity by Western blotting, and analysed for migratory and invasive potential using the xCELLigence System (Roche). To model the CRC microenvironment, we have utilised a range of co-culture techniques with CRC cell lines and colon fibroblasts. c-MET expression in FFPE tissues was measured using IHC in a tissue microarray (TMA) derived from early stage CRC patients. HCT116 and LoVo invasive subpopulations showed an EMT-like, mesenchymal, migratory/invasive phenotype. In addition, increased expression and activation levels of c-MET were found in these sublines, which was determined to be ligand independent. Inhibition of c-MET using RNAi abrogated both basal and HGF-induced migration and invasion in CRC cell lines. Co-culture of CRC cells with HGF-expressing colon-derived fibroblasts leads to activation of c-MET, inducing CRC migration, invasion, and resistance to targeted therapy, and this phenotype could be diminished using a HGF neutralising antibody. Significant increased expression levels of c-MET were also found in a CRC TMA compared to matched normal tissues, and also at the invasive edge of some CRC tumours, where it may be driving invasive biology. We are currently investigating the role of the tumour microenvironment in regulating migration/invasion and resistance to targeted therapy in CRC. The identification of key pathways driving metastasis has a huge potential to change treatment strategies in CRC. We intend to further investigate the role of c-MET in regulation of migration, invasion, and resistance to therapy in CRC. Characterisation of such novel mechanisms may provide a preclinical rationale for therapeutic c-MET inhibition in CRC, specifically in patient subpopulations which display enhanced c-MET expression and activation.
TOPOISOMERASE 2-α IS A NOVEL CDC7/DBF4 INTERACTING PROTEIN

KZL Wu¹,², G O’Brien¹,², MD Rainey¹,², C Santocanale¹,²
¹Centre for Chromosome Biology, NUI Galway, Galway
²National Centre for Biomedical Engineering Science, NUI Galway, Galway

CDC7 is an essential serine/threonine kinase required for the initiation of DNA replication in eukaryotic cells, where it triggers origin firing through phosphorylation of the MCM helicase complex. Formation of an active kinase requires the binding to either of its regulatory subunits, DBF4 or DRF1. Additional roles for CDC7 in the S-phase checkpoint, chromatin assembly, and transcriptional regulation have also been proposed.

Topoisomerase 2-α (TOP2A) mediates the decatenation of double stranded DNA molecules, and is therefore required for maintenance of proper chromatin structure, chromosome condensation, and accurate segregation of DNA into daughter cells. Topoisomerases are essential for cell division and are already a major target for many anti-tumour drugs.

We have identified a novel interaction between CDC7/DBF4 kinase and TOP2A. Mass spectrometry analysis of DBF4 FLAG immunoprecipitates lead to the initial identification of TOP2A as a potential novel interacting partner, and we confirmed this by co-immunoprecipitation with endogenous TOP2A. We showed that this interaction occurs during S-phase, and that it is abolished by treatment with CDC7 inhibitor, indicating that it is dependent on CDC7 kinase activity. By deletion analysis, we have also determined the regions of DBF4 which are important for this interaction. Immunofluorescence shows that DBF4 and TOP2A co-localise at distinct nuclear foci, the nature of which are currently being investigated.

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BRCA1 deficiency exacerbates estrogen-induced DNA damage and genomic instability
KB Matchett1, KI Savage1, EM Barros1, KM Cooper2, GW Irwin3, JJ Gorski2, KS Orr1, J Vohhodina1, JN Kavanagh1, AF Madden1, A Powell1, 2, L Manti3, SS McDade1, BH Park4, KM Prise1, SA McIntosh1, M Salto-Tellez1, DJ Richard5, CT Elliott2, DP Harkin1
1Centre for Cancer Research and Cell Biology, Queen’s University Belfast, Belfast, 2Institute for Global Food Security, Queen’s University Belfast, Belfast, 3Department of Physics, University of Naples Federico II, Naples, 4The Sidney Kimmel Comprehensive Cancer Centre, The Johns Hopkins University School of Medicine, Baltimore, 5Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane

Germline mutations in BRCA1 predispose carriers to a high incidence of breast and ovarian cancers. BRCA1 functions to maintain genomic stability through critical roles in DNA repair, chromatin remodeling, and transcriptional control. A major question has been why BRCA1 loss or mutation leads to tumours mainly in estrogen-regulated tissues, given that BRCA1 has essential functions in all cell types. Several observations suggest estrogen has an important role in the development of BRCA1 dependent breast cancer. Pre- or post-menopausal oophorectomy in BRCA1 mutation carriers significantly reduces the risk of breast cancer onset and reoccurrence 1-3. Furthermore, pregnancy increases the risk of early-onset breast cancer in BRCA1 mutation carriers, in contrast to non-carriers for whom pregnancy is protective 4. Estrogen is postulated to promote tumourigenesis directly through stimulation of estrogen receptor-alpha (ERalpha) and the downstream activation of pro-mitogenic transcriptional programs. However, this is confounded by observations that approximately 70-80% of BRCA1 mutated breast tumours are ERalpha-negative 5,6. Here, we report that estrogen and the predominant estrogen metabolites, 2-Hydroxyestradiol (2-OHE2) and 4-Hydroxyestradiol (4-OHE2), can cause DNA double-strand breaks (DSB) in ERalpha-negative breast cells and that BRCA1 is required to repair these DSBs to prevent metabolite-induced genomic instability. We discovered that BRCA1 also regulates estrogen metabolism and metabolite-mediated DNA damage by repressing the transcription of estrogen-metabolising enzymes, such as CYP1A1, in breast cells. Indeed, we found that CYP1A1 expression is significantly elevated in BRCA1 mutant breast tumours compared to wild-type breast tumours. Using LC-MS/MS, we observed that in addition to defective DNA repair of metabolite-induced DSBs, BRCA1 deficient cells also have increased production of 2-OHE2 and 4-OHE2. Finally, we used a knock-in human cell model with a heterozygous BRCA1 pathogenic mutation, and BRCA1+/− primary breast progenitor cells isolated from a BRCA1 mutation carrier undergoing a risk-reducing mastectomy, to show how BRCA1 haploinsufficiency affects these processes. Our findings provide pivotal new insights into why BRCA1 mutation drives the formation of tumours in estrogen-regulated tissues, despite the general role of BRCA1 in DNA repair in all cell types. Furthermore, our study suggests luteinizing hormone releasing hormone agonists may prove useful as chemopreventative agents in pre-menopausal BRCA1 mutation carriers. 1. Kauff et al. N Engl J Med 2002; 346:1609-15 2. Narod et al. Cancer Epidemiol Biomarker Prev 2012; 21:1089-96 3. Rebbeck et al. N Engl J Med 2002; 346:1616-22 4. Jernstrom et al. Lancet 1999; 354:1846-50 5. Atchley et al. J Clin Oncol 2008; 26:4282-8 6. Mavaddat et al. Cancer Epidemiol Biomarker Prev 2012; 21:134-47
RATIONAL TARGETING OF INHIBITOR OF APOPTOSIS PROTEINS (IAPS) FOR EFFECTIVE THERAPY OF COLORECTAL CANCER

NT Crawford, C McCann, DJJ Waugh, PG Johnston, DB Longley
1Centre for Cancer Research Biology, Queen's University, Belfast

Background: Inhibitor of Apoptosis Proteins (IAPs) are anti-apoptotic proteins which suppress the activation of caspases. IAP proteins have been shown to be overexpressed in several cancers, including colorectal cancer and correlate with poor prognosis. SMAC mimetics are a class of apoptosis inducing drugs that target IAPs by mimicking the N-terminal IAP-binding motif of SMAC. Colorectal cancer has a pro-inflammatory microenvironment with high levels of TNFa. The aims of this study were to assess the efficacy of SMAC mimetic treatment in colorectal cancer.

Methods: Colorectal cell lines were treated with SMAC mimetics and cell viability was determined using MTT assays. Apoptosis was assessed by Western blotting for caspase activation and PARP cleavage and Flow Cytometry using Annexin V/PI staining. Protein-protein interactions were assessed using co-immunoprecipitation experiments. The pro-inflammatory microenvironment was modelled by addition of exogenous TNFa and also media transfer from and direct co-cultures with macrophages (THP-1, monocytic cell line).

Results: Colorectal cell lines were resistant to SMAC mimetic treatment alone unless co-cultured with TNFa. Pretreatment of colorectal cancer cells with 5-FU and oxaliplatin sensitised the cells to SMAC mimetic treatment. Colorectal in vivo models were also sensitive to combination of SMAC mimetic and 5-FU/oxaliplatin. THP-1 cells were found to secrete soluble TNFa and co-culture with colorectal cell lines, sensitised the cancer cells to SMAC mimetic mediated cell death. Cell death under these conditions was found to be dependent on soluble TNFa through the utilisation of neutralising antibodies.

Conclusion: Our results to date suggest that the pro-inflammatory microenvironment of colorectal cancer could be exploited to target overexpressed IAP proteins leading to cell death.
HIGH-THROUGHPUT SCREEN IDENTIFIES A NOVEL SPECIFIC HISTONE DEACETYLASE 6 INHIBITOR THAT KILLS CHEMORESISTANT BREAST CANCER CELLS.

T Ní Chonghaile¹, B Smith², M Hemann³, JE Bradner², W Gallagher¹, A Letai³
¹The Conway Institute of Biomolecular and Biomedical Science, University College Dublin, Dublin
²The Department of Hematology and Oncology, Dana Farber Cancer Institute, Boston
³The Department of Biology, Massachusetts Institute of Technology, Boston

Triple negative breast cancer (TNBC) lacks expression of oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 (HER2). Importantly, there have been fewer advances in the treatment of TNBC with the mainstay of treatment being cytotoxic chemotherapy. All too often though tumor responds poorly to chemotherapy, or relapse and resistance follow an initial response. Most chemotherapeutic agents used to treat TNBC kill cells via the mitochondrial pathway of apoptosis. To identify novel therapeutics for chemoresistant breast cancer, we performed a high-throughput screen to identify small molecules that are cancer selective but can kill independent of the mitochondrial pathway of apoptosis. To perform this screen we used TNBC line MDA-MB-231 in which the pro-death proteins BAX/BAK were knocked down. BAX/BAK are essential for mitochondrial apoptosis. To enrich for compounds that were not non-specifically toxic, we simultaneously counter-screened against the non-transformed breast epithelial cell line MCF10a. We screened a total of 30,000 compounds in duplicate across the two cell lines. There was a hit rate of 0.3% in the screen and 85 compounds were retested in the validation cherry pick screen. From the cherry pick screen 18 compounds were further validated with low-throughput assessment for mitochondrial independent killing and selectivity for cancer cells across a panel of cell lines. To identify the mechanism of action of the lead compound we used a genetic approach to generate an RNAi signature for the compound. This signature enables classification of the compound into a known or a novel class of compounds. The lead compound signature was most like that of histone deacetylase inhibitors (HDAC). Using an in vitro HDAC inhibitor screen, we identified that the compound selectively inhibited HDAC6. Importantly, we validated that the lead compound caused an increase in acetylated tubulin in cells, a target of HDAC6. In conclusion, we have identified a novel HDAC6 specific inhibitor that selectively kills cancer cells independent of mitochondrial apoptosis.
The Education of MSCs by Prostate Cancer Cell Line Conditioned Media

Ms. Ridge¹, Ms. Shaw², Prof. Sullivan³, Prof. Giles¹, Dr. Glynn¹
¹Prostate Cancer Institute (PCI), NUI Galway, Galway
²Regenerative Medicine Institute (REMEDI), NUI Galway, Galway
³Radiation Oncology, Galway University Hospital, Galway

The tumour microenvironment is host to a heterogenous population of cells including cells of the immune system, fibroblasts, endothelial cells and mesenchymal stem cells (MSCs). MSCs are multipotent cells capable of differentiating into various cell types such as osteocytes, adipocytes and chondrocytes. Recent studies have shown that MSC can home to tumour sites and form part of the tumour stroma. Rather than exert a therapeutic effect, it has been suggested that MSCs contribute to tumour growth and progression. Emerging evidence suggests that MSCs exert this effect through the secretion of growth factors and cytokines. It has also been suggested that MSC are a possible precursor to cancer associated fibroblasts (CAF). We hypothesise that prostate cancer (PCa) cells reprogramme MSCs, inducing a phenotype that promotes PCa progression. We treated bone marrow derived MSCs with conditioned media from PCa cell lines PC3, DU145 and 22Rv1. MSCs treated with PC3 conditioned media were found to have upregulation in the expression of COX-2, the CAF marker ACTA2 (α-SMA) and pro-inflammatory factors IL6, IL8, MCP-1 and MIF. Interestingly, the most pronounced upregulation in genetic expression (>190 fold) was found in SPP1 (osteopontin). The migration and proliferation of these cells was found to be decreased in comparison to the control cells. The treated MSCs show an increased potential for osteogenic differentiation and a decreased potential for adipogenic differentiation. Taken together these results suggest that following prolonged exposure to factors released by the bone metastatic PC3 cell line, MSCs alter in their phenotype, releasing factors associated with cancer progression.
TAILORING APPROACHES FOR GLOBAL EPIGENOME ANALYSIS FROM ARCHIVAL FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUE SAMPLES

S Das¹, B Moran¹, R Klinger¹, B Fender², G Peutman³, D Smeets³, A Byrne⁴, M Ebert⁵, D Lambrechts³, WM Gallagher¹, DP O’Connor¹

¹Cancer Biology & therapeutics, Conway Institute, University College Dublin, Dublin, Ireland
²Oncomark Ltd., Nova UCD, Dublin, Ireland
³Department of Translational genetics, VIB, K.U., Leuven, Belgium
⁴Department of Physiology, Royal College of Surgeons in Ireland, Dublin, Ireland
⁵University of Heidelberg, University of Heidelberg, Manheim, Germany

Novel DNA extraction methodologies allow the use of archival material from formalin fixed paraffin embedded (FFPE) tissue samples in genomic studies. A major limitation of this source of DNA is the fragmented nature and low overall yield generally obtained from clinical materials, making downstream applications such as epigenetic analysis challenging. Previous published attempts have focussed on smaller regions of CpG islands, such as the use of Illumina 450k arrays (which measure methylation at approximately 485,000 sites). The objective of this study was to optimize experimental and analytical workflows that allow effective interrogation of global DNA methylation profiles from FFPE samples. Methylation capture was conducted on DNA from matched FFPE and fresh frozen samples from the same metastatic colorectal cancer patient using the SeqCap Epi (Roche) methyl capture system. The custom capture designed includes 5.5 million CpG sites across the genome, a greater than 10 fold increase compared to previously published studies. The wet-lab protocol was robustly optimized for several parameters, such as overall yield and bisulphite conversion efficiency (measured by shallow-read next generation sequencing). A data analysis pipeline composed of the bisulphite-converted DNA aligner Bismark, as well as in-house Perl and R scripts, was used to generate detailed methylation maps for individual sample types in order to identify differentially methylated regions (DMRs), which were further validated using targeted bisulphite sequencing for selected loci. Detailed analysis of the data revealed 98% bisulphite conversion efficiency and low PCR duplicate rate (4-5%) across both sample types. Intriguingly, we observe an 80% concordance between the overall DNA methylation profiles between FFPE and fresh frozen samples. Mapping overall methylation levels to CpG resorts (+/- 4kb of CpG islands) indicated ~10% of methylation occurred in these regions totalling 245MB. Known genomic features, including exons, promoters of coding/non-coding genes and enhancers, contained ~30% (1.5 million) of the methylated positions. Taken together, these results demonstrate a robust and novel approach to generate DNA methylation profiles from difficult-to-handle, but frequently available material, thus establishing a suitable platform for a whole methylome profiling from archival samples.
MicroRNA-10a is significantly down-regulated in breast cancer and regulated at least in part through Retinoic Acid
S Khan¹, D Wall², C Curran¹, J Newell², MJ Kerin¹, RM Dwyer¹

1Surgery, School of Medicine, National University of Ireland, Galway 2Clinical Research Facility and School of Mathematics, Statistics and Applied Mathematics, National University of Ireland, Galway

Introduction: MicroRNAs are short non-coding RNA molecules that play a critical role in mRNA cleavage and translational repression, and are known to be altered in many diseases including breast cancer. miR-10a has been shown to be deregulated in various cancer types. The aim of this study was to determine the relevance of miR-10a in breast cancer, and identify mechanisms of regulation.

Methods: Following ethical approval and informed patient consent, patient tissue samples were obtained during surgery. miR-10a was quantified in malignant (n=102), normal (n=30) and fibroadenoma (n=35) tissues by RQ-PCR. Gene expression of Retinoic Acid Receptor beta (RARB) and Thyroid Hormone receptor alpha (THRA) was also quantified in the same patient samples. The in vitro effects of all-trans Retinoic acid (ATRA) and L-Thyroxine (T4) both individually and in combination, on miR-10a expression was investigated in breast cancer T47D and SK-BR3 cell lines.

Results: The level of miR-10a expression was significantly decreased in tissues harvested from breast cancer patients (Mean(SEM) 2.1(0.07)) Log10 Relative Quantity (RQ)) compared to both normal (3.0(0.16) Log10 RQ, p<0.001) and benign tissues (2.6(0.17) Log10 RQ, p<0.05). The levels of both RARB and THRA gene expression were also found to be decreased in breast cancer patients compared to controls (p<0.001). A significant positive correlation was determined between miR-10a and RARB (r= 0.31, p<0.001) and also with THRA (r= 0.32, p<0.001). In vitro stimulation assays revealed miR-10a expression was increased in both T47D and SK-BR3 cells following addition of ATRA (2 fold(0.7)), with no change observed following stimulation with T4 alone. Combination treatment with ATRA and T4, showed a more robust increase of miR-10a expression in T47D cells (3.4 fold(0.9)) compared to SK-BR3 cells (2.7 fold(0.07)).

Conclusion: The initial data presented, highlights a role for miR-10a as a tumour suppressor in breast cancer. The data here presents a potential tumour suppressor role for miR-10a in breast cancer, and highlights retinoic acid as a positive regulator.
Investigation of the role of STAT3 signalling in obesity-associated adenocarcinoma of the oesophagus

KE O’ Sullivan¹, MC Cathcart¹, E O’ Regan¹, J Michaelson¹, N Gilmartin¹, A Cannon¹, G Moore¹, W Gao², UFearon², J Lysaght¹, J O’ Sullivan¹, JV Reynolds¹
¹Department of Surgery, Institute of Molecular Medicine, Dublin
²Education and Research Centre, Conway Institute of Bimolecular and Biomedical Research, Elm Park, Dublin

OBJECTIVE: Strong epidemiological evidence links obesity with the development of oesophageal adenocarcinoma (OAC). The inverse relationship is seen in squamous cell carcinoma (SCC). The molecular mechanisms underpinning this phenomenon are unknown. The objective of this study was to examine the role of the STAT3 signaling pathway.

METHODS: A series of co-culture experiments with OE33 (OAC) and OE21 (SCC) cells were carried out with visceral (vACM) and subcutaneous (sACM) adipose conditioned media. PhosphoSTAT3 quantification was performed on Tyr705 pSTAT3 assay (MesoScale Discovery). Western blotting for SOCS3, Total STAT3 and pSTAT3 was performed on matched samples. Quantification of total STAT3 in snap frozen OAC and SCC tissue was then performed (n=10 per subtype). Immunohistochemical staining for pSTAT3 and IL-6R was performed on tumour tissue from 154 patients with OAC and SCC in tissue microarrays.

RESULTS: Both OE33 and OE21 cells demonstrated a rapid increase in pSTAT3 post ACM exposure (p=0.02 and <0.0001 respectively). vACM treatment resulted in higher pSTAT3 levels in OE33 cells compared with sACM (p= 0.02). Furthermore, obese vACM resulted in a significantly greater increase in pSTAT3 than non-obese vACM (p=0.04) in OE33 cells only. Greater total STAT3 was demonstrated in tumor from obese OAC patients compared with non-obese (p= 0.01). TMA survival analysis revealed a protective effect with increased pSTAT3 expression in OAC (HR 0.74, p=0.02) and the inverse in SCC (HR 3.7, p=0.08). Western blotting results revealed no significant change in SOCS 3 following exposure to ACM in OE33 cells while OE21 cells demonstrated a significant decrease in SOCS 3 directly after ACM exposure (p=0.001).

CONCLUSION: Divergent STAT3 signalling exists in OAC and SCC in response to adiposity in vitro. These results are mirrored by in vivo survival analysis. Findings suggest this pathway is a potential adjunctive therapeutic target in oesophageal cancer.
FUNCTIONAL GENOMIC SCREENING IDENTIFIES USP11 AS A NOVEL THERAPEUTIC TARGET IN BREAST CANCER

L Dwane¹, AE O’Connor¹, L Mulrane¹, AM Dirac², K Jirstrom³, JP Crown⁴, R Bernards², WM Gallagher¹, DP O’Connor¹ UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Dublin 4, ²Division of Molecular Carcinogenesis, Netherlands Cancer Institute, Amsterdam, Netherlands, ³Department of Laboratory Medicine, Malmö University Hospital, Lund University, Malmö, Sweden

Approximately 70% of breast cancers overexpress the estrogen receptor alpha (ER-alpha) and depend on this key transcriptional regulator for growth and differentiation. The discovery of novel mechanisms controlling ER-alpha function represent major advances in our understanding of breast cancer progression and potentially offer attractive new therapeutic opportunities. Here, we investigated the role of deubiquitinating enzymes (DUBs), which act to remove ubiquitin moieties from proteins, in regulating transcriptional activity of the ER-alpha in breast cancer. To identify DUBs involved in the regulation of ER-alpha transcriptional activity, we performed an RNAi loss-of-function screen using a library of shRNA vectors targeting all human DUB genes. The DUB library consisted of pools of four non-overlapping shRNAs targeting all 108 known or putative DUBs (432 shRNAs in total). We found that suppression of a number of DUBs markedly repressed or enhanced the activity of an estrogen-response-element (ERE) luciferase reporter following estradiol stimulation. Of particular interest, suppression of the BRCA2-associated DUB, USP11, was found to down-regulate ER-alpha transcriptional activity. Subsequent validation using ZR-75-1 breast cancer cells with stably knocked-down USP11 and multiple independent hairpins revealed a notable reduction in expression of the endogenous ER-alpha target genes, as quantified using qRT-PCR. Growth assays and Western blot analysis also revealed a global decrease in cell survival and decreased ERK and AKT phosphorylation in USP11 knockdown cell lines. Immunoprecipitation of ER-alpha also revealed a physical interaction between USP11 and the receptor. In silico analysis of publically available breast cancer gene expression datasets revealed a highly significant correlation between high expression of USP11 mRNA in ER-positive patients and poor distant metastasis-free survival (HR 1.39, CI 1.1-1.76, p=0.006). This correlation was also significant in ER-positive patients who had received endocrine therapy (HR 1.84, CI 1.22-2.75, p=0.0023). To further investigate the prognostic relevance of USP11, immunohistochemical staining of a breast cancer tissue microarray (n= 144) was performed. Kaplan-Meier analysis of this cohort revealed a significant association between poor overall survival (p= 0.030) and poor breast cancer specific survival (p=0.041). These results suggest a role for USP11 in driving cellular growth and identify USP11 as novel therapeutic target in breast cancer.
IDENTIFICATION OF A NOVEL UBIQUITIN SPECIFIC PEPTIDASE INVOLVED IN MAINTAINING GENOMIC STABILITY

Edel McGarry¹, David Gaboriau¹, Umberto Restuccia², Michael D. Rainey¹, Angela Bachi² and Corrado Santocanale³

1 Centre for Chromosome Biology and National Centre for Biomedical Engineering Science, National University of Ireland, Galway
2 Functional Proteomics Unit, IFOM, FIRC Institute of Molecular Oncology, Milan, Italy

Cells possess checkpoint pathways which are important for maintaining genome stability and preventing cancer. These signalling pathways include the ATR and CHK1 kinases which are activated in response to DNA damage or replication stress. Activation of CHK1 by ATR requires the mediator protein Claspin.

Claspin also regulates the rate of fork progression during DNA replication. Claspin interacts with many replisome components including the MCM complex and the CDC7 kinase. The CDC7 kinase is essential for the initiation of DNA replication but also phosphorylates Claspin. CDC7 is up-regulated in many cancers and several CDC7 kinase inhibitors are being explored as anti-cancer agents.

The levels of Claspin are cell cycle regulated and importantly Claspin is stabilised during S-phase. A growing number of ubiquitin ligases and of deubiquitylating enzymes (DUBs) have been shown to affect Claspin stability, however how they cooperate in regulating Claspin levels remains unclear.

In this study we have tested the hypothesis that CDC7 dependent phosphorylation of Claspin is important to coordinate Claspin interaction with other cellular proteins. Using a quantitative proteomic approach, we have identified several proteins that differentially co-purify with Claspin when this is extracted from cells treated with a CDC7 kinase inhibitor or from control cells. Among these we find a novel DUB and we have confirmed by reciprocal immunoprecipitation experiments that it binds to Claspin in a CDC7 dependent manner. Using siRNA knock-down and pharmacological inhibition strategies, we find that this DUB controls Claspin stability in a phase specific manner, it affects DNA replication fork stability and checkpoint responses after replication stress.

Therefore we propose that this DUB is a novel player involved in the maintenance of genomic stability and in the DNA replication stress response pathway.
INVESTIGATING THE ROLE OF POLYMORPHISMS IN mir146a AND mir196a2 IN MODIFYING BREAST CANCER RISK AND PHENOTYPE
TP McVeigh¹, RJ Mulligan¹, KJ Sweeney², JB Weidhaas³, N Miller¹, MJ Kerin¹
¹Discipline of Surgery, NUI Galway, Galway, ³School of Medicine, Yale University, New Haven

Introduction and Aims: Micro (mi)RNAs are small non-coding RNA molecules bind with cis-regulatory regions in target messenger (m)RNA to exert effects on gene expression. miRNAs have a role in regulating a host of biological pathways. Polymorphisms in mature or precursor miRNAs may impact their function, thereby influencing disease risk. Variants associated with mir146a and mir196a2 have been shown to modify breast cancer risk and age of onset. The aim of this study was to investigate the role of these variants in breast cancer in an Irish cohort.

Methods: A case-control study was undertaken. Cancer-free controls were recruited from the community and cases were recruited from symptomatic and screening breast units. Patients with high-risk mutations in BRCA1/BRCA2 were excluded from analysis. Germline DNA was extracted from blood/buccal swabs using crystallisation precipitation and genotyping was performed using a Taqman probes. Data was analysed using SPSS v22.

Results: 635 (351 cases, 284 controls) were genotyped for mir196a2-variant rs11614913, and 719 (427 cases, 292 controls) for mir146a-variant rs2910164. The minor alleles at both loci were identified in this cohort, with minor allele frequency of 0.2 for mir146a-variant, and 0.56 for mir196a2-variant. There was not a significant difference in the frequency of the variants between cases and controls (p=0.189, mir-146a; p=0.931, mir-196-a-2, X²). Cases expressing the mir-146a-variant were affected at a significantly younger age than wild-type cases (57±12 v 55 ±11, p=0.04, t-test).

Conclusions: Mir-146a2 variant rs2910164 is associated with earlier age of onset of breast cancer in Irish patients.

Conflict of interest: nil Disclosures: nil
ELUCIDATING THE IMMUNOTHERAPEUTIC POTENTIAL OF GAMMA DELTA T CELL SUBSETS AND MAIT CELLS

MR Dunne¹, R Dunne¹, N Clarke¹, N Gilmartin¹, D O'Toole², F MacCarthy², N Ravi², JV Reynolds¹, JN O'Sullivan¹

¹Department of Surgery, Trinity College Dublin, Dublin
²Department of Clinical Medicine, St James's Hospital, Dublin

Gamma delta (gd) T cells and mucosal-associated invariant T (MAIT) cells are types of unconventional T cells with known cytotoxic potential. gd T cells, in particular the Vd2 subtype, show promise in clinical trials as cell-based immunotherapeutic anti-cancer agents. Little is known regarding the clinical potential of the Vd1 and Vd3 subtypes or MAIT cells however. MAIT cells possess the ability to lyse cells infected with bacteria but whether they kill tumour cells is unknown. MAIT cells have been observed in tumours however and possess drug efflux receptors which confer resistance to chemotherapy, making them interesting immunotherapy candidates. In this study we aimed to elucidate the role of unconventional T cells in tumour development by characterising subtype frequencies and function in blood and oesophageal tissue at normal, pre-malignant and tumour stages. Vd1, Vd2, Vd3 and MAIT (CD161hi/Va7.2+) cell frequencies were assessed by flow cytometry in whole blood and collagenase-dissociated oesophageal tissue biopsies from patients with Barrett’s oesophagus (n=31 blood, n=7 tissue) or oesophageal adenocarcinoma (n=15 blood, n=9 tissue). Blood from healthy age- and sex-matched donors (n=46) and adjacent normal tissue from Barrett’s oesophagus patients (n=7) was used for controls. Vd1, Vd2 and MAIT (but not Vd3) cells were significantly reduced (p<0.05, p<0.001, p<0.01, respectively) in circulation in patients with Barrett’s oesophagus or adenocarcinoma, compared to healthy control blood. In oesophageal tissues, T cell subset frequencies were similar between Barrett’s, tumour and control tissues, with the exception of Vd1 cells, which were significantly reduced in tumours compared to control (p<0.05) or Barrett’s oesophagus (p<0.05) tissue. All tissue resident T cell subsets showed significant expression of cytotoxicity-associated marker NKG2D and also inhibitory marker PD-1.

Current work involves assessing the implications of this and whether T cells remain functional in oesophageal tumours. We report for the first time, the frequency of specific gd T cell subsets and MAIT cells in circulation and oesophageal tissue during tumour development. Ongoing work is focused on evaluating the effects of tumour crosstalk and chemotherapy on the function of unconventional T cells. Elucidating factors which affect unconventional T cell cytotoxicity will aid development of improved immunotherapeutic strategies.
DEVELOPMENT OF AN EX-VIVO PRIMARY ACUTE MYELOID LEUKEMIA (AML) ASSAY TO PREDICT EFFICACY OF CHEMOTHERAPY
D Baev¹, J Krawczyk², M O'Dwyer ¹-², E Szegezdi¹
¹Apoptosis Research Center, National University of Ireland, Galway
²Hematology, Galway University Hospital, Galway

Introduction: Despite of the overall progress in cancer therapy the Acute Myeloid Leukemia remains the disease with more than 70% relapse rate within 5 years after the treatment. The AML relapses due to a small population of drug-resistant leukemia stem cells (LSC) acting as AML-initiating cells not reachable for therapeutic eradication. Bone marrow microenvironment is vital for LSC persistence and drug-resistance. Here we show results illustrating our progress in development of AML bone marrow-mimicking co-culture system for testing and predicting drug efficacy both for AML blast and for LSC populations.

Materials and Methods: The BM AML-stroma co-cultures were treated with clinically relevant doses of Cytarabine (AraC), Daunorubicine (or by fixed dose combinations of AraC and Daunorubicine), Clofarabine and 5-Azacytidine. Viability assays by flow cytometry using ToPro-3 staining were performed 24 and 48 h after drug administration. The percentage of LSCs as well as the proliferation/activation status of the cells in the drug-resistant fraction was determined by monitoring of CD38-CD34+CD117+/HLA-DR+/populations by multicolor flow cytometry.

Results: For the available patient cohort the drug responsiveness/resistance profile was reproduced in >70% of patients treated with daunorubicine-based regimen. We also confirmed previously reported 1.5 to 4 fold increase in percentages of the LSC subpopulation in drug-resistant patients and lower magnitudes of the increase in the percentage of LCSs in drug-responder after the treatment. For patients treated with 5-azacytidine and Clofarabine the reproducibility of drug effect was around 50% and further analysis with larger number of recruited patients is required.

Conclusions: Within an up-to-date available cohort of patients the developed ex-vivo assay reproduces the responsiveness/resistance of AML patients to therapy in approximately 70% of cases for Cytarabine-based therapy regimens. Further analysis of samples from 20-30 more patient is needed for validation of the assay and for the development into clinically usable platform.
THE ROLE OF ZEB1/ZEB2 AND βIII-TUBULIN IN MEDIATING DOCETAXEL-RESISTANT PROSTATE CANCER

K Hanrahan¹, M Prencipe¹, J Bugler¹, L Murphy¹, A O’Neill¹, RW Watson¹
¹UCD School of Medicine and Medical Science, Conway Institute of Biomolecular and Biomedical Research, University College Dublin

Docetaxel therapy is the gold standard treatment for advanced castrate-resistant prostate cancer (CRPC). However, patients either do not respond or develop resistance over time. Transcriptomic and proteomic analysis of docetaxel-resistant prostate cancer sub-lines developed by our group revealed multiple mechanisms of resistance in line with advanced disease, including over-expression of anti-apoptotic proteins and alterations of NF-κB activation. The sub-lines also demonstrated a coordinated loss and gain of epithelial and mesenchymal markers respectively; a process characteristic of Epithelial-Mesenchymal Transition (EMT). Studies have highlighted a role of EMT in prostate cancer progression, metastasis and docetaxel resistance. However, the role of EMT drivers in mediating resistance is not defined. We hypothesise EMT to be a central mechanism of apoptotic resistance in advanced docetaxel-resistant prostate cancer, representing a target for therapeutic manipulation.

EMT was characterised in the PC-3 D12 and DU145 R docetaxel-resistant sub-lines through an increased invasive capacity, MMP-1 secretion and protein expression of E-cadherin transcriptional repressors ZEB1 and ZEB2, in comparison to parental cell lines. This was associated with an increased expression of βIII-tubulin; a tubulin isotype linked to taxane resistance and tumour aggressiveness. Upon treatment with the apoptotic trigger docetaxel (20nM), the PC-3 D12 and DU145 R sub-lines demonstrated significant resistance compared to parental controls. Simultaneous siRNA knockdown of ZEB1 and ZEB2 resulted in both a partial reversal in apoptotic resistance and a decrease in cell viability; this was also associated with a down-regulation of βIII-tubulin and a re-expression of E-cadherin.

Our results provide evidence of the process of EMT in in vitro models of docetaxel-resistant prostate cancer, which is associated with differential susceptibility to docetaxel and is partially reversed through siRNA knockdown of the EMT drivers, ZEB1 and ZEB2. In addition, we have identified a link between EMT and βIII-tubulin in our models of docetaxel resistance. Current experiments are investigating the tissue expression of ZEB1 and βIII-tubulin in prostate cancer metastases following docetaxel therapy, which will determine the clinical relevance of EMT and βIII-tubulin as mediators of docetaxel resistance in CRPC.
DELINEATING AN EPIGENOMIC ROADMAP OF PROSTATE TUMORIGENESIS

CJ O’Rourke¹*, AL Walsh¹*, AV Tuzova¹, J Hanson², S O’Meachair³, B Hayes⁴, R Anney⁵, D Golden⁶, R Hennessy¹, L Shirren¹, S Madden⁷, A O’Neill⁸, C Morrissey⁹, J Thornhill¹⁰, B Loftus¹¹, R Vessella⁹, M Emmert-Buck², JJ O’Leary⁴, SP Finn⁴, D Hollywood¹, T Lynch¹, RWG Watson⁸, Antoinette S Perry¹

¹Prostate Molecular Oncology, Trinity College Dublin, ²Department of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, ³Dublin Centre for Clinical Research and Centre for Health Decision Science, School of Computer Science and Statistics, Trinity College Dublin, ⁴Department of Histopathology, Trinity College Dublin and St. James’s Hospital, ⁵Department of Psychiatry, Trinity College Dublin, ⁶Trinity Centre for High Performance Computing, Trinity College Dublin, ⁷Royal College of Surgeons in Ireland, ⁸Conway Institute of Biomolecular and Biomedical Research, University College Dublin, ⁹Department of Urology, University of Washington, Seattle, ¹⁰Department of urology, Tallaght Hospital, ¹¹Department of Histopathology, Tallaght Hospital

Facilitated by an atypically low mutation frequency and poorly delineated pathobiology, prostate cancer (CaP) has continued to evade any molecular taxonomic sub-classification. Accordingly, efficient prognostication of the worlds most prevalent non-cutaneous malignancy remains challenging with overtreatment of the indolent sub-type, as well as delayed early intervention of its aggressive counterpart, emerging as significant clinical issues. Analogous to traditional mutations, numerous epimutations have been reported by us and others in CaP. Here, we apply coupled genome-wide DNA methylation and miRNA profiling to unmask epigenomic events demarcating prostate tumorigenesis. Six discrete pathological states were chosen to reflect the stepwise progression of CaP: benign prostate (n=11), proliferative inflammatory atrophy (PIA, n=7), high-grade prostatic intraepithelial neoplasia (HGPIN, n=6), and indolent (n=7), aggressive (n=8) and metastatic (n=6) CaP. Laser capture microdissection was carried out using the Arcturus XT system (Life Technologies) to enrich for target epithelia. For each sample, the methylation status of > 485,000 CpG sites across the genome was profiled using Infinium 450k Human Methylation beadchip (Illumina) technology and analysed with the R-based package, RnBeads (Assenov et al., 2014). Matched expression data for 752 miRNAs was also generated using LNAqPCR (Exiqon). Epigenomic analyses support a role for PIA as a precursor lesion, detecting aberrant methylation of insidious tumour-targeted miR-205 and CDKN1A, amongst others, at this early stage for the first time. However, PIA and HGPIN appear to be largely independent precursor lesions with HGPIN sharing more epigenomic features in common with primary tumour. A hotspot of DNA hypomethylation, in contrast to predominant autosomal hypermethylation, was observed along the X chromosome at tumour induction. Comparison of the methylomes of indolent and aggressive tumours revealed that approximately 50% of epigenetic lesions are shared. Finally, analysis of the constituency of metastatic epigenetic aberrations found 12.7% indicative of aggressive-only disease and, unexpectedly, 5.5% resonant of indolent-only tumours. Here, we report the first epigenomic roadmap of prostate tumorigenesis, reconciling the variable stages of carcinogenesis into a sequential model. This data set is currently being exploited to identify an epigenetic biomarker panel capable of discriminating clinically indolent from aggressive disease.
RATIONAL DESIGN AND VALIDATION OF A TIP60 HISTONE ACETYLTRANSFERASE INHIBITOR FOR THE TREATMENT OF BREAST CANCER SUBTYPES

C Gao\textsuperscript{1,2}, E Bourke\textsuperscript{3}, M Scobie\textsuperscript{4}, MC Casey\textsuperscript{6}, A McGuire\textsuperscript{6}, A Shalaby\textsuperscript{3}, M Webber\textsuperscript{3}, C Curran\textsuperscript{6}, E Holian\textsuperscript{7}, MA Famme\textsuperscript{4}, T Koolmeister\textsuperscript{4}, G Callagy\textsuperscript{3}, M Kerin\textsuperscript{5}, T Helleday\textsuperscript{4}, LA Eriksson\textsuperscript{1,2}, NF Lowndes\textsuperscript{5}, JA Brown\textsuperscript{5,6}

\textsuperscript{1}School of Chemistry, National University of Ireland Galway, Galway, \textsuperscript{2}Department of Chemistry and Molecular Biology, University of Gothenburg, Göteborg, Sweden, \textsuperscript{3}Discipline of Pathology, National University of Ireland Galway, Galway, \textsuperscript{4}Division of Translational Medicine and Chemical Biology, Department of Medi, Karolinska Institute, Stockholm, Sweden, \textsuperscript{5}Centre for Chromosome Biology, School of Natural Sciences, National University of Ireland Galway, Galway, \textsuperscript{6}Department of Surgery, School of Medicine, National University of Ireland Galway, Galway, Ireland, \textsuperscript{7}School of Mathematics, Statistics and Applied Mathematics, National University of Ireland, Galway, Ireland.

Histone acetylation is required for many aspects of gene regulation, genome maintenance and metabolism and dysfunctional acetylation is implicated in numerous diseases, including cancer. Acetylation is regulated by histone acetyltransferases (HATs) and histone deacetylases and currently, few general HAT inhibitors have been described.

We identified the HAT Tip60 as an excellent candidate for targeted drug development, as Tip60 is a key mediator of the DNA damage response and transcriptional co-activator. Our modeling of Tip60 indicated that the active binding pocket possesses opposite charges at each end, with the positive charges attributed to two specific side chains. We used structure based drug design to develop a novel Tip60 inhibitor, TH1834, to fit this specific pocket\textsuperscript{1}.

We demonstrated that TH1834 significantly inhibits Tip60 activity in vitro and treating cells with TH1834 results in apoptosis and increased unrepaired DNA damage (following ionizing radiation treatment) in breast cancer but not control cell lines. Furthermore, TH1834 did not affect the activity of related HAT MOF, as indicated by H4K16Ac, demonstrating specificity\textsuperscript{4}.

Using tissue microarrays (TMA), with >700 breast cancer patient biopsies, we identified breast cancer subtypes with dis-regulated Tip60 levels. Identifying subtypes with lower Tip60 levels proves a subtype specific breast cancer therapeutic target for our inhibitor.

The modeling and validation of the small molecule inhibitor TH1834 represents a first step towards developing additional specific, targeted inhibitors of Tip60 that may lead to further improvements in the treatment of breast cancer.

Targeting colon cancer cell NF-kappaB promotes an anti-tumor M1-like macrophage phenotype and inhibits peritoneal metastasis

AE Ryan$^{1,2}$, A Colleran$^1$, A O’Gorman$^1$, L O’Flynn$^2$, J Pindjakova$^2$, P Lohan$^2$, G O’Malley$^{1,2}$, M Nosov$^2$, C Mureau$^1$, LJ Egan$^1$

$^1$Discipline of Pharmacology and Therapeutics, NUIG, Galway, $^2$Regenerative Medicine Institute, NUIG, Galway

Metastatic colorectal cancer poses a significant health problem both in terms of its poor prognosis and high incidence. Metastatic spread of colorectal cancer to the liver and peritoneal cavity is associated with particularly grave consequences. Better understanding of the molecular pathways that regulate peritoneal metastasis is essential in guiding the development of more effective therapies. In a model of peritoneal metastasis in immune competent mice, we show that NF-kappaB inhibition in CT26 colon cancer cells prevents metastasis. NF-kappaB inhibition, by stable over-expression of IkappaB-alpha super-repressor, induced differential polarization of co-cultured macrophages to an M1-like anti-tumor phenotype in vitro. NF-kappaB deficient cancer cell conditioned media (CT26/IkappaB-alpha SR) induced significant IL-12 and nitric oxide synthase expression (iNOS) in macrophages, and increased tumoricidal activity. Control cell (CT26/EV) conditioned media induced high levels of IL-10 and arginase in macrophages. In vivo, this effect translated to reduction in metastasis in mice injected with CT26/IkappaB-alpha SR cells, and was positively associated with increased CD8 and CD4 memory effector T cells. Furthermore, inhibition of NF-kappaB activity induced high levels of NO in infiltrating immune cells and decreases in matrix metalloproteinase (MMP)-9 expression, simultaneous with increases in tissue inhibitor of metalloproteinase (TIMP)-1,-2 within tumors. CT26/IkappaB-alpha SR tumors displayed increased pro-inflammatory gene expression, low levels of angiogenesis and extensive intra-tumoral apoptosis, consistent with the presence of an anti-tumor macrophage phenotype. Macrophage depletion following an initial period of tumour development reduced tumor size in CT26/EV injected animals and increased tumor size in CT26/IkappaB-alpha SR cells compared to untreated tumors. Our data demonstrates, for the first time, that an important implication of targeting tumor cell NF-kappaB is skewing of macrophage polarization to an anti-tumor phenotype. This knowledge offers novel therapeutic opportunities for anti-cancer treatment.
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A Novel Anti-Angiogenic Approach of EP Receptor Antagonism for the Prevention/Treatment of Oesophageal Adenocarcinoma
MC Lowry\textsuperscript{1}, BN Kennedy\textsuperscript{2}, NN Pullen\textsuperscript{3}, JV Reynolds\textsuperscript{1}, MC Cathcart\textsuperscript{1}
\textsuperscript{1}Department of Surgery, Trinity College Dublin/St. James's Hospital, Dublin 8, Ireland
\textsuperscript{2}Department of Biomedical Sciences, University College Dublin, Dublin 4, Ireland
\textsuperscript{3}Inflammation and Remodelling Research Unit, Pfizer Global Research and Development, Cambridge, MA, USA.

Introduction: Oesophageal adenocarcinoma (OAC) is the 7\textsuperscript{th} leading cause of cancer deaths worldwide, with its incidence increasing in Ireland. COX-2 is overexpressed in the progressive sequence from Barrett’s oesophagus to OAC. While COX-2 inhibitors hold promise for prevention/treatment, they have been associated with cardiovascular toxicity. Down-stream prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) and its corresponding EP receptors have been associated with angiogenesis and tumourigenesis in a number of cancers, including OAC. This study aimed to investigate PGE\textsubscript{2} receptor (EP) antagonism as a novel anti-angiogenic approach for OAC prevention and/or treatment.

Materials and Methods: The effect of selective EP blockade (with a panel of commercially available and novel EP1-4 antagonists) on blood vessel formation was investigated \textit{in-vivo} using a transgenic zebrafish model (\textit{TgEGFP fli-1}). Dose responses were carried out with the most effective ‘hits’ following preliminary screening. The most effective antagonists were brought forward for \textit{in-vitro} functional studies. EP receptor expression profiles were assessed in a panel of cell lines representing endothelial (EAHy.926, HUVEC), Barrett’s (QH) and OAC (OE-33, SKGT4) cells by qRT-PCR and western analysis. Cell survival analysis was carried out in all cell lines following EP antagonism using MTT (24 h, 48 h, 72 h). Tubule formation was also assessed in HUVECs \textit{(in-vitro angiogenesis assay)} following EP blockade.

Results: Selective EP antagonism significantly reduced intersegmental vessel formation \textit{in-vivo}. Significant anti-vascular effects were observed following EP1 antagonism (SC19220, SC51322), EP2 antagonism (PF-04418948) and EP4 antagonism (L161-982) (all \textit{p}<0.05). The EP3 antagonist (L798-106) had the greatest effect on vessel growth (\textit{p}<0.001). Significant anti-vascular effects (\textit{p}<0.05, \textit{p}<0.001) were also observed following dose response analysis. EP2 and EP4 were expressed in all cell lines, with EP4 expression the most abundant. EP1 and EP3 were expressed in selected cell lines only. With the exception of L-798-106 (EP-3 antagonist), no effect on cell viability was observed following treatment with commercial or novel selective EP antagonists. Additionally, no significant effect of treatment on tubule formation was recorded.

Conclusion: Selective EP antagonism has anti-angiogenic efficacy \textit{in-vivo}, with EP2, EP3 and EP4 blockade demonstrating the greatest anti-vascular effect. While selective targeting did not significantly affect cell function \textit{in-vitro}, its anti-angiogenic role may be more completely investigated with further \textit{in-vitro} studies.
5-LIPOXYGENASE AND 12-LIPOXYGENASE INCREASE ANGIOGENESIS IN OESOPHAGEAL CANCER VIA VEGF-MEDIATED SIGNALLING PATHWAYS

GY Moore¹, MCC Cathcart¹, N Tchrakian², DJ Maloney³, JV Reynolds¹, GP Pidgeon¹
¹Department of Surgery, TCD Centre for Health Sciences, St. James's Hospital, Dublin
²Department of Pathology, St. James's Hospital, Dublin
³Chemical Genomics Centre, NIH, Bethesda, Maryland, USA

Introduction: Lipoxygenases have been demonstrated to have a role in tumour angiogenesis in several malignancies including prostate and breast cancer (1,2). The importance of aberrant angiogenesis in oesophageal cancer is now appreciated (3), but clinical trials targeting VEGF have had mixed results to date. In this study we aim to investigate the role of 5-Lipoxygenase and platelet-type 12-Lipoxygenase as drivers of angiogenesis in oesophageal cancer both in vivo and in vitro.

Methods: 5-Lipoxygenase and 12-Lipoxygenase expression were investigated in oesophageal cancer tissue-microarrays (N=196; 20% squamous cell carcinoma (OSCC) and 80% adenocarcinoma (OAC)) and subsequently correlated with VEGF and VEGFR-2 and microvessel density (CD31). The effect of a panel of novel 5- and 12-Lipoxygenase inhibitors on proliferation and tubule formation of endothelial cells (HUVECs) was investigated. The effect of blocking 5-Lipoxygenase and 12-Lipoxygenase on the angiogenic gene profile of HUVECs was studied using qPCR RT2 profiler arrays.

Results: >85% of patients showed 12LO tumour epithelial and stromal expression, with stromal expression being higher in the OSCC cohort compared to the OAC cohort (p<0.001). 5LO expression was predominant in the stroma, with modest epithelial expression observed (<10% of patients). A strong correlation between OSCC 12LO tumour or stromal levels and VEGF was noted (p<0.01). Similarly, OAC 12LO tumour or stromal expression correlated with VEGF (p<0.001 and p<0.01). OAC 5LO stromal expression was shown to correlate with microvessel density (p<0.01). 5LO gene expression was significantly correlated with VEGF (p<0.0001) in a cohort of 38 OAC patients. The novel 5LO inhibitor NIH4 and 12LO inhibitor NIH15 (10uM) suppressed HUVEC proliferation and tubule formation (p<0.01). Following NIH4 or NIH15 treatment, genes regulating neo-angiogenesis and lymphoangiogenesis were down-regulated, including ANG, ANGPT1, ANGPTL4, NRP1, NRP2, KDR, VEGFA, VEGFB and VEGFC. The effect of the novel 5- and 12-Lipoxygenase inhibitors on these angiogenic gene profiles was also examined in the OE33 cancer cells.

Conclusion: Inhibitors of 5-Lipoxygenase and 12-Lipoxygenase show promise as anti-angiogenic agents, by down-regulating key angiogenic signalling pathways including VEGF. Combinatorial approaches targeting Lipoxygenases and VEGF may therefore have efficacy in the treatment of oesophageal cancer.

SRC-1 MEDIATION OF CANCER CELL REPROGRAMMING IN ENDOCRINE RESISTANT BREAST CANCER

AL Browne¹, J McBryan¹, A Fagan¹, ADK Hill¹, LS Young¹
¹Dept. of Endocrine Oncology, RCSI, Dublin

Endocrine resistance is a major clinical problem for breast cancer. This resistance can be acquired, whereby tumor cells initially respond to treatment but subsequently become resistant after prolonged treatment. Expression studies show that the steroid receptor co-activator SRC-1 plays a critical role in breast cancer cell proliferation, invasiveness, metastasis and endocrine resistance. To investigate the mechanism of SRC-1 action, RNA sequencing was carried out on tamoxifen treated LY2 SRC-1 shRNA and LY2 NT shRNA cells. This identified 1,731 up-regulated genes when SRC-1 is knocked out, of which, 153 were potential transcription factors. Three such transcription factors, E2F7, NFIA and FOSL1 have been confirmed as targets of SRC-1 and functional assays are currently assessing their contribution to driving tumor adaptability. Whether tumor cells acquire resistance by one cell changing or from a subpopulation taking over remains unclear. Recent data confirming the generation of pluripotent stem cells from differentiated adult somatic cells leads to the hypothesis that terminally differentiated ER positive breast cancer cells could reprogramme to a less differentiated state in response to their environment. To investigate this, we have taken one single, highly differentiated, luminal A MCF7 cell and are exposing it to long-term endocrine treatment. This will establish whether endocrine therapy can induce luminal cells to reprogramme to a more basal-like phenotype. Understanding how tumor cells adapt will be crucial to overcoming the clinical problem of endocrine resistance. Identifying the SRC-1 mediated network responsible for cellular reprogramming may help to identify novel targets to combat endocrine resistant breast cancer.
PDLIM2 is a cytoskeleton to nuclear courier protein for the IGF-1, Wnt and TGF beta signalling pathways in promotion of Epithelial to Mesenchymal Transition

MF Bustamante-Garrido¹, OT Cox¹, CH O’Flanagan¹, DA Buckley¹, PA Kiely², R O’Connor¹

¹School of Biochemistry and Cell Biology, University College Cork, Cork
²Department of Life Sciences, University of Limerick, Limerick

PDLIM2 is a PDZ-LIM domain protein that regulates the stability and activity of transcription factor families (including NF Kappa B, STATs and beta catenin). PDLIM2 expression is repressed in certain cancers but it is also highly expressed in Triple Negative Basal Breast cancers that are characterized by poor survival. Suppression of PDLIM2 reverses the EMT phenotype, inhibits polarized cell migration, and disrupts formation of polarized epithelial acini in 3D cell cultures. PDLIM2 shuttles from the cytoskeleton to the nucleus, but what mediates this nuclear translocation or activity in transcription factor regulation is unknown. The aim of this study was to identify the mechanisms governing PDLIM2 subcellular localization and nuclear translocation. We found that IGF-1 or TGF-Beta promotes PDLIM2 accumulation in the nucleus. Similarly, WNT3a stimulation enhances PDLIM2 accumulation in the nucleus while inhibition of WNT activity results in PDLIM2 stabilization in the cytoplasm. Cytoplasmic to nuclear translocation is associated with reduced phosphorylation on several serine residues in PDLIM2. The de-phosphorylation and subsequent nuclear translocation of PDLIM2 can be prevented by inhibiting the protein phosphatase PP1. In contrast, PDLIM2 phosphorylation can be enhanced by activation of protein kinase C, which is dependent on the presence of the focal adhesion scaffolding protein RACK1. Overall, the data indicate that PDLIM2 cytoplasmic to nuclear translocation in response to IGF-1, Wnt or TGF beta signalling is mediated by serine phosphorylation and de-phosphorylation by cytoskeleton-associated kinases and phosphatases. This PDLIM2 acts as a cytoskeleton-to-nucleus courier protein for these signalling pathways to promote cancer cell migration and EMT. Bowe RA, Cox OT, Ayllon V, Tresse E, Healy NC, Edmunds SJ, Huigsloot M, O’Connor R: PDLIM2 regulates transcription factor activity in epithelial-to-mesenchymal transition via the COP9 signalosome. Mol Biol Cell 2014, 25(1):184-195. Tanaka T, Soriano MA, Grusby MJ: SLIM Is a Nuclear Ubiquitin E3 Ligase that Negatively Regulates STAT Signaling. Immunity 2005, 22(6):729-736. Healy NC, O’Connor R: Sequestration of PDLIM2 in the cytoplasm of monocytic/macrophage cells is associated with adhesion and increased nuclear activity of NF-(kappa)B. J Leukoc Biol 2009, 85(3):481-490. Loughran G, Healy NC, Kiely PA, Huigsloot M, Kedersha NL, O’Connor R: Mystique is a new insulin-like growth factor-I-regulated PDZ-LIM domain protein that promotes cell attachment and migration and suppresses Anchorage-independent growth.

BIOMARKERS FOR METASTATIC COLORECTAL CANCER
CJ Hayes\textsuperscript{1,2}, PA Kiely\textsuperscript{1,2,3}, TM Dalton\textsuperscript{1}
\textsuperscript{1}Stokes Institute, University of Limerick, Limerick, \textsuperscript{2}Department of Life Sciences, University of Limerick, Limerick, \textsuperscript{3}Materials and Surface Science Institute, University of Limerick, Limerick

In patients who have been diagnosed with a cancer, biomarkers have many potential applications including differential diagnosis, prediction of response to treatment and monitoring of progression of disease. To identify potential biomarkers, large scale gene expression profiling has become an essential tool for the biological and medical investigations of pathological samples. However, this approach is often limited by the availability of large amounts of biological sample for RNA extraction.

Our approach is based upon the concept of using microfluidic droplets, to act as distinct miniature reactors, from which we will be able to quantify gene expression levels of both normal and cancer samples taken from a cohort of patients. We are focusing on genes that are regulating the extracellular matrix (ECM) environment. The ECM is a central component of the tumour microenvironment, housing several other cells including inflammatory cells and active fibroblasts, while feeding biological information to the cells to control cell differentiation and proliferation.

The gene expression profile being developed focuses on stroma related genes in colorectal samples. A dominant factor for biomarker discovery is the need for higher throughput with increased level of specificity coupled with reduced volumes of reagents. Microfluidics provides numerous advantages for this application including economies of scale, parallelisation, automation and increased sensitivity that come from utilising micro-volume reactions.

In a small patient cohort of matched benign, stage I, stage II, stage III and documented metastatic samples, we have demonstrated a pattern of ECM genes that are differentially expressed between normal and diseased tissue of the same patient. Integrin & matrix metalloproteinase (MMP) families are among those most dysregulated in the patient samples analysed. When expanded, this profile may help separate colorectal cancer into distinct ‘extracellular matrix protein expressing’ groups from which we will be able to develop robust diagnostic and prognostic tools that can be applied in a clinical setting.
Mechanisms of tumour invasion: Markers of EMT detected during 3D culture of patient breast tumour tissue
Jean McBryan, Arnold D.K. Hill and Leonie S. Young.
Dept Surgery, Royal College of Surgeons in Ireland, Dublin 2, Ireland.

Understanding the invasive progression from primary breast tumour to metastatic disease is key to improving clinical treatment strategies and reducing breast cancer mortality. A particular gap in our current knowledge is understanding how tumour cells separate themselves from the tumour mass to start migrating around the body. In vitro and in vivo models fail to accurately replicate this process as they can not reproduce the paracrine signalling and complex interactions between multiple cell types. The aim of this research is to culture human breast tumours ex vivo on a unique gelatin-rich matrix and assess the molecular changes occurring as cells migrate away from the tumour mass. This technique of ex vivo culture is sufficient to maintain the original tumour characteristics such as hormone receptor status. With time, cells start to migrate away from the tumour mass and invade the surrounding matrix. Results show evidence of epithelial to mesenchymal transition (EMT) as cells become more migratory. Altered expression of protein markers have been detected by immunohistochemistry as well as increased secretion of matrix metalloproteinases (MMPs) into the surrounding media. These findings add to our understanding of metastatic initiation. The detection of tumour-secreted paracrine signals may help to identify serum biomarkers suitable for monitoring disease progression in individual patients.
Epithelial to mesenchymal transition coincides with the development of cisplatin resistance in neuroblastoma cell line SK-N-AS.

JC Nolan$^{1,2}$, RA Conlon$^{1,2}$, H Harvey$^{1,2}$, P Dowling$^{3}$, F O'Sullivan$^{3}$, I Bray$^{1,2}$, RL Stallings$^{1,2}$, OPiskareva$^{1,2}$

$^1$Cancer Genetics Group, Department of Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin, $^2$Children's Research Centre, Our Lady's Children's Hospital, Dublin
$^3$National Institute for Cellular Biotechnology, Dublin City University, Dublin

Neuroblastoma is one of the most challenging childhood malignancies, accounting for ~15% of paediatric cancer deaths, emphasizing the need for novel therapeutic approaches. Acquired or innate resistance to chemotherapeutics agents is the single most important factor leading to therapy failure and recurrence of malignant cancer, including neuroblastoma.

In order to learn more about potential resistance mechanisms to cisplatin in neuroblastoma, we developed and characterised a drug resistant SK-N-AS neuroblastoma sub-line, named SK-N-ASCis24. This sub-line demonstrated a 5 fold increase in cisplatin resistance and also exhibited cross resistance to the unrelated chemotherapeutics etoposide, irinotecan and temozolomide.

A number of cytoskeletal proteins had altered abundance in SK-N-ASCis24 compared to SK-N-AS parental consistent with changes associated with epithelial-mesenchymal transition. These include decreases in cytokeratin proteins KRT18, KRT8 (8.414 and 18.371 fold, respectively) and increases in vimentin (4.831 fold). SK-N-ASCis24 acquired dramatic changes in cellular morphology and significant modifications in the expression of proteins involved with pathways such as actin cytoskeletal signalling (p=9.28E-10), integrin linked kinase signalling (p=4.01E-8), epithelial adherens junctions signalling (p=5.87E-8) and remodelling of epithelial adherens junctions (p=5.49E-8). All these pathways involve proteins of integrin [ITGB1 (2.7 fold), ITGA2 (2.1 fold)], actin [ACTN1 (3.1 fold), ACTN4 (2 fold), ACTC1 (1.6 fold), ACTB (1.4 fold)] and myosin [MYH9 (4.3 fold), MYL12B (3 fold), MYL6 (2.5 fold)] families which exhibit elevated levels in SK-N-ASCis24.

The increased expression of the genes on mRNA and protein levels without a change in their copy number suggests that their expression is regulated on post-transcriptional/translational level. As these proteins play important roles in mesenchymal cell migration, it is of interest that the SK-N-ASCis24 exhibits a very significant increase in migration potential relative to the parental line (2.5 fold, p< 0.005). Therefore, we can conclude that the epithelial to mesenchymal transition contributes to cisplatin resistance in neuroblastoma cell line SK-N-ASCis24.
SRC-1 MEDIATES TRANSCRIPTIONAL REPRESSION IN ENDOCRINE RESISTANCE

E Ward¹, A Fagan¹, D Vareslija¹, A Hill², L Young¹
¹Endocrine Oncology Lab, RCSI, Dublin
²Surgery, Beaumont Hospital, Dublin

Steroid receptor coactivators (SRCs) have been described as "the powerhouses of transcription" on the basis of their ability to bind across unrelated families of transcription factors and coordinate the regulation of many genes in multiple complex physiological states. There is now substantial evidence that SRC-1 is central to the ability of endocrine tumours to adapt and overcome targeted therapy thereby facilitating metastatic disease progression. Challenging the archetypal function of SRC-1 as a transcriptional activator, global bioinformatic analysis of direct SRC-1 targets carried out in the lab has revealed a significant number of repressed genes. Initial work identified a set of SRC-1 suppression targets which could be silenced through a combined recruitment of HDAC proteins and histone methylation. To further elucidate the mechanism of SRC-1 mediated transcriptional repression we examined the role of the SRC-1 interactome in DNA methylation utilising RNA-sequencing. We compared endocrine resistant LY2 cells transduced with either SRC-1 shRNA or a non-targeting control. Utilising this shRNA model we have been able to identify differentially expressed genes providing us with a transcriptional repression signature associated with SRC-1. To confirm SRC-1’s mechanism of action as a repressor we carried out Methylated DNA immunoprecipitation combined with next-generation sequencing on the same transduced cells. The functional significance of these repressed genes was further studied in a xenograft mouse model, in which LY2 shSRC-1 stable cells were implanted. The primary tumours were investigated to assess mechanisms driving metastasis. We have described that the coactivator protein SRC-1 can act as a transcriptional repressor and have proposed a model whereby SRC-1 can select genes for repression or activation in a promoter specific context. SRC-1’s ability to bi-directionally regulate key genes can drive functional change and enforce its end phenotype. This novel mechanism of coregulatory gene repression significantly alters the perception of SRC-1 in the molecular pathogenesis of cancer.

A.Walsh, C., 2012. The steroid receptor coactivator, SRC-1, can function as a transcriptional corepressor to selectively suppress anti-tumourigenic target genes in Tamoxifen resistant breast cancer.
PLATINUM-INDUCED DNA DAMAGE AND REPAIR MECHANISMS IN NON-SMALL CELL LUNG CANCER
Y He1, S Finn1,2, S Cuffe1, MP Barr1
1Thoracic Oncology Research Group, St James’s Hospital & TCD, Dublin
2Department of Histopathology, St James’s Hospital & TCD, Dublin

Introduction: In the absence of specific treatable mutations, cisplatin-based doublet chemotherapy remains the gold standard treatment for NSCLC patients1. However, its clinical efficacy is hindered in many patients due to both intrinsic and acquired resistance to this drug. Alterations in the DNA repair capacity of damaged cells is now recognised as an important factor in mediating this phenomenon2.

Methods: DNA Repair Pathway RT2 Profiler Arrays were used to elucidate the key DNA repair genes implicated in cisplatin resistant NSCLC cells using cisplatin resistant (CisR) and corresponding parental (PT) H460 NSCLC cells previously established in our laboratory3. The regulation of the trans-activation of p53 in response to DNA damage was studied by examining protein accumulation, post-translational modifications (p53Ser15) and whether depletion of the novel DNA repair protein, hSSB1, affects the regulation of p53 in response to cisplatin. The repair of cisplatin-induced double strand breaks (DSBs) was examined by immunofluorescence imaging of γH2AX foci. Expression of p53Ser15 (phosphorylated & total) in addition to hSSB1 was also assessed by HCA and Western blot analysis.

Results: We identified a number of critical DNA repair genes that were differentially regulated between parental and cisplatin resistant NSCLC cells, some of which are known to be implicated in the nucleotide and mismatch repair pathways. γH2AX was shown to be a reliable and specific marker of DNA double strand DNA breaks induced by platinum agents such as cisplatin. Cisplatin induced the translocation of p53 from the cytoplasmic compartment of H460 PT cells to the nuclear compartment, while significant levels of p53 were retained within the cytoplasmic compartment of CisR cells. Using both HCS and Western blot analysis, hSSB1 protein was undetectable.

Conclusions: To date, despite reports that differential expression of components of the various DNA repair pathways correlate with response to cisplatin, translation of such findings in the clinical setting are warranted. The identification of alterations in specific proteins and pathways that contribute to these unique DNA repair pathways in cisplatin resistant cancer cells may potentially lead to a renewed interest in the development of rational novel therapies for cisplatin resistant cancers, in particular, lung cancer.

The breast is an example of a stratified epithelium consisting of Estrogen Receptor-alpha (ER-alpha) positive milk-producing luminal cells and ER-alpha negative basal/myoepithelial cells that are in contact with the basement membrane and provide the contractile force to expel milk during lactation. Both cell types are thought to arise from a mammary stem cell which can give rise to bipotent progenitors, committed basal and luminal progenitors, which in turn give rise to the relevant mature cell types. The control of mammary differentiation is poorly understood but there is accumulating evidence that the hereditary breast and ovarian cancer susceptibility gene BRCA1 may play a key role in this process. Germline BRCA1 mutations are known to confer a lifetime risk of up to 80% for breast cancer. BRCA1 is a large multifunctional protein with well characterised roles in DNA damage repair, transcriptional regulation, cell cycle checkpoint control and ubiquitination. We have previously shown that BRCA1 regulates two key pathways involved in mammary stem cell regulation and differentiation, the p63 and Notch pathways, to enforce differentiation along basal and luminal lineages, respectively. We have recently identified the NF-kB pathway acts downstream of the BRCA1/p63/Notch signalling axis and show that BRCA1 regulates NF-kB activity levels through control of IkBalpha levels through Notch activation. This control appears to be crucial in the differentiation from stem to progenitor cells and onto mature breast epithelial cells as basal levels of NF-kB activity need to be altered in order to accommodate the new cell phenotypes. Inhibition of NF-kB either through siRNA or chemical inhibition leads to an accumulation of more primitive cell types and a loss of the normal differentiation program. We propose that loss of this signalling cascade, as seen in BRCA1 mutant tumours, leads to unregulated high NF-kB activity which in turn may drive tumourigenesis through the accumulation of stem-like, undifferentiated cells with high proliferative capacity and resistance to chemotherapy. Furthermore, high NF-kB activity has been shown to lead to the up-regulation and secretion of a number of chemokines which in turn play a key role in the tumour microenvironment enhancing cell migration, invasion and metastasis.
Golgi Phosphoprotein 2 (GOLPH2) is a novel bile acid-responsive modulator of oesophageal cell migration and invasion

AM Byrne¹, G Duggan¹, D Prichard¹, M Kirca¹, S Finn³, JV Reynolds², A Long¹
¹Clinical Medicine, Trinity College Dublin, Dublin
²Surgery, Trinity College Dublin, Dublin
³Histopathology, St James's Hospital, Dublin

The Golgi apparatus is the key organelle orchestrating protein processing trafficking and secretion. We previously demonstrated that the Golgi structure is fragmented in cancer and Golgi functions in regulating protein trafficking and secretion are impaired. In this study we identified that expression and intracellular localisation of a Golgi-associated protein GOLPH2, was altered in premalignant and malignant oesophageal tissue. GOLPH2 was found to be constitutively secreted from metaplastic, high-grade-dysplastic and adenocarcinoma cell lines but not squamous oesophageal epithelial cells. Treatment with Deoxycholic acid (DCA), a bile acid present in gastric refluxate implicated in oesophageal adenocarcinoma progression, caused disassembly of the Golgi complex. Moreover, GOLPH2 was cleaved at the Pro-protein convertase cleavage site and secreted in response to DCA. Knockdown of GOLPH2 by siRNA in oesophageal cancer cells decreased cell migration and invasion suggesting a novel function for this Golgi-associated protein. Expression of a non-cleavable GOLPH2 mutant impaired cell invasion. These results demonstrate the significance of GOLPH2 cleavage and secretion in regulating tumour cell invasion and underscore the importance of Golgi-associated proteins in tumourigenesis.
Inhibition of EGFR tyrosine kinase signalling pathway has emerged as an important treatment strategy available for solid tumours, with drugs approved and in clinical use against breast, lung, colon, and pancreatic cancers. The most commonly targeted members of this receptor family are EGFR (ErbB-1), HER2/c-neu (ErbB-2). EGFR+ patients have a reasonably high response rate of 60%, however the development of tumour resistance is rather common, thus driving the continual development of novel inhibitors and combination therapies. Cancer-associated cachexia syndrome is primarily associated with a degeneration of skeletal muscle and a profound reduction of lean body mass. It is highly prevalent, with an overall prevalence of up to 50% of cancer patients, and rising to 80% in patients with late-stage cancers. Cachexia is highly associated with morbidity and mortality. Few management options are available, and the most common treatments; anabolic steroids and progestins, are associated with rather severe side-effects and are generally used only in late-stage cancers. Here we present evidence that several clinical EGFR family inhibitors, including lapatinib and gefitinib, as well as several structural analogues and preclinical compounds, exhibit a profound inhibition of skeletal muscle differentiation in vitro. This inhibition is independent of generalized cell toxicity, but is associated with a large reduction in myogenic fusion, as well as the induction of phenotypic abnormalities. This evidence presented here suggests the possibility that EGFR inhibition may actually enhance the progression of cachexia during cancer treatment through the inhibition of normal muscle regeneration processes.
ANALYSIS OF GENE EXPRESSION AND COPY NUMBER VARIATION OF THE HISTONE VARIANT, H2AX, IN HUMAN CANCER CELLS
S Donohue¹, L Whelan¹,², R Lmfon¹,², A Flaus², H Dodson¹
¹Anatomy, School of Medicine, NUIG, Galway
²Centre for Chromosome Biology, Biochemistry, School of Natural Sciences, NUIG, Galway

H2AX is a variant of core histone H2A, an integral component of the nucleosome that wraps and packages eukaryotic DNA. H2AX plays an early role in the DNA damage response through phosphorylation on Ser 139 following a DNA double strand break to promote recruitment and retention of repair factors at the break site. The expression, distribution and abundance of H2AX in human cells is likely to influence the DNA damage response. We observed that 20-30% of all nucleosomes contain H2AX in a range of cultured human cell lines and investigated whether H2AFX gene copy number and expression is altered in breast and prostate cancer cells. Preliminary data from MDA Pca 2b, DU-145 and PC-3 prostate cancer cells lines shows a trend towards reduced H2AFX transcription compared to normal PWR-1E prostate cells. In contrast, parallel experiments for SK-BR-3, T47D and MDA-MB-231 breast cancer cells demonstrate elevated H2AFX expression compared to normal MCF10A breast cells. There is some evidence that the H2AFX gene is altered in tumours so we investigated gene copy number in several commonly used breast cancer cell lines. When normalised to the fully sequenced HeLa cell line known to have 2 copies of H2AFX, we found statistically significant copy number variation in MCF10A, T47D and MCF7 cell lines. These preliminary observations suggest that significant H2AFX gene copy number and expression variation may occur in tumour cell lines. Since H2AX is a highly abundant chromatin protein and an important participant in the DNA damage response, understanding these variations could lead to the development of H2AX as a potential biomarker and have implications for tumour development and progression.
RACK1 and Non Muscle Myosin IIa Promote Oesophageal Adenocarcinoma Cell Migration

DE Duff1, and A Long1
1Clinical Medicine, Institute Molecular Medicine, Trinity College Dublin, Dublin

RACK1 is a scaffolding protein which, by affecting the subcellular distribution and function of numerous proteins, can coordinate cell motility, proliferation and adhesion. RACK1 has been implicated in the progression of many cancers and can promote metastasis. The role of RACK1 in oesophageal adenocarcinoma has not been investigated. Non-muscle myosin IIa is an actin-binding motor protein which is important for migration and which has been implicated in metastasis. Using a proteomics approach we previously demonstrated a novel interaction between RACK1 and non-muscle myosin heavy chain IIa (NMHCIIa) in migrating T-lymphocytes. In this study we investigated if RACK1 and NMHCIIa interact during migration of Flo-1 oesophageal adenocarcinoma cells. However, no RACK1-NMHC2a interaction was identified by FRET microscopy. Furthermore, immunofluorescent staining and examination of NMHCIIa-mCherry and RACK1-EGFP localisation patterns using fluorescent ratiometric imaging showed that both proteins localise to different regions within migrating Flo-1 cells. NMHCIIa localises to the lamellipodium. RACK1 localises to a region which includes the lamella and an area of the cell body behind the lamella. The lamellipodium and the lamella are integral parts of the cell migration machinery; thus respective localisation of NMHCIIa and RACK1 to these structures indicated that both proteins are involved in FLO-1 migration.

To test this, we used siRNA to deplete RACK-1 or NMHCIIa expression from FLO-1 cells and assessed migration using a scratch-wound assay. We demonstrate a significant decrease in cell migration when RACK-1 or NMHCIIa expression is depleted compared to siRNA control. We have shown that both RACK1 and NMHCIIa are required for oesophageal adenocarcinoma cell migration. Both proteins localise to distinct subcellular locations in migrating oesophageal adenocarcinoma cells and thus their roles in migration appear to be independent of any interaction between them. Given their role in oesophageal adenocarcinoma cell migration, RACK1 and NMHC2a have potential as anti-metastatic targets for this malignancy.
THE ROLE OF ADAM-17 IN MEDIATING DRUG RESISTANCE AND CELL INVASION IN KRAS AND BRAF MUTANT COLORECTAL CANCER

N Forsythe\(^1\), K Redmond\(^1\), P Gilliland\(^1\), R Williams\(^1\), P Johnston\(^1\), SV Schaeybroeck\(^1\)

\(^1\)CCRCB, Queens University Belfast, Belfast

**Background:** ADAM-17 (TNF-alpha converting enzyme -TACE) is a membrane bound metalloprotease involved in the proteolytic shedding of several cytokines, growth factors and receptors. We have previously shown that ADAM-17 activity increases after chemotherapy, which leads to an increase in growth factor shedding and activation of several pro-survival receptors such as EGFR, IGF1-R, HER3, and VEGFR. Furthermore ADAM-17 activity has been shown to be regulated by mutant KRAS in a MEK1/2-dependent manner. Aim We now wish to examine the role of ADAM-17 in response to chemotherapy and radiotherapy in both a KRAS and BRAF mutant genetic background. In addition we will also investigate the involvement of ADAM-17 in mediating an invasive phenotype in a panel of cell line models.

**Methods:** ADAM-17 activity was measured using a fluorometric assay. Growth factor/cytokine shedding was measured by ELISA. Migration and invasion was measured using the xCELLigence system. Apoptosis was measured using flow cytometry. Cell lysates were analysed by western blotting.

**Results:** Chemotherapy and radiotherapy caused an increase in TGF-a shedding in KRAS and BRAF mutant colorectal cancer cell line but no significant increase was seen in the matched isogenic wild type cells. TGF-a shedding following chemotherapy/radiotherapy could be abrogated in a dose dependent manner using a small molecule inhibitor of ADAM-17. In addition targeting of ADAM-17 in combination with chemotherapy resulted in significant increases in apoptosis. Interestingly ADAM-17 targeting can reduce invasion rates in a KRAS dependent manner.

**Conclusion:** Increased ADAM-17 activity in KRAS and BRAF mutant CRC plays a key role in drug resistance and pro-survival signalling of CRC cells. These results suggest that inhibition of ADAM-17 activity in combination with chemotherapy particularly in a KRAS/BRAF mutant genetic background may have a therapeutic benefit.
ASSESSMENT OF AUTOPHAGY INDUCERS AND DIFFERENTIALLY EXPRESSED GENES AS MODULATORS OF CHEMO-SENSITIVITY IN OESOPHAGEAL CANCER

MK Healy1, TR O’Donovan1, MJ Nyhan,2, B Buckley2, SL Mckenna1
1Cork Cancer Research Centre, University College Cork, Cork
2Pharmacology, University College Cork, Cork

Background: Autophagy is a highly conserved cellular process, whereby components of the cytoplasm, such as protein aggregates, organelles and other macromolecules are digested. Our laboratory has previously shown that while chemo-sensitive cell lines (OE21 & OE33) display apoptotic cell death in response to treatment with 5-fluorouracil (5-FU), chemoresistant oesophageal cancer cell lines (OE19 & KYSE450) only induce autophagy [1]. Potential inducers of autophagy have been screened and tested to see if disruption of this process can chemosensitise resistant cells to 5-FU. Affymetrix GeneChip® array data was also examined to assess differential gene expression between the chemosensitive and resistant cell lines. A number of genes of interest have been analysed by rtPCR and siRNA knockdown in order to elucidate their functional role on chemo-sensitivity in these cell lines.

Results: (i) A number of compounds including amiodarone, trehalose, carbamazepine and valproic acid (VPA) were analysed for their ability to induce autophagy and the effect on chemo-sensitising cells to 5-FU. VPA (2.5mM & 5mM) as a single agent, negatively impacted clonogenic survival of KYSE450 cells. When tested in combination with 5-FU, valproic acid displayed a synergistic effect in decreasing clonogenic survival. The contribution of autophagy to this enhancement of cytotoxicity, with these agents, is currently under evaluation. (ii) Using various inclusion criteria (functional role in apoptosis/autophagy; vesicular trafficking; scientific novelty) and gene databases assembled in our lab, we selected and confirmed differential expression of several genes of interest including; TNFAIP3, PRKCA, Trim24 & NT5E.

Conclusion: In conclusion, valproic acid in combination with 5-FU may represent a novel treatment strategy for chemo-resistant oesophageal cancer cells. We have also created a database of genes of potential functional importance and are currently evaluating their role in chemo-sensitivity using siRNA knockdown studies.

FINE TUNING OF THE IRE1 AND ATF-6 ACTIVATION DURING UPR BY PERK IN MIR-424-503 DEPENDENT REGULATORY LOOP

A Gupta, MM Hossain, DE Read, S Gupta
1Pathology, National University of Ireland Galway, Galway

The endoplasmic reticulum (ER) responds to changes in intracellular homeostasis through activation of the unfolded protein response (UPR). UPR can facilitate the restoration of cellular homeostasis, via the concerted activation of three ER stress sensors namely IRE1, PERK and ATF6. UPR is an adaptive mechanism to compensate the protein misfolding and unfolding which ultimately results to a binary response either cell survival or cell death. Global approaches in several cellular contexts have revealed that UPR regulates the expression of many miRNAs that play an important role in the regulation of life and death decisions during UPR (1-3). Here we show that expression of miR-424-503 cluster is downregulated during UPR. IRE1 inhibitor (4µ8c) and deficiency of XBP1 had no effect on downregulation of miR-424-503 during UPR. Treatment of cells with CCT030312, a selective activator of E12AK3/PERK signalling leads to the downregulation of miR-424-503 expression. The repression of miR-424-503 cluster during conditions of ER stress is compromised in PERK-deficient mouse embryonic fibroblasts (MEFs). The miR-424 overexpression suppresses the ATF6 transcriptional activity during the ER stress response. Further miR-424 overexpression modulates the activity of IRE1-XBP1 axis during UPR as monitored by XBP1 mRNA splicing, transcriptional activity of spliced XBP1 and regulated IRE1-dependent decay (RIDD). The miR-424 had no effect on the activity of PERK pathway. Our results suggest that miR-424/322 constitutes an obligatory fine-tuning mechanism where PERK-mediated downregulation of miR-424-503 cluster regulates optimal activation of IRE1-XBP1 axis and ATF6 during conditions of ER stress.

CAN INSIGHT INTO THE LOCALISATION OF HUMAN DHFR AND DHFRL1 OFFER A NEW PERSPECTIVE ON ANTIFOLATE THERAPIES?

L Hughes¹, M Ozaki¹, F O’Sullivan² and A Parle-McDermott¹

1. School of Biotechnology, Dublin City University, Dublin 9, 2. National Institute for Cellular Biotechnology, Dublin City University, Dublin 9.

Antifolate drugs which target folate metabolism have been used for many years as treatments for parasitic, microbial and malignant diseases. Dihydrofolate reductase (DHFR), an essential enzyme in folate metabolism targeted by methotrexate, is considered to be exclusively cytoplasmic. In 2011 we and others reported that the formerly annotated pseudogene DHFRL1 was expressed and functional as well as participating in de novo dTMP synthesis in mammalian mitochondria. DHFRL1 has no mitochondrial targeting sequence therefore its localisation mechanism is not understood. Endogenous mitochondrial Dihydrofolate reductase activity has been reported in hepatocellular carcinoma (HepG2) cells.

Our aim was to understand how DHFRL1 localizes to the mitochondria by using site directed mutagenesis, Western blot and confocal microscopy.

The first three amino acids of DHFR and DHFRL1 were successfully mutated, DHFR to the equivalent amino acids in DHFRL1 and vice versa. Hek 293 cells were transfected with pCMV6-ac-GFP vectors containing DHFR, DHFRL1, and mutated versions of both genes. Transfected cells were fixed and stained with Mitotracker™ red and localisation studies were performed by confocal microscopy and Western blotting of extracted mitochondrial proteins. In both experiments all four proteins localized to the mitochondria. GFP from the transfected empty vector has a mitochondrial band on the Western blot but showed minimal localisation on confocal microscopy. Western blot analysis on mitochondrial extracts from untransfected Hek 293 cells showed the presence of an endogenous active Dihydrofolate reductase, specific activity 14.88 nmol/min/mg protein.

We have demonstrated that when coupled to GFP both DHFR and DHFRL1 localize to the mitochondria of HEK293 cells (as well as the cytoplasm) and that altering the first 3 amino acids of either protein does not affect localisation. We have also shown there is an endogenous active Dihydrofolate reductase present in the mitochondria of this non-cancerous cell line. Since there has been some dispute in past literature as to how Methotrexate interacts with the mitochondria and as current literature suggests that the up-regulation of some mitochondrial enzymes can be determinant’s of response to antifolate therapies, the localisation of DHFR and DHFRL1 in cancer cells is worth further investigation.
CELLULAR REGULATION OF THE UBIQUITIN CONJUGATING ENZYME UBC6

Lam SY\textsuperscript{1}, Murphy C\textsuperscript{1}, Foley LA\textsuperscript{1}, Ross SA\textsuperscript{1}, Wang TC\textsuperscript{2}, Fleming JV\textsuperscript{3}.

\textsuperscript{1}School of Biochemistry and Cell Biology, University College Cork, Cork, Ireland; School of Pharmacy, University College Cork, Cork, Ireland; Analytical and Biological Chemistry Research Facility, University College Cork, Cork, Ireland. \textsuperscript{2}Department of Medicine, Columbia University, New York, NY 10032, USA. \textsuperscript{3}School of Biochemistry and Cell Biology, University College Cork, Cork, Ireland; School of Pharmacy, University College Cork, Cork, Ireland; Analytical and Biological Chemistry Research Facility, University College Cork, Cork, Ireland.

The unfolded protein response (UPR) is recognised to play an important role in the early development and establishment of a number of different cancers. In particular, there is evidence to suggest that UPR induction can facilitate the epithelial to mesenchymal transition, and that it protects transformed cells in the deprived nutritional and hypoxic environment that exists before tumour vascularisation. There is also evidence to show that UPR induction can influence the response of cells to chemotherapeutic drugs. Bortezomib, for example, which is currently used in the treatment of multiple myeloma and mantle cell lymphoma, has significant UPR-related drug resistance associated with it. These data therefore point towards an important role for UPR signalling in the treatment of established tumours while at the same time highlighting the need to identify novel sites at which the UPR can be regulated and manipulated.

Endoplasmic reticulum associated degradation (ERAD) of proteins, which represents an important component of the UPR, is mediated by the ubiquitin proteasome pathway. We have looked at the ubiquitin conjugating enzyme, Ubc6, which is a key component of ERAD, and have shown that both ER localisation and cis-enzymatic activity are important in order for the protein to regulate its own degradation (Lam et al 2014, PMID; 25083800). Our previous studies gave no indication however as to the mechanism by which degradation occurs or the cellular conditions during which it might be regulated. Here we demonstrate that protein kinase C (PKC) signalling increases the stability of the protein and that this increase can be blocked by MEK inhibition. Our results suggest that regulation of Ubc6 stability by ERK activating factors may help to protect the cell during a variety of stressful conditions, including some that are associated with either the establishment and maintenance of cancerous cells, or the development of resistance to anticancer drugs. Ongoing studies aim to characterise the molecular basis for this regulation.
CANCER STEM CELLS: THE ROOT OF CISPLATIN RESISTANCE IN NSCLC

L MacDonagh¹, SG Gray³, S Cuffe³, SP Finn¹, KJ O'Byrne², MP Barr¹
¹Thoracic Oncology Research Group, Trinity College Dublin and St. James's Hospital, Dublin
²Cancer and Ageing Research Program, Queensland University of Technology, Brisbane, Queensland

Introduction: Lung cancer is the leading cause of cancer-related death worldwide, where non-small cell lung cancer (NSCLC) accounts for 85% of cases. Survival rates remain dismal with the current 5-year survival rate less than 16%. This is mainly due to the emergence of resistance to cisplatin. The root of this resistance is hypothesized to be due to the presence a rare cancer stem cell (CSC) population within the tumour cell population that can reform a heterogenic tumour, resulting in recurrence and subsequent resistance following cisplatin chemotherapy.

Methods: An isogenic model of cisplatin resistance was established by chronically exposing a panel of NSCLC cell lines to cisplatin for 12months, thereby creating cisplatin resistant (CisR) subline and their corresponding age-matched parental (PT) cells. CSC enrichment of CisR and PT cell lines was induced when cultured in selective CSC media. CSC isolation was performed using the Aldefluor assay (Stemcell Technologies). Cells were stained and separated in order to isolate the Aldehyde dehydrogenase 1 (ALDH1) positive (+ve) and negative (-ve) subpopulations by FACS, where the ALDH1+ve cells, at least in part, are a critical component of the CSC population. Cisplatin resistance was assessed using proliferation and clonogenic survival assays.

Results: CSC-enriched cells showed increased expression of a number of stem-like and CSC-specific markers, resistance to cisplatin, increased survivability and demonstrated the potential for a single cell to give rise to a mixed population of cells, a process known as asymmetric division, a key property of a stem cell. The Aldefluor assay identified a significantly increased ALDH1+ve population within the CisR sublines compared to their PT counterparts. Characterisation of this ALDH1+ve subpopulation confirmed enhanced stem-like properties compared to their ALDH1-ve counterparts. Importantly, inhibition of ALDH1 using a specific inhibitor (DEAB), re-sensitised the resistant cells to cisplatin.

Conclusion: We demonstrate the increased presence of a CSC population within our panel of resistant NSCLC cells and their potential role in modulating the cellular response to cisplatin. Our data also suggest a role for ALDH1 inhibition in the re-sensitisation of resistant lung cancer cells to cisplatin.
Evasion of apoptosis is one of the main hallmarks of cancer cells and therapeutic targeting of proteins that regulate apoptosis for the treatment of cancer is of high clinical relevance. Following ligation of the death receptor, the protein complex called the DISC (Death Inducing Signalling Complex) is formed, into which the initiator procaspase 8 is recruited, processed and activated. By binding to the DISC and dimerizing with procaspase 8, FLIP can inhibit procaspase 8 processing and activation therefore blocking the extrinsic apoptotic signalling cascade. The exact mode of the inter-molecular interactions that take place at the DISC is not fully understood limiting the development of the possible novel therapeutic strategy for targeting apoptosis. Using a combination of molecular modelling, mutagenesis and functional assays, we determined the preferential sites and mode of interaction of all three key apoptotic players at the DISC (FLIP, FADD and Caspase-8). We propose a novel model of DISC assembly in which procaspase 8 dimerizes between two FADD molecules to become activated at the DISC and in which FLIP blocks procaspase 8 dimerization by preferentially occupying one of the two dimerization sites. Our results provide a better understanding of the complex process of DISC formation and implications for the possible novel therapeutic strategy for targeting apoptosis.
EXPLOITING THE PRO-INFLAMMATORY TUMOUR MICROENVIRONMENT THROUGH RATIONAL TARGETING OF INHIBITOR OF APOPTOSIS PROTEINS
C McCann¹, N Crawford¹, P Johnston¹, D Waugh¹, D Longley¹
¹Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast

Introduction: Inhibitor of apoptosis proteins (IAPs) are overexpressed in multiple cancers, including prostate and colorectal. IAPs contribute to treatment resistance through the suppression of caspase activity and inhibition of apoptosis, hence IAPs are an attractive target for cancer therapy. SMAC mimetics are small molecule compounds which mimic the activity of the endogenous inhibitor of IAPs known as SMAC (Second Mitochondrial-derived Activator of Caspase). SMAC mimetic activity is dependent on TNFa signalling, and has also been shown to induce apoptosis in response to TRAIL therapy. Both TRAIL and TNFa are derived from inflammatory cells abundant in the tumour microenvironment, making SMAC mimetic therapy an attractive strategy for treating cancers associated with inflammation.

Methods: A panel of prostate and colorectal cancer cell lines were treated with SMAC mimetic alone, or in combination with exogenous TNFa or TRAIL. Cell viability was determined by MTT assay and apoptosis was assessed using western blotting and AnnexinV/PI flow cytometry. The addition of exogenous TNFa or TRAIL was used to model the pro-inflammatory microenvironment.

Results: Prostate cancer cell lines were resistant to SMAC mimetic therapy alone and in the presence of TNFa although the compound was shown to have on-target activity. Resistance to SMAC mimetics was dependent on the expression of the anti-apoptotic protein cFLIP. Cells displayed increased sensitivity to SMAC mimetic treatment as a result of depletion of cFLIP expression by siRNA. Colorectal cancer cell lines were sensitive to both SMAC mimetic/TNFa and SMAC mimetic/TRAIL combination therapy.

Conclusion: Prostate cancer cell lines are inherently resistant to SMAC mimetic therapy. Targeting of cFLIP through siRNA results in the sensitisation of prostate cancer cells to SMAC mimetics. Combined targeting of IAPs and FLIP in this way or using more clinically viable options may be an effective means of treating pro-inflammatory cancers.
UNTANGLING THE ROLE OF PROTEIN KINASE C IN BREAST CANCER DOWNSTREAM OF GROWTH FACTOR AND ADHESION SIGNALLING.

Mary McCumiskey\textsuperscript{1,2,3}, Catriona Dowling\textsuperscript{1,4}, Susan Dwane\textsuperscript{1,3}, Shona Tormey\textsuperscript{2}, Tara Dalton\textsuperscript{3}, Patrick A. Kiely\textsuperscript{1,4}

\textsuperscript{1} Department of Life Sciences, University of Limerick, Limerick, Ireland.
\textsuperscript{2} University Hospital Limerick and Graduate Entry Medical School, University of Limerick, Limerick, Ireland.
\textsuperscript{3} Stokes Institute, University of Limerick, Ireland.
\textsuperscript{4} Materials and Surface Science Institute, University of Limerick, Ireland.

Recent studies have revealed a correlation between higher RACK1 expression levels and shorter overall survival times of breast cancer patients. RACK1 is a 37 KDa protein with 7 WD repeat sequences which functions as a 7-sided Beta-propeller to shuttle proteins around the cell to their site of function. The scaffolding properties of RACK1 make it essential for integrating these signaling events and RACK1 stabilizes enzyme activity keeping specific pools of proteins in the ‘active’ state, thus facilitating the establishment and maintenance of signalling pathways. Suppression of RACK1 expression in MCF-7 cells severely disrupts cell adhesion and cell migration and has a dramatic effect on the overall activity and substrate specificity of enzymes such as PP2A and Src.

PKC is a family of serine/threonine kinases, consisting of at least 9 different isoforms. Isoforms of the PKC family have been implicated in the regulation of cell proliferation, differentiation and survival. Their activity is mediated by several factors including calcium, phorbol esters, and by binding to RACK1. PKC’s have a well-established role in cancer but their contribution to breast cancer in the context of transmitting signals from the ECM through integrin and IGF-I signalling pathways remains neglected. In this pilot study, we are looking at Protein Kinase C (PKC) expression and it's interaction with RACK1 in breast cancer downstream of growth factor and adhesion signalling. Using qRT-PCR, we have determined the expression profile of PKC's in breast cancer tissue of various breast cancer subtypes, and compared this to the expression profile in normal breast tissue. Combining this with patient data, we are correlating PKC expression with patient prognosis. Our work suggests that PKC expression may have potential use as a biomarker for prognosis and as a target for novel therapeutic agents.
GLIOBLASTOMA: THE ROLE OF AUTOPHAGY IN TREATMENT RESPONSIVENESS

J Noonan\textsuperscript{1}, ME Clerkin\textsuperscript{1}, S Tappuni\textsuperscript{1}, BM Murphy\textsuperscript{1}

\textsuperscript{1}Department of Physiology and Medical Physics, RSCI, Dublin

Despite advances in surgical technique and the addition of temozolomide (TMZ) to the armamentarium, glioblastoma (GBM) remains an essentially incurable disease. Among the various factors that can contribute to treatment failure, the role of autophagy is one of the most poorly understood. For example, in some cases, the induction of autophagy following use of anti-cancer drugs plays a pro-survival role, thereby hindering the apoptotic intention, while in other cases autophagy induction can be pro-death and thus contribute to the anti-cancer efficacy of the drug. In a bid to understand the role of autophagy in treatment responsiveness and resistance, we assessed the basal level of autophagy present in GBM cell lines in order to determine if this played a fundamental and correlative purpose. Furthermore, we investigated whether inhibition/induction of autophagy could sensitise resistant cell lines to various treatment strategies. Eleven GBM cell lines were used in this study: U251, U343, U373, U87, A172 (all commercially available) and MZ18, MZ294, MZ304, MZ256, MZ51, and MZ327 (patient-derived). In order to assess the basal level of acidic vesicle organelles (AVOs), cell lines were incubated with acridine orange (AO) for 15 min at 37 degrees and visualised using confocal microscopy/analysed using flow cytometry. LC3II immunocytochemistry was also performed. The AVO experiments were repeated following treatment of cell lines with TMZ (150\textmu M), TRAIL (100ng/ml), as well as TRAIL and TMZ combined for 48 h, while LC3II immunocytochemistry was repeated following treatment of cell lines with Bafilomycin A1 (BafA1) to determine the autophagic flux. Lastly, cell lines were pre-treated with 3-methyadenine (3-MA; 5mM), BafA1 (10 or 100nM) or Rapamycin (10nM) for 1 h prior to treatment with TMZ, TRAIL, or TMZ and TRAIL combined for 48 h in order to assess the potential of targeting autophagy as a mechanism to overcome treatment resistance. Our results suggest that basal levels of autophagy present in cell lines prior to treatment onset may determine the outcome of anti-cancer drugs, while manipulation of the autophagic flux (induction/inhibition) may sensitise resistant cell lines to treatment.
Microcalcifications in breast cancer: Novel insights into the molecular mechanism and functional consequences

S. O'Grady¹, M.P. Morgan¹

¹Molecular and Cellular Therapeutics, Royal College of Surgeons Ireland, Dublin

Microcalcifications are widely recognised as one of the most common mammographic indicators of malignant growth in breast tissue. Information regarding their size, shape, distribution and chemical composition can be predictive of several key clinical and pathological characteristics including malignancy, prognosis and HER2 status. Initially regarded as a form of cellular debris, evidence now supports a much more active role for these microscopic deposits in the promotion of cancer growth. Microcalcifications (in the form of hydroxyapatite) can increase proliferation, promote inflammation and may generate a more aggressive cancer phenotype. In addition, research from this group has shown that deposition of hydroxyapatite by breast cancer cells is an active process with similarities to osteoblast mediated physiological mineralisation. Despite the obvious importance of microcalcifications in breast cancer biology, many questions regarding the precise nature of their pro-tumouregenic effect remain. It is our aim to gain greater understanding of the role of microcalcifications in driving cancer growth and the mechanism by which they are deposited. Of particular interest is the relationship between microcalcifications and inflammation, as calcium phosphate crystals have been shown to promote inflammation not only in breast cancer but in several forms of arthropathy. We have begun to characterise the mineralisation potential of several commonly used breast cancer cell lines and will explore any links between the level of hydroxyapatite deposition and clinical prognostic factors, such as molecular subtype and metastatic potential. Using Raman spectroscopy, we will examine the chemical profile of our cell culture derived calcification samples and try to elucidate the molecular basis of why some cells will mineralise and others will not. Finally data will be presented regarding the relationship between microcalcifications and the body's inflammatory response to cancer. We will utilise microarray analysis to characterise the cytokine output of breast cancer cells and associated macrophages following stimulation with hydroxyapatite. It is hoped that this work will lead to increased insight into the regulatory mechanisms underlying hydroxyapatite deposition in malignant breast tissue and its effect on breast tumours.
The Prostate Cancer-Bone Marrow Mesenchymal Stem Cell Para-Endocrine Axis in the Metastatic Tumor Microenvironment

KO'L O'Leary¹, SR Ridge¹, GS Shaw², MM Murphy², SF Finn³, JO'L O'Leary³, FS Sullivan¹, SG Glynn¹

¹Prostate Cancer Institute, National University of Ireland, Galway
²Regenerative Medicine Institute, National University of Ireland, Galway
³Department of Histopathology, St James’s Hospital and Trinity College, Dublin
⁴Department of Radiation Oncology, University Hospital, Galway

Mesenchymal stem cells (MSCs) are self-renewing, multipotent cells capable of differentiating into multiple cell types including osteocytes, chondrocytes and adipocytes. They migrate to sites of injury and inflammation and have also been shown to home to primary tumors. Mounting evidence suggests that crosstalk between MSCs and tumor cells may contribute to enhanced migration, invasion and therapeutic resistance of the tumor. MSCs have been suggested as a source of cancer-associated fibroblasts (CAFs) in several primary tumors including prostate. Prostate cancers frequently metastasize to the bone, the primary residence of MSCs, however little is known of the role of these cells in the metastatic prostate cancer microenvironment. In addition, research to date has focused primarily on two-dimensional cell culture models which may not adequately reflect in vivo tumor biology. In the current study, we seek to address these issues using biologically relevant 3D co-culture models incorporating cell lines, bone marrow-derived MSCs and circulating tumor cells. We aim to use these models to characterize the effects of MSC-prostate cancer paracrine signaling on MSC differentiation and prostate cancer invasion, metastasis and therapeutic resistance.
Breast Cancer is the most common type of Cancer in women worldwide. Despite a dramatic improvement in survival rates over the last 40 years, the Basal-like Breast Cancer (BLBC) subtype has the poorest survival rates. The majority of BLBCs are triple negative; lacking expression of estrogen, progesterone and HER2 receptors, consequently, they are currently no targeted therapies. A DNA damaging combination chemotherapy treatment called FEC is the mainstay treatment for BLBC patients. However, chemoresistance to FEC is common in a considerable proportion of BLBC cases. Whilst BRCA1 mutant breast cancers fall within the BLBC subtype, they only represent a fraction of total BLBC cases. We currently have limited information on other key driver genes responsible for BLBCs but they are known to contain extremely high rates of mutation of the key anti-cancer gene p53 (mutation rate of approximately 85% compared to 15-20% in other breast cancer subtypes). Mutant p53 protein levels are often elevated in BLBCs compared to the wild-type protein and some mutations are thought to switch it from an anti-cancer to a pro-cancer gene (a so-called Gain-Of-Function, GOF). We strongly believe that mutant p53 proteins may be acting as oncogenic drivers in BLBCs. Previous research within our lab has shown that siRNA-mediated knockdown of GOF mutant p53 protein is detrimental to survival and proliferation BLBC cell lines. In this project we will outline how we plan to identify novel interactor and effector genes of both mutant and wild-type p53. We aim to identify pathways dysregulated as consequence of p53 mutations in BLBC cell lines and to assess their utility as chemomodulators of existing DNA damaging chemotherapies, or indeed, as novel targets for the design of entirely new chemotherapy drugs. In so doing, we hope to provide new insights into the design of more effective therapies against this particularly aggressive breast cancer subtype.
INHIBITION OF MYD88 FACILITATES PRIMED-STATE TRANSITION DURING DIFFERENTIATION OF NULLIPOTENT EMBRYONAL CARCINOMA CANCER STEM CELLS

G Sulaiman¹, ², B Ffrench¹, ², C Gasch¹, ², JJ O’Leary¹, ², MF Gallagher¹, ² ¹Histopathology, Trinity College Dublin, Dublin ²Pathology, Coombe Womens and Infants Hospital, Dublin

Background: Cancer Stem Cells (CSCs) are highly tumourigenic in the undifferentiated state, a property that is lost upon differentiation. With this in mind, forced differentiation of CSCs is being assessed in many malignancies as a potential clinical CSC-targeting mechanism. Historically, treatment with differentiation morphogen Retinoic Acid (RA) has yielded strong force-differentiation results in pre-clinical studies but its use does not translate to the clinic. We have previously described a role for Toll-Like Receptor (TLR) Signaling modulator MyD88 in malignancy pluripotency. The aim of this study was to further elucidate this mechanism with a view towards developing improved methods of forced differentiation. Approach MyD88 was inhibited (peptide-inhibitor) in nullipotent embryonal carcinoma (EC) CSC cell line 2102Ep and resulting cell types isolated and analysed via flow cytometry.

Results: Initial studies indicated that inhibition of MyD88 forced 2102Ep CSCs to transition into a Primed Undifferentiated State (PUS), which was responsive to differentiation by RA. Further analysis demonstrated that 2102Ep cells contain 2 sub-populations, only one of which is capable of RA-induced differentiation via PUS transition. The first population maintains nullipotency through maintained high expression of pluripotency markers SSEA4, Oct4, Sox2 and Nanog, which are lost during differentiation of the PUS sub-population. This mechanism is highly adaptive: PUS CSCs can return to the nullipotent, differentiation-resistant and highly-tumourigenic state if MyD88 inhibition is removed. Similar mechanisms are likely employed in vivo, which may explain the failure of RA treatment strategies in the clinic.

Conclusion: Our data indicates that MyD88 is a Differentiation-Gate-Keeper in pluripotent malignancy. It is likely that specific Gate-Keepers operate in other malignancies, acting to protect the CSC population from complete differentiation during tumorigenesis. Our data indicates that combining Gate-Keeper targeting with standard CSC morphogens increases force-differentiation efficiency. The addition of Gate-Keeper targeting strategies may facilitate the translation of CSC force-differentiation approaches to the clinic.
An investigation of the functional role of GRAB in the context of Rab3, Rab8 and Rab11

JA Timoney, CP Horgan, MW McCaffrey

1School of Biochemistry and Cell Biology, University College Cork, Cork

GRAB, also known as Rab3iL1 [Rab3A interacting protein (rabin3) Like 1], was identified as a guanine-nucleotide exchange factor (GEF) for Rab3A and bears high similarity to Rabin8 - a GEF for Rab8 and a downstream effector for Rab11A. In 2013, we reported that GRAB interacts with Rab11A and Rab11B via a carboxy-terminus domain of the GRAB protein, in a manner suggestive of it being an effector of Rab11A and Rab11B. Due to similarities between Rabin8 and GRAB we have hypothesised that GRAB may also act as a GEF for Rab8 and have a potential role in the formation of primary cilia. Data will be presented demonstrating the nucleotide dependence of GRAB's interaction with Rab3, Rab8 and Rab11 and the minimal region of GRAB required for such interactions. Furthermore, we have investigated the functional consequences of GRAB knockdown and will present data on the phenotypic outcome.

Invasive lobular carcinoma (ILC) is a subtype of breast cancer comprising 10% of tumours. The majority of ILCs are estrogen receptor (ER) positive and, as such, are candidates for anti-endocrine therapy. Unfortunately, one third of women with breast cancer are de novo resistant to anti-estrogen therapies, while as many as 50% of patients with recurrences display acquired resistance. Epigenetic phenomena control gene expression not by altering the gene sequence but by influencing chromatin structure. Epigenetically-mediated resistance to targeted therapies is a recurring theme in various cancer models. Previously, we identified the Bromodomain and Extraterminal (BET) family protein BRD2 as a putative therapeutic target for ILC. JQ1 (an epigenetic modifier that inhibits BET family proteins) has been reported to have therapeutic potential in the tamoxifen-resistant ductal breast cancer setting. Here, we aimed to test the efficacy of JQ1 as a therapeutic strategy for the treatment of ILC.

Ninety percent of primary ILC samples are ER positive. Two ILC cell lines which have been identified to be ER positive (SUM44-PE and MDA-MB-134VI) were chosen for our study. While both cell lines were relatively resistant to the anti-estrogen therapies tamoxifen and fulvestrant, compared to the positive control MCF-7 sensitive cell line, they displayed sensitivity to JQ1 as shown by decreased cell viability and increased apoptosis. Furthermore, preliminary investigations have shown that JQ1 treatment down-regulates the ER and progesterone receptor. Future work will include determining the efficacy of combination of anti-estrogen therapies with JQ1. We also aim to elucidate the mechanism of action of JQ1 in ILC cell lines using chromatin immunoprecipitation-sequencing, along with transcriptomic analysis, to enable illustration of altered signalling networks caused by JQ1 treatment.
miRNA PROFILING OF BREAST CANCER SUBTYPES.

JAL Brown¹, DP Joyce¹, K O'Brien¹, K Curran¹, E Ramphul¹, RM Dwyer¹, M Kerin¹

¹Discipline of Surgery, School of Medicine, Clinical Science Institute, National University of Ireland Galway, Galway, Ireland,

Currently, breast cancer affects approximately 1/8 women worldwide while the molecular subtyping of breast cancer, into 4 major categories, has advanced both the diagnosis and treatment of breast cancer. However, further insight into differences between each of the subtypes is needed.

Previously, miRNA have been shown to regulate processes involved in tumor initiation and progression. Investigating circulating miRNA, isolated from the blood of breast cancer patients, provides a method for scrutinizing differences between subtypes.

Here using bioinformatics, we identify profiles and clusters of miRNA deregulated in the breast cancer subtypes. In addition, we identify pathways commonly affected through miRNA deregulation. Using miRNA arrays and RQ PCR, we will define and explore the function of differentially expressed circulating miRNA from breast cancer subtypes. Future work involves examination of the function of differentially expressed miRNA using cell culture based models, exploring the effects of target genes and pathways.

Using this approach we believe we can gain important insights in molecular differences between breast cancer subtypes, which can potentially be exploited for the diagnosis or treatment of breast cancer.
**Correlating the expression of Protein Kinase C isozymes with the transformed phenotype in Colorectal Cancer**

CM Dowling\(^1,2\), JJ Phelan\(^4\), MC Cathcart\(^4\), B Mehigan\(^5\), P McCormick\(^5\), T Dalton\(^2\), JC Coffey\(^3\), J O’Sullivan\(^4\), PA Kiely\(^1,2\)

\(^1\)Department of Life Sciences, and Materials and Surface Science Institute, University of Limerick, Limerick, \(^2\)Stokes Research Institute, University of Limerick, Limerick, \(^3\)4i Centre for Interventions in Infection, Inflammation and Immunity, University of Limerick, Limerick, \(^4\)Department of Surgery, Trinity College Dublin, Dublin, \(^5\)GEMS, St.James Hospital, Dublin, Ireland

Protein Kinase C (PKC) is a family of serine/threonine kinases that are involved in almost every signal transduction cascade in cells. PKC enzymes are activated by second messengers and respond to specific environmental cues. The development of PKC modulators with anti-cancer therapeutic potential is a major target in cancer. However, this task is made difficult because PKC has an important role to play in normal processes and the PKC family consists of at least 9 different isozymes. Our hypothesis is that PKC expression, activity and localisation are altered as colon epithelial cells switch from normal to the transformed state. We are confident that being able to recognise these changes has the potential to be used as a biomarker and prognostic marker in the early detection of colon cancer. Real Time PCR was utilised to compare the gene expression profile of 9 PKC isozymes in matched normal and cancer tissue samples. We found differential expression of the conventional PKCs, with PRKCB (the gene coding for PKC Beta II) being significantly downregulated in the cancer tissue of over 90% of patients (p<0.01, n=22). This change was closely linked to the progression of the disease. Using tissue microarrays, we also demonstrated significant alterations in both epithelial and stromal levels of PKC Beta II between the patients normal and diseased tissue. The level of PKC Beta II in the diseased tissue was significantly decreased across patients (p<0.01, n=164) and we associate the decreased expression with tumour stage. In conclusion we suggest that differential expression of PKC Beta II may be an important mechanism regulating the change to the transformed phenotype in colon cancer.
USP18 siRNA RESTORES APOPTOTIC ACTIVITY TO DRUG RESISTANT OESOPHAGEAL CANCER CELLS

CM Falvey¹, TR O'Donovan¹, MJ Nyhan¹, S O'Reilly², SL McKenna¹
¹Cork Cancer Research Centre, University College Cork, Cork
²Department of Oncology, Mercy University Hospital, Cork

Oesophageal cancer is one of the most devastating, drug resistant cancers worldwide with a European five-year survival rate of less than 15%. In order to understand the mechanisms underlying this resistance, we used Affymetrix Micro-Array analysis to evaluate differential gene expression between apoptosis competent oesophageal cell lines which respond well to treatment (OE21,OE33) and apoptosis incompetent cell lines which are highly resistant (KYSE450,OE19). A number of differentially expressed genes were identified including several members of the ISG15-conjugation pathway. This ubiquitin-like protein modification system is stimulated by Type 1 Interferons and results in conjugation of ISG15 to up to 300 protein targets. It is believed that ISGylation stabilises target proteins by antagonising ubiquitination. This process is negatively regulated by the isopeptidase enzyme USP18. We have identified this protein as a crucial regulator of cell death in oesophageal cancer cells. USP18 knock-down in combination with Interferon alpha treatment leads to increased ISG15 conjugation of target proteins. These cells also exhibit decreased ubiquitination levels. Interestingly, this alteration in protein modification leads to the appearance of morphological markers of apoptosis such as cell shrinkage and nuclear fragmentation. Furthermore, this morphology was accompanied by significant elevation in active caspase-3 (from 0.7% to 33%). In clonogenic assays, cells treated with USP18 siRNA and Interferon alpha showed significantly less re-growth following drug removal than cells treated with 5-FU. Interferon alpha treatment alone had no effect on cell death or survival. Additionally, ISG15 knock-down in OE21 cells reverses their natural sensitivity to 5-FU, further supporting a role for this protein in response to chemotherapy. This data suggests that ISG15 conjugation of one or a number of protein targets is sufficient to re-open an apoptotic pathway in drug resistant oesophageal cancer cells and identifies USP18 as a potential drug target for the treatment of oesophageal cancer.
Elucidating the role exosomes play in acquired chemoresistance in triple negative breast cancer (TNBC)

L Gubbins¹, K Weiner-Gorzel¹, S Lindsay¹, S Sharma¹, L Cooke², A Salvati², J Simpson³, K Dawson², M Kell⁴, A McCann¹

¹UCD School of Medicine and Medical Science, University College Dublin, Dublin
²UCD Centre for Bionano Interactions, University College Dublin, Dublin
³UCD School of Biology & Environmental Science, University College Dublin, Dublin
⁴Department of Surgery, Mater Misericordiae Hospital, Dublin

Approximately 10-15% of invasive breast cancers can be described as triple negative (negative for protein expression of oestrogen receptors (ER-), progesterone receptors (PR-), and lack HER2 gene over-expression). Due to their large size, TNBC tumours are innately hypoxic, a tumour microenvironment (TME) that is inducive of cellular fates that are resistant to apoptosis such as senescence and autophagy. Our hypothesis is that following surgical resection of the tumour, remaining residual cancerous cells in the TME release exosomes which propagate a chemoresistance signature to surrounding non-resistant cells. Culturing of MDA-MB-231(MSL) cells in hypoxia (1% O2) for 0-96 hours resulted in the induction of p21, LC3, and TSG101, markers of cellular senescence, autophagy, and exosome release respectively. This result indicates that hypoxia can cause cells to enter senescence, a non-replicative cellular fate that is chemorefractory to cancer therapeutics that target actively dividing cells. Interestingly, hypoxia also resulted in increased protein expression levels of TSG101. Using Nanoparticle Tracking Analysis (NTA) (NS300 platform) to enumerate exosomes, hypoxic exposure (1%) resulted in an increase in the number of exosomes released from the MDA-MB-231(MSL) cell line. This could potentially act as an important avenue for inter-cellular communication. Interestingly, targeted siRNA knockdown of TSG101 resulted in a decrease in the amount of exosomes released by the MDA-MB-231(MSL) cells. Preliminary Raman spectroscopy data suggests that there is a fundamental difference in the biological composition of hypoxic versus normoxic derived exosomes from the ovarian cancer cell line A2780, suggesting a role for differential O2 levels on resultant exosome composition. To examine if exosomes could be involved in the transfer of senescence signals, normoxic cells were cultured in growth conditioned media (GCM) used to grow cells in hypoxia. This resulted in a decrease in phosphorylated-retinoblastoma (pRb) levels which is indicative of cells that are entering a non-replicative fate, possibly senescence. In summary, this work shows that exosomes may play a role in the transfer of senescent signals, which may result in a cellular fate unlikely to undergo cellular apoptosis when chemotherapeutically challenged.
Identification of TTBK1 as a novel RACK1 binding partner in breast cancer

S Hayes\textsuperscript{1}, M Kiely\textsuperscript{1}, M McCumiskey\textsuperscript{1,2}, S Tormey\textsuperscript{3,4}, T Dalton\textsuperscript{2}, PA Kiely\textsuperscript{1,2}

\textsuperscript{1}Department of Life Sciences, and Materials and Surface Science Institute, University of Limerick, Limerick
\textsuperscript{2}Stokes Research Institute, University of Limerick, Limerick
\textsuperscript{3}Graduate Entry Medical School, University of Limerick, Limerick
\textsuperscript{4}Department of Surgery, University of Limerick, Limerick

Increasing our knowledge of the mechanisms regulating cell adhesion and proliferation in breast cancer cells is central to understanding tumour progression and metastasis. RACK1 is a scaffolding protein with 7 WD repeats that mediates crosstalk between growth factor and adhesion receptor signalling and is a well-documented binding partner of PP2A. RACK1 plays a critical role in determining how PP2A functions in signalling pathways by modulating the activity and substrate specificity of PP2A, while regulating its distribution to specific cellular locations. A mass spectrometry screen of the RACK1/PP2A complex in breast cancer cells cultured in 3-Dimensional conditions identified 66 novel binding partners of the complex, a diverse group of proteins found in a variety of cellular locations. One of the proteins identified was Tau-Tubulin Kinase 1 (TTBK1). TTBK1 is a serine/ threonine/ tyrosine kinase and is a member of the casein kinase 1 superfamily, which has the ability to control tubulin activities, phosphorylate tau proteins and induce tau aggregation in human neuronal cells. We have found that the gene coding for TTBK1 is over-expressed in cancer cell lines and 70\% of the breast cancer patients we analysed. We established that in MDA-MB-231 cells, TTBK1 is found in the membrane fractions where it co-localises with RACK1. We used siRNA to knockdown TTBK1 in these cells and showed that TTBK1 plays a role in the regulation of IGF-1 mediated MAP Kinase activation. Using a Real Time Cell Analysis platform, we showed that TTBK1 is required for cell adhesion and proliferation in these cells. Taken together, this work suggests that TTBK1 has a role to play in breast cancer and that TTBK1s function is mediated in part by the interaction with RACK1. Future work in this space is directed at understanding the mechanism by which TTBK1 binds to RACK1 and may reveal the potential for the RACK1/TTBK1 interaction to be used as a novel therapy or diagnostic marker in triple negative breast cancer.
IDENTIFICATION OF NOVEL REGULATORS OF FLIP UBIQUITINATION

C Holohan, JS Riley, PG Johnston, DB Longley
1Centre for Cancer Research and Cell Biology, Queens University Belfast, Belfast

By suppressing the activation of caspase-8, FLIP is a critical regulator of cell fate that inhibits apoptosis induced by a variety of stimuli, including chemotherapy-induced DNA damage. FLIP has been reported by our research group and others to be overexpressed in colorectal (CRC), non-small cell lung (NSCLC) and prostate (CaP) cancers and also to mediate resistance to chemotherapy. In addition, inhibition of FLIP sensitizes pre-clinical models of CRC, NSCLC and CaP to apoptosis by removing its inhibitory effect on caspase-8. Both FLIPs main splice forms have extremely short half-lives and are known to be regulated by the ubiquitin-proteasome system (UPS). In order to identify regulators of FLIPs ubiquitination status we carried out an siRNA screen of all known deubiquitinating enzymes (DUBs). CSN5, a component of the COP9 signalosome (CSN) was identified as a potential DUB for FLIP and as this had also appeared on a yeast-2-hybrid screen for FLIPL, previously carried out in our group; it was taken forward for further investigation. By regulating the most prominent E3 ubiquitin ligase family, the cullin RING E3 ubiquitin ligases (CRLs) activity, CSN-mediated de-neddylation controls ubiquitination of proteins involved in apoptosis induction and DNA damage response (DDR). Moreover, the CSN has been linked to expression of the key apoptosis effector NOXA and the Wnt pathway. The CSN is composed of eight subunits (CSN1 to CSN8), of which CSN5 harbours the catalytic activity; however, CSN5 can also act independently of the CSN complex and has intrinsic DUB activity. CSN5 is overexpressed in many cancers and has been found to interact with a number of important cellular regulators, including p27, p53 and the Myc oncogene making it an attractive therapeutic target. In this current study CSN5 was found to interact with FLIP in colorectal cancer cell line models and regulate FLIP ubiquitination and sensitivity to TRAIL-induced apoptosis. Further studies are ongoing to determine whether CSN5 regulates FLIP via its de-neddylation activity and to identify potential E3 ligases which may also regulate ubiquitination of FLIP.
**Investigation into the pharmacological manipulation of PP2A signalling using novel peptides**

C Johnson\(^1\), F Giles\(^2,3\), F Sullivan\(^1,4\), MP Vitek\(^5\), S Glynn\(^1\)

\(^1\)Prostate Cancer Institute, NUI Galway, Galway
\(^2\)HRB clinical Research Facility, NUI Galway, Galway
\(^3\)Cancer Therapeutic, NUI Galway, TCD, Galway, Dublin
\(^4\)Radiation Oncology, Galway University Hospital, Galway
\(^5\)Division of Neurology, University Medical Center, Durham, NC 27710, Duke USA

**Background:** PP2A is a tumour suppressor protein, whose dysregulation/inhibition in cancer promotes activation of proliferation signalling pathways leading to tumour promotion. PP2A is comprised of a core dimer which consists of a catalytic subunit (PP2Ac) and a scaffold protein (A subunit). The A subunit is responsible for the binding of a wide variety of regulatory subunits (B subunits) to the core dimer. These B subunits determine the functional role of the heterotrimeric PP2A holoenzyme proteins. Peptide mimetics of Apolipoprotein E (apoE) have previously been shown to bind to and prevent SET activity, consequently increasing PP2A mediated phosphatase activity.

**Aims:** This study aims to investigate the role of a novel compound which targets the SET oncogene, an endogenous inhibitor of the tumour suppressing protein PP2A, and to determine its effects on the activity of PP2A by treating prostate cancer cell lines with an apoE mimetic peptide. The primary aim is to examine the expression and activity of SET, PP2A and also investigate the apoptotic effects and downstream signalling affected by modulation of PP2A in prostate cancer.

**Material & Methods:** Prostate cancer cell lines were treated with ApoE peptide mimetics of varying concentrations in order to determine IC\(_{50}\) values, cytotoxicity, and cell cycle interruption. Modulation of phosphatase activity was explored along with downstream signalling of PP2A.

**Results:** Results to date suggest that ApoE mimetics induce cell death by targeting a protein that is abundantly expressed in prostate cancer cells, making it an appealing candidate for the treatment of cancer. The data indicates up regulation of PP2A and consequential down regulation of AKT signalling, a major pathway implicated in cancer development and progression.
Characterisation of the prolyl isomerase, PIN1, as a novel biomarker and therapeutic target in BRCA1 deficient breast cancer

CN Knowlson¹, NE Buckley¹
¹CCRCB, Queen's University, Belfast

Triple negative (TNBCs) and the closely related Basal-like (BLBCs) breast cancers are a loosely defined collection of cancers with poor clinical outcomes. Both show strong similarities with BRCA1-mutant breast cancers and BRCA1 dysfunction, or "BRCAness", is observed in a large proportion of sporadic BLBCs. These observations suggest loss of BRCA1 function as a key step in their pathogenesis. Given the poor clinical outcomes and lack of targeted therapy for these subtypes, a better understanding of the biology underlying these diseases is required in order to develop novel therapeutic strategies. Phosphorylation of proteins in serine/threonine residues preceding proline (Ser/Thr-Pro) is a key signalling mechanism in breast development and breast cancer. Pin1 binds to and isomerises specific Ser/Thr motifs only after phosphorylation and thereby induces conformational changes to regulate function. Pin1 expression has been shown to be up-regulated in the absence of BRCA1 signalling and can impact on a number of signalling pathways known to be de-regulated in the absence of functional BRCA1 including cell cycle and growth control, DNA damage response, transcriptional regulation and NFκB signalling. Therefore the aim of this study is to investigate the function of Pin1 as a downstream effector of BRCA1. The mechanism of how BRCA1 regulates Pin1 will be investigated first using molecular biology techniques including transcriptional assays. Kinase arrays and kinase siRNA screens will then be used to identify key genes/pathways deregulated by Pin1 in a BRCA1-deficient background. These genes and pathways will then be assessed as potential biomarkers for BRCA1 deficiency. Potential novel therapeutic targets identified will be screened by siRNA and top hits validated in combination with existing conventional therapy for breast cancer. This project will, therefore, allow the identification of BRCA1/Pin1 regulated pathways, and to identify potential biomarkers and novel therapeutic strategies that can be used to treat BRCA1-deficient breast cancers.
The Impact of Expression of the Fusion Protein YWHAE-NUTM2 on Cell Signalling

N McDonagh¹,², E O'Meara¹,², MJ O'Sullivan¹,²
¹National Children's Research Centre, Our Lady's Children's Hospital Crumlin, Dublin
²School of Medicine, Trinity College Dublin, Dublin

Clear cell sarcoma of kidney [CCSK] is the second most common childhood renal cancer. CCSK is a diagnostic challenge, as well as being both aggressive and therapy-resistant. Three individual case reports of CCSKs with t(10;17)(q22;p13) prompted our characterization of the chromosomal translocation. This translocation results in rearrangement of YWHAE (encoding the adaptor protein 14-3-3 epsilon) on chromosome 17 and NUTM2 (encoding the protein NUTM2) on chromosome 10, producing an in-frame fusion transcript YWHAE-NUTM2. To investigate the cell biological effects of the fusion protein, we developed cell lines allowing inducible expression of HA-YWHAE-NUTM2 in HEK293 and NIH3T3 cells. We observed that HA-YWHAE-NUTM2 is present in both nuclear and cytoplasmic compartments of the cell, while wild-type 14-3-3e is localised primarily in the cytoplasm. Since 14-3-3e can mediate signal transduction by phospho-protein interactions, we reasoned that YWHAE-NUTM2, given its ability to enter the nucleus, could alter cell signalling events. Unfractionated, nuclear and cytoplasmic HEK293 cell lysates, induced to express HA-YWHAE-NUTM2 or mock-treated were applied to the Kinex Kam 850 microarray encompassing 850 total and phospho-specific antibodies. From the Kinex Kam 850 microarray heat-map, a short list of proteins was generated, which were up- or down-regulated after treatment, based on their loading intensities. These proteins include MAPK pathway members, heat shock proteins, apoptotic mediators, transcription factors and oncogenes. Of these, the levels of the anti-apoptotic proteins Mcl-1, Bcl-2 and Bcl-xL were validated by western blotting to show increase in cytoplasmic fractions (Mcl-1), or decrease in nuclear fractions (Bcl-2 and Bcl-xL). Hsp60 was validated by western blotting to show an increase following induction of HA-YWHAE-NUTM2 in unfractionated and both the nuclear and cytoplasmic fractions, as observed on the Kinex Kam microarray. Further validation of, and investigation into, the altered cell signalling events in response to YWHAE-NUTM2 expression is ongoing and will assist in identifying novel therapeutic targets for this treatment-resistant cancer.

Circulating microRNAs: Novel breast cancer biomarkers and their use for guiding and monitoring therapy
A McGuire¹, MC Casey¹, M Murphy¹, H Heneghan¹, A McDermott¹, N Miller¹, JAL Brown¹, M Kerin¹
¹Disciple of Surgery, NUIG, Galway

Chemotherapy and hormone therapy are key components in the treatment of breast cancer for many patients. However, response to these treatments can be unpredictable. There exists a cohort of patients who experience little or no benefit from treatment but still experience the side effects associated with it. MicroRNAs are small RNA molecules, detectable in the circulation, whose expression has been found to be deregulated in many cancers, including breast cancer. Our study aims to use a predetermined panel of microRNAs to determine if this signature can be used to predict which breast cancer patients would most likely to respond to treatment. In addition, we aim to discover a novel microRNA signature that could monitor or predict a patient’s response to chemotherapy or hormone therapy. Our prospective multicenter study, involves investigating patients from three distinct cohorts. The first, newly diagnosed breast cancer patients undergoing neo-adjuvant chemotherapy. The second, patients presenting with metastatic disease (either progression or recurrence) undergoing chemotherapy. The final cohort investigated includes patients with metastatic disease (either progression or recurrence) undergoing hormone therapy alone. The unique feature of this study is the collection and analysis of sequential patient blood samples, collected before, during and after treatment. Currently the recruitment for cohort 1 has finished with the final analysis to be completed shortly. Presently, recruitment for cohorts 2-3 is ongoing. Our goal is to investigate the relationship between changes in patients circulating miRNA expression levels over the course of their systemic therapy and their response to treatment. Additionally, any correlation between systemic miRNA levels and standard biomarkers of response (including serum CEA and CA 15-3 levels) will be assessed.
Primary cilia are solitary cellular antennae known to regulate cell proliferation, migration and differentiation and are surprisingly absent in a number of cancers. It has yet to be determined whether the loss or defect in primary cilia is a contributing cause, or secondary manifestation, in cancer progression. HDAC6 is an enzyme responsible for cilia disassembly and inhibition of this molecule has been shown to restore ciliogenesis. Therefore, this study aims to investigate the possibility of restoring cilia incidence in cancer models by inhibiting HDAC6 activity and thus analysing the effect may have on signalling pathways prominent in cancer. The MCF7, breast cancer cell line, was used as a model of cancer for this analysis alongside a control benign breast cell line, MCF10A. The prostate cancer cell line, PC-3, was also used as a model and RWPE-1 cells were used as a benign control. A HDAC6 inhibitor, Tubastatin A, was employed to restore ciliogenesis in the cancer models. Treated samples were maintained in media containing a series of Tubastatin A concentrations for 48 hours (1µM-10µM). Total RNA was extracted from all samples and analyses via qPCR. qPCR results presented a significant increase in IFT88 expression (required protein for cilia formation) for Tubastatin-A treated MCF7s (2.18 fold increase) and PC-3s (2.35 fold increase) in comparison to the untreated cancer cell lines suggesting restoration of cilia in these treated cancer cells. Canonical Wnt signalling biomarker, Axin-2, was down-regulated following HDAC6 inhibitory treatment; indicating abnormal Wnt activation in cancer model is reversed by potential restoration of cilia inferred by increased ITF88 expression. HDAC-6 treatment was also found to significantly alter expression levels of Hedgehog signalling biomarkers Patched and Gli1 which are known to have multifaceted, central roles in development and tumorigenesis. The potential link between ciliogenesis and oncogenic transformation is yet to be fully elucidated. This study indicates potential restoration of ciliary function using HDAC6 inhibitory treatment on cancer cell lines; therefore these findings warrant further investigation of HDAC6 inhibitory treatment as a potential cancer therapy and its role in cilia restoration.
DISSECTING THE MECHANISMS OF CART SIGNALLING THROUGH THE ESTROGEN RECEPTOR IN ER+ BREAST CANCER
BP Mooney¹, S Das¹, K Connor¹, L Mulrane¹, WM Gallagher¹, DP O Connor¹
¹Cancer Biology and Therapeutics, Conway Institute, University College Dublin, Belfield, Dublin

The cocaine- and amphetamine-regulated transcript (CART) was first discovered as a peptide up-regulated by the administration of cocaine and amphetamine to rats. CART peptides are involved in regulating physiological processes, such as feeding and neuro-protection. However, recent studies have associated high CART expression with worse overall survival in patients with small-bowel carcinoid tumours [1] and estrogen receptor-positive (ER+), lymph node-negative breast cancer. Interestingly, in ER+ breast cancer, CART was also shown to be associated with poor patient response to tamoxifen, suggesting CART may play a role in conferring tamoxifen resistance [2]. The aim of this study was to elucidate the mechanism(s) by which CART signals in ER+ breast cancer. In order to test whether CART could mediate the ligand-independent activation of ER-alpha, MAP-Kinase pathway activation and levels of downstream gene-targets of ER-alpha were assessed post CART stimulation. Additionally, the ability of CART to activate ER-alpha using three LXD motifs (nuclear receptor co-activator recognition motifs) present within the CART sequence was also assessed. This was achieved through selectively mutating these motifs and testing whether CART still possessed the ability to activate ER-alpha using an ERE luciferase reporter assay. Treatment of cells with CART demonstrated an increase in MAP-Kinase activity through the detection of increasing phosphorylated ERK levels, as well as an increase in levels of the progesterone receptor. Over-expression of CART within the cells also demonstrated an activation of ER-alpha via the luciferase reporter assay. Finally, the data suggests that LXD motifs play a role in the activation of ER-alpha, which may indicate a potential structure-function relationship between CART and ER-alpha. In conclusion, we suggest that CART can potentially activate ER-alpha in a ligand-independent manner through the MAP-Kinase pathway, and also through specific LXD motifs within its sequence. Further investigation into the relationship between CART and ER-alpha will help us gain a better understanding of, not only the potential structure-function relationship between CART and ER-alpha, but also the role CART plays with regards to tamoxifen resistance in ER+ breast cancer.

The Insulin-like Growth Factor Receptor (IGF-1R) has an essential function in normal cell survival and growth, and its deregulation is associated with cancer progression and Epithelial Mesenchymal Transition (EMT). Previously we showed that IGF-1R activity and signalling in cancer cells is regulated by its C terminal tail, and in particular by a motif that encompasses Tyrosines 1250 and 1251 flanked by Serines 1248 and 1252 (1248-SFYYS-1252). This motif is important for IGF-1 induced cell migration and for IGF-1R cooperation with Integrin signalling via association with the scaffolding protein RACK1. However, it is not known whether or how phosphorylation of this domain occurs in any cells, or whether this phosphorylation contributes to cancer phenotype. Here we investigated the phosphorylation status of Y1250/Y1251 and its contribution to IGF signalling in cancer cells. Mass spectrometry and an anti-phospho-Y1250 antibody demonstrated that Y1250/51 are both phosphorylated in non-transformed and cancer cell lines. Y1250 phosphorylation is clearly induced by IGF-1 stimulation, but not by insulin. However, the kinetics of response to IGF-1 indicate that phosphorylation of Y1250 is an autophosphorylation event that occurs significantly later than autophosphorylation of other Tyrosines in the IGF-1R. This suggests either a role in mediating specific signals from the receptor or alternatively in feedback regulation of receptor activity. To investigate proteins that interact with the IGF-1R when Y1250 is phosphorylated we explored the interactome of SH2 domain-containing proteins that were predicted to interact with phospho-Y1250 in peptide screens (1). From this we observed that BCAR3, SH2B, and RAS p21, which are involved in cytoskeleton dynamics, occlusion of phosphatases and negative regulation of RAS/MAPK signalling respectively, can be co-immunoprecipitated with the IGF-1R when Y1250 is phosphorylated. We conclude that differential interaction of these SH2 domain proteins with phospho-Y1250 is necessary for IGF-1R cooperation with adhesion signalling to determine cancer cell phenotype.

INHIBITION OF ONCOGENIC MAPK SIGNALING THROUGH DISRUPTION OF THE KSR1-MEK COMPLEX

J Rauch1, B McCann1, N Rauch1, M Auer2, W Kolch1,3

1Systems Biology Ireland, University College Dublin, Dublin
2Centre for Translational and Chemical Biology, The University of Edinburgh, Edinburgh
3The Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin

The EGFR-MAPK pathway regulates many fundamental cellular processes and its deregulation is implicated in 30% of all human cancers. While targeted therapies towards these tumours exist, many patients develop resistance and relapse. Kinase suppressor of Ras 1 (KSR1) is a scaffold protein that regulates MAPK signaling by assembling components of this pathway. Surprisingly, KSR1 knockout mice treated with oncogenic retrovirus do not develop cancer, suggesting that KSR1-mediated signalling plays a crucial role in tumourigenesis. Indeed these studies lead to the intriguing hypothesis that the scaffolding function of KSR1 may be a suitable target for anti-tumour therapy. Using quantitative interaction proteomics we identified approx. 300 protein interactions with KSR1 during a 2-hour time course following EGF stimulation. Several novel interactions were identified and validated, suggesting links with MST2/Hippo signalling and p53 activity. While Raf (Raf-1 or B-Raf) and ERK1/2 are recruited to KSR1 upon upstream mitogen stimulation, MEK is constitutively bound to KSR1. These results suggest, that disruption of the KSR1 interaction with MEK alone should be sufficient to inhibit oncogenic Ras signaling. We successfully identified novel small-molecule inhibitors, which not only disrupt KSR's interaction with MEK, but also inhibit Ras-mediated oncogenic transformation. As KSR function is crucial for oncogenic signaling in a large set of cancers, inhibitors of KSR function might be used alone or in combination with the currently used anticancer drugs, to overcome resistance.
The potential of exosomal Neuromedin U as a predictive biomarker in HER2-overexpressing breast cancer

S Breslin1, M Daly1, B Hennessy2, J Crown3, N O Donovan4, L O’Driscoll1

1School of Pharmacy and Pharmaceutical Sciences & TBSI, Trinity College Dublin, Dublin
2Department of Medical Oncology, Royal College of Surgeons, Beaumont Hospital, Dublin
3Department of Oncology, St. Vincent’s University Hospital, Dublin
4NICB, Dublin City University, Dublin

Recently we reported Neuromedin U (NmU) expression to be associated with poor prognosis in HER2-overexpressing patients and associated with drug-resistance and increased cell aggression. Exosomes are cell-derived nanovesicles, the nucleic acids and protein contents of which reflects their cells of origin and studies by ourselves and others indicate that exosomes contain potential biomarkers for cancer. Here, including 35 patients serum specimens from a randomised phase II study of neo-adjuvant docetaxel, carboplatin with trastuzumab and/or lapatinib in HER2-overexpressing breast cancer patients (ICORG 10-05), we aimed to establish if NmU can be detected in the peripheral circulation and, if so, if it is contained within exosomes. Exosomes were isolated from 250µl aliquots of sera procured from patients prior to, during, and after administration of neo-adjuvant treatment. Isolations were performed using filtration and ultracentrifugation techniques. Immunoblotting for exosomal markers TSG101 and Alix was performed. NanoSight was used to establish the size range of the isolated vesicles. Exosomes were quantified and NmU ELISAs were performed. In this study, blinded to the researchers, exosomes were successfully isolated from all serum specimens (n=224). This was established by detecting TSG101 and Alix; NanoSight analysis showed the sizes of vesicles isolated were within the expected range for exosomes (73.1±4.3nm). ELISA analysis indicated that circulating NmU is contained within these nano-sized vesicles. Both the quantities of exosomes and their NmU content changed over the course of this randomised trial. This study supports our hypothesis that NmU is contained in serum-derived exosomes from breast cancer patients, suggesting that exosomes and their NmU content may have potential as predictive biomarkers. Our findings will now be investigated, in the context of patients outcome, to establish if quantities of exosomes and/or their NmU contents are indicative of response to these neo-adjuvant treatments.

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PHENOTYPE BASED DEVELOPMENT OF NOVEL THERAPIES FOR THE TREATMENT OF COLORECTAL CANCER
C Butler¹, A Murphy¹, E Conroy¹, WM Gallagher³, J O'Sullivan², B Kennedy¹
¹School of Biomolecular and Biomedical Science, Conway Institute, UCD, Dublin
²School of Biomolecular and Biomedical Science, Conway Institute, UCD, Dublin
³School of Biomolecular and Biomedical Science, Conway Institute, UCD, Dublin
⁴School of Biomolecular and Biomedical Science, Conway Institute, UCD, Dublin
⁵Institute of Molecular Medicine, Department of Surgery, St. James' Hospital, TCD, Dublin
⁶School of Biomolecular and Biomedical Science, Conway Institute, UCD, Dublin

Introduction: Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide. Treatment of late stage CRC using Bevacizumab®, a targeted anti-VEGF, produces insufficient patient response rates and acquired tumour resistance. There is a clinical need for development of improved treatment options.

Materials and Methods: Previously, anti-angiogenic screens in Tgfli1:EGFP zebrafish identified Quininib, a small molecule drug as effective as Bevacizumab® at reducing tumour volume in vivo and which reduces tumour CD31 expression in xenografted mice. Here, structural analogues of Quininib were synthesised to identify novel chemical entities with higher anti-angiogenic and anti-inflammatory efficacy. Analogues were examined for anti-angiogenic effects in the zebrafish intersegmental-vessel assay. Hits were progressed for safety analysis in cytotoxic assays, anti-angiogenic efficacy in aortic explants, and effects on cell proliferation and tubule formation using HT29_Luc2 and HMEC cells. Leads were then tested in ex vivo human CRC tumour explants to determine effects on angiogenic and inflammatory factor secretion. A CRC-specific murine xenograft model was created by subcutaneous injection of HT29_Luc2 cells for anti-tumorigenic analysis of the Quininib analogues.

Results and Discussion: Five out of 12 drug candidates (CTB_1, CTB_2, WP_1, WP_2 and WP_11) reduced developmental angiogenesis in fli1:EGFP zebrafish significantly more than Quininib (98% vs 67%, ***P<0.01). These analogues were well-tolerated in human endothelial and CRC cell cytotoxic assays and were more effective than Quininib at reducing tubule formation and angiogenic factor secretion in HMEC cells. Two analogues reduced aortic ring vessel sprouting equally or more effectively than Quininib. Preliminary data suggests that certain analogues can reduce inflammatory and angiogenic factor secretion from ex vivo human CRC explants more so than Quininib. We successfully established a CRC-specific murine tumour xenograft model. Using a ranking efficacy table, we chose analogues to analyse their anti-tumorigenic and anti-metastatic effects, by i/p injection in an optimised model of HT29_Luc2-CRC tumourigenesis in Balb/c nude mice.

Conclusion: We identified 5 analogues which are significantly more effective than Quininib within in vitro and ex vivo models of inflammation and angiogenesis. Our highest ranking analogue will be tested in murine xenografts to determine effects on tumour burden and metastasis.
Preclinical evaluation of afatinib in triple negative breast cancer
A Canonici\textsuperscript{1}, M.F.K Ibrahim\textsuperscript{1}, I Kaupe\textsuperscript{2}, K Bosch\textsuperscript{2}, D Zach\textsuperscript{2}, D.M Collins\textsuperscript{1}, F Solca\textsuperscript{2}, J Crown\textsuperscript{1,3}, N O'Donovan\textsuperscript{1}
\textsuperscript{1}MTCI, National Institute for Cellular Biotechnology, Dublin City University, Dublin
\textsuperscript{2}Boehringer Ingelheim, Vienna
\textsuperscript{3}Dept of Medical Oncology, St. Vincent's University Hospital, Dublin

\textbf{Background:} Triple negative breast cancer (TNBC) lacks expression of oestrogen and progesterone receptors and amplification of HER2, and is associated with poor prognosis. There are currently no targeted therapies approved for TNBC. EGFR, a member of the HER family, is frequently overexpressed in TNBC. We have previously shown that Src is frequently expressed in TNBC and crosstalk between EGFR and Src kinase signalling has been reported in TNBC. The aim of this study is to assess the activity of afatinib, an irreversible pan-HER tyrosine kinase inhibitor, alone and in combination with dasatinib, a Src inhibitor, in TNBC.

\textbf{Methods:} Sensitivity to afatinib alone and in combination with dasatinib was assessed in TNBC cell lines using 2D proliferation assays and 3D matrigel assays. Combination index (CI) values were calculated using CalcuSyn. Apoptosis induction was examined using the TUNEL assay on the Guava EasyCyte.

\textbf{Results:} The IC\textsubscript{50} values for afatinib in the 8 TNBC cell lines ranged from 17.5 nM to 3.4 µM. No association was observed between response to afatinib and basal A versus basal B subtype, PI3K signalling status or EGFR expression. Dasatinib IC\textsubscript{50} values ranged from 9.6 nM to 3.1 µM in the TNBC cell lines. The HDQ-P1 cell line, classified as basal-like 2, was the only cell line that showed significant sensitivity to both afatinib (IC\textsubscript{50} = 17.5 ± 2.1 nM) and dasatinib (IC\textsubscript{50} = 16.6 ± 5.3 nM). However, the combination of afatinib and dasatinib was synergistic (CI<1) in 6 of the 8 cell lines tested in 2D and in 2/3 cell lines tested in 3D. Combined treatment with afatinib and dasatinib induced greater apoptosis than either of the single agents.

\textbf{Conclusion:} Our results suggest that afatinib in combination with dasatinib may be an effective therapeutic regimen for TNBC.
Characterisation of novel AKT inhibitor in treatment of androgen sensitive prostate cancer

David J. Cochrane¹, Katie M. O’Donovan¹, Heather Nesbitt¹, Stephanie R. McKeown¹, Jenny Worthington², Martin O’Rourke³, Iain James⁴, Declan J. McKenna¹

¹ Centre for Molecular Biosciences, University of Ulster, Coleraine, N. Ireland, UK
² Axis Bioservices, Coleraine, N. Ireland, UK
³ Charles River Laboratories, Essex, UK
⁴ Almac Discovery, Craigavon, N. Ireland, UK

Background
An upregulation of the AKT pathway has been associated with the progression to castrate resistant prostate cancer and loss of response to androgen deprivation therapy (ADT). Targeted AKT pathway inhibition is one strategy for increasing the clinical efficacy of prostate cancer therapeutics. Therefore, in this pre-clinical study, we investigated the effect of a novel AKT inhibitor ALM301 (ALMAC Discovery) on prostate tumour growth as a single agent and in combination with ADT.

Method
In vitro characterisation of ALM301 was performed using enzyme kinetic and drug biomarker studies. The in vitro effect of ALM301 on cell survival, proliferation, invasion, migration and tubule formation/angiogenic potential was analysed. The in vivo characterisation of ALM301 in singularity and in combination with ADT was performed in an LNCaP-luc Xenograft Balb-c (SCID) mouse model and the effects upon tumour biomarkers, tumour growth, metastasis and molecular markers of malignant progression were assessed.

Results
ALM301 was confirmed as an allosteric ATP-independent inhibitor of AKT, leading to repression of the PI3K/AKT pathway. In vitro, ALM301 treatment significantly reduced the survival, proliferation, migration and invasive capacity of LNCaP cancer cells, as well as impacting on the ability of endothelial cells to instigate tubule formation. In vivo, ALM301 was also shown to successfully inhibit AKT and the subsequent activation of the PI3K/AKT pathway. ALM301, in combination with ADT, was shown to down-regulate molecular markers of malignant progression and impact on a tumours ability to proliferate and metastasise to distal sites.

Conclusion
The AKT inhibitor ALM301 successfully targets AKT pathway activation in prostate cancer cells in vitro and in vivo, causing a potent inhibition of cell growth. Our results demonstrate that combination of ALM301 with ADT demonstrates significant delay of tumour growth and metastatic progression.
NOVEL SMALL MOLECULE INHIBITORS THAT ALTER ENERGY METABOLISM AND DNA REPAIR AND IMPROVE RADIORESPONSE IN OESOPHAGEAL AND COLORECTAL ADENOCARCINOMA

R Dunne¹, N Lynam-Lennon¹, JV Reynolds¹, B Kennedy², J O'Sullivan¹
¹Department of Surgery, Trinity Centre for Health Sciences, St James's Hospital, Dublin 8
²UCD School of Biomolecular and Biomedical Sciences, University College Dublin, Dublin 4

Introduction: Tumours display high levels of angiogenesis leading to leaky blood vessels and hypoxia, mechanisms of radioresistance. Altered energy metabolism and DNA repair in tumours can also result in radioresistance. Targeting tumour angiogenesis, metabolism and DNA repair may therefore increase radiosensitivity. Zebrafish intersegmental blood vessel screening identified a novel small molecule inhibitor, RUD1, with strong anti-angiogenic properties. Structural analogues of this compound (RUD2-4) were created. We examined the ability of these inhibitors to alter metabolism, DNA repair gene expression and radioresponse in oesophageal and colorectal cancer cell lines.

Methods: An isogenic model of oesophageal radioresistance, OE33P (sensitive) and OE33R (resistant), in addition to the radioresistant colorectal cancer cells, HT29-LUC, were treated with the inhibitors. Metabolism profiles were generated using Seahorse technology. Expression of DNA repair genes was assessed using qPCR. Radiosensitivity was assessed by clonogenic assay.

Results: Oxidative phosphorylation was reduced in OE33P treated with RUD2 and RUD3 (p<0.05), OE33R treated with RUD1-3 (p<0.0001) and HT29-LUC treated with RUD1,3,4 (p<0.05). Expression of several DNA repair genes was reduced following treatment with the inhibitors. MLH1 expression was reduced in OE33P and OE33R treated with RUD2-4 (p<0.0001). PARP1 expression was reduced in OE33P and OE33R treated with RUD1 (p<0.01). RUD1 also reduced expression of MMS19 in OE33R (p<0.01). MLH1 expression was also reduced in HT29-LUC treated with RUD4 (p<0.01). RUD4 increased radiosensitivity of OE33P and OE33R (p<0.0001) and HT29-LUC (p<0.05). RUD3 also increased radiosensitivity in OE33R (p<0.05).

Conclusion: We have identified a number of novel anti-angiogenic small molecules that alter energy metabolism and DNA repair gene expression, processes linked to radiation response, in both oesophageal and colorectal cancer cell lines. Two of these inhibitors also improve radiation response.
Poster No.58
Experimental Therapeutics

Pertuzumab alone or in combination with copanlisib displays limited proliferation inhibition in 2D assays; but significantly inhibits 3D colony formation of HER2-positive breast cancer (BC) cells, including those with acquired trastuzumab resistance

N Elster¹, W Thomas², NA O’Brien³, BT Hennessy¹, AJ Eustace¹
¹Medical Oncology Group, Department of Molecular Medicine, RCSI
²Molecular Medicine, Royal College of Surgeons in Ireland, Dublin
³Medicine, University of California Los Angeles, Los Angeles

Background: HER2-targeted therapies have improved outcomes for patients with HER2-positive BC¹, but resistance to these remains a clinical problem. Co-targeting HER2/PI3K could represent an improved treatment strategy for patients with HER2-positive BC.² Pertuzumab is a HER2 dimerization inhibitor which has been shown clinically to improve survival in patients with HER2-positive metastatic BC³.

Materials and Methods: Pertuzumab and the PI3K inhibitor copanlisib were tested alone and in combination using acid phosphatase assays in an 11-cell line panel which included models of acquired resistance to trastuzumab and/or lapatinib. 3D Soft agar assays were used to determine the effect of pertuzumab, copanlisib or a combination of both on the colony forming ability of BT474 and BT474-Res cells in 3D. Colonies were imaged using an LSM510 confocal microscope (Zeiss).

Results: Pertuzumab alone did not inhibit growth in 2D assays, whilst copanlisib achieved low nanomolar IC50s across all cell lines tested (3.9 ± 0.8 nM (BT474) to 29.4 ± 4.7 nM (MDA-MB-453)). Combining pertuzumab with copanlisib resulted in improved growth inhibition relative to copanlisib alone in only 2/6 cell lines. When tested in 3D assays, pertuzumab and copanlisib alone and in combination resulted in a statistically significant inhibition of colony formation (~20% relative to untreated controls for all treatments) in both BT474 and BT474-Res cells. However, combining the two treatments did not result in additivity or synergy. When visualised under the LSM510, colonies treated with pertuzumab or copanlisib were much smaller than untreated controls and colonies treated with combination pertuzumab:copanlisib showed increased signs of cellular stress relative to untreated controls or single treatments.

Conclusion: Pertuzumab showed no efficacy as a single agent and limited effects in combination with copanlisib in 2D assays. Pertuzumab and copanlisib significantly inhibited growth in 3D cultures, but did not have an additive or synergistic effect on colony forming growth when used in combination. Testing pertuzumab in 3D may better represent pertuzumabs clinical efficacy, but does not take into account its immunological effects.

¹ Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER
Electrochemotherapy and B7 family antibody therapy: Novel Combinational Therapy for the enhanced treatment in cancers of poor prognosis

M.A O’ Brien, P. Forde, D. Soden

1Cork Cancer Research Centre, University College Cork, Cork

The correlation between the level of immune involvement and immune cell infiltration within a treated tumour and the clinical outcome for patients has been well established in many solid tumour types [1]. High numbers of tumour infiltrating lymphocytes has been associated with a significantly improved prognosis. Some of the key cell types involved are CD3+ and CD8+ T Cells, B cells and APCs such as dendritic cells (DCs). Cork Cancer Research Centre (CCRC) has long utilised an ablative cancer treatment that involves delivering a pulse of electrical energy (electroporation) directly to the tumour. This treatment results in the formation of reversible pores in the cell membrane, allowing up to a 1000 fold increase in chemotherapy drug absorption (Electrochemotherapy) [2]. A significant benefit of this non thermal ablation is its ability to cause hallmarks of Immunogenic Cell Death, the release of Damage Associated Molecular Proteins (DAMPs) and tumour associated antigens into the microenvironment, leading to activation of APCs and subsequent cross presentation of the antigens to circulating T and B cells [3]. While ECT facilitates the priming of a favourable immune response, the combination of B7 antibodies, capable of stimulating T cell proliferation and prolonging immune responses, causes a very robust antigen mediated regression of treated tumours [4]. Preclinical work in aggressive murine models has yielded very encouraging results. The combinational therapy has achieved cures in both colorectal (CT26) and metastatic lung (LLC) cancer cell lines. Follow up immunohistochemical staining confirmed the presence of tumour infiltrating CD3+ and CD8+ T Cells, CD45R+ B cells and CD11c+ DCs, post treatment.

PLK1 and CDC7 inhibitors compromise the robustness of the spindle assembly checkpoint
A O'Connor¹, M.D. Rainey¹, S Maffini², A Kaczmarczyk¹, D Gaboriau¹, A Musacchio², C Santocanale¹
¹Centre for Chromosome Biology, National University of Ireland Galway, Galway, Ireland
²Department of Mechanistic Cell Biology, Max-Planck Institute of Molecular Physiology, Dortmund, Germany

Drugs which target microtubule dynamics elicit the activation of the spindle assembly checkpoint (SAC) resulting in a prolonged cell cycle arrest in mitosis. The SAC inhibits activation of the APC/C thus preventing inappropriate exit from mitosis until all kinetochore-microtubule attachments are correct. Disruption of SAC signalling allows cells to progress through the cell cycle without performing chromosome segregation, ultimately causing genome instability. PLK1, a master regulator of mitosis, is up-regulated in multiple cancer types and PLK1 kinase inhibitors have demonstrated anti-tumour activity in preclinical models with several of these inhibitors entering clinical trials. Recently PLK1 has also been reported to be involved in SAC signalling. CDC7 kinase instead is well known to be required for the initiation of DNA replication in S-phase and similarly, CDC7 inhibitors are being explored as anti-cancer agents. To date CDC7 kinase has not yet been reported to have a role in mitosis, however genetic evidence suggests that CDC7 may negatively regulate PLK1. In this study we show that CDC7 is an active kinase in cells arrested in mitosis where it undergoes auto-phosphorylation and we explore the relevance of both CDC7 and PLK1 kinase activities in maintaining robust SAC signalling. Using combinations of clinical and preclinical kinase inhibitors we show that simultaneous CDC7 and PLK1 inhibition promote mitotic slippage in cells with depolymerised microtubules. Furthermore, when PLK1 inhibitors are combined with partial inhibition of Aurora B, efficient mitotic slippage can occur. At the molecular level we found that PLK1 kinase activity is required for maintaining Aurora B activity at kinetochores and that Aurora B inhibition partially displaces PLK1 from kinetochores. These results indicate that PLK1 is indeed involved in maintaining efficient SAC signalling upon a prolonged cell cycle and suggest that it performs this function by cooperating with Aurora B in a positive feedback loop. We provide the first experimental evidence that the CDC7, DNA replication, kinase can contribute to prevent inappropriate exit from mitosis, possibly through PLK1, although the molecular mechanisms have to be further elucidated.
Development of Tumour Therapy and Imaging Strategies Utilising Bacteria

M Stanton, M Cronin, P Lehouritis, M Tangney

1Cork Cancer Research Centre, University College Cork, Cork
2Cork Cancer Research Centre, University College Cork, Cork
3Cork Cancer Research Centre, University College Cork, Cork
4Cork Cancer Research Centre, University College Cork, Cork

The ability of systemically administered bacteria to target and replicate to high numbers within solid tumours allows for the targeted activation of therapeutics and the confinement of therapeutic effect to the tumour site. For example, bacterial directed activation of a prodrug specifically within a tumour permits effective and specific localised tumour therapy. In order to monitor therapeutic activity, there is a requirement for the development of non-invasive imaging strategies to track bacteria within a live host. Optical imaging is the most commonly utilised preclinical imaging system. To date, the vast majority of non-invasive in vivo imaging systems for bacteria have relied on the use of bacteria that have been genetically modified or harbour genetic expression constructs. However, the use of engineered bacteria is often not experimentally appropriate or technologically feasible as engineering tools are not available for a number of bacterial strains. Therefore, there is a need for the development of optical imaging strategies using unmodified bacteria. Our work aims to develop novel therapeutic and non-invasive imaging strategies based on the endogenous enzymatic activity of tumour targeting bacteria. Nitroreductases are a family of bacteria-specific enzymes that are capable of activating the fluorescent probe CytoCy5S as well as the cytotoxic prodrug CB1954. In vitro and in vivo data demonstrate that endogenous levels of these enzymes are sufficient for probe activation and elicitation of a therapeutic effect in BALB/c mice bearing subcutaneous CT26 tumours. This study introduces the concept of utilising endogenous nitroreductases for therapeutic effect and as a reporter for wild-type bacteria. The system may be adapted for use with other imaging modalities or for other research fields such as infectious disease.
Inhibition of Multidrug Resistance Protein I (MRP1) improves chemotherapy drug response in primary and recurrent glioblastoma multiforme

Tivnan, A.1, Zakaria, Z.1, O'Leary, C.1, Pokorny, J.L.2, Sarkaria, J.N.2, Kögel, D.3, Prehn, J.H.M.1

1Centre for Systems Medicine, Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, York House, St. Stephens Green, Dublin 2, Ireland. amandativnan@rcsi.ie
2Department of Radiation Oncology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, United States of America. 3Experimental Neurosurgery, Neuroscience Center, Frankfurt University Clinics, Theodor-Stern-Kai 7, D-60590, Frankfurt en Main, Germany.

Glioblastoma multiforme (GBM) is a highly aggressive grade IV brain cancer with an extremely poor prognostic outcome despite intensive treatment regimes. GBM represent ~17% of all primary brain tumours diagnosed worldwide; and 60-75% of astrocytomas, increasing in frequency with age (1). The average five year survival is less than 3%, leading to the fact that GBM is the most lethal form of brain, or central nervous system (CNS), tumour. Despite surgical resection of GBM tumours, recurrence at distal sites is typically 6.9 months (2-4) and in instances where repeat resection is not a viable option, adjunct chemotherapy is ineffective at stopping tumour progression and, eventually, morbidity. All chemotherapeutic agents currently used; however, have no greater than 30-40% response rate and many fall into the range of 10-20%(5) with delivery across the blood brain barrier or chemoresistance contributing to the extremely poor patient outcomes despite treatment.

Increased expression of the multidrug resistance protein 1(MRP1) in high grade glioma, and it’s role in blood brain barrier active transport, renders this member of the ABC transporter family, a potential target for improving drug responses in this highly aggressive brain cancer. In this study we show that small molecule inhibitors of MRP1 had a significant effect on GBM cell drug responses to Temozolomide (150µM), Vincristine (100nM) and Etoposide (2µM). Pre-treatment with Reversan led to a significantly improved response in terms of Temozolomide, Vincristine and Etoposide-induced cell death, in both primary and recurrent GBM cell lines. The presence of MK571 led to an enhanced effect of Vincristine and Etoposide in reducing cell viability over a 72 hour period. MRP1 and MRP4 inhibition by MK571 did not have any effect on Temozolomide drug response in these cells. To ensure that the observed findings were MRP1 specific, an MRP1-targeting siRNA was used in three glioblastoma cell lines. Specific MRP1 inhibition led to a significant increase in Vincristine and Etoposide-induced cell death in all three cell lines assessed; (*p<0.05, **p<0.001) relative to single chemotherapy-induced cell death. Notably, specific MRP1 inhibition did not have any effect on Temozolomide-induced cell death. The findings of this study have significant implications in terms of providing researchers an opportunity to improve currently used chemotherapeutics for the initial treatment of primary GBM, and improved treatment for recurrent GBM patients.

1. WHO, IARC. http://globocan.iarc.fr/

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Exosomal miRNA expression profiling of in vitro and in vivo models of treatment resistant neuroblastoma.

RA Conlon¹, H Harvey¹, O Piskareva¹, J Nolan¹, S Miller-Delaney¹, I Bray¹, RL Stallings¹
¹Dept. of Molecular and Cellular Therapeutics, Cancer Genetics Group, Royal College of Surgeons in Ireland, York house, York St, Dublin 2

Neuroblastoma is the most common solid tumour in infants, and accounts for approximately 15% of childhood cancer deaths worldwide. The acquisition of drug resistance is the principal obstacle to the successful treatment of neuroblastoma. Thus, the elucidation of mechanisms involved in the development and modulation of drug resistance is vital to the discovery of novel biomarkers and therapeutics for this devastating disease. The aim of this work is to ascertain the contribution of miRNA to the development of drug resistance in neuroblastoma. To this end, we have developed three cell lines, KellyCis83, CHP212Cis100 and SK-N-ASCis24 that are significantly resistant to cisplatin and other agents. MicroRNA expression profiling of the lines identified a panel of miRNA predicted to target genes known to be involved with drug resistance, providing a solid basis for testing our hypothesis that these miRNA may also modulate drug resistance. Previous work revealed that exosomal microRNAs are present in the bloodstream and these circulating miRNA are remarkably stable, here we demonstrate that drug resistant and parental neuroblastoma cell lines export a significant amount of exosomal miRNAs into their culture medium. Taqman Low Density Array miRNA cards identified a number of miRNA differentially expressed in the 3 cell lines, when compared to their parental counterparts. Using an orthotopic xenograft murine model of neuroblastoma, KellyCis83luc and Kellyluc tumours were established in the renal capsule of athymic Nude-Foxn1nu mice. Tumour weights at three weeks post-implantation revealed a two-fold difference indicating drug resistant cells grow more rapidly in vivo than their parental equivalent. This finding replicates the clinical behaviour of treatment-resistant neuroblastomas. Treatment of tumours with 3.5mg/kg cisplatin every 3 days for 2 weeks resulted in resistant tumours that were four times less responsive than sensitive tumours. Exosomal profiling of these different tumour cohorts revealed several miRNA to be significantly differentially expressed. The list includes miRNAs which have previously been implicated in neuroblastoma pathogenesis and also those with prior associations with an acquired drug resistant phenotype in cancer. Our findings also lend further support to the idea of a novel miRNA trafficking system concurrent with the cell to cell communication hypothesis.
EPIGENETIC REGULATION OF THE COCAINE AND AMPHETAMINE REGULATED TRANSCRIPT (CART) GENE IN CANCER
K Connor¹, S Das¹, L Mulrane¹, WM Gallagher¹, DP O'Connor¹
¹Conway institute of Biomolecular and Biomedical Science, University College Dublin, Dublin

Introduction: The cocaine and amphetamine regulated transcript (CART) is well documented in the central nervous system for its role in appetite regulation and reward pathways. In recent studies CART has been demonstrated to be a promising marker of poor prognosis in lymph node-negative, estrogen receptor (ER)-positive breast cancer patients (1), and has been shown to be associated with worse survival in small bowel carcinoid tumours (2). The regulation of CART in these different malignancies, however remains largely unknown. The presence of a candidate CpG island in the putative CART promoter region and the lack of expression of CART across several cell lines, suggests a possible epigenetic mechanism of regulation.

Methods: A panel of cancer cell lines were assessed for endogenous CART expression via Western blot and qRT-PCR analyses. Cells were then treated with varying doses of two DNA methyltransferase inhibitors (5-Aza-2-deoxycytidine and RG108) and a histone deacetylase inhibitor (Trichostatin A), followed by examination of CART expression post treatment. In addition, whole methylome sequencing using a novel capture technology, followed by next-generation sequencing, was carried out in order to determine the global effects of treatment with epigenetic modifiers, and treatment with CART conditioned media, which has been previously shown to induce CART expression. Finally, bisulfite sequencing of the CpG island in the putative promoter region of CART was performed in all cell lines to confirm methylation status.

Results & Conclusion: Assessment of a panel of cell lines via Western blot and qRT-PCR demonstrates negligible CART expression at both mRNA and protein level across the panel. Treatment with 5-Aza-2-deoxycytidine results in increased CART mRNA levels in MCF7 cells, while methyl capture sequencing identified methylated regions within the CART promoter. Moreover, subsequent bisulfite sequencing of the promoter region of CART has confirmed dense methylation at the identified CpG island. Collectively this data suggests the potential involvement of DNA methylation in regulation of CART.

MYCN Integrative Omics Reveals Neuroblastoma Regulatory Networks, Enables Patient Stratification and Network-based Therapeutic Target Discovery

DJ Duffy¹, A Krstić¹, T Schwarzl¹, M Halasz¹, D Fey¹, JP Mehta¹, K Killick¹, F Westermann⁴, DG Higgins¹,²,³, W Kolch¹,²,³

¹Systems Biology Ireland, University College Dublin, Dublin
²Conway Institute of Biomolecular & Biomedical Research, University College Dublin, Dublin
³School of Medicine and Medical Science, University College Dublin, Dublin
⁴Division of NB Genomics, German Cancer Research Center (DKFZ), Heidelberg

The paucity of somatic mutations in the currently intractable MYCN-driven paediatric cancer neuroblastoma has meant that current therapeutic strategies and classical genetic approaches which rely on frequently altered oncogenic drivers to identify novel therapeutic options have been largely unsuccessful. To overcome this limitation, we conducted MYCN integrative omics, examining multiple molecular levels simultaneously. We have integrated data from both MYCN overexpression and amplification at multiple molecular levels, the protein-protein interactome, DNA binding, transcriptome and proteome, utilising a range of high-throughput methodologies.

**MYCN regulatory networks:** We showed that MYCN rewires its own protein interaction network by transcriptionally regulating its binding partners, that MYCN oncogenic functions are likely independent of its classical heterodimerisation partner MAX and that MYCN has different global effects on transcription depending on whether it is overexpressed or amplified. MYCN forms highly connected networks, with the activity of the majority of components and associated signalling pathways being suppressed in both MYCN overexpression and amplification scenarios. These results inform differences between the basic functioning of endogenous, overexpressed and amplified MYCN, helping to dispel long-standing MYCN contradictions.

**Network-based Therapeutic Target Discovery:** By mapping regulatory networks through which MYCN orchestrates cell fate decisions and cancerous outcomes, we identified network vulnerabilities amenable to therapeutic interventions. These were validated by patient data and functional studies, focussing primarily on Wnt/β-catenin signalling, as a targetable vulnerability of MYCN-amplified neuroblastoma. This work provides a systems-level approach for revealing network vulnerabilities in multifactorial diseases, integrating disparate omic data types, and identifying druggable therapeutic targets in the MYCN network.
Evolution of the CRPC Epigenome and its clinical application for delivering Precision Medicine

C Fahey¹, R Montella¹, S Finn², R McDermott³, A Perry¹

¹Prostate Molecular Oncology, Trinity College Dublin, Dublin
²Department of Histopathology, St James's Hospital & Trinity College Dublin, Dublin
³Department of Oncology, Tallaght Hospital and Trinity College Dublin, Dublin

Aberrant DNA methylation is a hallmark of prostate cancer and is known to occur as a seminal event during the tumour initiation. Less is understood of how the tumour methylome evolves during metastatic dissemination and during the emergence of castration resistance. The aim of this study is to establish the effects that specific therapeutic regimens has on modulating the methylome and conversely the role that epigenetic alterations play in treatment resistance. This will be achieved by longitudinal, real-time analysis of whole-genome DNA methylation patterns in men with metastatic prostate cancer. Serial samples from individual patients will be analysed to investigate evolution of the methylome during development of castrate resistance. Initial clinical sequencing will be carried out on circulating DNA isolated from plasma, and matched benign and primary tumor tissue from approximately four men who were diagnosed with metastatic castration resistant prostate cancer (mCRPC). Circulating DNA was isolated from peripheral blood plasma (3ml) obtained at diagnosis, using the ctDNA isolation kit (Qiagen). Matched benign and tumour TRUS-biopsy cores will be subjected to laser capture microdissection to enrich for epithelial cells and then used for DNA isolation (Qiagen microDNA kit). Sequencing will be carried out by Reduced Representation Bisulfite Sequencing (RRBS), and subsequent data analysis using Methylkit software (http://code.google.com/p/methylkit). Subsequent sequencing of ctDNA in these men will be achieved through enrollment into the iPROSPECT (Irish Programme for Stratified Prostate Cancer Therapies) translational study. Understanding the evolution of the metastatic epigenome will help to re-classify lethal CRPC into molecular subtypes, which can then be used to rationally guide personalized sequences /combinations of molecularly-targeted agents and generate a better understanding of the mechanistic landscape in CRPC and treatment resistance.
Ultraviolet radiation accelerates BRAF-driven melanomagenesis by targeting TP53
SJ Furney1,2, A Viros2, B Sanchez-Laorden2, M Pedersen3, J Rae3, K Hogan2, S Ejiama2, MR Girotti3, M Cook4, N Dhomen3, R Marais2
1School of Medicine & Medical Science, University College Dublin, Dublin
2Molecular Oncology Group, Cancer Research UK Manchester Institute, Manchester
3Signal Transduction Team, Institute of Cancer Research, London
4Histopathology, Royal Surrey County Hospital, Guildford

Cutaneous melanoma is epidemiologically linked to ultraviolet radiation (UVR), but the molecular mechanisms by which UVR drives melanomagenesis remain unclear (1, 2). The most common somatic mutation in melanoma is a V600E substitution in BRAF, which is an early event (3). To investigate how UVR accelerates oncogenic BRAF-driven melanomagenesis, we used a BRAF(V600E) mouse model. In mice expressing BRAF(V600E) in their melanocytes, a single dose of UVR that mimicked mild sunburn in humans induced clonal expansion of the melanocytes, and repeated doses of UVR increased melanoma burden. Here we show that sunscreen (UVA superior, UVB sun protection factor (SPF) 50) delayed the onset of UVR-driven melanoma, but only provided partial protection. The UVR-exposed tumours showed increased numbers of single nucleotide variants identified by whole exome sequencing and we observed mutations (H39Y, S124F, R245C, R270C, C272G) in the Trp53 tumour suppressor in approximately 40% of cases. TP53 is an accepted UVR target in human non-melanoma skin cancer, but is not thought to have a major role in melanoma (4). However, we show that, in mice, mutant Trp53 accelerated BRAF(V600E)-driven melanomagenesis, and that TP53 mutations are linked to evidence of UVR-induced DNA damage in human melanoma. Thus, we provide mechanistic insight into epidemiological data linking UVR to acquired naevi in humans (5). Furthermore, we identify TP53/Trp53 as a UVR-target gene that cooperates with BRAF(V600E) to induce melanoma, providing molecular insight into how UVR accelerates melanomagenesis. Our study validates public health campaigns that promote sunscreen protection for individuals at risk of melanoma.

Biological Consequences of Expression of the Fusion Protein YWHAE-NUTM2 in a Model of Clear Cell Sarcoma of Kidney

C Kenny¹, E O'Meara¹, M O'Sullivan¹,²,³
¹School of Medicine, Trinity College, Dublin 2
²Histopathology, Our Lady's Children's Hospital Crumlin (OLCHC), Dublin 12
³National Children's Research Centre (NCRC), Trinity College, Dublin 2

Clear Cell Sarcoma of Kidney (CCSK), the second commonest paediatric renal cancer, is aggressive, therapy-resistant and has poor outcomes. The oncogenic mechanisms underpinning CCSK are poorly understood and specific diagnostic/prognostic markers and effective therapies are lacking. Prompted by three independent case reports of a t(10;17)(q22;p13) in CCSK, we characterised this chromosomal translocation to show in-frame fusion of YWHAE and NUTM2 genes. To determine the biological consequence of YWHAE-NUTM2 expression, we generated stably transfected HEK293 and NIH3T3 cell lines with tetracycline/doxycycline inducible HA-tagged YWHAE-NUTM2. Using these inducible cell lines, cell viability and motility were examined on the real-time xCELLigence platform. Cell migration of HEK293 and NIH3T3 cells induced to express HA-YWHAE-NUTM2 was significantly higher than mock-treated cells, confirming an oncogenic effect. Additional molecular changes following YWHAE-NUTM2 expression will be investigated by gene expression profiling at a selected time-point based on the xCELLigence results. Furthermore, the occurrence of additional genetic mutations in both translocation-positive and -negative CCSK will be analysed by Whole Genome Sequencing. This will contribute to our understanding of CCSK tumourigenesis, and, in conjunction with ongoing signalling studies in the laboratory, will help to elucidate novel therapeutic targets.
Genome wide characterization reveals complex interplay between TP53 and TP63 in response to genotoxic stress

S McDade¹, D Patel¹, M Moran¹, J Campbell², K Fenwick², I Kozarewa², N Orr², C Lord², A Ashworth², D McCance³

¹CCRCB, QUB, Belfast, ²Breakthrough Breast Cancer Research Centre, ICR, London ³University of New Mexico, Albuquerque

In response to genotoxic stress the TP53 tumour suppressor activates target gene expression to induce cell cycle arrest or apoptosis depending on the extent of DNA damage. These canonical activities can be repressed by TP63 in normal stratifying epithelia to maintain proliferative capacity or drive proliferation of squamous cell carcinomas, where TP63 is frequently overexpressed/amplified. Here we use ChIP-seq sequencing, integrated with microarray analysis, to define the genome wide interplay between TP53 and TP63 in response to two types of genotoxic stress in normal cells. We reveal that TP53 and TP63 bind to overlapping but distinct cistromes of sites through utilization of distinctive consensus motifs. We demonstrate that cisplatin and adriamycin elicit distinct effects on TP53 and TP63 binding events, through which TP53 can induce or repress transcription of an extensive network of genes by direct binding and/or modulation of TP63 activity. Collectively, this results in a global TP53 dependent repression of cell cycle progression, mitosis and DNA damage repair concomitant with activation of anti-proliferative and pro-apoptotic canonical target genes. Further analyses reveals that in the absence of genotoxic stress TP63 plays an important role in maintaining expression of DNA repair genes, loss of which results in defective repair.
ELUCIDATING THE MECHANISMS OF SYNERGY BETWEEN P53 AGONISTS AND HISTONE DEACETYLASE INHIBITORS

AJ McIntyre¹, DB Longley¹, SS McDade¹
¹CCRCB, QUB, Belfast

The tumour-suppressive functions of the p53 transcription factor are lost in 50% of all human cancers via mutation. Unlike canonical tumour suppressors the majority of these mutations are missense, resulting in expression of a full-length p53 protein which is compromised in its ability to directly bind DNA and activate anti-proliferative and pro-apoptotic target genes. Furthermore, in tumours harbouring wild-type p53 its function is often compromised through non-mutational mechanisms. These include over-expression of negative regulators such as MDM2 and PPM1D, or inactivation by viral oncogenes. As such, mutant and wild-type p53 represent potential clinical targets. We are therefore interested in exploiting novel and existing therapies to modulate both wild-type and mutant p53 function to selectively kill cancer cells.

Recent data from our group and others has demonstrated synergistic induction of apoptosis as a result of combinations of histone deacetylase (HDAC) inhibition and genotoxic chemotherapeutic agents. Here we demonstrate that these synergies are WTp53-dependent, possibly due to switching of p53 promoter selectivity towards pro-apoptotic target genes. Our data suggest that these events, coupled with the HDACi-mediated depletion of pro-survival proteins such as c-FLIP collectively enhance apoptosis in our cellular models. Further studies are on-going to determine the genome-wide extent of these effects, the specific HDACs involved and whether HDACi-induced p53 acetylation can influence target switching and/or cellular signalling to enhance cell death.
Investigating the role of POL-I variant rs3730477 in breast cancer predisposition

TP McVeigh¹, RJ Mulligan¹, KJ Sweeney², JB Weidhaas³, N Miller¹, MJ Kerin¹

¹Discipline of Surgery NUI Galway
²Breast Check Western Unit
³School of Medicine, Yale University

Introduction: Recent discovery efforts in addressing the missing heritability of breast cancer have focused on genes involved in maintenance of genomic integrity. Polymerase (Pol)-I protein, has a role in dsDNA repair by re-synthesis of missing nucleotides during non-homologous end joining. In vitro work to date has shown that the polymorphism rs3730477 in the POL-I gene may interfere with dsDNA repair.

Aim: The aim of this study was to investigate the role of POL-I variant in breast cancer predisposition.

Methods: A case-control study was performed, including cases recruited via symptomatic and screening breast units and cancer-free controls from the community. DNA was extracted from by crystallisation precipitation from whole blood and buccal swabs. Taqman-based genotyping was performed. Data was analysed using SPSS v22.

Results: Samples from 235 cases and 268 controls were genotyped for the POL-I variant. The minor allele frequency (MAF) observed was 0.17. No significant difference in frequency was noted between cases and controls, or across stage or molecular phenotype of breast cancer.

Conclusion:

The POL-I variant rs3730477 was identified in this small Irish cohort, but was significant association was observed between the minor allele and breast cancer risk. Studies of increased statistical power may be required to elucidate the role of the POL-I variant in breast cancer susceptibility.
TACKLING NEUROBLASTOMA: DESIGN AND DEVELOPMENT OF PNA BASED MIR-34A MIMICS

V Piacenti¹, M Moccia¹, M Saviano², I Bray³, R Stallings³, MFA Adamo¹

¹Pharmaceutical and Medicinal Chemistry, RCSI, Dublin
²IC-Bari, Bari
³Cancer Genetics, RCSI, Dublin

Neuroblastoma is the most common extra-cranial paediatric solid tumour and responsible for 15% of childhood cancer deaths. Overexpression of the MYCN proto-oncogene remains the most significant biomarker of the disease and is found amplified in ~25% of neuroblastoma tumours. Its overexpression correlates with tumour progression and poor outcome. MYCN makes an ideal therapeutic target in neuroblastoma. The expression of MYCN is regulated by miR-34a resulting in inhibition of the MYCN protein and cancer cell growth. Peptide Nucleic Acids (PNA) have been identified as suitable analogues to develop miRNA-34a mimics with enhanced properties such as stability and resistance to endonucleases and proteases. The aim of the project is the identification of a small RNA sequence of minimum length that can be synthesized as a PNA and used to successfully inhibit MYCN. PNA constructs of different length have been designed including (a) a seed sequence mimicking the same of miR-34a (b) a peptide carrier able to improve the internalization of the construct (c) a linker (d) a fluorescent tag. The synthesised PNA-based mimics are currently undergoing in vitro characterization of uptake, cell viability and proliferation. The best peptide carrier identified includes tri-lysine fragment on the C-term and lysine-cysteine fragment on the N-term. The longest sequence, including 22 nucleobases, was internalized with a greater efficiency into the cell and it exerted the highest suppression of neuroblastoma cell growth. An increased internalization by 20% was observed using lipofectamine2000. Further optimization of the transfection conditions will be carried out to find the best suppressive effect. Target validation tests will be run to confirm that the PNA sequence exhibits the properties of tumour suppressive miR-34a. A computational study of the interaction between the active structures and mRNA along with structural analysis will be translated into the design of novel small molecules to be proposed for a high scale industrial drug manufacture.
**CRC-associated microbiota profiles are linked with distinct mucosal gene expression profiles**

Burkhardt Flemer\(^1,2\), Denise Lynch\(^1,2\), Jillian Brown\(^1,2\), Ian B Jeffery\(^1,2\), Marcus J Claesson\(^1,2\), Michael O’Riordain\(^3\), Fergus Shanahan\(^1,4\), Paul W O’Toole\(^1,2\)

\(^1\)Alimentary Pharmabiotic Centre, University College Cork, Ireland  
\(^2\)Department of Microbiology, University College Cork, Ireland  
\(^3\)Mercy University Hospital, Grenville Place, Cork, Ireland  
\(^4\)Department of Medicine, University College Cork, Ireland

**Background:** Although several bacteria such as *Fusobacterium nucleatum* have been linked with colorectal cancer (CRC), a unifying microbiota signature across multiple studies has not been identified. In addition to methodological variance, heterogeneity may be due to both microbial and host response differences, which were addressed in this study.

**Design and methodology:** We conducted a prospective study of the colonic microbiota and the expression of specific host response genes using paired faecal and mucosal samples (‘on’ and ‘off’ the tumor, proximal and distal) from 36 patients undergoing surgery for colorectal cancer and 37 controls. The microbiota profiles were analysed using 16S-rRNA amplicon sequencing, and expression of host genes involved in CRC progression and immune responses was quantified by RT-qPCR.

**Results:** The microbiota associated with distal and proximal cancers was significantly different. Six bacterial co-abundance groups (CAGs) associated with the gut mucosa were defined, at least one of which was of increased abundance in any CRC patient. Furthermore, the CAGs interacted in such a way that cancer individuals could be grouped into three clusters based on the abundance of each CAG in each individual. The different CAGs were also associated with different mucosal gene-expression profiles. Two of the CAGs defined (named after their most abundant member, *Bacteroides fragilis* and *Prevotella copri*, respectively) were both significantly more abundant in cancer patients, and were differentially correlated with the expression of host response genes for TLRs, CCL20 and STAT3.

**Conclusion:** CRC-associated microbiota profiles are linked with distinct mucosal gene-expression profiles. Compositional changes in the microbiota are not restricted to cancerous tissue and differ between right- and left-sided cancers.
Towards The Development Of A Microfluidic Cell Culture Instrument For Therapeutics

MC Keays\textsuperscript{1,2}, PA Kiely\textsuperscript{1,2}, T Dalton\textsuperscript{1}
\textsuperscript{1}Stokes Institute, University Of Limerick, Limerick
\textsuperscript{2}Department of Life Sciences, and Materials and Surface Science Institute, University Of Limerick, Limerick

The realisation of personalised medicine has been the target of many multidisciplinary scientific teams. A system that can individualise point of care diagnostics has the potential to revolutionise medical treatment. Currently, drug discovery uses 2-dimensional cell culture techniques as a test to monitor the cells reactions to drugs. These models have provided the platform for most toxicity and drug testing. However, the requirement of sample containers such as dishes or flasks are a limiting factor and do not lend themselves to automation. With a desire to understand and diagnose disease and infection at a patient specific and cellular level, there is a pressing need to modify procedures to facilitate multi-experimental approaches that are cost effective, automated and use small amounts of patient sample and reagents. This has encouraged us to develop a microfluidic droplet cell culture platform. The proposed system will create droplet cultures and facilitate incubation and imaging of the cultures on one platform. This system facilitates the establishment 3-dimensional culture conditions that more accurately mimics natural cell conditions and cell-cell communication. The culture droplets are created in a nano-liter range and multiple unique bioreactor droplets can be prepared and analysed on one system making this a time and cost effective instrument. This system can benefit cancer diagnosis as multiple assays can be mixed with individual culture droplets. This lends itself to drug cocktail testing tested against a specific sample making this a powerful instrument in understanding cells.
OvMark: a user-friendly system for the identification of prognostic biomarkers in publically available ovarian cancer gene expression datasets
SF Madden¹,², C Clarke², B Stordal³, MS Carey⁴, R Broaddus⁵, WM Gallagher¹, J Crown², GB Mills⁶, BT Hennessy⁷
¹Irish Cancer Society Collaborative Cancer Research Centre BREAST-PREDICT and Cancer Biology UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Dublin
²Molecular Therapeutics for Cancer Ireland, National Institute for Cellular Biotechnology, Dublin City University, Dublin 9, ³Department of Histopathology, St James' Hospital and Trinity College Dublin, Dublin 8, ⁴Department of Obstetrics and Gynecology, University of British Columbia, Vancouver, BC, Canada, ⁵Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, Tx, USA, ⁶Department of Systems Biology, The University of Texas MD Anderson Cancer Center, Houston, Tx, USA, ⁷Department of Medical Oncology, Beaumont Hospital and RCSI, Dublin 9

Ovarian cancer has the lowest survival rate of all gynaecologic cancers and is characterised by a lack of early symptoms and frequent late stage diagnosis. There is a paucity of robust molecular markers that are independent of and complementary to clinical parameters such as disease stage and tumour grade. We have developed a user-friendly, web-based system to evaluate the association of genes/miRNAs with outcome in ovarian cancer. The OvMark algorithm combines data from multiple microarray platforms (including probesets targeting miRNAs) and correlates them with clinical parameters (e.g. tumour grade, stage) and outcomes (disease free survival (DFS), overall survival). In total, OvMark combines 14 datasets from 7 different array platforms measuring the expression of ~17,000 genes and 341 miRNAs across 2,129 ovarian cancer samples. To demonstrate the utility of the system we confirmed the prognostic ability of 14 genes and 2 miRNAs known to play a role in ovarian cancer. Of these genes, CXCL12 was the most significant predictor of DFS (HR=1.42, p-value=2.42x10⁻⁶). Surprisingly, those genes found to have the greatest correlation with outcome have not been heavily studied in ovarian cancer, or in some cases in any cancer. For instance, the three genes with the greatest association with survival are SNAI3, VWA3A and DNAH12. OvMark is a powerful tool for examining putative gene/miRNA prognostic biomarkers in ovarian cancer (available at http://glados.ucd.ie/OvMark/index.html). The impact of this tool will be in the preliminary assessment of putative biomarkers in ovarian cancer, particularly for research groups with limited bioinformatics facilities.
A preclinical study to identify mechanisms of radio-resistance during the treatment of non-small cell lung cancer with advanced radiotherapy

VD Dunne¹, KM Redmond¹, AJ Cole¹, KM Prise¹, GG Hanna¹
¹Centre for Cancer Research and Cell Biology, Queens University, Belfast, Northern Ireland

**Background:** Non-small cell lung cancer (NSCLC) accounts for 75-85% of patients diagnosed with lung cancer. Radiotherapy is commonly used as a potentially curative treatment for patients unfit for surgical resection or whose cancer is situated in a position that surgical resection cannot be implemented. In spite of the generation of innovative technology including use of novel imaging techniques, local recurrence rates for NSCLC remains disappointing with up to 50% of patients experiencing local recurrence. It is therefore necessary to employ alternative treatment strategies to sensetise NSCLC tumours to radiotherapy. Previous in-vitro work suggests that a number of oncogenic pathways and tumour-suppressor genes act as mediators of radio-resistance in NSCLC as they can become dysfunctional during tumorigenesis. PTEN is a candidate tumor suppressor reported to be deleted, mutated or downregulated in a number of cancers including NSCLC. In the absence of PTEN, an increase in Bcl-2 and Akt phosphorylation occurs. Proteins including mTOR, Bad and TSC2 are also altered, causing PI3K signalling to become upregulated. This cascade leads to an increase in cell survival and the development of radio-resistance which may be a factor in cancer recurrence. Hypothesis: PTEN down regulation results in radio-resistance in NSCLC. Overcoming this with specific inhibitors may lead to reduced radio-resistance and ultimately provide a novel therapeutic strategy in the NSCLC treatment with radiotherapy.

**Methods/Results:** Western blotting was used to show the presence or absence of PTEN in A549, H1299, H157, H460 and BEAS-2B cell lines. Clonogenic survival assays were used to show that the PTEN-expressing H460 cells were more sensitive to IR than PTEN null H1299 cells. siRNA will be utilised to knockdown PTEN expression in A549 and H460 cell lines. Modulation of radiosensitivity will be assessed by clonogenic survival before and after knockdown of PTEN.
THE ROLE OF CLUSTERIN IN OESOPHAGEAL ADENOCARCINOMA RADIORESISTANCE

R Dunne¹, T Condon¹, N Lynam-Lennon¹, JV Reynolds¹, J O'Sullivan¹
¹Department of Surgery, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8

Introduction
Oesophageal adenocarcinoma (OAC) is treated with neoadjuvant chemoradiation in order to reduce tumour size prior to surgery, however only approximately 25% of patients have a complete pathological response. This means up to 75% of patients suffer side effects of treatment and delays to surgery with no clinical benefit. Response to treatment is scored using a tumour regression grade (TRG). Currently, there is no biomarker to indicate which patients will respond to treatment. Clusterin is a glycoprotein with multiple biological roles, including radiation response, DNA repair, angiogenesis and inflammation. Its role in oesophageal adenocarcinoma has not yet been investigated.

Methods
An isogenic cell line model of oesophageal cancer radioresistance was used for in vitro work. Clusterin expression, and expression of DNA repair genes, in cell lines and oesophageal tumour explants was examined by qPCR. Secretion of clusterin, and angiogenic and inflammatory markers from oesophageal tumour explants was assessed by ELISA.

Results
Expression of clusterin was significantly upregulated in radioresistant cells (p=0.01). There was significantly higher expression of clusterin in oesophageal cancer patients with a poor response to radiation (p=0.04). Expression of clusterin in explants also significantly correlated with expression of DNA repair genes associated with oesophageal cancer radioresistance, MMS19, SMUG1, PARP1 and MLH1 (p<0.01) [10]. Secretion of clusterin from explants significantly correlated with a number of angiogenic (ANG-2, bFGF, PAI-1, sICAM-1, sVCAM-1, VEGF) and inflammatory markers (GROα, IL-6, MCP-1, MMP-9) (p<0.05).

Conclusion
Clusterin may be useful as a biomarker to identify oesophageal adenocarcinoma radioresponders. Targeting clusterin could potentially improve response to radiation, perhaps through targeting DNA repair, angiogenesis and inflammation, mechanisms associated with radioresistance.
PRIMA-1MET (APR-246): A NOVEL TARGETED THERAPY FOR TRIPLE NEGATIVE BREAST CANCER?

NC Synnott¹,², PM McGowan¹,², A Pierce¹,², M Kiely³, N O'Donovan⁴, J Crown⁴,⁵, PA Kiely³, MJ Duffy¹,²

¹Education and Research Centre, St Vincent’s University Hospital, Dublin
²UCD School of Medicine and Medical Science, University College Dublin, Dublin
³Department of Life Sciences, University of Limerick, Limerick
⁴National Centre for Cellular Biotechnology, Dublin City University, Dublin
⁵Department of Medical Oncology, St Vincent’s University Hospital, Dublin

Background: Despite intensive efforts, a validated targeted therapy for triple-negative breast cancer (TNBC) remains elusive. One of the most frequent genetic alterations identified to date in TNBC is mutation in the p53 gene, which has been found in > 80 % of these samples [1]. The aim of this study was therefore to investigate mutant p53 as a potential target for the treatment of TNBC.

Methods: The low molecular weight compound PRIMA-1MET (APR-246), which has previously been shown to reactivate mutant p53 and convert it to a form with wild-type properties [2], was investigated in a panel of 23 breast cancer cell lines (TN = 11; non-TN = 12). Cytotoxicity was determined using both the MTT assay and colony formation assay. Proliferation was measured using the real-time xCELLigence system, while induction of apoptosis was measured using flow cytometry. p53 protein levels were determined by western blot and ELISA.

Results: Using the MTT assay, IC50 values across the panel of breast cancer cells ranged from 0.9 to 31.1 µM, for PRIMA-1MET. Inhibition of cell growth varied from 3.1 to 96.6 %, using concentration of inhibitor at 6.25 µM. PRIMA-1MET also reduced the ability of breast cancer cells to form colonies, in the 6 cell lines investigated, with IC50 values ranging from 0.69 to 6.4 µM. These findings were confirmed with the xCELLigence system. p53 mutated cell lines were significantly more sensitive to PRIMA-1MET than p53 WT cells (p = 0.0144, Student's t-test). Response to PRIMA-1MET correlated strongly and significantly with p53 protein level (p=0.0003, r= -0.7376, Spearman test). In addition to inhibiting cell proliferation, PRIMA-1MET also induced apoptosis in the p53 mutated cell lines MDA-MB-453 and MDA-MB-468 but not in the p53 WT cell line, MCF7.

Conclusion: Our preclinical results suggest that targeting mutant p53 with PRIMA-1MET (APR-246) is a potential new approach for treating p53-mutated breast cancer including the subgroup with triple-negative disease. Acknowledgement: This work was funded by the BREAST-PREDICT (CCRC13GAL) programme of the Irish Cancer Society.

TARGETING THE PI3K PATHWAY IN ANDROGEN-DRIVEN AROMATASE INHIBITOR RESISTANT BREAST CANCER

A Ali1,2, L Creevey1, F Bane1, ADK Hill1,2, LS Young1, M McIlroy1
1Endocrine Oncology Research Group, Royal College of Surgeons, Dublin 2
2Department of Surgery, Beaumont Hospital, Dublin 9

Breast cancer has the highest incidence and second highest mortality rate among all cancers in women worldwide (1). The gold standard treatment for post-menopausal breast cancer is aromatase inhibitor (AI) therapy. Unfortunately, resistance is inevitable in about 30-60% of these patients (2). Research from our lab has previously identified a transcription factor, HOXC11, to be associated with breast cancer resistance and metastases (3). Prosaposin (PSAP) was then identified as a putative HOXC11 target gene in the AI resistant setting. PSAP is a secreted protein that also plays a role in metastatic prostate cancer in both a ligand dependent and independent fashion (4,5). We have previously shown PSAP to be an androgen responsive gene that can upregulate androgen receptor (AR) expression in a ligand dependent manner in AI resistance. An AR antagonist was effective at slowing down cell proliferation in AI resistant cells in vitro. However, since PSAP can also potentially act in a ligand independent fashion via binding with a G protein coupled receptor 37 (GPR37), this current study is focused on evaluating the role of PSAP in PI3K pathway activation in the development of AI resistance. Immunofluorescence staining in a panel of cells showed that GPR37 expression is much higher in the resistant cells as compared to the sensitive cells with further increased expression when treated with a recombinant PSAP protein (rhPSAP). rhPSAP treatment in the AI resistant cells showed increasing expression of p-AKT in a dose-dependent manner. To target this pathway of resistance, we used a pan-class PI3K/mTOR inhibitor BEZ235 on our AI resistant cells. Functional studies with BEZ235 treatment showed significant reduction in both cell motility as well as cell proliferation. Treatment of AI resistant cells with BEZ235 resulted in decreased expression of p-AKT, with little or no effect on AR expression. These findings suggest that to prevent resistance to AI therapy, combination treatment regimens including AR antagonists plus a PI3K inhibitor may ensure sustained response to therapy.

CIGARETTE SMOKE EXTRACT ALTERS THE INFLAMMATORY TISSUE MICROENVIRONMENT, INDEPENDENT OF NFkB AND HIF-1α ACTIVITY, IN HUMAN AND MURINE MODELS OF COLITIS

A Cannon¹, R Horan², N Mahmud², N Ullah², F MacCarthy², J Lysaght¹, P Fallon², P Walsh², J O’Sullivan¹

¹Surgery, Trinity College Dublin, Dublin
²Clinical Medicine, Trinity College Dublin, Dublin

Introduction: Ulcerative Colitis (UC) is a form of inflammatory bowel disease, characterised by chronic or relapsing inflammation in the gastrointestinal tract. The severity of UC is higher in non-smokers than smokers; however, the biological mechanisms controlling this effect are unknown. This study is examining the effect of Cigarette Smoke Extract (CSE) using ex vivo and in vivo models of UC to determine if inflammatory mediators and transcription factors are altered by CSE.

Materials and Methods: Colonic biopsies were obtained from consenting UC patients. These ex vivo biopsies were cultured in the presence or absence of CSE. Multiplex ELISAs assessed the levels of inflammatory mediators. In mice, colitis was induced with 3% DSS. CSE was injected intraperitoneally. Disease activity index (DAI) scores and weight were recorded daily. Gene expression of inflammatory mediators was measured in murine colonic tissue. NFκB and HIF-1α expression was measured by ELISA in human and mouse colonic tissue.

Results: Secreted levels of CXCL1, IL-1β, TNF-α, IL-2, IL-6, IL-10, CCL2, CCL20, and from treated UC biopsies were decreased (all, p < 0.05). Mice treated with CSE had lower DAI scores (p<0.001). Gene expression of MMP9 and CCL2 were down-regulated in CSE treated mice (p < 0.001 and p < 0.05 respectively). This effect was specific to recto-sigmoid tissue. Expression of NFκB and HIF-1α were not significantly different between the treated and untreated groups in humans or mice.

Conclusion: CSE elicits similar anti-inflammatory effects in mouse and human models of UC. These changes are independent of NFκB and HIF-1α expression.
INVESTIGATING THE ROLE OF ARGONAUTE-2 IN BREAST CANCER

MC Casey¹, A McGuire¹, A Shalaby², M Weber², C Curran¹, E Bourke², JAL Brown¹, MJ Kerin¹
¹Discipline of Surgery, School of Medicine, Clinical Science Institute, National University of Ireland, Galway, Galway, ²Discipline of Pathology, School of Medicine, Clinical Science Institute, National University of Ireland, Galway, Galway

While many advances have been made in terms of our molecular understanding of breast cancer, further research is required to discover and validate novel biomarkers, particularly ones that further categorise the 4 main molecular subtypes. These new biomarkers will facilitate improvements in diagnosis and optimisation of treatment for this disease. MicroRNAs (miRNAs) have been proposed as one such biomarker, as their expression is known to be dysregulated in breast cancer[1]. Significantly, little work has been performed to examine the role of the miRNA processing machinery in the different breast cancer subtypes. Argonaute 2 (Ago2) forms an essential component of the miRNA-induced silencing complex (miRISC). Microarray studies have implicated aberrant Ago2 gene expression in tumourigenesis, with significant alterations found in breast cancer subtypes [2]. Significantly, the functional role of the Ago2 protein in the main molecular breast cancer subtypes has not yet been investigated. Using tissue microarrays from >700 breast cancer patients we examined the levels of Ago2 protein across all known breast cancer subtypes. In addition, we characterized the levels of Ago2 in several breast cancer cell lines used to represent these molecular subtypes in vitro. Furthermore we have investigated a panel of miRNAs bound to Ago2, both within and secreted from, these cultured breast cancer cells. We believe that investigating the functional consequences of changing Ago2 levels could provide important insights into the molecular differences between breast cancer subtypes. Furthermore, Ago2 could potentially serve as a new marker to aid in the diagnosis, or as a therapeutic target for the treatment of, breast cancer.

Elucidation of the Expression Profile and Role of the Thromboxane Pathway in Colorectal Cancer: A Novel Anti-Vascular Approach for Therapeutic Intervention?

MC Cathcart¹, B Mehigan¹, PMc Cormick¹, MC Lowry¹, Z Useckaite¹, GY Moore¹, JV Reynolds¹, EW Kay², JNO Sullivan¹, GPPidgeon¹

¹Department of Surgery, Trinity College Dublin/St. James's Hospital, Dublin 8.
²Department of Histopathology, Beaumont Hospital/RCSI, Dublin 9.

Introduction: Colorectal cancer (CRC) is the third most common cancer worldwide. VEGF targeting with avastin is an anti-angiogenic approach to treat metastatic CRC, although is associated with poor response. COX-2 inhibitors are promising, although associated with cardiovascular risk following chronic use. The downstream thromboxane (TX) signalling pathway may promote cancer growth via increased tumour-associated angiogenesis. This study aimed to examine the expression pattern of the thromboxane pathway in CRC and to investigate targeting this pathway for CRC treatment.

Methods: 200-patient colorectal tissue microarrays (TMAs) were stained for TXS, TP (whole protein and both isoforms), COX-2 and VEGF expression by IHC. Selected TX-pathway inhibitors (from in-vivo vascular screening studies in zebrafish; ozagrel, seratrodast, AH-23848) were used to treat fresh CRC explant tissue (ex-vivo human 3D-explant culture model) for 72 h. Angiogenic metabolite secretions from tumour explants were assessed using multi-plex ELISAs (MSD), and compared with avastin. Vascular stability following TX-pathway targeting was assessed by fluorescence staining of explant tissue for vWF and α-SMA.

Results: Expression of TP, TP-α, TP-β and COX-2 were significantly increased in tumour tissue, relative to matched normal colonic mucosa (p<0.0001, TP, TP-α, TP-β; p<0.05, COX-2), with no increase in TXS or VEGF observed. Levels of TXS, COX-2 and VEGF were significantly (p<0.05) higher in early-stage patients. TXS levels were significantly (p<0.05) associated with both COX-2 and VEGF in the same patients. While no association with overall patient survival was observed for TXS, COX-2, or VEGF in our cohort, a preliminary trend was observed for reduced survival with TP-β expression (p=0.3; n=100). Human explant culturing revealed that the secretion of a number of angiogenic metabolites from CRC tumour explants was significantly reduced following thromboxane pathway targeting, including VEGF, bFGF, Ang-2, IL-6 and IL-8 (all p<0.05). A significant reduction in the percentage of mature (stable) vessels was observed following TX-pathway targeting (p<0.05, ozagrel, AH-23848; p<0.001, seratrodast), an effect that was not seen with avastin.

Conclusion: TX pathway targeting has anti-vascular efficacy in-vivo. This may be mediated through a reduction in angiogenic protein secretions and increased vascular destabilisation. Targeting this pathway may be a novel anti-angiogenic approach for CRC treatment.
PP2A AND INNATE LAPATINIB RESISTANCE IN HER2-POSITIVE BREAST CANCER CELLS

NT Conlon1, M McDermott2, J Crown1, 3, N O'Donovan1

1National Institute for Cellular Biotechnology, Dublin City University, Dublin
2Department of Oncology, St. Vincent's University Hospital, Dublin
3South Carolina College of Pharmacy, University of South Carolina, Columbia

Background: Despite the success of HER2-targeted therapies, such as trastuzumab and lapatinib, de novo and acquired resistance remains an important clinical issue. Previously, we identified decreased levels of phosphorylated elongation factor 2 (phospho-eEF2) in two cell line models of acquired lapatinib resistance. This alteration was due to increased protein phosphatase 2A (PP2A) activity and these cell lines were more sensitive to PP2A inhibition than their parental cell line, suggesting that PP2A may mediate acquired lapatinib resistance. In this study we investigated the potential role of PP2A in innate lapatinib resistance.

Method: Immunoblotting was performed on a panel of HER2-positive cell lines, which were classified as sensitive or resistant (IC50 >1 µM lapatinib) to lapatinib, for PPP2CA (PP2A catalytic subunit), phospho-PPP2CA. Acid phosphatase assays were performed to assess sensitivity to lapatinib and, the PP2A inhibitor, okadaic acid, alone and in combination.

Results: PPP2CA was detected in all of the nine HER2 positive cell lines tested. No significant differences in PPP2CA (p=0.079) or phospho-PPP2CA (p=0.228) levels were observed between the lapatinib sensitive (n=6) and resistant (n=3) cell lines, and neither correlated with response to lapatinib. Five cell lines, including 2 innately resistant cell lines (JIMT1 and UACC732), were tested for sensitivity to okadaic acid alone and in combination with lapatinib. The JIMT1 cells showed sensitivity to okadaic acid alone, but the combination did not enhance response. The UACC732 cells also displayed sensitivity to okadaic acid and in this cell line dual treatment with okadaic acid and lapatinib significantly improved response compared to the single agents. Two of the three lapatinib sensitive cell lines were resistant to okadaic acid, however okadaic acid in combination with lapatinib enhanced response in 2 of the 3 sensitive cell lines.

Conclusion: PP2A is abundantly expressed in HER2 positive breast cancer cells and PP2A inhibition may represent a novel therapeutic strategy to overcome innate lapatinib resistance or enhance response to lapatinib in HER2 positive breast cancer.
The heterogeneous nature of breast cancer, exemplified by a wide range of subtype classifications, is well documented. In addition to the classical hormone receptors associated with breast cancer progression such as estrogen and progesterone receptors (ER, PR), the androgen receptor (AR) has recently come under renewed scrutiny due to its potential association with poor response to endocrine therapy. Aromatase Inhibitor (AI) therapy is the gold standard first line therapy for postmenopausal breast cancer. AIs function by abrogating the activity of the aromatase enzyme, which converts circulating androgens to estrogen. By preventing this step, the main steroid driving breast cancer growth is eradicated, resulting in a more androgenic environment. We hypothesise that AR may act as a pseudo-ER in AI resistant (MCF7-letrozole resistant) cells. Preliminary data demonstrates that AR is recruited to a number of classical ER target genes (CMYC and PS2) as well as AR target genes (Prosaposin (PSAP)) in MCF7-LetR cells. PSAP is a known AR agonist associated with metastatic ability therefore highlighting the potential of AR to act as an oncogene in this setting. AI resistant cells demonstrated higher levels of both AR and secreted PSAP protein versus sensitive cells. Recombinant PSAP (rhPSAP) increased both AR mRNA and protein levels and increased cell migration in AI resistant cells compared to sensitive cells. This migratory effect was inhibited through the use of bicalutamide, an AR antagonist. Nuclear translocation assays revealed significantly increased nuclear AR staining following rhPSAP treatment. Survival analysis revealed that the survival benefit classically associated with AR expression was lost in the AI resistant setting. Preliminary results demonstrated that ~20% of postmenopausal patients who have recurred on endocrine therapy express PSAP serum levels elevated above baseline. Given that the development of resistance to AI therapy is an emerging clinical issue, this study gives insight into a mechanism by which breast cancer cells may adapt to a more androgenic environment, which in turn can contribute to the failure off endocrine therapy.
CUX1 is upregulated and negatively regulates invasion in a cell line model of castrate resistant prostate cancer

ER Dorris1, A O’Neill1, A Treacy2, S Boyce1, M Prencipe1, H Klocker3, W Watson1

1Medicine and Medical Sciences, University College Dublin, Dublin
2Histopathology, St Vincent’s University Hospital, Dublin
3Urology, Medical University of Innsbruck, Innsbruck

Background: Non-organ confined prostate cancer is treated with androgen ablation therapy but can progresses to castrate resistant prostate cancer (CRPC), which represents a significant clinical challenge to treat with no effective therapeutic interventions. Our group previously combined transcriptomic analysis with bioinformatic prediction tools to identify transcription factors associated with a cell line model of the CRPC phenotype. Cut-like homeobox 1 (CUX1) was associated with castrate resistance and chosen for further functional analysis because it is reported to be involved in cellular processes relevant to cancer including cell proliferation, cell motility and invasiveness.

Methods: In androgen sensitive and castrate resistant prostate cancer cell lines CUX1 knockdown was undertaken using siRNA and cells assessed for proliferation, migration and invasion. Immunohistochemical (IHC) analysis of tissue microarrays were used to assess the clinical association of surrogate markers of CUX1 with CRPC.

Results: CUX1 knockdown had no effect on proliferation in in the models of castrate resistant disease. CUX1 knockdown significantly enhanced migration, but not the invasive capacity of androgen sensitive prostate cells. Conversely, the invasive but not the migration ability of CRPC cells was significantly increased following CUX1 knockdown in CRPC cells. IHC analysis did not provide any significant associations between CUX1 markers and CRPC.

Conclusions: In conclusion, CUX1 is upregulated in a CRPC cell line model and negatively regulates invasion. However, this association does not robustly translate to clinical samples.
INHIBITION OF CASPASE-3 AS A NEW THERAPEUTIC APPROACH IN COLON CANCER

L Flanagan1,2, S Curry3, J Fay3, O Bacon1,2, E.W Kay3, D.A McNamara4, J.H.M Prehn1,2

1Department of Physiology & Medical Physics, Royal College of Surgeons in Ireland, Dublin
2Centre for Systems Medicine, Royal College of Surgeons in Ireland, Dublin
3Departments of Pathology, Beaumont Hospital, Dublin
4Departments of Surgery, Beaumont Hospital, Dublin

Colon cancer is one of the commonest non-cutaneous cancers in Ireland. Novel targets in chemotherapy are sought constantly to improve adjuvant therapy and prevent resistance to current chemotherapy and radiotherapy regimes. The mainstay of treatment is with cytotoxic agents, such as FOLFOX. These treatments activate executioner caspases in target cells. Executioner caspases have long been recognised as key proteins involved in cell disassembly during apoptosis. More recently, studies have reported that activation of executioner caspases also plays a role in tissue regeneration by stimulating signal transduction and cell proliferation in neighbouring, non-apoptotic cells. Exploiting and antagonising this paracrine role of executioner caspases may be an interesting, novel approach for the treatment of tumours characterised by resistance and relapse. We constructed tissue micro arrays using tumour tissue from 93 colon cancer patients. Active Caspase-3 levels were analysed and levels were correlated with patient outcome. Low levels of active Caspase-3 gave a significant survival advantage, and patients with low levels of this protein had an increased disease free survival time. We next used a novel ex vivo technique to examine the effect of inhibition of Caspase-3, and its downstream effectors, in concert with chemotherapy on the proliferation and regeneration of tumour tissue. Our results confirmed chemotherapy increases expression of proliferation markers, such as Beta-Catenin, Ki-67 and COX-2. However, inhibition of Caspase-3, and particularly its downstream effectors, can significantly reduce the expression of these proliferation markers. These data indicate that absence or low levels of active Caspase-3 may represent an important predictor of chemotherapy responsiveness. More importantly, these data highlight that inhibition of Caspase-3, or antagonising downstream effectors of Caspase-3 paracrine signalling, such as PGE2 or components of the Wnt signalling pathway, may represent a novel approach to halt, or at least impede, tumour cell repopulation following chemotherapy.
PROSTATE CANCER RISK STRATIFICATION: THE CREATION OF A NOVEL RISK PREDICTION MODEL AND THE INTEGRATION OF NOVEL PROSTATE CANCER BIOMARKERS.

RW Foley¹, ², N Sharifi³, LV Gorman¹, K Murphy⁴, DJ Lundon⁵, A Tuzova⁶, A Perry⁶, TB Murphy⁴, RWG Watson¹, ²
¹UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin 4
²UCD School of Medicine and Medical Sciences, University College Dublin, Dublin 4
³Department of Biochemistry, Beaumont Hospital, Dublin 9
⁴UCD School of Mathematical Sciences, University College Dublin, Dublin 4
⁵Department of Urology, Mater Misericordiae University Hospital, Dublin 7
⁶Institute of Molecular Medicine, Trinity College Dublin, Dublin 2

Objectives

The gold standard for the diagnosis of prostate cancer (PCa) is a TRUS biopsy. The decision on who to send for prostate biopsy is a difficult one. It is clear that in order to make this decision, better use of the clinical information available as well as more accurate biomarkers of disease are needed. Our objective was to create a novel risk prediction model for the Irish male population and integrate novel biomarkers to improve PCa risk stratification pre-biopsy.

Methods

Patient information on 982 men under investigation for PCa was analysed. A subset of patients (n=250) for whom pre-biopsy serum samples were available underwent testing for a novel serum biomarker for PCa, namely proPSA. Risk prediction models were created via logistic regression with 10-fold cross validation. The performance of these models was assessed via calibration plots, ROC curves and decision curves.

Results

Our novel risk prediction model improved upon the current gold standard: the Prostate Cancer Prevention Trial calculator (PCPT). The AUC of our novel calculator was 0.732 and 0.763 for the prediction of PCa and high grade PCa (> Gleason 6) respectively, while the AUC was 0.700 and 0.730 for the PCPT. The novel model demonstrated superior calibration. Decision curve analysis confirmed a superior net benefit of our novel model across its entire range of risk predictions. The addition of proPSA, in the form of the phi test to our subset of patients increased the AUC of our risk prediction model to 0.77 for PCa and 0.79 for high grade disease. This improvement was also maintained when analysed via calibration and decision curve analysis.

Conclusion

A logical and standardised approach to the use of clinical risk factors can allow for more accurate risk stratification of men under investigation for PCa. The measurement of proPSA and the integration of this biomarker into a risk prediction model can further increase the accuracy of risk stratification, helping to better inform the decision for prostate biopsy in the Irish male population.
IDENTIFICATION OF THE CHEMORESISTANT COMPONENT OF A NOVEL OVARIAN CANCER STEM CELL HIERARCHY

C Gasch1, 2, B Ffrench1, 2, C Spillane1, 2, G Blackshields1, L Kehoe1, B Doyle1, JJ O'Leary1, 2, MF Gallagher1, 2
1Histopathology, Trinity College Dublin, Dublin
2Pathology, Coombe Womens and Infants Hospital, Dublin

Background: Ovarian cancer is characterised by advanced presentation and high rates of terminal, chemoresistant recurrence. Although chemoresistance is known to be a property of Cancer Stem Cells (CSCs), the mechanism is poorly understood. We have previously identified a novel four-member CSC Stem-Progenitor cell hierarchy for ovarian cancer, based around differential expression of stem cell marker ALDH. The aim of this study was to characterise the contribution of each member of the ovarian CSC hierarchy to chemoresistance. Approach Each member of the CSC hierarchy was assessed for tolerance to chemotherapy drug cisplatin (MTT assay) both as components of the parent population (A2780 cell line) and as isolated cell types. The hierarchy was additionally assessed in the long-term cisplatin-adapted "A2780cis" cell line. Cell types were analysed and isolated via flow cytometry and assessed for stem cell characteristic via single-cell asymmetric division and murine xenograft tumourigenicity assays, and molecularly characterised (whole transcriptome arrays).

Results: Cisplatin dose-response assays from A2780-derived CSC sub-populations indicated that only one of the four populations within the ALDH hierarchy had a high cisplatin-tolerance (IC50=10uM) compared to the other populations (IC50=4uM). This was notable as the relative cisplatin IC50s for the A2780 and A2780cis parent cell lines are 4uM and 11uM respectively. Treatment of the parent A2780 cell line with the IC50 (48 hours) resulted in a proportional 50% loss in each of the four cell types, suggesting that this specific CSC sub-population adapts to cisplatin over a longer period of time.

Conclusion: Although CSCs are known to be chemoresistant, the mechanism though which this is achieved is poorly understood. Our data indicates that only some members of a CSC hierarchy are responsible for chemoresistance. Notably, this sub-population appears to possess inherent chemoresistance in pre-treatment cells. As such, it should be possible to target these CSCs in the primary malignancy to prevent chemoresistant recurrence.
VALIDATION OF A PANEL OF SERUM BIOMARKERS TO INFORM SURGICAL INTERVENTION FOR PROSTATE CANCER

1L Gorman, 1L Murphy, 1S Boyce, 1R Foley, 1K Murphy, 1JM Fitzpatrick, 1J Clemments, 1H Klocker, 2TB Murphy, 1RWG Watson

1School of Medicine and Medical Science, University College Dublin, Ireland
2School of Mathematical Sciences, University College Dublin, Ireland
3Australian Prostate Cancer Research Centre Queensland, Queensland University of Technology, Australia
4Department of Urology, Innsbruck Medical University, Austria

BACKGROUND

The main dilemma faced by the patient and clinician once Prostate Cancer (PCa) has been diagnosed is determining the most appropriate treatment. The assessment of stage, i.e. organ confined (OC) versus non organ-confined (NOC) is centrally important as it informs the basis of clinical decision making. If the disease is significant but localized to the prostate, curative treatment of surgery or radiation therapy is offered. If not localised, hormone ablation therapy is the most appropriate treatment. Approximately 30% of men have NOC disease following radical prostatectomy. The availability of clinical adjunctive decision making tools such as our biomarker panel, to aid in the prediction of post radical prostectomy patient outcomes can assist therapeutic selection and planning while improving patient outcome and alleviating the pressures on already over-burdened healthcare systems.

METHODS

Using the Meso Scale Discovery multiplexing platform, we developed and analytically and clinically validated a panel of 9 serum biomarkers in an independent Irish patient cohort (n=197). A sub-panel of 4 biomarkers with improved predictive outcome was identified and further clinically validated in an Austrian (n=135) and Australian (n=177) cohort.

RESULTS

Using a combination of logistic regression and backwards feature selection, we established an optimal biomarker panel of 4 serum proteins which can more accurately predict PCa stage in an Irish cohort when combined with the clinical gold standard (Partin tables) (AUC=0.86), compared to the Partin tables alone (AUC=0.59). Further clinical validation of the panel in an Austrian and Australian cohort demonstrated predictive probabilities (AUC) of 0.70 and 0.72 respectively when combined with the Partin tables, compared to an AUC of 0.63 and 0.60 respectively for the gold standard alone.

CONCLUSIONS

Our biomarker panel in conjunction with the current Partin tables (which uses PSA, DRE and Biopsy Gleason grade) offers a significant improvement in PCa staging in an Irish, Australian and Austrian cohort and could significantly improve patient quality-adjusted life-years in terms of a reduction in treatment associated side effects as a consequence of over-diagnosis.
XBP1 regulates the expression of NCOA3/AIB1 during unfolded protein response in breast cancer cells

A GUPTA, MH MOSARAF, G CALLAGY, S GUPTA

1DISCIPLINE OF PATHOLOGY, SCHOOL OF MEDICINE, NUI GALWAY, GALWAY

The tumour microenvironment exerts a poorly characterized stress on cancer cells that is mediated by various factors, including hypoxia and nutrient deprivation. The UPR (or ER stress) has emerged as a marker for tumour microenvironment-induced stress, which can be triggered within cancer cells by hypoxia or nutrient deprivation (1). Although activation of the UPR has been reported in a variety of human cancers including high-grade breast carcinomas, the role of UPR in cancer is not yet fully characterized. The protein encoded by the nuclear receptor co-activator-3 (NCOA3; also known as AIB1 or SRC3), gene is a steroid receptor co-activator that enhances the transcriptional activity of ERα (2). NCOA3 is over-expressed in ~ 64% breast cancers. A high level of NCOA3 is associated with reduced survival and poor prognosis (2). Overexpression and increased phosphorylation of NCOA3 leads to enhanced ERα-mediated transcription (2). Although the important role of NCOA3 in breast cancer is well established, relatively little is known about how NCOA3 becomes over-expressed in human cancers. First we studied the endogenous levels of NCOA3 transcript in a panel of six breast cancer cell lines and found that NCOA3 was amplified in MCF7 cells. During conditions of UPR the levels of NCOA3 mRNA and protein was increased substantially in breast cancer cell lines (MCF7 and T47D). Further investigation showed that the IRE-1 arm of the UPR signalling upregulated the expression of NCOA3 during UPR via XBP1. Upregulation of NCOA3 during UPR was blocked by IRE-1 inhibitors. Ectopic overexpression of spliced XBP1 resulted in increased expression of NCOA3 in MCF7 and 293T cells. XBP1 binding sites were identified in the NCOA3 proximal promoter region. An NCOA3 promoter luciferase reporter construct responded to XBP1 overexpression and UPR signalling. Knockdown of NCOA3 in MCF7 cells compromised UPR signalling and reduced UPR induced cell death. mRNA Levels of NCOA3 and spliced XBP1 showed a very good correlation in breast cancer patient samples. These results suggest that UPR-mediated upregulation of NCOA3 can occur in breast cancer tumors and NCOA3 plays a role in UPR signalling.

Lipopolysaccharide (LPS)-induced tolerisation of colorectal cancer cells increases their metastatic potential in vitro and in vivo

D Hechtl¹, JH Wang¹, HP Redmond¹
¹Academic Surgical Research, University College Cork, Cork

Introduction: Endotoxin tolerance (ET) is a refractory state in which, immune cells show unresponsiveness to repeated LPS stimulation. Inflammation increases the incidence of tumour recurrence and metastases despite curative surgery in colorectal cancer. Toll-like receptor 4 (TLR4), which binds LPS, has an essential role in initiating the inflammatory cascade and mediating ET. Therefore TLR4-dependent signaling has a cardinal contribution in tumour cells adhesion, proliferation and invasion.

Aim: To investigate whether LPS pre-treatment influences human or murine colorectal cancer cells adhesion, proliferation and invasion in vitro. Also we evaluated LPS pre-treatment effects on murine colorectal tumour cells in vitro and in vivo.

Methods: Human (SW620, SW480) and murine (CT-26) colorectal cancer cells were pre-stimulated with LPS (100ng/ml) to induce tolerisation. Non-tolerised and tolerised cells were then assessed for cytokine profile, adhesion, invasion and proliferation or viability. For in vivo experiments, CT-26 cells were either inoculated into the flank of BALB/c mice (n=24) to assess primary tumour growth or (n=24) underwent intra-splenic injection of CT-26 cells to observe metastatic growth in the liver. Statistical significance was evaluated by Students t-test for comparisons between groups, and analysis of variance (ANOVA).

Results: Following LPS treatment SW620 cells demonstrated reduced (p=0.002) interleukin-8 and vascular endothelial growth factor release, confirming that LPS pre-stimulation induced tolerisation in these cells. Furthermore, LPS pre-treatment of SW480, SW620 and CT-26 cells resulted in increased (p=0.046) proliferation, adhesion and invasion in vitro. Similarly, LPS pre-stimulation of CT-26 cells in BALB/c mice exhibited marked primary and metastatic tumour growth.

Conclusion: These results suggest that endotoxaemia as a result of surgery or sepsis may play a critical role in colorectal cancer cell growth in the perioperative period.
Exosome mediated transport of miRNAs
DP Joyce¹, M Higgins¹, CL Glynn¹, JA Brown¹, E Holian², P Dockery³, MJ Kerin¹, RM Dwyer¹
¹Discipline of Surgery, National University of Ireland, Galway
²School of Mathematics, Statistics and Applied Mathematics, National University of Ireland, Galway
³Discipline of Anatomy, National University of Ireland, Galway

Introduction: Exosomes are membrane-derived vesicles that are actively secreted by cells. They have been implicated in cell-to-cell communication, with recent studies suggesting exosome-mediated trafficking of microRNAs between cells. The aim of this study was to identify the panel of exosome-encapsulated microRNAs secreted by breast cancer cells in vitro, and to apply these findings to circulating levels in breast cancer patients.

Methods: Cell-secreted exosomes were isolated from 4 breast cancer cell lines and characterized using Transmission Electron Microscopy (TEM) and Western Blot analysis. MicroRNA array analysis targeting 2089 miRNAs was performed to identify the panel of miRNAs that was secreted within exosomes, with targets of interest validated using RQ-PCR. The presence of these miRNAs in the circulation of patients with breast cancer was also investigated.

Results: TEM analysis of secreted exosomes revealed vesicular bodies of 40-100nm in size. Immunoblotting confirmed the presence of the exosome-associated protein CD63. Greater than 320 miRNAs were secreted within exosomes from each cell line. Exosome-mediated transport of miRNAs was demonstrated using confocal microscopy. miR744-5p, which was found to be secreted within exosomes, was detectable in the blood of breast cancer patients and healthy controls. Preliminary analysis supports an association between this miRNA and tumour epithelial subtype.

Conclusions: A distinct panel of miRNAs are selectively packaged into exosomes and secreted by breast cancer cells. This transfer of functional miRNAs between cells may play an important role in intercellular communication in the tumour microenvironment, and have implications in their use as circulating biomarkers of disease.

Crosstalk between Junctional Adhesion Molecule A (JAM-A) and HER2 in the Trastuzumab-resistant breast cancer phenotype

A Leech\(^1\), LS Young\(^1\), AD Hill\(^1\), AM Hopkins\(^1\)

\(^1\)Surgery, RCSI, Dublin

Junctional Adhesion Molecule-A (JAM-A) is an adhesion protein whose expression on breast tumour tissue positively correlates with poor patient prognosis. We have shown that JAM-A is frequently co-expressed with the human epidermal growth factor receptor 2 (HER2), and in fact regulates HER2 degradation and signalling in breast cell lines. Since HER2 signalling drives aggressive tumour behaviour, its antagonist Trastuzumab (Herceptin) has greatly improved patient outcomes. However, it remains a significant clinical challenge to identify the growing population of HER2-positive patients who will develop resistance to Trastuzumab. We hypothesised that JAM-A-dependent regulation of HER2 expression could participate in Trastuzumab resistance. To investigate this, we utilised two Trastuzumab-resistant breast cancer cell lines along with their matched Trastuzumab-sensitive controls. There was no significant difference in JAM-A protein expression between Trastuzumab-resistant versus-sensitive cells. Interestingly, JAM-A knockdown in Trastuzumab-resistant cells significantly decreased proliferation in a ligand-independent manner, suggesting that antagonism of JAM-A may benefit patients who have developed resistance to the drug. We are currently interrogating a breast cancer patient tissue microarray to investigate whether there is any correlation between JAM-A expression and/or localisation and response to Trastuzumab treatment. Furthermore, we have demonstrated in breast cells that JAM-A expression influences that of HER2 at two levels, both transcriptional and post-translational (via a proteasomal but not lysosomal pathway). Taken together, our data suggest that upstream targeting of HER2 at both levels via interference with JAM-A merits further study as an option to treat patients whose tumours have developed resistance to anti-HER2 therapy.
Breast cancer has classically been described as a heterogeneous disease. More recently, the molecular basis for this heterogeneity has started to be analysed at the genomic, transcriptomic and proteomic level allowing the development of accurate models in order to redefine the treatments by creating new precision therapies. Cancer cells showing therapeutic resistance frequently have an intrinsic deficiency in their ability to initiate or execute apoptosis. The BCL2 (B-cell lymphoma 2) family of proteins emerged as key regulator of the event termed Mitochondrial Outer Membrane Permeabilization (MOMP), which occurrence is vital to initiate intrinsic apoptosis. Previously, we developed a computational model to investigate the role of these proteins in the regulation of apoptosis. Applying DR_MOMP in colorectal cancer patients, we successfully identified patients not responding to genotoxic therapy. To analyze the contribution of BCL2 proteins in modulating apoptosis in breast cancer we validated DR_MOMP in a panel of Triple Negative Breast Cancer (TNBC) cell lines. Using quantitative Western blotting we determined the levels of 2 pro-apoptotic proteins (BAX and BAK) and 3 anti-apoptotic proteins (BCL2, BCL(X)L and MCL1). Although we did not find a correlation between BCL2 levels and cell survival after Cisplatin/Paclitaxel treatment, we found a correlation between cell survival and BCL(X)L levels (rho = 0.76). Using the protein levels as input for DR_MOMP, we found a strong correlation between model predictions and cells responses to Cisplatin (rho = 0.93) and Paclitaxel treatments (rho = 0.97). Moreover we performed synergy studies using two BCL2 antagonists (ABT-263 and ABT-199) showing that this class of antagonist can successfully be used in chemotherapeutic regimens against breast cancer showing high expressions levels of BCL(X)L. Our findings provide evidence that DR_MOMP can be deployed to predict the response of TNBC breast cancer patients to genotoxic therapy. We further showed that the model could be used to identify patients which are likely to benefit from BCL2 antagonists.

Neuromedin U enhances breast cancer cell resistance to HER2-targeted drugs through changes in immune mediators

VG Martinez1, S Rani1, C Corcoran1, L O'Driscoll1
1School of Pharmacy & Pharmaceutical Sciences and Trinity Biomedical Sciences Institute

Neuromedin U (NmU) is a neuropeptide belonging to the neuromedin family. Recently, a significant association between NmU signalling and cancer has been described, particularly correlated with increased aggressiveness and resistance to chemotherapy, although the mechanism through which it exerts this effect remains unexplained. Using stably transfected HER2-positive breast cancer cells, we show here that expression of NmU correlates with secreted levels of IL-6 (0 vs 0.330 ng/µg total protein in mock-transfected vs NmU-overexpressing cells, respectively), a cytokine involved in promoting migration, expansion of the cancer stem cell population and drug resistance in breast cancer cells. Results also show that NmU expression levels correlate with the proportion of cancer stem cells in the total cell population, by detecting CD44+/CD24- cells by flow cytometry (52.78 ± 5.765 in mock-transfected cells vs 67.42 ± 5.172 in NmU-overexpressing cells, p = 0.07; fold change, 1 vs 1.31 ± 0.14, p = 0.00015). Furthermore, over-expression of NmU in breast cancer cells also results in increased expression of the lymphocyte activation inhibitor PD-L1, as detected by flow cytometry (49.50 ± 4.213 vs 60.06 ± 1.628, p = 0.0327); this suggests that NmU over-expression could enhance evasion of the immune response in breast cancer cells. Confirming this hypothesis, NmU over-expressing HCC1954 cells were shown to display enhanced resistance to antibody-dependent cell cytotoxicity mediated by Trastuzumab (47.13 ± 3.31% lysis of mock-transfected cells vs 36.46 ± 4.33% lysis in NmU-overexpressing cells, p = 0.05). Treatment with antibodies that block NmU receptors NMUR1 and NMUR2 reduces cell migration and enhances toxicity of HER2-targeted drugs in NmU over-expressing HER2-positive breast cancer cell lines, suggesting that interaction of NmU with NMUR1 and NMUR2 are necessary for its effects. Altogether, our results show a new mechanism of action of NmU in HER2-positive breast cancer cells that enhances resistance to HER2-targeted drugs and is at least partially mediated by IL-6.

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MECHANISTIC INTERROGATION OF LOW DOSE ASPIRIN EFFECTS ON BREAST CANCER METASTASIS

IS Miller\textsuperscript{1}, LP Shiels\textsuperscript{1}, P Loadman\textsuperscript{2}, RS Kerbel\textsuperscript{3}, K Bennett\textsuperscript{4}, TI Barron\textsuperscript{4}, AT Byrne\textsuperscript{1}

\textsuperscript{1}Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin
\textsuperscript{2}Yorkshire Experimental Cancer Medicine Centre, University of Bradford, Bradford
\textsuperscript{3}Molecular and Cell Biology Research, Sunnybrook and Women's College Health Sciences Centre, Toronto
\textsuperscript{4}Department of Pharmacology and Therapeutics, Trinity College Dublin, Dublin

More than 1 in 3 women with breast cancer take aspirin, a statin, or both, prior to breast cancer diagnosis (Choi et al. Exp Mol Med. 2013). Preclinical and epidemiologic data from BREAST-PREDICT investigators (Barron et al. Cancer Res 2014) and others (Xin et al. Lab Invest 2014) indicate that these pre-diagnostic drug exposures can have significant effects on breast tumour biology, response to treatment and disease prognosis. Previous preclinical studies have demonstrated that tumour over-expression of lymphangiogenic factors VEGF-C/-D may inhibit prostaglandin degradation, induce lymphatic hyperplasia, and increase lymph node metastases. It has thus been suggested that inhibition of COX-2 by aspirin, may suppress lymphangiogenesis with downstream effects on the metastatic cascade (Xin et al Lab Invest 2014). Herein, we sought to reverse translate pre-treatment aspirin exposure in clinically relevant orthotopic surgical resection models of breast cancer to further elucidate mechanisms by which aspirin may influence outcome. NOD/SCID mice were surgically implanted with Her2+ MDA-MB-231-LUC2 cells in the right inguinal mammary fat pad. 48hrs after implantation, animals were commenced on a daily low dose [30mg/kg or 120mg/kg Aspirin] aspirin or vehicle regimen, until tumours were approximately 400mm\textsuperscript{3}. Aspirin therapy was halted and primary tumours resected. Post-resection metastatic spread was monitored weekly by bioluminescence imaging [IVIS Spectrum]. Animals were sacrificed 30 days post-resection. Tissues were fixed in formalin and analysed by immunohistochemistry to assess VEGF A, C, D, COX-2, PGE2 and CD34 expression. Initial results indicate a significant delay in tumour growth following treatment with 120mg/kg aspirin, as well as reduced tumour invasiveness at time of resection. Imaging studies to assess impact of aspirin pre-treatment on metastatic spread are currently ongoing (November 2014), as is the immunohistochemical analysis of lymphangiogenic factors to identify their putative role in aspirin’s mechanism of action.

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AN INTEGRATED APPROACH TO STUDY MICRO-RNA INVOLVEMENT IN ANTI-ENDOCRINE RESISTANCE IN BREAST CANCER

L Mulrane¹, M Terrile¹, K Connor¹, K Bryan², RL Stallings², R Clarke³, JP Crown⁴, WM Gallagher¹, DP O'Connor¹

¹School of Biomolecular and Biomedical Science, University College Dublin, Dublin
²Department of Molecular & Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin
³Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University, Washington
⁴Molecular Therapeutics for Cancer Ireland, Dublin City University, Dublin

Resistance to endocrine-directed therapy represents a significant problem in the management of breast cancer, with a substantial number of estrogen receptor (ER)-positive patients experiencing relapse post treatment. To further investigate the role of miRNAs in this phenomenon, TaqMan Human MicroRNA Arrays (Applied Biosystems) were used to profile global expression of 667 miRNAs from an isogenic cell line series derived from MCF7 cells (consisting of parental MCF7 cells, estrogen-independent anti-estrogen sensitive LCC1 cells and estrogen-independent anti-estrogen resistant LCC9 cells). Using an in-house developed Java (v6.0) software application, transcriptomic data from these cell lines were integrated with miRNA expression data, together with a miRNA-mRNA target site prediction database (compiled from TargetScan), to highlight networks of related genes/miRNAs. Gene Ontology (GO) categories were retrieved from DAVID. Hierarchical clustering produced two distinct clusters of genes which were negatively correlated with two groups of miRNAs differentially expressed >2 fold between the three cell lines. GO analysis revealed that Cluster 1 (2,146 genes negatively correlated with 37 miRNAs) was significantly associated with protein catabolism, chromatin modification and changes in Wnt, TGF-beta and insulin signalling. Cluster 2 (1,289 genes negatively correlated with 7 miRNAs) was associated with neuronal differentiation and cell motion, as well as changes to the ErbB and mTOR signalling pathways. Alterations to the mTOR pathway were validated in vitro and the cell line series was shown to display differential sensitivity to PI3K inhibitors GDC0941 and GDC0032, as well as dual PI3K/mTOR inhibitor BEZ235 (p<0.05). Furthermore, treatment of LCC9 cells with GDC0941 or BEZ235 partially reversed resistance to the anti-estrogen fulvestrant (p<0.05). Four miRNAs, three associated with Cluster 1 genes (miR-20b, -28-5p and -28-3p) and one associated with Cluster 2 genes (miR-31), were selected for further analysis owing to their increased expression in anti-endocrine resistant LCC9 cells. Ectopic expression of miR-31, but not miR-20b or -28, conferred resistance to tamoxifen treatment in both MCF7 and LCC1 cells (p<0.05). These data suggest that expression of miR-31 in ER+ breast cancer leads to poor response to anti-endocrine drugs and may represent a biomarker for addition of PI3K/mTOR targeted drugs to treatment regimens.
The Vitamin D Receptor: A Therapeutic Target for the Treatment of Breast Cancer?

AM Murray, NC Synnott, J Crown, N O'Donovan, MJ Duffy

1Breast Cancer Research Group, St Vincent's University Hospital, Dublin
2UCD School of Medicine and Medical Science, University College Dublin, Dublin
3National Institute for Cellular Biotechnology, Dublin City University, Dublin

Background: Recent evidence suggests that vitamin D plays a role in cancer cell growth with studies indicating that a deficiency can lead to a higher disease risk and a poorer outcome. It is the activation of the vitamin D receptor (VDR) by the active form of vitamin D, i.e., calcitriol that leads to the regulation of the anti-cancer genes. Therefore, we propose that VDR could be targeted as a potential therapeutic in the treatment of breast cancer.

Methods: The effects of calcitriol on breast cancer (BC) cell growth were investigated in a panel of 16 BC cell lines (TN = 7, Her2+ = 5, Luminal = 4). Cytotoxicity was determined using the MTT assay, while cell proliferation was determined using colony formation assays (CFA). VDR expression was measured using ELISA (USCN Life Science Inc.).

Results: IC50 concentrations across the panel of 16 cell lines ranged from 0.12 µM to >100 µM, using the MTT assay. These IC50 values were validated by CFA with a significant correlation between the 2 assays (p = 0.0046, r = 0.898). Sensitivity to calcitriol was observed to be higher in non-triple negative compared to triple negative cell lines (p= 0.0412). Levels of VDR expression in the cell line panel varied from undetectable to 10 pg/mg. A significant correlation was found between the expression of VDR and both calcitriol-induced growth inhibition (p = 0.0420, r = 0.5133) and IC50 value (p = 0.0076, r = -0.6401). Calcitriol when combined with doxorubicin or tamoxifen further enhance growth inhibition.

Conclusion: The preclinical results suggest that calcitriol can inhibit breast cancer cell line growth. VDR is therefore a potential target for the treatment of breast cancer.

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IMPACT OF PROTOCOLS EMPLOYED ON THE CLINICAL POTENTIAL OF MICRORNAS AS BIOMARKERS OF BREAST CANCER

KPOB O'Brien, DJ Joyce, ER Ramphul, MJK Kerin, RMD Dwyer
1Discipline of Surgery, School of Medicine, National University of Ireland, Galway, Galway

Introduction: MicroRNAs are short non-coding strands of RNA with fantastic potential as circulating biomarkers of breast cancer. However, variations are seen throughout this field in starting material (whole blood(WB), serum and plasma) methods of extraction, and endogenous controls(ECs) employed, all of which result in publication of contradictory results. The tremendous potential of these molecules as circulating biomarkers is thus not being realised. This study aims to investigate the variations in approaches used, and to determine their true impact on study outcome.

Methods: Initially, a literature review was performed focusing on studies analysing circulating microRNAs in breast cancer patients compared to healthy controls. This aimed to identify variations in approaches to analysis. WB, serum and plasma samples from the same individuals were then collected(n=90). WB was collected in either (1) PAXgene tubes with subsequent PreAnalytix kit(Qiagen) RNA extraction or (2) EDTA tubes with subsequent Trizol BD RNA extraction. Plasma and serum samples were collected in EDTA or serum-separating tubes respectively, followed by centrifugation and subsequent microRNA extraction using the miRCURY kit(Exiqon).

Results: In the 47 studies carried out on circulating microRNAs in breast cancer, all three starting materials, 16 different methods of extraction, and >20 different ECs were employed. Analysis of the same targets by different groups yielded conflicting results. The WB samples collected and analysed herein using PAXgene/PreAnalytix(Qiagen), were found to have relatively stable expression of the candidate EC miR-16, with a range of 14-16 Ct. In contrast, EDTA/Trizol BD processed samples showed a range of 14-30 Ct for the same microRNA target. A comparison of WB, serum and plasma originating from the same individual was performed targeting miR-16, miR-138, miR-504 and miR-379. miR-16 and miR-379 were detectable in all samples (n=90), although at varying levels depending on starting material. In contrast, miR-138 and miR-504 were detected in all WB samples, but undetectable in 32% of serum, and 38% of plasma samples.

Conclusion: The starting material and method of processing chosen has a significant impact on microRNA detection and profile of expression. Standardisation of protocols is critically important to support reliable identification of clinically relevant biomarkers.
Cetuximab belongs to the humanized, monoclonal antibody class of targeted therapies currently in use for the treatment of metastatic colorectal cancer, and head and neck cancer. Despite achieving blockbuster drug status, resistance to cetuximab limits its effectiveness in a large proportion of patients. While activating mutations in K-Ras, N-Ras, B-Raf and PI3KCA, have been shown to confer resistance to cetuximab, a significant fraction of patients with tumours expressing the wild type forms of these proteins (so-called quadruple negative tumours) still remain resistant to the drug. Therefore, the discovery of new biomarkers for cetuximab resistance has become a key mission in improving the effectiveness of colorectal cancer therapy. For this work, we have used high throughput, reverse phase protein array (RPPA) technology to quantify the relative expression of sixty key oncological proteins in a panel of 100 quadruple negative, metastatic colorectal cancer tumours from patient-derived xenografts, with known responses to cetuximab treatment. In line with other research, we show that 2% of the tumours over expressed HER2, and this over expression correlated with cetuximab resistance. We also demonstrate that resistance to cetuximab is not mediated through perturbations in the apoptotic machinery; tumours that regressed in response to treatment, and those that stabilized or progressed in response to treatment, each expressed similar levels of the key Bcl2 family proteins, effector caspases, and additional regulatory proteins in the apoptosis pathway. Instead, our findings show that resistance to cetuximab is likely achieved through a dysregulation of the Akt pathway or the MAPK cascade in some tissues, with higher expression levels of key proteins in each pathway detected in subsets of cetuximab-resistant tumours. This report therefore identifies which additional proteins could prove critical in determining patient response to cetuximab and which proteins might ultimately serve as clinically relevant biomarkers, useful for guiding the choice of treatment regimes for patients.
Mechanistic interrogation of off-target toxicities following treatment with sunitinib and sorafenib

AC O’Farrell¹, I Miller¹, R Evans¹, M Cary², D Murray¹,³, A Maratha³,⁴, G Mallya Udupi³,⁴, M Alamanou³, L Shiels¹, WM Gallagher³,⁴, AT Byrne¹

¹Physiology & Medical Physics, Royal College of Surgeons in Ireland, Dublin
²Pathology Experts GmBH, Basel, Switzerland
³OncoMark Ltd., Belfield, Dublin
⁴School of Biomolecular and Biomedical Science, University College Dublin, Conway Institute, Dublin

The tyrosine kinase inhibitors (TKIs) sunitinib and sorafenib are multi-targeted agents approved for treatment of a number of cancers[1]. However, since approval, data has continued to emerge relating to side effect profiles; including hypertension, hand-foot syndrome, fatigue, diarrhoea, mucositis, proteinuria and (rarely) congestive heart failure[2]. Underlying mechanisms are unresolved. It has been hypothesised that the observed multi-parameter toxicity profile is related to 'on-target' kinase inhibition in 'off-target' tissues[3]. To interrogate off-target effects, a reverse protein array (RPA) approach was used in tissues from drug treated animals. Mice were treated with sunitinib (40 mg/kg) or sorafenib (60 mg/kg) for 4 weeks, following which critical organs were removed and snap frozen. Additional tissue was collected for histological analysis. The Zeptosens RPA platform was used for protein expression analysis as previously described[4]. Differentially expressed proteins associated with damage and/or stress were found in the majority of organs. Of note, proteins differentially expressed in the heart following sunitinib treatment have previously shown association with myocardial hypertrophy, ischaemia/reperfusion and hypoxia. However, hypertrophy was not evidenced on histology, and microvessel density/HIF-1a levels were not significantly affected. Increased presence of lipid droplets was seen via electron microscopy in the hearts of sunitinib treated animals which has been shown to correlate with cardiac dysfunction. In skin, proteins associated with cutaneous inflammation, keratinocyte hyper-proliferation, and increased inflammatory response were differentially expressed, but histo-pathological evaluation revealed no changes. Nevertheless, animals treated with a higher dose of sunitinib showed some overt signs of skin toxicity. Serum levels of triiodothyronine and thyroxine (markers of fatigue) were unchanged. Mild proteinuria was observed in sunitinib treated animals; however, no changes in renal glomerular structure were visible via electron microscopy. Further analysis and validation of protein targets is ongoing. Implementation of a combined histopathological/RPA approach provides a sensitive method to mechanistically elucidate the off-target toxicity sequelae associated with TKIs approved in the oncology setting.

ELUCIDATING THE LINK BETWEEN METABOLISM, HYPOXIA, INFLAMMATION, P53 AND OBESITY IN BARRETT’S OESOPHAGUS

JJ Phelan¹, R Feighery¹, OS Eldin³, F MacCarthy², D O’Toole¹, JV Reynolds¹, JN O’Sullivan¹
¹Dept. Surgery, Trinity College Dublin, Dublin
²Dept. Clinical Medicine, Trinity College Dublin, Dublin
³Dept. Histopathology, St. James's Hospital, Dublin

Introduction: Recent research in our lab has demonstrated that both oxidative phosphorylation and glycolysis are reprogrammed early in the Barrett’s disease sequence and act mutually to promote disease progression in Barrett's oesophagus (BO) [1]. However, the link between energy metabolism and other central molecular processes involved in disease progression in BO have not yet been ascertained. Therefore, the aim of this study was to examine the relationship between metabolism, hypoxia, inflammation, p53 and obesity in in-vitro, in-vivo and ex-vivo BO models.

Methods: Barrett's (QH) and adenocarcinoma (OE33) cell lines were examined for hypoxia-induced (0.5% O2) genomic alteration in ATP5B, GAPDH, HSP60, PKM2, VEGF, IL1β and p53 expression using qPCR. Tissue microarrays consisting of in-vivo BO patient tissue (n=29) were screened for ATP5B, GAPDH, IL1β, SERPINA3, p53 and HIF1α protein markers. The expression of both metabolic protein markers was subsequently correlated with HIF1α, IL1β, SERPINA3 and p53 status. The link between hypoxia, inflammation and p53 in BO was additionally examined at the protein level. Moreover, levels of metabolism were correlated to obesity status (n=15). Barrett’s ex-vivo explant tissue (n=9) was cultured to generate tissue conditioned media, inflammatory and angiogenic cytokine levels were assessed by multiplex ELISAs and metabolism was assessed using qPCR.

Results: QH and OE33 cell lines exhibited significant hypoxia-induced alterations in ATP5B (P<0.01), PKM2 (P<0.05), GAPDH (P<0.01), VEGF (P<0.01) and IL1β (P<0.05) gene expression. In-vivo, ATP5B (r=0.71, P<0.0001) and p53 (r=0.455, P=0.015) strongly positively correlated with epithelial HIF1α expression. Levels of ATP5B (r=0.53, P=0.0031) and GAPDH (r=-0.46, P=0.012) positively correlated with p53 expression. Levels of epithelial ATP5B strongly positively correlated with SERPINA3 (r=0.6644, P=0.0001) and IL1β (r=0.8, P<0.0001). Additionally, glycolysis positively correlated with levels of epithelial IL1β (r=0.44, P=0.0167). Interestingly, obesity was negatively associated with oxidative phosphorylation (r=-0.6016, P=0.0177) but positively associated with glycolysis (r=0.743, P=0.0015). Comparable correlations were exhibited in ex-vivo explant tissue between metabolism (ATP5B, GAPDH), p53, hypoxia (HIF1α, VEGF) and secreted inflammatory (IL6, IL1β, TNFα) and angiogenic (bFGF, ANG1) markers (P<0.0001).

Conclusion: We have shown that energy metabolism is significantly associated with hypoxia, inflammation, p53 and obesity in BO. Identifying and exploring the underlying molecular mechanisms that link metabolism to these key cellular processes would significantly aid in understanding how these processes interact and may provide some insight into the development of targeted therapies influencing these processes.

Despite initial response to androgen ablation, patients with advanced disease relapse to develop castrate-resistant prostate cancer (CRPC) which is difficult to treat. We identified Serum Response Factor (SRF) as an important transcription factor in an in vitro model of CRPC. Since CRPC is associated with androgen receptor (AR) hypersensitivity, we investigated the relationship between SRF and AR in vitro and in vivo using tissue microarrays (TMA) which recapitulate prostate cancer progression.

To investigate AR involvement in SRF response to androgen stimulation, AR expression was down-regulated using siRNA. This resulted in the abrogation of SRF induction post-DHT. In addition, DHT stimulation failed to induce SRF transcriptional activity in AR-negative PC346-DCC cells, which was only restored following AR over-expression. Next, SRF expression was down-regulated by siRNA, resulting in AR increased transcriptional activity in castrate-resistant LNCaP-Abl cells but not in the parental LNCaP. This negative feedback loop in the resistant cells was confirmed by immunohistochemistry which showed a negative correlation between AR and SRF expression in CRPC metastases (n=42, Pearson correlation= -0.208, p=0.01) and a positive correlation in hormone-naïve prostatectomies (n=340, Pearson correlation=0.362, p<0.001). These results indicate a switch from a positive to a negative correlation between SRF and AR during prostate cancer progression. SRF association with biochemical recurrence (BCR) was also assessed using the hormone-naïve prostatectomies TMA (n=340). Kaplan-Meier analysis showed an association of SRF with BCR, with patients with higher SRF levels recurring earlier than patients with low SRF levels (Log Rank test, p=0.006). These results are currently being validated in an independent TMA of hormone-naïve prostatectomies (n=140).

We next assessed cell proliferation following SRF inhibition and demonstrated that SRF inhibition by siRNA or the small molecule inhibitor CCG-1423 was as effective as AR inhibition (siRNA or Enzalutamide) in decreasing cell proliferation. Moreover concomitant inhibition of AR and SRF (Enzalutamide+CCG-1423) showed a synergistic effect in decreasing proliferation.

In conclusion, our in vitro and in vivo data have demonstrated a key role for SRF in prostate cancer progression, supporting SRF as a promising therapeutic target alone or in combination with current treatments.
Identification of Heat Shock Factor 1 (HSF-1) as a potential therapeutic target for castration-resistant prostate cancer

S Lynch¹, A Fabre², K Murphy¹, K Klocker⁴, WM Gallagher⁵,⁶, RW Watson¹, M Prencipe¹
¹UCD School of Medicine and Medical Science, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, ²Department of Pathology, St Vincent’s University Hospital, Dublin, ³UCD School of Mathematical Sciences and Insight Centre for Data Analytics, Dublin
⁴Department of Urology, Innsbruck Medical University, Anichstraße 35, Innsbruck, Austria
⁵UCD School of Biomolecular and Biomedical Science, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, ⁶OncoMark Limited, NovaUCD, Belfield Innovation Park, Belfield, D4, Dublin

Despite initial response to androgen ablation, patients with advanced disease relapse to develop castrate-resistant prostate cancer (CRPC) which is difficult to treat. We previously identified Heat Shock Factor 1 (HSF-1) as an important transcription factor in an in vitro model of CRPC. HSF-1 has been implicated in affecting cellular migration in mouse embryonic fibroblasts. It has also been shown to affect the progression of aneuploidy in PC-3 prostate cancer cells. Both these functions are necessary for cancer progression, suggesting a role for HSF-1 in driving castration-resistant disease.

To confirm HSF-1 relevance in clinical samples, we performed IHC staining for HSF-1 on 31 hormone-naïve primary tumours and 20 tumours resected from men with CRPC. HSF-1 staining was localised to the nucleus of the epithelial cells of the prostate. Higher nuclear expression was detected in CR-tumours compared with local tumours, although this difference did not reach statistical significance (p= 0.086) probably due to the limited sample size.

We next assessed cell viability following HSF-1 silencing with siRNA, showing a modest decrease in cell viability in the CR-subline LNCaP Abl (13% decrease, p=0.026). Experiments analysing cellular migration and invasion following HSF-1 silencing are currently ongoing in our laboratory.

Our preliminary results suggest an active role for HSF-1 in key cellular processes leading to metastases and development of CRPC such as cell survival, cell migration and invasion. Further research will assess the potential use of HSF-1 as a therapeutic target for CRPC.
Pancreatic cancer survival rate is poor, with a 5 year survival rate of 7% in the Republic of Ireland. To date, cell-line models have proven to be insufficient as a guide to providing rational treatment regimes or predicting response. In collaboration with St Vincent’s University Hospital, Dublin, our group initiated the first Irish national patient-derived xenograft models of pancreatic cancer. By implanting freshly dissected, pathologically confirmed, pancreatic tumour material into SCID mice we developed xenograft models for investigating of pancreatic tumour development, progression and drug response. Herein we describe our experience after an 18 month pilot programme. Tumour material from candidate patients is resected with curative intent. After initial macroscopic pathological confirmation, material remaining after diagnostic sampling is cold transferred with a mean transfer time from surgery to implantation of 80 minutes. The material is dissected and implanted sub-cutaneous into CB17/Icr-Prkdcscid mice. To-date, 20 pancreatic patient samples have been implanted. Palpable tumours have developed in 14 in the F1 generation. Of the remaining 6; one was material implanted from an intraductal papillary mucinous neoplasm which did not produce a tumour and 5 implantations are too early to detect at time of writing. Nine derived tumours have been transplanted from F1 to F2 generation, 8 cases have a confirmed histopathology of pancreatic adenocarcinoma similar to the patient of origin. One tumour was confirmed to be a lymphoma, which was subsequently defined as human in origin. The remaining 5 PDX tumours, which have formed, have not yet been passaged and confirmed as similar to the patient origin. Our pilot study confirms the feasibility of our programme and our ability to generate and passage viable pancreatic PDX material, with a high success rate, that retains cellular characteristics similar to the tissue of origin. Based on this success we are now expanding the project, with the intent to include additional patient cohorts such as neo-adjuvant treatment or pre-operative radiation.
PANEL OF GENE EXPRESSION BIOMARKERS TO PREDICT RESPONSE TO LAPATINIB

S Roche\textsuperscript{1}, T Santra\textsuperscript{2}, W Kolch\textsuperscript{2}, R O’Connor\textsuperscript{1,3}

\textsuperscript{1}National Institute for Cellular Biotechnology, Dublin City University, Dublin 9
\textsuperscript{2}Systems Biology Ireland, University College Dublin, Dublin 4
\textsuperscript{3}School of Nursing and Human Sciences, Dublin City University, Dublin 9

A microarray dataset, generated by GSK, available within our group and now publicly available was analysed to derive potential gene expression biomarkers of lapatinib response. The dataset used originates from gene expression profiling experiment on four cell lines (MDA-MB-468, SKBR-3, T-47D and BT-474) using the U133A Affymetrix human 22,000-element microarray. The cell lines (two sensitive and two insensitive) were treated with lapatinib (0.1 µM and 1µM) for 6, 12 and 24hrs.

The key question was to identify genes which responded to Lapatinib in BT474, SKBR3 cell lines but do not respond to Lapatinib in MDA468, T47D. We compared the list generated to one previously compiled to identify genes of strong biological significance. The hypothesis being that any gene that was of interest when analysed by two distinct bioinformatic methods would be more likely to have a functional role in predicting response to lapatinib.

Twenty seven candidate genes identified to be validated. Initial validation of the gene target list was achieved on pre-seeded Taqman PCR plates that were specially selected to express the probe of greatest interest for each target genes. Two genes from the original 27 were particularly interesting a) Vitamin D Receptor (VDR) and b) Suppressor of cytokine signalling 2 (SOCS2). Recent population studies have suggested implications of VDR in Breast Cancer, with high serum levels of vitamin D being associated with decreased breast cancer risk. The Janus kinase (JAK) signal transducer and activator of transcription (STAT) signalling pathway was first discovered in a study of interferon signalling. SOCS2 mediates a negative feedback loop in the JAK-STAT pathway.

Recent publication has implied cross-talk between VDR and the JAK/STAT pathway. As both of these genes appeared altered in our study, biological validation and interrogation of this cross-talk in breast cancer is required.
The CDC7/CDK9 inhibitor PHA-767491 sensitizes AML cells to the BH3-mimetic ABT-737 by blocking microenvironment-driven Mcl-1 expression

A McCormick¹, D Baev¹, R Morrell¹, A Samali¹, C Santocanale², M O'Dwyer¹,³, E Szegezdi¹

¹Apoptosis Research Centre, National University of Ireland, Galway, Galway
²Centre for Chromosome Biology, National University of Ireland, Galway, Galway
³Haematology, University Hospital Galway, Galway

The aim of this study was to examine the role of the tumour microenvironment in AML treatment response. The bone marrow microenvironment consists of a network of growth factors, cytokines and stromal cells, creating a permissive environment for disease progression. Using the bone marrow-derived cell line HS5 we examined the effects of the bone marrow stroma had on drug responsiveness in established AML tumour cell lines and primary patient AML cells. Leukemic cells express high levels of anti-apoptotic Bcl2 proteins and recent studies with the BH3-mimetic Bcl-2+Bcl-XL inhibitor ABT-737 and the selective Bcl-2 inhibitor ABT-199 showed dependence on these proteins for survival.

We show that co-culture of AML cell lines with stromal cells induced the expression of the anti-apoptotic proteins Bcl-2, Bcl-xL as well as Mcl-1 and resulted in marked resistance to cytarabine in AML cell lines and primary AML. Despite previous indications of Bcl-2 upregulation as the causative, combination treatments with ABT-737 and cytarabine was unable to overcome the stromal-mediated resistance, suggesting that Mcl-1 may play an important role. Using a combination strategy we found that ABT-737 potently synergizes with the Cdc7/Cdk9 inhibitor PHA-767491 via transcriptional inhibition of Mcl-1. This combination also proved effective in primary AML, able to equally target the leukemic blast cells and the CD34+/CD38- leukemic stem cell (LSC) enriched population. These results together provide a rationale for combined targeting of the anti-apoptotic Bcl-2 family members, with emphasis on Mcl-1 in the treatment of AML. Overall this approach proved to be highly efficient and allowed for the removal of the LSC population, a major causative of disease relapse and remission.
A URINE DNA METHYLATION BIOMARKER PANEL FOR NON-INVASIVE DETECTION OF HIGH-RISK PROSTATE CANCER

AV Tuzova¹, L De Barra¹, S O'Meachair², J Clark³, C Cooper³, R Power⁴, K O'Malley⁵, RP Manecksha⁶, T Lynch⁶, AS Perry¹

¹Prostate Molecular Oncology, Institute of Molecular Medicine, Trinity College Dublin, Dublin
²Dublin Centre for Clinical Research and Centre for Health Decision Science, Trinity College Dublin, Dublin
³School of Biological Sciences, University of East Anglia, Norwich, ⁴Department of Urology, Beaumont Hospital, Dublin, ⁵Department of Urology, Mater Misericordiae Hospital, Dublin
⁶Department of Urology, St. James’s Hospital, Dublin

Introduction: Opportunistic PSA testing has increased prostate cancer (PCa) incidence, resulting in significant over-treatment of clinically indolent disease. The poor tumour-specificity of PSA causes unnecessary invasive biopsies, associated with substantial economic burden and trauma and anxiety to patients. Conversely, sampling bias means that needle-biopsies cannot conclusively rule-out the presence of PCa or the co-existence of more aggressive lesions. The aim of this study is to develop a liquid-biopsy DNA methylation biomarker panel to alleviate the problems surrounding early detection of PCa and improve non-invasive early detection of aggressive disease.

Methods: Post-DRE first catch urines from 156 men undergoing TRUS-biopsy were used for DNA isolation and methylation analysis by quantitative methylation specific PCR of six loci. Sensitivity and specificity of the methylation panel and serum PSA were determined using logistic regression. All analyses were done blinded from TRUS-biopsy findings.

Results: The patient cohort consisted of 48 biopsy-negative and 108 biopsy-positive (14 low-risk, 58 intermediate-risk and 36 high-risk) men. DNA methylation analysis revealed significantly higher frequencies and quantitatively higher levels in biopsy-positive men; most exemplified in the high-risk group. Urinary methylation discriminated between biopsy-positive and -negative men with an AUC of 0.86, compared with serum PSA, AUC of 0.56. Focusing specifically on high-risk PCa, the methylation panel gave an AUC of 0.87, compared with PSA, AUC of 0.54. Finally, combining DNA methylation of the 6-gene panel with PSA for non-invasive detection of high-risk PCa, produced an AUC of 0.96. Conclusion Early indications suggest that urinary profiling of DNA hypermethylation can selectively detect high-risk PCa, with improved specificity over PSA. This liquid biopsy may have utility in reducing unnecessary TRUS-biopsies and serially monitoring disease progression in an active surveillance setting.
GLYCOLYTIC PATHWAY INHIBITORS ARE POTENTIAL THERAPEUTIC TARGETS IN OBESITY-DRIVEN OESOPHAGEAL CANCER
Z Useckaite^1, C O’Malley^1, E Allott^1, H Sheridan^2, JJ Phelan^1, JV Reynolds^1, GP Pidgeon^1
^1Surgery, Trinity College Dublin, Dublin
^2School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin

Background: Oesophageal adenocarcinoma (OAC) is the fastest growing cancer in the developing world. Visceral obesity, and in particular factors secreted from visceral adipose tissue, may alter tumour glycolytic pathways resulting in a more aggressive phenotype. Strong preliminary data indicates glycolysis to be the most altered pathway in oesophageal cancer cells following culture with adipose explants. The aim: To delineate how excess adiposity affects tumour metabolism and investigate inhibitors of the glycolytic pathway as anti-cancer agents in obesity driven oesophageal cancer.

Methods: OE33, oesophageal adenocarcinoma cells were co-cultured with Adipose Conditioned Media (ACM) from obese (VFA>163.8cm^2 in males, VFA>80.1 cm^2 in females) (n=10) or non-obese (VFA<163.8cm^2 in males, VFA<80.1 cm^2 in females) patients, RNA isolated. A panel of genes and glucose transporters were assessed with quantitative polymerase chain reaction (qPCR). Seahorse XF analyzer was used to measure the effect of small molecule inhibitors on glycolytic activity of oesophageal cancer cells. The effect of small molecule inhibitors on ECAR and OCR rate investigated in both ACM co-cultured and media alone treated OE33 cells. Expression of the glycolytic enzymes was confirmed by Immunohistochemistry (IHC) in tumours of oesophageal cancer patients.

Results: qPCR analysis demonstrated a significant upregulation of PKM2, HK2, Glut1, Glut4 and ALDOC in OE33 cells co-cultured with ACM from obese patients and fat explants, compared to untreated controls. Treatment of the cells with the glycolytic pathway inhibitors, bromopyruvic acid (HK2 inhibitor) and sodium oxamate (PKM2 inhibitor), decreased ACM-induced cell proliferation. This effect was more pronounced under hypoxic, suggesting potential anti-glycolytic activity of the compounds. Glycolysis was highly upregulated following treatment of cells with ACM (24hr) from obese OAD patients. Small molecule inhibitor synergy with BPTES further reduce ECAR rate suggesting that glutaminase 1 is stimulating energy production in oesophageal cancer cells.

Conclusion: Secreted factors from adipose tissue from obese individuals activate glycolytic pathways in oesophageal cancer cells. Novel small molecule inhibitors decrease cancer cell glycolytic activity and the effect is further reduced when they are combined with BPTES. Glutaminase therefore is a promising therapeutic target for the prevention of obesity driven cancer and may starve the cancer cells of their energy source.
Strategies to Specifically Target Tumour Angiogenesis - Lessons to Be Learnt From Defibrotide

G Walsh¹, S Curley¹, A Costello¹, E Cahil¹, L Elliott², E Ryan², K Sheehan², G Doherty², W Sievert³, G Multhoff³, W Kolch¹, G Eissner¹

¹Systems Biology Ireland, University College Dublin, Dublin
²St. Vincent’s University Hospital, University College Dublin, Dublin
³Clinical Cooperation Group, Technical University of Munich, Munich

Overview - Personalised anti-angiogenic therapies require target specificity for endothelial cells residing in the tumour microenvironment. One promising drug candidate is Defibrotide. Defibrotide, a poly-disperse mixture of stable oligonucleotides, has demonstrated anti-angiogenic properties in vitro and in vivo. It has originally been described as an endothelial protective agent. Defibrotide has been approved by the EMA for the treatment of severe veno-occlusive disease post allogenic haematopoietic stem cell transplantation. We are interested in uncovering the anti-neoplastic properties of Defibrotide, specifically the impact on tumour-associated endothelial cells.

Methodology – To test this hypothesis we are isolating ECs from biopsies of human colorectal tumours and adjacent healthy stromal tissue from the same patient. We are applying a novel CD31 Immuno-magnetic bead separation technique, as published by W Sievert et al. The innovative step involves high affinity streptavidin-biotin competitive release of the CD31 bound magnetic beads from the cells following isolation. This enables the collection of CD31 positive cells without membrane-bound beads, a step necessary for the long term culturing of viable primary ECs. In addition we present recent data demonstrating the effect Defibrotide on reducing tube formation and migration of human microvascular endothelial cells (HMECs), when incubated with Pancreatic Cancer Conditioned medium (PCM), and primary Colorectal Cancer medium (CRCM).

Results - To characterise and compare isolated tumour and healthy ECs we are applying an 8-colour flow cytometry marker panel including CD31, CD45, CD102, CD105, CD54, CD34 and CD144. ECs are identified as CD31+, CD45- cells. Preliminary data show Tumour ECs have up regulated CD102 (ICAM-2) and CD105 (Endoglin) compared to normal ECs. Interestingly, this change in expression was exclusively attributable to CD34+ cells within the tumour EC population. CD34 is a haematopoietic marker largely known to exist on progenitor and stem cell populations. HMECs stimulated with PCM and CRCM showed reduced tube formation, migration and chemotaxis in the presence of Defibrotide.

Future Prospects – Successful isolation of tumour endothelial cells, and the optimisation of culture conditions for functional analysis, will enable further pathologically relevant investigations into the anti-angiogenic properties of Defibrotide.

Resistance to chemotherapy in ovarian cancer is mirrored by the microRNA miR-433-dependent dysregulation of the cell cycle

K Weiner-Gorzel¹, E Dempsey², M Milewska³, A McGoldrick¹, V Toh¹, A Walsh¹, S Lindsay¹, L Gubbins¹, A Cannon⁴, D Sharpe⁵, J O'Sullivan⁴, M Murphy¹, SF Madden⁶, M Keil⁷, A McCann¹, F Furlong⁵

¹UCD School of Medicine and Medical Science (SMMS), University College Dublin, Dublin
²UCD School of Biomolecular and Biomedical Science, University College Dublin, Dublin
³Systems Biology Ireland, University College Dublin, Dublin
⁴Molecular Department of Surgery, Trinity Centre for Health Sciences, St James's Hospital, Dublin
⁵School of Pharmacy, Queen's University of Belfast, Belfast
⁶Molecular Therapeutics for Cancer Ireland, Dublin City University, Glasnevin, Dublin
⁷Department of Surgery, Mater Misericordiae University Hospital, Dublin

An understanding of the mechanisms underlying the development of resistance to chemotherapy treatment is a gateway to the introduction of novel therapies and improved outcomes for women presenting with ovarian cancer (OC). The desired apoptotic death post-chemotherapy depends on an intact and fully functioning cell cycle machinery. In this study we demonstrate that stable expression of miR-433 renders OC cells more resistant to paclitaxel treatment. Interestingly, only cells with the highest miR-433 survived paclitaxel suggesting the possible role of miR-433 in cancer recurrence. Importantly, for the first time we demonstrate that miR-433 induces cellular senescence, exemplified by a flattened morphology, the downregulation of phosphorylated Retinoblastoma (p-Rb) and increased B-galactosidase activity. Surprisingly, miR-433 induced senescence was independent of two well recognised senescent drivers: p21 and p16. Further in silico analysis followed by in vitro experiments identified CKD6 as a novel miR-433 target gene possibly explaining the observed p21 and p16-independent induction of cellular senescence. Another in silico identified miR-433 target gene was CDC27, a protein involved in the regulation of the cell cycle during mitosis. We demonstrate that the overexpression of pre-miR-433 leads to the downregulation of CDC27 in vitro revealing a novel interaction between miR-433 and CDC27, an integral cell cycle regulating protein. Interestingly, miR-433 expressing cells also demonstrated an ability to impact their tumour microenvironment. We show that miR-433 is present in exosomes released from miR-433 overexpressing and high miR-433 naïve cells. Moreover, growth condition media (GCM) harvested from cells with high miR-433 have higher levels of IL-6 and IL-8, two key cytokines involved in the senescence associated secretory phenotype (SASP). Importantly, GCM from miR-433-enriched cells repressed the growth of co-cultured cells with initial studies showing a GCM-dependent induction of chemoresistance. In conclusion, data in this study highlights how the aberrant expression miR-433 contributes to chemoresistance in OC cells. We postulate that standard chemotherapy, particularly paclitaxel, used to treat women with OC may have an attenuated ability to kill cells harbouring increased levels of miR-433, allowing for a subsequent chemoresistant phenotype post-therapy.
MODULATION OF COLORECTAL CANCER TUMOURIGENESIS BY KHSRP THROUGH THE TUMOUR MICROENVIRONMENT

F Caiazza$^{1,2}$, R Power$^2$, L Elliott$^{1,2}$, M Tosetto$^1$, B Nolan$^1$, JN O'Sullivan$^3$, GA Doherty$^{1,2}$, EJ Ryan$^{1,2}$

$^1$Center for Colorectal Disease, Saint Vincent's University Hospital, Dublin
$^2$School of Medicine and Medical Science, UCD, Dublin
$^3$Institute of Molecular Medicine, Saint James's Hospital, TCD, Dublin

Inflammation plays a key role in the development of Colorectal Cancer (CRC). Patients with Inflammatory Bowel Disease (IBD) have an increased lifetime risk of developing CRC. The mechanisms by which the pro-tumorigenic inflammatory microenvironment is established are yet to be fully elucidated. The K-homology splicing regulatory protein (KHSRP) is a multifunctional RNA-binding protein which regulates a variety of cellular processes including transcription, miRNA maturation and mRNA trafficking, localisation and degradation. KHSRP has been implicated in different functions associated with cancer cell biology, such as inflammation, lipid metabolism, cell-fate determination, and response to DNA damage. Importantly, KHSRP regulates IL-6 and IL-8 expression, and mining of online CRC databases provided preliminary evidence for a potential role in CRC. Our aim was to determine if KHSRP plays a role in establishing the inflammatory environment of CRC. Expression of KHSRP was increased 2-fold in the inflamed tissue of IBD patients compared to normal controls. Analysis of a Tissue MicroArray of IBD patients who progressed to CRC showed that KHSRP was expressed in both the epithelial and stromal compartment, however dysplastic tissue had increased expression of KHSRP specifically in the stroma. Furthermore, tumour biopsies from patients with metastatic CRC had elevated KHSRP expression compared to matched adjacent normal tissue. Silencing of KHSRP in two CRC cell line models provided evidence that KHSRP is involved in modulating production of both pro-tumorigenic and anti-inflammatory cytokines, as well as regulating cell proliferation. Our preliminary data suggests a role for KHSRP in supporting a tumor-promoting microenvironment in CRC.
Targeting the chemokine pathways that guide T cell migration to the liver and visceral adipose tissue of obesity-associated cancer patients.

MJ Conroy, KC Galvin, R Fahey, K O'Sullivan, AM Mongan, A Cannon, M Kavanagh, G Moore, C O'Farrelly, J Geoghegan, JV Reynolds, J Lysaght

1Department of Surgery, Trinity Centre for Health Sciences, St. James's Hospital and TCD, Dublin 8
2School of Biochemistry and Immunology, Trinity Biomedical Science Institute, TCD, Dublin 2
3Liver Transplant Unit, St. Vincent's University Hospital, Dublin 4

Introduction: Obesity is a global health problem affecting over 500 million adults and 40 million children and is a serious risk factor for diseases such as cancer and liver disease. Oesophageal adenocarcinoma (OAC) is increasing rapidly in Ireland with a 5-year survival rate of approximately 15% and a strong association with obesity. We have previously shown that the omentum, part of visceral adipose tissue (VAT), is a rich source of activated pro-inflammatory T cells in OAC. More recently, we have shown that there are significant proportions of inflammatory T cells in the liver of these patients. We have identified MIP-1α, MIP-1β and IP-10 as key drivers of T cell trafficking to omentum and liver in OAC. We propose that blocking these pathways using chemokine receptor antagonists may reduce inflammatory T cell trafficking to these tissues and ultimately serve to reduce pathological inflammation in obesity.

Methods: The chemokine receptor expression profiles of T cells were examined in blood, liver and omentum of OAC patients, along with the levels of a panel of secreted chemokines using flow cytometry and MSD multiplex ELISAs. Pre-treatment of T cells with chemokine receptor antagonists was performed prior to chemotaxis assays using an in vitro transwell system, tissue conditioned media and recombinant chemokines.

Results: Both the chemokine levels and chemokine receptor expression profiles of T cells in the omentum and liver suggest that MIP-1α, MIP-1β and IP-10 are key drivers of T cell trafficking to these tissues in OAC. In addition, OAC-derived T cells preferentially migrate to the adipose and liver tissue conditioned media of obese OAC patients and this could be significantly reduced using novel antagonists against the MIP-1α receptor, CCR1.

Conclusion: MIP-1α-, MIP-1β- and IP-10-mediated recruitment of inflammatory T cells to omentum and liver contributes to obesity-associated inflammation in OAC. Blockade of the MIP-1α pathway can reduce T cell migration to omentum and liver in vitro and may be used to treat carcinogenic chronic inflammation and liver disease in obesity.
PDLIM2 IS REQUIRED FOR MACROPHAGE SUBTYPE DIFFERENTIATION WITH IMPLICATIONS FOR CYTOKINE PRODUCTION IN THE TUMOUR MICROENVIRONMENT

E Tresse¹, OT Cox¹, SJ Edmunds¹, M Coleman¹, R O'Connor¹
¹School of Biochemistry and Cell Biology, UCC, Cork

The PDZ-LIM domain protein PDLIM2 is associated with an invasive cancer phenotype[1] and integrates cytoskeleton signalling with regulation of gene expression during epithelial cell differentiation[2]. PDLIM2 is also expressed in hematopoietic cells and regulates NFkB and STAT transcription factor stability in macrophages and lymphocytes[3,4]. Macrophage infiltration may correlate with a poor prognosis in many types of human cancer. We hypothesized that PDLIM2 activity in macrophages may influence their phenotype and thereby, the cancer microenvironment. To test this, we investigated the contribution of PDLIM2 to the classically activated pro-inflammatory subtype (M1) and the alternatively activated (M2) macrophage subtypes using bone marrow derived macrophages (BMDM) from PDLIM2 WT and knockout (-/-) mice. We found that PDLIM2 translocates from the nucleus to the cytoplasm in response to M1 macrophage activation, although Nitric oxide (NO) and Nitric Oxide Synthase (iNOS) levels were similar in WT and PDLIM2-/- BMDM. However, BMDM from PDLIM2 -/- mice exhibited altered inflammatory cytokine profiles with increased IL-6 and decreased IL-1b compared to WT BMDM. Macrophage inducible factor (MIF) expression and nuclear retention of the Interferon Regulatory Factor 3/CREB-binding protein (IRF-3/CBP) complex were also enhanced in PDLIM2-/- macrophages. M2-polarized macrophages derived from PDLIM2-/- BMDM exhibited reduced migratory capacity, delayed pro-angiogenic cytokine production, and failed to suppress pro-inflammatory cytokine expression. Altogether, our results demonstrate that PDLIM2 is essential for complete M2 polarization and for suppression of the M1 phenotype. Thus, PDLIM2 expression in macrophages could impact cancer progression by controlling macrophage phenotype and cytokine production in the tumour microenvironment.[1]Gene expression profiles in cells transformed by overexpression of the IGF-I receptor. Loughran G, Huigsloot M, Kiely PA, Smith LM, Floyd S, Ayllon V, O'Connor R. Oncogene. 2005 Sep 8;24(40):6185-93. [2]PDLIM2 regulates transcription factor activity in epithelial-to-mesenchymal transition via the COP9 signalosome.

TUMOUR RESIDENT MYELOID CELLS IN COLORECTAL CANCER EXPRESS HIGH LEVELS OF THE CO STIMULATORY MOLECULES CD40 AND CD80

L Elliott1, K Sheahan1,2, G Doherty1,2, R Elizabeth1,2

1Centre for Colorectal Disease, St. Vincent's University Hospital, University City Dublin, Dublin
2Pathology Department, St. Vincent's University Hospital, Dublin
3Centre for Colorectal Disease, St. Vincent's University Hospital, St. Vincent's University Hospital, Dublin
4Centre for Colorectal Disease, St. Vincent's University Hospital, University City Dublin, Dublin

Introduction: Previous work published by our group has shown that the tumour tissue microenvironment in colorectal cancer can inhibit the maturation of monocyte derived dendritic cells. However, very little is known about the different myeloid cell subsets present within the tumour tissue in colorectal cancer along with their functional role. Understanding the roles of specific myeloid cells in tumour progression and immunity in colorectal cancer may offer significant suggestions for the efficacy of immunotherapeutic strategies that target endogenous myeloid cells in situ.

Aim: The aims of the present study were to identify and characterise myeloid cells in colorectal tumours and adjacent normal tissue. Method: First we used multi-colour flow cytometry to determine the frequency and phenotype of CD11c+ myeloid (mDC) and CD123+ plasmacytoid DCs (pDC) isolated from tumour and adjacent normal tissue from patients with colorectal cancer.

Results: Results showed a significant increase in the number of CD45+ cells in the tumour tissue (p<0.0233) when compared to normal adjacent tissue. Within the CD45+ population, we found that the tumour tissue exhibited a distinct HLA-DR+ CD11c+ CD64+ CD14+CD11b+ population of myeloid cells that was not as prevalent in the normal adjacent tissue (p<0.0001). Moreover, the recruited myeloid cells displayed a mature phenotype, determined by their increased expression for CD80 and CD40 although no difference in the levels of CD83 was observed. Interestingly, no change in the expression of several inhibitory markers, ILT-4, ILT-7, CD277, PD-L1 and HLA-G was notable on the recruited myeloid cells when compared to cells isolated from normal adjacent tissue.

Conclusion: It is most likely that a number of tumour-derived mediators are involved in the recruitment of this myeloid population to the tumour. However, additional work is required to establish the exact function of these cells and whether they are associated with immune suppression or anti-tumour immunity.
ALTERED CHEMOKINE PROFILE IN THE PROGRESSION OF OESOPHAGITIS TO OESOPHAGEAL ADENOCARCINOMA COULD REPRESENT TARGETS FOR INTERVENTION

M Kavanagh\(^1\), M Conroy\(^1\), N Gilmartin\(^1\), N Clarke\(^1\), N Ravi\(^1\), F MacCarthy\(^2\), D O’Toole\(^2\), J V Reynolds\(^1\), J O’ Sullivan\(^1\), J Lysaght\(^1\)

\(^1\)Department of Surgery, Trinity centre for health sciences, Trinity College Dublin, St James's Hospital, Dublin
\(^2\)Department of Clinical Medicine, Trinity College Dublin and St. James’s Hospital, Dublin

**Introduction:** The development of Oesophageal adenocarcinoma (OAC) provides an excellent model of an inflammatory driven neoplastic transformation, with chronic gastro oesophageal reflux disease (GORD) and Barrett's oesophagus (BO) being classified as major risk factors for OAC development. Studies have not only identified a role for T-cells in oesophageal inflammation but they have noted phenotypic switches in T-cell profile as the disease progresses, suggesting a key role in disease progression. The aim of this study was to characterise the chemokines responsible for T-cell trafficking to oesophagitis, BO and OAC tissue to identify possible immunotherapeutic targets.

**Methods:** Oesophageal biopsies were cultured to generate tissue conditioned media (TCM). Levels of chemokines in TCM and patient serum were assessed by ELISA. Chemokine receptor expression on T-cells was assessed by flow cytometry.

**Results and conclusion:** Chemokine receptor expression was examined in peripheral blood from healthy, oesophagitis, BO and OAC patients. Expression of CCR6, CXCR3, CCR5, CCR4 and CCR3 were assessed by flow cytometry. Significant alterations were identified along the disease sequence. There was a significant reduction in the proportion of T-cells expressing CXCR3 and CCR6 within OAC blood, suggesting that cells expressing these receptors may be recruited to OAC tissue. Results from the tumour microenvironment support this theory as increased levels of IP-10 and MIP3-\(\alpha\), ligands for CXCR3 and CCR6 respectively, were observed in tumour. Conversely, T-cells expressing CCR3 were significantly increased in BO and OAC blood compared to normal and oesophagitis. Levels of MIP1-\(\alpha\), MIP1-\(\beta\) and RANTES were also increased in tumour microenvironment. By characterising the chemokine/receptor profile within the tissue microenvironment, we can identify chemokines responsible for T-cell trafficking to diseased tissue and may identify possible immunotherapeutic targets to enhance protective or reduce pathological T-cells during oesophageal adenocarcinoma progression.
The Potential Role of the ST2/IL-33 axis, in Colon Cancer.
C O'Donnell\textsuperscript{1}, A Houston\textsuperscript{1,3}, E Brint\textsuperscript{2,3}

\textsuperscript{1}Department of Medicine, University College Cork, Cork
\textsuperscript{2}Department of Pathology, University College Cork, Cork
\textsuperscript{3}Alimentary Pharmabiotic Centre, University College Cork, Cork

The importance of inflammation in cancer is well established. Cancer cells have been shown to produce cytokines and chemokines that recruit immune cells and alter their activity. The recruitment and polarization of immune cells by the tumour cells has the potential to either inhibit or promote tumour growth. A cytokine potentially involved in this process is IL-33. IL-33 was recently identified as the ligand for ST2. ST2 is a member of the toll-like receptor/IL-1 receptor family. Three isoforms of ST2 exist: a trans-membrane receptor (ST2L), a secreted soluble form (sST2), and a variant form (ST2V). The IL-33/ST2 pathway has been implicated in inflammatory bowel disease, a risk factor for colon cancer with changes in expression of IL-33 and ST2 being now reported in several cancers. Aim: To investigate the role of IL-33 and ST2 in colon cancer. Results: HT29 and CT26 colon tumour cells express IL-33 and ST2, as assessed by both qRT-PCR and Western blotting. Expression of both ST2 and IL-33 increased upon stimulation with pro-inflammatory mediators (LPS, TNFa and PGE2). Stimulation with IL-33 did not induce proliferation of colon tumour cells. Colon cancer cells were stimulated with IL-33 and changes in a panel of cytokines/chemokines examined. However, only CXCL-1 and macrophage chemoattractant protein (MCP-1) were robustly induced by IL-33. To investigate the role of ST2 in vivo, ST2 knockdown cells were generated using ST2-specific shRNA. CT26ST2shRNA cells were injected subcutaneously into BALB/C mice. Knockdown of ST2 resulted in enhanced tumour growth (2.3 fold increase) compared to CT26scrshRNA cells in vivo. This was confirmed using 3 separate clones. Changes in tumour growth were associated with alterations in immune cell infiltration, including a decrease in macrophage and cytotoxic T cell infiltration. The decrease in macrophage numbers was confirmed by immunohistochemical analysis. Consistent with the induction of MCP-1 by IL-33, supernatant from IL-33-stimulated colon cancer cells was found to increase migration of macrophages. This increased migration was abrogated by treatment of the supernatant with anti-MCP-1. Conclusion: Our results indicate that IL-33 and ST2 may be protective against colon cancer. This finding merits further investigation. Stimulation of colon cancer cells with IL-33 results in augmented MCP-1 and that this may play a role in reducing tumour growth.
The effect of NF-kB tumour-stromal interactions in colorectal cancer

G O'Malley¹,², G McStravick², P Lohan², K Lynch¹, G Shaw², T Ritter², LJ Egan¹, A Ryan¹,²
¹Pharmacology, NUI Galway, Galway
²REMedi, NUI Galway, Galway

The tumour microenvironment is an important factor in determining colorectal tumour growth and metastasis, and can aid tumours in evading the immune response. It has recently been shown that MSCs are recruited to this environment and can acquire a distinct functional phenotype that serves to promote tumour growth. This study aims to elucidate the molecular regulation of the induced immunosuppressive phenotype of tumour-associated MSCs. NF-kB-deficient CT26 colon tumour cell lines were established by stable over-expression of an IkB-a super-repressor plasmid (CT26/IkB-a SR) with transfection of an empty vector as control (CT26/EV). By culturing MSCs in 60% conditioned medium (TCM) from these cells we have shown that the tumour-promoting properties of MSCs are influenced by NF-kB. After exposure to TCM for 72hrs, MSCs displayed an heightened immunosuppressive capacity which was partially dependent on NF-kB, and which was enhanced when the tumour cells had been pre-treated with TNF-a. Furthermore, TCM exposure caused MSCs to increase PD-L1 expression, independent of NF-kB, and MHC-II in an NF-kB-dependent manner, two molecules which may contribute to the enhanced immunosuppressive phenotype. In addition to suppressive effects on the immune system, MSCs can also directly affect the growth and metastasis of cancer cells. We have shown using co-culture experiments that exosomes isolated from MSCs increase the migratory capacity of tumour cells in vitro. This data demonstrates the importance of tumour-stromal interactions in the colorectal tumour microenvironment, and identifies novel potential targets for the development of more specific and efficacious cancer therapies.
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