IRISH ASSOCIATION FOR CANCER RESEARCH

Annual Meeting

Slieve Donard Resort and Spa, Newcastle, County Down

Friday 29th February and Saturday 1st March, 2008

President: Professor Mark Lawler (St James Hospital/Trinity)
Secretary: Professor William Watson (UCD)
Treasurer: Professor Rosemary O'Connor (UCC)

Council Members: Dr Michael Carty (NUI-Galway) Professor Elaine Kay (Beaumont Hospital/RCSI) Dr Ken O'Byrne (St James Hospital/Trinity) Dr Robert O'Connor (DCU) Dr Tracy Robson (QUB), Dr Sharon McKenna (UCC), Dr David Waugh (QUB)

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The Irish Association for Cancer Research would like to thank all the sponsors of this years meeting

Friday 29th February, 2008

10:30- 12:30 **SESSION I: Free Papers (8 x 15min)** Chairs: Dr Sharon McKenna

OR01. Sarah A. Penny[1], Catherine M. Kelly[1], Donal J. Brennan[1], Peter Holloway[2], Sallyann L. O'Brien[1], Amanda H. McCann[2], Ailis Fagan[2], Aedin C. Culhane[3], Desmond G. Higgins[2], Peter A. Dervan[2], Michael J. Duffy[3], Karin Jirstrom[4], Goran Landberg[4], Fredrik Ponten[5], Matthias Uhlen[6], and William M. Gallagher[1] Systematic Validation of Candidate Breast Cancer Biomarkers via High-Throughput Antibody Generation and the Application of Cell Line and Tissue Microarray Technology. [1]UCD School of Biomolecular and Biomedical Science, & [2]UCD School of Medicine and Medical Science, UCD Conway Institute, Dublin, Ireland; [2]Department of Pathology and Laboratory Medicine, St. Vincent's University Hospital, Ireland; [3]Dana Farber Cancer Institute, Boston, MA, USA; [4]Department of Pathology, Lund University, Sweden; [5]Uppsala University, Uppsala, Sweden; [6]Karolinska Institute, Stockholm, Sweden.

OR02. Lisa O' Connor, Stephen Langabeer, Shaun McCann, Eibhlin Conneally The role of BCR-ABL kinase domain mutations in mediating resistance to Imatinib & novel tyrosine kinase inhibitors in Chronic Myeloid Leukaemia. Department of Haematology, Trinity College Dublin & St James Hospital Dublin

OR03. Glenda J. McGonigle, Damian P.J. Finnegan, Mary Frances McMullin, Ken I. Mills, Terence R.J. Lappin, and Alexander Thompson.. Functional insights into the role of HOXA6 in Haematopoiesis and AML Haematology Research Group, CCRCB, Queen's University Belfast.

OR04. Helen O. McCarthy[1], J. Coulter[1], J. Worthington[2], T. Robson[1] & D.G. Hirst[1] Tissue Targeting in Metastatic Prostate Cancer. [1]School of Pharmacy, Queens University Belfast.

OR05. Alex D. Chacko[1], Nyree Crawford[1], Dario Barbone[3], Luciano Mutti[4], Courtney V. Broaddus[3], Giovanni Gaudino[5], Dean A. Fennell[1,2] NOXA-MCL-1-BAK Axis mediates Apoptosis following 20S Proteasome Inhibition by Bortezomib in Mesothelioma: Implications for Therapy [1] Queen's University Belfast, Centre for Cancer Research and Cell Biology. [2] Northern Ireland Cancer Centre. [3]Lung Biology Centre, University of California, San Francisco, USA. [4]Lab. di Oncologia Clinica, Borgosesia , Italy . [5] University of Piemonte orientale "A.Avogadro", DISCAFF & DFB Center, Italy.

OR06. Michael F. Gallagher, Elbaruni S, Heffron CCBB, Salley Y, Martin C, Sheils O & O'Leary JJ. Characterisation of Novel 'Early

Cancer Stemness' Gene Events in a Teratoma Model The Departments of Histopathology, University of Dublin, Trinity College.

OR07. Moya Cunningham, S.K.Brady, R.Preston, B.White, D.Hollywood, J.O'Donnell Radiotherapy and Chemotherapy induce procoagulant effects by modulating the ability of endothelial cells to regulate the protein C pathway. Haemostasis Research Group and Division of Radiation Therapy, IMM, Trinity College Dublin

OR08. Jennifer C. Byrne[a,b], Michelle R. Downes[a,b], Niaobh O'Donoghue[a], John Fitzpatrick[b], Michael J. Dunn[a] & R. William G. Watson[b] A Proteomics Approach to Identify Molecular Markers for Progression in Prostate Cancer. [a]Proteome Research Centre, [b]UCD School of Medicine and Medical Science, Mater Misericordiae University Hospital, UCD Conway Institute of Biomolecular and Biomedical Research, Belfield, Dublin 4, Ireland.

12:30 – 1:30 LUNCH

1:30 – 3:00 **SESSION II: Modeling Cancer** Chairs: Dr David Waugh and Dr Dean Fennell

1:30-2:00 **Professor Martin McMahon** – *"Exploring the initiation, progression and therapy of lung cancer and melanoma in genetically engineered mice".* UCSF Comprehensive Cancer Center

2:00-2:30 **Professor Johanna Joyce** – *"Understanding and targeting the functions of cathepsin proteases in the tumor microenvironment"* Memorial Sloan-Kettering Cancer Center

2:30-3:00 **Professor Liam Gallagher** – *"Isogenic Cell Line Models of Tumour Progression: From In Vitro to In Vivo to Clinical Studies"* University College Dublin

3:00 – 4:00 **Oral Poster Presentations (10x6min)** Chairs: Professor Elaine Kay and Professor John Fitzpatrick

OP1. John H. Bannon & Margaret M. Mc Gee Identification and functional characterisation of cyclophilin A as a novel regulator of genome stability. UCD School of Biomolecular and Biomedical Science, Conway Institute, UCD

OP2. Catherine M Dowling [a], S Cuffe [a], C Gill [a], M Tacke [b], JM Fitzpatrick [a], RWG Watson [a].Effects of Docetaxel and Novel Titanocene Analogues on Cell Death in Prostate

Cancer Following Down-regulation of Id-1and the IAPs. UCD School of Medicine & Medical Sciences [a] School of Chemistry and Chemical

Biology [b]UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin

OP3. William J. Faller[1], **Gabriela Gremel[1]**, Mairin Rafferty[1], Shauna Hegarty[1,2], Peter A. Dervan[2], and William M. Gallagher[1] MicroRNA Dysregulation in an Isogenic Human Cell Culture Model of Melanoma Progression. [1]UCD School of Biomolecular and Biomedical Science and [2]UCD School of Medicine and Medical Science, UCD Conway Institute, University College Dublin, Ireland.

OP4. Claire Grills, Alex Chacko, Nyree Crawford, Francis McCoy, Patrick Johnston, Francesca O'Rourke, Dean A. Fennell Dynamical Systems Analysis of Mitochondrial BAK Activation unifies Agonism/Dissociation Models and Predicts BH3 Mimetic Efficacy. Queen's University Belfast, [1]Department of Applied Mathematics and Theoretical Physics, [2]Centre for Cancer Research & Cell Biology

OP5. Clare Hodkinson[*], Galligan, L[*], Drain, S*, Catherwood MA[*+], Drake MB*, Kettle PJ*, Morris TCM[*], Alexander HD[*+]Cytogenetic aberrations detected by interphase FISH in CD138 purified plasma cells from MGUS and Multiple Myeloma patients. *Haemato-oncology Laboratory, Department of Haematology, Belfast City Hospital, Belfast BT9 7AB, Northern Ireland. School of Biomedical Sciences[+], University of Ulster, Coleraine BT52 1SA, Northern Ireland.

OP6. Joan Kyula, Sandra Van Schaeybroeck, Caitriona Holohan, Daniel Longley & Patrick Johnston. ADAM-17: a mediator of chemotherapy-induced EGFR activation. Queens University Belfast

OP7. Sinéad T. Loughran[1], Eva M. Campion[1], Brendan N. D'Souza[1, 4] Paul G. Murray[2], Georg Bornkamm[3] and Dermot Walls[1]. Bfl-1 is a crucial pro-survival Nuclear factor kappa B target gene in Hodgkin/ Reed-Sternberg cells of Hodgkin's Lymphoma.

[1]School of Biotechnology and National Centre for Sensor Research, Dublin City University, Dublin 9, Ireland; [2]Cancer Research UK Institute for Cancer Studies, The Medical School, University of Birmingham, Edgbaston, United Kingdom. [3]Institut fur Klinische Molekularbiologie und Tumorgenetik, GSF-Forschungszentrum fur Umwelt und Gesundheit, Marchioninistrasse 25, D-81377 Munchen, Germany.

OP8. Áine Prendergast[1], G. Shaw[2], F. Barry[2] and M.P. Carty[1] Characterisation of DNA damage response pathways in human mesenchymal stem cells (hMSCs). [1]DNA Damage Response laboratory, Department of Biochemistry, and [2]Regenerative Medicine Institute (REMEDI), NUI, Galway, Galway, Ireland.

OP9. Sandra Van Schaeybroeck, J. Kyula, C. Holohan, S. Moulik, D. Longley, P. Johnston. Role of Src-family kinases in chemotherapy

resistance. Centre for Cancer research and Cell Biology, Queens University Belfast

OP10. Garrett D Casey[1], MC Whelan[1], MP MacConmara[2], JA Lederer[2], M Tangeny[1] and GC O'Sullivan[1].Oral immune tolerance mediated by Tregulatory cells may be responsible for the poorer prognosis of foregut cancers. [1] Cork Cancer Research Centre, Mercy University Hospital, Cork, Ireland. [2] Dept of Surgery (Immunology), Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

4:00 – 4:30 **IACR AGM**

4:00 – 5:00 COFFEE AND POSTER VIEWING

5:00 – 6:00 **SESSION III: State of the Art lecture** Chair: Dr Michael Carty

Professor Alan Ashworth – *"Synthetic Lethal Approaches to the Development of New Therapies for Cancer".* Institute of Cancer Research, London

6:00 – 8:00 **SESSION IV: Poster presentations (wine reception)**
*(**Odd** numbered abstracts presenting 6:00-7:00pm and **Even** numbered abstracts presenting 7:00-8:00pm)*

8:00pm **BUFFET DINNER**
(Sponsored by the Irish Cancer Society)

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SATURDAY 1st MARCH, 2008

8:30 – 10:30 **SESSION V: Free Papers II (8 x 15min)** Chairs: Dr Robert O'Connor and Dr. Ray McDermott

OR09. Catherine R Wilson, T.R Wilson, P.G Johnston, D.B Longley, and D.J.J Waugh. Interleukin-8 /CXCR2 signaling plays an important role in conferring resistance of prostate cancer cells to chemotherapy.Centre for Cancer Research and Cell Biology, Queen's University Belfast, N.Ireland.

OR10. Annette Byrne[1], A. O'Connor[2], M.J. Hall[2], J. Murtagh[2], K. O'Neill[3], K. Curran[3], K. Mongrain[4], R. Lecomte[4], S. McGee[1], D.F. O'Shea[2] and W.M. Gallagher[1]
Vascular Targeted Photodynamic Therapy with ADPM Agents [1]UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland; [2]UCD School of Chemistry and Chemical Biology, Centre for Synthesis and Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland; [3]UCD School of Medicine and Medical Science, University College Dublin, Belfield, Dublin 4, Ireland; [4]Sherbrooke Molecular Imaging Centre, Etienne-LeBel Clinical Research Centre, Centre Hospitalier Universitaire de Sherbrooke and and Université de Sherbrooke Sherbrooke, Quebec, Canada.

OR11. Lynn Campbell, Sandra Van Schaeybroeck, Joan Kyula, Caitriona Holohan, Martin Eatock, Patrick Johnston. Role of Human Epidermal Receptor targeted therapies in chemo-sensitization of human gastro-oesophageal cancer cells. Centre for Cancer research and Cell Biology, Queens University Belfast

OR12. Scott McCloskey, MF McMullin, B Walker, AE Irvine. Proteasome activity profiles differ between acute and chronic phase BCR-ABL positive cell lines. CCRCB, Queen's University, Belfast

OR13. Shane Duggan, Behan, F; Vicente, R, Long, A, Kelleher, D. Tribbles homolog 3 (TRB3) a novel regulator of bile acid signaling in esophageal cells that may be lost in esophageal carcinogenesis. Institute of Molecular Medicine, St James Hospital, Dublin 8.

OR14. Rebecca Gallagher; PJ Maxwell; S Berlingeri; C Askin; A Seaton; C Wilson; P Scullin; J Pettigrew; IJ Stratford, KJ Williams, PG Johnston and DJJ Waugh. Inhibition of stress-induced interleukin-8 signaling sensitizes prostate cancer cells to ionizing radiation. Centre for Cancer Research and Cell Biology, Queen's University Belfast

OR15. Stephen G. Maher, Niamh Lynam-Lennon, John V. Reynolds Differential gene expression profiles as markers of radioresistance in oesophageal cancer Department of Surgery, Institute of Molecular Medicine, Trinity College Dublin.

OR16. Tim R. Wilson, D. Logan, K. McLaughlin, P.G. Johnston and D.B. Longley The role of Bax and XIAP in regulating c-FLIP silencing-induced cell death. Queen's University Belfast

10:30-11:00 COFFEE AND POSTER VIEWING

11:00–1:00 **SESSION VI:** ***Translational Research*** Chairs: Dr Tracy Robson and Professor Donal Hollywood

11:00 – 11:30 **Professor Jens Overgaard** - *"Lost in translation"* University of Aarhus

11:30 – 12:00 **Professor Penny Jeggo** - *"DNA non-homologous end-joining: the process and its cross talk with damage response signaling".* University of Sussex

12:00 – 12:30 **Professor Kevin Prise** - *"New mechanistic insights on radiation-induced intercellular signalling from targeted studies"* Queens University Belfast

12:30 – 1:30 LUNCH

1:30 – 3:00 **SESSION VII: Free Paper (6 x 15min)** Chairs: Professor William Watson and Dr Ken O'Byrne

OR17. Brendan Power, Philip Murphy, Judy Harmey SEMA3A decreases CXCR4 expression in B-cell Chronic Lymphocytic Leukaemia. Royal College of Surgeons Ireland.

OR18. Suzanne McFarlane, Ashleigh McClatchey, Patrick G. Johnston & David J. Waugh.Characterization of a cytoskeletal signaling pathway underpinning CD44-initiated, integrin-mediated adhesion of breast cancer cells to bone marrow endothelium CCRCB, Queen's Univeristy Belfast

OR19. Miriam O'Connor[1], McCormack O[2], Aherne S[3], Murphy H[2], Geraghty J [2], Rothwell J[2], Jeffers M[3], Walshe JM[1] Changes in hormonal receptor status in a series of breast cancer patients treated with neoadjuvant therapy. Department of Medical Oncology[1], Surgery[2] and Histopathology[3], The Adelaide and Meath Hospital, Tallaght, Dublin 24.

OR20. Eilis Foran and Laurence J. Egan Interleukin-6–stimulated DNA methylation in colon cancer cells: a mechanism of tumour

suppressor gene silencing. Department of Pharmacology and Therapeutics, National University of Ireland, Galway.

OR21. Cedric Favre & Rosemary O'Connor Effect of PNC1 on Epithelial-Mesenchymal Transition in transformed cells. Cell Biology Laboratory, Department of Biochemistry, University College Cork

OR22. Roberta Burden[1], Philip Snoddy[2], Richard Buick[2], James Johnston[3], Brian Walker[1], Christopher Scott[1].Cathepsin S propeptide attenuates cell invasion by inhibition of Cathepsin L-like proteases in the tumour microenvironment [1]School of Pharmacy, Queen's University of Belfast, 97 Lisburn Road, Belfast, BT9 7BL, [2]Fusion Antibodies Ltd., Springbank Industrial Estate, Pembroke Loop Road., Belfast, BT17 0QL and [3]Centre for Cancer Research and Cell Biology, Queen's University of Belfast, 97 Lisburn Road, Belfast, BT9 7BL.

3:00 – 3:30 COFFEE AND POSTER VIEWING

3:30 – 5:30 **Session VIII: Targeting the Tumour Microenvironment** Chairs: Professor Rosemary O'Connor and Professor Stephenie McKeown

3:30 – 4:00 **Professor Martin Brown** - *"Bone marrow derived cells: A novel target of the tumour microenvironment"* Stanford University

4:00 – 4:30 **Dr Kaye Williams** – *"Tumour vasculature and radiosensitisation"* The University of Manchester

4:30 – 5:00 **Professor Cormac Taylor** – *"Hypoxia, inflammation and cancer"* University College Dublin

5:00 – 5:30 **Professor David Hirst** – *"Nitric oxide in cancer biology: not too much, not too little, just right"* Queen University Belfast

5:30 – 6:30 **SESSION VII: IRISH CANCER SOCIETY LECTURE** Chair: Professor Mark Lawler

Professor Brian O'Sullivan *"The management of head and neck cancer in the era of molecular oncology"* Princess Margaret Hospital, University of Toronto

8:00 - LATE **BANQUET DINNER WITH PRESENTATION OF PRIZES**

Poster Presentations:

1. Aherne S, Smyth P, Flavin R, Russell S, Denning K, Li JH, Guenther S, O'Leary J, Sheils O. Geographical mapping of a multifocal thyroid tumour using genetic alteration analysis & miRNA profiling. Institute of Molecular Medicine, TCD

2. E. Allott, J. Howard, H. Roche, G.P. Pidgeon, J.V. Reynolds. Adipokine regulation of tumour cell survival in oesophageal and colorectal cancer cells Trinity College Dublin / St. James's Hospital

3. Anne-Marie Baird, Nael Al-Sarraf, Steven G. Gray, Kenneth J. O'Byrne Epigenetics underpinning the regulation of the CXC (ELR+) chemokines in NSCLC. Trinity College Dublin/St. James's Hospital

4. [1]Martin Barr, [2]Graham Pidgeon, [1]Kathy Gately, [1]Kenneth O'Byrne. Neuropilin-1 blockade inhibits hypoxia-induced Akt and MAPK phosphorylation and induces apoptosis of non-small cell lung cancer cells. [1]Thoracic Oncology Research Group, Departments of Oncology and [2]Surgery, Institute of Molecular Medicine, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8.

5. HL Barrett, R Cummins, EW Kay Genomic Analysis of Colorectal Cancer to Assess Intratumour Heterogeneity. RCSI ERC, Beaumont Hospital.

6. Barry A., O'Cearbhaill R., Griffin D., Donnellan P., Grimes H.Audit of the Calculated Carboplatin Dosage According to the Calvert Formula, using Different Equations Glomerular Filtration Rate Estimation. Galway University Hospital, Galway

7. Razvan Bocu. Drug medication and cancer evolution. University College Cork

8. Rachael Bowe, Orla Cox, Nollaig Healy and Rosemary O'Connor. Mystique is required for polarization and migration of prostate carcinoma cells. Cell Biology Laboratory, Department of Biochemistry, Biosciences Institute, University College Cork

9. Buckley N, Nic An tSaoir C, Tkocz D, Farmer H, Redmond K, Da Costa Z and Mullan P Is p63 a marker of basal breast cancer? CCRCB, Queens University Belfast

10. Vikki Campbell[1], Joanne Lysaght[3], Kathy Gately[2], Elaine Kay[4], John Reynolds[1], Graham Pidgeon[1], Kenneth J. O'Byrne[2] Mechanisms controlling survival and apoptosis induction following inhibition of 12-Lipoxygenase in lung cancer cells. Department of Clinical Surgery[1], Oncology[2] and Haematology[3], Institute of Molecular Medicine, TCD Health Sciences Centre, Trinity College Dublin / St. James Hospital and Dept. Pathology[4], Beaumont Hospital, Royal College of Surgeons Ireland, Dublin 9.

11. Cathcart, MC, Gately, K, Kay, E, Reynolds, JV, O' Byrne, KJ, Pidgeon, GP.An imbalance in the expression profiles of PGIS and TXS in NSCLC: Regulation of tumor cell growth and invasive potential St. James's Hospital/Trinity College Dublin.

12. Mark A. Catherwood[1,2], Drake MB[1], Kettle PJ[1], Morris TCM[1], El-Agnaf M[3], H.D Alexander[1,2]CHRONIC LYMPHOCYTIC LEUKAEMIA EXPRESSING IGHV4-34 IDENTIFIES A SUBSET WITH HIGHLY HOMOLOGOUS HEAVY AND LIGHT CHAIN THIRD COMPLEMENTARY DETERMINING REGION (HCDR3 & LCDR3) AND INDOLENT DISEASE.[1] Haemato-Oncology, Belfast HSC Trust, Level C, Belfast City Hospital, Northern Ireland. [2] School of Biomedical Sciences, University of Ulster, Coleraine, Northern Ireland, UK. [3] Ulster Hospital, Dundonald, Belfast.

13. Chang KH, Miller N, McNeill RE, Smith MJ, MacCarthy F, Regan M, McAnena OJ, Kerin MJ Identification of Differentially Expressed Mature MicroRNAs in Colorectal Cancer and Non-tumoral Tissues. Department of Surgery, National University of Ireland, Galway

14. T.Clarke[a], J.M. Fitzpatrick[b], A. McCann[a] ZEB1 – A POTENT REPRESSOR OF E-CADHERIN IN UROTHELIAL CARCINOMA OF THE BLADDER. [a] Conway Institute, University College Dublin [b] Department of Surgery, Mater Misericordiae Hospital, Dublin

15. Amy Colleran, Aideen Ryan, Eilis Foran, and Laurence Egan Long-term suppression of IκBα expression by inflammatory cytokines: Molecular mechanisms. Department of Pharmacology and Therapeutics, NUI, Galway, Ireland.

16. Corkery B[1,2], Crown J[1,2], Clynes M[1], O'Donovan N[1]Preclinical evaluation of EGFR in triple negative breast cancer. [1]National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9; [2]St. Vincent's University Hospital, Elm Park, Dublin 4.

17. Orla T. Cox, Nollaig Healy, Rachael Bowe and Rosemary O'Connor Differential localisation and function of Mystique in various cell types. Biosciences Institute, UCC.

18. VM Coyle, WL Allen, PV Jithesh, I Proutski, L Stevenson, G Stewart, C Fenning, DB Longley, RH Wilson and PG Johnston Identification of predictive signatures of response to chemotherapy in metastatic colorectal cancer. Drug Resistance Group, Centre for Cancer Research and Cell Biology, Queen's University Belfast.

19. Lisa Crawford, Brian Walker, Treen C. M. Morris, Alexandra Irvine Investigation of additional protease targets of proteasome inhibitors. CCRCB, Queen's University Belfast

20. Nyree Crawford , Alex Chacko, Francis McCoy, Gary Coleman, Patrick G. Johnston, Dean Fennell BH3 domain of BID interacts with VDAC1/Prohibitin Complex and Depolarizes Mitochondria in the absence of Cristae Remodelling. Centre for Cancer Research and Cell Biology, Queen's University Belfast, Northern Ireland.

21. Cuffe[a] S., Dowling[a] C., Gill[a] C., Tacke[b] M., Fitzpatrick[a] JM, Carthy[c] MP, Watson[a] RWG. Titanocene analogues induce apoptosis in prostate cancer epithelial cells via a DNA damage response. UCD School of Medicine and Medical Science[a] and UCD School of

Chemistry and Chemical Biology[b], Conway Institute of Biomolecular and Biomedical Research, University College Dublin and Department of Biochemistry, National University of Ireland, Galway.

22. D'Costa Z.C, Farmer H.L., Redmond K, O'Brien N, Nic An tSaoir C, Tkocz D & Mullan PB.The identification of transcriptional targets of TBX2 in breast cancer cell lines. Queen's University Belfast.

23. Denning K, Smyth P, Cahill S, Li JH, Flavin R, Aherne S, O'Leary J, Sheils O ret/PTC-1 alters the immunoprofile of thyroid follicular cells. Trinity College Dublin

24. RF Donnelly, DIJ Morrow, PA McCarron, AD Woolfson. Design of a novel drug delivery system for photodynamic and photodiagnostic methodologies in the colorectal region. School of Pharmacy, Queen's University Belfast, MBC, 97 Lisburn Road, Belfast BT9 7BL, N. Ireland.

25. Michelle R. Downes [a,b], Jennifer C. Byrne [a,b], Niaobh O'Donoghue [b], John M. Fitzpatrick [a], Mike J. Dunn [b], R. William G. Watson [a] Determination of Prostate Cancer Urinary Biomarkers using a 2D-DIGE Proteome platform. School of Medicine and Medical Science [a], Proteome Research Centre [b] UCD, Conway Institute of Biomolecular and Biomedical Research, Mater Misericordiae University Hospital, University College Dublin.

26. N Johnston, V Gunasekharan, P Johnston, M El-Tanani.A role for Ran GTPase in breast cancer metastasis and invasion. CCRCB, Queens University, Belfast

27. Vittal Venkatasatya Kurisetty[1], Patrick G. Johnston[1], Philip S. Rudland[2] and Mohamed K. El-Tanani[1].Identification of genes differentially expressed between benign and metastatic mammary epithelial cells [1]Centre for Cancer Research and Cell Biology (CCRCB), Queen's University Belfast, Belfast BT9 7BL and [2]Cancer and Polio Research Fund Laboratories, School of Biological Sciences, University of Liverpool, PO. Box 147, Liverpool L69 7ZB, United Kingdom.

28. Alex J. Eustace (1), John Crown (1, 2), Martin Clynes (1), Norma O'Donovan Effects of Src kinase inhibition by dasatinib in melanoma cell lines. (1) National Institute for Cellular Biotechnology, Dublin City University, Dublin 9, Ireland. (2) Dept of Medical Oncology, St Vincent's University Hospital, Dublin 4, Ireland.

29. William J. Faller[1], Mairin Rafferty[1], Shauna Hegarty[1,2], Mario F. Fraga[3], Manel Esteller[3], Peter A. Dervan[2], William M. Gallagher[1] etallothionein 1E (MT1E) Gene is Methylated in Both Primary and Metastatic Melanomas. CD School of Biomolecular and Biomedical Science and [2]UCD School of Medicine and Medical Science, UCD Conway Institute, University College Dublin, Ireland; [3]Centro Nacional de Investigaciones Oncologicas, Madrid, Spain.

30. Francois Fay, Paul A. McCarron, Chris Scott.Nanoparticle design for cytosolic delivery of peptide and protein drugs into tumour cells. chool of Pharmacy, Queen's University.

31. Finucane O, Behan F, Kelleher D, Duggan SP.Use of an Intestinal Filter for analysis of transcriptomic studies of Barrett's Oesophagus identifies a GATA-6 regulated network of gene regulation. Institute of Molecular Medicine, St James Hospital, Dublin 8.

32. Flavin R[1], Smyth P[1], Finn SP[3], Laois A[2], O'Toole S[2], Barrett C[1], Ring M[1], Denning K[1], Li J[1], Aherne S[1], Aziz NA[2], Alhadi A[2] , Sheppard B[2], Loda M[3], Martin C[1], Sheils O[1], O'Leary JJ[1]. LOW EIF6 EXPRESSION IS ASSOCIATED WITH REDUCED DISEASE-FREE SURVIVAL IN OVARIAN SEROUS CARCINOMA PATIENTS From the Departments of Histopathology[1], Obstetrics and Gynaecology[2], Trinity College Dublin, Ireland and The Dana Farber Cancer Institute[3], Harvard Medical School, Boston, MA, USA.

33. Ruth Foley, Laure Marignol, Mark Lawler Prodrug Activation Therapy Kills Prostate Cancer Cells in an Three-Dimensional Culture Model. Institution of Molecular Medicine, Trinity College Dublin

34. Fox, D.M.[1], McCoy, C.E.[2], Higgins, W.[1], Pickering M.[1] and Worrall, D.M.[1]Subcellular localization and analysis of tyrosine phosphorylation of the tumour suppressor protein, maspin. [1]UCD School of Biomolecular & Biomedical Research, Conway Institute, University College Dublin, Belfield, Dublin 4.
[2]School of Biochemistry and Immunology, Trinity College Dublin, Dublin 2

35. Gately K, Stewart DJ, Davies A, Edwards JG, Richardson D, Jones JL, Burke B, Waller DA, Ziegler-Heitbrock L, Wardlaw AJ, and O'Byrne KJ Investigating the link between hypoxia, AKT compartmentalization and cell survival. Institute of Molecular Medicine, St. James Hospital, Dublin 8

36. Dr Anna Gavin, Mrs Heather Kinnear Cancer Services. N. Ireland Cancer Registry, Queen's University Belfast.

37. David Connolly[a]*, Anna Gavin[c], Amanda Black[b], Liam J. Murray[b], Patrick F. Keane[a]. The value of PSA testing in men older than 65 years. [a]Department of Urology, Belfast City Hospital, [b]Cancer Epidemiology & Prevention Research Group, Centre for Clinical and Population Sciences, Queen's University Belfast, [c]Northern Ireland Cancer Registry, Queen's University Belfast.

38. S. Gorman, M. Tosetto, H. Mulcahy, O. Howe, F. Lyng, D. O'Donoghue, J. Hyland, Gibbons D, Winter D, K. Sheahan & J. O'Sullivan. Gamma ray-induced bystander effects in colorectal cancer: a specific study on anaphase bridge and micronuclei formations in unirradiated bystander cells. Centre for Colorectal Disease, St. Vincents University Hospital, Elm Park, Dublin 4, Ireland. Radiation & Environmental Science Centre, Dublin Institute of Technology and St Lukes Hospital, Dublin.

39. Julia J. Gorski, Colin R James, Jennifer E. Quinn, Gail E. Stewart, Alison Hosey, Paul B Mullan, Patrick. G. Johnston, Richard H. Wilson and D. Paul Harkin. BRCA1 transcriptionally regulates genes associated with the basal phenotype in breast cancer. CCRCB

40. Steven G. Gray, Nael Al-Sarraf, & Kenneth J. O'Byrne EP receptors in NSCLC, and their regulation by epigenetic modifications. St James's Hospital.

41. Gabriela Gremel[1], Mairin Rafferty[1], Kate Fitzgerald[2], William M. Gallagher[1] Differential Cell Adhesion within an Isogenic Model of Melanoma Progression Under Shear Flow Conditions Using a Microfluidic Cell-Based Assay [1]UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Belfield, Dublin 4; [2]Cellix Ltd, Institute of Molecular Medicine, James's Street, Dublin 8, Ireland.

42. S. Cruet-Hennequart, A. Kaczmarczyk, M.T. Glynn, and M.P. Carty. EFFECTS OF DNA POLYMERASE ETA EXPRESSION AND PIKK INHIBITION ON THE RESPONSE OF HUMAN CELLS TO CISPLATIN, OXALIPLATIN AND CARBOPLATIN. DNA Damage Response Laboratory, Department of Biochemistry, School of Natural Sciences, National University of Ireland, Galway, Ireland.

43. Wayne J. Higgins, Oliver E. Blacque and D. Margaret Worrall. The Tumour Suppressor and Angiogenesis Inhibitor Maspin Binds to the Glycosaminoglycan Heparin CD School of Biomolecular and Biomedicinal Sciences, UCD Conway Institute, University College Dublin.

44. Caitriona Holohan, Sandra Van Schaeybroeck, Joan Kyula, Owen McGrath, Patrick Johnston.The role of the HER2/HER3/PI3 Kinase survival pathway in colorectal cancer. Queens University Belfast

45. Paula Hyland, Naomi Pentland, Peter Hall, Hilary Russell.Septin 9_v1 stabilisation of HIF-1α in the absence of hypoxia mediates increased expression of COX-2 and VEGF-A in vitro. Centre for Cancer Research & Cell Biology, Queens' University Belfast.

46. D Kevans , LM Wang, M Gancarczyk-Biniecka, DP O'Donoghue , JH Hyland , H Mulcahy , K Sheahan,_J O'Sullivan Epithelial-MesenchymalTransition (EMT) protein expression and mis match repair profiles in Stage II colorectal cancer with tumour budding status. Centre for Colorectal Disease, St. Vincent's University Hospital, Elm Park, Dublin.

47. Kevans D, Foley J, O'Donoghue DP, Hyland JH, Sheahan K, Mulcahy H, O'Sullivan J High Clusterin expression is associated with poorer prognosis in a cohort of stage II Colorectal Cancer patients. Centre for Colorectal Disease, St. Vincents's University Hospital, Elm Park, Dublin.

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Systematic Validation of Candidate Breast Cancer Biomarkers via High-Throughput Antibody Generation and the Application of Cell Line and Tissue Microarray Technology

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There now exist vast quantities of DNA microarray data defining differences in gene expression between different subtypes of breast cancer, including variations in invasiveness and metastatic capabilities. However, this type of genetic assay is of limited prognostic or predictive value in most clinical settings due to general requirements for fresh/frozen tissue. The aim of this project is to translate the genetic data available into a more clinically relevant form – that of immunohistochemistry - to identify from these gene datasets any independent biomarkers that may be potential biomarkers and/or drug targets. Our approach involves the high-throughput validation of the affinity purified, mono-specific antibodies created by the Swedish Human Proteome Resource (SHPR, www.proteinatlas.com) against candidate breast cancer progression-associated biomarkers selected from publicly available and in-house transcriptomic and proteomic datasets. Initial validation of these antibodies was performed by the SHPR using a variety of normal and cancer tissues. Of the 137 targets selected for antibody production, 32 antibodies have begun specificity validation by Western blot analysis. Those that are successful at this stage of optimisation were moved forward to immunohistochemical (IHC) validation using cell pellet arrays derived from different human breast tumour cell lines. Successful IHC validation then leads to the use of tissue microarrays (TMAs) of patient samples to assess the clinical relevance of the putative biomarkers, either individually or as a panel. For efficient validation of the candidate biomarkers, a TMA constructed from a cohort of 512 consecutive breast cancer cases, diagnosed between 1988 and 1992, is being used. All invasive TNM stages are represented within the cohort. PDZK1, an estrogen-responsive gene, was previously found to be associated with good prognosis (interval to distant metastasis) at the transcript level in breast tumours. Our TMA IHC results showed PDZK1 protein to be associated with improved breast cancer-specific survival ($p=0.0247$), ER positivity ($p=0.041$) and low grade ($p=0.002$). Another promising putative biomarker undergoing validation according to this schema is PDZ-binding kinase (PBK). This and any further biomarkers found using this validation process may be put forward as prognostic indicators, or be investigated as a possible targets for biological therapeutics.

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Title: **The role of BCR-ABL kinase domain mutations in mediating resistance to Imatinib & novel tyrosine kinase inhibitors in Chronic Myeloid Leukaemia**

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Abstract:
The hybrid oncoprotein BCR-ABL has de-regulated tyrosine kinase activity and is pathognomic of Chronic Myeloid Leukaemia (CML). Imatinib Mesylate (IM) is a tyrosine kinase inhibitor that specifically binds BCR-ABL in its inactive conformation leading to cell cycle arrest has revolutionized therapy for patients with CML. Resistance develops in a significant proportion of cases and is predominantly mediated by single base-pair substitutions within the BCR-ABL kinase domain that inhibit IM binding whilst retaining BCR-ABL phosphorylation capacity. Two second generation tyrosine kinase inhibitors Dasatinib & Nilotinib retain activity in the majority of IM-resistant patients due to less stringent binding requirements and represent viable alternatives for IM-resistant or intolerant CML patients. We undertook to examine the molecular mechanisms underlying IM resistance; a cohort of 34 patients with primary or acquired resistance (n=32) or intolerance (n=2) to IM was identified by high or increasing BCR-ABL transcript levels. The Threonine to Isoleucine substitution at amino acid 315 (T315I) severely limits binding of all tyrosine kinase inhibitors, identified to date, to the kinase domain. An allele-specific PCR screen was used to sensitively detect the clinically significant T315I mutation: five (14.7%) IM resistant/intolerant patients were T315I positive. To further characterise the molecular mechanisms of resistance, the BCR-ABL kinase domain was screened for the presence of mutations using a sensitive denaturing high performance liquid chromatography (dHPLC) approach. dHPLC can detect a single base pair substitution within the BCR-ABL kinase domain. Mutated samples display reduced hybridization capacity to the dHPLC column and elute at an earlier time-point. Sensitivity of dHPLC (0.1-10%) is greater than that of sequencing (15-25%). Samples showing evidence of mutation were subsequently examined by sequencing to identify the mutation(s) present. Kinase domain mutations have been identified in 19 / 34 (56%) patients examined to date and these include p-loop mutations (M244V, G250E, Q252H), IM-binding domain mutations (T315I & F317L), catalytic domain mutations (M351T & E355G), and an activation-loop mutation (L387M). A previously unreported mutation, the L273M that may be associated with Nilotinib resistance was identified. The L273M positive patient also has a M244V mutation conferring IM-resistance. The identification of clinically significant mutations facilitates selection of alternative approaches to therapy such as IM dose escalation, second generation tyrosine kinase inhibitors or allogeneic stem cell transplantation, if eligible, facilitating patient specific approaches to therapy.

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Functional insights into the role of *HOXA6* in Haematopoiesis and AML

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Molecular profiling in AML has identified several candidate genes that may define prognosis and response to therapy including members of the Class I homeobox (*HOX*) gene network. *HOX* genes encode master regulators of haematopoiesis. DNA microarray expression analyses were carried out on 318 AML patient samples using the Affymetrix human U133 Plus 2.0 Array. We focused on a subset of 13 genes (12 *HOX* plus *MEIS1*) previously reported to be highly expressed in AML. Specific RQ-PCR analyses were performed for the same gene selection in twenty-four *de novo* AML patient samples. *HOXA6* was identified as the most consistently and highly expressed gene, substantially higher than *HOXA9*. Distinct gene expression signatures were found and high *HOXA6* expression was associated with the intermediate and poor prognostic groups. Furthermore *HOXA6* was highly expressed in $CD34^+$-enriched primary progenitors. Parallel studies with murine progenitors (c-Kit^+, Lin^-) and cell lines also showed a preponderance of *Hoxa6* expression over other family members.

Hoxa6 regulation following differentiation or growth factor stimuli was subsequently investigated in haematopoietic cell lines. Decreased expression of *Hoxa6* was observed following differentiation of EML and FDCP-Mix A4 cells by ATRA / IL-3 combination. Growth factor depletion followed by replenishment indicated cell-cycle regulation of *Hoxa6* in both 32Dcl3 and Ba/F3 cells. Direct evaluation of cell-cycle status identified peak expression of *Hoxa6* during S-phase in Ba/F3 cells.

To gain further insights into the potential role of HOXA6 in haematopoiesis, we overexpressed *HOXA6* in the FDCP-Mix A4 and Ba/F3 cell lines using Nucleofection technology. FDCP-Mix-A6 and Ba/F3-A6 cells were examined on the basis of proliferation, cell-cycle status, apoptosis, growth factor-dependence and lineage differentiation. Both FDCP-Mix-A6 and Ba/F3-A6 displayed growth advantage over control cells in the presence of IL-3 and cell-cycle analysis indicated a reduced number of cells in S-phase, with associated accumulation in the pre-G_1 phase, indicative of increased apoptosis. IL-3 depletion studies of FDCP-Mix-A6 and Ba/F3-A6 cells indicated substantial factor-independent growth compared to controls, implying oncogenic potential for *HOXA6*. FDCP-Mix-A6 cells also exhibited increased self-renewal as shown by colony replating assays in methylcellulose and less capacity for myeloid lineage differentiation when stimulated with appropriate growth factors in liquid culture compared to control cells.

Recent reports demonstrate that *HOXA6* is differentially expressed in AML patient samples and a potential co-factor for Meis1-induced AML in a murine model. This suggests that *HOXA6* should be included in the growing list of *HOX* genes associated with AML and future study of its function, specificity and importance in haematopoiesis is warranted.

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Title: **Tissue Targeting in Metastatic Prostate Cancer**

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Abstract: Conventional treatments for hormone refractory prostate cancer (HRPC) are mainly palliative and the prognosis is poor so there is a pressing need for new therapies. HRPC is typically characterized by metastatic deposits at bone sites where they display osteoblast-like characteristics. Gene therapy has been identified as a promising treatment option for disseminated prostate cancer. The human osteocalcin (hOC) promoter shows promise for gene therapy in this setting, as expression is limited to osteotropic tumours and mature calcified tissue. Previously we have demonstrated the cytotoxic effects induced by overexpression of the iNOS transgene, therefore, application of a hOC/iNOS construct may prove an attractive approach for specifically targeting HRPC.

We have cloned a hOC/EGFP-1 reporter vector and confirmed *in vitro* that the promoter is strongly activated in the androgen independent PC-3 and DU145 cell lines, but not in the androgen dependent LNCaP cell line, the HT29 cell line or HMEC-1 cell line. Using a hOC/iNOS construct we have demonstrated increased iNOS protein and total nitrite in PC-3 and DU145 cells, but not LNCaP or HT29, and this increase was not significantly different from that achieved with the constitutively expressed CMV/iNOS construct. Cytotoxicity was then assessed *in vitro* by clonogenic assay. Transfection with CMV/iNOS or hOC/iNOS resulted in no cytotoxicity in the androgen dependent LNCaP cell line or in the non-prostate cancer cell lines. However, transfection with either construct resulted in a greatly reduced cell survival (to 10-20%) in the androgen independent PC-3 and DU145 cell lines. Further *in vivo* studies have shown highly significant inhibition of tumour growth especially when a multiple injection regimen was administered in PC-3 metastatic prostate tumours.

Utilizing the tumour-type specific properties of the hOC promoter we have demonstrated target cell specificity resulting in significant cytotoxic effects in the androgen independent prostate cancer cell lines (PC-3 and DU145) as a result of high-level generation of $NO^{\cdot}$. This effect was not observed in androgen dependent cells (LNCaP), colon cancer (HT29) cells or normal human endothelial cells (HMEC-1). The levels of $NO^{\cdot}$ generated are comparable with those seen with constitutively (CMV) driven iNOS. These *in vitro* data have been effectively translated into a human tumour xenograft model (PC-3) *in vivo,* in which impressive growth inhibition was achieved with both single and multiple hOC/iNOS treatments. The data obtained from this study provide an encouraging basis for future development of hOC/iNOS gene therapy.

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OR5

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Title: NOXA-MCL-1-BAK Axis mediates Apoptosis following 20S Proteasome Inhibition by Bortezomib in Mesothelioma: Implications for Therapy

Authors:
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Abstract:
Malignant Mesothelioma (MM) is a highly lethal, apoptosis resistant cancer. Inhibition of the 20S proteosome by Bortezomib (Bz) is an approved anti-cancer strategy licenced for myeloma, that is under evaluation in a mesothelioma trial (ICORG 05-10) based on promising *in vivo* findings(*1*). Bz modulates the BCL-2 family during apoptosis(*2*). We have therefore explored the impact of Bz on the core apoptosis pathway in MM, in order to understand potential mechanisms of sensitivity and resistance relevant to our Phase II trial. Bz exhibits differential, concentration-dependent killing of MM cells with a logfold difference in EC50 between REN versus MMP cell lines. RNAi knockdown of BAK confirms its requirement for MM cell killing whereas BAX knockdown is ineffective in protecting MM cells. Bz causes mitochondrial cytochrome C and SMAC release, mitochondrial depolarization, and modulates both pro- & anti-apoptotic BCL-2 protein expression. Isolated state IV mitochondria from Bz primed MM cells are sensitized to proapopototic BID BH3 domain peptide consistent with modulation of mitochondrial apoptosis signalling. Accordingly, Bz treated MM cells were sensitized to TRAIL. In MMPs, Bz downregulates mitochondrial prosurvival BCL-2 and A1, but upregulates MCL-1. Proapoptotic BH3 only protein BIM is upregulated and translocates to mitochondrial outer membrane where it binds to MCL-1, to de-repress BAK. Proapoptotic BH3-only proteins BIK, BID, PUMA expression are not altered. In MMPs NOXA is neither constitutively expressed nor upregulated post Bz. In contrast, REN cells dramatically upregulate NOXA, which translocates to mitochondria and disrupts a high molecular weight MCL-1 complex with BAK. REN appear to MCL-1 addicted as evidenced by concentration dependent depolarization of isolated state IV mitochondria following exposure to peptide derived from NOXA BH3 domain. In summary, BIM-MCL-1-BAK axis mediates Bz induced toxicity in MM. NOXA facilitates Bz toxicity by releasing multidomain proapoptotic BAK (and BAX) from MCL-1. This model suggests that loss of critical proapoptotic components of this pathway may account for therapeutic drug resistance to 20S proteosome inhibition.

1. Sartore-Bianchi *et al.*, *Clin Cancer Res* **13**, 5942
2. Fennell et al, *Oncogene* (EPub ahead of Print).

OR6

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Title:

Characterisation of Novel 'Early Cancer Stemness' Gene Events in a Teratoma Model

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Abstract

It is widely accepted that extensive self-renewal and differentiation (defined as 'stemness') of cancer stem cells (CSCs), progenitor cells required for normal tissue renewal that appear most likely cells of origin of tumours, may drive tumourigenesis and that persistence of CSCs post-intervention may explain metastasis and recurrence. Furthermore, description of CSCs in breast, brain, head and neck, prostate and ovarian tumours suggest that CSCs are key components of malignancy. However, as CSCs clearly mirror normal stem cells (NSCs) of comparable potency, clinical inhibition of CSC stemness has not been achieved to date. Observing that both CSCs and NSCs are functional (can self-renew and differentiate to produce mature cell types), we hypothesised that CSCs were characterised by aberrant regulation of differentiation rather than of differentiation itself. Addressing this we have generated whole-genome profiles enriched for novel regulators of CSC stemness through assessment of early differentiation in a teratoma model.

Human teratocarcinoma ('classical stem cell' gonadal tumours) CSCs originally derived from well (pluripotent) and poorly-differentiated (nullipotent) tumours were retinoic acid-differentiated for 3 days, whole-genome array analysis performed and profiles validated through further analysis of 50 genes of interest by TaqMan real-time PCR analysis. Validated gene expression profiles were bioinformatically compared to published hES data, permitting identification of gene events exclusive to CSCs. As hypothesised, resultant data was enriched with novel regulatory genes previously unassociated with CSC stemness and included genes from stemness pathways such as Wnt, Snail, Notch and Shh and a novel marker of mesodermal differentiation in CSCs, ENO3. We hypothesise that these **early stemness genes** regulate key downstream stemness genes and pathways and postulate that specific CSC-targeting, in a manner not affecting NSCs, can now be achieved by their functional knockdown.

OR7

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Title: Radiotherapy and Chemotherapy induce procoagulant effects by modulating the ability of endothelial cells to regulate the protein C pathway

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Abstract: Venous thromboembolism (VTE) is a major complication of malignancy. Moreover, VTE in the form of pulmonary embolism represents the leading cause of death in cancer patients after the cancer itself. Radiotherapy and chemotherapy are both independent risk factors for VTE in cancer. The molecular mechanisms underlying this treatment related prothrombotic effect have not been elucidated. However previous studies have suggested that radiotherapy and chemotherapy directly influence normal endothelial cell function. In this study we have characterised the effects of radiation and chemotherapy on the ability of human endothelial cell (EC) surface to regulate the protein C anticoagulant pathway, through expression of thrombomodulin (TM) and the endothelial protein C receptor (EPCR).

All studies were performed using the EAhy 926 human endothelial cell line. Preliminary experiments demonstrated that EAhy 926 cells expressed both TM and EPCR on the cell surface. In order to investigate the effects of radiation EAhy 926 cells were grown to confluence in 6 well plates. The cells were irradiated over a range of clinically relevant doses (2Gy to 20Gy). Parallel sham irradiated EC were used as controls. At various time points following irradiation cells were harvested for analysis. TM and EPCR expression on the EC surface were analysed using specific monoclonal antibodies and flow cytometry. Irradiation was associated with a small but significant decrease in EPCR expression, but had no apparent effect on TM expression.

In parallel experiments, we also studied separately the effects of Doxorubicin (Dox) and Cisplatin (Cis) treatment on EC cells. In brief EAhy 926 cells were incubated with Dox (over a range of clinically relevant concentrations 5μg/ml to 10μg/ml) for 24 hours and then analysed as before. In the Cisplatin experiments EAhy 926 cells were incubated with Cisplatin at 10μmol/L for 24 hours. Dox resulted in a dose dependent down regulation in EPCR expression, up to 100% ($p<0.05$) and a 10% down regulation of TM which could not be explained by EC apoptosis. Cisplatin did not influence either TM or EPCR expression.

In conclusion we have demonstrated that both radiotherapy and chemotherapy directly influence the ability of human EC to regulate the protein C pathway. In view of the significant morbidity and mortality associated with the thrombotic complications of current therapeutic regimens, these findings are of direct clinical relevance. In addition, recent studies have also demonstrated that activated protein C has important anticoagulant-independent PAR-1 mediated cell signalling effects. As demonstrated from recent studies showing that Low Molecular Weight Heparin can improve overall survival in cancer patients, advances in understanding the interface between malignant cells, endothelial cells and the coagulation system may well offer future novel therapeutic opportunities.

OR8

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A Proteomics Approach to Identify Molecular Markers for Progression in Prostate Cancer

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Prostate cancer is the most common solid malignancy affecting men in the United States and Western Europe. Recent statistics released from the National Cancer Registry in Ireland predict a 275% increase in incidence of the disease between 2000 and 2020 [1]. Currently, the main diagnostic tools used to look for evidence of prostate cancer include physical examination using digital rectal exam (DRE), serum concentrations of prostate specific antigen (PSA) and biopsy. However, due to the low specificity of PSA in differentiating prostate cancer from other benign conditions, such as benign prostatic hyperplasia (BPH), many patients undergo radical therapeutic interventions. New serum markers are needed which could then be used in conjunction with PSA and other clinical tests to allow for a more specific diagnosis of disease and more appropriate clinical interventions.

We undertook proteomic analysis of serum from men with two grades of pathologically confirmed prostate cancer compared to men with BPH, to identify novel biomarker of disease.

Coherts of men with BPH (n=14), Gleason grade 5 (n=18) and 7 (n=18) prostate cancer, were identified from the Prostate Cancer Research Consortium BioResource. There corresponding serum samples were subjected to immunoaffinity depletion and protein expression analysis using 2D-DIGE. Image analysis isolated 63 spots that displayed differential expression between the three groups ($p<0.05$). Utilising LC-MS/MS, 35 of these proteins have been identified to date. Literature searches have demonstrated that a number of these proteins have known associations with prostate cancer. For example, we demonstrated an increase in zinc-α2-glycoprotein (ZAG) in the Gleason score 7 cohort whereas pigment epithelium-derived factor (PEDF), an extremely potent inhibitor of angiogenesis, was decreased. We have successfully validated the differential expression of these proteins across different grades of prostate cancer using western blotting and ELISAs. We have also examined protein expression in corresponding tissue samples and have validated decreased expression of PEDF, however ZAG tissue expression was decreased which is opposite to the changes in serum.

These studies have identified PEDF as a serum marker of Gleason grade in prostate cancer which reflects changes in tissue levels, however ZAG serum levels are not reflective of tissue expression but may be increased due to metabolomic alterations resulting in its release from the liver. These studies identify proteomic approaches to prostate cancer as useful and allow for some critical insights into the underlying molecular mechanisms of the disease and holds great promise for biomarker discovery.

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OR9

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Title: Interleukin-8 /CXCR2 signaling plays an important role in conferring resistance of prostate cancer cells to chemotherapy.

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Abstract:
Background: Androgen-independent prostate cancer (AIPC) is a chemoresistant disease. The current gold-standard therapy (docetaxel/prednisone combination) confers only a modest survival benefit over palliative care. Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) is a TNF gene-family member closely related to TNF-α and Fas-ligand. TRAIL induces apoptosis with varying sensitivity in prostate cancer, with advanced prostate tumour cells reportedly being more sensitive to this agent. Therefore, TRAIL may have future utility as a potential therapeutic strategy for treating advanced prostate cancer. Of interest to us, it has been shown that TRAIL induces the expression of the CXC-chemokine interleukin-8 (IL-8) in cancer cell lines and that IL-8 signaling attenuates the sensitivity of an ovarian carcinoma cell line to undergo TRAIL-induced death. In past studies we have determined that chemotherapy-induced IL-8 signaling modulates the expression of anti-apoptotic genes of the Bcl-2 and IAP families that act to regulate the intrinsic, mitochondrial-dependent apoptosis pathway. The objective of this study was to characterize a potential mechanism through which IL-8 signaling may modulate TRAIL- and chemotherapy-induced activation of the extrinsic apoptosis pathway.
Results: Experiments were conducted in LNCaP and PC3 cells. Administration of exogenous IL-8 failed to alter cell-surface death receptor (DR) or caspase-8 expression that co-mediate the apoptosis-inducing activity of TRAIL. However, real-time PCR and immunoblotting experiments revealed that IL-8 signaling increases the transcription and expression of the predominant splice forms of the native caspase-8 inhibitory protein c-FLIP in the androgen-independent PC3 and androgen-dependent LNCaP cell lines. Pre-treatment with the CXCR2 antagonist AZ10397767 significantly attenuated IL-8-induced c-FLIP mRNA expression in either cell line. Furthermore, inhibition of androgen receptor (AR)- and NF-κB-mediated transcription attenuated IL-8 induced c-FLIP expression in the LNCaP and PC3 cells, respectively. Although both cell lines were poorly sensitive to rTRAIL, co-administration of AZ10397767 increased the sensitivity of these cell lines to the cytotoxic effects of rTRAIL, increasing the efficacy of TRAIL-induced apoptosis. Immunoblotting also confirmed an enhanced cleavage of PARP that was co-incident with the down-regulation of c-FLIP following the co-administration of rTRAIL with AZ10397767 relative to the effect of rTRAIL alone. The role of c-FLIP in underpinning the chemoresistance of prostate cancer cells to chemotherapy was studied in further experiments. Using a non-isoform selective siRNA strategy to target c-FLIP expression, we observed that depletion of this protein induced spontaneous apoptosis and a loss of cell viability in prostate cancer cell populations. Co-administration of c-FLIP siRNA also resulted in a significant potentiation of chemotherapy-induced cytotoxicity in LNCaP and PC3 cells increasing oxaliplatin and docetaxel-induced apoptosis.
Conclusions: Our results suggest that IL-8 signaling can modulate the extrinsic apoptosis pathway in prostate cancer cells through direct transcriptional regulation of c-FLIP. Given the observed increases in sensitivity to TRAIL and cytotoxic chemotherapy agents, targeted inhibition of IL-8 signaling or c-FLIP expression in prostate cancer may be attractive therapeutic intervention to assist in sensitizing this disease to chemotherapy.

OR10

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Vascular Targeted Photodynamic Therapy with ADPM Agents

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Photodynamic therapy (PDT) is a treatment modality for a range of diseases including cancer. We have developed a new class of non-porphyrin PDT agent, the BF_2-chelated tetraaryl-azadipyrromethenes (ADPMs). Previously, we have demonstrated that the ADPM class of compounds displays excellent photochemical and photophysical properties for therapeutic application (1,2). *In vivo* studies now show that treatment of human tumour-bearing nude mice with ADPM06 and light (690nm) leads to eschar development, subsequent tumour ablation and lesion healing. Studies using the MDA-MB-231 GFP-expressing model of human breast cancer show tumour ablation in 86% of animals after I.V. delivery of ADPM06 (2 mg/kg) followed immediately by irradiation with 150 J/cm^2 light. We have utilised inherent drug fluorescent properties to describe organ bio-distribution patterns. Fluorescence images were acquired using an IVIS Spectrum imaging system (excitation/emission wavelengths: 640nm/720nm respectively). Peak fluorescence intensity was observed in the lungs, liver, kidneys, heart and spleen within one hour following drug administration. Fluorescence approached baseline levels within 24 hours and appeared to be completely cleared from the animal by 48 hours. Fluorescence from tumour tissue significantly declined 3 hours post-administration and reached baseline levels by 48 hours. We have postulated that ADPM06 is predominantly retained in tumour vasculature within the first few minutes following administration. Thus, using a short drug-light interval, we have sought to elicit a tumour vascular targeting response. In order to test this hypothesis directly, dynamic PET with continuous I.V. infusion of ^{18}F-FDG has been applied over a 2 hour period (3). After initial tracer uptake (~30 min), rats bearing 13762 mammary carcinoma tumours on both sides of the chest wall were treated by parenteral administration of ADPM06 (0.8 mg/kg), followed immediately by irradiation of one tumour with light. For comparison of effect, mice bearing two EMT-6 mammary tumours first received ADPM06 (2 mg/kg) and one tumour was then irradiated 30 min later while infusing FDG and imaging with PET. Dynamic list-mode PET data were sorted into 5 min frames and kinetic profiles plotted. Immediate irradiation after ADPM06 administration generally resulted in decreased ^{18}F-FDG tumour uptake over time. Such behaviour is compatible with a vascular targeting response to therapy. Our data continues to show the ADPM family of compounds to be an exciting new class of photosensitiser having significant potential for further translational development.

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1.Gorman A *et al.* J Am Chem Soc. 2004 Sep 1;126(34):10619-31. **2.**Gallagher *et al.* Br J Cancer. 2005 May 9;92(9):1702-10. **3.** Bérard et al, J Nucl Med, 2006 Jul;47(7):1119-26.

OR11

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Title: Role of Human Epidermal Receptor targeted therapies in chemo-sensitization of human gastro-oesophageal cancer cells.

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Abstract: **Background**: Over the last decade, major improvements in overall survival in patients with early stage and advanced oesophageal cancer have been achieved with the introduction of combined chemotherapy treatment. However, resistance to chemotherapy is a major barrier and sensitization of oesophageal cells to chemotherapy may be an important anticancer strategy. The aim of this study was to examine the effect of the epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI) gefitinib, EGFR-monoclonal antibody inhibitor panitumumab and dual EGFR/human epidermal receptor 2 (HER2)-TKI lapatinib on the sensitivity of human adenocarcinoma oesophageal cancer (EC) cell lines (OE19, OE33, SEG-1, FLO-1) to chemotherapy (5-FU and cisplatin). **Methods**: Cell viability was assessed using MTT assay. Apoptosis was measured by Flow Cytometry, PARP and caspase 8-cleavage. EGFR, HER2, HER3 and Akt expression/phosphorylation were determined by Western blotting. **Results**: All EC cell lines were found to be resistant to 5-FU and cisplatin treatment with IC_{50} doses for 5-FU ranging from 20μM to 50μM and for cisplatin between 40μM and 100μM. A synergistic interaction between chemotherapy and panitumumab, gefitinib or lapatinib was observed in both OE19 and OE33 cell lines and this was correlated with a dose dependent increase in EGFR, HER2, HER3 and Akt activation following 5-FU and cisplatin treatment. Furthermore, we found that panitumumab, gefitinib and lapatinib abrogated chemotherapy-induced EGFR/HER2/HER3 and Akt activation and this inhibition was most pronounced following the dual EGFR/HER2 inhibitor lapatinib. **Conclusions**: Our findings indicate that EC cell lines respond to chemotherapy with an EGFR/HER2/HER3-mediated survival response. Thus, inhibiting EGFR and HER2 may have therapeutic potential for sensitizing oesophageal tumours to chemotherapy. We are currently investigating the mediators of this anti-apoptotic stress response following chemotherapy treatment.

OR12

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Title: Proteasome activity profiles differ between acute and chronic phase BCR-ABL positive cell lines

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Abstract: Chronic myeloid leukaemia is a malignant proliferation of BCR-ABL positive cells. Imatinib induces remission, but not cure, by inhibiting the BCR-ABL tyrosine kinase. New strategies are required both to achieve cure and to overcome refractory disease. The proteasome degrades intracellular proteins by three proteolytic activities, the chymotrypsin-like (CT-L), trypsin-like (T-L) and caspase-like (PGPH) activities. These activities are associated with the β5, β2 and β1 subunits respectively. We have previously shown that BCR-ABL positive cells are more sensitive than normal haematopoietic cells to the anti-proliferative and pro-apoptotic effects of proteasome inhibition. In this study we measured the proteasomal activities of two different BCR-ABL positive cell lines. The K562 cell line is derived from a human chronic myeloid leukaemia and is of erythroid lineage. The SD-1 cell line is derived from a BCR-ABL positive acute lymphoid leukaemia. The component proteolytic activities were analysed in these cell lines using fluorogenic substrate assays and an active site-directed probe. Fluorogenic assays were performed by monitoring the release of the fluorophore AMC from peptide substrates specific for each activity (CT-L:Succ-LLVY-AMC, T-L:Z-ARR-AMC, PGPH:Z-LLE-AMC). In K562 cells the CT-L, T-L and PGPH activities were 13 ± 4.8, 79 ± 16 and 80 ± 14 AFU/min/50 µg of protein and in the SD-1 cells were 131 ± 39, 3 ± 0.6 and 25 ± 10.4 AFU/min/50 µg, respectively (n=3 in both cell lines). Catalytic activities were also labelled with the active site-directed probe, DansylAhx$_3$L$_3$VS. DansylAhx$_3$L$_3$VS is a cell permeable inhibitor that irreversibly binds to the catalytic activities of the proteasome. This probe has a dansyl tag attached to allow detection of the labelled active sites. Using immunoblotting, the dansyl labelled CT-L/PGPH sites accounted for 65 ± 10% of total activity in the SD-1 cell line and only 20 ± 11% in the K562 cell line.

Multiple proteasome inhibitors are in development which differentially inhibit the component proteolytic activities. The currently available proteasome inhibitor, bortezomib, is designed to inhibit the CT-L β5 subunit. Proteasome proteolytic profiling may be necessary to determine if a specific inhibitor will be efficacious in a given patient and individually tailor treatment.

OR13

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Title: Tribbles homolog 3 (TRB3) a novel regulator of bile acid signaling in esophageal cells that may be lost in esophageal carcinogenesis.

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Abstract:
Deoxycholic acid (DCA), has been shown to be present in the reflux aspirates of GERD patients and has been postulated to be involved in esophageal adenocarcinoma (EAC) promotion. Previous studies from our laboratory have demonstrated both increased levels of NF-kB activation and concomitant IL-8 expression in Barrett's esophagus (BE) and EAC. Using microarray technology and a bio-informatic approach we have identified a cohort of genes regulated by DCA and altered in the Metaplasia-Dysplasia-Adenocarcinoma (M-D-A) sequence (30% of Tt M-D-A genes). This cohort of DCA regulated genes which included inflammatory genes such as IL1, IL6 and IL8 could accurately classify between normal, Barrett's and adenocarcinoma tissue when hierarchically clustered supporting the role of bile acids in EAC. The pseudo-kinase TRB3 was identified as a gene specific to the non-transformed squamous HET-1A cells, absent in the SKGT4 cancer cells and down-regulated cancer sequence microarray data. This protein has numerous binding partners such as p65 subunit of NF-kB, CHOP, AKT, MEK1 and BMPRII commonly deregulated in BE and EAC and represented a potential master regulator of several pathways involved in carcinogenesis. TRB3, IL8, IL6, CHOP and GDF15 expression was assessed in both HET-1A and SKGT4 cells in response to DCA by real-time RT-PCR (ABI 7900HT). Inhibition of TRB3 was achieved through the use of a pool of siRNA molecules (Dharmacon). Bioinformatic analysis was performed using Genespring, Metacore and Ingenuity Pathway Analysis. We have now assessed the functional role of TRB3 in the regulation of cytokine expression regulated by pathways including NF-kB and MAPK (IL8+IL6). TRB3 expression could be significantly inhibited (80%) utilizing siRNA approaches in HET-1A cells. siRNA suppression of TRB3 in HET 1A cells resulted in increased levels of IL8 (5 fold) and IL6 (7 fold) over control cells. These findings are in keeping with the loss of TRB3 impacting on NF-kB and/or MAPK pathways leading to unchecked basal and inducible levels of cytokine gene expression as observed in BE, EAC and the cell line model. Additionally, DCA exposure of HET-1A cells resulted in the induction of CHOP mRNA expression (25 fold) which was reduced upon pre-treatment of HET-1A cells with siTRB3 (12 fold) demonstrating a role for this protein in DCA mediated ER stress in esophageal cells. Through a novel informatic and functional approach the relevance of bile acid regulated genes such as TRB3 in esophageal carcinogenesis has been assessed. This study strengths the role of secondary bile acids such as DCA in EAC the complete understanding of which may provide new avenues for therapies.

OR14

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Title: INHIBITION OF STRESS-INDUCED INTERLEUKIN-8 SIGNALING SENSITIZES PROSTATE CANCER CELLS TO IONIZING RADIATION

Authors: R Gallagher; PJ Maxwell; S Berlingeri; C Askin; A Seaton; C Wilson; P Scullin; J Pettigrew; IJ Stratford, KJ Williams, PG Johnston and DJJ Waugh
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Abstract

Clinically relevant levels of hypoxia are detected in 30-90 % of prostate cancers. Hypoxic cancer cells are resistant to radiotherapy leading to the selection of cells with a more malignant phenotype. The expression of interleukin-8 (IL-8) plays an important role in the tumorigenesis and metastasis of prostate cancer. Recently, we detected elevated expression of IL-8 and its receptors CXR1 and CXCR2 in prostate cancer tissue. The aims of this study were to (i) determine whether hypoxia is an environmental stress underpinning increased IL-8 and IL-8 receptor expression in prostate cancer cells and (ii) whether hypoxia-induced IL-8 signaling confers a survival advantage and renders hypoxic cells more resistant to radiation therapy. Exposure of PC3 cells to hypoxia resulted in time-dependent increases in IL-8, CXCR1 AND CXCR2 gene expression, detected using real-time PCR analysis. This was independently confirmed by ELISA demonstrating time-dependent increases in IL-8 secretion and by immunoprecipitation-immunoblotting and flow cytometry experiments that confirmed elevated total and cell-surface CXCR1 and CXCR2 expression in hypoxic PC3 cells. Mechanistic studies determined that inhibition of hypoxia–inducible factor (HIF-1) and nuclear factor-κB (NF-κB) transcriptional activity abrogated the hypoxia-induced transcription of CXCR1 and CXCR2 in PC3 cells. Chromatin-IP analysis also confirmed the binding of HIF-1 and NF-κB to the 5'-UTR region of the CXCR1 and CXCR2 genes. Therefore, hypoxia was shown to potentiate the constitutive autocrine/paracrine IL-8 signaling stimulus that prostate cancer cells are subject to. Similarly, exposure to ionizing radiation at clinically relevant doses (ie<2Gy) also potentiates the transcription of each of the IL-8, CXCR1 and CXCR2 genes in prostate cancer cell lines. In either case, the induction of IL-8 signaling is coupled to the activation of cell survival pathways. Consequently, siRNA-mediated inhibition of CXCR1 and CXCR2 expression renders hypoxic cells more sensitive to DNA damage and increases the cytotoxicity of ionizing radiation on prostate cancer cells. Attenuation of the IL-8 receptors, using an siRNA approach, resulted in a decrease in cell survival following exposure to ionising radiation.

OR15

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Title: Differential gene expression profiles as markers of radioresistance in oesophageal cancer

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Abstract: **Purpose** - Oesophageal cancer is an extremely aggressive disease with increasing annual incidence and an extremely poor prognosis. Current treatment regimes incorporate a multimodal approach, whereby patients receive neoadjuvant radiochemotherapy prior to surgery. Molecular predictors of response to therapy are essential to improve patient selection and ultimately therapeutic efficacy for this disease. The purpose of this study was to identify genes and gene sets indicative of resistance to radiation in oesophageal cancer cell lines. **Methods** - KYSE-410 human oesophageal squamous cell carcinoma cells were treated with fractionated doses of 2 Gy X-ray radiation (250 keV, 15 mA, 38 s) every 2 weeks until radioresistant cell lines were established. The parent line was maintained in a similar manner to the radioresistant cells except it received no radiation. Resistance to radiation was determined as a measure of sensitivity to radiation-induced apoptosis, which was measured by Annexin-V/PI staining and flow cytometry. The KYSE-410 parent and radioresistant clone was treated with or without 2 Gy radiation. After 24 h cells were harvested, total RNA isolated, and cDNA generated. cDNA from each treatment was applied to pathway-focused qRT-PCR arrays and differentially-induced gene expression analysed. Radioresistance-associated genes identified from the arrays were validated by semi-quantitative RT-PCR. **Results** - It was found that treatment of KYSE-410 cells with continuously fractionated doses of radiation resulted in the generation of radioresistant clones. Exposure of KYSE-410 parent to a bolus 10 Gy (250 keV, 15 mA, 3 min 5 s) resulted in 25.4±1.5% versus 5.3±1.5% in unstimulated control. Exposure of KYSE-410 radioresistant clone to 10 Gy radiation resulted in 7.7±1.3% apoptosis compared to 3.9±0.9% apoptosis in unstimulated control cells. Gene array analysis revealed that 9 genes (from a total of 84) were differentially regulated in response to radiation between parent and radioresistant cells. These genes were involved in cell cycle control (p16), apoptosis (Granzyme A, TNFα, TNFRSF25), invasion and metastasis (MMP1, S100A4 (also involved in cell cycle control)), angiogenesis (IFNβ1, TGFβ1), and gene transcription (c-Myc). In response to radiation in radioresistant cells p16, IFNβ1, MMP1, S100A4, and TNFRSF25 were upregulated, while granzyme A, c-Myc, TGFβ1, and TNFα were downregulated relative to parent controls. The effect of X-ray irradiation on the expression of these genes was subsequently validated by RT-PCR. **Conclusions** - These data indicate that genes involved in cell cycle control and apoptosis are differentially regulated in response to radiation in radioresistant versus radiosensitive oesophageal tumour cells. Future work will assess the involvement of the identified genes in resistance to radiation-induced apoptosis, and the expression of a number of these markers in patient biopsy material.

OR16

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Title: The role of Bax and XIAP in regulating c-FLIP silencing-induced cell death
Authors: T.R. Wilson, D. Logan, K. McLaughlin, P.G. Johnston and D.B. Longley
Institution: Queen's University Belfast
Abstract:
Death receptors (DR) such as Fas, DR4 and DR5 trigger apoptosis by activating caspase-8. A key inhibitor of DR signalling is c-FLIP. Previous studies have shown that c-FLIP is a key regulator of colorectal cancer cell survival and is a key mediator of resistance to death ligands and chemotherapy. In type I cells, sufficient caspase-8 is activated to induce apoptosis independently of the mitochondrial pathway. However, in type II cells, caspase-8 requires amplification through the mitochondria. In this regard, caspase-8 cleaves Bid, which translocates to the mitochondria resulting in Bax- and Bak-mediated release of pro-apoptotic molecules, including cytochrome c, part of the caspase-9 apoptosome, and SMAC, an inhibitor of the IAPs. The aim of this study was to investigate whether c-FLIP gene silencing can bypass the requirement for mitochondrial involvement in DR-mediated apoptosis by enhancing caspase-8 activation and thereby converting type II cells into type I cells.

To test this hypothesis, we utilised the HCT116 $Bax^{+/-}$ and $Bax^{-/-}$ colorectal cancer cell lines. In $Bax^{+/-}$ cells, silencing c-FLIP with c-FLIP-targeted siRNA (FT) induced caspase-8, -3 and -9 activation and cell death, however cell death was not observed in the $Bax^{-/-}$ cell line. Of note, in $Bax^{-/-}$ cells, full caspase-8 activation, but incomplete processing of caspase-3 to the p19 form (but not the fully active p17 form) was observed. In addition, FT siRNA sensitised the $Bax^{+/-}$, but not the $Bax^{-/-}$, cell line to TRAIL- and chemotherapy-induced cell death. We found that downregulating the expression of Bid abrogated the cell death induced by FT siRNA in both the HCT116 parental and HT29 cell lines, indicating that Bid mediates cross-talk between the extrinsic and intrinsic apoptotic pathways. Interestingly, inhibition of caspase-9 using siRNA failed to protect HCT116 and HT29 cells from FT siRNA-induced cell death. We found that co-treating $Bax^{-/-}$ cells with FT siRNA and a SMAC peptide induced cell death suggesting the involvement of the IAPs. Co-silencing XIAP and c-FLIP in $Bax^{-/-}$ cells induced full activation of caspase-3 and apoptosis. This study shows that silencing c-FLIP does not necessarily bypass the requirement for mitochondrial involvement in DR-mediated apoptosis in type II cells, and suggests multiple targeting of anti-apoptotic molecules may represent a therapeutic strategy for the treatment of colorectal cancers in which mitochondrial cell death is dysfunctional.

OR17

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Title: SEMA3A decreases CXCR4 expression in B-cell Chronic Lymphocytic Leukaemia
Authors:Brendan Power, Philip Murphy, Judy Harmey
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Abstract:
B-cell Chronic Lymphocytic Leukemia (B-CLL) is characterized by the accumulation of B-CLL lymphocytes in the blood, marrow and secondary lymphoid tissues. B-CLL cells have a long survival owing to alterations in the normal pathways of apoptosis. It has been shown that B-CLL cells rapidly undergo apoptosis during in-vitro culture indicating that signals from the micro-environment are of vital importance in maintaining resistance to apoptosis. In the marrow and lymphoid tissues CLL cells are in close contact with stromal cells that constitute distinct microenvironments. Co-culture of CLL cells with bone marrow stromal cells in-vitro can support long term cell survival. The chemokine receptor CXCR4 plays an important role in regulating the migration and survival of B-CLL cells through interactions with stromal cells. The secretion of the CXCR4 ligand CXCL12 by stromal cells attracts B-CLL cells and provides protection from spontaneous or induced apoptosis.
Vascular endothelial growth factor (VEGF) and it's receptors are also involved in the regulation of CLL cell migration and survival. Studies in other cell types have shown VEGF signaling to be involved in regulating CXCR4 levels. We have examined VEGF signalling in regulating CXCR4 levels in CLL cells.
Expression levels of VEGF receptors (VEGFRs)-1 and 2 in CLL samples were determined by flow cytometry. Expression of the VEGFR co-receptor Neuropilin-1 (NRP1) in CLL samples was examined by Western blot.
Treatment of CLL cells from patients with the NRP1 ligand SEMA3A which is a competitive inhibitor of VEGF binding to NRP1 resulted in decreased CXCR4 expression levels as determined by flow cytometry ($n=7$, $p<0.05$). Culture of CLL cells with a VEGF blocking antibody resulted in a variable change in CXCR4 levels which appears to correlate with VEGFR1 expression levels. Treatment of CLL cells with the VEGFR signalling inhibitor SU5416 caused a decrease in cell survival in a number of patient samples which also appears to correlate with VEGFR1 expression levels.
These results show that signalling through the VEGF co-receptor NRP1 plays a role in regulating CXCR4 levels in CLL cells. This pathway may therefore represent a target for future treatment in CLL.

OR18

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Title: Characterization of a cytoskeletal signaling pathway underpinning CD44-initiated, integrin-mediated adhesion of breast cancer cells to bone marrow endothelium

Authors: Suzanne McFarlane, Ashleigh McClatchey, Patrick G. Johnston & David J. Waugh.

Institution: CCRCB, Queen's Univeristy Belfast

Abstract:

Background: Bone metastasis is a frequent complication of breast cancer. It is estimated that up to 85% of breast cancers will metastasise to the bone. The selective metastasis of breast cancer to the bone is thought to result from the preferential adhesion of breast cancer cells to the bone marrow endothelial cells lining the bone marrow sinusoids. Our studies have shown that CD44 promotes the primary adhesion of breast cancer cells to bone marrow endothelium in vitro. The aim of this study was to further explore the cascade of events underpinning CD44-initiated adhesion.

Methods: Experiments using parental and bone-homing (BO) clones of the MDA-MB-231 breast cancer cell line established the importance of CD44 to integrin mediated adhesion to bone marrow endothelial cells (BMECs).

Results: MDA-MB-231BO cells displayed increased CD44 expression and adhesion to both BMECs and fibronectin, relative to parental cells. MDA-MB-231BO cells also displayed increased expression and activation of the β1-integrin subunit. In addition the bone homing cells exhibited elevated constitutive phosphorylation of the kinases Src and FAK and the cytoskeletal proteins cortactin and paxillin relative to the parental cells. Stimulation of MDA-MB-231BO cells with the CD44 ligand hyaluronan (HA) induced an increase in the expression of the β1-integrin chain, FAK and paxillin and furthermore, promoted a rapid increase in the activation status of the β1-integrin subunits, and the phosphorylation of Src, cortactin and paxillin in these cells. The HA-induced phosphorylation of paxillin was attenuated by depletion of CD44 and cortactin expression using selective RNAi strategies, suggesting that it is a downstream target of HA-CD44-cortactin signaling. MDA-MB-231BO cell adhesion to fibronectin or to hBMECs was attenuated by RNAi-mediated suppression of CD44, cortactin and paxillin expression or following administration of two neutralizing antibodies that inhibit β1-integrin and α4β1-integrin receptor signaling. Antibody-based inhibition of integrin signaling also attenuated the HA-induced phosphorylation of cortactin and paxillin suggesting that these proteins constitute a signaling cascade activated downstream of a CD44-initiated, integrin-dependent process.

Conclusion: Our results describe a molecular pathway promoting cytoskeletal reorganization that is activated downstream of a CD44 induced, integrin-dependent event and which is critical to efficient breast cancer cell adhesion to hBMECs.

OR19

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Title: Changes in hormonal receptor status in a series of breast cancer patients treated with neoadjuvant therapy.

Authors: **O'Connor M**[1], McCormack O[2], Aherne S[3], Murphy H[2], Geraghty J[2], Rothwell J[2], Jeffers M[3], Walshe JM[1]

Institution: Department of Medical Oncology[1], Surgery[2] and Histopathology[3], The Adelaide and Meath Hospital, Tallaght, Dublin 24

Background: Neoadjuvant therapy (NAT) using chemotherapy or hormonal therapy is used to treat inflammatory and locally advanced breast cancer. Hormonal receptor (HR) status and human epidermal growth factor receptor (HER2*neu*) status are two factors used to guide decisions regarding systemic therapies. While HER2*neu* status tends to be stable, alterations in HR status following NCT have been reported in the literature. The purpose of this study was to investigate the discordance rate of HR and HER2*neu* status between the initial diagnostic biopsy and the viable tumour remaining after NAT.

Methods: The records of all breast cancer patients (pts) who were recorded as having received neoadjuvant therapy during the period 2003-2007 were retrospectively identified and reviewed. Data collected included: age, menopausal status, neoadjuvant therapy administered, breast surgery performed, adjuvant therapy administered and disease status at last follow-up. HR and HER2*neu* status pre and post NCT were reviewed. HR status i.e. oestrogen receptor and progesterone receptor were determined by immunohistochemistry (IHC). HER2*neu* status was determined using IHC with FISH on equivocal cases (IHC 2+).

Results: During the period 2003-2007, 34 pts received NCT. At present complete data is available on 31 pts. At diagnosis, 22 pts had locally advanced disease, 4 pts had inflammatory breast cancer and 5 pts had metastatic breast cancer. The mean age at diagnosis is 49 years (range 31-69 years). At initial diagnosis, 25 (81%) pts were HR positive, 6 (19%) pts were HR negative, 6 (19%) pts were HER2*neu* positive, 25 (81%) pts were HER2*neu* negative. Following NCT, complete pathological response was documented in 6 (19%) pts. Repeat HR and HER2*neu* status was assessed in the remaining twenty five tumours. Five (16%) pts underwent a change in HR status and 1 pt (3%) underwent a change in HER2*neu* status. Of the five HR changes: 4 pts with HR positive disease initially became HR negative and 1 pt with HR negative disease initially became HR positive. The HER2*neu* change was from IHC negative (score 1+) to IHC positive (score 3+). At a median follow-up of 21 months (range 6-39mos) 22 pts (71%) are alive and 9 pts (29%) have died of metastatic disease.

Discussion: A discordant rate of 16% in HR status and 3% in HER2*neu* status was found in this single institution study which is consistent with the published literature. Decisions regarding systemic therapies tend to be based on initial HR and HER2*neu* status. The findings of this study emphasise the need to reassess HR and HER2*neu* status post NCT as alterations may lead to changes in systemic therapy.

OR20

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Title: **Interleukin-6–stimulated DNA methylation in colon cancer cells: a mechanism of tumour suppressor gene silencing.**

Authors: Eilis Foran and Laurence J. Egan

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Abstract:

It is not known how chronic gastrointestinal inflammatory diseases predispose to the development of cancer in affected organs. Our programme of research models this problem in the colon. Inflammatory Bowel Disease (IBD) affects 1.4 million people in the US and 2.2 million people in Europe, and results in chronic inflammation which increases the risk of colorectal cancer. IBD is associated with high concentrations of interleukin-6 (IL-6), a pro-inflammatory cytokine implicated in colon cancer. In patients with colitis-associated cancers, a CpG methylator phenotype has been described, indicating that a link may exist between chronic inflammation and gene silencing by promoter methylation in these patients. Moreover, elevated expression of DNA methyltransferase 1 (DNMT1) has been observed in many cancers. We tested the idea that IL-6 exposure might affect tumour suppressor gene expression through effects on epigenetic gene silencing. We found that IL-6 treatment of HCT116 colon cancer cells increased methylation of the promoter regions of a panel of 13 genes associated with tumour suppression, adhesion and apoptosis resistance. Expression levels of 4 transcripts (IRF-7, Maspin, PAI-1 and IL-4) were subsequently confirmed to be down-regulated between 2 and 5-fold following treatment with IL-6. Moreover, IL-6-stimulated down-regulation of these transcripts could be prevented by pre-incubation with DNMT inhibitor 5-aza-cytidine, indicating that the effect of IL-6 on expression levels of these genes is methylation-dependent. IL-6 treatment increased DNMT1 protein expression by about 2 to 3-fold in HCT116 cells and lesser effects were seen on DNMT1 mRNA levels. JAK2 and STAT3 were phosphorylated in response to IL-6 treatment, but luciferase assays on the DNMT1 P1–P4 promoter regions showed no activation of the DNMT1 promoter activity by IL-6. Neither cell cycle arrest nor inhibition of protein synthesis affected the IL-6-induced increase in DNMT1 expression, suggesting that IL-6 may regulate the stability of DNMT1 protein. Further experiments are underway to assess this possibility. Current work is focused on establishing coordinate expression of IL-6 and DNMT1 in sporadic and colitis-associated colon cancers. Our results indicate that IL-6, a component of the inflammatory tumour microenvironment may promote colonic tumorigenesis through DNMT1-mediated tumour suppressor gene silencing. Similar pathways may also exist in other inflammation-associated tumours of the gastrointestinal tract.

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OR21

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Title: Effect of PNC1 on Epithelial-Mesenchymal Transition in transformed cells.
Authors: Cedric Favre & Rosemary O'Connor
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Abstract:
The Insulin-like Growth Factor-I (IGF-IR) is a transmembrane receptor tyrosine kinase which has important functions in regulating metabolism, survival, and carcinogenesis. Although activation of the PI3-kinase/Akt/mTOR signalling pathway may largely mediate the effects of this receptor on cell proliferation and survival, it is not clear that this pathway mediates all of the effects of IGF-IR signalling on cancer progression, in particular cell migration and invasion. We recently identified a new inner mitochondrial membrane carrier protein whose expression is induced by IGF-I or Insulin in a PI3-kinase and mTOR-dependant manner. This carrier was designated Pyrimidine Nucleotide Carrier 1 (PNC1) based on its ability to transport pyrimidine nucleotides (UTP) into mitochondria. Over-expression of PNC1 enhances cell size, while a reduction in PNC1 expression causes reduced cell size. The objective of this study was to investigate the mechanism of action of PNC1 in cancer cell growth and migration. To do this we used cells in which PNC1 was either over-expressed or suppressed with siRNA. Suppression of PNC1 in MCF-7 and HeLa cells results in a drastic change of cell morphology with the acquisition of an elongated shape and protrusions at the periphery. This correlated with a decrease in adhesion to both collagen and fibronectin; an increase in migration; and greatly increased colony formation in soft agar assays. The cells exhibited the features of an Epithelial-Mesenchymal Transition (EMT) phenotype that was demonstrated by reduced E-cadherin at cell-cell contacts, as well as increased Vimentin, N-cadherin and β-catenin expression. EMT can be induced by Reactive Oxygen Species (ROS), which can mediate a retrograde signalling pathway from mitochondria that has previously been associated with phenotypic changes including EMT. Since PNC1 is a mitochondria carrier protein we investigated whether the effects of PNC1 on EMT were due to mitochondria-derived ROS. We found that cells over-expressing PNC1 had reduced cellular ROS levels, whereas cells with suppressed PNC1 expression had increased ROS levels. Mitochondria were shown to be the main source of this ROS. Overall our data indicate that PNC1 levels influence mitochondrial retrograde signalling and production of ROS. The data suggest that PNC1 may be induced by the IGF-I/mTOR signalling pathway to regulate production of ROS from the mitochondria and thereby to determine whether acquire transformed cells acquire an invasive phenotype characterized by EMT.

OR22

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Cathepsin S propeptide attenuates cell invasion by inhibition of Cathepsin L-like proteases in the tumour microenvironment

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The lysosomal cysteine cathepsins have been implicated in tumour invasion and metastasis due to their ability to degrade components of the extracellular matrix, when secreted by tumour cells. This has lead to much interest in the development of inhibitors specifically targeting this family, as potential anti-invasive therapeutic agents.

Each cathepsin contains an N-terminal propeptide domain which functions as a molecular chaperone and also as an inhibitor, rendering the protease inactive until it has reached the lysosome. Previous research has shown how application of these synthetic propeptides has the ability to inhibit their cognate protease activities, in addition to other proteases within the CatL-like sub-family.

In this investigation we have produced a recombinant form of the CatS propeptide and have shown that it is a potent inhibitor of the peptidolytic, elastinolytic and gelatinolytic activities of the CatL-like proteases. In addition, we have also demonstrated that this recombinant propeptide is capable of significantly attenuating the rate of tumour cell invasion in a range of human cancer cells. *In vitro* invasion assays were performed using astrocytoma, prostate, breast and colorectal carcinoma cell lines, where up to 50% reduction in tumour invasion was observed with a 200 nM dose over 24 hrs..

Furthermore, fusion of an IgG_2 Fc-domain to the C-terminus of the recombinant propeptide resulted in a fusion protein with a significantly enhanced ability to block tumour cell invasion in comparison to the original protein. This Fc-fusion protein was found to have a much improved half-live in cell-based assays in comparison to the unmodified propeptide species.

In conclusion, based on our research findings, we believe that the simultaneous inhibition of multiple cysteine cathepsins may represent a potential therapeutic target and that this propeptide species may represent the basis for novel therapeutics to attenuate tumorigenesis in a range of human malignancies.

OP1

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Title: **Identification and functional characterisation of cyclophilin A as a novel regulator genome stability**

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Abstract:
Cyclophilins belong to a group of proteins that have peptidyl-prolyl isomerase (PPIase) activity and also includes the structurally distinct FK-506 binding proteins and the parvulin, pin 1. The PPIase family catalyse the *cis-trans* isomerisation of peptide bonds located to the N-terminal o proline residues in polypeptide chains thereby altering protein conformation [1]. As such PPIas are believed to play a role in protein folding and transport, however their true cellular function remains unclear. In recent years pin1 has been shown to regulate cell growth and signalling and is overexpressed in a variety of tumours, implicating a role in cancer.

Recently cyclophilin A (cypA) was found to be overexpressed in pancreatic and lung cancer cells suggesting a possible role during tumorigenesis [2], however its function during tumor development and progression is unknown. We have recently found that cypA is overexpressed in cells derived from a number of solid tumours including breast, prostate and cervix, and haematopoietic malignancies such as chronic myeloid leukaemia (CML), Jurkat T lymphoma and HL-60 pro-myelocytic leukaemia [3].

In this study we have found that cypA is localised to the nucleus and centrosome in interphase haematopoietic cells. During mitosis, cypA concentrates at the spindle poles and lat migrates to form part of the intracellular bridge during cytokinesis. Centrosomal localisation o cypA was confirmed by double staining of cells with anti-cypA and anti-γ-tubulin, an importan component of the conserved γ-tubulin ring complex (γ-TuRC) that regulates microtubule nucleation and function during mitosis. Merged images illustrate co-localisation of cypA and γ tubulin thereby suggesting a potential role for cyp A during cell division. In support of this, leukaemia and lymphoma cells that do not express cypA undergo defective cell division and display a weakened spindle checkpoint response when treated with the microtubule targeting agents, taxol and nocodazole. Measurement of mitotic index using phosphorylated histone H3 a mitotic marker has revealed that cells that lack cypA exit mitosis prematurely without completing cytokinesis and subsequently re-enter mitosis leading to the accumulation of cells with >4N DNA and exhibiting the morphological features of polyploidy, a characteristic commonly associated with transformation. Collectively, our data strongly suggests a novel role of cypA in the maintenance of genome stability in haematopoietic cells.

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OP2

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Title: **Effects of Docetaxel and Novel Titanocene Analogues on Cell Death in Prostate Cancer Following Down-regulation of Id-1and the IAPs.**

Authors: CM Dowling [a], S Cuffe [a], C Gill [a], M Tacke [b], JM Fitzpatrick [a], RWG Watson [a].
Institution: UCD School of Medicine & Medical Sciences [a] School of Chemistry and Chemical Biology [b]UCD Conway Institute of Biomolecular and Biomedical Research,
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Introduction:

Docetaxel is the standard treatment strategy for androgen-independent metastatic prostate cancer, but only provides a short survival advantage. This indicates a need to both increase the sensitivity of cancer cells to triggers of apoptosis and identify new chemotherapeutic options. Androgen independent prostate cancer cells are associated with significant resistance to apoptosis mediated by a number of anti-apoptotic proteins including: Id-1 (inhibitor of differentiation) which protects against TNF-α- and JNK-induced apoptosis and the IAPs (Inhibitors of Apoptosis Proteins). Working in collaboration with the Centre of Synthesis and Chemical Biology, novel titanocene analogues have been synthesised and we have previously shown that they have promising cytotoxic and apoptotic activity against androgen independent prostate cancer cells mediate through a DNA damage response independent of JNK activity.

Objectives:

To investigate if down-regulation of Id-1 and the IAPs in androgen-independent prostate cancer cells can increase their sensitivity to docetaxel and novel titanocene analogue-induced apoptosis.

Methods:

PC-3 cells were cultured in supplemented RPMI medium containing 10% fetal bovine serum (normal) or 1% FBS (serum depleted). Id-1 and the IAPs were knocked down using siRNA to Id-1 or xIAP, cIAP-1 and cIAP-2 (Triple knockdown). PC-3 cells were assessed for apoptosis and viability using propidium iodide DNA staining by flow cytometry following treatment with docetaxel and the titanocene analogues.

Total protein was isolated from treated cells to confirm knockdown of the respective proteins by western blotting.

Results:

Docetaxel and the novel titanocene analogues induced apoptosis in a time and dose dependent manner in the PC-3 cells. Direct (siRNA) and indirect (serum depletion) down-regulation of Id-1 expression increased apoptotic susceptibility to titanocene- induced apoptosis, however not to docetaxel induced apoptosis. Triple IAP knockdown (cIAP-1, cIAP-2 and xIAP) resulted in the same differential effects.

Conclusions:

The novel titanocene analogues induce apoptosis in androgen-independent prostate cancer cells. Down-regulation of Id-1 and the IAPs increase sensitivity to these novel titanocence DNA damaging analogues but not the spindle inhibitor, docetaxel. In the treatment of any cancer you must not only understand the anti-apoptotic phenotype of the tumour but also the mechanisms of actions of specific treatment strategies.

OP3

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MicroRNA Dysregulation in an Isogenic Human Cell Culture Model of Melanoma Progression

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MicroRNAs (miRNAs) are small non-coding RNAs that function by regulating the translation and degradation of target mRNAs. They play a critical role in developmental and physiologic processes and are implicated in the pathogenesis of several human diseases including cancer. In this study, we analysed the expression of 179 miRNAs in an isogenic series of human melanoma cell lines that mimic key stages in progression of melanoma towards metastasis. This cell line series consists of a parental cell line (WM793), and three derivatives (WM793-P1, WM793-P2, 1205-Lu), each more advanced in tumourigenic, and in some cases metastatic, potential than the parental cell line. To quantify miRNA expression, we employed a highly sensitive TaqMan technique that uses stem-loop primers for reverse transcription followed by real-time PCR, allowing the detection of only the mature and active form of the miRNA. Exon array analysis also allowed us to interrogate several miRNAs, albeit at a lower level of specificity as this platform cannot differentiate between precursor and mature miRNAs. Finally, we also used a new Agilent Bioanalyzer kit to assess the global levels of miRNA in the cell lines. Presently, we have observed a difference in global miRNA levels between cell lines, a phenomenon previously noted in several studies. This global alteration was represented by a 1.6- to 2-fold increase in miRNA concentration between WM793 and derivative cell lines. The study has also revealed a number of miRNAs that are up- or down-regulated in the more tumourgenic cell lines compared to levels in the parental cell line. Of the 179 miRNAs tested, 29 showed a 2-fold increase or decrease in expression between WM793 cells and its derivatives. Interestingly, several of the altered miRNAs map to a single locus on chromosome 14, all of which are up-regulated, an observation that was mirrored in the exon array data. This region has previously been shown to be under the control of imprinting and contains the CTCF-regulated DLK1 and MEG3 genes. Further analysis of these genes revealed an expression pattern that suggests a loss of imprinting. We suspect that this loss of imprinting plays a major role in the up-regulation of miRNAs found in this region.

Funding is acknowledged from the Health Research Board and IRCSET.

OP4

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Title: **Dynamical Systems Analysis of Mitochondrial BAK Activation unifies Agonism/Dissociation Models and Predicts BH3 Mimetic Efficacy**

Authors:
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Abstract:
Mitochondrial outer membrane permeabilization mediated by multidomain BCL-2 proteins (MBPs) BAX and BAK is a pivotal checkpoint during cell killing by anticancer drugs, but is commonly blocked by prosurvival BCL-2 proteins (PBPs), accounting for apoptosis resistance. Proapoptotic BH3 only BCL-2 proteins trigger MBP activation, however considerable controversy surrounds how this activation actually occurs. Understanding this process could increase our understanding of sensitivity and resistance to emerging BH3 peptidomimetic drugs (eg. ABT737 or GX15-070) now entering the clinic. The agonist model states that activator BH3 proteins (ABPs) are essential for driving conformation change of BAK, whereas in the dissociator model, agonism is unnecessary and dissociator BH3s (DBs) achieve MBP activation by releasing ABPs/MBP from PBPs. We have addressed this apparent contradiction through heuristic mathematical modelling of mitochondrial BAK activation. Our results yield robust, experimentally verifiable models of increasing complexity that reveal multiple simultaneous molecular interactions between prosurvival, multidomain and BH3 only BCL-2 family proteins over time. Open BAK conformation (B*) was used as a reaction endpoint, and systems of equations derived by the law of mass action were solved either algebraically or graphically using numerical methods. Where B* is driven by instantaneous concentration jump of an ABP, PBB (called A1) arrests B* formation whereas DBs derepress B*, as observed experimentally. Importantly, we show that in the absence of ABP, a pool of B* must be generated spontaneously and be bound by PBPs in an adenoviral E1B like mechanism. This is essential to 1) maintain system stability 2) to enable dissociator BH3s to drive B*. Critically, the efficacy of DBs is antagonized by a second non-binding PBP in a linear concentration dependence due to occupancy of A2 with both MBP and ABP. B* activation requires another A2-targeting-DB as verified by formation of uncrosslinked BMH monomer in state IV isolated mitochondria following BAD BH3 with increasing levels of NOXA in the presence of BCL-2 and MCL-1. In summary, BAK activation kinetics modelling can predict activity of therapeutically relevant BH3 mimetics and may be a tool for predicting and surmounting resistance mechanisms for novel BH3 mimietics such as ABT737 or GX15-070.

OP5

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Title: Cytogenetic aberrations detected by interphase FISH in CD138 purified plasma cells from MGUS and Multiple Myeloma patients.

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Abstract: BACKGROUND: The success of traditional cytogenetics and interphase FISH in plasma cell dyscrasias has been limited in samples with very low levels of bone marrow involvement, hampering exploration of the relationship between clinical parameters and cytogenetic aberrations in such cases. This study investigated the routine application of CD138 plasma cell purification in the detection of cytogenetic aberrations in plasma cell dyscrasias by interphase FISH.

AIM: To evaluate the success rate of FISH in CD138 purified plasma cells; to report the frequency of cytogenetic aberrations; and to explore relationships between cytogenetic aberrations and clinical parameters.

METHOD: Bone marrow samples, from individuals identified as having a clonal plasma cell population by morphology/flow cytometry were purified using CD138 magnetic microbead autoMACS system (Miltenyi Biotec). Cytospins were prepared from the CD138-positive fraction and analysed by interphase FISH.

RESULTS: An 89% (29/35) success rate for detection of cytogenetic aberrations was observed. The frequency of cytogenetic aberrations in MGUS and myeloma PC were: chromosome 11+, 10% vs. 42%; Monosomy 13(q), 60% vs. 50%; monoallelic del13q14, 11% vs. 0%; monosomy 17(p), 0% vs. 11%; mono/biallelic del17p13.1, 14% vs.18%; IgH translocation, 24% vs. 12%; t(11;14), 24% vs. 0%; t(4;14), 0% vs. 6%; and t(14;16), 0% vs. 6%. No significant difference in the frequency of cytogenetic aberrations was observed between MGUS and myeloma patients. Mean (±SD) LDH and B2M were 385 (± 184) U/l and 6.38 (± 6.86) mg/l. LDH and B2M were positively associated with marrow %PC infiltration ($p < .001$). B2M was positively associated with the presence of t(4;14) in myeloma PC ($p = .033$).

CONCLUSION: CD138 purification provides a high success rate for interphase FISH. Previous studies have shown that all cytogenetic aberrations in MM are also seen in MGUS; however, the prevalence of specific aberrations may differ for MGUS and MM. Although the findings the current study did not reach statistical significance, improved detection rates in aspirates with very low marrow involvement may help to resolve this issue. As expected LDH and B2M were associated with cell proliferation, but only B2M was associated with a specific cytogenetic aberration.

OP6

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Title: ADAM-17: a mediator of chemotherapy-induced EGFR activation

Authors: Joan Kyula, Sandra Van Schaeybroeck, Caitriona Holohan, Daniel Longley & Patrick Johnston.
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Abstract:
Background: Human cancer cells may respond to chemotherapy by activating the epidermal growth factor receptor (EGFR) and survival pathways. Recently, we have shown that colorectal cancer (CRC) and non-small cell lung carcinoma (NSCLC) cells respond to chemotherapy by activating EGFR and are thereby sensitized to EGFR inhibitors. In light of these results, we have investigated the mechanism by which EGFR is activated following chemotherapy treatment in CRC and NSCLC cells.
Methods: Apoptosis was measured by Flow Cytometry and PARP cleavage. EGFR phosphorylation, ADAM-17 and PARP were assessed by Western blotting. Inhibition of ADAM-17 or TGF-α expression was achieved by siRNA and measured by real-time PCR.
Results: We found that the MMP (matrix-metalloprotease) and ADAM (a desintegrin and metalloprotease) inhibitor GM6001 abrogated chemotherapy-induced EGFR phosphorylation in CRC and NSCLC cells, indicating that EGFR activation was mediated by metalloproteases. Further studies indicated that ADAM-17 was the principal ADAM involved in chemotherapy-induced EGFR activation. Furthermore, we found that ADAM-17 regulated TGF-α shedding following chemotherapy treatment. In addition, silencing of ADAM-17 or TGF-α sensitized CRC and NSCLC cells to chemotherapy-mediated apoptosis. The importance of ligand shedding for chemotherapy-induced EGFR activation was further demonstrated by the use of the EGFR-targeted monoclonal antibody cetuximab (C225), which blocks ligand binding. Co-treatment with C225 attenuated chemotherapy-induced activation of EGFR and sensitized CRC and NSCLC cells to chemotherapy.
Conclusions: Our findings indicate that increased EGFR activity following chemotherapy is regulated by ADAM-17-mediated shedding of TGF-α, suggesting that inhibiting specific metalloproteases in combination with chemotherapy may enhance the response of CRC and NSCLC tumours to chemotherapy.

OP7

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Title: *Bfl-1* is a crucial pro-survival Nuclear factor kappa B target gene in Hodgkin/ Reed-Sternberg cells of Hodgkin's Lymphoma.

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Abstract: Hodgkin's lymphoma (HL) is characterized by the presence of mononuclear Hodgkin cells and multinucleated Hodgkin/Reed-Sternberg (H/RS) cells, comprising less than 1 % of the lymphoma tissue. These malignant B cells are surrounded by lymphocytes, plasma cells, eosinophils, histiocytes and stromal cells in the affected lymph nodes. The factors responsible for the neoplastic transformation of H/RS cell precursors remain elusive, and in particular those molecular events that lead to their inappropriate escape from apoptosis. A central role for Nuclear Factor-kappa B (NF-κB) in the pathogenesis of HL has been established and it is now clear that aberrant constitutive NF-κB activity controls a pro-proliferative and pro-survival signaling network in H/RS cells. It has been estimated that 30-50 % of all HL cases contain Epstein-Barr virus (EBV) DNA in the H/RS cells. We have already shown that EBV latent proteins promote resistance to apoptosis in virus-infected B cells and Burkitt's lymphoma cells by regulating *bfl-1* expression via components of the cellular CD40 and Notch signalling pathways (D'Souza *et al*, *J.Virol,* 2000, 74, 6652-6658; D'Souza *et al*, *J.Virol*, 2004, 78, 1800-1816; Pegman *et al*, *J.Virol,* 2006, 80, 8133-8144). Bfl-1 is an anti-apoptotic protein of the Bcl-2 family, whose preferential expression in hematopoietic and endothelial cells is controlled by inflammatory stimuli, and we reasoned that *bfl-1* was therefore a candidate gene that might play an important role in the pathogenesis of HL. Here, we present evidence that (a) *bfl-1* is expressed in both primary H/RS cells from HL tumour biopsies and cultured H/RS cells irrespective of their EBV status; (b) *bfl-1* is an NF-κB target gene in these cells whose regulation is effected through a consensus p65-binding DNA element located in its upstream transcriptional regulatory region; (c) importantly, exogenous Bfl-1 expression rescues cultured H/RS cells from apoptosis induced by anti-NF-κB inhibitors, and (d) knockdown of *bfl-1* potentiates the chemotherapeutic effect of these agents, implying that it is a crucial anti-apoptotic NF-κB target gene in this context. Targeting of Bfl-1 in H/RS cells, by means of its functional blockade or inhibition of its expression, could potentially restore the apoptotic machinery in these cells and increase the sensitivity of HL tumours to chemo- and radiotherapies.

OP8

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Characterisation of DNA damage response pathways in human mesenchymal stem cells (hMSCs).

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Human mesenchymal cells stem cells (hMSCs), which are a key component of the stromal compartment of bone marrow, are progenitor cells for other cell lineages including osteoblasts, chondrocytes and adipocytes. hMSCs also play an important role in maintaining the growth and proliferation of haematopoietic stem cells (HSCs), and may therefore be important in leukaemia. Given that many cancer therapies that act by damaging DNA have side effects on bone marrow function, and can lead to the development of secondary cancers in other tissues as a result of treatment, a better understanding of the function of hMSCs could provide new insights into cancer treatment. Little is known about the effects of DNA damaging agents used in cancer therapy on the hMSC population, or about the effects of differentiation on the sensitivity of hMSCs to such agents. The response of hMSCs to leukaemia treatments, such as chemotherapy using the anthracycline doxorubicin, or ionising radiation prior to bone marrow transplantation, is not well understood on a molecular level. In the present study we have investigated (i) the effects of ionising radiation, doxorubicin and cisplatin on cell survival and activation of DNA damage response pathways in proliferating hMSCs, and (ii) whether osteogenic differentiation alters the response of hMSCs to these agents. The endpoints of cell survival, and activation of a series of DNA damage response proteins, including the tumour suppressor p53, the DNA binding protein replication protein A (RPA), and DNA polymerase eta, a protein required for replication of DNA containing DNA damage, are being examined. hMSCs are relatively resistant to the cytotoxic effects of doxorubicin, cisplatin and ionising radiation, as determined using the trypan blue dye-exclusion assay. Following exposure to 10μg/ml cisplatin for 48 hours, 63% of differentiated hMSCs and 72% of proliferating hMSCs are viable. Following exposure to 10μg/ml doxorubicin for 48 hours, 65% of differentiated and 78% of proliferating hMSCs are viable. 48h following exposure to a single dose of 10 Gy gamma irradiation, 70% of differentiated hMSCs, and 65% of proliferating hMSCs are viable. Treatment with doxorubicin, cisplatin and γ-irradiation increases the level of the tumour suppressor protein p53, in both proliferating and osteogenically differentiated hMSCs. Exposure of hMSCs to cisplatin and doxorubicin also leads to phosphorylation of p53 on serine 392, as determined by western blotting using an anti-p53 phosphoserine 392 antibody. The level of the p53-inducible cell cycle inhibitor p21 increases in response to γ-irradiation. PI-3 kinase related protein kinase (PIKK) signalling is activated in response to DNA damage in hMSCs, as determined by using phosphospecific antibodies to detect phosphorylation of known PIKK substrates, including histone H2AX, and the 34kDa subunit of of the single-stranded DNA-binding protein replication protein A (RPA). Using a series of phosphospecific antibodies, we have found that the 34 kDa subunit RPA is phosphorylated on serines 4, 8 and 33, in response to both doxorubicin and cisplatin, providing evidence of activation of PIKK signalling. Using western blotting, the level of the lesion bypass DNA polymerase, pol eta, is increased following exposure of hMSCs to cisplatin (10μg/ml for 24 hours), doxorubicin (20μg/ml for 24 hours) in proliferating but not in differentiated hMSCs. Thus, hMSCs respond to DNA damaging agents used in cancer therapy by induction of keey DNA damage response proteins including p53 and p21, and activation of PIKK-dependent signalling pathways. These responses may be influenced by the differentiation state of the cells at the time of treatment.

OP9

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Title: Role of Src-family kinases in chemotherapy resistance

Authors: S. Van Schaeybroeck, J. Kyula, C. Holohan, S. Moulik, D. Longley, P. Johnston

Institution: Centre for Cancer research and Cell Biology, Queens University Belfast

Abstract: **Background:** Recent phase III studies in patients with chemo-naïve (CRYSTAL) and chemo-resistant (EPIC trial) metastatic colorectal cancer (CRC) have shown an improvement in response rate and progression-free survival with the addition of the EGFR-monoclonal antibody cetuximab to irinotecan based therapy. The identification of patients who will most benefit from this combined therapy is a crucial issue. Recently, we have shown that CRC cells respond to chemotherapy by activating the EGFR pro-survival pathway and are thereby sensitized to EGFR inhibitors. In light of these results, we investigated the mechanism by which EGFR is activated following chemotherapy treatment in CRC. Previous data of our group have shown that ADAM-17 and TGF-α are critical mediators of chemotherapy-induced EGFR activation. **Methods:** EGFR and SFK phosphorylation were measured by Western blotting. Cell cycle distribution was measured by flow cytometry. Inhibition of c-Src was obtained using the dual Src/Abl inhibitor AZD0530 and specific siRNA for c-Src. TGF-α shedding was measured with an ELISA assay and ADAM-17 activity with a TACE activity kit. **Results:** Following treatment with chemotherapy (5-FU, SN-38 and oxaliplatin), we found that increased EGFR activation was associated with increased Src-family kinase (SFK) activity. Using the SFK inhibitor AZD0530 and c-Src siRNA, we found that chemotherapy-activated EGFR phosphorylation was abolished, indicating that EGFR activation was mediated by c-Src. Furthermore, we found that cetuximab had no effect on chemo-induced activation of SFK, indicating that Src act upstream of EGFR. Moreover, following treatment with AZD0530 and c-Src siRNA, we found complete inhibition of chemotherapy-induced ADAM-17 activity and TGF-α shedding. In addition, when AZD0530 was combined with chemotherapy, we found an additive or synergistic induction of chemo-induced cell death. **Conclusions**: Our findings indicate that chemotherapy induces EGFR activation via a ligand dependent mechanism. We propose a model in which chemotherapy leads to SFK activation that in turn activates ADAM17-mediated shedding of TGF-α. Thus inhibiting SFKs may have therapeutic potential for sensitizing CRC tumours to chemotherapy. Furthermore, detecting TGF-α expression levels into serum of patients may predict response to combination of chemotherapy with EGFR targeted therapies or SFK inhibitors.

OP10

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Title: **Oral immune tolerance mediated by Tregulatory cells may be responsible for the poorer prognosis of foregut cancers**

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Abstract:
Aim
When patients with similar stages of disease at the time of diagnosis are compared, those with cancer of the oesophagus and stomach have a poorer outcome than those with cancer of the rectum and colon. We hypothesise, that the processing of immunogenic tumour cells by the mucosal immune system of the upper gastrointestinal tract, reduces the antitumour immune response, through Tregulatory cells, facilitating a tumour growth advantage.
Methods
Balb/C mice were gavage fed fibrosarcoma tumour in PBS or PBS alone for 14 days. On day 15, subcutaneous tumours were induced. Anti-Treg antibody was administered i.p. at various time points with regard to feeding. Spleen tissue was excised and stained for CD3, CD4, CD8, CD25 and foxp3, to monitor the alterations of immune sub-populations, via flow cytometry, in response to feeding and therapy.
Results
Subcutaneous tumours, in the groups fed tumour antigen, appeared earlier and grew at a faster rate versus those receiving PBS ($p<0.05$). Systemic Treg cell numbers were significantly higher in those exposed to tumour antigen ($p< 0.002$). The administration of anti Treg antibody following feeding overcame the effects of oral tolerance, with 100% of mice curing of tumour. Continuous Treg inactivation during feeding and tumour challenge also resulted in 100% cure. These mice were resistant to tumour re-challenge 90 days post tumour clearance, when fully Treg competent.
Conclusion
Elimination of the effects of oral tolerance by immune-based therapy is now a realizable goal which could confer a relative survival advantage on patients with oesophageal and gastric cancers.

P1

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Title: Geographical mapping of a multifocal thyroid tumour using genetic alteration analysis & miRNA profiling.

Authors: Aherne S, Smyth P, Flavin R, Russell S, Denning K, Li JH, Guenther S, O'Leary J, Sheils O.
Institution: Institute of Molecular Medicine, TCD

INTRODUCTION: Papillary thyroid carcinoma (PTC) frequently presents as multiple tumour-foci within a single thyroid gland. In addition, a significant proportion of PTC are also pluriform, with synchronous tumours comprising different histological variants. This raises the question of the clonal origin of PTC. Among genetic aberrations described in PTC, BRAF V600E mutation and ret/PTC activation occur most commonly. Several studies have investigated the genetic alteration status of multifocal thyroid tumours, producing conflicting results.
To expand on this question of clonality the objective of this study was to examine disparate geographical and morphological areas from a single PTC for the presence of ret/PTC or BRAF mutations and correlate it with miRNA expression profiles.

METHODS: Laser Capture Microdissection was used to harvest cells from areas of classic PTC, insular, and anaplastic carcinoma in addition to tumour cells adjacent to vascular invasion and lymphocytic infiltrate. DNA and RNA were co-extracted and genetic alteration analysis was performed using TaqMan based PCR/RT-PCR. The expression of a panel of 330 miRNAs was examined using stem loop primers for reverse transcription followed by real time TaqMan PCR.

RESULTS: All of the tumour areas proved negative for ret/PTC 1 rearrangement. Two distinct foci with classic morphology harboured the BRAF mutation. All other tumour areas, including the insular and anaplastic were negative for the mutation.
MiRNA profiles were found to distinguish classic PTC from the other tumour types, and differentiate between the more aggressive insular and anaplastic tumours. Profiles included miRNAs previously discovered in this carcinoma, and miRNAs linked to various processes involved in tumour growth and proliferation. Putative gene targets were obtained for the differentially regulated miRNAs. Pathways such as TGF-β and cytoskeletal regulation were significantly over-represented in classic PTC miRNA target lists, and angiogenesis, Wnt, and Ras pathways were significantly over-represented in anaplastic miRNA gene target lists compared to insular cancer.

CONCLUSION: These results suggest that pluriform PTC does not necessarily evolve from classic PTC progenitor foci. Both the differentially expressed miRNAs and their putative targets suggest an image of how PTC obtains its classic papillary structure and indolent state, and depict anaplastic carcinoma as more aggressive than insular cancer with hints at how the cells dedifferentiate, and how the tumour may metastasise.

P2

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Title: Adipokine regulation of tumour cell survival in oesophageal and colorectal cancer cells
Authors: E. Allott, J. Howard, H. Roche, G.P. Pidgeon, J.V. Reynolds.
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Background: The prevalence of obesity has increased markedly over the past two decades and is predicted to rise by at least 1% per annum, contributing to the world's health problems in the form of increasing rates of CVD, diabetes and now cancer. Obesity has been identified as an independent risk factor for both oesophageal and colorectal cancer, and adipose tissue is an important multifunctional endocrine organ secreting numerous protein factors with a range of biological activities. A detailed investigation of the mechanisms linking obesity and cancer is required.
Methods: The effect of leptin and adiponectin on tumour survival was assessed by dose response in oesophageal (OE21 and OE33) and colorectal (SW480 and HCT-15) cell lines. Adipokine and adipokine receptor expression was assessed in each cell line using RT-PCR.
Visceral and peripheral fat and matched serum was collected from (30) patients undergoing resective surgery for either oesophageal (15) or colorectal (15) malignancy. Adipocyte conditioned media (ACM), from aged-matched normal and viscerally obese cancer patients, was prepared and its effects on survival of each cancer cell line was examined. The differences between central and peripheral ACM were evaluated for each cancer type.
Results: Oesophageal and colorectal cell lines expressed detectable levels of leptin, visfatin, adipsin and resistin. Treatment of oesophageal and colorectal cell lines with leptin (1-500ng/ml) resulted in a dose dependent increase in cell proliferation, while adiponectin (100-5000ng/ml) decreased survival in each of the cell lines. The cancer cell lines expressed robust levels of both leptin and adiponectin receptors. Following serum withdrawl, adiponectin receptor-2 expression was upregulated and cells were more sensitive to adiponectin, with a greater reduction in cell survival. When oesophageal and colorectal cell lines were cultured with ACM from visceral adipose tissue (VAT), increased proliferation of the cells was observed compared to media prepared from peripheral fat. There was no difference in total adiponectin levels between visceral and peripheral adipose tissue, while leptin mRNA and protein was elevated in VAT.
Conclusion:
Oesophageal and colorectal cells are sensitive to leptin and adiponectin through altered receptor expression. Conditioned media from VAT promoted tumour growth to a greater extent than media prepared from peripheral fat. With the growing epidemic of obesity in western society and epidemiological studies linking this to increased cancer incidence, an understanding of the biological mechanisms responsible for this association may lead to the development of novel therapeutic strategies and interventions.

P3

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Title: Epigenetics underpinning the regulation of the CXC (ELR+) chemokines in NSCLC
Authors: Anne-Marie Baird, Nael Al-Sarraf, Steven G. Gray, Kenneth J. O'Byrne
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Abstract:
Introduction: Chemokines are a group of small mostly basic, structurally related molecules that regulate cell trafficking of various types of leukocytes. The CXC (ELR+) chemokines have been demonstrated to play a variety of significant roles in oncogenesis. They have effects on endothelial cells involved in angiogenesis, and have been implicated in breast cancer metastasis to the lung. Histone deacetylase inhibitors have been shown to inhibit angiogensis and are currently undergoing clinical trials for the treatment of cancer. We are interested in examining the expression of the CXC (ELR+) family in normal and lung cancer cells and their epigenetic regulation.
Methods: A panel of normal and lung cancer cell lines were screened for the following CXC (ELR+) chemokines; CXCL1, 2, 3, 8 (chemokines) and CXCR2 (receptor) at the mRNA level by RT-PCR. The epigenetic mechanisms regulating their expression was examined using a) two HDAC inhibitors Phenylbutyrate (PB-10 mM) and Trichostatin A (TSA-250ng/ml, and b) DNA methyltransferase inhibition using 5-aza-2-deoxycytidine (DAC-1 μM). The chemokine expression in matched tumour/normal samples from patients with NSCLC was also examined.
Results: mRNA for all chemokines was detected in both normal and lung cancer cell lines. In an adenocarcinoma cell line (A549), TSA down-regulated the majority of the chemokines, bar *CXCL8* and *CXCR2*, which were both induced by TSA. In contrast, phenylbutyrate (PB) a drug with pleiotropic activities (including the ability to inhibit HDACs), was found to increase the expression of all chemokines tested including *CXCR2*. A similar pattern of expression was observed in a squamous carcinoma cell line (SK-MES-1) where TSA exerted a significant down-regulation of *CXCL1, 2, 3*. Both HDAC inhibitors caused a considerable upregulation of *CXCL8* and *CXCR2*. Chromatin immunoprecipitation (ChIP) confirmed the upregulation of *CXCL8* by TSA was a direct response to HDAC inhibition. Treatment with 5-aza-2-deoxycytidine significantly increased the expression of *CXCR2* indicating that this receptor is regulated at the level of DNA CpG methylation. Finally, in primary NSCLC patients mRNA for the CXC family was decreased in the majority of adenocarcinoma tumour samples, but was increased in the bulk of squamous tumour samples with one exception, *CXCL2*.
Conclusions: This study indicates that members of the CXC family are epigenetically regulated by histone post-translational modifications and DNA CpG methylation. It remains to be determined whether or not this represents a viable therapeutic target in NSCLC. Further investigations are required to further delineate the vital balance of these chemokines in NSCLC, and to determine if aberrant epigenetic regulation of these genes plays a role in NSCLC pathogenesis. Should this prove true, targeting the epigenetic mechanisms underpinning this pathway may be of therapeutic value in the treatment of NSCLC.

P4

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Title: Neuropilin-1 blockade inhibits hypoxia-induced Akt and MAPK phosphorylation and induces apoptosis of non-small cell lung cancer cells.

Authors: [1]Martin Barr, [2]Graham Pidgeon, [1]Kathy Gately, [1]Kenneth O'Byrne.

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Abstract:

Introduction: Lung cancer is the leading cause of cancer mortality worldwide. Despite advances in anti-cancer therapies such as chemotherapy, radiotherapy and surgery, the overall five-year survival for patients remains poor. Neuropilin-1 (NP1), originally identified as a receptor for the semaphorin/collapsin family of neuronal mediators, has recently been documented as an isoform-specific receptor for $VEGF_{165}$ on a number of tumour cell types. At present, it is unclear as to the mechanism of NP1 signalling in lung tumour cells. We investigated the role of the phosphatidylinositol-3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) cell signalling pathways in hypoxic lung tumour cells and the effect of blocking the NP1 receptor on these survival pathways.

Methods: A panel of lung cancer cell lines (H460, H647, A549 and SKMES1) were screened for NP1 at the mRNA and protein levels by RT-PCR and Western blotting, respectively. NP1 expression was further examined by immunocytochemistry. Regulation of NP1 expression under hypoxia (0.1% O_2) was examined by Western blot over a time-course of 0h-72h. The effect of blocking NP1 under hypoxia using NP1 neutralising antibodies (1µg/ml) was assessed using the BrdU proliferation assay. Phospho-Akt (pAkt) and phospho-MAPK (pErk1/2) expression was examined in a panel of retrospective resected lung tumours and matched normal lung tissues. A549 (adenocarcinoma) and SKMES1 (squamous cell carcinoma) cells were treated with neutralising antibodies to NP1 under hypoxia in the presence or absence of VEGF (100ng/ml). Phospho-Akt and phospho-MAPK expression were examined by Western blot. Apoptosis was measured by Annexin-V/Propidium iodide staining using FACS analysis. Expression levels and localisation of both phosphorylated proteins in response to NP1 blockade under hypoxia were examined by confocal microscopy.

Results: All lung cancer cell lines expressed NP1, with the exception of the H460 cell line. NP1 blockade inhibited the survival/proliferation of lung adenocarcinoma cells significantly under hypoxia relative to normoxia. In a panel of adenocarcinoma and squamous cell lung carcinomas, lung tumours expressed increased levels of pAkt and pErk1/Erk2 relative to their matched normal tissue. In A549 and SKMES1 cells, hypoxia increased pAkt relative to cells grown under normoxia. NP1 blockade abrogated this hypoxia-induced increase in pAkt. A similar effect was seen on Erk1/2 levels in SKMES1 cells, with little or no effect in A549 cells. Hypoxia decreased cell apoptosis in both lung cancer cell lines relative to normoxic cells, while blocking NP1 reversed this effect, inducing cell death.

Conclusion: These results suggest a role for PI3K and MAPK signalling pathways in the survival of lung tumour cells under hypoxia, an effect that can be inhibited by blocking NP1.

P5

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Title: **Genomic Analysis of Colorectal Cancer to Assess Intratumour Heterogeneity**
Authors: HL Barrett, R Cummins, EW Kay
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Abstract:
Colorectal carcinoma remains the second most common malignancy in the western world, with 5year crude survival rates after curative resection ranging between 40% and 60% in most large series. The standard therapy for colorectal carcinoma is surgical resection, with or without adjuvant chemo-radiotherapy. 'Targeted therapy' refers to a new generation of cancer drugs designed to interfere with a specific molecular target, generally a protein, that is believed to have a critical role in tumour growth or progression. Three such therapies have recently been approved for use in the management of colorectal cancer. The effectiveness of 'targeted therapies' depends on homogenous expression of the targeted molecule throughout a tumour. However, it remains unclear whether the molecular profile of tumour cells can vary within a given tumour.

Aim & Methods:
To compare and contrast the gene expression profile of tumour cells from three different areas of 25 Dukes' C (stage III) colorectal carcinomas. Samples from the superficial part of the tumour, the invasive front and a nodal metastasis were analysed by a cDNA microarray [Colorectal Cancer Disease Specific Array (DSA)] created by Almac Diagnostics Ltd.

Results:
The Colorectal Cancer DSA detects 60380 transcripts. Comparative analyses of the results showed the following significant differences:

- Deep v Superficial samples – 270 genes
- Superficial v LN samples– 744 genes
- Deep v LN samples – 521 genes

Many of these transcripts code for structural proteins and inflammatory mediators. Specific targets such as epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) did not show significant variation in gene expression for the three different areas, suggesting intratumour heterogeneity may not influence the approved targeted therapies. These findings will now be confirmed by RT-PCR array and immunohistochemistry.

P6

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Title: Audit of the Calculated Carboplatin Dosage According to the Calvert Formula, using Different Equations Glomerular Filtration Rate Estimation

Authors: Barry A., O'Cearbhaill R., Griffin D., Donnellan P., Grimes H.
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Abstract:
Introduction: Carboplatin is a platinum based alkylating chemotherapeutic agent used in the treatment of a wide range of malignancies, mainly lung, ovarian, head and neck cancers. Dosage calculations are based on formulae which incorporate some estimate of glomerular filtration rate (eGFR). Traditionally, the Cockcroft-Gault and the Jelliffe equations are used to calculate creatinine clearance (CrCl) and carboplatin dosage using the Calvert formula. In this audit we reviewed the effect on GFR and on carboplatin dosage when using the recently readily available 4-variable MDRD (Modification of diet in renal disease) equation to calculate the GFR.
Methods: Data was collected retrospectively from the pharmacy records at Galway University Hospital on 36 patients with a confirmed diagnosis of cancer who were treated with carboplatin chemotherapy alone or in combination with another chemotherapy agent between December 2005 and October 2006. The eCrCl, calculated using either the Cockcroft-Gault or Jelliffe formulae, was compared with the eGFR, calculated using the 4-variable MDRD equation.
Results: There were 91 creatinine clearances calculated. In patients with normal renal function (<80μmol/L) there was significant variation in carboplatin dosage when the MDRD-derived eGFR and the eCrCl calculated using the Cockcroft-Gault/ Jeliffe formulae were compared. At serum creatinine levels >80μmol/L there was tight correlation between actual and projected carboplatin dosage. At these higher creatinine levels the range of difference in carboplatin dosage was -130 to 155mg.
Discussion: To date the use of the MDRD equation has not been validated in oncology patients. We compared the MDRD-derived eGFR and the Cockcroft-Gault or Jelliffe eCrCl and its effect on carboplatin dosage. There is no gold standard to determine which dosage calculation is the most accurate. In cases of impaired renal function our data would support the substitution of MDRD-derived eGFR for the traditional Cockcroft-Gault or Jelliffe formulae.

P7

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Title: **Drug medication and cancer evolution**
Authors: **Razvan Bocu**
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Abstract:

The article analyses the relationship between the physician-prescribed drug medication treatment and the cancer evolution, using an interesting mathematical model. It also includes, in its first part, a review for the main approaches regarding the mathematical modelling of cancer evolution. The first section comprises the following subsections: *Mathematics and biology*, which describes how biology could benefit by using mathematical apparatus and abstraction power; the second subsection comprises some *basic considerations related to cancer biology*. The last subsection of the first chapter along with the entire second section reviews the main approaches towards the cancer modelling. The following approaches are dealt with: *multiscale simulation*, *tumour-induced angiogenesis and mathematical modelling*, *the blood flow model*.

The last section of the paper deals with drug administration optimization and treatment efficacy. The entire model is based on the following Fister and Panetta's formula:

$$\frac{dN}{dt} = rN \ln \frac{}{N} - G\ N, U\ t$$

It is further slightly modified and explained and then used for producing numerical values and therefore graphs that analyze the tumour evolution in both of the following two situations: the physician prescribes a treatment intended to maximize the patient's overall physical comfort or the treatment is intended to minimize the overall expenses occasioned by the necessity for drug provisioning. As a consequence, some quite intuitive visualizations of the cancerous tumour in these given condition are presented and explained.

The paper ends with some conclusions regarding the cancer modelling and drug treatment optimization.

P8

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Mystique is required for polarization and migration of prostate carcinoma cells

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Mystique is a PDZ-LIM domain containing protein that associates with the actin cytoskeleton via the actin cross-linking proteins α-actinin and filamin A. Mystique is also known as PDLIM2 and acts in the nucleus of hemopoietic cells where it promotes the degradation of the transcription factors NFκB and STATs. We have previously shown that Mystique is required for epithelial cell attachment and migration; however the mechanism of action at the cytoskeleton remains unknown. The objective of this study was to investigate this mechanism, using the DU145 androgen-independent human prostate carcinoma cell line stably expressing a specific shorthairpin RNA (shRNA) targeting the Mystique gene or expressing a control shRNA. Cell adhesion, migration, and integrin-mediated signalling were examined in these cells. DU145 with suppressed Mystique expression (DU145-shMystique) had decreased ability to spread upon contact with extracellular matrix proteins, but displayed increased adhesion compared with control cells. In live cell imaging assays these cells had greatly impaired directional cell migration and could not fill in wounds in monolayer culture. However, DU145-shMystique cells displayed increased proliferative rates in monolayer culture, although they were greatly deficient in colony formation in soft agarose. Increased β1 integrin expression levels accompanied by increased tyrosine phosphorylation of Focal Adhesion Kinase were observed in DU145-shMystique cells compared with controls. This increase in integrin signalling is consistent with decreased cell motility and increased adhesion. Overall these data indicate that Mystique is required for directional cell migration and anchorage-independent growth in DU145 cells and suggests that Mystique mediates these effects through regulation of β1 integrin expression and integrin-dependent signaling.

P9

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Title: Is p63 a marker of basal breast cancer?

Authors: Buckley N, Nic An tSaoir C, Tkocz D, Farmer H, Redmond K, Da Costa Z and Mullan P
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Abstract:
BRCA1 encodes a tumour suppressor gene that is mutated in the germline of women with a genetic predisposition to breast and ovarian cancer. BRCA1 is known to play a role in a number of important cellular functions including DNA damage repair, cell cycle control, ubiquitination and transcriptional regulation. BRCA1 mutant breast cancers have a characteristic pathology including being poorly differentiated, highly proliferative, high levels of p53 mutation, and ERα/PR/HER2 receptor negativity (the so-called 'triple negative' phenotype). These features are very similar to another subset of breast cancers called the 'basal' subtype. Indeed microarray analyses of BRCA1 mutant and basal tumours strongly suggest that both types of tumours show highly similar transcriptional profiles implicating BRCA1 in the basal subtype. Both subtypes also show the poorest overall survival amongst all breast cancer subtypes suggesting they are a common subtype. Therefore, there is clearly a need for novel biomarkers and therapies for the treatment of this breast cancer subtype. Using an Affymetrix U133 Plus 2 microarray comparing mutant HCC1937 cells to HCC1937 cells reconstituted with wildtype BRCA1, p63 was identified as a BRCA1 transcriptional target. p63 is a member of the p53-like family of transcription factors. It encodes 2 major gene products (TA- and ΔN) from 2 distinct promoters and each product can be alternatively spliced to give rise to 6 isoforms. P63 has been previously been implicated in basal breast cancer and has also been reported to be overexpressed in BRCA1 mutant tumours. It had therefore been postulated to be a marker of the basal subtype. However, we now demonstrate positive regulation of p63 by BRCA1 in a number of cell line models using real time PCR and western blotting. We have identified that BRCA1 preferentially upregulates the ΔNp63α isoform (which is also the commonest form of p63 found in epithelial tissues). Investigation of BRCA1 regulation of p63 using luciferase reporter constructs appears to show that BRCA1 does not regulate p63 through the classical TA- and ΔN promoters. In addition, following screening of basal and luminal breast cancer cell lines for p63 expression we found no correlation between p63 expression and basal/luminal status. These observations call into question the putative role of p63 as a true marker of basal breast cancer.

P10

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Title:
Mechanisms controlling survival and apoptosis induction following inhibition of 12-Lipoxygenase in lung cancer cells

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Abstract:
Background: Platelet-type 12-Lipoxygenase is an arachidonic acid metabolising enzyme that results in the formation of 12(S)-HETE. 12(S)-HETE is proangiogenic, and has been shown to stimulate tumour cell adhesion, invasion and metastasis. Inhibitors of 12-LOX are currently undergoing extensive investigation. In this study we examined the expression profile of 12-LOX in human lung cancer cell lines and resected tissue. We also examined the mechanisms responsible for cell death following selective inhibition of 12-LOX with baicalein.
Methods: A549 (adenocarcinoma, AC), SK-MES1 (squamous cell lung carcinoma, SCC), H460 and H647 (large cell lung carcinoma) were grown in serum depleted media (0.5%) and screened for 12-LOX expression by RT-PCR and western blot analysis. The cells were treated with baicalein (10μM), a specific inhibitor of 12-LOX, or 12(S)-HETE (100ng/ml) and cell survival / proliferation determined by BrdU assay. Apoptosis was determined using the multi-parameter apoptosis kit and In-cell Analyser, FACS and DNA laddering. Gene alterations following 12-LOX inhibition in both A549 and SKMES-1 cells were assessed by quantitative PCR arrays and validated by RT-PCR. A panel of retrospective resected lung tumours and matched normal samples were stained for 12-LOX expression by immunhistochemistry IHC.
Results: All lung cancer cells lines expressed moderate levels of platelet-type 12-LOX following treatment for 24h with the 12-LOX inhibitor, baicalein. Baicalein decreased cancer survival in all cell lines, while 12(s)-HETE increased cellular proliferation. Inhibition of 12-LOX induced apoptosis in all cell lines in a dose dependent manner, with decreased f-actin filaments and a loss in mitochondrial mass potential. Induction of apoptosis was also confirmed by DNA laddering and Annexin-V FACS labelling. QPCR array data implicated a number of genes regulating these effects, many of which control apoptosis and angiogenesis. The subset of genes downregulated included bcl-2, VEGF, integrin α2 and α4, which were identified previously in prostate cancer cells. A number of these genes were validated by RT-PCR in response to baicalein treatment. 12-LOX expression was observed in a variety of human lung cancers with different histological subtypes. We are currently silencing 12-LOX expression in these cells, using shRNA technology, to further examine these mechanisms.
Conclusions: 12-LOX is a survival factor in NSCLC. 12-LOX inhibition decreased NSCLC survival, inducing apoptosis through mechanisms including downregulation of the bcl family of proteins, integrin receptor and angiogenic growth factors. Expression of 12-LOX in fresh resected and retrospective tissue suggests that inhibition of this enzyme is a potential therapeutic strategy in the treatment of lung cancer.

P11

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Title: **An imbalance in the expression profiles of PGIS and TXS in NSCLC: Regulation of tumor cell growth and invasive potential**

Authors: Cathcart, MC, Gately, K, Kay, E, Reynolds, JV, O' Byrne, KJ, Pidgeon, GP.
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Abstract:

Background: Prostacyclin Synthase (PGIS) and Thromboxane synthase (TXS) metabolize the cyclooxygenase product, prostaglandin H (2), into prostacyclin (PGI_2) and thromboxane (TXA_2) respectively. PGI_2 and TXA_2 have opposing actions, and changes in their ratio underlie many pathological conditions. PGIS over-expression inhibits cancer growth in a murine model, while over-expression of TXS has the opposite effects. TXS over-expression has been reported in a number of cancers and is associated with a poor prognosis and increased metastasis. The aim of this study was to examine expression patterns of PGIS and TXS in NSCLC, relative to normal and to determine the individual contributions of these enzymes to the development and progression of the disease.

Methods: PGIS and TXS expression was examined in human lung tumours (adenocarcinoma and squamous cell carcinoma) by both western analysis and IHC. A stable cell line over-expressing PGIS and TXS was generated and the effect on tumour cell survival was examined by BrdU, flow cytometry, and cell invasion assay. Cell proliferation/survival was examined in A-549 and SKMES-1 cell lines, following 24h selective TXS inhibition with ozagrel (50 nM, 500 nM 5 μM). Apoptosis was assessed following TXS inhibition by DNA laddering, Cell Death ELISA, and High Content Screening (HCS). RT^2 Profiler PCR Arrays were used to analyse the expression of genes regulating tumourigenesis following TXS inhibition, relative to untreated controls.

Results: Western and IHC analysis revealed PGIS to be down-regulated or absent in human tumour protein samples relative to normal, while TXS was up-regulated in tumours. SKMES-1 cells over-expressing PGIS proliferated at a significantly slower rate than null transfected controls, were less invasive, and were also more sensitive to apoptosis following serum-starvation. In contrast, over-expression of TXS resulted in a significant increase in proliferation, increased invasive potential, and a reduction in apoptosis. Selective TXS inhibition significantly reduced tumour cell proliferation/survival ($p<0.05$) and increased apoptosis in both cell lines. HCS for multi-parameter apoptosis showed enlarged nuclei, decreased f-actin staining and a loss in mitochondrial mass potential following TXS inhibition. TXS inhibition (A-549 cell line) resulted in down-regulation in the expression of a number of genes, including VEGF, Integrin alpha 4, and CDC25A.

Conclusion: Expression profiles of PGIS and TXS are altered in NSCLC, relative to normal. Overexpression of TXS increased proliferation and invasiveness of lung cancer cells, while PGIS overexpression had contrasting effects. The balance in expression of these enzymes may underlie the pathogenesis of lung cancer. TXS is a potential therapeutic target for the treatment of NSCLC, while PGIS over-expression may be a novel strategy for chemoprevention.

P12

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CHRONIC LYMPHOCYTIC LEUKAEMIA EXPRESSING IGHV4-34 IDENTIFIES A SUBSET WITH HIGHLY HOMOLOGOUS HEAVY AND LIGHT CHAIN THIRD COMPLEMENTARY DETERMINING REGION (HCDR3 & LCDR3) AND INDOLENT DISEASE.

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Background
Previous studies have shown that Immunoglobulin (Ig) heavy and light chain variable region repertoire is skewed in B-CLL. This suggests that antigens/superantigens may be involved in the stimulation of B cells that are encoded in particular Ig genes. Certain Ig genes are preferentially used in unmutated (IGHV1-69) and mutated (IGHV4-34) rearrangements. This feature is therefore thought to be CLL-biased. The IGHV4-34 gene encodes antibodies that are autoreactive and are infrequent in the sera of healthy individuals, although the IGHV4-34 gene is frequent in the repertoire of normal peripheral B cells.

Aims
To explore the antigen-driven selection in CLL, we assessed the heavy and light chain CDR3 in a large cohort of CLL's expressing IGHV4-34.

Methods
Three hundred and seventy patients were recruited from the Haematology Outpatient Clinic and surrounding regional hospitals. Clinical staging, immunophenotyping, lymphocyte doubling time (LDT) and time to treatment (TTT) were available on all patients. IGHV and IGL mutational status were determined using multiplex BIOMED-2 primers and sequence analysis. Chromosomal abnormalities were determined using interphase fluorescence in-situ hybridisation (FISH).

Results
IGHV4-34 rearrangements were detected in 40 cases (10.8%). Nineteen patients were female and twenty-one were male. Interestingly, all patients had mutated IGHV genes. FISH analysis revealed: no detectable abnormality (n=18) and monoallelic or biallelic del13q14 (n=22).Thirty-six patients presented with stage A disease and have not required treatment with a mean time from diagnosis of 5 years (range 1-24yrs).
Four patients presented with more advanced clinical stage (Binet B or C) and these 4 patients received treatment.
Among the 40 cases expressing IGHV4-34, specific subsets were identified. The main subset was associated with the IGKV2-30 gene displaying a highly homologous LCDR3 and HCDR3 sequence.

Summary / Conclusions
In our B-CLL cohort expressing IGHV4-34 we demonstrated a population characterized mainly by indolent disease with good risk cytogenetics. All patients possessed mutated IGHV4-34 Ig genes and only 4 required chemotherapeutic intervention.
In conclusion, our subset displayed skewed HCDR3 and LCDR3 and suggests that distinct antigens drive a subset of IGHV4-34 CLL patients.

P13

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Title: **Identification of Differentially Expressed Mature MicroRNAs in Colorectal Cancer and Non-tumoral Tissues.**

Authors: Chang KH, Miller N, McNeill RE, Smith MJ, MacCarthy F, Regan M, McAnena OJ, Kerin MJ
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Abstract:

Background. MicroRNAs (miRNAs) are a class of endogenous, non-coding RNAs of 18-24 nucleotides in length. They are involved in the regulation of various cellular processes and tumorigenesis. Certain miRNAs have been displayed aberrant expression in human cancers, indicative of both tumour suppressor and oncogenic functions. Deregulation of several miRNAs in colorectal cancer tissues and cell lines has previously been demonstrated. The purpose of this study is to establish a bio-repository of patient material from colorectal cancer patients on which to investigate the miRNA expression profiles in tumour and adjacent tumour-associated normal tissue samples.

Methods. Blood and tissue samples from patients undergoing surgery for early stage, locally advanced and metastatic disease have been banked further to informed consent and ethical approval. Full clinicopathological data including family history has been gathered on all patients. To date, 30 colorectal cancer and corresponding normal tissue samples have been collected at the time of surgery subsequent to histopathological review. RNA has been extracted from tissue samples using a modification of the Qiagen RNeasy® system. Samples were analysed for miRNA expression using quantitative real-time RQ-PCR. We examined the aberrant miRNAs in tumour and normal tissues; and sought for correlation with clinicopathological parameters.

Results. Our study cohort consists of 23 males and 7 females, with a median age of 66.5. Location and TNM stage of tumours range from caecum to rectum, and from stage I to IV respectively. To date, relative quantification of *let-7a*, *miR-10b*, *miR-16*, *miR-21*, *miR-143* and *miR-145* has been performed on 14 tissue samples. Expression of *miR-10b* was found to be consistently reduced in tumour samples.

Conclusion. To our knowledge, this is the first report demonstrating that *miR-10b* is down-regulated in colorectal cancer. *MiR-10b* has also been found to be down-regulated in breast cancer. Our findings are consistent with previously reported tumour suppressor role of *miR-10b*. Further analysis will be performed for correlation with clinicopathological variables.

P14

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ZEB1 – A POTENT REPRESSOR OF E-CADHERIN IN UROTHELIAL CARCINOMA OF THE BLADDER.

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E-cadherin is known to be a frequent target of transcriptional repression, promoter hypermethylation and loss of heterozygosity. In this study established transcriptional repressors, namely SNAIL, TWIST and ZEB1 were Western profiled on a panel of bladder cancer cell lines of known E-cadherin status. This was to establish a) which of these repressors plays the most significant role in E-cadherin regulation in UCB, and b) can siRNA specific approaches be used to restore E-cadherin expression, thus rendering cells less invasive.

Western blot analysis for SNAIL, TWIST, ZEB1 and E-cadherin were carried out on a panel of bladder cancer cell lines (RT-4, T-24, Cal-29, HT-1376, TCCSUP, RT-112) displaying differential invasive and growth properties.
Methylation sensitive PCR specific for E-cadherin was carried out on those cell lines found to be negative by Western Blotting to exclude epigenetic regulation.
In those cell lines shown to have transcriptional repression of E-cadherin siRNA approaches were used to determine the effect on E-cadherin expression.

Western Blot analysis has revealed an inverse relationship between Zeb1 and E-cadherin expression at the protein level. A similar relationship was demonstrated at the mRNA level using reverse transcription PCR. Targeted siRNA experiments resulted in re-expression of E-cadherin and restoration of the epithelial phenotype.

Our studies show that Zeb1 may be the key player in E-cadherin regulation in urothelial carcinoma of the bladder. Zeb1 may therefore be an important indicator of disease progression and may also provide a potential target for therapeutic strategies.

P15

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Title: Long-term suppression of IκBα expression by inflammatory cytokines: Molecular mechanisms.

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Abstract:

Background: Inflammatory bowel disease (IBD) has a well established association with the development of colorectal cancer (CRC). Transcription factor, NF-κB has been reported to be activated in biopsy samples and cultured cells of patients with IBD and colorectal cancer, while levels in adjacent normal tissue remain normal. While much is known about the acute regulation of NF-κB by proinflammatory cytokines, less is understood about how chronic NF-κB activation is sustained.

Methods and Aims: We sought to characterise the biochemical mechanisms regulating the long-term i.e. greater than 4 hours, activation of NF-κB. Chronic intestinal inflammation was modelled *in vitro* using cytokine-stimulated HT29 colonic epithelial cells.

Results and Discussion: 1. TNF-α stimulation resulted in a marked decrease in IκBα protein expression after 30mins, which was restored to baseline at 1hr. However, a second decrease in IκBα protein expression was seen at 8hr sustained for up to 48hr. TNF-α stimulation resulted an increase in IκBα mRNA expression and NF-κB activation. **2.** In contrast, chronic IL-1β stimulation resulted in no significant changes in IκBα expression. **3.** Proteasome inhibitors, MG132 and ALLN, blocked the early but not the late IκBα protein degradation **4.** Ongoing experiments are assessing the role of CK2, calpain and caspases in late suppression of IκBα following chronic TNF-α stimulation.

Conclusion: Chronic inflammation, modelled by long-term TNF-α stimulation, resulted in a previously unrecognised late suppression of IκBα protein expression. This late decrease in IκBα protein appears to be proteasome independent. Elucidation of the mechanism responsible for long term regulation of IκBα may provide novel insights into the pathogenesis of chronic inflammation.

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P16

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Title: Preclinical evaluation of EGFR in triple negative breast cancer

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Abstract:

Introduction: Triple-negative breast cancer (TNBC) lacks expression of HER-2 and hormone receptors, but frequently over-expresses epidermal growth factor receptor (EGFR) and is associated with earlier age of onset and increased risk of metastasis. Given the current lack of targeted therapeutic options, we sought to assess the potential benefits of EGFR inhibition in TNBC.

Methods: EGFR levels were measured in TNBC cell lines (BT20, HCC1937, MDA-MB-231) by ELISA. Sensitivity to EGFR inhibitors (gefitinib, erlotinib, cetuximab), and chemotherapy drugs (carboplatin, docetaxel, doxorubicin) was measured by the acid phosphatase assay. Two HER-2 positive breast cancer cell lines, BT474 and SKBR3 which express low levels of EGFR were used as controls. Cell cycle analysis was performed using the Guava EasyCyte. Inhibition of the key signalling molecules Akt and MAPK was assessed by western blot with phospho-specific antibodies.

Results: EGFR is significantly over-expressed in the TNBC cell lines. We have previously shown that the TNBC cell lines are less sensitive to EGFR inhibition than the HER2 positive cell lines. One of the proposed mechanisms of action of EGFR inhibition is induction of G1 arrest. We found that gefitinib did not cause significant G1 arrest in the TNBC cell lines, in contrast to the sensitive HER-2 positive SKBR3 cell line. Analysis of Akt and MAPK in the TNBC cells suggests that inhibition of Akt and MAPK phosphorylation does not predict response to gefitinib. Although the TNBC cells are not as sensitive to EGFR inhibition as the HER2 positive cells, gefitinib improved response to chemotherapy in the TNBC cell lines. We have previously shown that combined treatment with gefitinib and docetaxel, carboplatin or doxorubicin, showed improved response compared to either drug alone. To develop a rational therapeutic combination for testing in TNBC patients, we tested the triple combination of gefitinib, carboplatin and docetaxel in HCC1937 cells and the combination showed greater inhibition of proliferation than each of the drugs alone.

Conclusion: Although the TNBC cell lines are less sensitive to EGFR inhibition than the control cell lines, the addition of gefitinib to chemotherapy enhances response and the triple combination of gefitinib, paclitaxel and carboplatin warrants further preclinical and clinical investigation in TNBC.

P17

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Title: Differential localisation and function of Mystique in various cell types

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Institution: Biosciences Institute, UCC

Abstract:

Mystique is a PDZ-LIM domain protein which we have previously shown to regulate cell adhesion and migration when located at the cell cytoskeleton of epithelial cells. Interestingly, in normal T cells Mystique is localised in the nuclei, where it has been shown to regulate levels of STATs and the p65 subunit of NFκB. These data indicate that Mystique has differential roles in various cell types.

The aim of this study was to investigate whether function of Mystique is dependent on its subcellular localisation. We examined Mystique expression patterns in non-transformed and transformed cells using immunofluorescence cytology and subcellular fractionations. We found that Mystique was predominantly located in the nuclei of the non-transformed MCF10A mammary epithelial cells, MCF7 breast carcinoma cells and haemopoietic cells. In addition, treatment with leptomycin B, which blocks nuclear export function enhanced nuclear accumulation in these cells. However, in Ras-transformed MCF10A cells, in highly invasive DU145 cells and in MCF7 cells overexpressing GFP-tagged Mystique, Mystique was predominantly located in the cytoplasm and colocalised with the actin cytoskeleton. Furthermore, we found that cell adhesion was necessary for cytoplasmic localisation of Mystique in these cells. Finally, live cell imaging of MCF7 cells overexpressing GFP-tagged Mystique demonstrated that Mystique behaves in a dynamic manner within the cell, with clear evidence of continuous movement throughout the cell cytoplasm/cytoskeleton.

Overall, this study suggests that Mystique is predominantly nuclear in non-transformed and non-adherent cells whereas it is sequestered in the cytoplasm of adherent transformed cells, where it is necessary for cell adhesion and migration. The differential nuclear and cytoplasmic functions of Mystique in these various cells types is the focus of current studies.

P18

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Title: Identification of predictive signatures of response to chemotherapy in metastatic colorectal cancer

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Abstract:

Introduction: Colorectal cancer (CRC) is a leading cause of cancer-related death in the Western world. Despite recent improvements in the treatment of advanced CRC drug resistance remains a major factor limiting the effectiveness of chemotherapy.

Methods: DNA microarray technology (Affymetrix HGU133 Plus 2.0 array/Almac-Diagnostics Colorectal Disease-Specific array) was used to identify novel determinants of response to 5FU and Irinotecan both *in vitro*, using a panel of drug-sensitive and drug-resistant Hct116 CRC cell lines, and *in vivo,* in pre-treatment metastatic tumour biopsies from patients with advanced CRC who received Irinotecan/5-FU chemotherapy. Data analysis was carried out using Genespring GX v7.3 (Agilent Technologies).

Results: Data analysis identified panels of *in vitro* genes whose expression is acutely altered in the parental setting following drug treatment and also basally deregulated between parental and resistant cells. The tumour-derived transcriptional profiles were correlated with response to treatment and a three step classification approach used to build predictive signatures of response to irinotecan/5-FU chemotherapy. In addition, an assessment of the feasibility of using *in vitro* expression data to separate patient samples based on their response to treatment has been assessed using principal components analysis (PCA). Finally, using both the *in vitro* genes and the clinically-derived genes, signatures have been generated that predict patient response using different class prediction methods (Support Vector Machines and K-Nearest Neighbour). The overall accuracy, sensitivity and specificity of these models have been assessed using cross-validation procedures.

Conclusions: The overall aim of this study is to design a prospective trial, in which treatment for CRC is administered on the basis of the molecular profile of both tumour and patient.

P19

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Title: Investigation of additional protease targets of proteasome inhibitors
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Abstract: The 26S proteasome is a multicatalytic enzyme responsible for regulated degradation of the majority of cellular proteins, including those involved in cell cycle control, transcription and apoptosis. Its function is mediated by three main catalytic activities: chymotrypsin-like (CT-L), trypsin-like (T-L) and peptidylglutamyl peptide hydrolysing (PGPH). Inhibition of the catalytic activity of the proteasome is an emerging treatment for many cancers. Bortezomib, the first proteasome inhibitor to be used in clinical practice, is approved for the treatment of multiple myeloma (MM). We have previously investigated the effect of Bortezomib along with two structurally distinct proteasome inhibitors, MG-132 and BzLLLCOCHO, on total proteasome activity and compared this with their ability to induce apoptosis in MM cell lines. We found that the degree of apoptosis induced by Bortezomib and MG-132 does not correlate with the extent of functional inhibition of proteasome activity. This implies that there must be other factors contributing to the cytotoxicity of these compounds. Many proteasome inhibitors are designed to target the CT-L activity of the proteasome, therefore we have used an active site-directed probe to investigate whether any of the inhibitors have affinity towards other CT-L proteases, aside from the proteasome. MM cell lines were cultured in the presence of Bortezomib, MG-132 or BzLLLCOCHO and cell lysates prepared. Lysates were incubated with active site-directed probe [Biotin-Peg-Succ-Phe (OPh)2] at 37°C for 30 mins to label CT-L proteases. The samples were subsequently separated by SDS-PAGE and biotinylated proteins were visualised using streptavidin-HRP. A band of molecular weight between 51-64 kDa was found to be decreased in response to treatment with Bortezomib and MG-132, suggest that the corresponding protein or protease may be inhibited in response to treatment with these compounds. Additional investigations to identify this protein/protease are underway and should help to provide a better understanding of the mechanisms of action of the proteasome inhibitors. Such information could be exploited for the development of more specific and targeted proteasome inhibitors.

P20

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Title: **BH3 domain of BID interacts with VDAC1/Prohibitin Complex and Depolarizes Mitochondria in the absence of Cristae Remodelling**

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Abstract:
The multidomain proapoptotic BCL-2 family proteins BAX and BAK are pivotal regulators of cell death, are activated by BH3 proteins, and mediate outer mitochondrial membrane permeabilization (MOMP). Complete release of cytochrome C activates caspases and uncouples respiration by inhibiting electron transport between complex III and IV. Remodelling of cristae junctions by BID mobilizes cytochrome C in a cyclosporin A inhibitable and BH3 independent manner. We have observed however that cell permeable $BH3^{BID}$ triggers rapid mitochondrial depolarization (MD) of lung cancer cells prior to permeability transition, and kills in a caspase and calcium independent manner. L90A mutation or BAX/BAK double siRNA knockdown abolished MD in isolated state IV mitochondria. Ultrastructural damage and cardiolipin perxoxidation accompanied MD. Although endogenous BID, BIM localized to the outer mitochondrial membrane, their double knockdown by siRNA was insufficient to inhibit MD. Neither BAD/ NOXA or BADY105I BH3s were able to induce MD. Depolarized mitochondria retained cytochrome C after MOMP and retained high molecular weight OPA1 oligomers consistent with an absence of cristae remodelling. Artificially induced MOMP by C2 ceramide failed to induce MD. $BH3^{BID}$ interactions at the outer mitochondrial membrane were therefore explored by crosslinking, coimmunoprecipitation, and peptide mass fingerprinting, and identified VDAC1/prohibitin 1/prohibitin 2 complex as the principle target. $BH3^{BID}$ depolarized FCCP driven, ATP and complex V dependent membrane potential. Full length BID has been reported to reduce ADP permeability of the outer membrane which we propose is via a BH3-VDAC1 interaction, which may be linked to generation of oxidative stress, MD and cell death in the absence of complete cytochrome C mobilization.

P21

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Title: **TITANOCENE ANALOGUES INDUCE APOPTOSIS IN PROSTATE CANCER EPITHELIAL CELLS VIA A DNA DAMAGE RESPONSE**

Authors: Cuffe[a] S., Dowling[a] C., Gill[a] C., Tacke[b] M., Fitzpatrick[a] JM, Carthy[c] MP, Watson[a] RWG.
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Abstract:
Introduction: Treatment options for locally advanced metastatic prostate cancer are extremely limited with Taxotere being the standard chemotherapy but only providing a three month survival advantage. The objectives of this study are to investigate novel titanocene analogues as possible alternative chemotherapies for advanced disease. The primary aims are to investigate the apoptotic effects of these novel titanocene analogues on prostate cells and to examine their mechanism of action.

Methods: PwR-1E and PC-3 cell lines were grown in optimal conditions and treated with titanocene analogues at different doses and times. Apoptosis and viability were assessed by propidium iodide staining and flow cytometry and PARP cleavage. Cellular uptake and DNA binding of Titanium was measured by atomic absorption spectroscopy (Dr. JL Beltramo, Université de Bourgogne, France). Replication Protein A (RPA) and p53 phosphorylation was assessed by western blotting. Knock-down of p53 was achieved by si-RNA and assessed by western blotting.

Results: PwR-1E and PC-3 cells undergo apoptosis in a dose dependent manner following treatment with a range of titanocene analogues as determined by PI DNA staining and PARP cleavage. These compounds enter both cell lines and bind to DNA as confirmed by atomic absorption spectroscopy. These results confirm a correlation of increased Titanium-DNA binding and apoptotic responses. The differential apoptotic response between the PwR-1E and PC-3 cell lines correlates with the uptake of Titanium into the cells and consequently the level of DNA binding.
Induction of a DNA damage response is indicated by the phosphorylation of p53 and RPA. However induction of apoptosis by the titanocene compounds is not p53 dependent as demonstrated by knock-down of p53 by si-RNA in the PwR-1E cell line and no expression in the PC-3 cells.

Conclusion: These pre-clinical studies demonstrate for the first time that these novel titanocene analogues induce apoptosis in prostate cancer cell lines. Further evaluating the mechanism of action will indicate their appropriate clinical use in different stages of prostate cancer development.

P22

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Title: **The identification of transcriptional targets of TBX2 in breast cancer cell lines**
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Institution: Queen's University Belfast.
Abstract:

TBX2 is transcription factor located on 17q23, a region which is amplified in approximately 15% of breast cancers (usually the most aggressive forms including BRCA1- and BRCA2-linked breast cancers). TBX2 acts as a transcriptional repressor and switches off a number of key cell cycle regulatory molecules such as $p21^{WAF1}$ and $p14^{ARF}$. In this study we have used the MCF-7 breast cancer cell line (which has 17q23 amplification and expresses high levels of TBX2 protein) to identify transcriptional targets of TBX2 which play a role in growth control. We performed siRNA knockdown of TBX2 in MCF-7 cells and using a Breast Cancer Disease Specific Array (DSA) we identified 603 potential TBX2 regulated genes showing 2-fold or greater upregulation. Many of these genes have been reported to play roles in regulating cell proliferation, invasion, metastasis, angiogenesis and apoptosis but had not been identifed as TBX2 targets following analysis of identical RNA samples by a Affymetrix U133 plus 2.0 array. These genes included a cysteine protease inhibitor expressed in normal breast epithelium but not in metastatic breast cancer cell lines. Also present were genes involved in plasma lipoprotein metabolism, signal transduction proteins, a Rho GDP dissociation inhibitor, a retinoid regulated type II tumor suppressor gene and a protein that recognizes and binds to some phosphorylated proteins promoting their ubiquitination and degradation. We are in the process of validating many of these targets by real time PCR and Northern Blotting. The ultimate aim of our work is to use this expression profiling information from TBX2 overexpressing cells to develop therapeutic strategies to target this tumor subtype.

P23

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Title: *ret*/PTC-1 alters the immunoprofile of thyroid follicular cells

Authors: Denning K, Smyth P, Cahill S, Li JH, Flavin R, Aherne S, O'Leary J, Sheils O
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Abstract:

Background:

ret/PTC-1 has been described in autoimmune thyroid disease (AITD) and thyroid neoplasia . A common morphologic feature in each is the presence of a florid lymphocytic infiltrate. It is unclear if the presence of *ret*/PTC-1 facilitates cross-talk between the infiltrate and thyrocytes. Moreover the extent to which *ret*/PTC-1 may be involved with molecular pathobiology and disease progression remains to be uncovered.

Methods:

RNA from *ret*/PTC-1 positive and negative thyrocytes was analysed over a time course to identify variations in immunoprofile following co-culture with activated T cell lymphocyte supernatant from Hashimoto Thyroiditis (H.T.) and normal donors. Expression analysis was performed using TaqMan® Immune profiling Low-Density Arrays (Applied Biosystems, CA, USA) comprising gene expression markers for 93 immune related targets plus 3 endogenous controls.

Results:

Stimulation of normal thyrocytes with activated T cell supernatant from the H.T. donor yielded global up-regulation of immune targets compared with base line expression. In particular, targets associated with cytotoxic cell death, TCR and T cell signaling were up-regulated in normal thyrocytes. These targets were significantly down-regulated in corresponding *ret*/PTC-1 harboring thyrocytes exposed to the same stimulus.

Discussion:

Activation of the c-ret oncogene down-regulates a subset of immune targets including granzyme B, CD3, CD25, CD152 and CD45; genes which are involved in apoptosis, T cell signalling and T cell activation. Down-regulation of these targets could compromise immunogenicity in the thyroid and facilitate papillary thyroid carcinoma development.

P24

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Design of a novel drug delivery system for photodynamic and photodiagnostic methodologies in the colorectal region.

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Serious cellular abnormalities in the colorectal region are a leading cause of morbidity and mortality in industrialised countries, with an estimated 300,000 new cases and 200,000 related deaths annually in Europe and the United States. The development of technologies to improve the detection process or enhance treatment would be a welcomed addition to current treatment methods. Two such promising procedures are photodynamic therapy (PDT) and photodiagnosis (PD). The techniques rely on specific accumulation of photosensitiser in a neoplastic lesion with the former therapy used to bring about selective destruction and the latter only making it more conspicuous upon fluorescent emission. Administration of 5-aminolevulinic acid (ALA) lead sto selective accumulation of the photosensitiser protoporphyrin IX in neoplastic tissue. However, systemic administration of ALA is associated with significant side effects.

In this study, ALA-loaded, poly(ethylene glycol) (PEG) discs prepared using three molecular weights (1000, 6000 and 10000) were shown to be of potential for rectal administration as part of photodynamic and photodiagnostic colorectal procedures. The disc-shaped delivery system was mechanically robust, as judged by friability measurements. Calorimetric analysis confirmed that low concentrations of ALA (1% w/w) were dispersed completely throughout the PEG matrix, but higher concentrations (5% w/w and 10% w/w) formed crystalline suspensions. The molecular weight of the PEG determined the melting temperature, with PEG 1000 being suitable for melting around body temperature. The drug release kinetics were shown to be a function of both molecular weight and drug loading. Although the higher molecular weight PEG discs were resistant to surface erosion arising from an aqueous receptor phase, this effect was counterbalanced by more rapid and complete release when the ALA loading was increased. The lowest loading used (1% w/w) produced incomplete release, often not exceeding 30% of the total amount of drug. Results suggest that this simple formulation containing ALA can be administered directly to the colorectal area and is a feasible alternative to peroral dosing of ALA.

P25

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Title: **DETERMINATION OF PROSTATE CANCER URINARY BIOMARKERS USING A 2D-DIGE PROTEOME PLATFORM**

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Abstract: **Introduction:** Prostate cancer is the commonest solid organ malignancy to affect men in Europe and the United States [1]. Current screening relies on a combination of digital rectal examination with a serum prostate specific antigen test (PSA). PSA exhibits poor specificity, thus, more appropriate markers are urgently required. Urine is easily obtained and readily available and may allow earlier identification of prostatic malignancy than serum markers [2]. We utilised a novel approach consisting of a 2D-DIGE proteome platform to determine differential urinary protein expression between Gleason 5 & 7 prostate cancer cohorts.
Materials & Methods: Analysis of the Prostate Cancer Research Consortium patient database identified 8 matched patients for this pilot project. Following initial precipitation procedures, the urinary proteins were labelled using commercially available CyDye fluors (GE Healthcare). Image analysis was performed with the Progenesis PG240 software suite (NonLinear Dynamics). The differentially expressed proteins were identified using LC-MS/MS (Finnigan LTQ).
Results: Eleven gel spots were noted to be differentially expressed using the above platform. Mass spectrometry successfully identified ten of these. In total, 17 different proteins were identified. Literature searches revealed the known association of six of these with prostate cancer. Three of these (IGFBP-7, Clusterin, CD-14) have been taken forward for preliminary validation across a larger cohort of urine samples.
Discussion: 2-D DIGE is a suitable platform for the determination of differentially expressed urinary proteins in a prostate cancer cohort. We have successfully identified a number of potential urinary biomarkers of prostate cancer utilising this promising technique.

[1] Jemal A et al. Cancer statistics, 2005. CA Cancer J Clin. 2005, 55(1): 10-30

[2] Downes MR et al. Application of proteomic strategies to the identification of urinary biomarkers for prostate cancer: a review. Biomarkers. 2006, 11(5): 406-16

P26

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Title: A role for Ran GTPase in breast cancer metastasis and invasion.

Authors: N Johnston, V Gunasekharan, P Johnston, M El-Tanani.

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Abstract:
Osteopontin (OPN) overexpression in human breast cancer is correlated with metastatic disease, leading to a poor prognosis for patients. Suppressive subtractive hybridization was used to identify genes expressed due to OPN activation of the Rama 37 rat mammary cell line. One of the genes that demonstrated highly expression in association with OPN was RAN GTPase (RAN). Here we show that stable transfection of non-invasive R37 cells with an expression vector for RAN resulted in R37-RAN cells with an invasive phenotype both *in vitro* and *in vivo*. We identify RAN as a novel enhancer of proliferation, anchorage-independent growth, adhesion, invasion and metastasis. We also present a novel mechanism of RAN activity; signal transduction through the c-Met receptor/PI3kinase pathway. Evidence of comparable RAN activity in a human mammary model system is also presented.

P27

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Title: **Identification of genes differentially expressed between benign and metastatic mammary epithelial cells**

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Abstract:

Osteopontin is a secreted, integrin-binding and phosphorylated acidic glycoprotein which has an important role in tumor progression. In this study, we have utilized suppressive subtractive hybridization (SSH) to evaluate OPN regulated gene expressions, using the Rama 37 benign non-invasive rat mammary cell line and a subclone, Rama 37-OPN, with increased malignant properties *in vitro* produced by stable transfection with an expression vector for OPN. These properties include increased cellular adhesion to fibronectin, anchorage-independent growth in soft agar and invasion through Matrigel. Sequence and expression array analysis of the respective cDNA libraries of over 1600 subtracted cDNA fragments revealed 982 ESTs, 45 novel sequences and 659 known genes. The known up-regulated genes in the Rama 37-OPN library code for proteins with a variety of functions including those involved in metabolism, cell adhesion and migration, signal transduction and in apoptosis. Four of the most diffentially expressed genes between the benign and *in vitro* malignant rat mammary cell lines using reverse Northern hybridizations and between the less aggressively and more aggressively malignant human breast cancer cell lines using quantitative real time Reverse Transcription Polymerase Chain Reactions are tumour protein translationally controlled I (TPTI), aryl hydrocarbon receptor nuclear translocator (ARNT), ataxia telangiectasia mutated (ATM) and RAN GTPase (RAN). The largest difference (ca 10,000 fold) between the less aggressively (MCF-7, ZR-75) and more aggressively malignant (MDA MB 231, MDA MB 435S) human breast cancer cell lines is that due to RAN, the next is that due to osteopontin itself. These results suggest that overexpression of osteopontin and properties associated with the malignant state *in vitro* may be due to overexpression of RAN GTPase.

P28

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Title: Effects of Src kinase inhibition by dasatinib in melanoma cell lines

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Abstract:
Metastatic melanoma is notoriously resistant to common chemotherapeutic treatments. With increasing incidence of the disease, the use of newer targeted therapies alone and in combination with chemotherapy has increasing importance. Dasatinib a multi-target kinase inhibitor is currently approved for the treatment of chronic myeloid leukaemia and has shown promising results in preclinical studies in a number of solid tumors. We tested dasatinib alone in a panel of melanoma cell lines and found that four of the six cell lines were responsive to dasatinib. Dasatinib was tested in combination with temozolomide and the combination was more effective than either drug alone. Dasatinib also significantly inhibited cell migration and invasion of HT144 and M14 cells and induced apoptosis in two of the cell lines tested, LOX-IMVI and Malme-3M. Src kinase expression does not appear to predict response to dasatinib in the melanoma cell lines but dasatinib treatment reduces phosphorylation of Src kinase in sensitive cell lines. A six-gene predictor of dasatinib sensitivity, which was previously developed using breast cancer cell lines, was tested in the melanoma panel by q-RT-PCR. Five of the six genes showed similar trends in expression in the dasatinib-sensitive melanoma cell lines, to that observed in the breast cancer cell lines. Dasatinib has anti-proliferative, pro-apoptotic and anti-invasive effects in dasatinib-sensitive melanoma cells. Therefore combining dasatinib with chemotherapy, such as temozolomide, may improve response to treatment in these tumors.

P29

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Metallothionein 1E (MT1E) Gene is Methylated in Both Primary and Metastatic Melanomas

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DNA methylation is known to play a major role in many cancers by facilitating the silencing of tumour suppressor genes and for malignant melanoma, only a small percentage of the total number of methylated genes are believed to have been identified. Previously, we identified a cohort of genes whose altered expression in melanoma cell lines is modulated following treatment with the DNA methyltransferase inhibitor, 2'-deoxy-5-azacytidine (DAC), indicating that they are directly or indirectly regulated via methylation (1). In this study, we identified which of these genes were directly methylated genes in human melanoma cell lines and determined their methylation status in a range of benign nevi and melanoma tissues. Firstly, we pre-selected genes that contained a CpG island in their promoter and first exonic region, before performing sodium bisulphite sequencing. In all, we examined the methylation status of 13 genes (CDKN2A, CYBA, FABP5, G1P3, HSPB1, LGALS3, MCAM, MT1E, MX1, PTN, RGS3, RPL37A and TAC1) in an isogenic cell line model series that mimics key stages of melanoma progression, which consists of a poorly tumourigenic parental cell line (WM793) and 3 derivative lines that display increased tumourigenicity and, in some cases, metastatic capability. Of the 13 genes studied, only four genes (CYBA, FABP5, MT1E and TAC) were shown to be methylated, and specifically displayed increasing methylation in the more aggressive derivative cells compared to the parental cells. Further analysis of primary/metastatic melanoma tumour pairs, using methylation-specific PCR (MSP), revealed that CYBA was not generally methylated in tumour samples (1/40), but methylation was detectable for FABP5 (5/28) and MT1E (11/18). MT1E methylation was then examined in a larger panel of samples, which showed that 7% of benign nevi, 40% primary tumours and 46% of metastases were methylated (n=74, p=0.025). Using RNA extracted from formalin-fixed, paraffin-embedded tissues, real-time reverse transcriptase-PCR analysis of MT1E mRNA expression showed a significant down-regulation of expression in both primary and metastatic tumours as compared to benign nevi (n=113, p=<0.0009). In addition, analysis of matched tumour pairs showed that 5/6 pairs displayed a decrease of MT1E mRNA expression in the metastatic sample compared to the primary tumour. Overall, these studies indicate that MT1E is methylated in melanoma samples compared to benign nevi and that expression is decreased in a progression-associated manner, implicating MT1E as a candidate tumour suppressor gene in melanoma.

Funding is acknowledged from the Health Research Board and IRCSET.

1. Gallagher WM *et al.* (2005). Multiple markers for melanoma progression regulated by DNA methylation: insights from transcriptomic studies. Carcinogenesis. 2005 Nov; 26(11): 1856-67.

P30

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Title: Nanoparticle design for cytosolic delivery of peptide and protein drugs into tumour cells.

Authors: Francois Fay, Paul A. McCarron, Chris Scott.
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Abstract:

Nanoparticle mediated drug delivery is a promising approach for the delivery of various drug types to the site of disease in an effort to improve efficacies. Development of polymeric nanoparticles with slow drug release kinetics coated with targeting antibodies or peptides enables specific drug delivery to tumour cells, whilst minimising off-target side effects.

Currently, we are examining the application of poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles to deliver bioactive peptides and proteins into the cytoplasm of target cells. Our work is based on the modulation of physical characteristics such as particle size and surface charge to control their release from endosomal compartments into the cytosol after endocytosis into the tumour cell. This is in an effort evade the premature destruction of these labile molecules in the harsh environment within the endosomal lumen before they can elicit their biological effect. We demonstrate the formulation of PLGA nanoparticles loaded with either a 16 residue peptide, albumin or recombinant eGFP, using the water-in-oil-in-water (w/o/w) emulsification and solvent evaporation process. We have successfully generated homogenous populations of 200 nm nanoparticles with more than 40 % entrapment efficiency. Using fluorescent coumarin 6 labelled nanoparticles and confocal scanning laser microscopy we have been able to monitor the cellular uptake of these particles to endosomes in various tumour cell lines. Currently, by dual staining microscopy, we are investigating the sub-cellular localisation of our various nanoparticle species and formulations to observe if surface charge on these particles can result in destabilisation of endosomal membranes resulting in release of the nanoparticles into the cytoplasm of tumour cells. In parallel we are also examining if modulation of the sub-cellular localisation of these peptide/protein containing nanoparticles impacts on the half life of these labile molecules.

P31

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Title: Use of an Intestinal Filter for analysis of transcriptomic studies of Barrett's Oesophagus identifies a GATA-6 regulated network of gene regulation

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Abstract:
Barrett's oesophagus (BO), a precancerous condition associated with oesophageal adenocarcinoma (OAC) is characterised by the replacement of the normal squamous epithelium with an intestinal metaplasia above the gastro-oesophageal junction. In the progress of OAC through BO many intestinal markers are observed including CDX2, Villin and various MUC and Keratin staining patterns. Intestinal markers in BO are frequently lost in the cancer sequence and thus may not be the driving factors behind either the observed genomic instability or the progress to adenocarcinoma. Therefore a genomic comparison between BO intestinal metaplasia from cancer patients and tissue samples of the normal intestine may accurately classify the intestinal specific gene expression of BO and highlight genes involved in driving tumourogenicity. We have utilised online publicly available depository sites for microarray data (GEO and Array express) to retrieve and re- interpret pre-existing studies and generate a novel meta-analysis of genomic data. Two such transcriptomic studies have been obtained, one charting the BO to OAC cancer sequence (Kimchi *et al.,* 2005, GDS 1321) and one examining gene expression of the duodenum (Troost *et al.,* 2006). We utilised the duodenal array data to generate an intestinal filter for the oesophageal cancer sequence. This filter was successful in accurately classifying intestinal gene expression in BO the (160 genes) removal of which highlighted Barrett's specific genes (478 genes). These gene lists were subsequently analysed in a gene networking and pathway analysis programme (Metacore) to unravel potential functional relationships. This system categorised the gene lists obtained from the oesophageal cancer sequence with and without the intestinal filter applied and identified key differences in cell adhesion/ECM remodelling networks and in cytokine-mediated adhesion networks. GATA6 and PPARg/RXR transcription factor driven networks were clearly identified as networks involved in the BO-OAC sequence following application of the intestinal filter. These networks displayed potentially biologically important interactions and regulations that may have relevance to the oesophageal cancer sequence including relationships with inflammatory genes. This study has developed a novel intestinal filter in the analysis of oesophageal carcinogenesis and proposed potentially important interactions of these genes through gene networking. Further studies with more diverse duodenal material may further increase the stringency of the intestinal filter developed in this study. GATA-6 and RXR-regulated pathways warrant further study in oesophageal carcinogenesis.

P32

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Title: LOW EIF6 EXPRESSION IS ASSOCIATED WITH REDUCED DISEASE-FREE SURVIVAL IN OVARIAN SEROUS CARCINOMA PATIENTS

Authors: Flavin R[1], Smyth P[1], Finn SP[3], Laois A[2], O'Toole S[2], Barrett C[1], Ring M[1], Denning K[1], Li J[1], Aherne S[1], Aziz NA[2], Alhadi A[2], Sheppard B[2], Loda M[3], Martin C[1], Sheils O[1], O'Leary JJ[1].
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Abstract: MicroRNAs (miR) are a group of small non-coding RNA's approximately 22 nt in length. Recent work has shown differential expression of mature miR in human cancers. Production and function of miR requires coordinated processing by proteins of the miR machinery. Dicer and Drosha (RNase III endonucleases) are essential components of the miR machinery. Recently, the ribosome anti-association factor eIF6 has also been found to have a role in miR-mediated post-transcriptional silencing. We characterized the alterations in expression of genes encoding proteins of miR machinery in ovarian serous carcinoma. Protein expression of eIF6 and Dicer was quantified in a TMA of 66 ovarian serous carcinomas. Dicer, Drosha and eIF6 mRNA expression was analysed using qRT-PCR on an independent set of 50 FFPE ovarian serous carcinomas. Expression profiles of eIF6 and Dicer were correlated with clinico-pathological and patient survival data. We provide definitive evidence that eIF6 and Dicer are both upregulated in a significant proportion of OSC and are associated with specific clinico-pathological features of OSC, most notably low eIF6 expression being associated with reduced disease-free survival. The status of eIF6 and proteins of the miR machinery may help predict toxicity and susceptibility to future RNAi based therapy.

P33

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Title: Prodrug Activation Therapy Kills Prostate Cancer Cells in an Three-Dimensional Culture Model

Authors: Ruth Foley, Laure Marignol, Mark Lawler
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Abstract:

Prostate cancer is a leading cause of morbidity and mortality among older men, and novel therapies are needed particularly for advanced disease. This study investigates a prodrug activation gene therapy approach for prostate cancer treatment in a three-dimensional culture format. Cell lines grown in this format can form spheroids, thus offering significant advantages over two-dimensional monolayer cultures in relevance to clinical tumours.

The prodrug activation system uses the bacterial gene cytosine deaminase (CD) to convert the prodrug 5-fluorocytosine (5-FC) to 5-fluorouracil. Mammalian expression vectors were generated in which transcription of this gene was regulated by the constitutive cytomegalovirus (CMV) and prostate-specific antigen (PSA) promoters. These plasmids were transiently transfected into the prostate cancer cell line 22Rv1. Transfected cells were plated in a basement membrane matrix (Matrigel®) and treated with 5-FC. Cells were assayed for survival by an MTT assay and spheroid sizes were measured.

22Rv1 cells formed three-dimensional spheroids within three days when grown in Matrigel. Transgene expression was maintained for at least six days after transfection in three-dimensional cultures as indicated by GFP fluorescence. Cell survival was reduced by 70% and 20% respectively in cultures transfected with CMV-CD and PSA-CD vectors and treated with 5-FC. Average volumes of spheroids were reduced by up to tenfold by CD transfection and 5-FC treatment.

Three-dimensional cultures offer a realistic method of testing gene therapy and other approaches to prostate cancer treatment in vitro, and the CD/5-FC prodrug activation system shows efficacy in this format in addition to monolayer cultures.

P34

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Title: Subcellular localization and analysis of tyrosine phosphorylation of the tumour suppressor protein, maspin.

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Abstract:
The tumour suppressor protein, maspin (serpin B5), prevents tumour growth and metastasis through inhibition of angiogenesis and tumour cell migration. It was originally identified in breast epithelial cells[1], and down-regulation of maspin expression correlates with tumour progression and metastasis in both mammary and prostate cancers. Thus, it is of interest both as a cancer therapeutic and a diagnostic tool. However, maspin is a non-inhibitory member of the serine protease inhibitor (serpin) family, and the molecular mechanisms by which it carries out its actions are not well understood.
In this study we set out to establish the subcellular localization and tyrosine phosphorylation of maspin, in order to subsequently examine their importance for tumour suppression. Using HEK293 cells transiently transfected with an EGFP-maspin construct, we have shown that maspin has a nucleocytoplasmic distribution and can be actively imported into the nucleus. This result clearly indicates active nuclear import is taking place, as the fusion protein is too large for passive diffusion. We have carried out relative quantification of subcellular localisation using EBImage to analyse LSM images; the Fn/c ratio calculated is 1.65, indicating that EGFP-maspin protein was predominantly found in the nucleus. To date the nuclear import and export pathways have yet to elucidated/described. We have made a preliminary study of its nuclear export pathway; a Leptomycin B treatment showed that maspin is not exported via the most common route, the Crm1-dependent pathway.
Previous studies suggest that maspin is phosphorylated on one or more tyrosine residues[2]; our aim is to identify the modified residues. We have performed site-directed mutagenesis to individually and conservatively mutate tyrosines to phenylalanines. Expression in HEK 293 and immunoprecipitation of the wild type and mutant maspin proteins was followed by immunoblot analysis with anti-phosphotyrosine antibodies. *In vitro* kinase assays were carried out to identify the modified residues and mass spectrometry analysis of the modified protein will be used to confirm these results.

1. Zou et al (1994) Science 263, 526–529.
2. Odero-Marah et al (2002) BBRC 295, 800–805.

P35

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Title: Investigating the link between hypoxia, AKT compartmentalization and cell survival.

Authors: Gately K, Stewart DJ, Davies A, Edwards JG, Richardson D, Jones JL, Burke B, Waller DA, Ziegler-Heitbrock L, Wardlaw AJ, and O'Byrne KJ

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Abstract:

INTRODUCTION – The Phosphatidylinositol-3 kinase (PI3K)/Akt (PKB) pathway is activated in a wide range of tumour types, and plays a central role in cell survival. Recent evidence indicates that hypoxia induces upregulation of Akt and activation of the Akt/PKB pathway. Once activated, Akt phosphorylates a variety of downstream substrates, including FOXO3a, facilitating cell survival by blocking apoptosis. Evidence from patient tumour samples suggests that nuclear localization may be the most important aspect of the cell survival activity of pAkt. We have shown that nuclear pAkt expression is associated with more advanced disease or poor prognosis in non-small cell lung cancer (NSCLC) and malignant pleural mesothelioma (MPM). Carbonic Anhydrase (CA)-IX, a tumour-specific member of the carbonic anhydrase family, is a surrogate marker of hypoxia overexpressed in solid tumours. This study examines the expression of CA-IX and phosphorylated Akt (pAkt) in tumour samples from patients with MPM, correlating expression with established prognostic factors. Changes in the subcellular localisation of pAkt and FOXO3a, in a panel of MPM cells, over time under both normoxia and hypoxia were quantified. The role of pAkt in the survival of MPM cell lines exposed to both normoxic and hypoxic conditions was also examined.

METHODS – Tumour samples from 200 patients with MPM were stained using pAkt and CA-IX specific antibodies. Western blot analysis was used to examine the effect of hypoxia on Akt and pAkt expression in 4 MPM cell lines in the presence or absence of the phosphatidylinositol-3-kinase inhibitor, LY294002. High-content screening (HCS) analyses, using the Incell 1000, was used to quantify any changes in subcellular localisation of pAkt and FOXO3a in the cells. FACs and HCS were used to quantify levels of apoptosis.

RESULTS - There was a positive association between the level of CA-IX and pAkt staining, implying that intra-tumoural hypoxia may be a stimulus for Akt phosphorylation. On multivariate analysis increased expression of nuclear phospho-Akt (pAkt) was found to be associated with a poor survival. Hypoxia induced the activation of Akt in the panel of cell lines with CRL5915 cells showing very low levels of pAkt expression. Changes in the subcellular localisation of pAkt and FOXO3a were quantified. JU77 cells had a significant increase in the level of apoptosis under hypoxic conditions when the phosphorylation of Akt was blocked by LY294002.

CONCLUSION - This work provides evidence for the anti-apoptotic role of pAkt in hypoxic conditions in solid human malignancies. Phospho-Akt may represent a novel therapeutic target in MPM.

P36

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Title: Cancer Services
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Abstract:

There have been many recommendations re improvement in cancer services. We report the results of tracking service change in Northern Ireland for Oesophageal and Stomach cancers from a baseline in 1996 to 2001 and 2005.

Each year approximately 200 oesophageal cancers occurred with $^{2}/_{3}$ in males, 35% were smokers, 30% ex-smokers, 55% were current drinkers and 5% ex-drinkers, 12% had a positive history of Barrett's Oesophagus. Endoscopy and CT rates increased markedly, with 69% surgery patients, 50% all patients having a PET scan, staging increased from 41% in 1996 to 78% patients in 2005 with higher levels among surgery patients. The number of patients with more than 15 lymph nodes examined increased from 3% in 1996 to 41% in 2005. 61% of patients were discussed at MDT compared with 1% in 1996. There were 49 resections undertaken by 12 operators, this represented 20 fewer resection than in 1996/2001. Those recorded as receiving 'no active treatment' fell from 39% to 10%. There was a large increase in referrals for dietetic advice from 45% to 80% of patients. There was a significant improvement in total survival driven by the improved observed survival at 1 year in resection patients from 69% - 79% (<0.05). This reflects improved patient selection.

For stomach cancer, CT scan and endoscopy use increased as did staging, discussions at MDT from 2% - 42%. 49 resections (a reduction from 74 in 1996) were undertaken by 22 operators. By 2001 18% of patients had 'no active treatment' recorded compared with 36% in 1996. There were 14 single operators in 2005. Survival rates remained unchanged. The recommendations included a need for further service centralisation. The report of this and other similar audits on lung, breast, colorectal, cervix & ovary, thyroid, pancreas and prostate cancers are available at www.qub.ac.uk/nicr

P37

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Title: The value of PSA testing in men older than 65 years

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Abstract:

Questions about the significance of raised PSA levels in older men remain unresolved. We assessed initial PSA levels and the risk of clinical prostate cancer and prostate specific mortality in men over 65 years, in a region where PSA screening is not recommended.

Methods: A register of PSA tests were linked to the Cancer Registry database. All men with a first PSA between 1994 and 1998 were included with follow-up for prostate cancer diagnosis and mortality until end 2003. Absolute cancer risk, hazard ratios for prostate cancer and mortality based on initial PSA levels were calculated using Cox proportional hazards models adjusted for age.

Results: Of 36003 men, 2153 (6.0%) were diagnosed with prostate cancer within follow-up period. Mean [SD] age was 74.9 [6.7] yrs and mean [SD] follow-up was 5.4 [2.9] yrs. 13074 (36.3%) men died, with prostate cancer the primary cause in 673 men (5.1% all deaths). Within age groups, the absolute risk and hazard ratio of cancer increased with PSA level. Prostate specific mortality remained low (<5/1000 person yrs) at PSA categories <15.0ng/ml. All cause mortality was similar in PSA categories <10.0ng/ml, and was much greater than prostate specific mortality in all PSA categories.

Conclusions: Prostate cancer risk and prostate specific mortality is related to initial PSA level. However, in those over 65 years, death from prostate cancer was infrequent compared to other causes, even when initial PSA was elevated (≤20.0ng/ml). A conservative approach to invasive investigation is appropriate in the majority of older men.

P38

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Gamma ray-induced bystander effects in colorectal cancer: a specific study on anaphase bridge and micronuclei formations in unirradiated bystander cells

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Introduction: The bystander effect is a term in radiation biology, describing how irradiated cells can signal to neighbouring un-irradiated cells via secreted factors. Our hypothesis is that the bystander effect may accelerate early genomic instability events which in turn could influence radiation treatment responses and disease recurrence.

Method: A novel ex vivo colorectal cancer explant model was used to examine the radiation bystander response in tumour and matched normal resected tissue in 8 patients. Explant tumour and matching normal tissue were treated with radiation alone (2Gy, 5Gy and 10 Gy) or with FOLFOX. Anaphase bridge formation and micronuclei cells were scored as markers of cellular genomic instability by H&E assessment.

Results: There was a significant increase in anaphase bridge formation in bystander cells exposed to conditioned media from both tumour and normal treated tissue for the different radiation dosages $\pm$ FOLFOX (all p values<0.01). These bridges represent lagging chromosomes not resolve following mitosis. The levels of micronuclei formation in bystander cells did not differ between treatments.

Conclusion: This novel finding of increased anaphase bridge formation induce by radiation bystander events imply that radiation bystander responses may play a major role in driving early genomic instability and possibly disease recurrence in colorectal cancer.

P39

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Title: **BRCA1 transcriptionally regulates genes associated with the basal phenotype in breast cancer**.

Authors: Julia J. Gorski, Colin R James, Jennifer E. Quinn, Gail E. Stewart, Alison Hosey, Paul B Mullan, Patrick. G. Johnston, Richard H. Wilson and D. Paul Harkin.

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Abstract:

BRCA1 encodes a tumour suppressor gene that is mutated in the germline of women with a genetic predisposition to both breast and ovarian cancer. A number of recent studies have demonstrated that BRCA1-deficient tumours exhibit a genotype similar to basal-like breast tumours including triple negative receptor status (low ERα, PR, HER2 expression) and strong expression of the basal cytokeratins 5, 6 and 17 and p-cadherin. In order to investigate the potential mechanisms underpinning this observed similarity we investigated the direct impact of BRCA1 modulation on the expression of a number of well characterised basal markers. We demonstrated that functional BRCA1 repressed the expression of KRT5, KRT17 and p-cadherin in HCC1937, T47D and MDA468 breast cancer cells at both the mRNA and protein levels. Furthermore, chromatin immunoprecipitation (ChIP) assays demonstrated that BRCA1 is recruited to the promoters of KRT5, KRT17 and p-cadherin and re-ChIP assays confirmed that BRCA1 is present within a complex with c-Myc and SP1 on the p-cadherin promoter. In addition we demonstrated that siRNA mediated inhibition of endogenous c-Myc resulted in a marked increase in p-cadherin expression analogous to that observed following inhibition of endogenous BRCA1. Finally we confirm the interaction between BRCA1 and c-Myc by co-immunoprecipitation and demonstrated that this interaction is lost in BRCA1 mutant cells. The data provided suggests a model whereby BRCA1 and c-Myc can form a repressor complex on the promoters of defined basal genes and provides a novel mechanism to explain the observed overexpression of key basal markers in BRCA1 deficient tumours.

P40

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Title: **EP receptors in NSCLC, and their regulation by epigenetic modifications**
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Abstract:
Introduction: PGE_2 exerts its effects through binding to specific receptors. There are at least four subtypes of PGE_2 receptor, designated as EP1, EP2, EP3, and EP4, according to their pharmacological profiles and signal transduction pathways. Although the role of each EP receptor in cancer biology remains complex, evidence is building that these receptors may be relevant therapeutic targets, and may also have predictive and or prognostic value in non-small cell lung cancer (NSCLC).
Methods: A panel of normal and lung cancer cell lines were screened for expression of EP1-4, by RT-PCR under conditions of normoxia and hypoxia (0.5%). Their expression in matched tumor/normal samples from patients with NSCLC was also examined. Epigenetic mechanisms regulating their expression was examined using a) two HDAC inhibitors Phenylbutyrate (PB-10 mM) and Trichostatin A (TSA-250ng/ml, and b) DNA methyltransferase inhibition using 5-aza-2-deoxycytidine (DAC-1 μM).
Results: Expression of all four EP receptors could be readily detected in all cell lines with the following exceptions; Beas-2B and A549 did not express EP3, and H1299 did not express any EP2. In primary NSCLC lung tumour samples (n=20) with matched normal tissue, altered expression for EP receptors was observed in all tumours specimens. Epigenetics mechanisms regulating the expression EP1-4 were studied. A549 (adenocarcinoma) and SK-MES-1 (squamous cell carcinoma) cell lines were treated with 5-Aza-2- deoxycytidine. A clear upregulation of EP1 mRNA was observed. Bioinformatic analysis of the genomic DNA containing the EP1 gene, indicates that the introns for this gene are extremely CpG rich and may be a hot-spot for DNA CpG methylation. Cells were also treated with the histone deacetylase inhibitors TSA and PB. EP-2 and EP-3 were robustly inducible by histone deacetylase inhibition, while EP4 was slightly inducible. In contrast, EP1 expression was downregulated following treatment with TSA. When compared to cells grown under normoxic conditions, EP1 expression was induced in A549 cells following 24 hour exposure to hypoxia. We are currently evaluating the effect of hypoxia on the other EP receptors in these samples.
Conclusions: Our data indicates that aberrant expression of the EP receptors is a common event in NSCLC. We show that EP receptors are epigenetically regulated via histone post-translational modifications and DNA CpG methylation. In addition, the expression of these receptors is affected by hypoxia. Further investigations are required to delineate the role of these receptors in NSCLC, and whether aberrant epigenetic regulation of these genes is important in NSCLC pathogenesis. Should this prove true, targeting the epigenetic mechanisms underpinning this pathway may be of therapeutic value in the treatment of NSCLC.

P41

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Differential Cell Adhesion within an Isogenic Model of Melanoma Progression Under Shear Flow Conditions Using a Microfluidic Cell-Based Assay

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Melanoma is the most aggressive form of skin cancer, which invades into deeper layers of the skin and has a propensity to metastasise early. There are several steps in this metastasis process, including: intravasation, survival in circulation, arrest in a specific organ, extravasation, growth and secondary tumour formation. The key steps involved in extravasation (exit of tumour cells from blood vessels), is the attachment of tumour cells to the endothelial cells lining the blood vessels via adhesion proteins, whilst under constant blood flow, and invasion to the tissue underneath. In this study, we modelled survival in circulation and adhesion to endothelial cell-derived proteins, using Cellix's Microfluidic Platform, to determine if these steps in extravasation differed in an isogenic melanoma cell line model of progression. The isogenic cell line model series was comprised of the poorly tumourigenic parental cell line WM793 and its derivatives WM793-P2 (obtained via Matrigel-assisted in vivo passaging in mice) and 1205-Lu (obtained from spontaneous metastasis to the lung) which display increased growth and invasion in vitro, as well as tumourigenicity in vivo, compared to the parental line. Firstly, Cellix's Vena8^{TM} Biochip micro-capillaries were coated with three different adhesion molecules, V-CAM, I-CAM and fibronectin, and melanoma cells were subjected to a constant shear stress of 0.5 dyne/cm^2, mimicking microvascular conditions. We found that WM793 and WM793-P2 cells did not adhere to any of the specified adhesion molecules, whereas 1205-Lu cells adhered to V-CAM under defined flow conditions. To examine this further, we applied a decreasing gradient shear stress of 5, 2, 1, 0.5, 0.25 and 0.1 dyne/cm^2 which resulted in increasing adhesion of 1205-Lu cells to VCAM at shear stresses lower than 2 dyne/cm^2. This ability of 1205-Lu cells to attach to V-Cam under high shear stresses may contribute to its extravasation abilities, thus contributing to its high metastatic potential. Currently, we are exploring the use of Cellix's VenaEC^{TM} Biochip which facilitates the growth of endothelial cells. GFP-labelled tumour cells will be flowed over endothelial cells and will be subjected to defined shear stresses to mimic the full extravasation process.

Funding is acknowledged from IRCSET.

P42

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Title: EFFECTS OF DNA POLYMERASE ETA EXPRESSION AND PIKK INHIBITION ON THE RESPONSE OF HUMAN CELLS TO CISPLATIN, OXALIPLATIN AND CARBOPLATIN

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Abstract: The platinum-based drugs cisplatin, oxaliplatin and carboplatin induce DNA damage, inhibit DNA replication, and lead to cell death. Because the three compounds differ in chemical structure, the molecular response of cells to DNA damage induced by each agent may also differ. The capacity of cells to carry out replication of damaged DNA, using the process of translesion synthesis (TLS), is one mechanism by which cells can tolerate DNA damage. We have investigated the effect of expression of DNA polymerase η (polη), a translesion synthesis (TLS) enzyme, on the response of human cell lines to cisplatin, oxaliplatin and carboplatin, in terms of replication arrest and activation of DNA damage responses mediated by the phosphatidylinositol-3-kinase-related protein kinases (PIKKs) ATM, ATR, DNA-PK. In S-phase cells, ssDNA generated following DNA damage activates ATR, leading to arrest of cell cycle progression. One PIKK substrate is replication protein A (RPA), a heterotrimeric protein that plays a key role in maintaining the stability of DNA containing single-stranded regions. We focused on DNA damage-induced phosphorylation of RPA2, the 34kDa subunit of RPA, in response to cisplatin, oxaliplatin and carboplatin. To investigate the relationship between lesion bypass, replication arrest and activation of DNA damage responses, responses have been compared between polη-deficient XP30RO cells and normal GM00637 cells. Cells lacking polη are more sensitive to killing by cisplatin, oxaliplatin and carboplatin. In polη-deficient cells, drug treatment leads to prolonged S-phase arrest and increased phosphorylation of chk1, p95/Nbs1 and RPA2. To characterise the sequence of PIKK-dependent phosphorylation events, cells have been co-treated with platinum-based drugs and a series of specific inhibitors of individual PIKKs. Cisplatin-induced phosphorylation of RPA2 on serine 4/serine 8, but not on serine 33, was inhibited by the DNA-PK inhibitor, NU7441. DNA-PK-dependent hyperphosphorylation of RPA2 on serine 4/serine 8 occurs after recruitment of RPA to chromatin, as determined by immunofluorescence and by subcellular fractionation. In contrast, inhibition of ATR using the small molecule CGK733 blocks both cisplatin-induced phosphorylation of RPA2 on serine 33, and recruitment of RPA to chromatin. Thus, in response to cisplatin, RPA2 is first phosphorylated on serine 33 by ATR, followed by recruitment of RPA to chromatin, and by DNA-PK-dependent phosphorylation on serines 4/8.

While cisplatin and oxaliplatin both strongly induced phosphorylation of RPA2 on serine4/serine8 in polη-deficient cells, exposure to an equitoxic dose of carboplatin failed to induce RPA2 phosphorylation after 18h. Comparison of the kinetics of RPA2 phosphorylation in response to the three platinum agents with their effects on cell cycle progression, indicates that RPA2 hyperphosphorylation on serine4/serine8 correlates with the extent of replication arrest and checkpoint activation in polη-deficient cells. Elucidation of the precise sequence of PIKK-dependent phosphorylation events that occur in response to cisplatin, oxaliplatin and carboplatin should lead to a better understanding of the molecular basis of the tumour specificity of individual platinum-based drugs.

P43

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Title: The Tumour Suppressor and Angiogenesis Inhibitor Maspin Binds to the Glycosaminoglycan Heparin

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Abstract:
Over recent years a number of members of the serpin (serine protease inhibitor) superfamily have been shown to possess potent anti-angiogenic effects including α-1 antitrypsin, antithrombin III, PEDF and maspin. Both *in vitro* and *in vivo* models have shown that these serpins can reduce tumour cell growth, migration and tube formation *in vitro* as well as reducing tumour size and microvessel density in *vivo*. In addition, mutation of the heparin-binding site of antithrombin III has been shown to abrogate its antiangiogenic effect.

Maspin (Serpin B5) is a tumour suppressor protein down regulated in breast and prostate cancer. Maspin has been shown to inhibit angiogenesis exogenously and endogenously and previous studies have shown maspin binds to the cell surface. To date, it is unclear how maspin mediates its cell surface effects. We suggest that maspin inhibits angiogenesis via attachment to heparin sulphate proteoglycans where it either activates a cell surface receptor (possibly integrins) or is internalised and inhibits angiogenesis endogenously.

We have expressed maspin as a His-tagged fusion protein in E.coli and purified it by IMAC chromatography. We have shown that maspin is capable of binding to heparin agarose and its elution requires high concentrations of salt or addition of free heparin Chemical modification of maspin by biotinylation greatly reduced its capacity for binding heparin, indicating the importance of lysine residues for heparin binding. We have shown that addition of heparin shifts the electromobility of maspin in a native PAGE gel. Finally, in a pulldown experiment we have shown that only heparin and heparin sulphate can elute maspin from heparin agarose beads but not other glycosaminoglycans (De-N-Sulphated heparin, N-Acetylheparin or the Chondroitin sulphates A and B). These preliminary results taken together strongly suggest an interaction between maspin and the glycosaminoglycan heparin and support the hypothesis that serpins inhibit angiogenesis through a heparin/heparan sulphate dependent mechanism.

P44

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Title: The role of the HER2/HER3/PI3 Kinase survival pathway in colorectal cancer

Authors: Caitriona Holohan, Sandra Van Schaeybroeck, Joan Kyula, Owen McGrath, Patrick Johnston.
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Abstract: **Background:** Human cancer cells may respond to chemotherapy by activating survival pathways such as the human epidermal receptors (HER). Previous studies carried out by our group have shown that colorectal cancer cells which respond to chemotherapy with increased HER1 phosphorylation, were sensitized to HER1-targeted therapies. Moreover, we found that TGF-α and ADAM-17 are critical mediators of chemotherapy-induced HER1 activation. The aim of the present study was to investigate the role of the HER family ligands and HER2-HER3-PI3K-Akt pathway as an underlying mechanism of chemotherapy-resistance in CRC cells. **Methods**: Apoptosis was measured by Flow Cytometry. HER1, HER2, HER3 and Akt phosphorylation were assessed by Western blotting. Inhibition of ligand expression was achieved by siRNA and measured by real-time PCR. **Results**: We have found that increased HER1 activation was associated with increased HER2, HER3 and Akt activation following chemotherapy treatment. Furthermore, treatment with 5-FU resulted in the increased expression of TGF-α, amphiregulin, epiregulin and heregulin at the mRNA level. Interestingly, silencing of TGF-α, amphiregulin and heregulin resulted in a complete abrogation of the 5-FU-induced PI3K/Akt pro-survival pathway. Furthermore, inhibition of this HER1/HER2/HER3 survival response using the HER1/HER2 tyrosine kinase inhibitor lapatinib, resulted in a synergistic interaction in CRC cells. **Conclusions**: Our findings indicate that the HER1/HER2/HER3 pathway is an important survival pathway following chemotherapy treatment and that TGF-α, amphiregulin and heregulin are critical mediators of this anti-apoptotic stress response. Moreover, combining the HER1/HER2 inhibitor lapatinib with chemotherapy may have therapeutic potential for the treatment of colorectal cancer.

P45

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Title: **Septin 9_v1 stabilisation of HIF-1α in the absence of hypoxia mediates increased expression of COX-2 and VEGF-A *in vitro***

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Institution: Centre **for Cancer Research & Cell Biology, Queens' University Belfast.**

Abstract: Several sets of data indicate that there are marked alterations in Septin 9 (SEPT9) transcript expression in cancer. In particular there is increased expression of SEPT9_v1 and v4*. Previously, we have shown that overexpression of the SEPT9 _v4 isoform leads to enhanced motility, loss of polarity and resistance to microtubule-interacting drugs. Recent experiments have now focused on SEPT9_v1 and in particular its involvement in the HIF-1alpha (HIF-1α) pathway. HIF-1α is an oxygen responsive transcription factor that under normoxic conditions is rapidly degraded by a ubiquitin-mediated pathway. It has been shown that SEPT9_v1 can bind HIF-1α and we demonstrate that the minimal region required to interact with HIF-1α is SEPT9_v1 (1-164) and that only SEPT9_v1 (which contains a bipartitie NLS in its unique N terminus) is capable of entering the nucleus with HIF-1α. Over-expression of SEPT9_v1 leads to stabilisation of HIF-1α even under normoxic conditions which then translocates to the nucleus resulting in the transactivation of downstream hypoxia response elements (HRE) genes. Comparing SEPT9_v1 over-expressing cells with control cells the expression of the HIF-1 responsive gene VEGF-A was shown to be upregulated at the mRNA level. Also, the more potently angiogenic and mitogenic secreted (soluble) VEGF-A-121 protein isoform was shown to be increased by SEPT9_v1 expressing cells relative to controls. In addition, SEPT9_v1 over-expressing cells show increased expression of cyclooxygenase (COX)-2 compared to controls when grown under normoxia and hypoxia. COX-2 is highly expressed in a number of cancers and tumour cell lines and its expression induces the synthesis of prostaglandin E2 (PGE2) which can increase HIF-α protein levels under normoxia, potentiate hypoxia-induced HIF-1α-expression and nuclear localisation. In this study we demonstrate that over-expression of SEPT9_v1 in human breast and ovarian cancer cell lines results in COX-2 induction in these cells, and that stabilisation of HIF-1α protein under normoxia (and hypoxia) leads to increased expression and alteration of specific VEGF-A isoforms. Our data suggests for the first time that SEPT9_v1 stabilisation of HIF-1α in the absence of hypoxia may be mediated via an NFkB/COX-2 pathway and suggests a mechanism by which overexpression of this isoform contributes to the malignant phenotype.

P46

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Title: Epithelial-MesenchymalTransition (EMT) protein expression and mis match repair profiles in Stage II colorectal cancer with tumour budding status

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Abstract:

Introduction: Tumour budding occurs when tumour cells become detached from neoplastic glands either singly or in small aggregates of less than five cells
. Limited reports suggest that different degrees of budding are observed in microsatellite stable (MSS) versus microsatellite instable (MSI) in Colorectal Cancer (CRC) subtypes. Epithelial-Mesenchymal transition (EMT) is the process whereby epithelial cells de-differentiate and acquire a more invasive phenotype and this process is hypothesised to be the molecular basis for tumour budding.

Aims & Background: To define the mismatch repair protein status and assess expression of EMT proteins: LAMC2, E-Cadherin & Cathepsin L in a cohort of patients with stage II(T3N0) CRC with well characterised tumour budding status and extensive patient survival data.

Method: Degree of tumour budding was assessed by 2 independent pathologists in 128 stage II (T3N0) CRCs. Immunohistochemistry was performed using mouse monoclonal antibodies to LAMC2, E Cadherin, Cathepsin L, MLH1 & MSH2 and data was correlated with tumour budding status and survival.

Results: High tumour budding index significantly correlates with a poorer prognosis in stage II (T3N0) CRC (HR 4.7; $p<0.001$).
Tumour budding occurred more frequently in MSS versus MSI tumours 48% versus 26% respectively. LAMC2 positivity was associated with tumour budding phenotype ($p=0.01$) and correlated with cancer specific survival ($p=0.017$).

Conclusion: Tumour budding status is more common in MSS versus MSI tumours and may play a role in distinguishing prognoses in different CRC subtypes. LAMC2 expression strongly correlates with the tumour budding phenotype and survival, further work will validate its functional role in tumour de-differentiation, cellular invasion and as a prognostic marker in colorectal cancer.

P47

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Title: High Clusterin expression is associated with poorer prognosis in a cohort of stage II Colorectal Cancer patients

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Abstract:

Introduction:
Clusterin is a disulfide-linked secreted glycoprotein. Its role in tumourigenesis has remained elusive with reports supporting both a pro and anti-apoptotic effects. No study has evaluated Clusterin expression and cancer specific survival in colorectal cancer(CRC).

Aims & Background:The aim of this study was to assess Clusterin expression in a cohort of Stage II CRC patients and correlate with cancer specific survival.

Methods: Tumour tissue from 251 chemotherapy naïve stage II CRC cases was analysed. Matched adjacent normal colonic tissue was available for analysis in 210 cases. Tissue microarrays were constructed and immunohistochemistry was performed. Survival data was retrieved from institutional CRC database. Univariate survival analyses were performed using Kaplan-Meir method. Multivariate analyses were performed using Cox-regression.

Results: Median age 70.6 years (range 32.9 – 88.8); Male 136(54%); Median Follow-up 4.85 years (Range 0.02-13.79). High Clusterin expression correlated significantly with impaired survival across the following parameters: Tumour epithelial cytoplasmic % positivity and intensity($p<0.005$); Tumour stromal cytoplasmic % positivity and intensity ($p<0.001$); Adjacent Normal epithelial cytoplasmic intensity ($p<0.01$); Adjacent Normal stromal cytoplasmic % positivity and intensity ($p<0.005$). Multivariate analysis demonstrated Tumour epithelial cytoplasmic intensity($p<0.001$) and Adjacent Normal epithelial cytoplasmic intensity($p<0.01$) to be independently prognostic.

Conclusion: This is the first study to demonstrate a correlation between Clusterin expression and cancer-specific survival in CRC. This data suggests clusterin may be of value as a prognostic biomarker in stage II CRC. Further assessment of clusterin in CRC is required to determine its precise role in tumourigenesis and potential as a novel therapeutic target.

P48

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Title: **Investigation of miR-9,miR-101 and miR-21 as candidate tumor suppressors or oncogenes in cancer**.

Authors: Prasad KOVVURU[1], Grace MARTIN[1], Duygu SELCUKLU[1,2], Katherine SCHOUEST[1], Rachel CLIFTON[1] and Charles SPILLANE[1]

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Abstract: MicroRNAs (miRNAs) are endogenous non-coding RNAs, ~ 22nt in length which play important regulatory roles in multicellular organisms. MicroRNAs regulate gene expression by either translational repression or mRNA cleavage based on their complementarity to the 3'UTR regions of target genes. Recent studies suggest that more than 50% of miRNA genes are located in cancer-associated genomic regions or fragile sites. Up to 30% of protein encoding genes are potentially regulated by miRNAs. Reflecting their role in multicellular organisms and the fact that cancer is limited to multicellular organisms, miRNAs have been shown to be differentially expressed in several cancer types. Misregulated miRNA themselves may act as an oncogene or tumor suppressor genes, where they target tumor suppressors or oncogenes, respectively. Although there are a range of bioinformatics programs developed to predict candidate downstream targets of miRNAs, few targets of miRNAs have been experimentally validated. We have taken all of the known microRNAs in the human genome and conducted a bioinformatics screen of these against 363 genes known to be associated with cancer (derived from Sanger Cancer Gene Census).We used three programs, miRanda, Target Scan, and PicTar to identify cancer genes that could potentially be targeted by one or more miRNAs. The miRNA target genes were divided into two groups – those in which the targeting miRNAs are expressed in HeLa or MCF7 (the cell line platforms which we are using for functional studies), and those in which they are not. Three miRNAs, miR-9, miR-101, and miR-21 were chosen for functional analysis using two different research approaches. Firstly, in case of miR-9 and miR-101, the precursor miRNAs were cloned into pcDNA3.0 mammalian expression vector. These constructs are used to overexpress mature miRNAs in the cell lines where they are not endogenously expressed. The effect of overexpression on predicted targets at the mRNA level is tested by RT-PCR and at the protein level by luciferase reporter based assays/western blot techniques. In the case of mir-21, which we have shown the mature form by qRT-PCR to be endogenously expressed in HeLa and MCF-7 cells, we test predicted targets by luciferase reporter assays. For this assay, predicted targets are cloned into the 3'UTR of luciferase gene of the pMIR report luciferase vector. These constructs are transfected into HeLa and MCF-7 cells and luciferase assays are performed to determine if the miRNA interacts with its predicted targets. If down regulation of luciferase is seen, transfection of a specific antimir-miRNA inhibitor can subsequently confirm if the down regulation is due to the predicted miRNA. Our research aims to identify novel miRNA: target gene interactions that are relevant to understanding cancer. The research highlights the role of RNA signalling pathways whereby some miRNAs can act as oncogenes or tumour supressors.
This research is supported by Cancer Research Ireland and the Irish Research Council for Science, Engineering and Technology.

P49

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Title: Investigating the HOXA9/MEIS1 axis in Leukaemia

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Abstract:

HOX genes encode proteins which function as master regulators of embryogenesis and are involved in haematopoietic cell development. Some 39 HOX genes exist in clusters (A-D) on 4 chromosomes. HOXA9 is highly expressed in acute myeloid leukaemia (AML) and is a fusion partner of NUP98 encoding the leukaemogenic protein NUP98-HOXA9. In murine models over-expression of Hoxa9 and its cofactor Meis1 results in a transplantable and fatally aggressive leukaemia.

We set out to identify target genes associated with HOXA9 over-expression. To investigate this further we used the benign Rama37 epithelial cell line which expresses low levels of Hoxa9 (~150 copies/50ng RNA) and high levels of Meis1 (~105,000 copies/50ng RNA). Transient transfection of Rama37 yielded ~100,000 copies of Hoxa9/50ng RNA thereby recapitulating the expression of Hoxa9 and Meis1 typically found in the murine model. These changes were associated with an increase in invasiveness compared to Rama37 control cells. Rama37 cells stably transfected with Osteopontin, a known downstream target of Hoxa9, also showed a 50% increase in invasiveness and a 59% reduction in adhesiveness compared to the Rama37 parental cell line.

Microarray data generated from 330 patients with AML showed a positive correlation between the expression of HOXA9 and β-integrin (ITG) family of adhesion molecules and vascular endothelial growth factor (VEGF). Thus ITGB1, ITGA2B, ITGA6, ITGA9 and VEGFA all showed increased expression in the presence of raised levels of HOXA9. Future work will attempt to unravel the biochemical pathways involved in the increased invasiveness associated with HOXA9 over-expression.

P50

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Title: **Dysregulation of miR-223 and miR-9 in recurrent ovarian cancer.**
Authors: Laios A, O'Toole SA, Flavin R, Kelly L, Sheppard B, Martin C, Ring M, D'Arcy T, McGuinness E, Gleeson N, Sheils O, O'Leary JJ.
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Abstract:
Background: MicroRNAs (miRNAs) are small, noncoding RNAs that negatively regulate gene expression by binding to target mRNAs. Dicer and Drosha constitute essential proteins in miRNA processing machinery. miRNAs have not been comprehensively studied in recurrent ovarian cancer, yet an incurable disease. Our recent profiling of 180 miRNAs in a set of recurrent versus primary serous papillary adenocarcinomas identified miR-223 and miR-9 as the top up and downregulated miRNAs respectively. Predicted targets for these miRNAs were previously identified in our transcriptomic approach. The aim of this study was to validate these miRNAs in an independent set of primary and recurrent ovarian cancers in fresh frozen and FFPE archival tissue and elucidate a potential mechanism for miRNA deregulation.

Design: Total RNA was extracted from 12 primary advanced and 8 recurrent fresh frozen ovarian tumours using the Ambion mirVana™ miRNA isolation kit. We also extracted total RNA from FFPE material available for the above tumours using the Ambion RecoverAll™ Total Nucleic Acid Isolation Kit. miRNA expression levels were examined using the AB stemloop RT/PCR kit (ABI). Quantification of recurrent samples was carried out relative to primary using the ΔΔCt method. Let-7a was used as an endogenous control. Dicer and Drosha expression was also analysed in the independent set using qRT-PCR. Relative quantitation was carried out as previously and with 18S ribosomal RNA as an endogenous control.

Results: miR-223 was upregulated in recurrent versus primary fresh frozen tumours and miR-9 downregulated by more than two fold change. Additional validation of miR-223 expression in FFPE samples also demonstrated similar upregulation and proves miRNA amenability to detection in archival material. Fold changes <2 were observed for Dicer and Drosha in the independent validation set.

Conclusions: Dysregulated miR-223 and miR-9 may be important in recurrent ovarian cancer. Dicer and Drosha are not responsible for miRNA deregulation in recurrent ovarian cancer.

P51

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CCN3 reduces the clonogenic potential of Chronic Myeloid Leukaemia cells

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Chronic Myeloid Leukemia (CML) is characterized by expression of the constitutively active BCR-ABL tyrosine kinase. Previously, we have identified down-regulation of the negative growth regulator, CCN3, as a result of BCR-ABL kinase activity and detected reduced CCN3 expression in human CML cell lines and primary human CML cells. We now report the growth inhibitory effect of CCN3 expression in human CML cells.

Colony formation assays were performed over 7 days to determine clonogenicity of CML cells expressing CCN3 and compared to cells treated with imatinib (1 micromolar). Human K562 CML cells were transfected with vector alone or vector containing CCN3 using Amaxa nucleofector technology or treated with imatinib, for 24h prior to plating in methyl cellulose cultures. Increased CCN3 expression in K562 cells significantly reduced colony formation by 65.4% plus/minus SD 18.8 when compared to cells transfected with vector alone ($p=0.027$, $n=3$). Treatment with imatinib also reduced colony formation (75% plus/minus SD 8.2; $p=0.001$, $n=3$) compared to untreated cells. We next assessed the clonogenic effects of CCN3 and imatinib on primary human CD34+ progenitor cells derived from CML peripheral blood samples at diagnosis ($n=3$). Cells were treated with exogenous addition of CCN3 (1 nanomolar) or imatinib (1 micromolar) for 24h prior to plating in methyl cellulose. CCN3 reduced clonogenic capacity by 25.5% plus/minus SD 3.9 ($p=0.011$) whilst treatment with imatinib reduced colony formation by 37.9% plus/minus SD 19.9 ($p=0.010$).

CCN3 is known to be a negative growth regulator and increased expression of CCN3 in BCR-ABL+ cells decreased cell clonogenic potential. Thus CCN3 down-regulation mediated by BCR-ABL offers growth advantage to hematopoietic cells.

P52

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Title: **Investigation of the role of Bcl-2 proteins during caspase independent cell death induced following microtubule disruption in chronic myeloid leukaemia cells**

Authors: Seán MacFhearraigh and Margaret M. Mc Gee
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Programmed cell death has been shown to occur in both a caspase-dependent and caspase-independent manner. While caspase-dependent apoptosis has been well characterised, our understanding of caspase-independent cell death remains incomplete.

The mitochondrion plays an important regulatory role during caspase-dependent and caspase-independent cell death, through the release of apoptogenic proteins such as cytochrome C, Smac/Diablo, AIF, Omi/Htra and Endonuclease G from the intermembrane space.

Mitochondrial release of apoptogenic proteins is regulated by the Bcl-2 protein family that is made up of both pro-apoptotic and anti-apoptotic members. Post-translational modification of the Bcl-2 protein family members such as phosphorylation and proteolytic cleavage plays an important part in regulating their activity.[1]

The BH3 only pro-apoptotic family member,Bim, a proapoptotic member of the Bcl-2 family is phosphoryled by MAP kinases Erk and JNK1/2. Erk phosphorylates Bim resulting in its targeting for proteasomal degradation.[2] Regulation of Bim by JNK1/2 occurs at both the transcriptional level; through the phosphorylation of the transcription factor c-jun causing the upregulation of Bim, and also through post translational direct phosphorylation of Bim on serine and threonine residues.[3,4]

It has been previously shown that chronic myeloid leukaemia (CML) cells undergo caspase-indepdent cell death following disruption of the microtubule network by microtubule targeting agents including taxol.[1 and unpublished results]

In this study it has been found that Bim resides in the mitochondria of CML cells. Also it has been found that the two Bim isoforms, Bim EL and L, undergo phosphorylation following treatment with taxol. Phosphoryation of Bim occurs in a time- and dose-dependent manner and precedes taxol induced apoptosis in CML cells. On further examination it has been found that phosphorylation of Bim EL occurs within 4 hours treatment with taxol, whereas phosphorylation of Bim L does not occur until 12 hours after treatment. Synchronisation of K562 CML cells by double thymidine block and treatment with taxol, has revealed that phosphorylation of Bim occurs during M phase of the cell cycle.

Inhibiton of Erk with PD98059 did not block taxol induced phosphorylation of Bim at the mitochondria or the cell death induced. However,in contrast inhibition of JNKmap kinase with SP600125 was found to block taxol induced phosphorylation of Bim and significantly reduces the cell death induced. Furthermore , inhibition of JNK causes downregulation of endogenous Bim in K562 CML cells.

Collectively these results suggest that the MAP kinase JNK plays an important role in the reglation of Bim during caspase-independent cell death.

P53

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Pyrrolo-1,5-Benzoxazepine (PBOX)-15-Induced Apoptosis of Multiple Myeloma Cells *In Vitro* is Caspase-8-Dependent and Potentiated by Bim

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Multiple myeloma (MM) is an incurable B cell malignancy accounting for ~15% of haematological malignancies. It is characterized by the accumulation of plasma cells with low proliferative capacity and defective apoptotic mechanisms. A mean life expectancy of 3-5 years, even with existing intensive chemotherapy, highlights the need for development of novel therapies. Ideally, such agents should selectively induce malignant cell death via mechanisms distinct from existing chemotherapies. Pyrrolo-1,5-benzoxazepine (PBOX) compounds are a series of novel tubulin depolymerising agents which display anti-cancer activity against a variety of malignant cell types. Importantly, PBOX compounds display minimal toxicity against normal blood and bone marrow cells. The mechanism(s) by which these agents induce cell death remains incompletely defined, and may be cell-type specific. Here we investigate PBOX-15 as a potential novel anti-myeloma agent, and investigate its mechanism of action in these cells.

Flow cytometry analysis of propidium iodide stained cells demonstrated that PBOX-15 arrested two myeloma cell lines, H929 and U266, in the G_2/M phase of the cell cycle. However, the appearance of a sub-G_0 peak, which is indicative of apoptosis, was seen only in the PBOX-15-treated NCI-H929 cells. The induction of apoptosis in PBOX-15-treated H929 cells was confirmed by DNA laddering and Annexin-V/propidium iodide staining. After 24hr of treatment, PBOX-15-induced apoptosis (35.83% ± 2.55%) was measured at higher than, or comparable levels as induced in H929 cells with vincristine (42.16% ± 2.8%), dexamethasone (14.42% ± 2.9%), and As_2O_3 (13.41% ± 5.72%).

PBOX-15-induced apoptosis of H929 cells was found to be caspase-8-dependent. However, inhibition of caspase-8 did not prevent mitochondrial membrane depolarization, and only partially prevented release of cytochrome c from the mitochondria, suggesting the independent activation of more than one apoptotic pathway in H929 cells following PBOX-15 treatment. A decrease in expression of pro-apoptotic Bim was detected in PBOX-15-treated H929 cells after 6hrs, and preceded downregulation of anti-apoptotic Mcl-1 and Bcl-2, and pro-apoptotic Bax and Bid. PBOX-15-induced apoptosis of H929 cells was reduced in cells transfected with Bim siRNA, suggesting a role for Bim in potentiation of apoptosis in PBOX-15-treated H929 cells.

Gene expression profiling of H929 and U266 cells, carried out using ABI TaqMan Low Density Arrays, identified a number of apoptosis-related genes with differential expression in H929 and U266 cells. Of the 20 pro-apoptotic genes displaying significantly greater expression in H929 cells, 6 are members of the TNF receptor superfamily. Expression of TNFR, DR6, TNFRSF1A, and TNFRSF1B was >25-fold that detected in U266 cells. In U266 cells, expression of anti-apoptotic DIAP1, IAP1/2, and Bcl-xL were detected at levels >10-fold that present in H929 cells. These results suggest the differential response of H929 and U266 cells to PBOX-15 treatment is the result of inherent differences in the apoptotic machinery of the cell lines.

This study highlights PBOX compounds as potential novel anti-myeloma agents, and identifies downregulation of Bim as an early event in potentiation of PBOX-15-induced apoptosis. Work is ongoing to further elucidate the mechanism(s) of action of PBOX-15-induced apoptosis of H929 cells, and to understand the differential responses of H929 and U266 cells to PBOX treatment.

P54

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Title: Defining the microvasculature of Invasive Ductal Carcinoma using advanced Stereological techniques

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Abstract: Microvessel density (MVD) is a commonly used adjective to describe vascular beds. Other descriptors of vascularity have been developed and have been applied to a wide variety of systems including the reproductive and nervous systems.

These Stereological approaches are providing objective unbiased assessment of structural change. In this presentation, we elucidate the basic principles of the stereological approach and their implementation in the analysis of vascular beds.

We illustrate this approach to the analysis of the microvasculature of breast tumours. The samples studied come from a cohort of 60 pre-menopausal invasive ductal carcinomas with moderate NPI. The stereological parameters described are length density and radial diffusion distance. Length density Lv. is a simple method of estimating the length of an object (e.g. a blood vessel) embedded in a tissue. From this the radial diffusion distance can be calculated this provides a simple, robust indication of a cylindrical zone of diffusion around a vessel. This may be potentially physiologically relevant parameter.

This presentation will outline the basic steps involved in the stereological assessment of microvasculature beds.

P55

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Title: Study of the Functional Effects of Lentiviral-Mediated RNAi Knockdown of Novel Gene PLAC8 in Breast Cancer Progression.

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Abstract: PLAC8 was first identified in mouse embryos as a placental-enriched gene and has also been referred to as C-15 and Onzin. Affymetrix microarray analysis of an isogenic cell line model generated for increasingly invasive breast cancer, identified PLAC8 as being strikingly altered at the mRNA expression level and the most up-regulated gene. PLAC8 showed a 32-fold increase in expression in the invasive variant, Hs578Ts(i)$_8$, relative to the Hs578T parental cell line. Expression of PLAC8 mRNA was investigated in a panel of 17 human cancer cell lines of breast, prostate, colon, lung, liver, bladder and leukemic origin, where high levels of PLAC8 mRNA were detected in the more invasive cell lines. RNA interference (RNAi) technology was then employed to elucidate the role of PLAC8 in breast cancer progression.

PLAC8 mRNA was silenced using a lentiviral-mediated delivery system to facilitate stable, sustained gene suppression. Using this approach, PLAC8 mRNA expression was suppressed to 20% of the expression levels detected in the Hs578Ts(i)$_8$ empty vector control cell line. Lentiviral transduction efficiency was determined as >90%, as determined via flow cytometric analysis of the co-expressed GFP. Suppression of PLAC8 expression has no discernable effect on growth rate or doxorubicin-mediated apoptosis, when cells were grown as a monolayer culture. There was a marginal reduction in the invasive capacity of the knockdown cell line and there was a significant decrease in the ability of this cell line to form colonies when cells were cultured in soft agar. The control cell line formed 28% more colonies in soft agar (>50μm) with 19% of colonies formed growing to sizes over 100μm compared to 15% in the knockdown cell line.

Despite reducing PLAC8 mRNA levels by 80% in the Hs578Ts(i)$_8$ cell line, expression levels are still higher than the endogenous levels in the parental cell line. Therefore, lentiviral-mediated RNAi knockdown was applied to the Hs578T cell line. Transduction efficiency was determined as >86% by flow cytometry and RT-PCR shows a suppression of PLAC8 mRNA by 68%. Immediately, a slower growth rate was evident in the knockdown cell line via MTS absorbance. Trypan viability showed a cell doubling time of 18.3hours for the control cell line compared to 37.7hours for the knockdown cell line. Further *in vitro* and *in vivo* studies will be conducted on these cell lines. In addition, an inducible cell line has been developed to further manipulate and investigate the functional effects of PLAC8 in breast cancer progression.

P56

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Title: Evaluating the effects of monoclonal antibody therapies on pro-angiogenic growth factors in individual human colorectal cancer explants.

Authors: J Marry**,** M Tosetto, H. Mulcahy, J Hyland, D O'Donoghue, K Sheahan, D Fennelly, J O'Sullivan
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Abstract:

Background

Conventional *in-vitro* single cell lines cannot adequately assess the cellular effects of molecular targeted therapies since they lack peritumoral stroma and thus a vascular milieu. The monoclonal antibodies bevacizumab (anti-VEGF) and cetuximab (anti-EGFR) are in clinical use, though we lack markers predictive of response. We have developed a colorectal cancer explant model that allows us to assess the effects of anti-angiogenic therapies on individual patient's tumors.

Methods

Tumor and matching normal tissue was obtained at surgery from 18 patients and subsequently cultured and treated with bevacizumab and cetuximab for 72 hours. Media from treated explant tissue was screened by ELISA for the secreted factors described above. 7 patients received palliative chemotherapy containing bevacizumab while one received cetuximab.

Results

VEGF levels were lower in both tumor ($p<0.01$) and normal ($p<0.01$) tissue secretions treated with bevacizumab, while MMP levels were lower in normal ($p=0.04$), but not tumor media. There was a trend towards higher IL-8 levels in tumor secretions following bevacizumab treatment ($p=0.06$), but not in normal tissue explants. Cetuximab treatment resulted in reduced EGFR in tumor ($p<0.01$) and normal ($p<0.01$) secretions, but cetuximab had no significant effect on other secretion profiles.

Normal tissue EGFR secretions were increased ($p=0.03$) and MMP9 levels reduced ($p<0.01$) by telomerase inhibition, with no effects seen in tumor secretions. In contrast, tumor co-treatment with telomerase inhibitor and bevacizumab resulted in increased VEGF secretion compared to bevacizumab alone ($p<0.01$). Normal tissue EGFR secretions increased when telomerase inhibitor was combined with Cetuximab compared to Cetuximab alone ($p=0.05$).

Conclusions

Ex-vivo tissue explant models have the ability to assess the biological effects of molecular treatments in individual colorectal cancer patients. Combining these results may allow us to develop a profile indicating response/resistance to these expensive treatments.

P57

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Sequence Effect on the Survival of Prostate Cancer Cells May Potentiate Daily Radiation Therapy Delivery

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INTRODUCTION: Recent *in vitro* studies have shown that not all 2Gy dose prescriptions are equivalent. The typical daily dose of radiation is split into a number of smaller partial fractions (PFs) delivered as separate fields, building up to a total daily dose of 2Gy. The order in which these PFs are delivered could potentially influence tumour response.

PURPOSE: To test in prostate tumour cells *in vitro* whether the cytotoxic effect of 2Gy radiotherapy prescriptions depends on the delivery sequence of the PFs, and how this effect be altered by (a) prolonged treatment times, and (b) concurrent treatment with cisplatin.

METHODS: Clonogenic assays were performed to test cell survival of DU145 and 22RV1, following irradiation sequences, designed to imitate prostate cancer radiotherapy plans. Survival following these sequences was also tested with a prolonged treatment time of 15 minutes, and concurrent treatment with 0.1 and 1μM cisplatin.

RESULTS: The order in which the PFs were delivered in the theoretical and 3-field plans resulted in significantly different survival in both cell lines ($P<0.05$). In response to the S-L delivery of the 3-field protocol survival was significantly increased in DU145 cells but decreased in 22RV1 cells ($P<0.05$). Survival was significantly decreased when DU145 cells were irradiated with the S-L IMRT protocol ($P=0.055$). In 22RV1 cells, survival was significantly decreased with the S-L boost protocol ($P=0.030$). Extending the treatment delivery time to 15 minutes and combining radiation exposure with cisplatin resulted in increased survival in 22RV1 cells. A similar effect was seen in DU145 cells.

CONCLUSIONS: S-L sequencing was found to increase cell kill in 22RV1 cells, suggesting this strategy may improve the daily efficiency of radiation therapy of primary prostate cancer. Extending the treatment time to 15 minutes appears to negate the advantage of this approach and so it is unlikely to benefit IMRT treatment plans. The delivery of concurrent cisplatin increased survival, suggesting its use as a radiosensitiser in these plans is of no benefit.

P58

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Bcr-Abl Escapes Growth Regulation by Reducing CCN3 Expression in Chronic Myeloid Leukaemia.

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Chronic Myeloid Leukemia (CML) is characterized by expression of the constitutively active BCR-ABL tyrosine kinase. Previously, microarray analysis identified down-regulation of CCN3 as a result of BCR-ABL kinase activity. We now show that CCN3 and BCR-ABL have a reciprocal relationship of expression and CCN3 expression exerts negative growth regulation in CML cells.

Real-time PCR was used to examine gene expression levels for CCN3 and BCR-ABL. K562 cells showed high expression of BCR-ABL whilst CCN3 expression was not detected. Treatment with siRNA directed against BCR-ABL resulted in a 3.7 fold decrease in BCR-ABL and 6.1 fold increase in CCN3 expression (n=3, p=0.001). Similarly, K562 cells treated with imatinib (1 mM, 96 h) showed a 5.9 fold decrease in BCR-ABL expression and a 4.2 fold increase in CCN3 expression (n=3, p=0.001).

CCN3 function was investigated in K562 cells using flow cytometry and colony formation assays. CCN3 expression in BCR-ABL+ cells caused an accumulation of cells in the subG$_0$ phase of cell cycle (mean for subG$_0$ 9.9% ± 4.6 and 21.8% ± 0.7 for the vector alone and vector containing CCN3 construct respectively). In addition, CCN3 expression reduced the clonogenic capacity of K562 cells; cells transfected with CCN3 formed significantly less colonies in methyl cellulose in comparison to cells transfected with vector only (n=3, p=0.027).

CCN3 is known to be a negative growth regulator and increased expression of CCN3 in BCR-ABL+ cells inhibits proliferation and decreases clonogenic potential. Thus CCN3 down-regulation mediated by BCR-ABL offers growth advantage to hematopoietic cells.

P59

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Title: Implementation of adjuvant Trastuzumab in Northern Ireland: Patient tolerability and experience in comparison to clinical trial data.

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Abstract:

Background: Recent large multicentre trials have demonstrated a survival advantage with adjuvant Trastuzumab but have also highlighted potential side effects especially cardiotoxicity. We have audited the first year of adjuvant Trastuzumab examining adherence to guidelines and toxicity in Northern Ireland patients in comparison to the trial population.

Method: 117 patients were identified by cancer unit pharmacists as starting adjuvant treatment in the first year. The regional guideline (based on the HERA protocol) advised Trastuzumab for patients who were HER2 positive, who had received adjuvant chemotherapy and had either node-positive tumours or node-negative tumours with a primary larger than 1 cm. Trastuzumab was given on a 21 day cycle for 18 doses and patients were reviewed at cycle 1,2,5,9,13 and 18 with echocardiogram at baseline and repeated at 3 month intervals. Adverse events and reasons for deferring or stopping treatment were documented.

Results: From December 2005 to November 2006, 117 patients started treatment and all met the criteria set out in regional guidelines. At analysis in December 2007, 27 patients (23%) had not remained on schedule. Five patients had developed relapse and had moved to a metastatic disease protocol. Thirteen patients (11%) suffered a decline in left ventricular ejection fraction (LVEF), with 4 recovering after a four week break and 9 (7%) stopping, one of whom subsequently died from cardiac failure. Two patients stopped because of arrhythmia, without decline in LVEF, two stopped because of severe allergic reaction and a further two had neurological symptoms that led to early discontinuation. One was deferred for one month due to dyspnoea and palpitations, but with no LVEF change. One patient was deferred whilst having reconstructive surgery. The remaining 90 patients have either completed treatment (87 patients) or are continuing on their planned schedule. Service impact was in very close accord with the pre-implementation estimates in the business case.

Conclusion: The introduction of adjuvant Trastuzumab in Northern Ireland has been shown to accord with agreed regional guidelines and was associated with toxicity comparable to that described in the registration clinical trials.

P60

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Title: The role of nucleophosmin in keratinocyte differentiation
Authors: Rachel McCloskey, Adam Pickard, Dennis McCance
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Abstract:

The E6 and E7 oncoproteins of high-risk HPVs are together sufficient to cause cellular transformation. The E6 protein is best known for its ability to bind and cause degradation the tumour suppressor p53, whilst the E7 protein, can bind and degrade the retinoblastoma tumour suppressor, pRb. Recent data indicates that E7 may target proteins other than pRb that play a role in the development of cervical cancer. Nucleophosmin was identified as a protein with increased levels in 2-D gel analysis of HFKs expressing E7 following methyl-cellulose induced differentiation.

NPM is a nucleolar phosphoprotein capable of shuttling between nucleus and cytoplasm and is abundant in tumour and proliferating cells. It has been shown to act as a molecular chaperone, participate in ribosome biogenesis and more recent data indicates a role in regulation of proliferation in apoptosis. Analysis of NPM expression in E7 expressing cells in culture and in organotypic rafts confirms the increase observed in 2d-gel analysis. Knock down of NPM expression with shRNA indicates that differentiation-specific markers are increased in organotypic raft culture and in-vitro models of differentiation. We are further investigating NPMs role in differentiation and trying to determine whether the levels are mediated by pRb or its related family members p107/p130. Preliminary data shows NPM at least binds to pRb during differentiation.

P61

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Title: Knockdown of ΔNp63α inhibits keratinocyte differentiation
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Abstract:

p63 is a multi-isoform member of the p53 family of transcription factors. It is highly expressed in the basal epithelium of adult skin, although its role in the differentiation process is unclear. Knockdown of all isoforms causes cell arrest and inhibition of stratification and differentiation. HPV-16 E6 or E6/E7 expression in primary human foreskin keratinocytes (HFKs) leads to expression of p63 in the upper layers of the epithelium in organotypic raft culture, however which isoforms are involved is unclear. This altered distribution of p63 expression is dependent of the ability of E6 to bind and degrade p53. In an attempt to determine the function of p63 isoforms we have depleted total and p63α using shRNA and found that it results in a hypo-proliferative epithelium with an inhibition of the early (K1) and late (filaggrin) differentiation-specific markers. We are investigating if ΔNp63α is required for commitment to differentiation, if other isoforms play a role and if so, which pathways they controls.

P62

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Title: The role of FKBPL-associated ER/Hsp90 chaperone complexes in breast cancer growth and survival

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Abstract:

Hsp90 chaperone complexes are involved in maintaining the stability and signalling of Hsp90 client proteins such as the oestrogen receptor (ER). ER is the primary mediator of breast cancer proliferation in response to oestrogen. Since increased ER levels and transcriptional activation are associated with over 50% of breast cancers, ER is an attractive target for cancer treatment strategies. The novel gene, FKBPL, has recently been isolated as a gene that shares homology with FKBPs; a family of immunophilin co-chaperones known to be important in the Hsp90/steroid hormone receptor complexes. Using the mammalian two-hybrid assay and co-immunoprecipitations, we have identified FKBPL as an Hsp90 co-chaperone associated with the ER and dynein motor protein complex. Furthermore, using the biomolecular complementation assay, we have identified that the PPIase domain of FKBPL is required for binding to dynamitin, a subunit of the dynein complex. To elucidate the consequences of modulating FKBPL levels, we constructed MCF7 (ER+) and MDA-MB-231 (ER-) cell lines which stably overexpress FKBPL. Cell growth in both MCF7/FKBPL and MDA-MB-231/FKBPL was inhibited by 31% and 35% respectively compared to controls. Furthermore, clonogenic survival assays determined that both cell lines displayed increased radioresistance up to 6Gy. Western blot analysis revealed that higher levels of ER alpha were present in the MCF7/FKBPL cell line therefore an ERE luciferase reporter plasmid was used to analyse effects on ER transactivation. ER transactivity increased at least 2-fold over parental controls in the presence of 17β-estradiol (10^{-6}M-10^{-8}M). Moreover, in this stable cell line, ER alpha demonstrated increased binding to its coactivator proteins. FKBPL obviously plays a key role in ER signalling and since most tumours become refractory to current hormonal therapies within a year of starting treatment it represents a novel target which would enable the disruption of signalling pathways integral in maintaining ER-mediated tumour growth and survival.

P63

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Title: Scalp Cooling- The St. James Experience
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Abstract:
CHEMOTHERAPY INDUCED ALOPECIA IS ONE OF THE MOST DISTRESSING SIDE EFFECTS OF CHEMOTHERAPY (PICKARD-HOLLY S, 1995). THE USE OF SCALP COOLING REDUCES OR ELIMINATES CHEMOTHERAPY INDUCED ALOPECIA IN SOME CASES (DOUGHERTY, 1996). THE AIM WAS TO OFFER SCALP COOLING TO PATIENTS THAT WERE RECEIVING PALLITATIVE CHEMOTHERAPY.
THE COHORT OF PATIENTS PREVIOUSLY EXPERIENCED CHEMOTHERAPY INDUCED HAIR LOSS AND EXPRESSED A DESIRE TO AVOID FURTHER ALTERED BODY IMAGE.
THE OBJECTIVES TO PATIENTS INCLUDED MINIMISING AND AVOIDING FURTHER ALTERED BODY IMAGE. THE PRESERVATION OF EXISTING HAIR AND PREVENTION OF FURTHER HAIR LOSS WHILE UNDERGOING CHEMOTHERAPY.
STAFF OBJECTIVES INCLUDED THE PROVISION OF OPTIUM TREATMENT TO PATIENTS WHILE PREVENTING/MINIMISING ADDITIONAL ALTERED BODY IMAGE.
TO PARTAKE IN CLINICAL RESEARCH/AUDIT. TO FACILITATE CLINICAL TEAMWORK AND ONGOING CLINICAL LEARNING.
THIS POSTER DEMONSTRATES THE EFFECTIVE USE OF THE SCALP COOLING MACHINE WHEN USED ON PATIENTS RECEIVING CERTAIN DRUGS SUCH AS TAXANES AND SOME ANTI-TUMOUR ANTIBIOTICS.
SUBSTANTIAL RESEARCH HAS HIGHLIGHTED THE BENEFITS OF SCALP COOLING (DOUGHERTY, 1996).
SCALP COOLING WAS INTRODUCED INTO THE ONCOLOGY DAY CARE UNIT IN OCTOBER 2006.
RESULTS TO DATE ARE VERY ENCOURAGING, WITH 88% COMPLIANCE AND WITH VARYING DEGREES OF SUCCESS.

P64

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Title:
Vascular endothelial growth factor decreased by Camptothecin in a breast cancer model.

Authors:
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Abstract:
Neo-angiogenesis contributes to the metastatic phenotype of breast cancer. Cyclooxygenase-2 (COX-2) and Vascular Endothelial Growth Factor (VEGF) have been implicated in this disease process. Blockade of the COX-2 enzyme is thought to inhibit angiogenesis via reduction of VEGF. This study investigated the effect of two organic compounds; Phorbol-12myristate-13-acetate (PMA) and Camptothecin on COX-2 and VEGF production in a breast cancer cell model.

MDA-MB-231 (COX-2 positive) and MDA-MB-468 (relatively COX-2 negative) breast cancer cell lines were treated with PMA or Camptothecin. Cell viability was measured by MTT assay. VEGF was quantified by ELISA. Cells were saturated with arachadonic acid and Prostaglandin E2 (PGE2) measured, by ELISA, to assay COX-2 activity. Statistical significance was calculated using unpaired t test.

PMA decreased cell viability of MDA-MB-231 by 29% ($p<0.001$) but was not cytotoxic to MBA-MB-468 cells. Camptothecin reduced viability by 13% and 28%, respectively ($p<0.001$).

PGE2 production was increased 1.47 (4h) and 1.64 fold (24h) by PMA in MDA-MB-231 ($p<0.01$) but there was no sustained increase in MDA-MB-468. A similar pattern was observed with VEGF (3.2 (4h) and a 5.4 fold (24h) increase in MDA-MB-231 cells ($p<0.001$).

Camptothecin had no significant impact on PGE2 levels in either cell line but reduced VEGF by 42% in MDA-MB-231 and by 62% in MDA-MB-468 at 24h ($p<0.01$).

PMA increased VEGF production only in the COX-2 positive cell line, supporting a causative link, as suggested by previous studies. Camptothecin markedly decreased VEGF production, regardless of COX-2 expression, thus this agent has significant promise for treating breast cancer. Combined with the aforementioned results this indicates the necessity for a multimodal approach in the prevention of angiogenesis in breast cancer.

P65

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Title: Clonal methylation profiling as a risk biomarker for colitis-associated colorectal cancer

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Abstract:
Ulcerative colitis (UC) is a form of inflammatory bowel disease, with approximately 250 new cases presenting each year in Northern Ireland alone. UC patients have an elevated risk of cancer development, indeed, 50% of patients with prolonged disease duration will develop colorectal cancer (CRC). Currently these individuals undergo cancer screening on a yearly basis by colonoscopy and histological analysis, however this method is ineffective. Therefore, the identification of a biomarker of risk for colitis-associated CRC (CACRC) would be invaluable.
To this end, we aim to determine if the DNA methylation profile of several non-expressed genes in colon biopsies from UC patients is a marker of CACRC risk and if so, to identify the optimal biopsy strategy for this purpose.
We have collected a number of normal, UC and CACRC colons and obtained 10-18 biopsy samples from each. DNA has been extracted from these samples and bisulphite treated. Following this, polymerase chain reaction (PCR) is performed to amplify CpG-rich regions of 3 genes, namely cardiac-specific homeobox (CSX), myogenic factor 3 (MYOD1) and estrogen receptor alpha (ERα). All of these genes have been shown to undergo accelerated age-related methylation in UC. We initially employed bisulphite-sequencing to assess methylation density and pattern of these genes. Preliminary data suggest that methylation density of CSX is elevated in tumour tissue with respect to colitis and normal tissue. More recently we have used pyrosequencing due to its greater suitability to high-throughput screening.
This study is ongoing, and if the potential of these markers is confirmed, we will compare the biopsy strategy/methylation profiles with clinical cancer surveillance methods to determine CACRC risk in UC patients. This work may provide the scientific basis for more accurate cancer risk assessment and effective cancer prevention in colitis patients.

P66

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Title: Immunohistochemical profiling of death receptor expression in resected stage II and III colorectal tumours: comparison with matched normal tissue and correlation with survival

Authors: McLornan DP[1], Barrett HL[2], Cummins R[2], Treacy A[2], Johnston PG[1], Kay EW[2] and Longley DB[1]

Institution: (1) CCRCB, Queen's University Belfast & (2) Department of Pathology, Beaumont Hospital and Royal College of Surgeons in Ireland, Dublin.

Aims & Methods

We sought to determine the expression of apoptosis-related proteins in patients with colorectal carcinoma and to correlate this with survival end points. Tissue Microarrays (TMAs) were constructed from matched normal and tumour tissue derived from a large cohort of patients (n=254) enrolled in a Phase III trial of adjuvant 5-FU-based chemotherapy versus observation alone. The protein expression of tumour necrosis factor- related apoptosis inducing ligand (TRAIL), the TRAIL receptors DR4 and DR5, the Fas death receptor and the death receptor inhibitor c-FLIP were determined by immunohistochemical (IHC) techniques and scored by two independent observers. In addition, Bcl-2, p53 and the proliferation marker Ki67 were assessed. Expression was correlated with the endpoints of recurrence-free survival (RFS) and overall survival (OS) via Cox regression analyses.

Results

Colorectal carcinoma cells displayed significantly higher expression of DR4 (p=0.01), DR5 (p=<0.001), Total FLIP (p=<0.001), $FLIP_L$ (p=<0.001), p53 (p=<0.001) and Ki67 (p=<0.001) but lower Bcl2 (p=0.002) and TRAIL (p=<0.001) expression when compared with matched normal tissue as determined via IHC. There was no difference in Fas expression between matched normal and tumour tissue. In univariate analysis, higher TRAIL expression in the tumour was associated with improved OS with a trend to improved RFS. High Ki67 expression in both normal and tumour tissue had a significant adverse effect on both OS and RFS. The remainder of the biomarkers had no significant impact on either OS or RFS via univariate analysis. Utilising multivariate predictive modelling via Cox regression and backward elimination for OS and RFS in all patients and including all biomarkers, age, treatment for survival and Dukes' Stage, we found that the overall predictive model was significant after 19 backward steps when only the Mean Ki67 expression (Normal Tissue), Mean Ki67 expression (Tumour Tissue) and Dukes' code were included.

Conclusions

High Ki67 expression in both colorectal tumour and surrounding normal tissue has an adverse affect on both OS and RFS. High TRAIL expression in colorectal carcinoma is associated with significantly improved OS and enhances RFS.

P67

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Title: The effect of siRNA mediated knockdown of CTNNA3 on cell adhesion and migration in UCB cell lines.

Authors: Maria Meehan,[1] Emma Gallagher,[1] James Smith,[1] Alo Mc Goldrick,[1] Steven Goossens,[2,3] Michele Harrison,[4] Elaine Kay,[5] John Fitzpatrick,[6] Peter Dervan,[4] and Amanda Mc Cann[1]

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Abstract:
Our recent work focused on the imprinting status of CTNNA3 in UCB. A total of 96 samples were analysed and included 22 paired normal and tumour UCB cases, 38 superficial (pTa and pT1) UCB cases and 14 cell lines of various lineages. RTPCR analysis of 35 heterozygous samples followed by sequence analysis allowed monoallelic versus biallelic patterns to be assigned. We have demonstrated novelly that CTNNA3 displays differing allelic expression patterns in UCB. Specifically 35% (7/20) of informative UCBs showed monoallelic expression, a feature confined to the tumour, with normal urothelial samples displaying biallelic expression. In addition we have demonstrated by RTPCR analysis that there is differential levels of expression of CTNNA3 in these cell lines.

To investigate the role that loss of CTNNA3 may be playing in UCB, knockdowns of CTNNA3 have been performed in a number of these UCB cell lines. The migratory and invasive potentials of the transfectant cells are to be assessed by scratch wound, adhesion and in vitro transwell migration assays.

Considering the potential role alterations in CTNNA3 play in EMT, loss of this key molecule by epigenetic alterations such as monoallelic expression have the ability to compromise CTNNA3's stabilizing role in the adherens junction, theoretically leading to a weakening of binding with beta catenin, which in turn is bound to the cytoplasmic domain of E-cadherin, the gate-keeper of the epithelial phenotype.

P68

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Title: Promoter hypermethylation and reduced expression of MGMT in oligodendroglial tumours

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Abstract: Gliomas constitute a heterogenous group of tumours with differing therapeutic responses to chemotherapy with alkylating agents. O^6-Methylguanine-DNA methyltransferase (MGMT), a DNA repair enzyme, inhibits the killing of tumour cells by alkylating agents. MGMT activity is controlled by a promoter and epigenetic silencing of the MGMT gene by promoter methylation comprises DNA repair and has been associated with longer survival in patients receiving alkylaing agents.

Aims
To assess the degree of MGMT promoter methylation and levels of gene expression in a group of gliomas from formalin-fixed, paraffin-embedded (FFPE) brain tumour.

Methods
We investigated 9 tumours (5 female and 4 male; Age range 39-72yrs). The group consisted of 4 grade III anaplastic oligodendrogliomas (AOIII), 1 grade IV gliosarcoma (GSIV), 2 grade III anaplastic astrocytoma (AAIII) and 2 grade IV glioblastomas (GBIV). DNA was extracted from FFPE material using QIAamp DNA micro kit. Sodium bisulfite treatment of DNA was performed followed by methylation specific PCR. The expression of MGMT transcripts was determined by RT-PCR using SYBR.

Results
Amplifiable DNA was obtained from all cases. In 7 of 9 cases (77%), we detected MGMT promoter methylation. Two cases (1 GSIV and 1 GBIV) showed no hypermethylation. RT-PCR revealed MGMT mRNA levels were considerably reduced in those hypermethylated tumours in comparison to unmethylated tumours.

Summary / Conclusions
Our data demonstrates that MGMT hypermethylation and reduced gene expression are frequent in glioma tumours. As this approach is applicable to FFPE tissue it could be easily incorporated into routine molecular pathology practice.

P69

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Title: Silicon Microneedles for Topical Delivery of 5-Aminolevulinic acid and Preformed Photosensitisers: Potential for Enhanced Treatment of Skin Cancers.

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Abstract:
Photodynamic therapy, based upon topical administration of aminolevulinic acid (ALA), is a novel treatment for premalignant and malignant skin lesions. Application of exogenous ALA stimulates the over-production of protoporphyrin IX (PpIX), an effective endogenous photosensitiser. This effect is prevalent in rapidly proliferating neoplastic cells and explains selective lethality of the therapy. However, ALA is a small hydrophilic (167.6 dalton) molecule and zwitterionic at physiological pH. Consequently, permeation across intact *stratum corneum* is poor (Casas et al 2000, Malik et al 1995). Due to this reason, a number of methods have been investigated and employed to improve ALA penetration. These include tape stripping, permeation enhancers, ion pairing, iontophoresis, laser SC ablation, and formulation development. In this study, we aimed to enhance topical delivery of both ALA and preformed photosensitisers using novel microneedle (MN) technology. A silicon MN is a mechanical approach to bypass the outermost layer of the skin, the stratum corneum (SC) and is a physical technique to increase drug delivery through skin, working simply by puncturing the SC. Importantly though, these solid needles do not protrude far enough to reach pain receptors, ensuring that application is without sensation.

ALA and porphyrin loaded, bioadhesive films were cast from drug containing aqueous blends of poly(methylvinylether.maleic anhyhydride) (PMVE/MA), suitably plasticised using tripropylene glycol methyl ether (TPM). Silicon MN arrays were fabricated using wet etch technology to produce needles of approximately 270 μm in length and 240 μm in base diameter with interspacing of approximately 750 μm. *In vitro* permeation studies were performed using the Franz cell model, employing both silicone and excised mouse skin as model membranes. Animal experiments were approved by the animal department of the Norwegian Radium Hospital. *In vivo* PpIX accumulation studies were performed by first puncturing the dorsal skin of anaesthetised female nude mice for 30 seconds using the MN array. Subsequently formulations were applied for 4 hours. Upon removal of the vehicle, fluorescence *in-vivo* was measured using a fiber-optic probe coupled to a Perkin-Elmer LS50B luminescence spectrometer (excitation 407 nm, emission 635 nm).

In vitro permeation studies showed significant increases in ALA and TMP penetration across both the model silicone membrane and excised mouse skin ($p = < 0.05$). The accumulation kinetics of PpIX followed a similar profile for all drug formulations. Following removal of the vehicle, PpIX fluorescence peaked at 3-6 hours and reduced to baseline levels at 24 hours. Puncturing the skin using MN arrays was shown to enhance photosensitiser production significantly when compared to control ($p = < 0.05$).

To date, topical PDT has been restricted due to the relatively poor penetration of ALA and pre-formed photosensitisers into lesions. This study illustrates that MN technology is a novel strategy of overcoming the principle barrier to drug penetration into skin.

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Malik, Z., Kostenich,G., Roitman,L., Ehrenberg,B., Orenstein,A. (1995) *J.Photochem.Photobiol.B.* **28**: 213-218.

P70

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Title: The Role of Histone Modifications in the DNA Damage Response
Authors: Jennifer FitzGerald, Sylvie Moureau and Noel F.Lowndes
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DNA damage can be caused by a wide range of both exogenous and endogenous sources. It is imperative that DNA damage is detected and repaired before the cell divides, to prevent propagation of mutations that could lead to cancer. The DNA damage checkpoint pathway allows the cell to detect and respond to DNA damage, and thus plays a major role in maintaining genomic integrity.

53Bp1 is a DNA damage response protein involved in both cell cycle arrest and DNA repair. After genotoxic insult, this protein is rapidly recruited to DNA double strand breaks. However, the mechanism of recruitment remains unclear, with several reports presenting conflicting results on the roles of histone H3 methylation, histone H4 methylation, and histone H2AX phosphorylation in this process.

We have generated a cell line deficient in histone H3 lysine 79 methylation by knocking out the histone methyltransferase Dot1, in the chicken DT40 model system. Here we report that 53Bp1 recruitment to DNA damage is not dependent on Dot1, as assayed by immunofluorescence microscopy. Preliminary results also suggest that DNA repair is not affected by the absence of Dot1.

Currently we are generating cell lines deficient in histone H3 lysine 79 methylation, histone H4 lysine 20 methylation, and histone H2AX phosphorylation. By studying 53Bp1 recruitment and its downstream functions in these cell lines, we aim to dissect the role of these histone modifications in the functions of 53Bp1.

P71

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Title: Increased Incidence of Hypertension Associated with Tyrosine Kinase Inhibitors (TKIs)

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Abstract:

Background: Sunitinib and sorafenib are oral multi-kinase inhibitors. They exert their effect by targeting tyrosine kinases such as platelet-derived growth factor receptor and vascular endothelial growth factor receptors. Sunitinib was the first cancer drug simulataneously approved for two different indications. It has been approved for use in renal cell cancer and imatinib-resistant gastroinstestinal stromal tumours. Its efficacy is currently being evaluated in a broad-range of solid tumours including breast, lung and colorectal cancer. Sorafenib is licensed for second-line treatment of renal cell cancer and most recently for hepatocellular cancer. These novel agents have a more favourable side-effect profile compared to traditional non-specific cytotoxins. The incidence of hypertension associated with these agents has been reported in the literature to be less than 30%.

Materials and Methods: We studied a population of all oncology patients treated with sunitinib and sorafenib in a large teaching hospital. Our study period was from January to December 2007. We report all associated vascular events identified and their subsequent management.

Results: A total of 13 patients received sunitinib and 4 of these also received 2nd line sorafenib during the study period. All patients had biopsy confirmed diagnosis of metastatic renal cell cancer. The median age was 66 years (range 47-74yrs). Twelve patients had a prior nephrectomy. Four patients had pre-existing hypertension and 1 ischaemic heart disease. The mean number of cycles of sunitinib was 4 (range 1-9 cycles) and of sorafenib was 1-2 cycles. The mean baseline blood pressure (BP) was 128/73mmHg and the mean BP post 1 cycle of sunitinib was 152/83mmHg. All of the 10 patients who were not on prior anti-hypertensive agents developed at least grade 1 hypertension during the course of treatment with a TKI. Seven of these patients developed grade 3 hypertension but three patients did not have appropriate anti-hypertensive treatment initiated/altered. One patient required hospitalisation secondary to uncontrolled hypertension. There were no other vascular events recorded.

Conclusions: Our findings suggest a much higher incidence of hypertension than reported in the literature associated with tyrosine kinase inhibitors. This requires the continuous monitoring and appropriate management of blood pressure in all patients requiring tyrosine kinase inhibitors.

P72

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Title: Investigating promoter hypermethylation of apoptotic genes in prostate cancer.

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Abstract:
It is now well established that cancer cells exhibit a number of genetic defects in the machinery that governs programmed cell death and that sabotage of apoptosis is one of the principal factors aiding in the evolution of the carcinogenic phenotype. A number of studies have implicated aberrant DNA methylation as a key survival mechanism in cancer, whereby promoter hypermethylation silences genes essential for many processes including apoptosis. To date, studies on the methylation profile of apoptotic genes have largely focused on cancers of the breast, colon and stomach, with only limited data available on prostate cancer. The aim of this study was to profile methylation of apoptotic-related genes in order to generate a prostate cancer "apoptotic methylation signature", which could have utility as diagnostic and prognostic biomarkers to aid early detection and treatment choice for prostate cancer. A bioinformatics approach was first applied to generate a list of apoptotic genes. Relevant genes were identified based on the following criteria: 1) biological role in apoptosis, 2) the presence of CpG Island (determined using the March 2006 human genome assembly, accessed at http://genome.ucsc.edu/ 3) susceptibility to promoter hypermethylation in other cancer types as assessed by extensive review of the literature via Pubmed and OMIM and 4) possible down-regulation in prostate cancer. A number of published microarray studies that compared and listed gene expression changes between different stages and grades of prostate cancer were examined. Under these criteria, a list of 22 genes was identified as possible targets of methylation in prostate cancer. PCR assays have now been designed to amplify whole CpG islands in the gene promoters. Genes will be screened for CpG methylation in a panel of prostate cancer cell lines (LNCaP, DU145, PC-3, 22RV1, RC58) and in a test set of tumour specimens (n = 20) using an automated Denaturing High Performance Liquid Chromatography (DHPLC) instrument (WAVE®, Transgenomic Inc).To date, screening of Apaf1 revealed no evidence of promoter methylation in the 5 cell lines. Currently we are screening TMS1, BNIP3 and FAS for promoter hypermethylation.

Genes of interest will be further validated through bisulfite sequencing and methylation levels quantified using quantitative methylation specific PCR in a prostate cancer biorepository that we have generated in Ireland, representing prostate cancer, normal adjacent prostate and benign prostatic hyperplasia. Deciphering the methylation profile of an integral cellular process such as apoptosis, whose dysregulation is key for tumour progression, could yield a biomarker signature for early disease and disease progression in prostate cancer.

P73

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Title: Regulation of Cyclin D1 by the BRCA1-BARD1 complex

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Abstract:

BRCA1 and cyclin D1 are both essential for normal breast development and mutation or aberration of their expression is associated with breast cancer. The BRCA1 tumour suppressor gene is mutated in up to 45% of inherited breast cancers and its expression is downregulated in approximately 30% of invasive sporadic tumours. Cyclin D1 is an oncogene that is overexpressed in almost 50% of sporadic breast tumours. Interestingly, these proteins often have opposing functions e.g. cyclin D1 is responsible for driving cell proliferation, whereas BRCA1 is involved in differentiation.

We propose that cyclin D1 is a novel substrate for BRCA1 ubiquitination and that this targets cyclin D1 for proteasomal–mediated degradation. We initially identified cyclin D1 as a binding partner of BARD1 in a yeast-2-hybrid screen and defined the minimal binding region as the N-terminus of BARD1. This region also binds BRCA1 and imparts ubiquitin ligase activity to the complex. Covalent modification of proteins with ubiquitin is a common regulatory mechanism in eukaryotic cells. Traditionally, polyubiquitin chains linked through lysine 48 targets proteins for degradation by the 26 S proteasome. We have demonstrated that cyclin D1 protein levels are inversely related to BRCA1 and BARD1 levels using transient transfection, antisense and siRNA technology. Interestingly, this post-transcriptional mechanism occurs in G2/M. Additional experiments indicate that the regulation of cyclin D1 levels by BRCA1 is driven by the ligase activity of BRCA1 itself. Finally co-immunoprecipitation of endogenous proteins show that poly-ubiquitinated cyclin D1 associates with BRCA1 and BARD1 *in vivo*. Future work will focus on ascertaining the functional consequence of cyclin D1 regulation by the BRCA1-BARD1 complex.

P74

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Title: DNA methylation in Prostate Cancer is related to extraction procedure.

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Introduction: Epigenetic silencing of genes through hypermethylation has been demonstrated during the development of many cancers including prostate cancer (PCa). Genes such as GSTP1 and IGFBP3 are frequently hypermethylated in PCa leading to the suggestion that methylation status of such genes could be used as cancer diagnosis markers alone or in support of histopathology. Most pathology specimens are formalin-fixed and paraffin embedded (FFPE) tissues which are invaluable resources for conducting retrospective investigations in PCa. Methylation specific PCR (MSP) analyses DNA methylation status but has shown varying degrees of sensitivity in FFPE material, possibly due to the DNA extraction procedure.

Aim: To compare two routine methods for the extraction of DNA from FFPE and assess the subsequent sodium bisulphite modification by MSP.

Materials and Methods: Following pathology review, DNA was extracted from FFPE specimens of normal prostate, BPH, PIN and PCa by two methods: 1) Proteinase K digestion and phenol/chloroform extraction (n=30) or 2) QIAamp DNA micro kit (n=10). DNA concentration was calculated by spectrophotomtry (Nanodrop) and quality was by a specimen control size ladder. DNA Methylation status was evaluated by real time quantitative methylation specific PCR (QMSP).

Results: FFPE DNA was successfully extracted by both methods with no differences in DNA yields between methods (DNA concentration ranging from 40ng-365ng/ul). FFPE DNA was of adequate quality being able to amplify up to 500bp using the specimen control ladder. However QMSP revealed that DNA extracted using the phenol/chloroform method failed to undergo bisulphite modified DNA in 80% of cases. In contrast, DNA prepared from QIAamp kit showed successful bisulphite modification.

Conclusion: MSP is a bisulphite-based method for the assessment of DNA methylation status in PCa with false negative results frequently occurring when using FFPE material. Our study compares two common extraction methods for DNA extraction from FFPE. We show similar yield and quality from both methods. However DNA extracted by method 1 failed to produce bisulphite modified DNA in 80% of cases when assessed by QMSP which is possibly due to chemical modification by the organic extraction procedure. Method 2 produced bisulphite modified DNA in all cases as assessed by QMSP.
Our results show that QMSP is easily achievable in when using FFPE material but is dependent on the extraction procedure. This has important implications in retrospective studies as most pathology material is stored in this format.

P75

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Title: **Investigating the role of BRCA1 as a stem cell regulator**

Authors: Caoimhe Nic An Tsaoir, Niamh O'Brien, Hannah L. Farmer, Keara L. Redmond, Dorota Tkocz, Zenobia D'Costa and Paul B. Mullan.
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Abstract:
BRCA1 was identified by linkage analysis in 1994 to be the first breast and ovarian cancer hereditary predisposition gene. It is a multifunctional protein and is known to have roles in DNA damage repair, transcriptional regulation, cell cycle control and ubiquitination. Mutations in the tumour suppressor gene BRCA1 account for 40-45% of hereditary breast cancers and 80% of hereditary breast and ovarian cancer cases. Typical features of BRCA1 associated breast cancers are poor differentiation, high proliferation, TP53 mutation, and negative for expression of HER2 receptor, oestrogen receptor and progesterone receptor (all features similar to the basal subclass of breast cancers). BRCA1 has been suggested as a potential breast stem cell regulator and has been shown to regulate luminal/basal marker expression in breast cells and may be important for breast cell lineage specification. One of the stem cell pathways which has been shown to be important for cell lineage determination and differentiation in a number of tissues is the Notch pathway. Notch signalling plays a role in vertebrate embryogenesis, post-natal development and adult tissue homeostasis. Three main functions of Notch signalling are lateral inhibition, boundary formation, and cell fate specification. Aberrant Notch signalling has been associated with a vast spectrum of cancers including leukaemia, colon cancer and breast cancer. However, whilst most attention has been given to the link between aberrant or constitutive notch activation in cancer it is also clear that the Notch pathway is an important regulator in normal breast tissue.

Microarray analysis using the BRCA1 mutant (transcriptionally inactive) HCC1937 breast cancer cell line reconstituted with wild-type BRCA1 showed that BRCA1 transcriptionally upregulated components of the Notch pathway including the receptor Notch3 and ligands JAG1 and DLL1. Our hypothesis is that BRCA1 regulates Notch signalling to suppress basal gene expression and promote a luminal cell fate in the mammary gland epithelium. Using real time PCR we have found that BRCA1 actually upregulates the transcription of notch receptors 1,2 and 3 as well as the ligands JAG1 and DLL1. We have also used a notch-responsive β-globin luciferase reporter construct to assess the role of BRCA1 in activation of the notch pathway in breast cancer cell models. By using a synthetic DSL peptide (Delta Serrate Lag) we can mimic notch ligand mediated activation. Using this system we have found that knockdown of BRCA1 using siRNA leads to almost complete abrogation of notch reporter activation in response to the DSL peptide. This indicates that BRCA1 is required for proper notch activation, most likely through its ability to transcriptionally upregulate components of the notch pathway. By performing promoter analyses we are currently investigating how BRCA1 upregulates notch genes and what the consequences of this is for breast cell differentiation and growth.

P76

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Title: Identification of the BRD7 bromodomain gene as a novel BRCA1 interacting protein
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Abstract: BRCA1 is a tumour suppressor gene involved in the maintenance of genome integrity and has been shown to be mutated in women who are genetically predisposed to ovarian and breast cancer [1]. We recently identified BRD7 as a novel BRCA1-interacting protein by yeast-2-hybrid analysis, and confirmed this interaction in mammalian cells by endogenous co-immunoprecipitation. BRD7 is a bromodomain containing protein which has been shown to bind to the acetylated histones H3 and H4. Acetylation of histones opens up DNA to allow protein access and has been shown to be important in both transcription and DNA damage repair. BRD7 has been shown to regulate the expression of a variety of genes including those involved in cell-cycle checkpoint and cell growth [2].

BRCA1 has been shown to regulate the transcription of a variety of target genes. We have used a siRNA approach to investigate the role of BRD7 in BRCA1-dependent transcription. BRD7 downregulation led to an increase in the expression level of the DNA damage-inducible gene Psoriasin (S100A7) which is normally repressed by BRCA1. We also show that loss of BRD7 function leads to a downregulation of the endogenous luminal marker ERalpha and an upregulation of the basal marker p-cadherin which are downregulated and upregulated respectively by BRCA1. Thus our results suggest that BRD7 may play an important role in BRCA1-dependent transcription. We are currently investigating the mechanism by which BRD7 modulates BRCA1 dependent transcription.

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P77

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Title: BiomaRker Analyses in serum samples from Breast Cancer patients using a Novel Assay

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Abstract:

Introduction

Breast cancer is the commonest cause of death in middle-aged women in Western countries, with an estimated 1 in 8 women being affected in their lifetime. A biomarker for breast cancer would be useful in terms of assessment of treatment response and in early detection of local recurrence and distant metastases.

Research to date has shown that breast cancer patients have an elevated level of a novel surface expressed protein expression in their tissues. This novel protein is known to be present in low levels in normal breast tissue but is over-expressed in the membranes of breast cancer cell lines where it appears to play a key role in tumour invasion. To date the activity of this potential biomarker has not been analysed in serum samples of breast cancer patients.

The aim of this work was to investigate and measure the expression pattern of this novel biomarker in patients with breast cancer using a newly developed serum assay.

Materials and Methods

Blood samples were taken prior to surgery from patients who had histologically proven ductal carcinoma *in situ* (DCIS), invasive lobular carcinoma (ILC), invasive ductal carcinoma (IDC) and from control patients who were admitted electively for varicose vein surgery. These samples were centrifuged and the serum was removed for analysis. The expression pattern of the novel protein in serum was monitored using a highly sensitive and specific assay.

Results

Results show that there is a highly significant statistical difference between the novel protein levels in the control patients and those patients that had confirmed cases of breast cancer ($p=0.0001$). On average cancer patients have a 2-fold increase in Seprase levels compared to non-cancer patients. Analyses of the results show that the assay is 89% sensitive and has a specificity of 70%.

Conclusions

The tumour markers CEA, CA125 and CA15-3 have shown sensitivity (%) and specificity (%) values of 47.2/81.8, 50/48.5 and 59.5/63.6 respectively for differentiating malignant from benign. The best sensitivity of PSA for the detection of cancer ranges between 67.5-80% with a specificity range of 60-70%. Therefore, the current cancer biomarkers are not as sensitive or specific as our novel biomarker.

These results indicate that levels of this surface expressed protein are elevated in patients with breast cancer. To date there are no serum tumour marker(s) for breast cancer screening. The novel biomarker assay used in this study has the potential to be used initially as a kit for breast cancer screening, diagnosis and for monitoring progression of the disease.

P78

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Title: IκB-α as a Target for Epigenetic Silencing in Colon Cancer
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Abstract: Constitutively active nuclear factor kappa B (NF-κB) contributes to cancer development by opposing apoptosis and controlling the inflammatory response. IκBα is a target gene of NF-κB, and in normal cells, post-activation, NF-κB repression is re-established by increased IκBα gene expression. NF-κB activity is elevated in colon cancer cells and since many genes are epigenetically silenced in colon cancer, we speculated that high NF-κB activity in those cells may be due to lowering of IκBα levels by gene silencing. In support of this idea, we found that *NFKBIA*, the gene encoding IκBα contains two CpG islands in its promoter region. The aim of this project is to determine if DNA methylation regulates IκBα expression in colon cancer and if so, to determine the mechanism of this regulation. HCT116 cells were treated with 5-aza-deoxycytidine (5-aza), a nucleoside methyltransferase inhibitor for 72hrs. IκBα mRNA and protein expression levels were measured by RT-PCR and western blotting. 5-aza treatment caused a 2-fold elevation of IκBα mRNA, and a slight increase in IκBα protein expression. HCT116 cells lacking DNA methyltransferase (DNMT3b) had an approximately 2-3 fold increase in IκBα mRNA expression and a similar elevation of protein levels, compared with the parental cell line. Bisulfite sequencing of the 2 CpG islands in the promoter region of *NFKBIA* showed that these islands are unmethylated in HCT116 cells. Together, these data demonstrate that IκBα expression is lowered by DNMT in a colon cancer cell line, but because the *NFKBIA* promoter is unmethylated, this suggests that the effect of methylation on IκBα expression is indirect. Ongoing experiments are directed at elucidating the mechanism of repression of IκBα by DNMT. Supported by Science Foundation Ireland.

P79

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Hypoxia leaves its mark on the epigenome

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Hypoxia is a well known characteristic of the tumour microenvironment. Recent studies of tumour hypoxia have shown that these conditions significantly alter the global transcriptome, resulting in the differential expression of many transcription factors and their targets. Such changes may be attributable to the impact of hypoxia on patterns of epigenetic modifications. The aim of this study was to identify alterations in the global epigenetic patterns of histone 3 lysine 9 acetylation (AcH3K9) and DNA methylation as a result of chronic hypoxia.

PWR-1E prostatic cells were grown under normoxic and hypoxic conditions (sublines derived following over 30 passages at 10, 3 and 1% O_2). Flow cytometry was employed to measure changes in epigenetic modifications and total histone H3 levels. Immunofluorescent imaging was used to illustrate these results and for the assessment of distributional changes across PWR-1E cell populations. Monoclonal anti-AcH3K9 (Abcam), anti-5'Methylcytidine (Eurogentec) and anti-pan Histone H3 (Upstate) antibodies labelled with FITC were used for both analyses.

Low density microarrays have previously demonstrated significant alterations in the proliferation and apoptotic transcriptional phenotype of the chronic hypoxic cultured PWR-1E sub-cells. Our results have identified concurrent disruption of global epigenetic profiles, which include a significant H3K9 hyperacetylation ($p=0.0001$) and DNA hypermethylation ($p=0.001$) relative to PWR-1E cells cultured in a normoxic environment. Interestingly, chronic hypoxia was also associated with an enlargement of the PWR-1E nuclei despite levels of histone H3 remaining largely unchanged. This suggests that a genome-wide de-condensation of nuclear chromatin may occur as a result of hyperacetylation during hypoxia, thus promoting the aberrant expression of regulatory genes and the activation of well characterised adaptive pathways.

The impact of hypoxic influences on global and gene-specific epigenetic programming will provide important insights into the mechanisms of hypoxia-induced cellular changes. This may provide new therapeutic possibilities for previously unmanageable cases of advanced neoplasia.

P80

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Title: **A NOVEL PSMA-DRIVEN GENE THERAPY APPROACH FOR THE TREATMENT OF PROSTATE CANCER.**

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Abstract:

Introduction.

The probability that a man will develop clinically diagnosed prostate cancer is around 13% although the likelihood of disease related death is only 3% (Albertsen et al, 2005). Early stages of prostate cancer are hormone sensitive (HS) and are responsive to hormone therapy, however, this is not curative and most progress to hormone refractory (HR) cancer within 12-18 months of hormone therapy, usually resulting in progression to metastatic disease (Oh and Kantoff, 1998). Novel gene therapy-based strategies aim to increase tumour cell killing by specific targeting of toxic transgenes. Prostate Specific Membrane Antigen (PSMA) is a type II membrane protein, whose presence is almost exclusively restricted to prostate tissue. The precise function of PSMA is unknown, however, the literature would denote a role in the suppression of prostate cancer invasiveness (Ghosh et al, 2005). PSMA expression is in fact up-regulated after androgen ablation therapy and therefore poses an attractive therapeutic target for advanced prostate cancer (O'Keefe et al 2000). $NO^{\bullet}$ has previously been shown to be an extremely versatile molecule, implicated in many physiological processes, essentially as a signaling molecule. Whilst small doses of $NO^{\bullet}$ are shown to be cytoprotective, large doses are known to be cytotoxic, chiefly through the generation of other highly reactive free radicals. Previous work within our group has illustrated the extensive cytotoxicity achieved by generating elevated levels of $NO^{\bullet}$ *in vivo*, by means of a constitutive CMV promoter. This strategy resulted in a significant delay in tumour growth compared with untreated controls (Worthington et al, 2002, 2004).

Results.

We report here, the use of a tissue specific promoter, PSMA, to drive iNOS gene therapy as a potential anti-cancer treatment. In order to assess the effectiveness of PSMA to drive iNOS gene therapy, a PSMA/iNOS vector was created and LNCaP cells were transfected with this construct coupled with a known iNOS co-factor BH4. The results demonstrated a significant reduction ($p< 0.05$) in clonogenic cell survival compared to vector only controls. Similar effects were seen in DU145 and PC3 cells. However, non-prostate cancer cell lines e.g. MCF-7 and HT29, showed no iNOS expression and PSMA/iNOS treated cells showed little variation in cell survival compared with vector only controls ($p>0.05$). Initial i*n vivo* studies showed a highly significant ($p<0.01$) delay in tumour growth in PSMA/iNOS treated tumours (18.9 days) compared with vector treated controls (13.3 days), following single dose treatment.

Conclusions.

Our initial results suggest that the tissue specificity of the PSMA promoter coupled with the cytotoxic potential of $NO^{\bullet}$ presents an attractive therapeutic option for the treatment of both localized and metastatic prostate cancer.

This work was supported by Cancer Research UK and The Department of Education and Learning

P81

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Title: Interleukin-8 promoted CXCR4 expression potentiates migration of prostate cancer cells to stromal-derived factor-1: implications for metastasis to bone.

Authors: Johanna R. Pettigrew*, Pamela Maxwell*, Angela Seaton, Christopher F. MacManus, Patrick G. Johnston, David J.J. Waugh.

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Abstract:

Interleukin-8 (IL-8) is a CXC-chemokine implicated in the angiogenesis and metastasis of prostate cancer. We have reported elevated expression of IL-8 and its two receptors CXCR1 and CXCR2 in cancer cells of prostate biopsy sections, indicating the potential for increased autocrine signalling in these cells. The aim of the current *in vitro* studies was to identify mechanisms by which increased IL-8 signalling in prostate cancer may account for the prevalence of metastasis to bone observed in advanced disease. Our experiments focused on the role of IL-8 signalling in regulating the expression of CXCR4, a receptor for SDF-1, a further CXC-chemokine strongly associated with promoting the metastasis of prostate cancer to bone. Experiments were conducted using the parental androgen-independent, bone-derived PC-3 cell line and 2 clonal derivatives PC-3-56 and PC-3-11 that express high and low levels of IL-8, respectively. Quantitative-PCR assays confirmed that IL-8 signaling increased the CXCR4 mRNA transcript levels in PC3 cells in a time-dependent response. Luciferase reporter assays employing a pGL3 plasmid encoding the CXCR4 promoter upstream of the luciferase gene also confirmed that IL-8 signaling potentiated the transcriptional regulation of the CXCR4 gene. Immunoblotting experiments also demonstrated that IL-8 signalling potentiates the expression of CXCR4, through promotion of both transcription and translation-dependent mechanisms. The functional significance of elevated CXCR4 expression was demonstrated in cell motility assays in which PC-3 cells stimulated with IL-8 increased migration towards SDF-1 compared to untreated controls ($p<0.05$). Our current data suggest that the elevated IL-8 expression within prostate cancer cells *in situ* may enhance their metastatic potential via increasing the cell surface expression of CXCR4, consequently potentiating the chemotactic and selective homing of prostate cancer cells to organs that constitutively express SDF-1.

P82

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Title: Acetylation of the retinoblastoma protein is induced during differentiation of human keratinocytes

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Abstract:

Human cancers of epithelial origin are often poorly differentiated, suggesting that loss of differentiation capacity is a key step in tumor development. The function of the tumour suppressor retinoblastoma protein (pRb) has been extensively investigated with regards its regulation of the cell cycle and proliferation. However, pRb has also been implicated as a regulator of differentiation. Many models have demonstrated that loss of pRb expression inhibits differentiation. The activity of pRb is also regulated by phosphorylation and acetylation events. These modifications are differentially regulated during various cellular processes, including the cell cycle, DNA damage and differentiation. Previously, pRb has been shown to be acetylated during differentiation of muscle cells and myeloid leukemia cells. In the present study, it is demonstrated that pRb is acetylated during calcium-mediated differentiation of primary human foreskin keratinocytes (HFKs) and oral keratinocytes (HOFs). Previously acetylation of pRb has been attributed to p300 and its associated factor P/CAF. Knockdown of either p300 or P/CAF resulted in reduced pRb acetylation during calcium-mediated differentiation, suggesting that both contribute to pRb acetylation during differentiation. These results establish that acetylation is an important post-translational modification of the retinoblastoma protein that occurs during the differentiation of human keratinocytes.

P83

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Title: Investigating promoter methylation of Wnt signalling antagonists in CLL

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Abstract: The Wnt signal transduction pathway is involved in regulating many cellular processes including fate specification, cellular proliferation, differentiation and apoptosis. The classical Wnt signalling cascade inhibits the enzyme activity of glycogen synthase kinase-3β, leading to translocation of β-catenin to the nucleus where it results in transcriptional activation of a panoply of genes that promote cell proliferation and survival. Two functional classes of Wnt antagonists have been identified: the Secreted Frizzled-Related Protein (sFRP) class and the Dickkopf (Dkk) class. By binding directly to Wnt or to the Wnt receptor complex, these molecules block Wnt signalling, resulting in phosphorylation and degradation of β-catenin, and repression of its target genes. Aberrant activation of the Wnt signalling pathway has been well documented in solid tumours and haematological malignancies, and as a result, Wnt antagonists have been proposed as tumour suppressor genes. Promoter hypermethylation associated transcriptional silencing of Wnt antagonists has been reported in a variety of cancer types, but less is known of the importance of this event in haematological malignancies. The aim of this study was to investigate the significance of epigenetic silencing of Wnt antagonists in Chronic Lymphocytic Leukaemia (CLL). CpG islands (the target of promoter methylation) were characterised for 8 Wnt antagonists (*SFRP1*, *SFRP2*, *FRZB*, *SFRP4*, *SFRP5*, *WIF1*, *DKK1* and *DKK2*). Methylation of the *SFRP* class of antagonists was investigated by methylation specific PCR (MSP) and bisulfite sequencing in peripheral blood samples from 136 patients with CLL, stage A (n = 77), stage B (n = 21) and stage C (n = 36). For control purposes, 20 normal blood donor controls were also included. The overall methylation frequencies in CLL were: 48.74% (*SFRP1*), 36.10% (*SFRP2*), 2.94% (*SFRP4*) and 4.65% (*SFRP5*)**.** Bisulfite sequencing confirmed extensive methylation in the *SFRP1* promoter in CLL samples. There was no evidence of *SFRP1* methylation in any of the 20 normal blood donor controls. Identifying methylated genes that are correlated with poor clinicopathological features could potentially identify those patients who may benefit from more aggressive treatment. Hypermethylation of *SFRP1* was detected at similar frequencies in patients with mutated and unmutated (associated with a poorer prognosis) IgVH genes, but occurred in higher frequencies in patients with advanced disease (Binet B/C). In addition, *SFRP1* methylation was detected in significantly more patients with nodular bone marrow histology, the least aggressive form of the disease, than in patients with interstitial or diffuse bone marrow histology ($P < 0.0001$). Quantitative RT-PCR revealed that 53.16% (42/79) CLL samples did not express the *SFRP1* gene and pharmacological demethylation of CLL cell line EHEB with 0.5 μM 5-aza-2'deoxycytidine induced *SFRP1* expression. Although we find *SFRP* methylation in CLL at more moderate frequencies than have previously been reported, these results demonstrate that hypermethylation of the *SFRP* family of Wnt antagonists is frequent in CLL and could represent an important mechanism of Wnt signalling deregulation. We are currently designing a multiplex real time quantitative MSP approach to quantify methylation of the remaining 4 Wnt antagonists. This may provide further insights into the molecular mechanisms behind deregulation of the Wnt signalling cascade in CLL.

P84

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Title: **MAD about Taxol: a role for BRCA1**

Authors: Maria Prencipe(i), Wen Yuan Chung (i), Fiona Furlong (i), Peter A. Dervan(ii), Desmond Carney (iii), Amanda McCann(i).
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Abstract: Taxol is increasingly being used as a microtubule inhibitory (MI) treatment for breast cancer. However, up to a third of patients will not respond to this treatment. The mechanisms underlying this *de novo* resistance involve many pathways, the subject of a recent review by us (McGrogan B.,*et al.,* BBA Reviews in Cancer). We are particularly interested in alterations in key components of the Spindle Assembly Checkpoint (SAC) system involving proteins such as the mitotic arrest deficiency protein 2 (MAD2). As part of this MI response, BRCA1 also plays a role, being required in the activation of the SAC by virtue of its transcriptioanl regulation not only of MAD2 but also the SAC protein BubR1.

The purpose of this study is to define the relationship between two key cellular checkpoint proteins, BRCA1 and MAD2, to further understand the mechanisms underlying cellular resistance to Taxol.

Our experimental system is based on three cell lines, with a differing BRCA1 status; 1) MCF7 (wt BRCA1), 2) HCC1937 (mutated BRCA1) and 3) UACC3199 (methylated BRCA1). The cells are routinely treated with 100 nM Taxol over a 72 hour period. Cultures are subsequently trypsinized and fixed in 70% methanol. The expression levels of MAD2 are analyzed by Western Blotting and flow cytometric analysis of DNA content and percentage of apoptosis are also evaluated following propidium iodide staining.

Results to date have demonstrated that in the cell lines having compromised BRCA1, either through mutation (HCC1937) or hypermethylation (UACC3199), MAD2 expression is lower than in the cells with wt BRCA1 (MCF7). This confirms the known transcriptional regulation by BRCA1 of MAD2. Following Taxol, MAD2 levels increase only in the UACC3199 and in the HCC1937, while remaining at a baseline in the MCF7, cells. This reflects the fact that the majority of cells will not progress to mitosis after Taxol treatment when BRCA1 is functional. However, when BRCA1 is compromised, MAD2 levels increase to ensure that entry to mitosis is impeded. For all cell lines a prominent G2/M arrest was demonstrated as long as BRCA1 or MAD2 were functional.
In summary, a number of human malignancies display reduced levels of MAD2 conferring resistance to microtubule inhibitors. We suggest that if patients have compromised levels of BRCA1 through mutation or hypermethylation, MAD2 levels increase to compensate for any lack of checkpoint control. However, if MAD2 levels are functionally decreased in addition to alterations in BRCA1, these patients are unlikely to respond to Taxol treatment.
Reference:- Barbara T. McGrogan, Breege Gilmartin, Desmond N. Carney, Amanda McCann.Taxanes, microtubules and the chemoresistant breast cancer. Biochim. Biophys. Acta, 2007

P85

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Title: **PDF (Prostate Derived Factor) is a novel modulator of drug response in colorectal cancer cells**

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Abstract:
Prostate derived factor - a member of TGFβ superfamily – appears to be involved in tumour progression and development. Initial DNA microarray experiments were carried out in the HCT116 sensitive and resistant colorectal cancer (CRC) cell lines and aimed to identify genes that are involved in resistance to chemotherapy in CRC. The results from the microarray studies demonstrated that PDF is highly inducible after treatment with Oxaliplatin, 5-Fluorouracil and SN38 (active component of Irinotecan). Further studies in HCT116 p53 wild type cells showed increased PDF expression at both mRNA and protein. No upregulation of PDF expression was observed in HCT116 p53 null cells and other p53 mutant cell lines (H630, HT29).
PDF silencing prior to drug treatment in HCT116 wild type cells led to increased cell death as measured by PARP cleavage and flow cytometric analysis. The same effect was observed in HCT116 drug resistant cell lines after transfection with siRNA followed by treatment with chemotherapy, suggesting that PDF knock-out sensitizes drug-resistant cells to treatment. Overexpression of PDF in the HCT116 p53 wild type cell line on the other hand was shown to rescue cells from apoptosis as demonstrated by decreased PARP cleavage. Thus, PDF appears to be an important factor in the development of drug resistance and may be an attractive target to prevent it.

P86

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Title: Interleukin-8 Signalling Contributes to Chemotherapy Resistance in Colorectal Cancer Cells.

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Abstract: We have previously demonstrated that androgen independent prostate cancer (AIPC) cell lines possess a constitutive interleukin-8 (IL-8) signalling loop and that potentiation of this signalling in response to treatment with the 3rd generation platinum compound, oxaliplatin (L-OHP), confers a chemoresistant phenotype to these cells via NF-κB mediated induction of anti-apoptotic proteins. Current studies are investigating whether a similar mechanism is present in colorectal cancer (CRC) cell lines. This work has clinical relevance as L-OHP is a mainstay of treatment for patients with advanced or metastatic CRC. Initial *in vitro* studies using a panel of CRC cell lines have demonstrated that the majority of cell lines tested secrete IL-8 into culture medium under resting conditions, with the highest secretion observed in metastatic cell lines, and that this secretion is increased following treatment with L-OHP. The presence of the IL-8 cell surface receptors CXCR1 and CXCR2 in the same panel of cell lines has been demonstrated by flow cytometry. Further experiments using the HCT116 CRC cell line have shown that treatment with L-OHP induces NF-κB activation and increases the expression of anti-apoptotic proteins including Bcl-xL and survivin as well as IL-8 and CXCR2 protein expression. Interestingly, treatment of the same cell line with recombinant IL-8 also induces NF-κB activation and anti-apoptotic protein expression. Administration of AZ10397767, a pharmacological antagonist of CXCR2, following treatment of HCT116 cells with L-OHP results in attenuation of L-OHP induced Bcl-xL and survivin protein expression and increases the sensitivity of the cells to L-OHP, as determined by cell count and clonogenic assays. Collectively, these studies suggest the presence of an IL-8 autocrine/paracrine signalling loop in HCT116 cells leading to anti-apoptotic signalling and increased survival in response to L-OHP treatment. In addition, inhibition of IL-8 signalling may be an appropriate intervention to sensitise CRC cells to L-OHP treatment. Ongoing and future studies aim to determine in more detail the role of NF-κB and other transcription factors in the signalling pathways described and also evaluate the effect of employing alternate strategies for inhibiting IL-8 signalling on the sensitivity of CRC cells to L-OHP.

P87

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Title: Investigation into methylation of the Secreted Frizzled Related Proteins (*SFRP*) family of Wnt antagonists in prostate cancer.

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Abstract: In the era of prevalent use of PSA as a screening test for Prostate cancer (CaP), there has been a dramatic increase in the incidence of the disease. Promoter hypermethylation associated silencing of tumour suppressor genes and genes with important cell regulatory functions is widespread in cancer, including prostate cancer. An increasing body of evidence advocates that DNA hypermethylation may be useful for the early detection and diagnosis of CaP. Wnt signalling plays diverse roles in embryo development, cell differentiation, proliferation and apoptosis. Wnt signalling causes intracellular stabilisation of β-catenin, enabling it to translocate to the nucleus where it interacts with transcription factors to stimulate the expression of cell proliferation and pro-survival genes. Aberrant activation of Wnt signalling has become a hallmark of many human cancers. We are investigating whether promoter hypermethylation and subsequent epigenetic silencing of the Secreted Frizzled-Related Protein (*SFRP*) family of Wnt signalling antagonists is a cause of abnormal Wnt signalling in prostate cancer. We first demonstrated the presence of a promoter CpG island (the target of promoter methylation) in four *SFRP* genes, *SFRP1*, *SFRP2*, *SFRP4* and *SFRP5* through a bioinformatics approach. DNA methylation was investigated by both conventional and quantitative methylation specific PCR and bisulfite sequencing in CaP cell lines (LNCaP, DU145, RC58/T, PC-3, 22Rv1, PWR1E and RWPE1) and tissue samples of CaP (n=40), benign prostatic hyperplasia (BPH) (n=37), histologically normal prostate (n=39) and preinvasive high-grade prostatic intraepithelial neoplasia (HGPIN) (n=15). The methylation frequencies of the four genes in prostate cancer were 11.11% (*SFRP1*), 72% (*SFRP2*), 0% (*SFRP4*) and 30% (*SFRP5*)**.** *In vitro* studies revealed frequent hypermethylation of *SFRP2* in prostate cancer cell lines (LNCaP, DU145, PC-3 and 22Rv1). *SFRP1* and *SFRP5* were both methylated in DU145 and PC-3 androgen dependent cell lines only. *SFRP4* was completely unmethylated. Further investigation into *SFRP2* showed significantly lower frequencies and quantitatively lower levels of methylation in histologically normal prostate (10.52%; relative methylation score (RMS) = 0.35), BPH (11.54%; RMS = 0.05), and HGPIN (15.38%. RMS = 1.39) compared with prostate cancer (72%; RMS = 56.64), $P < 0.0001$, Kruskall-Wallis test. Methylation of *SFRP2* was not significantly associated with tumour grade (Gleason score, $P = 0.47$, Fisher's exact test) or TNM classification ($P = 0.38$, Fisher's exact test), indicating that methylation of this gene may occur as a frequent event throughout all stages and grades of this disease. We have shown that promoter hypermethylation of the *SFRP2* Wnt signalling antagonist is a frequent epignetic hit in prostate cancer. We are further investigating the functional effect of promoter methylation on *SFRP2* expression by quantitative reverse transcription PCR in a sample of tissue specimens and in the PC-3 cell line following pharmacological demethylation with 5-aza-2'-deoxycytidine. This may provide further insights into the molecular mechanisms behind deregulation of the Wnt signalling cascade in prostate cancer.

P88

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Title: **The identification of transcriptional targets of TBX2 and their role in breast cancer proliferation**

Authors: Keara Redmond, Hannah Farmer, Zenobia D'Costa, Niamh O'Brien, Caoimhe Nic An tSaoir, Dorota Tkocz and Paul B. Mullan.
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Abstract:
TBX2 is a member of a family of genes encoding developmental transcription factors which play a crucial role in the development of many tissues, and mutations in these genes have been implicated in multiple human disorders. TBX2 is a transcriptional repressor and has been shown in vitro to repress a number of key growth regulatory genes such as $p14^{ARF}$, $p21^{WAF1}$ and the Gap Junction Protein connexion 43. Previous work has shown that the TBX2 locus is found on a region of chromosome 17 (17q23) which is amplified in up to 20% of primary breast cancers. This amplification has been shown to result in TBX2 overexpression and this may be important for tumour development and/or progression. Indeed, TBX2 is associated with highly aggressive breast cancer and its expression has been shown to correlate strongly with hereditary breast cancers (50% of BRCA1 and 90% of BRCA2 mutant breast cancers).
We have found that knockdown of TBX2 by siRNA leads to profound growth inhibition in a number of breast cell lines. We have also developed an inducible dominant negative MCF-7 cell line which when induced leads to dramatic cell growth inhibition, again suggesting that TBX2 is driving cell proliferation. To identify targets that are transcriptionally regulated by TBX2 we carried out microarray analysis using siRNA targeting TBX2 in MCF-7 cells. This resulted in a list of over 600 genes either up- or down-regulated at least 2-fold following TBX2 knockdown. We have validated a number of these targets by real time PCR and northern blotting in a several breast cancer cell lines which are known to express TBX2. We are currently performing promoter studies for a number of these targets including NDRG1 and IGFBP3. We are interested in identifying the minimal responsive element in these promoters as well as defining the mechanism of TBX2 transcriptional repression. Studies in C.elegans have suggested that TBX2 is sumoylated and that this may be crucial for its activity. We have also preliminary evidence that KAP1 and HP1 proteins may also be involved. All of these potential mechanisms are being investigated. Ultimately we hope to define a pathway downstream of TBX2 which may be amenable to chemotherapeutic intervention.

P89

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Title: The Role of c-FLIP in regulating non-small cell lung cancer cell death
Authors: K.M. Redmond, T.R. Wilson, K.M. McLaughlin, P.G. Johnston, D.B Longley
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Abstract: Non-small cell lung carcinoma (NSCLC) is the leading cause of cancer related deaths in Ireland, with five-year survival rates of only 9% in females and 7% in males. Classically, platinum-based therapies are used in the treatment of advanced NSCLC, with response rates of between 17-32%. Clearly a major factor limiting the efficacy of chemotherapy in this disease is drug resistance.

Death receptors are transmembrane proteins which can initiate cell death via activation of caspase-8. c-FLIP is a key regulator of death receptor induced apoptosis. c-FLIP is expressed as long (c-$FLIP_L$) and short (c-$FLIP_S$) splice variants and both act by binding to FADD (a death receptor adaptor molecule) and preventing caspase-8 activation and subsequent cell death. We have previously shown that c-FLIP is a key regulator of chemotherapy- and death ligand-induced cell death in colorectal cancer.

The aim of this study was to elucidate the role of c-FLIP in chemotherapy-induced apoptosis in NSCLC. Interestingly, *c-FLIP* gene silencing induced spontaneous apoptosis in a panel of NSCLC cell lines. Pretreatment with c-FLIP-targeted siRNA also sensitized these cell lines to the death ligand TRAIL. Furthermore, treatment with c-FLIP targeted siRNA sensitized our panel of NSCLC cell lines to taxol- and cisplatin-induced cell death. Isoform specific gene silencing of *c-FLIP* indicated that both c-FLIP splice variants must be silenced for maximal induction of apoptosis and sensitisation to TRAIL and chemotherapy. Importantly, *c-FLIP* silencing *in vivo* significantly reduced xenograft growth compared to control siRNA treated xenografts and enhanced sensitivity of the xenografts to cisplatin treatment.

Interestingly, we found that c-FLIP silencing in normal lung cell lines did not induce spontaneous apoptosis, did not significantly sensitise these cells to TRAIL- or chemotherapy-induced cell death. Finally, we found that FT siRNA-induced cell death is mediated by caspase-8 and the death receptor DR5 in non-small cell lung cancer cells. However, it is not dependent on DR5 binding by its ligand TRAIL. Interestingly, FT siRNA does not induce caspase-8 activation in the normal lung cell lines.

Collectively, our results indicate that c-FLIP is an important regulator of chemoresistance in NSCLC and that c-FLIP may be an important therapeutic target in the treatment of this disease.

P90

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Title: Potential Therapeutic Targets in Invasive Pancreatic Cancer Identified by Gene Expression Profiling.

Authors: A. Rogers[1], J. Murphy[1], E. Manahan[1], D.P. Toomey[1], K.C. Conlon[1].
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Abstract:

Introduction: Pancreatic cancer remains a lethal disease and is the fifth most common cause of cancer death in Ireland. The Irish incidence (386 cases in 2005), correlates closely to the death rate (379 in 2005) on an annual basis. Current management of pancreatic cancer is mired by late presentation and lack of effective adjuvant therapy. We investigated the genome-wide expression profiles of two pancreatic cancer cell lines, AsPC-1 and BxPC-3, to identify diagnostic markers and therapeutic targets for this disease.

Methods: Cells were treated with Camptothecin (pro-apoptotic) or phorbol 12-myristate 13-acetate (PMA - pro-inflammatory) for 4h. Non-treated cells were used as control. RNA was extracted and hybridised to Affymetrix arrays. Differentially expressed genes were identified using ArrayAssist®. Significantly interacting genes were linked and pathways mapped using Pathway Studio®. Genes were selected for validation by quantitative RT-PCR based on pathway significance and fold change.

Results: Genesets for each condition displayed a 1.5 fold differential expression with p values <0.02. Pathway analysis revealed that camptothecin was primarily involved in signal transduction via MAP kinase pathways. PMA induced apoptotic signalling through a family of receptors known collectively as 'death receptors' including Fas, DR3 and DR4-5. Quantitative RT-PCR confirmed microarray expression profiles. Genes selected included those already implicated in pancreatic cancer (SMAD3, BRCA2, MMP-1, IL1-R1) and also several not previously reported. Although camptothecin and PMA had distinct expression profiles, 3 genes (ATF3, uPA and SOD2) were ≥ 10 fold up- and down-regulated in AsPC-1 and BxPC-3, respectively.

Conclusion: Three genes, ATF3, uPA and SOD2, have been identified in pancreatic cancer for evaluation as screening and therapeutic targets. These genes are involved in early stage invasion and cell dissociation. Both SOD2 and uPA up-regulate the expression of matrix metalloproteinases (MMPs -2 and -9), which are present in the vast majority of pancreatic adenocarcinomas and are important for invasion, metastasis and angiogenesis. In conclusion, ATF3, uPA and SOD2 have potential as specific tumour markers or molecular targets in pancreatic cancer.

P91

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Title: Inhibition of NF-κB in colon cancer cells significantly decreases tumour burden and increases survival time in a mouse model of peritoneal metastasis

Authors: Aideen Ryan, A Colleran, A O'Gorman, E Foran and Laurence J. Egan
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Abstract:
Colorectal carcinoma (CRC) is the third leading cause of cancer related deaths worldwide. In 25% of recurrent CRC the peritoneal cavity is the site of metastatic disease. Younger patients present more frequently with peritoneal carcinomas, which prevents the possibility of a curative treatment. Therefore, the control of metastatic spread of CRC remains a critical issue for cancer treatment. The pathogenesis of CRC peritoneal metastasis involves tumour cell proliferation, angiogenesis, detachment, survival in the circulation, extravasation and growth in distant organs. Several lines of evidence suggest that the transcription factor, NF-κB, may play an important role in tumour metastasis since many NF-κB regulated genes have been shown to be associated with tumour progression and metastasis in diverse models of cancer. However, to date, it is not known if NF-κB plays a functionally important role in CRC metastasis. We established an experimental murine model in which a colon adenocarcinoma cell line, CT26, generates peritoneal metastases. To investigate the role of NF-κB in CRC peritoneal metastasis, we generated CT26 cells lacking NF-κB activity by stable expression of an NF-κB super-repressor (SR). Control cells (CT26-EV), transfected with the plasmid backbone, were used as a control for all subsequent *in vitro* and *in vivo* experiments. Characterization of the cell lines by Western blotting, PCR and NF-κB luciferase assay confirmed the stable inhibition of NF-κB activity following stimulation with TNF-α. Mice injected with various concentrations of NF-κB inactive cells (CT26-SR) survived for significantly longer (≥70%) when compared to those injected with control CT26-EV cells ($p < 0.001$, $n=8$). In a separate experiment, we found that mice injected with CT26-SR cells had significantly less tumour burden and showed up to 50% less intra-abdominal spread when compared to control mice ($p = 0.004$). These results indicate an important role for NF-κB in CRC metastasis. Therefore, pharmacological inhibition of NF-κB could represent a possible therapeutic modality in this disease. Ongoing work will enable us to determine the precise molecular pathways involved in the suppression of peritoneal metastasis in this model. This work is supported by Cancer Research Ireland and Science Foundation Ireland.

P92

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MSX2 as a Prognostic Marker of Primary Cutaneous Melanoma

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Malignant melanoma represents an aggressive and lethal form of cancer. Unfortunately, incidence rates are increasing rapidly each year, and current treatments do not provide much relief, with few contributing to the overall survival of patients with advanced metastatic disease. We previously performed DNA microarray-based gene expression profling on an isogenic panel of human melanoma cell lines, called the WM793 series, which mimicked key steps in tumour progression; this work identified a cohort of 66 genes that were differentially expressed between the poorly tumourigenic parental cell line and 3 more aggressive derivative lines (1). From this analysis, MSX2 emerged as an upregulated transcript in the derivative lines, as compared to parental cells. Here, we evaluated MSX2 protein expression in two separate cohorts of human melanoma tissues via tissue microarray (TMA) technology. The first cohort consisted of 161 primary cutaneous melanomas, 54 benign nevi and 31 metastatic tumours, obtained from patients visiting the Mater Misericordiae Hospital. Clinical information available on this cohort included Breslow thickness, Clarks level and ulceration; however, survival and treatment information were not available. To determine if MSX2 has associations with survival, we also analysed a separate TMA, comprised of 157 patient samples and obtained from University Hospital, Uppsala. We probed two TMAs with an anti-MSX2 antibody and assessed antibody-antigen interaction via standard immunohistochemistry. The stained TMAs were manually scored by a pathologist and additional trained reviewer, with all data examined for statistical significance via SPSS. From this analysis, MSX2 was found to be a promising marker of melanoma progression, with its expression being associated with several clinical parameters. Importantly, MSX2 positivity (intensity of staining 1-3) for Cohort 1, was found to be associated with increased Breslow thickness ($p=<0.001$), increased Clarkes level ($p=<0.001$) and ulceration. As these clinical variables are known prognostic factors for melanoma, this provides support for MSX2 as a poor prognostic marker. In addition, MSX2 expression was also highly correlated with nodular tumours, opposed to superficial spreading melanomas. We are still in the process of evaluating the MSX2 expression pattern within Cohort 2. Initial functional studies, whereby MSX2 is ectopically overexpressed in cell culture models, shows a potential role for MSX2 in controlling cell survival and morphology. Overall, this study provides further evidence for MSX2 as playing a possible role in melanoma progression. Moreover, it implicates MSX2 as a promising prognostic marker for human melanoma.

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(1) Gallagher WM *et al.* (2005). Multiple markers for melanoma progression regulated by DNA methylation: insights from transcriptomic studies. Carcinogenesis. 2005 Nov; 26(11): 1856-67.

P93

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Title: **Application of Electroporation-Driven Intraluminal Gene Delivery**

Authors: M. Sadadcharam, P. Forde, L. Cogan, D. Soden, G.C. O'Sullivan
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Abstract:

AIMS: Gene therapy of several diseases, including cancer, is close to clinical application. Recent research has focused on the development of non-viral systems that function effectively episomally and do not integrate into host DNA. The Cork Cancer Research Centre has achieved success with various gene delivery methods in laboratory and murine models *in vitro, in vivo* and *ex vivo*. Specifically, we have found that plasmid vectors coding for immunogenes can be delivered locally into solid tumours by electroporation. Our aim in this study is to examine the ability to detect expression of the reporter gene, pCMVβ, following delivery directly into intraluminal tissue.

METHODS: To date, electroporation-based therapies have been limited to the treatment of externally accessible tumours. To overcome these limitations, we have developed an electroporation device, the EndoVac, for use in electrochemotherapy / electrogenetherapy of previously inaccessible tumours. Using this device, we plan to assess the efficacy of intraluminal gene delivery using electroporation. We have obtained ethics and license approval for work with 20 pigs. 0.2mg pCMVβ in 100µl injection volume is injected into specific sites e.g. oesophagus. Tissue is then electroporated with the EndoVac using optimised parameters from the ESOPE study. Relevant sites are marked with India ink. Repeat endoscopy and tissue biopsy is carried out after 48 hours. Tissue is then sectioned and stained for pCMVβ and Haemotoxylin and Eosin. Blood samples are taken pre- and 48 hours post-treatment to look for pCMVβ expression using ELISA.

CONCLUSIONS: Although the efficacy of electroporation on *in-vitro* cell based systems has been well established, the final product will be designed for *in-vivo* human use. If effective, this system could pave the way for delivery of therapeutic genes directly into intraluminal tumour nodules, rendering many cancers, which are presently deemed inoperable or unresponsive to conventional therapy, accessible to electrochemotherapy/electrogenetherapy.

P94

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Title: **Increased HER2 testing and trastuzumab access in metastatic breast cancer in Northern Ireland from 2004 to 2007: the audit effect?**

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Abstract:

Background: Clinical trials have clearly established that patients receiving taxane-based chemotherapy for metastatic breast cancer (MBC) should be treated with trastuzumab if their tumour is shown to overexpress the human epidermal growth factor receptor 2 (HER2) receptor. A previous audit carried out in Northern Ireland throughout 2004 identified that some patients who were receiving chemotherapy for MBC did not have HER2 testing or trastuzumab where indicated and that the time to obtain a result averaged 41.5 days. This re-audit examined the same issues given that results of the previous audit were widely disseminated and that testing of HER2 status is now performed at diagnosis.

Materials and Methods: Patients commencing chemotherapy for MBC in Northern Ireland in the first quarter of 2007 were identified from pharmacy records. Their case notes were retrospectively reviewed to determine whether patients in routine clinical practice had HER2 testing and trastuzumab treatment if indicated.

Results: Fifty six patients commenced chemotherapy. HER2 data is available for 52 patients, of whom 51(98%) had HER2 testing, compared to 93% in 2004. The patient who did not have HER2 testing had cardiac dysfunction and was not suitable for trastuzumab therapy. In 50(98%) patients the HER2 result was already available at the time of this relapse compared with 44% in 2004. In the remaining 1 patient the result became available in 9 days compared with a median of 41.5 days in 76 (49%) patients in 2004. Of those tested, 21 patients (41%) were HER2 positive (immuno-histochemistry 3+ or fluorescence in situ hybridization positive). Eighteen of these patients were treated with trastuzumab, either as a single agent or in combination with chemotherapy. There were valid reasons for trastuzumab omission in the 3 patients not given trastuzumab (2 given first line anthracycline-based regimen and 1 had cardiac dysfunction).

Discussion: In our region all of the 52 patients who received chemotherapy for MBC and for whom data is currently available for, were tested for overexpression of the HER2 receptor. Of those patients who were eligible to receive trastuzumab all received trastuzumab, compared with 91% of patients in the 2004 audit. In most cases the HER2 status was known at the time of relapse and the time required to obtain a HER2 result was 9 days compared with a median of 41.5 days in 2004. The move to "up-front" testing of HER2 status at time of original diagnosis has streamlined management of HER2 positive metastatic disease.

P95

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Title: Interleukin-8 signalling regulates the sensitivity of prostate cancer cells to bicalutamide through induction of androgen receptor expression and activity

Authors: Angela Seaton, Paula Scullin, Pamela Maxwell, Catherine Wilson, Johanna Pettigrew, Rebecca Gallagher, Joe O'Sullivan, Patrick Johnston and David Waugh

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Abstract: The aim of our study was to assess the importance of the CXC chemokine, interleukin-8 (IL-8) in promoting the transition of prostate cancer (CaP) to the androgen-independent state. Stimulation of the androgen-dependent cell line, LNCaP, with exogenous recombinant-human IL-8 increased androgen receptor (AR) gene expression at the mRNA and protein level, assessed by quantitative real time PCR and immunoblotting, respectively. Using an ARE-luciferase construct we demonstrated that IL-8 treatment also resulted in increased AR transcriptional activity, and a subsequent up-regulation of PSA and Cdk2 mRNA transcript levels in LNCaP cells. Blockade of CXCR2 receptor signalling using a small molecule antagonist (AZ10397767) attenuated the IL-8 induced increases in AR expression and transcriptional activity. Furthermore, in MTT assays, co-administration of AZ10397767 reduced the viability of LNCaP cells exposed to bicalutamide. Our data shows that IL-8 signaling increases AR expression and promotes ligand-independent activation of this receptor in LNCaP cells, describing two mechanisms by which this chemokine may assist in promoting the transition of CaP to the androgen-independent state. In addition, our data shows that IL-8 promoted regulation of the AR attenuates the effectiveness of the AR antagonist bicalutamide in reducing CaP cell viability.

P96

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Title: Investigation of hsa-miR-21 (MIRN21) targets by bioinformatic analyses and by microarray gene expression profiling in the breast cancer cell line MCF7

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Abstract:
MicroRNAs are small non-coding RNAs binding to complementary 3'UTR regions of mRNAs and regulate gene expression negatively through mRNA cleavage, translational repression or recently discovered mRNA degradation by deadenylation. MicroRNAs contribute to diseases such as cancer directly (regulating disease specific genes) or indirectly (regulating other regulators). Thus, they can potentially act as oncogenes or tumour suppressors in cancer mechanism. To date, only a few microRNAs have been experimentally shown to target cancer related genes. For example, hsa-mir-21 (MIRN21), targets *PTEN* in hepatocellular cancer and *PCDC4* apoptotic gene in breast cancer, while let-7 targets *KRAS* in lung cancer, hsa-mir-15 and hsa-mir-16 targets *BCL-2* in CLL. In this study, our aim is to identify gene targets of MIRN21 that are regulated through mRNA downregulation by cleavage or deadenylation. Firstly, we tested for high expression of MIRN21 in cancer cell lines such as the breast cancer cell line MCF7 by qRT-PCR, and verified its relatively high expression compared to the housekeeping gene U6. To investigate the potential targets of MIRN21, we used two approaches: The first approach involved bioinformatic analysis of the targets where we searched predicted targets of MIRN21 available online by microRNA target prediction programs such as PicTar, MiRanda, TargetScan or miRBase. We identified more than 30 candidate genes with high scores which were predicted by more than two of the prediction programs and further classified these candidates based on their potential functions in cancer-relevant pathways (tumour suppressors, oncogenes, kinases, apoptotic factors, etc.). Our second approach involved an unbiased approach to experimental discovery of MIRN21 targets. To block MIRN21 expression, MCF7 cells were transfected with anti-mir-21 inhibitor oligos. Transfection optimizations were conducted and tested using pmir-REPORT luciferase constructs specifically designed to elicit decreased luciferase signals when the anti-mir inhibitor is operational. Following transfection, anti-mir-21 oligo transfected cells in duplicate as well as negative controls of oligo transfections were hybridized to Affymetrix Microarrays (Human Genome U133 Plus 2.0) containing ~47,000 transcripts to profile the effects of MIRN21 silencing on mRNA transcripts. Statistical and clustering analyses of microarray data will identify potential target mRNAs cleaved or deadenylated by MIRN21, and also secondary effects of MIRN21 expression knockdown on MCF7 transcriptome. Common targets found by both approaches will be compared and further tested by qRT-PCR to show downregulation (upregulation) of a particular mRNA based on presence (absence) of MIRN21. The results of this genome wide screen will identify gene targets of MIRN21 regulated by mRNA cleavage or deadenylation. These studies will offer new cancer biomarkers under regulation by microRNAs that could be used for better diagnostic and therapeutical applications for breast cancer.
This research was funded by Cancer Research Ireland (CRI), Irish Research Council for Science, Engineering and Technology (IRCSET) and Turkish Academy of Sciences (TUBA).

P97

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Title: **Differential Expression of the HOXD Cluster in Normal and Neoplastic Oral Epithelial Cells**

Authors: **Daniel J. Sharpe, Perry Maxwell, Alexander Thompson, Terence R.J. Lappin, and Jacqueline A. James**
Institution: **CCRCB, Queen's University, Belfast**

Abstract:

Class I Homeobox (HOX) genes encode a family of transcription factors, defined by a highly conserved 61 aa-motif (the homeodomain). HOX proteins control a variety of important cellular functions, including proliferation, differentiation and apoptosis. During embryogenesis they regulate morphogenesis of the body structures. HOX genes are also expressed in adult tissues, in patterns characteristic of that tissue, often distinct from that found in their embryonic precursors. These adult expression patterns are often altered in cancers and in some cases can be used as prognostic indicators. Changes in the HOX gene expression profile have been found in every cancer type investigated. To date, the expression profile of all 39 HOX genes has not been investigated in cancers of the oral cavity. Approximately 90% of all 'Head and Neck' cancer cases including those of the oral cavity are squamous cell carcinomas.

HOX gene expression profiles of oral keratinocyte cell lines derived from normal buccal mucosa or gingiva, and four squamous cell carcinoma cell lines derived from malignancies of the hypopharynx (BICR6) or tongue (H357, PE/CA, SCC15), were determined by real-time quantitative-PCR (Q-PCR). Comparison of these profiles showed an increase in the expression of all of the HOX genes in the OSCC cell lines. A subset of the HOXD genes (HOXD8-HOXD11) showed considerably higher expression levels in OSCC cell lines compared to the other HOX genes and were selected for further investigation. Preliminary western blots and immunocytochemistry for these HOX proteins show strong correlation with the Q-PCR results.

Modulation of the expression levels of the HOXD gene subset is currently underway using gene expression constructs, siRNA oligonucleotides and shRNA vectors, and successful knockdown has been achieved. The functional consequences of altering HOXD8-11 gene expression is being investigated by assays of invasion, migration, proliferation and adhesion utilising standard and organotypic cell culture techniques.

Recently a decrease in the global methylation of DNA in Head and Neck cancer patients has been reported as a biomarker for the disease. The methylation status of the promoter regions of these HOXD cluster genes is under investigation to determine if hypo-methylation has a role in the aberrant expression of these genes. Investigation of the expression of these genes in a longitudinal study of patient tissue samples could potentially provide valuable insights into their role in the development and progression of oral malignancy.

P98

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Title: Silencing of HPV Viral Oncogenes E6 and E7 in Cervical Cancer.
Authors: CD Spillane, L Kehoe, H Keegan, O Sheils, CM Martin, JJ O'Leary.
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Abstract: Cervical cancer is the second most common cancer in women worldwide. Human papillomavirus (HPV) is the main etiological agent in invasive cancer of the cervix, with high-risk HPV types 16 and 18 being detected in more than 95% of cervical cancers. Integration of high-risk HPV genomes and subsequent expression of the two main viral oncogenes, E6 and E7, are considered to be critical steps in the development of cervical cancer. The protein products of E6 and E7 interfere with essential cell cycle pathways including those governed by tumour suppressor proteins, p53 and retinoblastoma (Rb).

Previous studies have demonstrated the potential of using RNA interference (RNAi) targeted towards HPV16 E6 and/or E7 as a novel molecular therapeutic approach. While the majority of these studies showed that the down-regulation of HPV16 E6 and E7 resulted in retarded growth and/or apoptosis of HPV16 positive cells, none have examined the changes occurring at the transcriptome or proteome level, for pathways other than those p53 and Rb. The aim of this project was to use short interfering RNA (siRNA) targeted towards the E6/E7 oncogenes and to establish the downstream effects of this knockdown in HPV positive cells using microarray and immunofluorescent proteomic analysis.

The HPV16 transformed SiHa and CaSki cells are being used as a model system. The most efficient transfection agent for our cell lines was established using a high throughput 96-well set up. With controls a central issue in RNAi experiments it was essential to initially optimise transfection conditions for the positive control siRNA, GAPDH. Efficiency of the knockdown process was validated at the RNA and protein level, using RT-PCR and western blotting approaches respectively. Silencing of on average 87% at the RNA level has been achieved using the optimal conditions. We are currently treating the cells with the E6 and E7 siRNA with >70% knockdown at the RNA level and a corresponding significant down-regulation at the protein level as a criteria for a true silencing event.

This study will identify a subset of dysregulated genes in cervical cancer, which are translated into functional protein. Thus, pinpointing genes and pathways that are functionally important in the development of cervical cancer.

P99

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Title: The role of Calretinin as a novel modulator of chemotherapy-induced cell death in colorectal cancer cells

Authors: Leanne Stevenson, Wendy L. Allen, Irina Proutski, Vicky Coyle, Puthen Jithesh, Cathy Fenning, Gail Stewart, Daniel B. Longley, Patrick G. Johnston.
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Abstract:
A panel of HCT116 CRC cell lines resistant to 5FU, oxaliplatin and SN-38 were generated by repeated exposure to stepwise increasing concentrations of drug over a period of months. These model cell lines were used in conjunction with DNA microarray technology to identify novel determinants of response to these chemotherapies. The expression profiles of these cell lines were examined using the Affymetrix HGU133 plus2.0 array and, more recently, the Almac-Diagnostics Colorectal Disease-Specific array (DSA). Calcium-binding protein calretinin was identified as being constitutively altered in drug resistant cells relative to sensitive parental cells and induced following acute exposure of parental cells to drug.

Further analysis of the HCT116 $p53^{+/+}$ parental cells by real time RT-PCR and western blot showed calretinin to be inducible in response to 5-FU, oxaliplatin and SN-38 at an mRNA and protein level respectively. An over-expression of calretinin basal mRNA and protein was observed in the drug-resistant HCT116 $p53^{+/+}$ daughter cell lines compared to the HCT116 $p53^{+/+}$ parental cells. Calretinin mRNA expression was also induced in a panel of 3 other CRC cell lines (HCT116 $p53^{-/-}$, LoVo and H630) following treatment with oxaliplatin and SN-38. 5-FU treatment induced calretinin mRNA expression in the HCT116 $p53^{-/-}$ and H630 cell lines but not in the LoVo cell line. The effect of calretinin on the apoptotic response to chemotherapeutic agents in the HCT116 $p53^{+/+}$ and $p53^{-/-}$ cell lines was then investigated. Western blot analyses and flow cytometry studies showed that calretinin knock down with siRNA attenuated the sensitivity to 5-FU but did not appear to effect sensitivity to oxaliplatin or SN-38.

Taken together, these results suggest that calretinin may be a key mediator of 5-FU induced cytotoxicity of CRC cells.

P100

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Title: Prostate Cancer Bio-resource, St James's Hospital: *Prostate Cancer Research Consortium.*

Authors: Linda Sullivan, Antoinette S Powell, Ruth Foley, Rustom Manecksha, Barbara Dunne, Eoin Gaffney, Thomas Lynch, R William G Watson, Donal Hollywood, Mark Lawler.

Institution: Institute of Molecular Medicine, Trinity Centre for Health Sciences.

Abstract: The incidence of prostate cancer (CaP) among Irish men is increasing annually, and there is a need for improved diagnostic procedures and novel treatment strategies. One of the principal objectives of the Prostate Cancer Research Consortium (PCRC), a Dublin Molecular Medicine Centre (DMMC) programme founded in 2003, was to establish a shared CaP Bio-resource that would house high quality bio-specimens and well-annotated anonymised patient data in order to facilitate trans-institutional research. St James's hospital and the Trinity Centre for Health Sciences are now among five Dublin-based hospitals and three research institutes to become part of the PCRC.

To date 83 patients undergoing radical prostatectomy at St James's hospital have consented to donate bio-specimens. These include urine and blood, which are processed for prospective genomic (DNA and RNA) and proteomic (plasma and serum) research. In addition, suspected tumour and benign tissue samples, as indicated by TRUS-biopsy, are collected from the prostate gland post-surgery. All samples are coded with a unique anonymous identification code and stored appropriately. Information of interest regarding bio-specimens and clinical/biomedical data can then be accessed by researchers within the consortium via a password protected Bio-resource Information Management System.

High quality bio-specimens are fundamental in the generation of meaningful data. To achieve uniformity in the quality of samples, Standard Operating Procedures for collection, processing and storage are strictly followed. There is a need, however, to further investigate the effect of handling procedures on bio-specimens. To this end prospective work on the St James's Bio-resource will include a comparison of sample preservation methods, and investigation into the effect of the freeze-thaw process on RNA yield and integrity.

P101

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Title: **The identification of pathways responsible for driving the proliferation of basal breast cancers.**

Authors: Tkocz DM, O'Brien N, Nic An tSaoir C, Farmer HL, Redmond KL, D'Costa ZC and Mullan PB.
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Abstract:
Recent advances in expression profiling using DNA microarray technology has shown that breast cancer can be classified into five subtypes: Luminal A, Luminal B, Normal-like, HER2 and Basal. Each of these subtypes also has distinct clinical outcomes. Basal breast cancers are a group of breast cancers often referred to as 'triple negative' because they lack the oestrogen, progesterone, and HER2 receptors. This triple negative receptor phenotype means that current therapies such as anti-oestrogen or Herceptin will be ineffective. Interestingly, most or possibly all basal cancers are characterised by decreased expression of BRCA1 protein and resemble BRCA1 mutant breast cancers. Several recent DNA microarray studies of tumour samples revealed a subset of genes which appear to be specifically upregulated in this type of cancer, and importantly, have also been implicated in cell proliferation or survival. The aim of this project is to identify and characterise the genes responsible for driving basal breast cancer which could represent novel targets for treatment. We have compared datasets from a number of published microarray studies of basal breast cancer with some of our own datasets in which we have used models of BRCA1 to identify transcriptional targets. From this we have compiled a list of target genes overexpressed in basal and BRCA1 mutant breast cancer. We have generated a siRNA library targeting 32 basal/BRCA1 genes (in triplicate) and we now plan to interrogate this library using three different cell models. Two already available cell lines are the MDA468 and HCC1937 cell lines both stably transfected with either an empty vector construct (EV) or a vector containing full-length wild-type BRCA1 (BR). A third cell model is currently being generated in the MDA231 basal cell line. We will measure cell proliferation as a readout of our siRNA panel using the Roche WST-1 kit. Using this library all three cell models will be compared for the inhibition of proliferation following treatment with the siRNA library. The library will also be used to screen non-basal cell lines such as the non-tumorigenic MCF10A and the luminal T47D cell lines (thus identifying false positives which may represent viability genes). Candidate genes will be further assessed by real-time quantitative PCR analysis followed by in-depth promoter analysis (including luciferase reporter and chromatin immunoprecipitation studies) to identify the BRCA1 responsive elements. Ultimately we aim to identify genes which are driving the proliferation of basal and BRCA1 mutant breast cancers and represent targets for future therapies.

P102

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Therapeutic potential of OSU-03012, a Celecoxib Derivative, in Pancreatic Cancer.

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Introduction:
Pancreatic Cancer is a lethal disease.There is increasing evidence that Non Steroidal Anti-inflammatory Drugs (NSAIDs) have Cyclooxygenase-2 (COX-2) independent actions in cancer. These effects occur regardless of COX-2 expression and may be beneficial or detrimental. This study compared the actions of specific COX-2 inhibitors (Celecoxib, NS398) with that of OSU-03012, a celecoxib derivative, in pancreatic cancer.

Methods:
A previous study confirmed COX-2 levels in two pancreatic cancer cell lines. BxPC-3 has consistently high COX-2 expression and AsPC-1 has no detectable COX-2 despite pro-inflammatory and pro-apoptotic stimulation. Proliferating cells were treated with NS398, celecoxib or OSU-03012 and LC50's determined using MTT assay. Prostaglandin E2 (PGE2) and Vascular Endothelial Growth Factor (VEGF) production were measured by ELISA. Significance was calculated using Student's t test.

Results:
Each of the reagents had a concentration dependant effect on cell viability regardless of cellular COX-2. The LC50 for NS398 was >100μM and for Celecoxib >50μM. OSU-03012 was cytotoxic at 10μM in AsPC-1 cells and had a LC50 of 15-20μM in both cell lines ($P<0.05$). Of note, proliferating cells seemed more susceptible than confluent.
COX-2 was inhibited by Celecoxib [1μM] or NS398 [1μM] ($p<0.05$). OSU-03012 resulted in less inhibition of COX-2 activity. NS398 did not effect VEGF levels however VEGF was increased 1.8 (AsPC-1) and 2.1 (BxPC-3) fold by Celecoxib [50μM] ($p<0.01$). OSU-03012 [10μM] stimulated a 1.2 fold increase in VEGF from AsPC-1 cells ($p<0.05$) but not from BxPC-3 cells.

Conclusion:
Although high dose Celecoxib is cytotoxic to pancreatic cancer cells, it stimulates production of VEGF, a growth factor associated with worse prognosis. OSU-03012, a celecoxib derivative, has similar cytotoxicity but at lower, physiologically achievable concentrations with minimal effect on VEGF. Thus OSU-03012 has exciting potential for pancreatic cancer therapy.

P103

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Title: **Management of colorectal liver metastases: a single institution experience**
Authors: D. G. Power, A. Treacy, A. T. Behebehani, G. P. McEntee, J. A. McCaffrey
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Abstract:
Introduction: Metastatic colorectal cancer (mCRC) to liver has been associated with poor outcome. As CRC is a common disease with approximately 25% of patients presenting with liver metastases, and an additional 25% developing liver metastases, management of these patients has been the focus of much research. Recent advances in surgery and chemotherapy has lead to improved survival and changed the way we view advanced CRC.
Methods:Using our hospital database, we identified all new patients with CRC who presented with or developed liver metastases from July 1st 2005-June 30th 2007. All cases were discussed at multi-disciplinary team (MDT) meetings. Resectable patients had upfront liver resections, and unresectable patients were assessed for 'neoadjuvant' chemotherapy and targeted therapy. Response to chemotherapy was evaluated using response evaluation criteria in solid tumours (RECIST) and then liver resection was revisited. All findings at surgery were discussed and concordance with radiology assessed.
Results:185 patients were diagnosed with CRC. 25 patients had liver metastases at diagnosis and 15 patients developed liver metastases. 29 (73%) of these patients were unresectable due to poor performance score, excess number or location of metastases, and presence of extra-hepatic disease. 18 patients with unresectable disease received 'neo-adjuvant' treatment with FOLFOX6 and Bevacizumab. 13 of these patients went for resection with 11 having a partial response (PR) and 2 patients having a complete response (CR) on imaging. 11 patients had 'curative' resections. Discordance between surgery and radiology was seen in 3 cases. Chemotherapy was well tolerated in the majority of cases and there was no significant postoperative morbidity.
Conclusion:Liver is the most common site of colorectal metastases. All patients should be considered for metastectomy. Recent advances in liver surgery, chemotherapy and targeted therapy can render unresectable liver disease resectable, thus providing a chance of cure or longer survival. Our experience in the management of liver metastases from CRC shows that 38% of unresectable liver disease is rendered resectable with modern chemotherapy. MDT involvement is of paramount importance.

P104

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Title: **Analysis of the immunoglobulin heavy chain gene rearrangements in Nodular lymphocyte predominant Hodgkin lymphoma**

Authors: L Venkatraman, M A Catherwood, P Kettle, TCM Morris
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Abstract: **Background and aims**: Analysis of Immunoglobulin gene rearrangements enhances understanding of lymphomagenesis as expression of a functional B-cell receptor is common to many B-cell lymphomas. We analysed the immunoglobulin heavy chain gene (IgH) to determine the gene usage in 10 cases of nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) and 2 cases each of T-cell rich B-cell lymphoma (TCRBCL) and Classical Hodgkin lymphoma (CHL).

Material and methods: Between January 2000 and December 2005, 15 cases diagnosed as NLPHL or TCRBCL were studied. Following histopathology review, laser dissected CD20 stained tumour cells were isolated. Clonality studies and analyses of the IgH gene were done by DNA PCR according to the BIOMED-2 protocol using primers against FR2, FR3 regions of the IgH gene and VH1-6 family specific primers. The sequences were compared with published databases to determine presence and pattern of somatic hypermutation, H-CDR3 structure and gene segment usage.

Results: Histological review yielded 10 cases of NLPHL, 2 cases of TCRBCL, 2 cases of CHL and 1 case of small lymphocytic lymphoma. The latter was excluded from further studies. B-cell clonality was established in all cases; functional IgH gene rearrangements were obtained in 7/10 NLPHL, 2/2 TCRBCL and 2/2 CHL. The VH gene segments used in the productive rearrangements in NLPHL were VH 3-23 and VH4-34 in 2 cases each; VH3-33, VH4-61, VH4-30 were each used once. The mutation frequency varied from 3-23% (6-30 nucleotide substitutions) with an average of 11%. The replacement to silent mutation ratio (R/S) ranged from 1.4-6. Five of the ten NLPHL had R/S ratio of 1.3-1.6 implying antigen selection while in the other 2 NLPHL the R/S was large indicating escape from antigen selection. A subset of 4/10 NLPHL had identical H-CDR3 length. 3 NLPHL had considerable homology in H-CDR3 amino acid composition and 2 of these had identical VH, DH, JH gene usage and D-region reading frames.
The VH, DH, JH gene usage was unbiased in TCRBCL and CHL The TCRBCL and CHL samples also displayed somatic hypermutation with high R/S ratios and H-CDR3 lengths of 16-20 amino acids. The CHL samples used identical VH, DH, JH gene segments and had similarity in CDR3 composition but used different D-region reading frames.

Conclusions: NLPHL, TCRBCL and CHL are somatically hypermutated and display unbiased gene usage. The antigen selection mechanism is heterogeneous in NLPHL. Up to 40% NLPHL have identity of H-CDR3 lengths implying similarity in antigen binding structure. Subsets of NLPHL and CHL have identical gene usage and H-CDR3 composition indicating these two diseases are related.

P105

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Cell cycle-dependence of the activation of DNA damage responses by the chemotherapeutic drug, cisplatin in human cell lines

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Cisplatin is a widely-used cancer chemotherapeutic agent which acts primarily by induction of DNA damage, leading to inhibition of DNA replication, and cell death. In human cells, replication arrest leads to activation of the phosphatidylinositol-3-kinase-related protein kinases (PIKKs), ATM, ATR, DNA-PK, which in turn phosphorylates a number of downstream protein substrates. One such substrate is replication protein A (RPA), a heterotrimeric single-stranded DNA binding protein that is required for DNA replication, repair and homologous recombination. RPA is hyperphosphorylated on RPA2, the 34 kDa subunit, following exposure of cells to a variety of DNA damaging agents including UV light, ionising radiation and cisplatin. To understand the relationship between DNA replication arrest, activation of cell cycle checkpoints and RPA2 phosphorylation, we have investigated the effect of cell cycle phase at the time of cisplatin exposure on activation of PIKK-dependent DNA damage responses. As a model system, experiments have been carried out in DNA polymerase η (polη)-deficient XP30RO cells. XP30RO cells have a mutation in the *POLH* gene encoding polη, which normally carries out translesion synthesis during replication of DNA containing UV or cisplatin-induced DNA damage. As a result XP30RO cells are defective in replication of UV and cisplatin-damaged DNA. To obtain cells in specific cell cycle stages at the time of cisplatin exposure, XP30RO cells were treated with the microtubule inhibitor nocodazole (0.3μM), and cells in G2/M phase were collected by mitotic shake-off. Following removal of nocodazole, cells were released into the cell cycle for either eight hours to generate cells in G1 phase, or for thirteen hours to generate cells in S phase. Cells in G1 or S phase, respectively, were treated with cisplatin (5μg/ml). Cisplatin did not induce G1 arrest but induced a strong S-phase delay in XP30RO cells, consistent with a role for polη in replication of cisplatin-damaged DNA, as determined by flow cytometric analysis of cell cycle progression. The timing of PIKK-dependent phosphorylation of chk1 and RPA2 was determined by western blotting using a series of phosphospecific antibodies. After DNA damage, chk1 is phosphorylated on serine 317 by ATR, while RPA2 is phosphorylated on serines 4/8 in a DNA-PK-dependent manner, and on serine 33 by ATR. Cisplatin-induced phosphorylation of chk1 on serine 317 occurs within 8h of exposure of cells in G1 phase. In contrast, the peak of cisplatin-induced RPA2 phosphorylation on serine 4/serine 8 and serine 33 occurred 15h after exposure of cells in G1 phase. When cells in S phase cells were exposed to cisplatin, RPA2 phosphorylation on serine 4/8 was delayed to 24h. Thus, the timing of RPA2 phosphorylation differs depending on whether cells are in G1 or S phase at the time of cisplatin treatment. Comparison of the kinetics of cisplatin-induced RPA2 hyperphosphorylation on serine4/8 with cell cycle progression as determined by flow cytometry, indicates that this PIKK-dependent event correlates with resumption of DNA replication in polη-deficient cells. Further investigation of the relationship between the timing of cisplatin-induced cell cycle arrest and the activation of PIKK-dependent phosphorylation of specific proteins, will provide insights into the precise molecular events that determine the outcome of drug exposure in individual cells.

P106

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Title: **Proteomic analysis of secreted invasive factors in conditioned media of pancreatic cancer cells**

Authors: Naomi Walsh, Norma O'Donovan, Paula Meleady, Michael Henry, Martin Clynes and Paul Dowling
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Abstract:

Conditioned media from clonal populations of the pancreatic cancer cell line, MiaPaCa-2 with differing invasive abilities, Clone #3 (CM#3) high invasion and Clone #8 (CM#8) low invasion, modulated invasion through the *in vitro* Boyden chamber invasion assay. 2D DIGE MALDI-TOF MS analysis of CM#3 and CM#8 identified 41 secreted proteins differentially regulated in our model, 27 proteins down-regulated and 14 proteins up-regulated in the high invasion promoting CM#3. Western blotting analysis validated two down-regulated secreted proteins, and two up-regulated secreted proteins in our model. Functional studies of a cytoskeleton protein and a protein involved in oxidoreducatase activity in the CM by siRNA transfection revealed an important involvement of these secreted proteins in inhibiting and promoting invasion in pancreatic cancer. The analysis of secreted proteins could identify critical targets involved in the invasive progression and could be potentially used for research into circulating serum biomarkers of pancreatic cancer.

P107

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Title: **Lack of association of the heparanase gene single nucleotide polymorphism Arg307Lys with acute lymphoblastic leukaemia in patients from Northern Ireland**
Authors: PC Winter, MF McMullin and MA Catherwood
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Abstract: Heparanase is an endoglycosidase that cleaves the heparan sulphate component of the extracellular matrix (ECM). Expression of heparanase activity is associated with normal and pathological processes that involve degradation and remodelling of the ECM including wound healing, inflammation, neovascularisation, and tumour metastasis. Heparanase is widely expressed in solid tumours where high levels of expression are correlated with advanced tumour progression and reduced post-operative survival of cancer patients. Expression of heparanase also occurs in blasts of human leukaemias however its involvement in haematological malignancies has not been systematically assessed. Ostrovsky *et al*.(1) have recently shown that a single nucleotide polymorphism (SNP) of the heparanase gene (rs11099592) that causes the substitution Arg307Lys was significantly associated with ALL. They reported that genotype and allele frequencies of the SNP in a group of 43 Israeli ALL patients were significantly different from a group of 103 healthy controls (χ^2_2 =6.384, p=0.041; for genotype comparison and χ^2_1 =4.96, p= 0.026 for allele comparison). We sought to confirm this association by comparing the genotype and allele frequencies of the heparanase Arg307Lys SNP in a group of 58 Northern Irish ALL patients and a group of 45 healthy controls. In contrast with the findings of Ostrovosky *et al.* (1), we found no significant differences in the genotype and allele frequencies for the Arg307Lys SNP in our population (χ^2_2 = 2.866 p= 0.260 for genotype comparison and χ^2_1 = 2.629, p= 0.105 for allele comparison). Furthermore, a comparison of our data with those reported by Ostrovosky *et al*. revealed substantial differences in genotype and allele frequencies between the Northern Irish and Israeli ALL and control populations.
The lack of concordance between our findings and those reported by Ostrovosky *et al*. may be related to the heterogeneity of the Israeli population studied and ethnic variations in the prevalence of the Arg307Lys SNP. Due to the relative genetic homogeneity of the Irish population, the influence of genetic factors predisposing to complex conditions such as ALL may be more apparent in our population.

1. Ostrovsky O, Korostishevsky M, Levite I, Leiba M, Galski H, Vlodavsky I et al. Association of heparanase gene (HPSE) single nucleotide polymorphisms with hematological malignancies. Leukemia 2007; **21(11)**: 2296-303.

P108

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Title: Gene Expression Profiling to Identify Gene Signatures within the MDS Subgroups
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Abstract:
Myelodysplastic syndrome (MDS) is a set of haematopoietic stem cell disorders characterised by ineffective haematopoiesis. Patients with MDS fall into one of two groups; those with increased apoptosis of bone marrow progenitors, and those with progressive blast proliferation and transformation to acute myeloid leukaemia (AML). Currently the only known cytogenetic lesion in MDS is the 5q syndrome, otherwise classification of MDS is based on clinical and morphological criteria.

An international multicentre gene expression profiling consortium, the Microarray Innovations in Leukaemia (MILE) study, profiled approximately 2900 leukaemia samples from patients within 18 subclasses. These were compared with the original diagnosis as defined by the current "Gold Standard" tests. Within the study, 175 MDS samples were included. Gene expression classification of the MDS samples showed ~50% had a "true" MDS signature, whilst ~26% were called as MDS with a "non-leukaemia-like" signature and ~24% called as MDS with an "AML-like" signature. Survival data for MDS patients (n=122) indicated that those patients with a call of "MDS-like AML" had a shorter survival than those called as "MDS" or "MDS like non-leukaemia".

Data from the MILE study indicated that further molecular definition of MDS was required, leading to the development of the Microarray Innovations in Myelodysplastic Syndromes (MIMS) study. AML and high-risk MDS samples will be profiled to determine the diagnostic call of the samples and identify lists of significant genes between 5 subgroups of MDS; Normal bone marrow, MDS with normal signature, MDS, MDS with an AML-like signature, and *de novo* AML. This study will give an insight into the relationship between different molecular events within the context of a clinical trial and may have the potential to identify novel genes or pathways as therapeutic targets.

P109

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Title: Virtual Breast Cancer Biology
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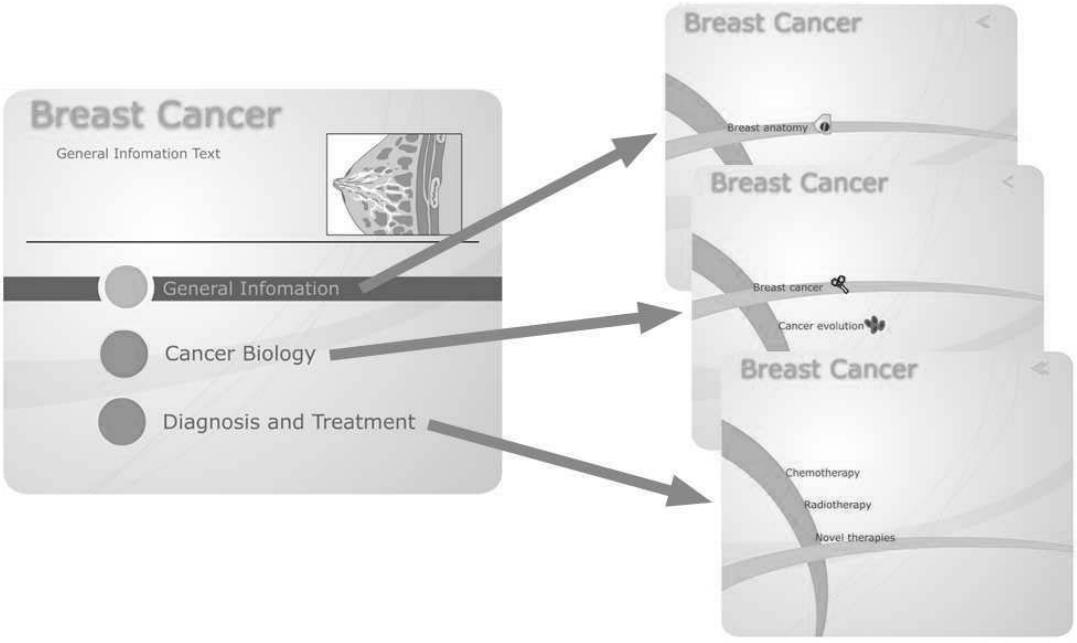
Institution: Computing Resources for Research Group
Abstract:

Multimedia represents a set of powerful tools for dissemination of data, in the context of teaching, public awareness education, and interpretation of research results. The Computing for Research Resources group at UCC is involved in the development of data visualisation systems for life science research. Building on the groups' skills in MM, IT & Learning and Cancer Research, we develop sophisticated multimedia content for researchers and practitioners in the life sciences. We have recently built a suite of animations on Breast Cancer aimed at patients, clinicians and researchers.

The application portrays breast cancer under three distinct areas, that of General Information, Diagnosis and Treatment, and Cancer Biology.



P110

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Title: Role of TNF-α in the development of colitis-associated cancer
Authors: Violeta Zaric and Laurence Egan
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Abstract:
Ulcerative colitis, a chronic inflammatory state, is associated with an increased risk for colorectal cancer. Proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1-beta (IL-1-β) and interleukin-6 (IL-6) have been shown to be over-expressed in the colonic mucosa of patients with ulcerative colitis. Recently a study indirectly supported the implication of TNF-α in the development of cancer through the anti-apoptotic nuclear factor-κB (NF-κB) pathway (Greten et al, 2004). TNF-α is becoming an increasingly important therapeutic target in colitis patients, so deeper understanding of the role of this cytokine in the pathogenesis of cancer is clinically very important. In relation to these studies, we are assessing whether TNF-α plays a major role for the development of colitis-induced cancer in a mouse model of inflammation-related colon carcinogenesis.
TNFα (-/-) (n=12) and wild type mice (n=12) were injected intraperitoneally with 12.5 mg/kg of the procarcinogen azoxymethane and exposed 5 days later to 3 cycles of dextran sulfate sodium (DSS), with each cycle consisting of 2% DSS for 5 days followed by water for 14 days. In TNFα (-/-) mice the body weight decrease to 80% of baseline value was measured, compared to 93% in wild type mice. Polyp number and size in the colon will be assessed macroscopically after staining with 0.2% of methylene blue. Histological examination of the adenoma (polyps) and the severity of colitis will be evaluated on haematoxylin and eosin stained colon sections. Gene expression of pro-inflammatory cytokines for IL-6, IL-1-β and TNF-α, CXCL1 and matrix metalloproteinase-9 will be assessed by real-time PCR. NF-κB expression and apoptosis will be analyzed using immunohistochemistry and by western blots. These results will be available at the time of the meeting. This study could potentially elucidate the involvement of TNFα in the development of tumours in an inflamed colon.

Reference:

Greten, F. R., L. Eckmann, et al. (2004). "IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer." *Cell* 118(3): 285-96.

P111

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Title: Evaluation of the therapeutic potential of pro-apoptotic pyrrolo-1,5-benzoxazepine (PBOX) compounds in the treatment of P-glycoprotein-associated multi-drug resistant (MDR) cancer.

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Abstract: Expression of the drug-efflux transporters, P-glycoprotein and breast cancer resistant protein (BCRP), are a major contributing factor to the development of multi-drug resistant (MDR) cancers. This phenotype renders cells resistant to many clinically used anti-cancer agents. We have recently developed a novel series of microtubule depolymerising, pro-apoptotic pyrrolo-1,5-benzoxazepine (PBOX) compounds. A number of members of this novel set of PBOX compounds have been proposed as potential new chemotherapeutic agents, due to their success in inducing apoptosis in a variety of human chemotherapy resistant cancerous cells *in vitro*, in *ex vivo* patient samples and in an *in vivo* mouse model of breast cancer. The purpose of this study was to investigate if representative PBOX compounds, PBOX-6, -15 and -16, which have potential as anti-cancer agents, were effective in treating P-glycoprotein-expressing and BCRP-expressing MDR cancer cells.

The human acute promyelocytic leukemia cell line HL60 along with adriamycin-selected sub-lines HL60-MDR (expressing p-glycoprotein) and HL60-ABCG2 (expressing BCRP) were treated with either PBOX-6, PBOX-15, PBOX-16, other microtubule-targeting agents (MTAs) paclitaxel or vincristine, topoisomerase inhibitor mitoxantrone or DNA-intercalating anthracycline antibiotic adriamycin. General cell survival, arrest in the G2/M phase of the cell cycle and apoptosis were evaluated in each cell line in the presence or absence of the P-glycoprotein inhibitor verapamil or the BCRP inhibitor fumitremorgin c.

We established that while p-glycoprotein-expressing HL60-MDR cells displayed resistance to common microtubule-targeting drugs paclitaxel and vincristine, they did not exhibit resistance to PBOX-6, -15 or -16. Inhibition of p-glycoprotein by pre-treatment with verapamil, appeared to reverse this resistance to paclitaxel and vincristine. Likewise, BCRP-expressing HL60-ABCG2 cells displayed no resistance to the PBOX compounds. In agreement with these findings we also established that a human ovarian cancer cell line, A2780, and its p-glycoprotein-expressing, adriamycin-resistant sub-line A2780-ADR were equally sensitive to PBOX compounds.

These results suggest that while the efficacy of other common chemotherapeutic agents appear to be affected by the expression of P-glycoprotein or BCRP, the efficacy of PBOX-6, -15 and -16 do not. This indicates that pro-apoptotic PBOX compounds may have the potential to treat p-glycoprotein- and BCRP-associated MDR cancers.

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P112

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Title: STI-571 (imatinib mesylate) enhances the apoptotic efficacy of pyrrolo-1,5-benzoxazepine-6, a novel microtubule-targeting agent, in both STI-571-sensitive and -resistant Bcr-Abl-positive human chronic myeloid leukemia cells.

Authors: Greene L.M., Kelly, L., Onnis, V., Campiani, G., Lawler, M., Williams, D.C. & Zisterer D.M.
Institution: School of Biochemistry and Immunology, Trinity College Dublin.

Abstract: We are developing a novel series of microtubule targeting agents, pyrrolobenzoxazepine compounds (PBOXs), as potential anti-cancer therapeutics. Interactions between the Bcr-Abl kinase inhibitor STI-571 (Gleevec) and a representative PBOX compound, PBOX-6, were investigated in STI-571-sensitive and –resistant human chronic myeloid leukemia (CML) cells. Co-treatment of PBOX-6 with STI-571 induced significantly more apoptosis in Bcr-Abl^{+ve} CML cell lines than either drug alone. STI-571 significantly reduced PBOX-6-induced G_2M arrest with a concomitant increase in apoptosis. However, enhanced levels of apoptosis by STI-571 and PBOX-6 was not observed in leukemia cells that do not express the Bcr-Abl oncoprotein (HL-60), or normal peripheral blood mononuclear cells. Co-exposure to STI-571 also enhanced the levels of PBOX-6-induced apoptosis in STI-571-resistant K562 cells expressing increased levels of the Bcr-Abl protein. STI-571 inhibited PBOX-6-induced endoreplication following microtubule disruption in the CML cells. In addition, the combined treatment of STI-571 and PBOX-6 greatly reduced the expression of Bcr-Abl, Mcl-1 and Bcl-x_L, but not Bcl-2, as compared to either drug alone. Together, these findings indicate that co-treatment of STI-571 and PBOX-6 may represent an effective anti-leukemic therapy for the treatment of both STI-571-sensitive and –resistant CML cells.

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