# Irish Association for Cancer Research

## Annual Meeting

**1-2 March 2012**

## Culloden Hotel, Belfast

***Incorporating:***

***Prostate Cancer Symposium: 29 February 2012***

MEN AGAINST CANCER

irish cancer society

# IACR COMMITTEE MEMBERS 2012

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**Jacintha O'Sullivan**

**Corrado Santocanale**

**Leonie Young**

# Contents

# Irish Association for Cancer Research

## Annual Meeting - 1-2 March 2012

### Culloden Hotel, Belfast

Dear Colleagues,

It is a great pleasure to extend a warm welcome to our 2012 Annual Meeting. As always, the Annual Meeting provides us with an opportunity to meet up with friends and to learn of research being conducted within and beyond our shores. The Council of the IACR is very grateful to all of our speakers for accepting our invitation and appreciative of the time they have taken from their busy schedules to attend this meeting.

The Annual Meeting is the focal point of the Association's activities and despite the significant challenges that the academic research environment is experiencing, it is encouraging that there has been such an enthusiastic response and an increase in the number of delegates attending the meeting. We are also very appreciative of our many sponsors. This meeting would not be possible without your generous financial assistance. As frontline researchers, we are keenly aware of how the downturn in funding is impacting on everyone within the sector, including the laboratory support sector. We are deeply indebted to your continued support of this meeting and trust that as we work in partnership now in this difficult time, hopefully brighter times are not too far off for us all. I strongly encourage all of our delegates to take the opportunity to visit all of the trade stands and discuss their research needs with each of our sponsors.

In particular, the IACR Council would like to extend our sincere thanks to the Irish Cancer Society who year-on year, serve as not only a generous sponsor of the meeting but are a tremendous partner of the IACR and its entire membership. As one exemplar of their commitment to young researchers-in-training, the Irish Cancer Society increased their financial support of the meeting in order to permit us to significantly reduce the registration fees for all PhD students attending this meeting. Allied with their financial support of several fellowship programmes and their response-mode grants, the Irish Cancer Society have established not only a track record of advocacy for research, but have provided considerable financial support to build the Irish cancer research community. John McCormick and his team at the Irish Cancer Society deserve our sincere and grateful thanks, and we look forward to hearing more about their plans to support the research environment under the leadership of John Fitzpatrick.

Finally, this is your association and I would encourage all of our members to attend the Annual General Meeting so that you can hear and influence how the Council intends to take the Association forward over the subsequent years.

With best wishes,

Sincerely,

*Elaine Kay*

Prof. Elaine Kay
President

*David Waugh*

Dr. David Waugh
Hon. Secretary

*Sharon McKenna*

Dr. Sharon McKenna
Hon. Treasurer

# PROSTATE CANCER SYMPOSIUM
## February 29th 2012, Culloden Hotel, Cultra, Northern Ireland
## PROGRAMME

| | |
|---|---|
| **09.00-09.45** | **Registration – Tea/Coffee – Networking** |
| **09.45-10.00** | **Welcome** |
| **10.00-12.15** | **Session I: The Challenges with Prostate Cancer: Detecting Disease Early and Informing Treatment Choice** |
| | **Chairperson: Prof John Fitzpatrick, Director of Research, Irish Cancer Society** |
| 10.00-10.30 | ***MECHANISMS OF PROGRESSION TO CASTRATE RESISTANT PROSTATE CANCER***<br>**Prof Colleen Nelson, Professor and Chair, Prostate Cancer Research, Institute of Health and Biomedical Innovation, Queensland University of Technology, Australia** |
| 10.30-11.00 | ***METABOLIC ALTERATIONS IN PROSTATE CANCER***<br>**Prof Massimo Loda, Harvard Medical School, Boston** |
| 11.00-11.30 | ***BIOMARKER DISCOVERY AND VALIDATION FOR PROSTATE CANCER: A PROGRAMME OF THE PROSTATE CANCER RESEARCH CONSORTIUM ADDRESSING THE CLINICAL DILEMMA***<br>**Prof William Watson, Associate Professor of Cancer Biology, UCD School of Medicine and Medical Science, Conway Institute, University College Dublin** |
| 11.30-12.00 | ***IDENTIFICATION OF A PROGNOSTIC MOLECULAR SUBTYPE IN PROSTATE CANCER***<br>**Prof Richard Kennedy, McClay Professor of Experimental Cancer Medicine, CCRCB, Queen's University Belfast** |
| 12.00-12.15 | ***'MEN AGAINST CANCER - PAST AND FUTURE CANCER RESEARCH FUNDING'***<br>***Mr Eric Cairns, Mr Patrick Keane & Prof Joe O'Sullivan*** |
| 12.15-13.30 | **LUNCH/POSTERS Detailing All-Ireland Prostate Cancer Research Programmes** |
| **13.30-16.00** | **Session II: New Options for Treating Castrate-Resistant Disease** |
| | **Chairperson: Dr David Gallagher, Mater Hospital, Dublin** |
| 13.30-14.00 | ***ABIRATERONE ACETATE: THE BEGINNING OF A NEW ERA IN THE TREATMENT OF CASTRATION RESISTANT PROSTATE CANCER***<br>**Dr Diletta Bianchini MD, The Institute of Cancer Research and the Royal Marsden NHS Foundation Trust, London UK** |
| 14.00-14.30 | ***EARLY EVALUATION OF RADIONUCLIDE THERAPY IN CASTRATE-RESISTANT PATIENTS***<br>***Prof Joe O'Sullivan, CCRCB, Queen's University Belfast*** |
| 14.30-15.00 | ***CYTOTOXIC THERAPY IN ADVANCED PROSTATE CANCER – DO WE REALLY NEED ANOTHER PARADIGM SHIFT?***<br>***Prof Rob Jones, CRUK Beatson Institute, Glasgow*** |
| 15.00-15.30 | ***RADIOTHERAPY FOR PROSTATE CANCER: WHERE TO NEXT?***<br>**Prof David Dearnaley, Royal Marsden, London** |
| 15.45-16.00 | ***PANEL DISCUSSION*** |
| 16.00-16.30 | ***TEA/COFFEE*** |

# PROSTATE CANCER SYMPOSIUM
## February 29th 2012, Culloden Hotel, Cultra, Northern Ireland
## PROGRAMME

| | |
|---|---|
| **16.30-19.00** | **Session III: The Underlying Tumour Biology and Novel Target Discovery for Prostate Cancer Treatment** |
| | **Chairperson: Dr William Watson, Conway Institute, UCD** |
| 16.30-17.00 | ***IN VIVO MODELING OF PROSTATE ADENOCARCINOMA TUMOR INITIATING CELLS***<br>**Prof Kathleen Kelly**, Branch Chief, Cell and Cancer Biology Branch, NCI, NIH, Bethesda MD, USA |
| 17.00-17.30 | ***CYTOKINE SIGNALING IMBALANCE IN PROSTATE CANCER PROGRESSION***<br>**Prof Zoran Culig, Medical University, Innsbruck** |
| 17.30-18.00 | ***CHEMOKINE SIGNALLING IN THEPROSTATE MICROENVIRONMENT***<br>**Dr David Waugh, CCRCB, Queen's University Belfast** |
| | **Chairperson: Dr David Waugh, CCRCB,**<br>**Proffered Papers from the Irish Prostate Research Strategy Group** |
| 18.00-18.15 | **EXOSOMES - POTENTIAL MEDIATORS OF PROSTATE CANCER PROGRESSION?**<br>**C Corcoran[1]**, S Rani[1], A O'Brien[1], A O'Neill[2], K O'Brien[1], M Prencipe[2], J Crown[3], W Watson[2], L O'Driscoll[1, 1]School of Pharmacy & Pharmaceutical Sciences, Trinity College Dublin, Dublin 2, Dublin [2]UCD School of Medicine and Medical Science, University College Dublin, Dublin 4, Dublin [3]Molecular Therapeutics for Cancer Ireland, (MTCI) , St. Vincent's University Hospital, Dublin 4, Dublin |
| 18.15-18.30 | **PARAMETRIC DIFFUSION WEIGHTED IMAGING AT 3 TESLA PREDICTS - HISTOLOGICAL OUTCOMES IN MEN PRESENTING FOR A PROSTATE BIOPSY**<br>**DC Moran[1]**, L Marignol[1], A Perry[1], JF Meaney[2], TH Lynch[3], DH Hollywood[4]<br>[1]Radiation Therapy, Trinity College Dublin, Dublin, [2]Radiology, St James Hospital, Dublin, [3]Urology, St James Hospital, Dublin, [4]Radiation Oncology, Trinity College Dublin, Dublin |
| 18.30-18.45 | **IDENTIFICATION OF SERUM RESPONSIVE FACTOR (SRF) AS A POTENTIAL THERAPEUTIC TARGET FOR CASTRATION-RESISTANT PROSTATE CANCER**<br>**M Prencipe[1]**, S Madden[2], AJ O'Neill[1], A Culhane[3], D O'Connor[4], H Klocker[5], WM Gallagher[4], RWG Watson[1, 1]UCD School of Medicine and Medical Science, Conway Institute , University College Dublin, [2]National Institute for Cellular Biotechnology, Dublin City University, Dublin, [3]Department of Biostatistics, Dana-Farber Cancer Institute, Boston, Massachusetts, US [4]UCD School of Biomolecular and Biomedcial Sciences, Conway Institute , University College Dublin, Dublin, [5]Department of Urology, Innsbruck Medical University, Innsbruck, Austria |
| 18.45-19.00 | **GENE-DIRECTED ENZYME PRODRUG THERAPY IN A SPHEROID MODEL OF PROSTATE CANCER**<br>**A Vajda[1]**, A Perry[1], R Foley[1], AM Davies[2], D Hollywood[1, 3], L Marignol[1]<br>[1]Prostate Molecular Oncology, Trinity College , Dublin, [2]High Content Research Facility, Trinity College , Dublin, [3]Academic Unit of Clinical and Molecular Oncology, Trinity College , Dublin |
| 19.15-20.00 | **Drinks Reception followed by Speakers Dinner (By Invitation Only)** |

# IRISH ASSOCIATION FOR CANCER RESEARCH

## *2012 IACR ANNUAL MEETING*

## THURSDAY MARCH 1st

**08.45** **Welcome Address – Professor Elaine Kay, President IACR**

**9.00am–10.30am** **PROFFERED PAPERS 1 SESSION**
**(6 x 15 minute talks)** **Chairpersons: Dr David Waugh & Dr Sharon McKenna**

09.00-09.15 **MODULATION OF CD44 SUB-CELLULAR LOCALIZATION DECREASES BREAST CANCER CELL MOTILITY**
**Irina S Babina**, AM Hopkins, [1]Surgery, Royal College of Surgeons in Ireland, Dublin

09.15-09.30 **INVESTIGATING THE ROLE OF HUMAN CDC7 KINASE AND CLASPIN IN THE DNA REPLICATION CHECKPOINT**
**Michael D Rainey[1]**, B Harhen[1], G O'Brien[1], C Santocanale[1], [1]Centre for Chromosome Biology and National Centre for Biomedical Engineering Science, National University of Ireland Galway, Galway

09.30-09.45 **IDENTIFICATION OF THE TRANSCRIPTIONAL COMPLEX THROUGH WHICH TBX2 DRIVES THE PROLIFERATION OF BREAST CANCER CELLS**
**NT Crawford**, N Dickson, KL Redmond, PB Mullan, [1]CCRCB, QUB, Belfast

09.45-10.00 **PROTEIN TYROSINE PHOSPHATASE RECEPTOR DELTA ACTS AS A NEUROBLASTOMA TUMOUR SUPPRESSOR BY DEPHOSPHORYLATING AND DESTABILISING THE AURORA KINASE A ONCOGENE**
**MH Meehan[1, 2]**, L Parthasarathi[1, 2], N Moran[1], CA Jefferies[1], N Foley[1, 2], E Lazzari[1], D Murphy[3], J Ryan[1, 2], B Oritz[4], A Fabius[4], TA Chan[4], RL Stallings[1, 2]
[1]Department of Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin 2, [2]National Children's Research Centre, Our Lady's Children's Hospital, Dublin 12, [3]Centre for Human Proteomics, Royal College of Surgeons in Ireland, Dublin 2, [4]Department of Radiation Oncology, Memorial Sloan-Kettering Cancer Center, New York, USA

10.00-10.15 **DNA MEDIATED CHROMATIN PULL-DOWN: A NOVEL METHOD FOR THE ANALYSIS OF NEWLY REPLICATED CHROMATIN**
**A E Kliszczak[1]**, M D Rainey[1], B Harhen[1], C Santocanale[1], [1]Centre for Chromosome Biology and National Centre for Biomedical Engineering Science, National University of Ireland Galway

10.15-10.30 **MIR-204 IS SIGNIFICANTLY ASSOCIATED WITH SURVIVAL IN NEUROBLASTOMA PATIENTS AND INCREASES SENSITIVITY OF NEUROBLASTOMA CELLS TO CHEMOTHERAPY IN VITRO THROUGH REGULATION OF TRKB AND BCL2.**
**JM Ryan[1]**, L Creevey[1], J Fay[1], AM Tivnan[1], I Bray[1], M Meehan[1], K Bryan[1], A Davidoff[2], L Tracey[2], R Stallings[1], [1]Cancer Genetics, MCT, Royal College of Surgeons and National Children's Research Centre, Dublin, [2]Surgery, St. Jude Children's Research Hospital, Memphis, TN, USA

**10.30-11.00** **Tea/Coffee/Posters & Meet the Sponsors**

# IRISH ASSOCIATION FOR CANCER RESEARCH

## *2012 IACR ANNUAL MEETING*

## THURSDAY MARCH 1st

**11.00-12.30** **PLENARY SESSION 1: Chromatin Biology and Regulation of Senescence**
**Chairpersons: Dr Corrado Santocanale & Dr Amanda McCann**

11.00-11.30 ***CELLULAR SENESCENCE IN CANCER, DEVELOPMENT AND REPROGRAMMING***
Prof. Manuel Collado, Spanish National Cancer Research Center CNIO, Spain

11.30-12.00 ***MOLECULAR MECHANISMS OF CELLULAR SENESCENCE***
Prof. Fabrizio d'Adda di Fagagna, Principal Investigator, IFOM Foundation - The FIRC Institute of Molecular Oncology Foundation, Milan, Italy

12.00-12.30 ***TRANSCRIPTIONAL MECHANISM AND PHARMACOLOGICAL INHIBITION OF PARACRINE TUMOR-PROMOTING ACTIVITIES OF CHEMOTHERAPY-DAMAGED AND SENESCENT CELLS***
Prof. Igor B. Roninson[1,2], Donald C. Porter[2], Eugenia V. Broude[1], and Hippokratis Kiaris[3]
[1]Department of Pharmaceutical and Biomedical Sciences, South Carolina College of Pharmacy, University of South Carolina, Columbia, SC, USA, [2]Senex Biotechnology, Inc., Columbia, SC, USA, [3]University of Athens Medical School, Athens, Greece

12.30-13.30 LUNCH (Poster Viewing)

**13.30-14.00** ****Technology Presentation from Sponsors****

**14.00-15.30** **PLENARY SESSION 2: Regulation of Cell Adhesion**
**Chairpersons: Dr Ann Hopkins & Dr Jacintha O'Sullivan**

14.00-14.30 ***SIGNALLING AT TIGHT JUNCTIONS IN EPITHELIAL PROLIFERATION, DIFFERENTIATION AND REPAIR***
Prof. Karl Matter, Department of Cell Biology, UCL Institute of Ophthalmology, University College London, London, UK

14.30-15.00 ***AN IN VIVO FUNCTIONAL SCREEN TO IDENTIFY NOVEL BREAST CANCER METASTASIS SUPPRESSOR GENES***
Prof. Clare Isacke, Interim Director, The Breakthrough Toby Robins Breast Cancer Research Centre, The Institute of Cancer Research, London

15.00-15.30 ***INTEGRINS AND POLARITY IN BREAST EPITHELIA***
Prof. Charles Streuli, Professor of Cell Biology, Wellcome Trust Centre for Cell-Matrix Research, University of Manchester, UK

**15.30-16.00** **Tea/Coffee/Posters & Meet the Sponsors**

**16.00-17.05** **ORAL POSTER PRESENTATIONS (9 x 7 minute presentations)**
**Chairperson: *Prof. John Fitzpatrick, Head of Research, Irish Cancer Society***

16.00-16.07 **FKBPL: SECRETION AND REGULATION OF ANGIOGENESIS**
**A Yakkundi[1]**, A Valentine[1], MG O'Rourke[1], J Worthington[3], H McKeen[1], H Dyer[1], L McClements[1], G Cotton[2], T Harrison[2], I James[2], T Robson[1,1]School of Pharmacy, Queen's University , Belfast, [2]Almac Discovery, Almac, Craigavon, UK, [3]Biomedical Sciences, University of Ulster, Coleraine, N. Ireland

# IRISH ASSOCIATION FOR CANCER RESEARCH

## *2012 IACR ANNUAL MEETING*
## THURSDAY MARCH 1st

16.07-16.14 **MIR-31 CONFERS RESISTANCE TO TAMOXIFEN IN BREAST CANCER CELL LINES**
**L Mulrane**[1], R Clarke[2], WM Gallagher[1], D O'Connor[1,] [1]UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Belfield, Dublin 4, [2]Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University School of Medicine, Washington, USA

16.14-16.21 **INVESTIGATION OF ADAM17 AS A NOVEL TARGET IN KRAS MUTANT AND WILD TYPE COLORECTAL CANCER**
**L Stevenson**[1], P Dunne, W Allen, M Kalimutho, C Fenning, P Johnston, S Van Schaeybroeck, [1]CCRCB, Queen's University Belfast, Belfast, N.Ireland

16.21-16.28 **MECHANISMS OF EXPRESSION AND REGULATION OF SOX2 AND ITS TARGETS IN TWO EMBRYONAL CARCINOMA CELL LINES**
**SF Vencken**[1, 2], MF Gallagher[1, 2], CM Martin[1, 2], OM Shiels[1], JJ O'Leary[1, 2,] [1]Department of Histopathology and Morbid Anatomy, Trinity College Dublin, Dublin, Ireland, [2]Molecular Pathology Research Lab, Coombe Women and Infants University Hospital, Dublin

16.28-16.35 **JUNCTIONAL ADHESION MOLECULE-A: A NOVEL REGULATOR OF HER2 PROTEIN DEGRADATION**
**K Brennan**[1], EA McSherry[1, 3], L Hudson[1], EW Kay[2], ADK Hill[1], LS Young[1], AM Hopkins[1] [1]Surgery, Royal College of Surgeons in Ireland, Dublin, [2]Pathology, Royal College of Surgeons in Ireland, Dublin, [3]Ontario Cancer Institute, University of Toronto, Toronto, Canada

16.35-16.42 **AVEN: CELL CYCLE AND APOPTOSIS REGULATION IN MEIOSIS AND MITOSIS**
LOS O'Shea[1], CMG McGarry[1], TF Fair[2], **CH Hensey**[1]
[1]UCD School of Biomolecular and Biomedical Science, University College Dublin, Dublin, [2]UCD School of Agriculture, Food Science and Veterinary Medicine, Dublin

16.42-16.49 **ASSESSMENT OF CARDIOVASCULAR TOXICITIES ASSOCIATED WITH THE RECEPTOR TYROSINE KINASE INHIBITOR SUNITINIB: A MULTI-MODALITY IMAGING (MMI) APPROACH**
**RL Evans**[1], E Conroy[2], L Shiels[1], A O'Connor[2,5], A Zagozdzon[2,5], D Hughes[1], N Corrigan[2], J Rousseau[3], W Gallagher[2,5], F McAuliffe[2], M Cary[4], R Lecomte[3], At Byrne[1,2,] [1]Physiology & Medical Physics, Royal College Of Surgeons In Ireland, Dublin, [2]Biomolecular & Biomedical Research, University College Dublin, [3]Department Of Nuclear Medicine, Universite De Sherbrooke, Sherbrooke, QC, Canada, [4], Pathology Experts Gmbh, Basel, Switzerland, [5] Oncomark Ltd, Dublin

16.49-16.56 **STROMAL AKT2 CONTROLS EPITHELIAL INVASIONS**
**AC Cichon**[1], A Pickard[1], D Patel[1], D McCance[1]
[1]Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, UK

16.56-17.02 **CYCLIN D1, CLUSTERIN, CYTOCHROME P4504F2 AND LIPID PEROXIDATION LEVELS SEGREGATE GOOD AND POOR STAGE II COLORECTAL CANCER TUMOURS AND REGULATE MITOCHONDRIA FUNCTION**
**C Dunne**[1], A Maguire[1], M Biniecka[3], D Kevans[1], M Tosetto[1], J Hyland[1], K Sheahan[1], D O'Donoghue[1], H Mulcahy[1], J O'Sullivan[2,] [1]Centre for Colorectal Disease, St Vincent's University Hopsital, Dublin, Ireland, [2]Department of Surgery, Institute of Molecular Medicine, Trinity Health Centre, Dublin, Ireland, [3]Education and Research Centre, St Vincent's University Hospital, Dublin, Ireland

# IRISH ASSOCIATION FOR CANCER RESEARCH

## *2012 IACR ANNUAL MEETING*
## THURSDAY MARCH 1st

17.10-18.00 **IRISH CANCER SOCIETY LECTURE**

***Introduced by: Prof. John Fitzpatrick, Head of Research, Irish Cancer Society***

***FUTURE FRONTIERS AND CHALLENGES IN MODERN CANCER MEDICINE***
***Prof. Patrick Johnston, CCRCB, Queen's University of Belfast, Belfast, N. Ireland***

18.00-20.00 **POSTERS, TRADE EXHIBITION AND WINE RECEPTION**

**20.00-22.00 Buffet Dinner – Sponsored by Irish Cancer Society**

# IRISH ASSOCIATION FOR CANCER RESEARCH

## *2012 IACR ANNUAL MEETING*

## FRIDAY 2nd MARCH

**08.30 – 10.00** **PLENARY SESSION 3: PRINCIPLES AND APPLICATION OF RADIATION THERAPY**
**Chairpersons: Dr Daniel Longley & Dr Joe O'Sullivan**

08.30-09.00 ***WHAT HAPPENS AFTER TUMOURS ARE IRRADIATED – A CASE OF PHOENIX RISING?***
Prof. Tim Illidge, Professor of Targeted Therapy and Oncology, School of Cancer and Imaging Sciences, University of Manchester

09.00-09.30 ***RADIONUCLIDE THERAPY OF BONE METASTASES: PAST, PRESENT AND FUTURE***
Prof. John de Klerk, Director of the Dept of Nuclear Medicine, Meander Medical Center, Amersfoort, The Netherlands

09.30-10.00 ***DEVELOPMENT OF NEW BIOLOGICAL-BASED MODELS FOR ADVANCED RADIOTHERAPIES***
Prof. Kevin Prise, CCRCB, Queen's University of Belfast, Belfast, Northern Ireland

10.00-10.30 **Tea/Coffee/Posters & Meet the Sponsors**

**PROFFERED PAPERS 2 SESSION (6 x 15 minute presentations)**
**Chairpersons: Dr Patrick Kiely & Dr Sinead Walsh**

10.30-10.45 **P63 BINDING ANALYSIS REVEALS A ROLE FOR AP-2 FAMILY CO-TRANSCRIPTIONAL REGULATION**
**AE Henry[1]**, S McDade[1], D Patel[1], D McCance[1, 1]CCRCB, Queen's University, Belfast, UK

10.45-11.00 **ASSESSING THE INFLUENCE OF EXOSOMES IN TRIPLE-NEGATIVE BREAST CANCER**
**Keith O'Brien[1]**, S Rani[1], S McDonnell [2], L Hughes [2], M Radomski [1], J Crown [3], L O'Driscoll [1]
[1]School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, [2]of Chemical and Bioprocess Engineering, University College Dublin, [3]Molecular Therapeutics for Cancer Ireland (MTCI) & St. Vincent's University, St. Vincent's University Hospital, Dublin

11.00-11.15 **ELUCIDATING MECHANISMS UNDERLYING TBX2 PATHOGENESIS IN BREAST CANCER IN ORDER TO DEVELOP NOVEL TARGETED THERAPIES**
**ZC D'Costa [1]**, C Higgins[1], NE Buckley[1], NT Crawford[1], R Williams[1], PB Mullan[1]
[1]Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, Northern Ireland

11.15-11.30 **SATB2 IS AN INDEPENDENT PREDICTOR OF OUTCOME IN COLORECTAL CANCER**
**K Naicker[1]**, DJ Brennan[1], R Klinger[1], F Ponten[2], D O'Donoghue[3], J O'Sullivan[4], K Jirstrom[5], WM Gallagher[1], DP O'Connor[1, 1]UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, [2]Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden, [3]Department of Gastroenterology, St. Vincent's University Hospital, Dublin, [4]Department of Surgery, Institute of Molecular Medicine, Trinity College Dublin, Dublin, [5]Department of Clinical Sciences, Division of Pathology, Lund University, Malmo, Sweden

# IRISH ASSOCIATION FOR CANCER RESEARCH

## *2012 IACR ANNUAL MEETING*

## FRIDAY 2nd MARCH

11.30-11.45 **MARKERS OF OXIDATIVE DAMAGE AND CIRCULATING IL6 LEVELS CORRELATE WITH SURVIVAL FOLLOWING TREATMENT WITH BEVACIZUMAB IN METASTATIC COLORECTAL CANCER PATIENTS**

**S Noonan[1]**, P Martin[1], A Maguire[1], M Binieka[1], M Tosetto[1], J Hyland[1], K Sheahan[1], D O'Donoghue[1], H Mulcahy[1], D Fennelly[2], J O'Sullivan, [1]Colorectal Centre for Disease, University College Dublin, Dublin , [2]Institute for Molecular Medicine, Trinity Centre for Health Sciences, Dublin

11.45-12.00 **FKBPL: AN IMMUNOPHILIN-LIKE PROTEIN WITH POTENTIAL AS A BREAST CANCER BIOMARKER**

**HD McKeen**, C Byrne, C Donley, A Yakkundi, H McCarthy, T Robson, [1]School of Pharmacy, Queen's University, Belfast, Northern Ireland

**12.00-12.50** **EACR YOUNG SCIENTIST AWARDS (2 x 25 Minute Presentations)**

12.00-12.25 **GLOBAL CHARACTERIZATION OF THE SRC-1 TRANSCRIPTOME INDENTIFIES ADAM22 AS AN ER-INDEPENDENT MEDIATOR OF ENDOCRINE RESISTANT BREAST CANCER.**

**C Byrne[1]**, D McCartan[1], JC Bolger[1], A Fagan[2], Y Hao[2], L Qin[3], M McIlroy[1], J Xu[3], AD Hill[1], P O'Gaora[2], LS Young[1,] [1]Surgery, Royal College of Surgeons in Ireland, Dublin, Ireland
[2]Medicine and Medical Science, Conway Institute, UCD, Dublin, Ireland
[3]Molecular and Cellular Biology and Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, USA

12.25-12.50 **BRCA1 – Master Regulator Of Mammary Cell Differentiation**

**N Buckley[1]**, C Nic An tSaoir[1], L Oram[1], N Crawford[1], P Mullan[1]
[1]Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, Northern Ireland

**12.50-14.00** **LUNCH**

**14.00-15.45** **PLENARY SESSION 4: REGULATION OF STEROID AND HORMONE SIGNALLING**

**Chairpersons: Dr Leonie Young & Dr Paul Mullan**

14.00-14.30 ***TUMOUR MICROENVIRONMENT AND BREAST CANCER PROGRESSION IN RELATION TO THE PRESENCE OF OESTROGEN RECEPTOR***

Prof. Göran Landberg MD PhD, Professor Molecular Pathology and Pathology, University of Manchester, UK/ University of Gothenburg, Sweden

14.30-15.00 ***NEW INSIGHTS INTO THE REGULATION OF HISTONE DEACETYLASE COMPLEXES***

Prof. John Schwabe, Professor of Structural Biology, Department of Biochemistry, University of Leicester, UK

15.00-15.30 ***THE ESTROGEN RECEPTOR/CHROMATIN AND EPIGENETIC LANDSCAPE THAT DEFINES AROMATASE INHIBITOR RESISTANCE IN PRIMARY TUMORS***

Prof. Wilbert Zwart, Junior Group Leader, department of Molecular Pathology,
The Netherlands Cancer Institute, Amsterdam

# IRISH ASSOCIATION FOR CANCER RESEARCH

## *2012 IACR ANNUAL MEETING*

## FRIDAY 2nd MARCH

15.30-15.45 **THE ROLE OF THE STEROID RECEPTOR COACTIVATOR, SRC-1 AND ITS FUNCTIONAL PARTNER HOXC11 IN THE DEVELOPMENT OF ENDOCRINE RESISTANCE IN BREAST CANCER**
C Walsh[1], D McCartan[1], **M McIlroy[1]**, ADK Hill[1], J Xu[2], LS Young[1]
[1]Endocrine Oncology Research Group, Royal College of Surgeons in Ireland, Dublin, Ireland,
[2]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas

**15.45-16.15 Tea/Coffee/Posters & Meet the Sponsors**

**16.15-18.15 PLENARY SESSION 5: ANGIOGENESIS & INFLAMMATION**
**Chairpersons: Dr Darran O'Connor & Dr Lorraine O'Driscoll**

16.15-16.45 ***MECHANISMS OF TOXICITY ASSOCIATED WITH CLINICALLY APPROVED ANGIOGENESIS INHIBITORS***
Prof. Annette Byrne, *Lecturer, Physiology & Medical Physics, Royal College of Surgeons in Ireland*

16.45-17.15 ***GENETIC MARKERS OF RESPONSE FOR THE ANTI-ANGIOGENIC AGENT BEVACIZUMAB***
Prof. Diether Lambrechts, VIB Vesalius Research Center, K.U. Leuven

17.15-17.45 ***NOVEL WAYS OF ANGIOGENESIS INHIBITION FOR CANCER THERAPY***
Prof. Arjan W Griffioen and Judy van Beijnum, Angiogenesis Laboratory, Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands

17.45-18.15 ***TARGETING THE TUMOR IMMUNE MICROENVIRONMENT TO OVERCOME CHEMO-RESISTANCE IN BREAST CANCER***
Prof. David DeNardo, Assistant Professor of Medicine and Immunology
Washington University St. Louis, School of Medicine, Saint Louis, MO, United States

**19.30 GALA AND AWARDS DINNER**

**Professor Colleen Nelson**

Professor Colleen Nelson is the founding Executive Director of the Australian Prostate Cancer Research Centre - Queensland and Chair of Prostate Cancer Research at Queensland University of Technology. Prof Nelson is also the founder and Director of the Australian-Canadian Prostate Cancer Research Alliance, an initiative developed to coordinate national and international network interactions of >200 prostate cancer scientists and clinicians in Australia and Canada, facilitating access to state-of-the-art infrastructure and clinical trials to assist in the translation of a wide range of discoveries in both countries.

Prof Nelson's expertise is in translational prostate cancer research, specifically in identification of potential therapeutic targets, their in vitro and in vivo validation, clinical validation through molecular pathology approaches and their translation into potential clinical application. These outcomes are derived from her expertise in high throughput applications in high throughput gene expression, gene regulation, animal models, prostate cancer, steroid hormones, molecular endocrinology and targeted therapeutics. She has long studied androgen action and effects of androgen deprivation and progression to castrate resistant prostate cancer. Her laboratory made the seminal discovery that castrate resistant prostate tumours can synthesize their own androgens de novo from cholesterol. Recently, these findings have been extended to investigate the inter relationships of androgen synthesis, prostate cancer progression, and metabolic syndrome. She has been on a number of Strategic and Scientific Advisory Boards for Biotech, NGOs and for Government. Prof Nelson has also been the inaugural Director of the Microarray Platform for Genome Canada 2000-2009. In 2010 she was selected to Chair the Global Scientific Committee of Movember's Global Action Plan.

She works closely with the Prostate Cancer Foundation of Australia and has been a co-organiser of their international conferences and assists in their fund raising activities. She is passionate about prostate cancer advocacy and regularly disseminates information to men's and prostate cancer support groups, travelling to regional of Queensland for prostate cancer education to the community. She has been a driver in helping establish the first Multi-Disciplinary Team Clinic for Advanced Prostate Cancer in Australia the linked MDT Prostate Cancer Trials unit.

**Massimo F. Loda, MD**

Prof. Loda received his M.D. summa cum laude at the University of Milan, Italy. He is a physician-scientist, Professor of Pathology at Harvard Medical School, an independent investigator at Dana Farber Cancer Institute, Associate Member of the Broad Institute and senior surgical pathologist at the Brigham & Women's Hospital in Boston. Dr. Loda conceived, built and directs a state of the art Center for Molecular Oncologic Pathology at the Dana Faber Cancer Institute. Dr. Loda is one of the pioneer molecular pathologists in the world and has discovered, and explored the mechanism of function of several important cancer biomarkers. His basic research is focused on prostate cancer carcinogenesis and his most important accomplishments include the definition of cell lineage in the prostate in a blastocyst complementation murine model; the discovery of targeted ubiquitination and inactivation of the cyclin dependent kinase inhibitor p27 in human cancer; the discovery of the role of a de-ubiquitinating enzyme (USP2a) in stabilizing a key metabolic enzyme, subsequently proving that this enzyme (Fatty Acid Synthase) is a "metabolic oncogene". This led, most recently, to the discovery of specific metabolomic profiles associated with driving oncogenes in genetically engineered prostate cells, transgenic mice and human tumors.

**William Watson**

Professor Watson received his PhD degree in Biochemistry from the Department of Biochemistry, University College Cork in 1995. He then undertook his post-doctoral research in University of Toronto and the Toronto General Hospital in Canada, before returning in 1997, to the Department of Surgery, Mater Misericordiae University Hospital, University College Dublin and is now Associate Professor of Cancer Biology in the UCD School of Medicine and Medical Science. Apart from his undergraduate and post-graduate teaching responsibilities, he has an active internationally recognised research group. As a translational biologist based in the Conway Institute he utilises latest technologies to study cellular and molecular pathways and clinical collaboration through the Prostate Cancer Research Consortium (of which he is the lead PI) to expand the understanding of the initiation and progression of prostate cancer in order to identify diagnostic and prognostic biomarkers and therapeutic sites for manipulation. Professor Watson is Section editor of the Scientific Discovery section of the BJUInternational, Chair of the Basic Research Section of the European Association for Urology Research Foundation, Principal Investigator in Molecular Medicine Ireland.

**Richard Kennedy**

Professor Richard Kennedy Is the McClay Professor in Experimental Cancer Medicine at the Centre for Cancer Research and Cell Biology, Queen's University of Belfast. He graduated in medicine from Queen's University Belfast in 1995. As a post-graduate he trained as a medical oncologist and was entered on the General Medical Council Specialist Register in 2007. In 2004 he was awarded a PhD in molecular biology from The Queen's University of Belfast. In recognition of this work he was given an American Association of Cancer Research (AACR) Scholar in Training Award in 2003 and the JMS Irish Doctor's Award for Excellence in Research in 2004. From 2004-2007 he worked as an instructor in oncology at Harvard Medical School, USA, where he identified novel biomarkers and drug targets for cancer treatment. This work was published in several high impact journals and the associated patent was in-licensed by a Boston-basedstart up company (DNAR) in 2007. In August 2007 he joined Almac Diagnostics as Vice President where he managed the internal research programme and the application of biomarkers to several Pharmaceutical Company sponsored clinical trials. He continues as the Medical director at Almac and is responsible a CLIA compliant diagnostics laboratory for biomarker delivery to patients. In June 2011 he joined Queen's University as Professor of Experimental Cancer Medicine and has established a research group focussed on various aspects of stratified medicine. He also has an Honorary Consultant position at Belfast City Hospital and continues to manage cancer patients.

He is currently a member of the scientific advisory board for the Breast Cancer Campaign and has previously served on the CR-UK Biomarker panel and Enterprise Ireland life sciences technology panel.

**Diletta Bianchini**

Dr Diletta Bianchini is currently a Medical Oncologist working as Associate Specialist at the Royal Marsden NHS Foundation Trust and the Institute of Cancer Research. Her main research interest is the development of novel therapeutics for castration resistant prostate cancer (CRPC) and she has been directly involved in the clinical development of abiraterone acetate, a novel inhibitor of CYP17.

**Joe O'Sullivan**

Professor Joe O'Sullivan graduated from UCD medical school in 1993 and following training as a radiation oncologist in St. Luke's Hospital in Dublin moved to the Academic Urology Unit of the Royal Marsden Hospital London where he completed an MD on the subject of Radionuclide therapy in Castration Resistant Prostate Cancer under the supervision of Professor David Dearnaley. In 2004 he took up post as Senior Lecturer in Clinical Oncology at Queen's University Belfast and the Northern Ireland Cancer Centre where he has developed and led the prostate cancer and radiotherapy clinical trial programme. Particular areas of interest include bone-seeking radionuclide therapy and translational studies in castration resistant prostate cancer. In 2011 he was appointed to the post of Professor of Radiation Oncology at QUB.

**Rob Jones**

Professor Rob Jones is currently Senior Lecturer in Medical Oncology at the University of Glasgow and the Beatson West of Scotland Cancer Centre, Scotland, UK. His background includes a molecular biology PhD at the Beatson Institute for Cancer Research.

His clinical practice is the medical management of cancers of the prostate, bladder and kidney. His research commitments include the management of a large portfolio of phase I, II and III trials as well as collaborative translational research with scientists from the Beatson Institute. He is the Chief Investigator of the TOUCAN and PLUTO trials in urothelial cancer, the SAPROCAN trial in prostate cancer and the UK component of ASPEN, a trial in renal cell cancer. He is also interim director of the CRUK Clinical Trials Unit in Glasgow.

He is an active member of the UK NCRI Clinical study groups in urology.

**David Dearnaley**

Professor David Dearnaley joined the Academic Unit of Radiotherapy at the Royal Marsden Hospital (RMH) and Institute of Cancer Research (ICR) as Bob Champion Senior Lecturer and Honorary Consultant in 1987. He subsequently became Reader in Prostate Cancer Studies in 1999 and Professor of Uro-Oncology in 2003. He was Head of the Royal Marsden NHS Trust Urology Unit from 1994 to 2006 and the SWLondon Cancer Network Urology Tumour Working Group (2006 - 2010) He is currently Chairman of the RMH/ICR Committee for Clinical Research and Prostate Cancer Lead for the NIHR Biomedical Research Centre at the RMH. He has Chaired both the MRC and NCRI Prostate Study Groups and also the MRC Radiotherapy Clinical Trials Group. He leads several national and international trials of radiotherapy and systemic treatments in prostate and other urological cancers. Principal areas of research have been in the development of conformal and, subsequently, intensity modulated radiotherapy (IMRT) treatments for prostate cancer. He has led phase III randomised trials to establish these in clinical practice and is the Chief Investigator leading a multicentre trial of intensity modulated radiotherapy using hypofractionated schedules.

**Kathleen Kelly**

Professor Kelly received her Ph.D. from the University of California, Irvine. She completed her postdoctoral training in the laboratory of Philip Leder, Harvard Medical School, and she has maintained an independent research program at the NCI since 1984. Dr. Kelly is the chief of the Cell and Cancer Biology Branch, Center for Cancer Research, NCI. She is involved in a variety of intramural NCI activities including chairing the Cancer Stem Cell Consortium. Dr. Kelly's interests are focused on the genetic regulation of prostate cancer progression and metastasis. Her group has made use of both human xenograft and genetically engineered mouse models to investigate contributions of autonomous and microenvironment-dependent signaling pathways to prostate cancer growth and metastasis.

**Zoran Culig**

Professor Zoran Culig is an Associate Professor of Molecular Pathology at the Innsbruck Medical University, Austria. His main research focus is on the androgen signaling pathway and cytokines in prostate cancer. His laboratory has published several important publications describing the mechanisms which are relevant to prostate cancer progression. His publications on androgen receptor mutations, ligand-independent activation of androgen receptor and androgen receptor expression in metastases have been cited in a large number of scientific articles. Zoran Culig´s laboratory explained importance of androgen receptor activation by interleukin-6 and analyzed functional role of suppressors of cytokine signaling in prostate cancer. His laboratory also contributed to the development of experimental therapies in prostate cancer (anti-interleukin-6 therapy and anti-p300 therapy). His publications appeared in the journals of the AACR, American Society for Investigative Pathology, Endocrine Society, Society of Endocrinology and several others. Zoran Culig has received prestigious awards of the Society for Basic Urological Research, the European Association of Urology as well as the Alken Award and the Heinrich Warner Award.

**David Waugh**

Dr David Waugh is a Reader in Molecular Oncology within the Centre for Cancer Research and Cell Biology at Queen's University Belfast. A former Associate Direction for Postgraduate studies, he currently serves Head of the Cancer Cell and Molecular Biology Division and Academic Lead of the Prostate Cancer Research Group within the Centre. His principal research interest lies in understanding the importance of pro-inflammatory chemokines in promoting tumour progression and modulating their sensitivity to therapeutic interventions.

**Manuel Collado**

Professor Manuel Collado obtained his PhD in 1997 at the Universidad Autónoma de Madrid for research on vaccines against HIV under the supervision of Mariano Esteban. From 1997 to 1999, Manuel worked as postdoctoral fellow in the laboratory of Eric W Lam, at the Ludwig Institute for Cancer Research (St Mary´s branch, London, UK), on p27Kip1 and its regulation by PI3K/AKT during cellular senescence. From 1999 to 2001, Manuel moved to the laboratory of Andrew Koff, at Memorial Sloan Kettering Cancer Center (New York, USA), where he continued working of p27Kip1 and the PI3K/AKT pathway. In 2001, Manuel returned to Spain to join the laboratory of Manuel Serrano, then at the Spanish National Biotechnology Center (CNB-CSIC, Madrid) and that later on moved to the recently created Spanish National Cancer Research Center (CNIO, Madrid). During this time, Manuel worked in cancer and ageing, and made important contributions in the field of cellular senescence having demonstrated that this response occurs in vivo during cancer development to block tumour progression. More recently, he has been involved in defining tumour suppressors Ink4a/Arf as a barrier against cellular reprogramming.

**Fabrizio d'Adda di Fagagna**

Professor Fabrizio d'Adda di Fagagna received his PhD in Molecular Genetics at the International School for Advanced Studies (ISAS-SISSA) in Trieste, Italy. He then worked as a Research Associate in the group of Prof. Steve Jackson at the University of Cambridge, UK, where he studied, among other things, the interplay between components of the DNA damage response (DDR) apparatus and the ends of linear chromosomes, the telomeres, culminating with the demonstration that replicative cellular senescence is triggered by the direct recognition of critically short telomeres and consequent DDR activation.

In 2003 became Principal Investigator at IFOM (The FIRC Institute of Molecular Oncology), in Milan, Italy and in 2009 was tenured. In 2005 he demonstrated that oncogene activation is an intrinsically genotoxic event caused by altered DNA replication, demonstrating the tumor suppressive role of DDR genes and suggesting a model of oncogene-induced DNA damage generation and genome destabilization. His group is studying the impact of DNA damage generation genomewide and the impact DNA damage in different cell types, including stem cells, from different organisms and the contribution of non coding RNAs to genome stability.

**Igor Roninson**

Professor Roninson obtained his Ph.D. in 1982 from Massachusetts Institute of Technology, where he also completed his postdoctoral training. From 1984 to 2003, Dr. Roninson was on the faculty of the Department of Molecular Genetics, University of Illinois at Chicago, where his final position was Distinguished University Professor and Head of the Division of Molecular Oncology. From 2003 to 2011, he was Director of Cancer Center at Ordway Research Institute in Albany, New York. In 2011, he joined the University of South Carolina, where he holds an Endowed Chair in Translational Cancer Therapeutics. He is an author of 160 articles and chapters, an inventor of 39 issued US patents, and the editor of books on multidrug resistance and non-apoptotic responses of tumor cells to anticancer drugs. Dr. Roninson's works include the development of the in-gel DNA renaturation technique for detecting and cloning amplified genes; cloning and characterization of the MDR1 (ABCB1) multidrug resistance gene; development of the Genetic Suppressor Element (GSE) methodology for function-based gene identification and the use of this approach to identify novel molecular targets for cancer therapy; elucidation of tumor cell senescence as a general antiproliferative effect of cancer therapeutics; and the discovery of the effect of cyclin-dependent kinase inhibitors on the expression of genes implicated in cancer and age-related diseases. Among his awards are Rhoads Award from the American Association for Cancer Research, MERIT Award from the National Cancer Institute and Life Extension Prize from Regenerative Medicine Secretariat.

**Karl Matter**

Professor Karl Matter received his PhD in Biochemistry from the Biozentrum, University of Basel, Switzerland, in 1989. After a postdoctoral fellowship in the Department of Cell Biology at Yale University, USA, he returned to Switzerland where he started his independent research programme at the University of Geneva, supported by a senior research fellowship from the Swiss National Science Foundation. In 2001, he moved to London and is now a Professor of Cell Biology at the UCL Institute of Ophthalmology. The research of his laboratory focuses on the structure and function of epithelial cell-cell junctions, and the molecular mechanisms that guide epithelial cell polarisation and differentiation in health and disease.

**Clare Isacke**

Professor Clare Isacke studied for her BA in Biochemistry and DPhil in Developmental Biology at the University of Oxford. She then moved to Tony Hunter's laboratory at the Salk Institute in San Diego to work on growth factor receptor signalling as a postdoctoral fellow. On returning to England, she started her own research laboratory first in the Department of Biochemistry and then in the Department of Biology at Imperial College London. She was appointed Professor of Molecular Cell Biology in 2000.

In 2001, Professor Isacke moved to the Institute of Cancer Research (ICR) in London to take up an appointment as Professor of Molecular Cell Biology in the Breakthrough Breast Cancer Research Centre. In 2004, Professor Isacke was appointed Deputy Director of the Centre and in 2011, she was appointed Interim Director of both the Breakthrough Breast Cancer Research Centre and the Division of Breast Cancer Research.
Professor Isacke is a Medical Research Council  Molecular and Cellular Medicine board member, a European Association of Cancer Research executive committee member, a Susan G. Komen for the Cure committee member and a Director of The Company of Biologists.

**Charles Streuli**

Professor Streuli is Professor of Cell Biology and Director of the Wellcome Trust Centre for Cell-Matrix Research. He is also a founding member of the Manchester Breast Centre and the Breakthrough Research Unit in Manchester
He obtained his first degree in Biochemistry at the University of Cambridge, and then studied for a PhD degree with David Critchley at the University of Leicester. This was followed by postdoctoral work with Beverly Griffin at the Imperial Cancer Research Fund Laboratories in London (now called the CR-UK London Research Institute), and then Mina Bissell at the Lawrence Berkeley National Laboratory in California. In 1992, he was awarded a Senior Research Fellowship in Basic Biomedical Science by the Wellcome Trust to establish his own laboratory at the University of Manchester. Since 2005, he was engaged with establishing the Manchester Breast Centre, a pan-Manchester organisation that brings together basic and clinical scientists working on mammary gland biology and breast cancer. In 2007, he co-founded and became Research Director of the Breakthrough Breast Cancer Research Unit at the University of Manchester, which is translating discoveries about the causes of early breast cancer to novel prevention and treatment strategies. He has also been associated with the Wellcome Trust Centre for Cell-Matrix Research since its inception in the mid-90s, and was appointed to the position of Director of the Cell-Matrix Centre in 2009.

**Patrick Johnston**

Professor Johnston is Dean of the School of Medicine, Dentistry and Biomedical Sciences and Director of the Institute of Health Sciences at Queen's University Belfast. Prof. Johnston's research is focused on cellular signalling pathways in human cancer, primarily related to molecular targeted cancer, therapeutics, personalised cancer medicine and mechanisms of drug resistance. He received his medical degree with distinction from University College Dublin in 1982, followed by his PhD in Medicine in 1988. He obtained a fellowship at the National Cancer Institute (NCI USA) in 1987 where he pursued further clinical training in medical oncology and doctoral studies in molecular pharmacology, drug resistance and drug development. He was promoted to Senior Investigator at the NCI in 1991. In 1997 he moved to Queen's University Belfast as Professor of Oncology and subsequently became Director of the Centre for Cancer Research and Cell Biology in 2004 at the same institution. He has been Dean of the Medical School since 2007. He sits on many influential national and international scientific and government advisory boards. He is the Founder of the Society for Translational Oncology and the biotechnology company, Almac Diagnostics.

**Tim Illidge**

Professor Tim Illidge is Professor of Targeted Therapy and Oncology; School of Cancer and Imaging Sciences, Manchester University since 2004. He is group leader for the CRUK Targeted Therapy Group based at Paterson Institute and leads the clinical nuclear medicine therapy group at the Christie Hospital. After completing his undergraduate degree in Biochemistry (BSc) at London University, Dr Illidge received his medical degree (MB BS) from Guy's Hospital Medical School, London, and subsequently was awarded a PhD from the University of Southampton, for his work in the field of targeted radiation and immunotherapy applied to lymphoma. He was awarded British Oncology Young cancer researcher of the year in 1997 and British Nuclear medicine researcher of the year in 1998. His post graduate qualifications include Diploma in Royal College of Gynaecologists 1990, Member of the Royal College of Physicians (1993), Fellowship of the Royal College of Radiologists (1997) and Fellowship of the Royal College of Pathologists ( 2009)

Dr Illidge completed research fellowships as CRUK Senior Clinical Research Fellow and was appointed Senior Lecturer and Honorary Consultant in Oncology at Southampton University Hospitals NHS Trust in 1999. He was a awarded a US Senior Fulbright fellowship and a Winston Churchill Fellowship which enabled him to work as part of the lymphoma team at Stanford University, California 1998. He moved his research group to the Paterson Institute and Christie hospital in 2004 and developed the multidisciplinary Radiation Related Research Group of the Manchester Cancer Research Centre (MCRC) in 2007 which have become established as a centre of excellence. Dr Illidge is currently the chair of the National Cancer Research Institute Clinical and Translational Radiotherapy Group (CTRad). His clinical interest is in lymphoma were he has led many early phase and late clinical trials and is recognised as an international expert in antibodies and radioimmunotherapy applied to lymphoma, and has published extensively in this area. He serves on the central steering group of the National Cancer Research lymphoma groups and has represented the UK at International Workshops in Lymphoma.

He is married and has 3 children, is passionate about sport, music (especially classical guitar), photography and hill walking.

**John de Klerk**

Professor John de Klerk was born in 1958. After receiving his medical master's degree at the Free University in Amsterdam, he started his Nuclear Medicine training at the University Medical Center Utrecht. Certification as a nuclear medicine specialist was obtained in 1990. Since this time he was a staff member of the Department of Nuclear Medicine of the University Medical Center Utrecht. In May 1st 2003 he became director of the Department of Nuclear Medicine of the Meander Medical Center Amersfoort. In 1995 he completed his PhD thesis on Rhenium-186-HEDP in metastatic bone pain. He has been involved as a principal- or co-investigator in the research projects conducted at the Department of Nuclear Medicine of the Meander Medical Center and in multicenter trials. John de Klerk has a close collaboration with the Queens University Hospital in Belfast (Professor Joe O' Sullivan).

His work was mentioned several times at highlights presentations of international scientific meetings like annuals meeting of the EANM and SNM.
His special interest is Nuclear Medicine therapy and Oncology in Nuclear Medicine. He has been is a member of the radionuclide therapy committee of the EANM for many years. He is reviewer for the Journal of Nuclear Medicine and several other medical journals. He is author and co-author of many peer-reviewed journal articles, book chapters and abstracts. He lectures widely on a national and an international basis on the therapeutic use of radiopharmaceuticals.

**Kevin Prise**

Professor Kevin Prise is Professor of Radiation Biology and Deputy Director at the Centre for Cancer Research & Cell Biology at Queen's University Belfast. A graduate from Aberdeen University in Biochemistry, he spent over 20 years as a radiation biologist at the Gray Cancer Institute in London before moving to Queen's in 2007. He has longstanding interests in the mechanisms of action of ionising radiation including the application of microbeam technologies. Since joining CCRCB he has been developing translational studies aimed at developing new biological based models for the application of advanced radiotherapies in collaboration with Prof Joe O'Sullivan and Dr Alan Hounsell from the Northern Ireland Cancer Centre.

**Göran Landberg**

Professor Landberg studied Medicine at Umeå University in Sweden and received an MD 1988 and a PhD 1991. After postdoctoral research at The Scripps Research Institute in La Jolla, California he returned to Sweden and was promoted associate professor in pathology and consultant in clinical pathology. 2000 he set up a laboratory in Malmö and became professor in clinical pathology at Lund University. Between 2003 and 2006 he was head of laboratory medicine at Malmö University Hospital. 2008 he was recruited to University of Manchester, UK as a professor in Molecular Pathology and established a Breakthrough Breast Cancer research Unit at the Paterson Institute for Cancer Research. Since 2011 he is also professor in Pathology at the University of Gothenburg in Sweden. His research has been focused on breast cancer and molecular pathology and specifically the delineation and targeting of subgroups of breast cancer. His laboratories currently study how the tumour microenvironment and hypoxia influences key tumour properties in breast cancer with a special interest in developing novel treatment schedules. Besides his research interest he has contributed to the dissemination and establishment of translational cancer research platforms and centres in Sweden and England. His research is supported by Breakthrough Breast Cancer, MCRC and the Swedish Cancer Society.

**John Schwabe**

Professor Schwabe studied for his PhD degree (awarded by the University of Cambridge) with Dr Daniela Rhodes at the MRC Laboratory of Molecular Biology where he received the Max Perutz Prize in 1991. He was then awarded an MRC Training Fellowship to continue postdoctoral studies in Cambridge. In 1995 he joined the Salk Institute in La Jolla, California as a Staff Scientist in Professor Ron Evans' laboratory. He returned to the MRC Laboratory of Molecular Biology in Cambridge in 1997 where he was a Group Leader / Senior Scientist. In 2006, he was appointed Professor of Structural Biology at the University of Leicester and since 2010 Professor Schwabe is also Head of the Department of Biochemistry. His research focusses on using structural and functional approaches to understand signalling by nuclear receptors and associated coregulator complexes. His research is funded by the Wellcome Trust and BBSRC.

**Wilbert Zwart**

Professor Zwart received his PhD Cum Laude from the Faculty of Medicine of the Leiden University in 2009, based on work he performed at the Netherlands Cancer Institute, Amsterdam. He then received his postdoctoral training at Cancer Research UK, Cambridge, in the research group of dr. Jason Carroll. In 2011 he returned to Amsterdam, where he started working as a junior group leader (assisted professor level) in November that year. His group is currently based at the department of Molecular Pathology, where a strong synergy exists between the translational research groups and medical specialists. His research group studies the cell biological, genomic and pathological criteria of anti-estrogen resistance in breast cancer, with a strong focus on finding predictive biomarkers and detection methods for endocrine response.

**Annette Byrne**

Professor Byrne lectures in Human Physiology and Medical Physics at the RCSI. She is involved in the pre-clinical development of novel anti-cancer therapeutics and elucidation of cancer biomarkers, with a particular interest in applying a multi-modality molecular imaging approach towards elucidation of drug mechanism of action in vivo. Dr Byrne's work involves the screening and identification of novel targets in vivo with specific expertise in identifying anti-angiogenic targets. In addition, she is also involved in the interrogation of new safety and efficacy biomarkers for clinically approved angiogenesis inhibitors. Technologies include optical and nuclear imaging strategies, development of imageable disease models & collaborative 'omics' discovery approaches.

**Research Interests**

Professor Byrne's main research interests are Angiogenesis Inhibitors, in vivo Cancer Models, Multi-modality in vivo molecular imaging, pre-clinical drug development and identification of biomarkers of angiogenesis inhibitor toxicity and efficacy. Dr Byrne co-ordinates two large scale EU FP7 Funded programmes in the angiogenesis inhibitor space: AngioTox [c.1.7 million Euros over 4 years; www.angiotox.com] and the recently commenced ANGIOPREDICT project [c.6 million Euros over 4 years] who's focus is to identify genomic biomarker methods for combination Avastin therapy in metastatic colorectal cancer.

**Diether Lambrechts**

Professor Diether Lambrechts graduated in Biological Engineering at the University of Leuven, Belgium, and worked with Peter Carmeliet on the role of vascular endothelial growth factor (VEGF) in amyotrophic lateral sclerosis for his PhD and postdoc until 2007. For in-depth training in human genetics, he worked at the Wellcome Trust Centre for Human Genetics at the University of Oxford, UK. In 2009, he established the Laboratory for Translational Genetics as an independent VIB group leader within VRC. His current research is focused on conducting large-scale cancer genetic studies and discovering pharmacogenomic biomarkers for targeted cancer therapies, in particular anti-angiogenic therapies.

**Arjan W. Griffioen, PhD**

Professor Arjan W. Griffioen studied medical biology at the Free University in Amsterdam and got his PhD in 1991 from the Utrecht University for his thesis on human B lymphocyte activation. In this year he also received his SMBWO-registration as immunologist. After a postdoctoral fellowship in the Department of Pathology of the Academic Medical Center in Amsterdam, he became Assistant Professor in the Department of Internal Medicine at the Utrecht Medical Center. In 1997 he was appointed at the Department of Internal Medicine of the University Hospital Maastricht. After a 6-months sabbatical leave to the University of Minnesota in Minneapolis in 2001, he was appointed at the Department of Pathology as Associate Professor, and in 2005 as Full Professor in Experimental Oncology and Angiogenesis.

The Angiogenesis Laboratory is currently located at the VU University Medical Center in Amsterdam, and hosts now over 20 researchers working on the mechanisms of angiogenesis and the development of novel treatment strategies for cancer (www.angiogenesis.nl). The major research lines are (i) gene expression profiling of tumor endothelial cells, (ii) the development of new angiogenesis inhibitors by the *de novo* design of cytokine-like peptides, (iii) the interplay between angiogenesis and immune functions, (iv) tumor cell plasticity and vasculogenic mimicry. Dr. Griffioen has authored over 150 peer-reviewed scientific publications, joins the editorial board of several international scientific journals and is Editor-in-Chief of the journal Angiogenesis.

**David DeNardo**

Professor DeNardo received his PhD degree in Cell Biology from the Department of Molecular Cell Biology at the Baylor College of Medicine in 1999. He then undertook his post-doctoral research training at the University of California, San Francisco in Dr. Lisa Coussens labrotory. The primary focus of his research there was on understanding the mechanisms by which tumor infiltrating T cells and macropahges concominantly regulate metastatic progression in breast cancer. His data support the notion that CD4 cells can have a role in modulating both the invasive nature of cancers as well as their ability to metastasize. In 2011, he joined Washington University as a faculty member and has a joint appointment in both the Department of Medicine and the Department of Pathology and Immunolgy. Dr DeNardo's current research focuses on the interaction between tumor infiltrating leukocytes and chemoresistance in malignant breast and pancreatic cancers.

# PLENARY SPEAKER ABSTRACTS

**METABOLIC ALTERATIONS IN PROSTATE CANCER**

**Massimo F. Loda, MD,** *Professor of Pathology, Harvard Medical School*

Metabolites are seen as the end products of the biological hierarchy starting with activated genes (genome) and extending over the collection of gene transcripts (transcriptome) and proteins (proteome). Uncontrolled proliferation does not occur in normal cells because these do not take up nutrients from their environment unless stimulated to do so by growth factors. Cancer cells overcome this growth factor dependence either by acquiring genetic mutations that result in altered metabolic pathways or by affecting metabolic pathways *de novo* with targeted mutations in critical metabolic enzymes. Altered metabolic pathways, in turn, stimulate cell growth by either providing fuel for energy or by efficiently incorporating nutrients into biomass.

Thus, metabolic alterations occur as a result of altered pathways, in turn a consequence of genetic events or metabolic alterations may be primary events in cancer but require genetic alterations in critical pathways for oncogenesis

We used immortalized human prostate epithelial cells transformed by the MYC and Akt oncogenes, transgenic mice driven by the same oncogenes and human tumors characterized for the expression of these proteins. Metabolic profiling was performed using fresh (cells) and OCT-embedded samples (murine and human tissue). Following organic and aqueous extractions, samples were split into equal parts for analysis on the GC/MS and LC/MS/MS platforms. Identification of known chemical entities was based on comparison to purified standards. Following log transformation and imputation with minimum observed values for each compound, Welch's two-sample *t*-tests were used to identify biochemicals that differed significantly between different states (e.g. tumor and non-tumor tissue; Akt versus Myc). An estimate of the false discovery rate (q-value) was calculated to take into account the multiple comparisons.

Through large-scale metabolite analyses and isotopic labeling approaches, we found that AKT1 drives primarily aerobic glycolysis while MYC does not elicit a Warburg-like effect and significantly enhances glycerophospholipid synthesis instead. This regulation is Gleason grade- and pathological stage-independent while Gleason grade itself is associated with a distinct metabolic and gene expression signature in human tumors. Metabolic profiling can also be assessed in serum samples and correlates with that in corresponding tumors.

Our data provides evidence that prostate tumors exhibit metabolic fingerprints of their molecular phenotypes, which may have high impact on metabolic diagnostics and targeted therapeutics.

**BIOMARKER DISCOVERY AND VALIDATION FOR PROSTATE CANCER: A PROGRAMME OF THE PROSTATE CANCER RESEARCH CONSORTIUM ADDRESSING THE CLINICAL DILEMMA**

**William Watson**, Associate Professor of Cancer Biology, UCD School of Medicine and Medical Science, Conway Institute, University College Dublin

Prostate cancer remains the most common form of male cancer in the US and Europe. Despite PSA screening decreasing cancer mortality it has been associate with the over detection and over treatment of the disease, impacting on patients quality of life. Determining the most appropriate treatment strategy represents a significant dilemma for the patient and clinician. The Prostate Cancer Research Consortium focus of research has been driven by the clinical need to identify biomarkers that will inform appropriate treatment options. The consortiums approach is to interrogate serum, tissue and urine at the genomic, transcriptomic and proteomic level using novel statistical and bioinformatics approaches to identify panels of biomarkers.

Preclinical serum samples were collected from men with different grades and stages of prostate cancer as part of the Prostate Cancer Research Consortium Bioresource. Their analysis by 2-D DIGE and label-free LC-MS/MS and the use of novel statistical software has identified panels of proteins with acceptable predictability for further validation. This panel has also been informed by our epigenetic, transcriptomics and other proteomics projects. Using multiplex antibody assays and multiple reaction monitoring assays these panels are being validated in independent samples from the Prostate Cancer Research Consortium Bioresource and our international collaborators and show AUC values in line for clinical utility. With careful validation these panels, in combination with current clinical tools, could improve diagnosis and thus patient outcome through the selection of appropriate treatment options.

**IDENTIFICATION OF A PROGNOSTIC MOLECULAR SUBTYPE IN PROSTATE CANCER**

**Richard Kennedy,** McClay Professor of Experimental Cancer Medicine, CCRCB, Queen's University of Belfast

Traditional prognostic markers for prostate cancer are relatively weak at predicting those who will develop metastatic disease after presenting with a primary tumour. Clinicians therefore likely undertreat some and over-treat the majority of patients. We have therefore analysed over 150 archived samples, including primary prostate cancers, normal prostate samples, metastatic lymph node samples and normal lymph nodes using a DNA microarray-based approach. An unsupervised hierarchical clustering analysis has been used to identify a distinct molecular subtype of primary prostate cancer that is molecularly similar to metastatic disease. This group is largely defined by loss of a significant number of molecular pathways. Aclassifying signature has been developed which prospectively identifies this molecular group and this has been validated in an independent publically available prostatectomy dataset. We now aim to apply this signature to 250 biopsy samples at Queen’s University, which has resulted in some technical challenges that I will discuss.

**ABIRATERONE ACETATE: THE BEGINNING OF A NEW ERA IN THE TREATMENT OF CASTRATION RESISTANT PROSTATE CANCER**

Diletta Bianchini MD, The Institute of Cancer Research and the Royal Marsden NHS Foundation Trust,London UK.

Abiraterone acetate (AA) is a rationally designed specific inhibitor of CYP17, the key enzyme along the biosynthesis of androgens and estrogens resulting in a significant suppression of serum androgenic steroids and estrogen[1]. Declines in prostate-specific antigen (PSA) by ≥50% and ≥90% have been reported in early clinical trials respectively in 50-60% and 20-30% of CRPC patients treated with AA[2,3,4,5]. These preliminary data prompted the start up of a large randomized, double blind, placebo controlled Phase III trial (COU-AA-301) investigating the effect on survival of AA versus placebo in patients with castration resistant prostate cancer (CRPC) in the post-docetaxel setting. The median overall survival was 14.8 months and 10.9 months in patients receiving AA and placebo respectively (HR 0.65, $p<0.001$). These results were published in early 2011 and they led to the FDA (Food and Drug Administration) approval of AA (6).

Similarly, MDV-3100 is a potent androgen receptor (AR) antagonist which has recently proven to improve survival in CRPC patients that have failed docetaxel chemotherapy[6]. This anti-tumour activity in heavily pre-treated (standard anti-androgens, low-dose steroids, estrogens and docetaxel) CRPC patients suggests these agents are more effective at inhibiting the AR signalling axis and importantly, confirms that CRPC remains hormone-driven disease. Interestingly, patients with CRPC almost invariably progress with a rise in PSA, suggesting reactivation of the AR-signalling as main mechanism of resistance. The future development of therapeutics for CRPC should be informed by an understanding of the mechanisms underlying disease progression following treatment with these novel agents[8].

The clinical data from abiraterone acetate and MDV-3100 confirm continued androgen receptor (AR) addiction in a significant proportion of castration-resistant prostate cancers (CRPC).

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***EARLY EVALUATION OF RADIONUCLIDE THERAPY IN CASTRATE-RESISTANT PATIENTS***

**Joe O'Sullivan,** *CCRCB, Queen's University of Belfast*

Bone-seeking radionuclide therapy has traditionally been used in metastatic castration resistant prostate cancer to palliate bone pain. In recent years there has been increasing interest in these agents as disease modifiers when combined with chemotherapy or used in innovative ways. In the past year new data from a global phase 3 trial of a 1$^{st}$ in class alpha-emitting radionuclide, Alpharadin (Radium-223) has demonstrated a significant survival benefit in men with symptomatic CRPC metastatic to bone. In this presentation I will summarise the recent data and postulate on future developments in the field.

***CYTOTOXIC THERAPY IN ADVANCED PROSTATE CANCER – DO WE REALLY NEED ANOTHER PARADIGM SHIFT?***

**Rob Jones**, Glasgow University, Beatson West of Scotland Cancer Centre, Scotland

Prostate cancer patients are elderly and frail and would never tolerate chemotherapy.

Or so it went, for many years. Partly as a result, it is only in the last 8 years that chemotherapy has had a significant role in managing these patients and there are still only two licensed cytotoxic drugs in use in metastatic, castration-resistant prostate cancer (mCRPC). As abiraterone, radium and MDV3100 become available to the same group of patients we will be forced to reconsider the role for cytotoxic chemotherapy. Whilst these newer treatments are undoubtedly less toxic, their mechanisms of action are sufficiently different from cytotoxics that we should assume that their activities are complementary, and for some men, the challenge will be how best to obtain the maximum advantage from not one, but all of these treatments. It seems likely that sequential, rather than combination, therapy will remain the standard of care, and so there are two important questions: first, when to start treatment for mCRPC, and second in which order to use them.

**RADIOTHERAPY FOR PROSTATE CANCER: WHERE TO NEXT?**

**David Dearnaley**, *Academic Radiotherapy, Institute of Cancer Research, Royal Marsden Hospital, London*

Recent long term results of surgical and radiotherapy phase III trials with dose escalation and combined modality therapy have informed the magnitude of the potential impact of treatment on localised and advanced localised disease. It is clear that local control of prostate cancer matters but that hormonal therapy has a major impact on outcome and survival in those patients with higher risk disease. Potential increases in treatment intensity (eg. combined modality with surgery/radiotherapy, radiotherapy/hormonal treatment, dose escalation) may all bring improvements in outcome but the potential benefits need to be weighted against the increased side effects of therapy and strategies designed to minimise acute and late toxicity. In radiotherapy, personalised treatment using intensity modulated techniques and focal high dose functional imaging guided boosts are realisable and biomarkers using TMA and other technologies hold promise for biological as well as physical optimisation on an individual basis.

**IN VIVO MODELING OF PROSTATE ADENOCARCINOMA TUMOR INITIATING CELLS**

**Kathleen Kelly**, Branch Chief, Cell and Cancer Biology Branch, NCI, NIH, Bethesda MD, USA

The phenotype of prostate adenocarcinoma tumor propagating cells has not been determined for primary prostate tumors. Both multipotent progenitors and luminal progenitor cells, residing in the basal or luminal compartments respectively, can act as prostate cancer cells of origin. Tumor propagating cells may be similar to or differentiated progeny of the cancer cell of origin. We have analyzed the phenotype of tumor propagating cells in a mouse model of aggressive prostate cancer initiated by Probasin promoter-driven Cre (Pb-Cre) dependent deletion of *Pten* and *TP53.* Pten deletion results in the development of PIN/adenocarcinoma, while combined *Pten*/*TP53* deletion results in more aggressive progression, associated with significant tumor heterogeneity. The heterogeneity associated with the Pb-Cre4 $Pten^{-/-}TP53^{-/-}$ mouse model is manifested in tumors consisting primarily of adenocarcinoma with foci of basal squamous carcinoma. The cellular source of this heterogeneity is unknown but may result from transformation of multipotent progenitors, or alternatively, from independent transformations of committed progenitors to the basal and luminal lineages.

The aim of the study to be presented was to interrogate the tumor initiating activity of the basal and luminal compartments in $Pten^{-/-}TP53^{-/-}$ prostate tissue. In order to separate the basal and luminal lineages, we devised a fractionation scheme using markers preferentially expressed by either lineage. Lineage negative, $EPCAM^{+}$ primary prostate tumor cells were labeled with CD49f and Prominin-1 (Prom-1) in order to differentiate basal and luminal populations respectively. In addition, the effect of short-term castration upon tumor initiating activity was determined. Our findings demonstrate that prostate adenocarcinoma tumor propagating cells reside in a population of self-renewing, castration-resistant progenitors expressing a luminal and not a basal phenotype. This model serves as a discovery platform for castration-resistant signaling associated with survival following *PTEN* loss.

**CYTOKINE SIGNALING IMBALANCE IN PROSTATE CANCER PROGRESSION**

**Zoran Culig**, Experimental Urology, Department of Urology, Innsbruck Medical University

Interleukin-6 (IL-6) levels are elevated in sera and tissues of patients with advanced prostate cancer. In order to understand the mechanisms by which IL-6 regulates tumor progression, we have investigated its effects in multiple *in vitro* and *in vivo* models. IL-6 activation of androgen receptor may result in either stimulation or inhibition of prostate cancer cell growth. The growth-inhibitory effects were seen in LNCaP cells in which the phosphorylation of STAT3 is induced by IL-6. In contrast, IL-6 growth-promoting effect was seen in the MDA PCa 2b cell line. Our further studies on IL-6 signaling in prostate cancer were focused on suppressors of cytokine signaling (SOCS) and protein inhibitors of activated STAT (PIAS). SOCS-3 is highly expressed in prostate cancer and inhibits apoptosis through interaction with extrinsic and intrinsic pathways. However, in the presence of basic fibroblast growth factor SOCS-3 caused inhibition of mitogen-activated protein kinases phosphorylation. Thus, this regulator of cytokine signaling has multiple effects in prostate cancer depending on the cellular context. Another member of the SOCS family, SOCS-1, is also increasingly expressed in prostate cancer tissues. However, it has a tumor suppressive role and inhibits expression of cyclin-dependent kinases and cyclins. We also found that PIAS-1, which potentiates androgen receptor activity, is expressed in tissue specimens from prostate cancer patients and in cell lines. Its expression is higher in tumor than in benign tissue. PIAS-1 promotes cell cycle progression through inhibition of the tumor suppressor p21. Taken together, our research shows that three regulators of cytokine signaling have an important role in modulation of proliferative and apoptotic responses in human prostate cancer. This knowledge is important for the development of a more rational approach for anti-IL-6 therapy in prostate cancer.

**CELLULAR SENESCENCE IN CANCER, DEVELOPMENT AND REPROGRAMMING**

Manuel Collado, Spanish National Cancer Research Center CNIO, 3 Melchor Fernandez Almagro, E28029 Madrid, Spain

Cells respond to stress by undergoing either apoptosis or senescence. High levels of cellular stress are intrinsically associated to the process of tumorigenesis. Current accumulated evidence indicates that, besides apoptosis, senescence is a relevant cellular response to stress, implicated both in protecting from cancer emergence, as well as in triggering cancer regression. I will review current knowledge about senescence as a tumour suppressor response and I will present our most recent data on the interplay between inflammation and senescence.

Apoptosis is known to be involved also in embryonic development. In contrast, cellular senescence has not been shown to play any role during development yet. I will present new and exciting data on the possible role of cellular senescence on embryo development.

Finally, reprogramming of differentiated cells into induced pluripotent stem cells (iPSCs) also entails cellular stress that is detected by tumour suppressors and triggers senescence. We have previously reported that the p16/Arf tumour suppressor locus is a main barrier for reprogramming. I will present data extending this paradigm to other tumour suppressors.

**MOLECULAR MECHANISMS OF CELLULAR SENESCENCE**

Fabrizio d'Adda di Fagagna, Principal Investigator, IFOM Foundation - The FIRC Institute of Molecular Oncology Foundation, Milan, Italy

Early tumorigenesis is associated with the engagement of the DNA-damage checkpoint response (DDR). Cell proliferation and transformation induced by oncogene activation are restrained by cellular senescence. We have previously shown that expression of an activated oncogene in cultured normal human cells results in a permanent cell-cycle arrest caused by the activation of a robust DDR. Experimental inactivation of DDR abrogates senescence and promotes cell transformation. Oncogene-induced senescence is also associated with a global heterochromatinization of nuclear DNA. Our most recent results on the interplay between DDR and heterochromatin formation, the differential repair of the human genome, the regulation of DDR in stem cells and our search for novel pathways regulating genome stability will be discussed.

**TRANSCRIPTIONAL MECHANISM AND PHARMACOLOGICAL INHIBITION OF PARACRINE TUMOR-PROMOTING ACTIVITIES OF CHEMOTHERAPY-DAMAGED AND SENESCENT CELLS**

Igor B. Roninson[1,2], Donald C. Porter[2], Eugenia V. Broude[1], and Hippokratis Kiaris[3]
[1]Department of Pharmaceutical and Biomedical Sciences, South Carolina College of Pharmacy, University of South Carolina, Columbia, SC, USA, [2]Senex Biotechnology, Inc., Columbia, SC, USA, [3]University of Athens Medical School, Athens, Greece

Cellular damage by different factors, including chemotherapy or ionizing radiation, triggers a program of damage response that includes cell cycle arrest by cyclin-dependent kinase inhibitor proteins, in particular p21 (CDKN1A). Some of the damaged cells die, others recover and resume proliferation, and still others undergo terminal cell cycle arrest through the program of senescence. Cellular damage that induces p21, and in some cases p21 expression alone, not only cause cell cycle arrest and senescence but also have profound effects on cellular gene expression. These effects include both downregulation of genes involved in cell cycle progression and upregulation of genes encoding numerous secreted proteins, including various tumor-promoting factors with mitogenic, anti-apoptotic, angiogenic and pro-invasive activities. p21-dependent transcriptional activation of tumor-promoting genes is induced by damage in both normal and tumor cells, and it is “fixed” at the highest levels in cells that become senescent upon damage. The damaged cells and tissues develop multiple tumor-promoting paracrine activities, which can be detected by various assays both in vitro and in vivo.

Using high-throughput screening followed by chemical lead optimization, we have developed a novel small-molecule inhibitor of p21- and damage-induced transcription. This compound blocks the induction of tumor-promoting cytokines in damaged cells, inhibits paracrine anti-apoptotic and mitogenic activities of damaged tumor cells and normal fibroblasts in vitro, blocks chemotherapy-induced potentiation of tumor growth in mice, and sensitizes tumors to chemotherapy in vivo, without apparent systemic toxicity. The target of this compound has been identified (and verified by shRNA knockdown) as a kinase involved in the regulation of transcription and implicated in carcinogenesis. Pharmaceutical inhibition of this target would be expected to increase the efficacy of cancer therapy.

**SIGNALLING AT TIGHT JUNCTIONS IN EPITHELIAL PROLIFERATION, DIFFERENTIATION AND REPAIR**

Karl Matter, Department of Cell Biology, UCL Institute of Ophthalmology, University College London, London, UK.

Epithelial cells form polarised cellular sheets by adhering to each other via intercellular junctions. Tight junctions are the most apical intercellular junction and are essential for ephthelial barrier formation. Tight junctions also guide multiple signal transduction mechanisms that regulate epithelial proliferation, polarisation, and differentiation. Many of these mechanisms play important roles during epithelial stress responses and repair, and, if deregualted, contribute to epithelial degeneration and disease. The aim of our research is to understand the molecular mechanisms by which tight junctions guide epithelial cell behaviour and how these mechanisms contribute to chronic and acute diseases affecting epithelia. My presentation will focus on the interplay between RhoGTPase signalling and epithelial junctions during junction assembly and cell migration.

**AN IN VIVO FUNCTIONAL SCREEN TO IDENTIFY NOVEL BREAST CANCER METASTASIS SUPPRESSOR GENES**

Clare Isacke, Interim Director, The Breakthrough Toby Robins Breast Cancer Research Centre, The Institute of Cancer Research, London

Genetic screens provide an unbiased approach to the identification of genes associated with a phenotype of interest. Although cell-based screens have been highly informative in identifying genes involved in tumour cell survival, migration and invasion, these *in vitro* approaches are largely unsuitable for interrogating the later stages of the metastatic process, in particular the processes of cell dissemination, tumour cell extravasation from the circulation and colonisation of secondary sites.

Using the 4T1 mouse mammary carcinoma model, we performed an *in vivo* functional metastasis screen that integrates RNAi technology and massively parallel sequencing. 12 'hits' that suppress tumour cell colonisation of the lungs were identified. 3 of the top 5 hits have been validated as novel metastasis suppressor genes in both mouse and human cell lines and functional studies have been undertaken to interrogate mechanism of action. Critically, these findings are clinically relevant in primary breast cancers where there is a significant correlation between elevated expression levels of these suppressor genes and reduced frequency of metastatic events.

This study demonstrates the value of adopting an unbiased methodology to discover novel metastatic genes and establishes, for the first time, that an *in vivo* metastasis screen can be combined with next generation sequencing to identify novel components of the metastatic process.

**INTEGRINS AND POLARITY IN BREAST EPITHELIA**

Charles Streuli, Professor of Cell Biology, Wellcome Trust Centre for Cell-Matrix Research, University of Manchester, UK

The aim of our research is to determine the molecular mechanisms by which cell-matrix interactions control epithelial cell phenotype. We have discovered that integrin receptors are essential for mammary gland development and function, and they determine the capacity for breast epithelial cells to both proliferate and express tissue-specific genes. In this talk, I will show that they also have an essential role in determining the orientation of polarity in breast epithelia and will reveal the mechanisms involved.

**RADIONUCLIDE THERAPY OF BONE METASTASES: PAST, PRESENT AND FUTURE**

John M.H. de Klerk, Department of Nuclear Medicine, Meander Medical Center Amersfoort, The Netherlands

Several bone seeking radiopharmaceuticals has been used for palliative treatment of painful bone metastases, especially in patients with hormone refractory prostate cancer. Beta rays from the specific radionuclide, bound to its carrier ligand, result in the therapeutic effect. Besides beta ray emitters, alpha ray emitters are currently under investigation. Toxicity of bone seeking radiopharmaceuticals is limited to a reversible decline in total platelet count and in a lesser degree to a reversible leucopenia [1].
Pain relief will occur in a high percentage of patients (70-80%). Although the efficacy is high, as well as cost-effective, it is of paramount importance to explore the possibilities of improving efficacy even further. Enhancement of therapeutic efficacy can be achieved by: Increasing the administered dose, repeated treatment schedules, chemosensitization and combined treatment with other modalities like external beam radiotherapy.

Several studies showed a better clinical result using combined chemotherapy and radiopharmaceuticals. In collaboration with the Queens University Hospital in Belfast, we conducted a dose escalation study using docetaxel and repeated dosages Rhenium-186-HEDP[2]. This combination proved to be safe and a randomized phase II study has been initiated.

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**DEVELOPMENT OF NEW BIOLOGICAL-BASED MODELS FOR ADVANCED RADIOTHERAPIES**

Kevin M. Prise[1], Karl Butterworth[1], Conor McGarry[1,2], Stephen McMahon[1], Colman Trainor, Giuseppe Schettino[1], Alan D Hounsell[1,2] and Joe O'Sullivan[1,3]
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[2]Radiotherapy Physics, Northern Ireland Cancer Centre, Belfast, UK
[3]Clinical Oncology, Northern Ireland Cancer Centre, Belfast, UK

Our understanding of the mechanisms of radiation response in biological systems has been changing from studying the role of radiation damage at the DNA and cellular level to integrated models of tissue and whole organism responses. Key to this has been a growing appreciation of bystander responses where cells respond to their neighbours being irradiated and intercellular signalling mechanisms play a role. Bystander responses have been observed locally between cells but also over longer ranges including between tissues in the intact organism. Microbeam approaches, where localised radiation beams can be delivered, have been key experimental tools which are allowing the delineation of mechanisms underpinning bystander signalling in a range of models.

For advanced radiotherapies, delivery of dose into tumours is highly modulated in both space and time. A major consequence is the production of dose-gradients across tumours and normal tissues as dose is "painted" into the treatment volume. Little is known about the underlying biological rationale optimising these approaches and the role, if any bystander signalling will play. More recently studies with modulated photon beams have begun to address these issues to develop biologically driven models for radiotherapies which can take into account any impact of bystander signalling.

**TUMOUR MICROENVIRONMENT AND BREAST CANCER PROGRESSION IN RELATION TO THE PRESENCE OF OESTROGEN RECEPTOR**

Göran Landberg MD PhD, Professor Molecular Pathology and Pathology, University of Manchester, UK/University of Gothenburg, Sweden

Breast cancer is a highly heterogeneous disease with contrasting clinical behaviours. Tumours can be subdivided into relevant subgroups according to epithelial as well stromal properties and it is clear that the tumour microenvironment profoundly effects tumour behaviour. The potential for hypoxia to influence tumour growth has been addressed by analysing large sets of breast cancer cell lines and primary breast cancer samples and the results suggest that hypoxia has contrasting effects on the amount of cancer initiating cells and that this effect is partly mediated by ER and the notch pathway. The presence of the ER also seems to be important in defining the relation between key tumour properties as migration, proliferation and the fraction of cancer initiating cells. The presented findings support and illustrate fundamental differences in behaviours for ER-positive and ER-negative breast cancer which needs to be considered in future treatment approaches.

**NEW INSIGHTS INTO THE REGULATION OF HISTONE DEACETYLASE COMPLEXES**

John Schwabe, Professor of Structural Biology, Department of Biochemistry, University of Leicester. UK.

Histone deacetylases (HDACs) are increasingly recognised as important targets for the treatment of cancer and other diseases including Alzheimer's. They are essential enzymes required for human development & homeostasis. Histone deacetylases regulate gene expression through controlling the acetylation state of histones and hence chromatin structure. Class I histone deacetylases are recruited to chromatin, by repressive transcription factors, as complexes with cognate co-repressor proteins. These complexes are essential for cell viability and are particularly important for determination of cell fate and lineage commitment during development. Importantly, most class I HDACs are only enzymatically active when recruited to their respective large multi-subunit co-repressor complexes. To understand the structural and functional basis for this activation, we determined the structure of HDAC3 in complex with the interacting portion of the SMRT co-repressor. This is the first structure of an HDAC:corepressor complex and it reveals the molecular basis for the specific assembly and provides clues as to the activation mechanism. Unexpectedly a small signalling molecule, inositol-(1,4,5,6)-tetrakisphosphate (IP4), was observed at the interface between HDAC3 and the SMRT corepressor. The IP4 appears to act as an “intermolecular glue” between the two proteins. Functional studies show that the IP4 is essential for both co-repressor interaction and for HDAC3 activation. The finding that IP4 is required for class I HDAC activity opens new opportunities for therapeutic intervention.


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## THE ESTROGEN RECEPTOR/CHROMATIN AND EPIGENETIC LANDSCAPE THAT DEFINES AROMATASE INHIBITOR RESISTANCE IN PRIMARY TUMORS

Wilbert Zwart, Junior Group Leader, department of Molecular Pathology,
The Netherlands Cancer Institute, Amsterdam

About 75% of all breast cancer cases fall within the luminal subgroup of tumors.
In luminal breast cancer, the Estrogen Receptor is not only the key role player in the proliferative potential of tumor cells, but also the main target for treatment. When choosing treatment options, the menopausal status of the patient is a major determining factor. For premenopausal patients, tamoxifen is the first-line of treatment. When treating postmenopausal disease, the vast majority of patients receives any kind of aromatase inhibitor treatment either as a monotherapy, or sequentially combined with tamoxifen. For both tamoxifen and aromatase inhibitors, resistance to treatment is still commonly observed, and predictive biomarkers are required that could guide the endocrine treatment selection for the individual patient.

To identify biomarkers for outcome after aromatase inhibitor treatment, we analyzed tumor material that was removed during surgery, after which the patients received treatment with any of the clinically available aromatase inhibitors. From the primary tumor material, we determined the genome-wide chromatin binding patterns of the Estrogen Receptor, as well as the histone modifications H3K4me3 (an active histone mark) and H3K27me3 (an inactive histone mark) using ChIP-seq. Both the Estrogen Receptor binding patterns as well as the epigenetic landscape were correlated with disease progression, to find patterns that could identify patients with a poor or favorable outcome after aromatase inhibitor treatment.

## GENETIC MARKERS OF RESPONSE FOR THE ANTI-ANGIOGENIC AGENT BEVACIZUMAB

Diether Lambrechts, VIB Vesalius Research Center, K.U. Leuven

Bevacizumab is the first anti-angiogenic therapy proven to slow metastatic disease progression in cancer patients. Although it has changed clinical practice in several tumor types, some patients do not respond or eventually become unresponsive, resulting in rather modest gains in terms of overall survival. An urgent issue is therefore to understand why some patients respond well to anti-angiogenic therapies, and based on these insights, to select patients amenable for bevacizumab treatment. Here, we discuss some of the challenges in finding markers predictive of treatment outcome, and suggest what can be done to tackle these problems. Based on recent findings in large clinical studies, I propose that genetic variants in VEGF receptors are among the most promising markers and discuss how they may become implemented in clinical practice.

**NOVEL WAYS OF ANGIOGENESIS INHIBITION FOR CANCER THERAPY**

Arjan W Griffioen and Judy van Beijnum, Angiogenesis Laboratory, Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands
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Current angiostatic compounds in the clinic have moderate effect on survival, while displaying several disadvantages, e.g. side-effects, induction of resistance. Therefore, anti-angiogenesis therapy needs new strategies that are different from VEGF targeting. To identify tumor-specific angiogenesis markers, we performed yeast 2-hybrid screening techniques using existing angiogenesis inhibitors as bait, and genomic transcriptome subtraction techniques using RNA from angiogenic endothelial cells isolated from both malignant and non-malignant angiogenic tissues and of resting endothelial cells. We identified a series of genes that show specific overexpression in tumor endothelium but not in angiogenic endothelium of normal tissues, creating a therapeutic window for tumor vasculature specific targeting. Antibody targeting of four cell-surface expressed or secreted products (vimentin, galectin-1, HMGB1 and IGFBP7) inhibited angiogenesis *in vitro* and *in vivo*. Targeting endothelial vimentin or galectin-1 in a mouse tumor model significantly inhibited tumor growth and reduced microvessel density. These results demonstrate the utility of the identification and subsequent targeting of specific tumor endothelial markers for anticancer therapy.

Next to targeting of these markers for direct therapeutic use, it is possible to use ligands of these markers for targeted delivery of drugs or tracers for imaging. A therapeutic ligand of galectin-1 was successfully used for delivery of a fluorescent dye and of a gadolinium-based tracer for visualization of tumor angiogenesis by fluorescence microscopy and by magnetic resonance imaging.

**TARGETING THE TUMOR IMMUNE MICROENVIRONMENT TO OVERCOME CHEMO-RESISTANCE IN BREAST CANCER**

David DeNardo, Assistant Professor of Medicine and Immunology
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Immune-regulated pathways influence multiple aspects of cancer development. Herein we demonstrate that macrophage and T cell abundance in breast cancer (BC) represent prognostic indicators for recurrence-free and overall survival. We provide evidence that response to chemotherapy is in part regulated by these leukocytes - cytotoxic therapies induce mammary epithelial cells to produce monocyte/macrophage recruitment factors including colony stimulating factor (CSF)1 and interleukin-34 that together enhance CSF1 receptor-dependent macrophage infiltration. Blockade of macrophage recruitment with CSF1R-signaling antagonists, in combination with Paclitaxel improved survival of mammary tumor-bearing mice by slowing primary tumor development and reducing pulmonary metastasis. These improved aspects of mammary carcinogenesis were accompanied by decreased vessel density and appearance of anti-tumor immune programs fostering tumor suppression in $CD8^+$ T cell-dependent manners. These data provide rationale for targeting macrophage recruitment/response pathways, notably CSF1R, in combination with cytotoxic therapy, and identification of a BC population likely to benefit from this novel therapeutic approach.

# PROSTATE CANCER SYMPOSIUM PROFFERED PAPERS
# (As per programme)

**EXOSOMES - POTENTIAL MEDIATORS OF PROSTATE CANCER PROGRESSION?**

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Recent studies suggest that phenotypic changes associated with cancer may be transferred from cell-to-cell via microvesicles/exosomes [1, 2]. Exosomes have been described as nano-sized membrane-bound vesicles of endocytic origin that are expelled from a range of cell types [3]. Hormone-refractory prostate cancer treatment remains hindered by inevitable progression of resistance to first-line treatment with Docetaxel.
In order to help determine the complexity of this problem, this study aimed to (i) characterize in vitro models of docetaxel-resistance from three prostate cancer cell lines: DU145, PC3 and 22Rv1 compared to their aged-parent populations; (ii) isolate exosomes from medium conditioned by these cell line models and investigate their effects on motility, invasion, proliferation and docetaxel resistance of secondary cells; and to (iii) perform a proof-of-principle translational investigation of the clinical relevance of exosomes isolated from prostate cancer serum.
We successfully generated docetaxel-resistant prostate cancer cell lines that were found to be substantially more resistant to docetaxel than age-matched sensitive cells (109±7.4 fold for DU145RD; 71±8.4 fold for 22Rv1RD; 19±2.3 for PC3-D12). In addition, all resistant variants displayed cross-resistance to the anthracycline Doxorubicin (4.3±1.0 for DU145RD; 8.3±1.2 for 22Rv1RD; 4.2±1.2). Docetaxel-resistance was associated with alterations in motility, migration, invasion, proliferation, anchorage-independent growth and response to TRAIL-induced cell death. In the presence of DU145RD exosomes, a significant increase in docetaxel-resistance was observed in both DU145 cells (~22%; $p<0.01$) and 22RV1 cells (~15%; $p<0.001$); effects that were independent of proliferation influences. Furthermore, MDR-1/P-gp expression associated with docetaxel-resistance (detected in two of the three resistant cell lines [4]) was also detectable in corresponding exosomes secreted from those cells. In order to explore the potential clinical relevance of exosomes in prostate cancer, we performed a pilot study involving serum from docetaxel-treatment naive prostate cancer patients and healthy age-matched controls. We observed a significant increase of invasion of DU145 cells (~20%; $p<0.05$) and proliferation of 22Rv1 cells (~10%; $p<0.05$) in the presence of prostate cancer exosomes. Our in vitro observations and preliminary clinical studies indicate that exosomes play an important role in prostate cancer and may offer potential as vehicles containing predictive biomarkers and new therapeutic targets.

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## PARAMETRIC DIFFUSION WEIGHTED IMAGING AT 3 TESLA PREDICTS HISTOLOGICAL OUTCOMES IN MEN PRESENTING FOR A PROSTATE BIOPSY

**DC Moran**[1], L Marignol[1], A Perry[1], JF Meaney[2], TH Lynch[3], DH Hollywood[4]
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Background:

Diffusion Weighted Imaging (DWI-MRI) has potential to improve the specificity and sensitivity of current diagnostic tools used to detect peripheral zone (PZ) prostate cancer(PCa).

Study Objectives:

To determine the accuracy of quantitative (parametric) DWI-MRI at 3.0 Tesla inpredicting histological outcome in men presenting for TRUS-guided prostate biopsy.

Methods:

Sixty men with a clinical suspicion of PCa underwent DWI-MRI prior to 12-core systematic TRUS-guided biopsy. ADC maps were subjected to parametric analysis using three sequentially applied threshold values (tADC) of 1.0, 1.2 and 1.4 mm2/sec. After each tADC was applied, remaining pixels below each threshold value were evaluated as potential malignant lesions. Area size and mean ADC of these regions were recorded. Segment based and patient-specific performance of DWI-MRI for PCa detection was correlated with biopsy.

Results:

At a tADC of 1.2mm2/sec, the sensitivity and specificity for PCa detection was 91% and 89% respectively, with a negative predictive value of 97% for segment based analysis. For patient-specific analysis the sensitivity and specificity were 94% and 70%. Gleason score and mean ADC were negatively correlated. The mean ADC values of DWI-MRI identified lesions were significantly lower in biopsy-positive segments compared to those that were biopsy-negative. At a tADC of 1.2 mm2/sec, the area under the curve (AUC) for PCa detection was 0.83 with the best performing cut-off values established at an ADC value of 1.036mm2/sec and a lesion size of ≥0.26cm.

Conclusions:
Parametric evaluation of DWI-MRI at 3.0 Tesla can accurately detect PZ PCa. We outline a potential role for this imaging modality in future PCa diagnostic algorithms.

**IDENTIFICATION OF SERUM RESPONSIVE FACTOR (SRF) AS A POTENTIAL THERAPEUTIC TARGET FOR CASTRATION-RESISTANT PROSTATE CANCER**

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Advanced prostate cancer is treated by hormone ablation therapy. However, despite an initial response, the majority of men relapse to develop a castration-resistant disease for which there are no effective treatments; therefore defining the mechanisms of resistance represents a significant question.

Previous studies in our laboratory have shown a complex interplay between changes in pro and anti-apoptotic proteins in the development of this resistance. Manipulating individual genes has only minor alterations in the resistant phenotype so we hypothesise that targeting the central signalling pathways and transcription factors (TFs) would represent a better therapeutic approach. We have undertaken a transcriptomic analysis to study differences in gene expression between the androgen-dependent (AD) LNCaP parental cells, and the androgen-independent (AI) LNCaP Abl and Hof sublines, revealing 900 genes associated with androgen resistance. Using innovative bioinformatics techniques, these transcriptomic data were integrated with known and predicted TFs binding sites resulting in a list of TFs including HSF1, CDP, VDR-RXR, PBX1 and SRF, which may be responsible for the differential gene expression observed. These TFs and associated down stream genes and their protein products were validated by RT-PCR and western blotting.

SRF transcriptional activity was up-regulated by DHT, while bicalutamide treatment decreased SRF transcriptional activity, demonstrating a role for SRF in response to androgens. SRF inhibition by siRNA and using the small molecule inhibitor, CCG1423, resulted in decreased cell viability and proliferation.

We propose that SRF activation represents a novel mechanism by which resistant cells bypass current therapies and survive with very low concentrations of androgens representing a target for therapeutic manipulation.

**GENE-DIRECTED ENZYME PRODRUG THERAPY IN A SPHEROID MODEL OF PROSTATE CANCER**

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Spheroid cell models of cancer have been recognised as a useful technique, bridging the gap between standard monolayer cell line work and animal models. They allow for the assessment of cell behaviour and drug toxicity in an environment closer to tumour conditions in vivo. However current spheroid culture techniques can be time-consuming and are not always compatible with high throughput methodologies. Here we present work on the optimisation of a new method of spheroid formation using suspension media developed in Trinity College and the prostate cancer cell line 22Rv1. High-content screening and confocal imaging has demonstrated that the resulting spheroids are densely packed collections of viable cells with a necrotic core. The spheroids form as cells cluster over time, followed by subsequent cell division, increasing spheroid size. Secreted levels of prostate specific antigen (PSA) normalised to cell number are significantly higher for the 22Rv1 spheroids compared to the adherent cells, suggesting they may act as an improved model for the prostate tumour microenvironment. Therefore, this technique is being used to test a form of gene-directed enzyme prodrug therapy (GDEPT) previously demonstrated to show potential in 22Rv1 cells grown in a monolayer. In this GDEPT system a prodrug activating enzyme, cytosine deaminase (CD), is regulated by the PSA promoter. We have confirmed the efficacy of this system following dihydrotestosterone stimulation and demonstrated further upregulation of CD expression in response to hypoxia and radiation treatment using adherent 22Rv1 cells. We are currently testing the utility of the system using 22Rv1 spheroids.

# IACR Proffered Papers 1 Session – Abstracts
# (As per programme)

**MODULATION OF CD44 SUB-CELLULAR LOCALIZATION DECREASES BREAST CANCER CELL MOTILITY**

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The majority of breast cancer-related deaths result from metastasis, a process which requires dynamic regulation of tumour cell adhesive and migratory properties. The adhesion protein CD44 acts as an important regulator of cell migration. Alterations in CD44 expression have been linked to tumour progression in several carcinomas including breast, yet the contribution of CD44 to cancer metastasis remains incompletely understood. A pool of CD44 localizes in cholesterol-enriched regions of the cell membrane termed lipid rafts. Our previous findings suggested that this pool of CD44 moves outside lipid rafts during cancer cell migration (Donatello et al, submitted). Thus the aim of our study was to determine how lipid rafts regulate the localization and function of CD44 in migrating breast cancer cells. We first compared the raft affiliation status of CD44 and its binding partner ezrin in a panel of variably-invasive breast cancer cell lines under basal migratory conditions or after induction of CD44-specific migration using its extracellular matrix ligand hyaluronan. Raft affiliation of CD44 increased during migration of the non-invasive breast cancer cell line MCF-10a. In contrast, raft-affiliation of CD44 was significantly reduced during migration of the highly-invasive cell lines Hs578T and MDA-MB-231, in parallel with increased recovery of CD44 outside rafts. Ezrin was detected exclusively in non-raft fractions of all cell lines under all conditions tested. To investigate whether CD44 re-localization outside lipid rafts was sufficient to drive cancer cell migration, we introduced point-mutations into two CD44 palmitoylation sites (Cys286 and Cys295) which target it to lipid rafts. CD44 raft affiliation was significantly decreased in cells transiently transfected with all CD44 palmitoylation-impaired mutants. Furthermore, these cells gained a more motile and invasive phenotype compared to control cells. This phenotype was reversible upon termination of selection for mutant CD44. In conclusion, our results support a novel mechanism whereby sub-membranous trafficking of CD44 outside lipid rafts is sufficient to promote migration in invasive breast cancer cells. We suggest that pharmacological sequestration of CD44 within rafts could act as a novel strategy to reduce breast cancer cell migration and potentially metastasis.

**INVESTIGATING THE ROLE OF HUMAN CDC7 KINASE AND CLASPIN IN THE DNA REPLICATION CHECKPOINT**

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In eukaryotic cells, DNA replication is a multi-step process that requires origins of replication become licensed by formation of the pre-replicative complex. DNA synthesis is initiated by temporal activation of licensed origins, during S-phase of the cell cycle, in a manner that requires the co-ordinate action of both Cdc7 and Cyclin-dependent kinases.

Cdc7 is also reported to play a role in the replication block and recovery checkpoint response, which is activated in response to DNA damage/replication stress. However, the role of Cdc7 in this signal-transduction cascade remains controversial. Importantly, in human cells, we have demonstrated that Cdc7 is active during DNA replication blocks caused by either Hydroxyurea or Etoposide, and Cdc7 downregulation increases cell death in response to genotoxic agents.

Claspin is considered a sensor of ongoing DNA replication and acts as a mediator protein in the replication checkpoint signal-transduction pathway. Interestingly, human Cdc7 and Claspin have been shown to physically interact in vivo, and Cdc7 phosphorylates Claspin in vitro.

A clear mechanistic link between Cdc7 and the replication checkpoint has not been established. In our work, we have shown that Cdc7 kinase is required for efficient induction of replication checkpoint signaling in response to replication stress. We have identified Cdc7-dependent phosphorylation sites on Claspin using in vitro kinase assays and tandem-MS/MS in electron transfer dissociation mode. Finally, we have devised tools to investigate whether Cdc7-mediated phosphorylation of Claspin occurs in vivo at specific phosphorylation sites and if these phosphorylation events are biologically relevant for replication checkpoint signaling.

This work is supported by a Science Foundation Ireland grant 08/RFP/NSC1235

**IDENTIFICATION OF THE TRANSCRIPTIONAL COMPLEX THROUGH WHICH TBX2 DRIVES THE PROLIFERATION OF BREAST CANCER CELLS**

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The T-box (TBX) family of transcription factors play an important role in developmental gene regulation, binding to target promoters through highly conserved T-box domains. TBX2 is located on chromosome 17q23, a region amplified in a subset of breast cancers and associated with poor prognosis. Few TBX2 target genes have been identified but its role in tumorigenesis has been linked to its ability to repress key growth regulatory genes such as p14ARF, p21WAF1 and Connexin 43. Gene silencing of TBX2 resulted in a dramatic inhibition of proliferation of TBX2 expressing breast cancer cells. NDRG1 (N-myc DownRegulated Gene 1), a growth control gene, was identified by microarray analysis as a TBX2 target gene. The mechanism through which TBX2 represses genes involved in growth control, apoptosis and senescence is poorly understood. NDRG1 promoter studies resulted in the identification of a novel mechanism by which TBX2 targets this gene, through the association with the Early Growth Response 1 (EGR1) transcription factor. Through Co-IP experiments we have identified two novel co-repressors; KAP1 and HP1, interacting with TBX2. KAP1 is the universal co-repressor for the KRAB domain containing zinc finger superfamily of transcriptional repressors. HP1 is considered the 'gatekeeper' of methyl-K9-H3 mediated silencing. Although neither TBX2 or EGR1 contain KRAB domains (usually a requirement for KAP1 binding), the interaction between KAP1 and HP1 has been well documented. Downregulation of KAP1 and HP1, similarly to TBX2 and EGR1, results in a decreased ability of breast cancer cells to proliferate. Through site directed mutagenesis we have found two HP1 binding sites on TBX2 essential for its ability to recruit HP1 and KAP1 to target promoters and subsequently repress NDRG1 through trimethylation of K9-H3. Downregulation of KAP1 and HP1, similarly to TBX2 and EGR1, results in a decreased ability of breast cancer cells to proliferate.

TBX2 is an oncogene promoting the proliferation and survival of breast cancer cells. We have identified a novel mechanism of transcriptional repression by TBX2 through interaction with the putative tumour suppressor EGR1, allowing TBX2 to repress some of the major growth inhibitory pathways. We have also proposed a mechanism though which TBX2 represses transcription of target genes. Abrogating either this TBX2-EGR1 interaction or TBX2 repression complexes may present opportunities for the development of novel breast cancer treatments.

**PROTEIN TYROSINE PHOSPHATASE RECEPTOR DELTA ACTS AS A NEUROBLASTOMA TUMOUR SUPPRESSOR BY DEPHOSPHORYLATING AND DESTABILISING THE AURORA KINASE A ONCOGENE**

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Protein tyrosine phosphatase receptor delta (PTPRD) is a member of a large family of protein tyrosine phosphatases which negatively regulate tyrosine phosphorylation. Neuroblastoma is a major childhood cancer arising from precursor cells of the sympathetic nervous system which is known to acquire deletions and alterations in the expression patterns of PTPRD [1], indicating a potential tumour suppressor function for this gene. The molecular mechanism, however, by which PTPRD renders a tumour suppressor effect in neuroblastoma is unknown.
We demonstrate that low PTPRD expression in primary tumours is significantly associated with both poor overall and event free survival of neuroblastoma patients and that experimental up-regulation of this gene in neuroblastoma cell lines significantly decreases cell growth and increases apoptosis. As a molecular mechanism, we demonstrate by co-immunoprecipitation that PTPRD interacts with aurora kinase A (AURKA), an oncogenic protein that is over-expressed in multiple forms of cancer including neuroblastoma and that PTPRD negatively regulates AURKA by its dephosphorylation. Furthermore, ectopic up-regulation of PTPRD in neuroblastoma cells results in a decrease in the protein half-life of AURKA, along with one of AURKA's primary functions in neuroblastoma involving the stabilisation of MYCN protein, the gene of which is amplified in approximately 25 to 30% of high risk neuroblastoma.

We conclude that PTPRD has a tumour suppressor function in neuroblastoma through AURKA desphosphorylation and protein destabilisation and a downstream destabilisation of MYCN protein, representing a novel mechanism for the function of PTPRD in neuroblastoma.

1. Protein Tyrosine Phosphatase Receptor Delta Acts as a Neuroblastoma Tumor Suppressor By Destabilizing the Aurora Kinase A Oncogene

## DNA MEDIATED CHROMATIN PULL-DOWN: A NOVEL METHOD FOR THE ANALYSIS OF NEWLY REPLICATED CHROMATIN

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Replication of DNA and duplication of the epigenetic information are critical to the transmission of the genetic material from the parental cell to the two daughter cells and for specifying cell-type identity of the two daughter cells.

To investigate how these processes are regulated and coordinated, it is important to determine the identity of the proteins that are either stably or transiently associated with newly synthesized DNA. While the capture of newly synthesized naked DNA by BrdU labeling is possible and has yielded important information related to DNA replication dynamics, the protein component that binds to newly replicated DNA has remained elusive due to technical hurdles.

We have devised an experimental procedure that allows newly synthesized chromatin to be efficiently captured and analyzed, which we have termed “DNA mediated chromatin pull-down” (Dm-ChP). Here we show that Dm-ChP is a highly specific and flexible technique with the potential of becoming a leading methodology for the study of chromatin editing and maturation and for investigating how these processes are linked to the duplication of DNA.

Current work is focused on identifying the protein component of newly synthesized chromatin and in assessing qualitative and quantitative changes in this protein fraction caused by the normal temporal program of DNA replication as well as by perturbation of the DNA replication machinery.
This work is supported by Science Foundation Ireland grant 08/IN.1/B2064 and 08/RFP/NSC1235

**MIR-204 IS SIGNIFICANTLY ASSOCIATED WITH SURVIVAL IN NEUROBLASTOMA PATIENTS AND INCREASES SENSITIVITY OF NEUROBLASTOMA CELLS TO CHEMOTHERAPY IN VITRO THROUGH REGULATION OF TRKB AND BCL2.**

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Neuroblastoma (NB) is a leading cause of cancer linked mortality in children. Previously, we identified microRNAs significantly associated with patient survival (1). In particular, lower miR-204 expression at diagnosis in our NB tumour cohort (n=147) was significantly associated with poor EFS ($P < 0.0001$), indicating a potential tumour suppressive role. Here we present our findings from the study of functional effects of exogenous miR-204 expression in NB cell lines, and we identify the importance of TrkB and BCL2 (genes which facilitate increased resistance to chemotherapy treatment) as direct targets of miR-204.

Ectopic over-expression of miR-204 in NB cell lines (Kelly, SKNAS, SHSY5Y, NB1691) had no significant effect on cell viability or apoptosis. In addition, transfection of miR-204 into either NB1691 or SKNAS cells prior to retroperitoneal implantation of the cells into immune deficient mice had no effect on tumour growth (n=4). A candidate target, NTRK2/TRKB, expressed at high levels in unfavourable NB, and promotes cell survival and chemotherapy resistance through P13K/AKT pathway. TrkB is detectable in 30-40% of primary NBs but, rarely found in NB cell lines. By inducing NB cell lines to express full length TrkB, we determined that miR-204 expression resulted in decreased TrkB protein. Direct targeting of miR-204 with the 3'UTR of TrkB was validated by a luciferase reporter assay.

Counter-intuitive to expectations, ectopic up-regulation of miR-204 resulted in statistically significant increases in motility and invasion ($P < 0.05$). We identified BCL2 as an additional target of mir-204 (validated with luciferase assays). The role of BCL2 in the inhibition of motility and invasion in other cancers has only been recently described (2). We identified that siRNA knockdown of BCL2 in Neuroblastoma cells did in fact result in increased invasion in NB cells in vitro, identifying an alternative role of BCL2 in NB. However, despite increases in invasive capacity of the cells, siRNA knockdown of BCL2 and ectopic over-expression of miR-204 resulted in increased sensitivity to cisplatin treatment of NB cell lines.

We conclude that miR-204 associated increased survival in NB patients can be attributed in part to TrkB and BCL2 targeting thereby increasing sensitivity to chemotherapy treatment.

1.Bray et al, 2009 Plos one 2.Ke et al, 2010 Cell Research

# Oral Poster Presentations
## (Listed as scheduled)

**FKBPL: SECRETION AND REGULATION OF ANGIOGENESIS**

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FK506 binding protein-like (FKBPL) is an intracellular protein with a role in Hsp90-associated steroid hormone receptor complexes. Its regulation of oestrogen signalling supported a role for FKBPL as a prognostic and predictive biomarker of response to endocrine therapy in breast cancer patients (1). We have recently reported that recombinant FKBPL and its peptide derivatives exert potent anti-angiogenic activity in vitro and in vivo and control both breast and prostate tumour growth in xenograft models (2). The active anti-angiogenic domain was localised to a region distinct from the TPR domain and appears to target a pathway dependant on CD44. A therapeutic peptide, AD-01, spanning this domain was developed and a preclinical candidate based on AD-01 is currently undergoing toxicology studies with a view to initiating phase I clinical trials for solid tumours.
Here in support of FKBPL's role as a secreted anti-angiogenic protein, we report that FKBPL is secreted from human microvascular endothelial cells, HMEC-1. Interestingly, this secretion is inhibited under angiogenic stimuli such as hypoxia. Additionally, we were able to demonstrate that inhibition of extracellular, secreted FKBPL using a blocking antibody specific to the active site of FKBPL accelerated the migration of HMEC-1. This was further supported using FKBPL specific siRNA-mediated knockdown. Furthermore, MDA-MB-231 tumour cells stably overexpressing FKBPL were able to inhibit vascular development in vivo which correlated with perturbed tumour growth, suggesting that FKBPL secreted from tumour cells inhibited angiogenesis in these animals. To clarify the mechanism, we demonstrated that endogenous FKBPL showed binding to the CD44 receptor. FKBPL overexpression and knockdown or treatment with its peptide derivative, AD-01, regulated CD44 expression, suggesting a co-regulatory pathway for these two proteins. Downstream targets that mediate the anti-migratory activity of FKBPL peptide derivative AD-01, suggest an AD-01-mediated inhibition of Rac-1 activity and up-regulation of RhoA and the actin binding proteins, profilin and vinculin. This was associated with alterations in the actin cytoskeleton and a lack of cell spreading and communication explaining the anti-migratory phenotype. Thus endogenous FKBPL is a natural secreted protein and regulates angiogenesis by its effects on CD44 and the actin cytoskeleton.

1.McKeen et al, FKBPL regulates estrogen receptor signaling and determines response to endocrine therapy. Cancer Res. 2010 Feb 1; 70(3):1090-100. 2.Valentine et al, FKBPL and peptide derivatives: novel biological agents that inhibit angiogenesis by a CD44-dependent mechanism. Clin Cancer Res. 2011 Mar 1; 17(5):1044-56.

**MIR-31 CONFERS RESISTANCE TO TAMOXIFEN IN BREAST CANCER CELL LINES**

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Resistance to endocrine-directed therapy represents a significant problem in the management of breast cancer, with up to 50% of estrogen receptor (ER)-positive breast cancer recurrences displaying resistance to tamoxifen treatment. Recently, microRNAs (miRNAs) have been implicated in not only the initiation and progression of cancer, but also in the development of resistance to therapy. To further investigate the role of miRNAs in endocrine resistance in breast cancer, TaqMan Human MicroRNA Arrays v2.0 (Applied Biosystems) were used to profile global expression of 667 miRNAs from a cell line series developed from ER-positive MCF7 cells (consisting of the parental MCF7 cells, estrogen-independent LCC1 cells and antiestrogen-resistant LCC9 cells). From this screen, miR-20b, miR-28 and miR-28-3p were found to be expressed at low levels in MCF7 cells, displayed slightly increased expression in LCC1 cells and significantly increased expression in antiestrogen-resistant LCC9 cells. Additionally, miR-31 was found to be expressed at very low levels in both MCF7 and LCC1 cells with significantly increased expression in LCC9 cells. Moreover, ectopic expression of miR-31 in both MCF7 and LCC1 cells conferred partial resistance to tamoxifen treatment ($p<0.05$). miR-31 has been previously shown to bind directly to the mRNA transcripts of both LATS2, a serine-threonine protein kinase, and forkhead box protein P3 (FOXP3), thus negatively regulating their expression. Ectopic expression of miR-31 in both MCF7 and LCC1 cells was shown to decrease the expression of both LATS2 and FOXP3. Furthermore, when evaluated in a transcriptomics dataset consisting of 155 patients treated with tamoxifen, low levels of both LATS2 and FOXP3 correlated with relapse in these patients ($p=0.001$ and $p=0.034$, respectively). As such, we postulate that miR-31 may confer resistance to tamoxifen through the downregulation of both FOXP3 and LATS2.

## INVESTIGATION OF ADAM17 AS A NOVEL TARGET IN KRAS MUTANT AND WILD TYPE COLORECTAL CANCER

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Background.
A major factor limiting the effectiveness of chemotherapeutic management of colorectal cancer (CRC) is drug resistance. We have shown that chemotherapy results in an acute increase in ADAM17 activity in vitro and in vivo CRC models. Blocking ADAM17 activity, using siRNA, significantly increased apoptosis following chemotherapy treatment. We have also shown that overexpression of ADAM17 increases activity of a number of pro-survival receptors (eg EGFR, HER3, IGF-1R, VEGFR) resulting in resistance to chemotherapy in CRC in vivo models [1]. We have also demonstrated that mutant Kras regulates ADAM17 activity and thereby growth factor shedding in a MEK1/2/Erk1/2-dependent manner [2]. We hypothesise that targeting ADAM17 (and thereby the activity of multiple RTKs, such as EGFR, HER3, IGF-1R and VEGFR) may improve survival rates compared to those obtained with EGFR mAB inhibition (the current “best” treatment strategy in KrasWT patients) and maybe active in a Kras mutant setting. The aim of this study was to investigate further the role of ADAM17 as a novel therapeutic in KrasMT and KrasWT CRC using both small molecule and human monoclonal antibody approaches.
Materials and Methods.
ADAM17 inhibition was obtained using small molecule approach and a fully human monoclonal antibody, AD12 [3]. Analysis of cell viability was carried out using MTT assay, apoptosis was measured using Western blotting and Flow cytometry. Biflank subcutaneous H630 KrasWT xenograft models were used.
Results.
Using both the small molecule inhibitor GW280264X and the fully human monoclonal antibody AD12, we found strong synergistic inhibition in cell viability when combined with 5-FU chemotherapy treatment in both KrasMT and KrasWT CRC cell line models. Furthermore, we also found strong increases in apoptosis when ADAM17 inhibition was combined with chemotherapeutic agents 5-FU, SN-38 and oxaliplatin in both KrasMT and KrasWT CRC cells. In addition, treatment of KrasWT H630 xenograft models with the fully human ADAM17 monoclonal antibody (AD12) resulted in strong inhibition in serum human amphiregulin expression levels and this was associated with strong decrease in tumour growth. We are now investigating the effect of AD12 in a KrasMT CRC in vivo model. We are also further investigating the underlying mechanism by which ADAM17 regulates cell death in both KrasMT and KrasWT CRC cell line models.
Conclusions.
These results indicate that the combination of ADAM17 inhibition with standard chemotherapeutic agents could be a novel treatment strategy for both KrasMT and KrasWT CRC tumours.

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## MECHANISMS OF EXPRESSION AND REGULATION OF SOX2 AND ITS TARGETS IN TWO EMBRYONAL CARCINOMA CELL LINES

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SOX2 is a conserved pluripotency-associated transcription factor considered to be crucial for embryonal stem cell (ESC) and embryonal carcinoma cell (ECC) maintenance. In addition to its ability to autoregulate its expression, it closely regulates the expression of a wide range of genes important to 'stemness' and is also activated in various types of cancer stem cells (CSCs).[1–5]
MicroRNA (miRNA) are short, non-coding RNAs of approximately 23 nucleotides in length. Their most important documented capability is the regulation gene expression post-transcriptionally by preventing the translation of specific target mRNAs.[6–8]
We silenced SOX2 in two human ECC lines, NTera-2 and 2102Ep, which resulted in the initiation of the differentiation program of these cell lines. We measured whole-genome and whole-miRNAome expression after three days. Hundreds of genes were differentially deregulated in both cell lines, revealing pathways directly and indirectly regulated by SOX2. Besides a large overlap in differential gene expression of NTera-2 and 2102Ep cells, large discrepancies were also found, including in the expression of core pluripotency markers, POU5F1/OCT4 and NANOG. This could possibly indicate a difference in roles SOX2 plays in these cells. Compared to previous data from a third, widely used ECC line, NCCIT, 2102Ep showed a higher overlap with this cell line than NTera-2.[3]
Whole-miRNAome analysis revealed tens of differentially regulated miRNAs in both cell lines. Overlap was found but important discrepancies between NTera-2 and 2102Ep were also recorded, with the most notable difference being the downregulation of the C19MC polycistronic miRNA cluster in 2102Ep but not in NTera-2. This cluster has been associated with cancer and also with the differentiation program of ESCs.[9–11]
Our results showed that SOX2 directly and indirectly regulates hundreds of genes and tens of miRNAs in ECCs, but has distinctive roles in different ECC types. Further analysis of this data and future research could reveal what these precise roles are.

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**JUNCTIONAL ADHESION MOLECULE-A: A NOVEL REGULATOR OF HER2 PROTEIN DEGRADATION**

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**Background**: Each year breast cancer is diagnosed in approximately 1 million women worldwide. During malignant transformation, previously-normal breast epithelial cells often lose the expression of cell adhesion proteins. This confers a motile or migratory advantage which could contribute to early events in metastasis. Among the cell adhesion proteins potentially affected by this transformation is Junctional Adhesion Molecule A (JAM-A); a cell-cell adhesion protein involved in tight junction formation in epithelial and endothelial cells. We have previously linked JAM-A gene and protein over-expression in breast tumours with an increased risk of metastasis. In this study we sought to identify if JAM-A over-expression was associated with specific subtypes of breast cancer as defined by expression of human epidermal growth factor receptor-2 (HER2), estrogen receptor (ER) and progesterone receptor (PR).
**Approach**: JAM-A immunohistochemistry was performed in two breast cancer tissue microarrays (TMA). In parallel, cross-talk between JAM-A, HER2 and ER was examined in multiple breast cell lines using complementary genetic and pharmacological approaches.
**Results**: High JAM-A expression in the TMAs correlated significantly with HER2 protein expression ($p=0.019$), ER-negativity ($p=0.001$), high grade breast cancers ($p=0.001$) and aggressive luminal B, HER2 and basal subtypes of breast cancer ($p=0.005$). JAM-A and HER2 were co-expressed at high levels *in vitro* in SKBR3, UACC-812, UACC-893 and MCF7-HER2 cells. Knockdown or functional antagonism of HER2 (using siRNA and AG825 respectively) did not alter JAM-A expression in any cell line tested. Interestingly however, siRNA knockdown of JAM-A decreased HER2 and ER-α expression and reduced AKT phosphorylation without affecting ERK phosphorylation. The downstream effects of JAM-A knockdown on HER2 and pAKT protein levels were reversed upon treatment with the proteasomal inhibitor MG132, suggesting that JAM-A regulates HER2 degradation.
**Conclusions**: JAM-A is co-expressed with HER2 and associates with aggressive breast cancers. We have shown for the first time that JAM-A regulates HER2 degradation and downstream AKT activity, suggesting that JAM-A positively regulates oncogenic HER2 signalling. We therefore speculate that drugs targeting JAM-A may offer promise as novel therapeutic targets in HER2-positive breast cancers.

**AVEN: CELL CYCLE AND APOPTOSIS REGULATION IN MEIOSIS AND MITOSIS**

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Aven is a regulator of apoptosis whose expression is associated with poor prognosis in several cancers, including childhood acute lymphoblastic leukemia, acute myeloid leukemia and breast cancer [1,2]. Aven is also overexpressed in an ovarian carcinoma cell line resistant to the chemotherapeutic agent vincristine [3]. Following DNA damage, it was shown that Aven activates ataxia-telangiectasia-mutated (ATM) kinase to inhibit G2/M cell cycle progression [4]. Aven also inhibits the mitochondrial apoptosis pathway by binding to and inhibiting the self-association of pro-apoptotic Apaf-1 and binding to and enhancing Bcl-xL activity [5,6,7,8]. *Xenopus laevis* oocytes and embryos provide a useful model for studying meiotic and mitotic cell cycles. To understand Aven's role in oocyte and embryo survival and its regulation during meiosis/mitosis and the DNA damage response we have studied the expression and function of Aven during progesterone induced oocyte maturation and the early embryonic cell divisions [9]. The progesterone induced onset of meiotic maturation results in an initial loss of Aven followed by a gradual increase in the protein during metaphase I & II. Bioinformatic analysis of the Aven promoter has revealed progesterone response elements indicating the gene may be a direct target for steroid hormone regulation. Knockdown of Aven in the oocyte using an antisense morpholino oligonuceotide resulted in precocious oocyte meiotic maturation, determined by the onset of germinal vesicle breakdown (GVBD). Following GVBD the oocytes lacking Aven protein subsequently underwent apoptosis. DNA damage induced apoptosis in oocytes resulted in the dissociation of Bcl-xl from Aven as determined by immunoprecipitation studies, thus oocyte survival may be maintained by the binding of Aven to anti-apoptotic Bcl-xl. Aven protein displays a cyclical regulation in cycling egg extracts and as in oocytes the interaction with BCl-xl is disrupted following gamma-radiation induced DNA damage. In summary, Aven is dynamically regulated during meiotic maturation and early embryoinc cell cycles with peak expression during metaphase in meiosis and mitosis. Aven protein is required for oocyte survival and a dissociation of Aven from Bcl-xl underlies the onset of DNA damage induced apoptosis. Additionally we provide evidence that Aven may be a direct target for progesterone regulation.

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## ASSESSMENT OF CARDIOVASCULAR TOXICITIES ASSOCIATED WITH THE RECEPTOR TYROSINE KINASE INHIBITOR SUNITINIB: A MULTI-MODALITY IMAGING (MMI) APPROACH

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The multi-targeted tyrosine kinase inhibitor sunitinib is approved in several oncology indications. Associated cardiovascular toxicities include hypertension and congestive heart failure. Thus, cardiovascular function is closely monitored in at-risk patient populations. Nevertheless, an unmet need currently exists for reliable methods to facilitate early identification of cardiac toxicities. To this end, we have attempted to 'reverse translate' sunitinib clinical treatment protocols in mice and have interrogated resultant toxicities using MMI. Female Balb/CJ mice were treated orally with sunitinib over a clinically relevant dose range (20-40 mg/kg). Treatment protocols were: 5 days dosing, 2 days recovery or 4 weeks dosing, 2 weeks recovery. Cardiovascular function was assessed by cardiac ultrasound [Biomicroscope Vevo 770]. 18F-FDG-PET [LabPet/Triumph] scans were acquired to determine metabolic rate of glucose. Blood pressure measurements were taken at regular intervals. No significant change in blood pressure or cardiovascular physiology parameters were observed in the acute group. A significant elevation in blood pressure was noted following four weeks of treatment (40 mg/kg). Changes in cardiovascular physiology parameters and increase in glucose metabolism were observed following 40 mg/kg sunitinib treatment over the same period. Nevertheless, initial histopathologic analysis of cardiac (and other) tissues has shown no observable effects. Sunitinib may affect left ventricular function in two ways: Initially, following elevation of systemic blood pressure, afterload is increased and left ventricular ejection fraction falls. Subsequently, through off-target kinase inhibition cardiomyocyte apoptosis and hypertrophy is induced, evidenced much later at histopathology examination. Our data support these kinetics. Plasticity in cardiac energy substrate usage has previously been demonstrated during hypertension. This may explain the increase in FDG-PET signal observed following treatment .Further investigation of FDG PET as an early safety biomarker is warranted. Finally, feasibility studies undertaken in the current therapeutic setting using novel cardiac PET tracers (13N-ammonia and 11C-acetate) indicate several important considerations must be taken into account when implementing mouse models.

**STROMAL AKT2 CONTROLS EPITHELIAL INVASIONS**

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The tumour microenvironment is gaining importance in the regulation of tumourigenesis, invasion and metastasis. Loss of PTEN, a negative regulator of AKT, in stromal fibroblasts results in an increased tumour occurrence in ErbB2 expressing murine mammary tumours. Furthermore, we have shown that activation of AKT in stromal fibroblasts, following loss of the Retinoblastoma Protein (Rb), induces epithelial invasions in organotypic raft cultures, via an increase in Keratinocyte Growth Factor (KGF) expression. In addition, we have shown that loss of the PTEN tumour suppressor in stromal fibroblasts and subsequent activation of AKT, increases invasive potential of Human Papilloma Virus Type 16 E6 and E7 expressing keratinocytes. This is mediated by an increase in KGF expression. There are three isoforms of AKT expressed in human fibroblasts, AKT1, AKT2 and AKT3. By depleting each individual AKT isoform in stromal fibroblasts we have been able to establish that AKT2 is essential in regulating the cross talk between epithelium and stroma, and depletion of stromal AKT2 inhibits invasions. However, loss of stromal AKT2 does not function exclusively through regulation of KGF levels as it activates a second, protective pathway, which is able to counteract the effects of KGF. This protective pathway is mediated by increased Interleukin1 beta levels produced by the fibroblasts. Preliminary data from head and neck cancer specimens show that AKT is activated in stroma of cells associated with tumour but not normal tissue. Future work will determine the AKT dependent pathways that lead to increased KGF and Interleukin1 beta expression as well as how these factors mediate invasions.

## CYCLIN D1, CLUSTERIN, CYTOCHROME P4504F2 AND LIPID PEROXIDATION LEVELS SEGREGATE GOOD AND POOR STAGE II COLORECTAL CANCER TUMOURS AND REGULATE MITOCHONDRIA FUNCTION

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Background: 30% of stage II Colorectal Cancer (CRC) patients relapse within 5 years with survival rates similar to Stage III disease. Molecular markers to segregate Stage II patients likely to relapse are warranted. Using array screening, we identified significant alterations in cyclin D1, cytochromeP450 4F2 and clusterin at the gene level between good versus poor stage II tumours which are hypothesised to regulate both cell cycle and mitochondria function.
Aim: The aim of this study was to examine the expression of cyclin D1, clusterin, cytochrome P450 4F2 and a mitochondrial lipid peroxidation marker, 4HNE in 260 stage II cases by immunohistochemistry and correlate with patient outcome and secondly examine the functional role of cyclin D1 and clusterin in regulating mitochondrial function.
Method: We assessed cyclin D1, clusterin, cytochrome P450 4F2, and 4HNE in 260 stage II cases and the percentage positivity and intensity was graded. In parallel, we developed stable CRC lines overexpressing cyclin D1 and Clusterin to examine their role in regulating mitochondrial function. Mitochondrial function was measured by ROS levels, mitochondrial membrane potential, mitochondrial mass and ATPase levels using florescence based assays.
Results: High levels of stroma nuclear Cyclin D1 expression correlated with poor patient survival ($p=0.001$). High levels of stroma cytoplasmic cytochrome P450 4F2 and clusterin in correlated with poor survival ($p=0.016$, $p<0.001$ respectively). High levels of nuclear 4HNE expression in epithelium cells of stage II tumours correlated with poor patient survival ($p=0.003$). A strong association was detected between Cyclin D1, cytochrome P450 4F2 and clusterin ($p<0.001$, 0.001 respectively). Our mechanistic studies showed no significant change in mitochondrial membrane potential. However, overexpression of cyclin D1 and clusterin both caused a significant increase in ROS levels (p values $<0.05$), a reduction in mitochondrial mass (p values $<0.001$) and a reduction in ATPase production (p values $<0.001$). Addition of antioxidants reversed these effects.
Conclusion: High cyclin D1, clusterin, cytochrome P450 4F2, and 4HNE lipid peroxidation levels segregates good and poor stage II colorectal tumours. Over expression of cyclin D1 and clusterin both significantly regulate mitochondria function in colorectal cancer and the balance of antioxidants can influence this mitochondria dysfunction.

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# PROFFERED PAPERS 2 SESSION

**(Listed as Scheduled)**

**P63 BINDING ANALYSIS REVEALS A ROLE FOR AP-2 FAMILY CO-TRANSCRIPTIONAL REGULATION**

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p63 is a transcription factor belonging to the p53 family of proteins with a key role in epidermal differentiation. Previously, we have identified genome wide p63 binding sites by ChIP-Seq in primary human foreskin keratinocytes (HFKs). The potential p63 transcriptional targets were enriched for genes involved in epidermal development, differentiation and tumourigenesis. Interestingly, subsets of genes implicated in cleft palate were found to have p63 binding sites. In silico analysis indicated that Activator Protein-2 family binding motifs were significantly more abundant in the identified p63 binding regions. Mutations of one family member, AP-2alpha, are associated with severe craniofacial clefting. In addition a SNP in the AP-2alpha binding site on the IRF6 enhancer (adjacent to a p63 binding site) predisposes to non-syndromic cleft palate.
We were able to demonstrate that AP-2alpha acts as a co-factor with p63 both in primary HFKs and H1299 cells to enhance activation of the IRF6 enhancer and the PVRL1 promoter. Furthermore another member of the AP-2 family, AP-2gamma, was shown to have similar activity with p63. Our results reveal a novel interplay between p63 and AP-2 factors in the regulation of genes important in palatal fusion. This is intriguing since palatal fusion requires coordinated control of apoptosis, growth, migration and epithelial-to-mesenchymal transition (EMT), processes which are subverted during carcinogenesis. Increased AP-2alpha and p63 expression have been documented in Head and Neck Squamous Cell Carcinoma (HNSCC) patients. Future experiments hope to correlate expression of p63 isoforms and AP-2 family members in HNSCC samples to further explore the role of p63 in tumourigenesis.

**ASSESSING THE INFLUENCE OF EXOSOMES IN TRIPLE-NEGATIVE BREAST CANCER**

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Triple-negative breast cancer (TNBC) is responsible for 15-20% of breast cancers; is associated with occurrence in younger women; and is responsible for a disproportionate number of breast cancer deaths. Our analysis investigated the relevance of exosomes (i.e. membrane-bound nano-sized vesicles expelled from cells and found extracellularly which may play a role in cell-to-cell communication (1)) in TNBC. Specifically, we aimed to compare the effects of exosomes derived from Hs578T, its more invasive sub-clone, Hs578Ts(i)8 variant (2), and from TNBC compared to normal serum.

Exosomes were isolated from the conditioned media (CM) of Hs578T and Hs578Ts(i)8 cells and from sera specimens by filtration and ultracentrifugation (3). Detection of the exosome marker, Tsg101, confirmed successful isolation from both CM and sera. Investigating possible functional relevance, our analysis demonstrated that these exosomes alter secondary cells. Specifically, Hs578T(i)8-derived versus Hs578T exosomes increased the proliferation of SKBR3, MDA-MB-231 and HCC1954 cells by 53% (p=0.0163), 94% (p=0.0179) and 58% (p=0.0476), respectively. Similarly, Hs578T(i)8 exosomes also increased the motility of SKBR3, MDA-MB-231 and HCC1954 cells by 164% (p=0.033), 292% (p=0.0095) and 27% (p=0.001), respectively. Hs578Ts(i)8 derived exosomes increased the invasiveness of SKBR3 by 42% (p=0.0164); MDA-MB-231 by 52% (p=0.0004); and HCC1954 by 19% (p=0.0210) compared to Hs578T exosomes. Hs578Ts(i)8 compared to Hs578T- exosomes reduced SKBR3 anoikis resistance by 13% (p=0.006); a results consistent with the observation that Hs578Ts(i)8 cells are innately less resistant to anoikis (by 14%; p=0.009) than Hs578T cells. Preliminary data showed that Hs578Ts(i)8 exosomes reduce MDA-MB-231 anoikis resistance by 7%. Furthermore, Hs578Ts(i)8- versus Hs578T-derived exosomes also stimulated 166% (p=0.0004) more tubules formation during vasculogenesis. Considering their clinical relevance, exosomes from TNBC serum increased SKBR3 invasion by 123% (p=0.013) compared to exosomes from control sera (n=12).

This study supports the hypothesis that exosomes may be involved in cell-to-cell communication with exosomes from primary cells causing adverse effects on secondary cells, reflecting the characteristics of the cells from where they originated. Future studies planned include assessing the relevance of these exosomes in vivo.

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**ELUCIDATING MECHANISMS UNDERLYING TBX2 PATHOGENESIS IN BREAST CANCER IN ORDER TO DEVELOP NOVEL TARGETED THERAPIES**

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TBX2 is a transcriptional repressor known to drive breast cancer proliferation. It is known to switch off a number of key cell cycle regulatory molecules such as p21WAF1 and p14ARF. A breast cancer Disease Specific Array identified >700 potential TBX2 regulated genes. We have validated one particular target, Cystatin 6 (CST6), to be consistently repressed by TBX2 in a panel of breast cancer cell lines. CST6 is a putative breast tumour suppressor and we observe that exogenous expression of CST6 resulted in cell death of breast cancer cells but not in non-tumourigenic breast cell lines. Mechanistically, we show that TBX2 transcriptionally represses CST6 in an EGR-1 dependent manner rather than through direct CST6 promoter binding.

CST6 is a cysteine protease inhibitor known to repress several members of Clan CA (papain family) and Legumain (LGMN), an asparginyl endopeptidase (from the Clan CD family). LGMN has previously been reported to be highly expressed in breast cancers compared to normal breast tissue. We show that a point mutation in the LGMN inhibitory domain of CST6 prevented its ability to induce cell death in breast cancer cell lines. Consequently, knockdown of LGMN by siRNA resulted in cell death in a panel of breast cancer cell lines. We have also generated small molecule inhibitors which show growth inhibitory effects on breast cancer cells but not in non-tumorigenic MCF10A cells. Novel inhibitors targeting LGMN activity may be used for targeted treatment for breast and other tumours types showing high levels of LGMN expression where it is known to play roles in control of ECM remodelling/turnover, tumour growth and in the development of metastases. LGMN is also known to activate a number of downstream oncogenic proteins such as MMP-2 and TLR9. We are currently investigating the role of MMP2 in contributing to the LGMN mediated proliferation of breast cancer cells.

**SATB2 IS AN INDEPENDENT PREDICTOR OF OUTCOME IN COLORECTAL CANCER**

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SATB2 is a nuclear matrix-associated transcription factor that orchestrates gene expression in a tissue-specific manner by regulating higher chromatin structure. Using antibody-based screening of 48 normal human tissues and 20 cancer types, we identified SATB2 as a protein almost exclusively expressed in gastrointestinal tissue. Differential expression of SATB2 was observed in colorectal cancer, with loss of expression occurring along the adenoma-carcinoma sequence. We further confirmed this observation at the mRNA level in 10,533 colorectal cancer samples. Additionally, SATB2 expression was markedly decreased in a metastatic variant of SW480 colon cancer cells and siRNA knockdown of SATB2 expression in parental SW480 cells increased their growth and migratory capacity. Ectopic expression of SATB2 in the metastatic variant reversed the observed phenotype. Using tissue microarray and automated image analysis of SATB2 expression in colorectal cancers (n=309), SATB2 was demonstrated by multivariate Cox regression analysis to be an independent predictor of disease-specific survival (HR=0.52, 95% CI 0.32-0.83, p=0.006) and loss of SATB2 expression significantly correlated with poor differentiation (p=0.001). SATB2 mRNA levels were examined in a second cohort (n=290) and again, SATB2 was demonstrated to be an independent predictor of disease-specific survival (HR=0.40, 95% CI 0.18-0.92, p=0.031). Interestingly, in colorectal cancer patients, SATB2 levels significantly correlate with CD3+ T-cell infiltrates in the tumours (p=0.006) and inversely correlate with COX2 expression (p=0.019). Gene set enrichment analysis in two independent colorectal cancer cohorts (n>500) revealed that SATB2 low tumours demonstrate altered immune signalling with significant increases in IFNγ(p=0.001), IL6 (p=0.021) TFGβ (p<0.001). Furthermore, in patients with inflammatory bowel disease (n>1000), we observed a significant correlation between loss of SATB2 expression and occurrence of future cancers (p=0.013). We therefore postulate that SATB2 acts as a master regulator of the inflammatory response in the gut and loss of expression is significantly associated with the progression of colorectal cancer.

**MARKERS OF OXIDATIVE DAMAGE AND CIRCULATING IL6 LEVELS CORRELATE WITH SURVIVAL FOLLOWING TREATMENT WITH BEVACIZUMAB IN METASTATIC COLORECTAL CANCER PATIENTS**

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**Introduction:**

Angiogenesis drives cancer growth, tumour progression and metastases. Hypoxic tumours initiate recruitment of their own blood supply and enhance expression of vascular endothelial growth factor (VEGF). Bevacizumab is a recombinant humanised monoclonal anti-VEGF antibody which prevents VEGF binding to its receptors and improves overall survival in metastatic colorectal cancer patients when combined with cytotoxic chemotherapy. Currently, Bevacizumab is indicated as a first line treatment in all metastatic colorectal cancer patients, however only 38-44% of these patients will have a response to treatment. However, there is no good marker to predict treatment response. The role of inflammation and oxidative damage in driving angiogenesis and clinical response to Bevacizumab is poorly understood. The aim of this study was to investigate the levels of oxidative damage and inflammation in tissue and in the circulation in patients receiving Bevacizumab.

**Methods:**

Tissue from 80 patients was constructed into tissue microarrays (TMAs) and screened by immunohistochemistry for the levels of a DNA adduct marker 8oxodG and a lipid peroxidation marker 4HNE in addition to Ki67 status. Serum was screened for 8-oxo-dG, 4HNE and the inflammatory cytokines; IL1β, IL6, IL8, TNFα and pro-angiogenic factors; Ang 2, TGFβ, VEGF using ELISA. Data was correlated with patient survival following Bevacizumab treatment.

**Results:**

Following multivariate analysis, 8oxodG stromal nuclear positivity significantly correlated with survival following Bevacizumab ($p=0.035$) and this was independent of cell proliferation status. Circulating IL6 levels also significantly correlated with survival following Bevacizumab treatment ($p=0.01$). Using linear regression, circulating levels of 8oxodG correlated with IL6 levels ($p=0.016$). Circulating Ang 2 levels correlated with IL6 ($p=0.006$).

**Conclusion:**

We have shown for the first time that levels of a DNA adduct marker 8oxodG and circulating levels of IL6 correlate with survival in metastatic patients receiving Bevacizumab.

**FKBPL: AN IMMUNOPHILIN-LIKE PROTEIN WITH POTENTIAL AS A BREAST CANCER BIOMARKER.**

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Biomarkers provide important information on patient survival or likelihood of disease recurrence as well as predicting response to cancer therapies. Several important biomarkers in breast cancer, the oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), are routinely assessed to assign appropriate treatments for patients. ER-positive breast cancers are often assigned endocrine therapy however approximately 40% of patients do not respond or become resistant during treatment. It has therefore become important to identify additional biomarkers which could further inform treatment choice. We have identified an oestrogen responsive Hsp90 co-chaperone and immunophilin, FKBPL, which affects the stability and signalling of ER with implications for breast cancer growth and sensitivity to endocrine therapies. MCF7 cells stably overexpressing FKBPL demonstrate a slower rate of proliferation and become highly dependent on oestrogen for their growth. More importantly, this dependence on oestrogen renders these cells dramatically more sensitive to the anti-oestrogens, tamoxifen and fulvestrant. These FKBPL overexpressing cells also exhibit decreased levels of ER and the oestrogen responsive gene, cathepsin D, critical for breast cancer growth, survival and invasion, while knockdown of FKBPL using a targeted siRNA approach reverses these effects. Moreover, FKBPL knockdown is associated with decreased p21 expression and increased ER phosphorylation on Ser118 following treatment with 17β-estradiol or tamoxifen. Loss of p21 has been associated with a tamoxifen growth-inducing phenotype and hyperphosphorylation of ER at Ser118, with subsequent increased expression of ER-regulated genes. Our in vitro data support a model in which high levels of FKBPL would stabilise p21, decrease ER phosphorylation and abrogate tamoxifen-induced agonist potency thereby increasing drug sensitivity and suggest that FKBPL may have prognostic value that might impact upon tumour proliferative capacity and improve patient outcome. In support of this hypothesis, screening of a breast cancer tissue microarray demonstrated that high FKBPL expression was associated with smaller, low-grade tumours and prolonged recurrence-free survival (P=0.005). Overall FKBPL represents an important protein in p21 and ER signalling pathways which may have potential as a prognostic and predictive biomarker in breast cancer.

## THE ROLE OF THE STEROID RECEPTOR COACTIVATOR, SRC-1 AND ITS FUNCTIONAL PARTNER HOXC11 IN THE DEVELOPMENT OF ENDOCRINE RESISTANCE IN BREAST CANCER

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The steroid receptor coactivator; SRC1, has been well described in the development of endocrine resistant breast cancer[1]. SRC1 associates with clinically aggressive tumours [2] and promotion of distant metastasis [3]. SRC1 directly interacts with the developmental transcription factor, HOXC11 and together they are found to strongly predict poor disease-free survival in breast cancer patients (hazard ratios: 5.79; $P < 0.0001$) [4]. In this study, we investigate the mechanism of SRC1 and HOXC11 action in tumour adaptability and subsequent resistance to endocrine therapy.
Cells which are resistant to tamoxifen (LY2s) have enhanced SRC1 and HOXC11 mRNA and protein expression in comparison to their endocrine sensitive parent cells (MCF-7s). ChIP-sequencing data for SRC1- and HOXC11- DNA interactions in conjunction with DNA microarray and RNA-sequencing data identified potential signalling targets at play in the LY2 model of endocrine resistance. Real-time analysis and flow cytometry confirmed these interactions at a transcriptional and protein level. These observations were further confirmed in primary breast cancer cultures using flow cytometry. ChIP-sequencing data shows the ability of SRC-1 to repress the transcriptional activity of the luminal maker CD24 as well as the Hedgehog signalling pathway inhibitor, PTCH-1 in the endocrine resistant phenotype. SRC-1 also partners with HOXC11 and together they downregulate the common downstream targets; the tumour suppressor PAWR and the ETS-like transcription factor, ELK4. These observations may be very informative with regards to describing a new tumourigenic mechanism where SRC-1 can mediate the repression of well differentiated markers within a tumour cell whilst concomitantly instigating the recactivation of developmental pathways to enable the tumour to readapt itself and survive as it strives to become metastatic. This synergistic effect enables tumour cells to become increasingly steroid independent and thus better able to circumvent common targeted therapies such as Tamoxifen.

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# EACR YOUNG SCIENTISTS PRESENTATION ABSTRACTS

**GLOBAL CHARACTERIZATION OF THE SRC-1 TRANSCRIPTOME INDENTIFIES ADAM22 AS AN ER-INDEPENDENT MEDIATOR OF ENDOCRINE RESISTANT BREAST CANCER.**

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The p160 steroid coactivator protein SRC-1 is central to the development of endocrine resistant metastasis in breast cancer and is an independent predictor of disease free survival in tamoxifen-treated patients (1,2).

In this study SRC-1 ChIP-seq was performed in endocrine resistant (LY2) cells treated with tamoxifen to determine the SRC-1 regulated genes that mediate the resistant phenotype. Tamoxifen significantly increased the number of ChIP-enriched intervals detected. Combining ChIP-seq and microarray data, a list of 2,065 genes were identified as significant targets of SRC-1. From this list, ADAM22, a non-protease member of the ADAM family of disintegrins, was selected for validation as a potential novel metastatic oncogene in breast cancer (3).

We confirmed that tamoxifen treatment of resistant cells results in substantial recruitment of SRC-1 to the ADAM22 promoter. Cells derived from mammary tumours of the SRC-1-/-/PyMT mouse lacked ADAM22 compared to high expression observed in the wild type PyMT mouse. Furthermore, ADAM22 knockdown reduced cell migration and restored cell differentiation in resistant cells. In xenograft studies, tamoxifen treatment induced ADAM22 expression and increased volume of endocrine resistant tumours. In a TMA of breast cancer patient tissue (n=560) Kaplan–Meier estimates of disease free survival show ($p<0.0001$) that SRC-1 and ADAM22 are significant independent predictors of disease recurrence (odds ratios 2.18 and 2.4). Moreover, RNA-Seq analysis of matched primary and distant mets of breast cancer patients (n=3) revealed increased ADAM22 transcript levels with disease progression. We have developed an inhibitory peptide modelled on a natural ligand of ADAM22 (LGI-1) which can inhibit cellular migration of endocrine resistant cells in vitro. This peptide is now ready for in vivo testing using a murine xenograft model. Our discovery studies have uncovered a steroid independent SRC-1 mediated network in endocrine resistant breast cancer and has identified a new SRC-1 target, ADAM22. ADAM22 represents a rational new therapeutic target with a robust companion biomarker for treatment of endocrine resistant tumours.

1. Coassociation of estrogen receptor and p160 proteins predicts resistance to endocrine treatment; SRC-1 is an independent predictor of breast cancer recurrence. Clin Cancer Res. 2009 Mar 15;15(6):2098-106. Epub 2009 Mar 10. 2. Metastatic progression with resistance to aromatase inhibitors is driven by the steroid receptor coactivator SRC-1. McBryan et al. Cancer Res. 2011 Nov 22. [Epub ahead of print] 3. Global Characterization of the SRC-1 Transcriptome Identifies ADAM22 as an ER-Independent Mediator of Endocrine-Resistant Breast Cancer. Cancer Res. 2012 Jan 1;72(1):220-9. Research funded by Science Foundation Ireland B1853 (LY) the NIH (CA112403) (JX).

**BRCA1 – MASTER REGULATOR OF MAMMARY CELL DIFFERENTIATION**

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BRCA1 encodes a tumour suppressor gene that is mutated in the germline of women with a genetic predisposition to breast and ovarian cancer. It is a multifunctional protein with known roles in DNA damage repair, cell cycle control, ubiquitination and transcriptional regulation. More recently a role for BRCA1 in stem cell regulation and differentiation has been proposed. The breast is an example of a stratified epithelium consisting of (i) Estrogen Receptor-α (ER-α) positive milk-producing luminal cells and (ii) ER-α negative basal/myoepithelial cells which are in contact with the basement membrane and provide the contractile force to expel milk during lactation. Both cell types are thought to arise from a mammary stem cell which can give rise to bipotent progenitors, committed basal and luminal progenitors and ultimately mature basal and luminal cell types. Loss of BRCA1 leads to impaired differentiation and an accumulation of less differentiated cells resembling mammary progenitor cells which retain some stem cell like features. This phenomenon is even apparent in BRCA1 heterozygous mutant carriers implying it is an early event which may lead to subsequent tumourigenesis.

We propose that BRCA1 is acting as a master regulator of differentiation with a crucial role in the differentiation of progenitor cells into the more mature basal and luminal cells. This is achieved through the ability of BRCA1 to transcriptionally activate key regulatory factors in determining basal and luminal differentiation. We show that BRCA1 drives basal differentiation through its ability to transcriptionally upregulate p63, especially the ΔNp63 isoforms, while luminal differentiation is enforced by transcriptional regulation of the Notch pathway. This is reinforced by the regulation of other key luminal determination genes such as ERα and GATA3.

Loss of BRCA1 or the downstream p63 and/or Notch signalling pathways lead to impaired differentiation, an accumulation of immature stem cell like cells with an increase in cells possessing markers of basal-like breast cancer. Together these data suggest that BRCA1 functions to co-ordinate normal mammary differentiation and loss of this regulation through BRCA1 mutation or downregulation leads to an increase in immature stem-like cells that may drive basal-like breast cancer.

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## CHARACTERISATION OF A NOVEL PRECLINICAL MODEL OF EARLY STAGE COLORECTAL CANCER HIGHLIGHTS A KEY ROLE FOR THE RECEPTOR TYROSINE KINASE AXL IN CELL INVASION AND MIGRATION

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Background
Colorectal cancer (CRC) is the third most common cancer in the UK with around 80% of patients undergoing surgery followed by adjuvant chemotherapy treatment. Among patients with clinicopathologically defined poor stage II and stage III CRC, there are subsets of patients who will not benefit from adjuvant 5-FU or 5-FU/oxaliplatin treatment. In fact, there is preclinical evidence to suggest that some of these patients may actually do worse when treated with adjuvant chemotherapy. The aim of this study was to develop in vitro adjuvant colon cancer models with the ultimate goal to identify novel treatment strategies for early stage CRC.

Method
Progressively invasive populations from parental HCT116 were generated using invasion chambers. Analysis of migration and invasion was carried out using the XCELLigence system, protein activity/expression using receptor tyrosine kinase array (RTK) and Western blotting. Cell surface markers were analysed by flow cytometry and western blotting.

Results
Six sublines of HCT116 cells were generated (I1-6) which displayed an increasing invasive/migratory phenotype compared to the parental cell line. At the molecular level, we found increases in basal activity of a number of RTK and kinases in the invasive sublines, such as Axl (a phenomenon which is characterized with a more aggressive nature in cancers) and STAT3. In addition, exposure to sub-lethal 5-FU(IC20) doses resulted in significant increases in migration in these cell line models. Interestingly these sub-populations displayed characteristics associated with cancer stem cells with increases in CD44 expression and tumour initiating abilities. Silencing of Axl resulted in significant decreases in migration of the invasive subpopulations even in the presence of 5-FU. The generation of invasive cells lines was also carried out in a number of other cell lines which confirmed that an increase in Axl expression could increase the migratory phenotype regardless of the genetic background of the cell.

Conclusion
We have generated invasive CRC cells with an EMT and stem cell-like phenotype and altered RTK/kinase profile. Using siRNA and small molecule approaches, we are now using these models to identify novel treatment strategies in this disease setting.

**INHIBITION OF RIBOSOME BIOGENESIS INDUCES APOPTOSIS IN p53-/- CELLS**

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The nucleolus is an important region of the nucleus and the main site of rRNA transcription by RNA polymerase I (Pol-I). The number and size of nucleoli is one of the most prominent morphological markers of proliferative and invasive cancers. Pol-I transcription is the key rate limiting stage of ribosome biogenesis and upregulated rRNA synthesis fuels rapid cell growth and proliferation of cancer cells. It has been suggested recently that Pol-I transcription is a viable target for the development of anticancer therapeutics, as abrogation of Pol-I transcription leads to cessation of cell growth and eventual cell death. However, the exact mechanisms which link rRNA transcription and cell death are poorly understood.
We have found that ellipticine derivative 9-Hydroxyellipticine (9HE), is an efficient inhibitor of Pol-I transcription which efficiently represses rRNA synthesis by p53 independent mechanisms. Using 9HE and Actinomycin D (the only previously known Pol-I inhibitor) we investigated the effects of Pol-I inhibition in a p53-null background. Short-term treatments (≤24 h) led to decreased cell viability and a block of proliferation. At initial stages (2-4 hours) we observed activation of caspases 8 and 9, suggesting involvement of intrinsic and extrinsic cell death pathways. Consequently at the later stages (8 – 12 hours), we observed caspase 3 activation. Interestingly, repression of rRNA transcription also led to increased levels of reactive oxygen species. Long-term treatment (≥24 h) led to cellular senescence (as indicated by cellular morphology and the presence of senescence markers) and apoptotic cell death. Interestingly, the senescence is not accompanied by induction of autophagy. Following 24 h treatment we observed an accumulation of cells in the G0/G1 phase of the cell cycle and down-regulation of G1/S transition associated cyclins.
These data suggest that inhibition of Pol-I transcription in p53-null cells led to activation of cellular senescence and apoptosis. However, the mechanism(s) leading to the activation of these pathways remains unknown. Future work will be focused on investigation of the role of death receptors, involvement of mitochondrial signals and the role of Bcl2 family member proteins.

## IDENTIFICATION OF JAK2/STAT3 AS A NOVEL THERAPEUTIC TARGET IN KRAS MUTANT COLORECTAL CANCER

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Background: STAT3 is activated by Janus kinases (JAKs), which are recruited and activated by numerous cytokine receptors, receptor tyrosine kinases (including EGFR) and non-receptor tyrosine kinases (such as Src). A recent study has shown that high STAT3 activation is positively associated with adverse outcome in colorectal cancer, supporting its potential role as a therapeutic target. Kras mutations occur in 40%-45% of colorectal cancer (CRC) patients and confer resistance to EGFR mAb therapies. The aim of this study was to evaluate JAK2/STAT3 signalling as a novel Kras synthetic lethal pathway in CRC.
Methods: STAT3 and JAK2 inhibition was obtained using siRNA and small molecule approaches. Analysis of cell viability was carried out using MTT assay, apoptosis was measured using Western blotting and Flow cytometry and migration using the xCELLigence system. The isogenic KrasMT/WT HCT116 cell line model and a panel of KrasWT&MT CRC cells were used.
Results: Using different siRNA sequences, we found that silencing of STAT3 and JAK2 was lethal in the KrasMT HCT116 cell line compared to its KrasWT clone, and these results were confirmed using the small molecule JAK2/STAT3 inhibitor cucurbitacin. Similar data were obtained in our panel of KrasMT CRC cells. Interestingly, significant higher constitutive levels of pSTAT3 were observed in the KrasMT HCT116 cells compared to its WT clone. Combination of STAT3 or JAK2 silencing with MEK1/2 inhibition or chemotherapy (5-FU, oxaliplatin) resulted in synergistic decreases in cell viability and increase in apoptosis in KrasMT HCT116 cell line and this was associated with potent increase in STAT3 activity following MEK1/2i or chemotherapy. Furthermore, STAT3 silencing resulted in strong decreases in cell migration in KrasMT HCT116 cell line.

Conclusions: These results indicate that KrasMT CRC models are dependent on JAK2/STAT3 pathway for survival. We are now further evaluating the effect of JAK2/STAT3 inhibition in combination with MEKi/chemotherapy in in vivo models.

**TOWARDS NEW INHIBITORS OF PROTEIN KINASES: GUANIDINE ANALOGUES OF SORAFENIB**

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The drug Sorafenib (Fig. 1) is used for the treatment of kidney and liver carcinomas. It is the first oral multi- kinase inhibitor that targets Serine/ Threonine and receptor Tyrosine kinases in both the tumour cell and the tumour vasculature. These kinases play a key role in the regulation of cellular proliferation and death. Sorafenib has a dual action to stop the growth of cancer cells: It induces tumour cell apoptosis by targeting the MAP Kinase pathway involving the Raf/Mek/Erk[1] kinases and it also inhibits tumour angiogenesis by targeting receptors such as VEGFR 2, 3 and PDGFR.

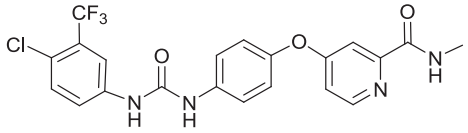


**Figure 1 Sorafenib structure**

Rozas' group has developed a family of compounds that are potential DNA minor groove binders and some of these compounds can also induce apoptosis in promyelocytic leukaemia HL-60, neuroblastoma Kelly and breast carcinoma MCF-7 cells. Considering the structural similarities between these compounds and Sorafenib, taking into account the 'rational' multi-target approach for the treatment of cancer and as a consequence of the promising results previously obtained we have prepared a number of analogues of Sorafenib.

During the synthesis of these molecules a novel simultaneous reduction of nitro and carbonyl groups was discovered. [2] Moreover, viability assays and CK-1δ and GSK-3 inhibition tests have been performed to assess their cytotoxicity and to evaluate their role as potential protein kinase inhibitors. A number of these novel compounds were found to inhibit HL-60 cell viability in the mM range, similar to efficacy of Sorafenib. Additionally they display 86-99% inhibition of the RAF-1/MEK-1 kinase pathway. Furthermore, one of these compounds shows 20% anti-angiogenesis activity by inhibiting the VEGFR at 10 mM. The inhibitory effect of these compounds was also tested on ERK-1/2 and p-38 MAPK and a moderate inhibition (20%) of both kinases was found.

We now plan to test their ability to induce apoptosis of a number of cancer cell lines. Computational docking studies will be executed to analyse the binding interaction of the compounds with the different targets in order to rationalise our biological results and allow for improvements in the anticancer activity of future compounds.

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**DEFINING A NOVEL BRCA1-EGR1 INTERACTION IN BREAST CANCER**

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Breast cancer arises as a consequence of loss of growth control mechanisms often as a result of loss of tumour suppressor function or aberrant activation of oncogenes. The familial breast and ovarian cancer tumour suppressor gene Breast Cancer associated gene 1 (BRCA1) is a multifunctional protein and germline mutations in BRCA1 predispose to early onset breast and ovarian cancers. BRCA1 is known to be a key player in orchestrating repair and cell cycle arrest following DNA damage and its ability to regulate transcription is central to this. Importantly, since BRCA1 cannot bind DNA in a sequence-specific manner it relies on its ability to interact with other transcription factors to target promoters. Another breast tumour suppressor is Early Growth Response 1 (EGR1), a zinc finger transcription factor possessing both activation and repression domains. EGR1 is thought to function as a convergence point for many signalling cascades with roles in growth control, stress responses and apoptosis and like BRCA1 has been shown to be downregulated in breast cancers. EGR1 has also been shown to be a direct transcriptional regulator of many other important growth regulatory genes including p53, $p21^{WAF1}$, TGFβ, and PTEN. Here we report a novel interaction between BRCA1 and EGR1, an interaction which is strongly enhanced following DNA damaging chemotherapy. We observe that loss of either BRCA1 or EGR1 results in the upregulation of markers of DNA damage and the induction of cell senescence. We show that the upregulation of some DNA damage response genes such as NDRG1 is abrogated upon BRCA1 or EGR1 loss. We will show how we plan to investigate this interaction further by minimally defining the interacting regions on both proteins and by describing the mechanism through which DNA damage signalling drives this association. Finally we will discuss implications for the development of novel targeted therapies for BRCA1 deficient breast cancers.

**EXPRESSION, DISTRIBUTION AND ABUNDANCE OF H2AX IN HUMAN CELLS.**

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DNA repair mechanisms are essential to combat genome damage which can lead to cancer. H2AX is phosphorylated in response to DNA damage and crucially acts as a trigger to activate the DNA damage response and recruit repair factors. Despite much investigation of this DNA damage response-related phosphorylation event, the relative abundance and distribution of unmodified H2AX has not been accurately determined. Experiments in human cells addressing the expression, distribution and abundance of unmodified H2AX are presented.
To address protein abundance, we are using quantitative western blotting of H2A and H2AX from human cells and mass spectrometry of $^{15}N$ labeled recombinantly expressed human H2A and H2AX as quantitation standards to compare with H2A and H2AX levels in human cells. The distribution of H2AX throughout the human genome is being explored using ChIP followed by next generation sequencing.
The relative cell cycle timing of H2AX and H2A expression is being investigated by quantitative PCR. We show that the peak timing of H2A and H2AX differ, as do the alternative transcripts from the H2AFX gene. These results suggest an explanation for the bias in H2AX localisation.

**INDUCTION OF AUTOPHAGY BY IMATINIB SEQUESTERS BCR-ABL IN AUTOPHAGOSOMES AND REDUCES BCR-ABL EXPRESSION**

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[2]Department of Haematology, Cork University Hospital, Cork, Introduction

Chronic Myeloid Leukemia (CML) is a disease of haematopoietic stem cells characterized by the Philadelphia Chromosome, which harbours the chimeric gene BCR-ABL. The resultant oncoprotein Bcr-Abl is a constitutively active tyrosine kinase. The expression levels of Bcr-Abl protein are critical for both the response to treatment with tyrosine kinase inhibitors and also disease progression, yet the regulation of protein stability is poorly understood.

Results

The distribution of Bcr-Abl in both murine and human CML cell lines was examined by cell fractionation and immunofluorescence. Bcr-Abl protein does not sediment with other soluble cytosolic proteins and its distribution is particulate in nature. As large macro-molecular complexes and abnormal proteins are often cleared by autophagy – we examined the potential contribution of autophagy to Bcr-Abl protein turnover. We have previously demonstrated that Imatinib can induce autophagy in Bcr-Abl expressing cells (1), however the contribution of autophagy to the turnover of Bcr-Abl protein has never been investigated. In this study we show that following Imatinib treatment, Bcr-Abl expression is down-regulated and it is sequestered into vesicular structures that co-localise with the autophagy marker LC3 and / or GABARAP. This co-localisation is inhibited by siRNA mediated knockdown of autophagy regulators (ATG7/Beclin1). Pharmacological inhibition of autophagy initiation with 3-methyladenine also reduced Bcr-Abl/LC3 co-localisation in both K562 and CML patient cells and increased the levels of Bcr-Abl protein.

Conclusion

The elevation of autophagy by Imatinib enhances the association of Bcr-Abl protein with autophagosomes and promotes protein down-regulation through autophagy. This may be an additional and important feature of the activity of Imatinib.

(1)Autophagy induction by Bcr-Abl-expressing cells facilitates their recovery from a targeted or nontargeted treatment. Crowley LC, Elzinga BM, O'Sullivan GC, McKenna SL. Am J Hematol. 2011 Jan;86(1):38-47.PMID: 21132731

## HSA-LET-7B AND HSA-MIR-100* ARE NOVEL MEDIATORS OF P21-REGULATED CELLULAR SENESCENCE

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Cellular senescence was first defined as the finite replicative lifespan of human fibroblasts in culture. Since then, it has emerged that senescence can be induced via multiple stimuli, including various stress signals, oncogenic signals or upregulation of cyclin-dependent kinase inhibitors, such as p21. Induction of p21 expression in HT1080 fibrosarcoma cells causes a senescent phenotype characterised by an inability to divide, activation of senescence-associated β-galactosidase expression, increased granularity and a large, flattened morphology. Mounting evidence demonstrates the crucial regulatory role of microRNA (miRNAs) in the wide range of vital biological processes, including cellular senescence. Here we describe a profiling study to identify novel miRNAs that are differentially expressed during p21-mediated senescence. MicroRNA profiles in HT1080 cells were compared in proliferating versus senescent cells using TaqMan Human MicroRNA Arrays (Applied Biosystems) to uncover expression level changes depending on p21-associated promotion of senescence. Several statistically significant ($p \leq 0.05$) microRNAs displaying altered abundance in senescent cells were identified. Among them, of particular interest were those that were exclusively expressed in cycling (hsa-miR-100*, hsa-let-7b, hsa-miR-30d, hsa-miR-422a) or senescent (hsa-miR-188-5p, hsa-miR-345, hsa-miR-425) cells. All of these miRNAs have previously been related to cancer in the literature, but there are no findings yet directly linking them to senescence. Decreased expression levels of hsa-miR-100*, hsa-let-7b were subsequently verified in senescent cells using individual Taqman assays. Functional miRNA inhibition using specific Miridian inhibitors was then performed in HT1080 cells, in order to validate the role of hsa-miR-100* and hsa-let-7b as regulators of senescence. The downregulation of either miRNA by Miridian inhibitors was demonstrated to promote cellular senescence in HT1080 cells by over 11-fold in both cases. Although role of hsa-let-7b and hsa-miR-100* as regulators of senescence requires further investigation, for example, identification of their potential target genes, we believe these miRNAs represent novel mediators of p21-regulated senescence.

**OBESITY DRIVES MITOCHONDRIAL DYSFUNCTION IN OESOPHAGEAL CANCER**

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**Introduction:** Obesity is a major health risk in western society and has a strong association with oesophageal cancer, which has a poor prognosis. How obesity drives the underlying cellular and molecular events associated with the development of oesophageal cancer is not fully understood. We hypothesise that mitochondrial dysfunction may be driven by factors released by viseral fat tissue in obese oesophageal patients which may accelerate disease progression.

**Materials and Methods:** Adipose conditioned media (ACM) was prepared from omental adipose tissue taken from viscerally obese and non-obese oesophageal adenocarcinoma patients. OE33 oesophageal adenocarcinoma cells were incubated with ACM from obese and non-obese patients for 24 h. Mitochondrial function was assessed by measurement of mitochondrial mass, mitochondrial membrane potential and reactive oxygen species generation by fluorimetry. Expression of 84 mitochondrial-associated genes was assessed using qPCR-based arrays. For each patient, visceral fat area (VFA) measurements were documented.

**Results:** Incubation of OE33 cells with ACM from obese individuals induced mitochondrial dysfunction, significantly increasing mitochondrial mass ($p = 0.03$) and mitochondrial membrane potential ($p = 0.02$), when compared to ACM from non-obese individuals. These alterations in mitochondrial mass and membrane potential also correlated with VFA ($R^2 = 0.5$, $p = 0.03$ and $R^2 = 0.6$, $p = 0.01$, respectively). ACM treatment also altered the expression of 4 mitochondrial-associated genes (*BNIP3*, *SLC25A1*, *SLC25A10* and *TOMM40*) > 3-fold in OE33 cells.

**Conclusion:** We have shown that obesity can significantly drive mitochondrial dysfunction and alter the expression of mitochondrial-associated genes. This suggests a molecular mechanism by which obesity may accelerate disease progression in oesophageal adenocarcinoma.

**MAPPING THE INTERACTION BETWEEN FLIP AND FADD**

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cFLIP is a major anti-apoptotic protein that blocks the apoptotic pathway mediated by death receptors. It is overexpressed in many cancers resulting in chemoresistance and limiting the effectiveness of commonly used anticancer therapies. Both the long (FLIPL) and the short (FLIPS) splice forms compete with procaspase 8 for binding with the adaptor protein FADD at the death inducing signaling complex (DISC), which is formed after stimulation of death receptors by their ligands. These interactions between FLIP, FADD and/or procaspase 8 respectively are mediated via their Death Effector Domain (DED) motifs. Developing a strategy to prevent recruitment of cFLIP to the DISC, thereby enhancing caspase-8 activation would be of potential therapeutic value.

The crystallographic structure of cFLIPS remains unresolved, limiting our understanding of the cFLIP-FADD interaction and hence the design of potential inhibitors of this protein-protein interaction. Using our knowledge of the DEDs in both FLIP and FADD, we have now performed computer modeling to map the possible interactions between cFLIP and FADD This modeling identified key residues which appeared to be important for mediating the cFLIP-FADD interaction. Site directed mutagenesis was performed against these key residues and a panel of cFLIP and FADD mutants was generated. These mutants were then screened for their ability to interact with either FADD or cFLIP using GST-pulldown assays. A functional assay for DISC recruitment was also carried out to confirm which of these mutations resulted in impaired recruitment. In conclusion we have established the critical orientation and important amino acids which mediate DED interactions between c-FLIP and FADD, forming a strong basis for our subsequent anti-cancer drug discovery studies.

**INVESTIGATING THE ROLE OF P53 GAIN-OF-FUNCTION MUTATIONS IN THE PATHOGENESIS OF BASAL-LIKE BREAST CANCER**

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Breast cancer is the most common cancer in women worldwide with the basal-like breast cancer (BLBC) sub-type displaying poorest clinical outcome. BLBCs are characterised by lack of expression of ERα, PR with normal HER2 status resulting in a lack of targeted therapies for these tumours. p53 is mutated in approximately 30% of breast cancers overall, however, BLBC and BRCA1 mutant tumours exhibit extremely high rates of p53 mutations (>80%), in particular gain-of-function (GOF) p53 mutations compared with other subtypes.

Mutation of tumour suppressor genes in breast cancer, for example BRCA1 or BRCA2, typically incur insertion or deletion mutations which commonly results in protein truncation or loss of expression. p53, however, incurs missense mutations resulting in expression of full length mutated proteins. These are often expressed at much higher levels than wild-type (WT) p53 implicating a role for mutant p53 in tumour progression. Mutant p53 proteins exhibit altered transcriptional regulation of target genes which results in loss of growth control and apoptosis.

Almost all p53 mutant proteins have lost ability to interact directly with DNA since most mutations occur in the central DNA binding domain. However, they are postulated to drive proliferation by modulating transcription of target genes, independently of p53 binding sites, by interacting with other transcription factors such as NF-Y, Sp1 or Ets1. Our preliminary data shows that mutant GOFp53 may rely on interaction with Ets1 for pathogenesis in BLBC. We have found that both p53siRNA and Ets1siRNA knockdowns in BLBC cell lines caused profound growth inhibition, an effect which was not seen in luminal breast cancer cell lines. We will use microarray profiling and e-siRNA screening to identify GOF mutant p53/Ets1 co-regulated targets with functional roles in BLBC pathogenesis which may represent novel therapeutic targets.

## THE IDENTIFICATION OF TBXA2R AS A DRIVER OF PROLIFERATION IN TRIPLE NEGATIVE BREAST CANCER

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Triple Negative Breast Cancers (TNBC) is defined by the lack of the ERα, PR and HER2 receptors. The majority of TNBCs are also basal-like breast cancers (BLBCs) which express basal cytokeratins. BRCA1 mutant breast cancers closely resemble the TNBC/BLBC subtypes and indeed BRCA1 expression is downregulated in up to 30% of sporadic invasive BLBCs. TNBCs have no specific therapy designed against them and therefore represent the subtype with the poorest clinical outcomes.Our aim is to identify genes involved in pathogenic events driving TNBC proliferation which could also represent novel therapeutic targets.
We have performed microarray profiling of 16 triple negative breast tumours (8 good and 8 poor FEC response tumours).Elevated levels of the thromboxane A2 receptor gene (TBXA2R) were observed in good outcome TNBCs,and triplicate siRNAknockdowns of TBXA2R consistently showed dramatic growth inhibition in BLBC cell lines but not in 'normal' HME-1 basal cells. TBXA2R is a G-protein coupled receptor with a well-established role in platelet activation and haemostasis and is known to regulate diverse cellular processes such as angiogenesis, cell survival and cytoskeletal arrangement. Upregulation of TBXA2R in inflammatory breast cancer has previously been shown.TBXA2R mRNA was higher in both Basal A and B lines than other subtypes and both Basal A and B cell lines were sensitive to knockdowns suggesting that TBXA2R may represent a novel driver of TNBC proliferation. We have also shown that TBXA2R mRNA is upregulated following BRCA1 siRNA (BRsi) in MCF10-A and MCF7 cells compared to scrambled controls and TBXA2R promoter activity is enhanced by knockdown of BRCA1 in T47D cells. We are currently performing promoter analysis on the proximal TBXA2R promoter region to identify the minimal BRCA1-responsive region and the transcription factor(s) responsible. We will then assess the role of TBXA2R in the pathogenesis of TNBC using in vitro cell models and tissue mircoarrays of TNBC.

In summary, our current understanding of the mechanisms underlying the pathogenesis of TNBC is lacking. Here we report a novel gene required for the proliferation of poor outcome TNBCs which could provide us with the opportunity to improve current therapy or develop novel, more effective treatments.

**THE CHROMATIN MODIFYING HMOF COMPLEX IN CANCER PROGRESSION**

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In humans, regulation of fundamental DNA-templated events such as transcription, replication, repair and recombination is largely managed through the modification of histone proteins. Histones physically associate with the DNA to form chromatin, and can be modified by the addition or removal of molecules, including: phosphate, acetyl and methyl chemical groups, as well as ubiquitin peptides. Such molecules can alter the interaction of histones with DNA or can act as binding sites for machines that carry out the above events at particular regions of chromatin.
Our Lab is particularly interested in the modification of histones by acetylation and ubiquitin. We are studying the human MOF complex [1]. This complex contains hMOF, which is a histone acetyl-transferase that is responsible for the acetylation of histone H4 at lysine 16 [2]. Such acetylation correlates with an increase in transcription, but recently, it was found that loss of hMOF and also reduction in H4K16 acetylation levels are frequent occurrences in various human cancers [3]. This complex also contains hMSL2, an ubiquitin ligase that can transfer a molecule of Ubiquitin to histone H2B at lysine 34 [4]. The role of this particular modification is still unclear, but initial studies suggest that it may be involved in transcription regulation and in DNA repair.

Thus, studying this hMOF complex allows us to investigate the roles of both these enzymes and the ensuing histone modifications in fundamental cellular events, and the development of cancer.

More at: www.chromosome.ie

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**INVESTIGATION OF POST-TRANSLATIONAL MODIFICATIONS OF C-FLIP**

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Cellular FLICE-inhibitory protein (c-FLIP) is an anti-apoptotic protein, which is over-expressed in multiple types of cancer and is associated with chemotherapy resistance and poor prognosis. It acts by inhibiting the membrane-proximal steps of death receptor-mediated apoptosis at the death inducing signalling complex (DISC). Both the long (c-$FLIP_L$) and short (c-$FLIP_S$) splice forms contain two tandem death effector domains (DEDs), homologous to procaspase 8. c-FLIP preferentially binds to FADD via its DEDs, inhibiting the recruitment and processing of procaspase 8 to its active form.
Post-translational modification of proteins can alter their activity, localisation within the cell and interactions with other cellular molecules. We have previously shown that HDAC inhibitors such as vorinostat (SAHA) down-regulate c-FLIP in various cancer types. To further understand the underlying mechanism, we have shown that c-FLIP down-regulation is blocked when cells are treated with the proteosome inhibitor MG-132. We have also shown that vorinostat treatment induces c-FLIP ubiquitination. Through an siRNA screen, we aim to identify the E3 ligases and deubiquitinating enzymes (DUBs) that regulate c-FLIP protein turnover. In addition, we have shown that c-FLIP is sumoylated and aim to identify the functional consequences of this modification.

Identifying and understanding the roles of the post-translational modifications involved in regulating c-FLIP protein turnover, localisation and anti-apoptotic activity may lead to a therapeutic strategy aimed at overcoming chemotherapy resistance by abrogating c-FLIP function.

**IDENTIFICATION OF AN ACETYLATION-DEPENDENT KU70/FLIP COMPLEX THAT REGULATES FLIP EXPRESSION AND HDAC INHIBITOR-INDUCED APOPTOSIS**

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FLIP is a potential anti-cancer therapeutic target that inhibits apoptosis by blocking caspase 8 activation by death receptors. We report a novel interaction between FLIP and the DNA repair protein Ku70 that regulates FLIP protein stability by inhibiting its polyubiquitination. Furthermore, we found that the histone deacetylase (HDAC) inhibitor Vorinostat (SAHA) enhances acetylation of Ku70, thereby disrupting the FLIP/Ku70 complex and triggering FLIP polyubiquitination and degradation via the proteasome. Using in vitro and in vivo colorectal cancer models, we further demonstrated that SAHA-induced apoptosis is dependent on FLIP down-regulation and caspase 8 activation. In addition, an HDAC6-specific inhibitor Tubacin recapitulated the effects of SAHA, suggesting that HDAC6 is a key regulator of Ku70 acetylation and FLIP protein stability. Thus, HDAC inhibitors with anti-HDAC6 activity act as efficient post-transcriptional suppressors of FLIP expression and may therefore effectively act as 'FLIP inhibitors'.

**IS RIBOSOME BIOGENESIS HORMONE INDUCIBLE?**

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Recently developed model of transcriptional activation of hormone dependent genes suggests that induction of transcription of such genes, for example by stimulating prostate or breast cancer cells with androgens or estrogens, respectively, may involve the formation of TopoII-beta induced transient DNA double stranded breaks (DSB), followed by recruitment of DNA repair proteins, chromatin remodelling factors and movement of the hormone induced genes to transcriptional hubs in the nucleus.

In light of these studies it is worthwhile to investigate if activation of steroid receptors by appropriate ligands would induce activation of ribosome biogenesis, the fundamental cellular process which is intimately linked to cells growth and proliferation.

Our preliminary results based on cellular models show that the treatment of androgen receptor (AR) positive prostate cancer cells with the synthetic AR agonist R1181 activates the key and rate limiting stage of ribosome biogenesis – the synthesis of rDNA within 2 hours of treatment. Importantly, this effect is receptor-dependent since Casodex (bicalutamide), a specific AR inhibitor, abrogates the activation of pre-rRNA synthesis. Similar effect was also observed in estrogen receptor (ER) positive MCF7 breast cancer cells when treated with estrogen.

We hypothesise that in steroid receptor positive cancer cells, transcriptional activation is not limited to hormone responsive genes transcribed by Pol-II, but it also affects transcription of rDNA by Pol-I. We propose that Pol-I associated TopoII-alpha might play a role in the activation of rRNA transcription similar to the role of TopoII-beta. In this case TOP2 recruitment can be utilised as a biomarker for identifying hormone dependent cancers and TOP2 inhibitors can be administered along with a hormone cycling therapy to treat such cancers.

Future work will be focused on the study of interactions of ligand-activated receptors (AR & ER) with the Pol-I machinery and delineation of the signalling pathways which link rRNA transcription and receptor's activation.

**AMPHETAMINE ANALOGUES – A NEW DIRECTION FOR HAEMATOLOGICAL MALIGNANCIES?**

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Amphetamines were used in the 1960s as antidepressants but today they are often used for illegal street highs. 4-MTA (4-methylthioamphetamine) an illegal amphetamine analogue has been previously shown to have anticancer effects in vitro. In this study we report the synthesis of a library of novel analogues and structural derivatives of 4-MTA; 1,3-bis(aryl)-2-nitro-1-propene derivatives, which contain a classic nitrostyrene structure, and assess their potential as anti-cancer agents.

Using the Alamar blue viability assay, a number of these compounds were shown to have antiproliferative activities in vitro in the haematological malignancies Burkitt's lymphoma (BL) and Chronic lymphocytic leukaemia (CLL). Both diseases are characterised by specific chromosomal translocations and involve clonal expansion of abnormal B cells in the lymph nodes (BL) and blood (BL and CLL). Some compounds also showed reduced toxicity against 'normal' peripheral blood mononuclear cells (PBMCs). Propidium iodide fluorescence-activated cell sorting (FACS) analysis and the involvement of caspases confirmed the cells were undergoing programmed cell death or apoptosis. A previous study suggests that such effects appear to be independent of the serotonin transporter, a high affinity target for amphetamines analogues. Compounds also displayed potent activity in the highly chemoresistant BL cell line (DG-75) and an unmutated $IgV_H$, poor prognosis CLL cell line (HG-3). Some compounds also showed potent activity in a cell line overexpressing the multi-drug resistant (MDR) proteins P-glycoprotein (P-gp) and BCRP suggesting the ability of these compounds to overcome resistance against conventional chemotherapies.

Enhanced antiproliferative activity was associated with a crucial nitrostyrene alkene bond. Hydroxy substitution also significantly increased potency when compared to chlorine whilst reduced efficacy was observed in the absence of a halogen or hydroxy substituent. It is hoped that such structure-activity information will aid in the design, synthesis and biochemical evaluation of a new library of second and third generation nitrostyrene amphetamine analogues with enhanced potency in cancer cells and reduced psychoactive effects.

## USE OF REOVIRUS, A SELECTIVE ONCOLYTIC VIRUS, IN THE TREATMENT AND PREVENTION OF METASTATIC CANCERS

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The need for novel therapy in the successful and curative treatment of cancer is rapidly growing. Oncolytic virology has long been noted as a potentially effective and non-toxic method of treatment but its full potential has only recently begun to be exploited.
Reovirus is a double-stranded RNA virus that has been repeatedly shown to be successful in the selective killing of transformed cells, specifically those that are mutated in the Ras oncogene or its affecter or effector pathways. More importantly, it is only mildly toxic, even when administered at high doses intravenously to patients.
Several previous studies have demonstrated the efficacy of Reovirus both in animal models and in patients, when delivered intratumourally. In this study, we have demonstrated the remission of B16, a mouse melanoma and a particularly aggressive and metastatic tumour, in a C57 mouse model. Induced tumours were treated at $1x10^7$ PFU twice weekly for three weeks. Remission was demonstrated by overall health of the mouse and measurement of the primary tumour.
In addition, we undertook to investigate if Reovirus could be utilised as a therapeutic vaccine in the treatment of B16, CT26, a mouse colon carcinoma and Lewis Lung carcinoma (LLC) models. A number of cells, considered to be ten times the tumourigenic dose for each model were infected *in vitro* with Reovirus at an MOI of 10. Following overnight infection the cells were then injected subcutaneously into the appropriate mouse model and monitored for an extended period of time to ensure no tumour development as compared to an uninfected control. This approach was successful in both the B16 and LLC models and successful in 66% of CT26 models. The mice were then re-exposed to each cell line and their immunity observed.

**IN VIVO BIOLUMINESCENCE IMAGING FOR CANCER AND GENE THERAPY**

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The ability to track a therapeutic in real time in the body is of enormous value for both preclinical and clinical investigations. Real-time in vivo analysis of reporter gene expression empowers the investigator with the ability to non-invasively assess tumour growth, and/or in the context of gene therapy, gene delivery over time, as well as host responses to therapeutic interventions. Various viruses and bacteria are in use as gene therapy vectors for cancer. Bioluminescent Imaging (BLI) represents a powerful tool for use with vectors or tumours engineered to express reporter genes such as firefly luciferase (FLuc) or bacterial lux.
In this study, activity of viral vectors and bacteria were examined in vivo by BLI. Viral vector trafficking and gene delivery in tumour bearing mice was examined using recombinant AAV2 or Ad5 vectors expressing FLuc from constitutive or tumour-selective promoters. We demonstrated the ability of the C-X-C chemokine receptor type 4 (CXCR4) promoter to restrict AAV expression to tumour cells in subcutaneous (s.c.) xenograft mouse models and in patient samples, using BLI. Various bacteria expressing the luxABCDE operon were intravenously (IV) administered to mice bearing subcutaneous (s.c) FLuc-expressing xenograft tumours. Bacterial lux signal was detected specifically in tumours of mice post IV-administration and bioluminescence correlated with the numbers of bacteria recovered from tissue. Through whole body imaging for both lux and FLuc, bacteria and tumour cells were co-localised. 3D BLI and micro-Computed Tomography (μCT) image analysis revealed a pattern of multiple clusters of bacteria within tumours. This study demonstrates for the first time the potential to simultaneously image multiple BLI reporter genes three dimensionally in vivo using approaches that provide unique information on spatial locations.

**LACTAM BRIDGE STABILISED BIM PEPTIDES PROMOTE APOPTOSIS IN PC3 PROSTATE CANCER CELLS THROUGH BAX ACTIVATION AT A NOVEL TRIGGER SITE: A PROMISSING STRATEGY FOR CANCER THERAPY.**

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The refractory nature of cancer cells towards mitochondrial-driven apoptosis is a key resistance mechanism to traditional chemotherapeutics. The mitochondrial apoptosis pathway is finely tuned by the BCl-2 family proteins, which play a pivotal role in the permeabilisation of the mitochondrial outer membrane and the subsequent release of apoptogenic factors from the mitochondrial inter-membrane space to cytoplasm. The family is subdivided into pro- and anti-apoptotic members the ratio of which will determine cell fate. Cancer cells increase their apoptotic threshold through the up-regulation of anti-apoptotic proteins.
The activation of BAX (a pro-apoptotic BCl-2 member) is known to promote mitochondrial apoptosis, a desirable objective for anti-cancer strategies. Gavathiotis *et al.* (2008) identified a new trigger site for Bax activation using stabilized alpha-helix Bim BH3 domain (SAHB) 20-mer peptides [1] and suggested a detailed activation mechanism in 2010 [2].
Based on this study, we have identified the shortest linear Bim peptide (10-mer) (truncated Bim, t-Bim) that can still activate Bax [3].

We now wish to report on the generation of lactam stapled alpha-helical Bim analogues of t-Bim (t-LSAB) that exhibit the ability to promote cell death in a range of tumor cell lines. The EC50 for those was determined, where t-LSAB (A) exhibits EC50 of 4.91 μM compared to those of linear t-Bim (5.26 μM) and linear Bim 20-mer peptide (4.75 μM).

Studies are currently in progress to indentify other key possible designs to stabilize the alpha-helicity of the t-Bim peptide and enhance its apoptogenic activity, a promising lead compound for cancer therapy.

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**FLUORESCENT RUTHENIUM-POLYPYRIDYL COMPLEXES INDUCE APOPTOSIS IN HELA CELLS IN A LIGHT DEPENDENT MANNER.**

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Photodynamic therapy (PDT) represents a new method of treatment in cancer, characterized by the combination of a light source with a photosensitizing agent. The therapeutic effect is mediated by the generation of reactive oxygen species (ROS) and/or the formation of singlet oxygen.

We have recently developed a novel Ruthenium(II) polypyridyl metal complex, RE37 ([Ru(phen)2(dppz)]2+) which exhibits photophysical properties due to the interaction of the triplet sensitizer with surrounding molecules. This results in photo-oxidative reactions; a positively charged ion which binds tightly to DNA via electrostatic binding to the phosphate backbone and water solubility. Electron accepting ligands confer a strong oxidizing excited state on the complex. These different properties make RE37 an excellent candidate for potential cancer therapeutics and the compound was thus evaluated for its ability to induce apoptosis in the cervical cancer cell line HeLa.

Localisation studies in HeLa cells using Confocal Microscopy showed the compound localised in the cytoplasm after 4 hours, at the edge of the nucleus after 8 hours and mostly in the nucleus after 24 hours. These results demonstrate the rapid uptake of the compound into cells and confirm the ability of the RE37 to bind to DNA. Flow cytometry analysis demonstrated RE37-induced apoptotic cell death in HeLa cells immediately after light exposure followed by an increase in cell death over time. Cell death occurred in a light dependent manner with little dark toxicity, underlining the importance of the interaction of photosensitizing agents with light and oxygen to obtain an effective biological response in PDT. In order to determine part of the mechanism of cell death, caspase inhibitor studies were conducted. These results showed a reduction of cell death in cells pre-treated with a general caspase inhibitor, indicating the involvement of caspases in RE37-induced apoptosis. Similarly, pretreatment of cells with N-acetyl-L-cysteine, a ROS scavenger, also showed reduced cell death in cells confirming the link between ROS production and the efficacy of photosensitisation.

The lack of toxicity in non-irradiated cells together with the above results suggest RE37 to be a good candidate for cancer therapy.

**INVESTIGATION INTO THE ANTI-CANCER EFFECTS OF NOVEL PYRROLOBENZOXAZEPINES IN NEUROBLASTOMA**

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**Background:** Neuroblastoma is the most common extra cranial solid tumor in early childhood accounting for 15% of cancer deaths in young children. Despite both advances in understanding the biology of the cancer, it continues to be one of the most difficult pediatric cancers to treat. A major obstacle in successful treatment is the development of multidrug resistance (MDR) to a range of cytotoxic drugs. This MDR phenotype often includes amplification of the proto oncogene MYCN, mutation/deletion of p53 and over expression of MDR proteins. We have recently demonstrated that members of a novel series of pyrrolo-1,5-benzoxazepine (PBOX) compounds are microtubule-depolymerising agents that possess the ability to potently induce apoptosis in several cancerous cell lines whilst eliciting minimal toxicity to normal cells. The PBOXs also induce apoptosis in *ex vivo* chronic lymphocytic leukaemia and chronic myeloid leukaemia (CML) patient samples including those that are resistant to imatinib. Furthermore, we have shown that PBOXs impair the growth of tumours in breast carcinoma and CML tumour models suggesting that the PBOXs are a promising group of potential anti-cancer therapeutics.
Previous studies have shown that PBOX-6 and 15 are not substrates for these MDR proteins therefore may have the potential to successfully treat MDR neuroblastoma. PBOX compounds may enhance apoptosis induced by conventional chemotherapeutic drugs such as vincristine, doxorubicin, etoposide and carboplatin.

**Methods**: Alamar Blue viability assay was utilized to determine the IC50 value for all drugs. The IC50 value is a measure of the decrease in cell proliferation. Propidium iodine stained cells were analysed by flow cytometry and the percentage of apoptotic cells was determined by quantification of the pre G1 peak. Colonies of SHSY5Y cells were counted using a Colcount.

**Results:** The IC50 values for PBOX-6 (1-2μM), PBOX-15 (60-100nM), vincristine (20-100nM), doxorubicin (180-250nM), etoposide (350-650nM) and carboplatin (15-40μM) in a number of neuroblastoma cell lines have been determined. PBOX-6/15 were shown to induce apoptosis in a number of neuroblastoma cell lines (including the MDR CHLA-90 line). PBOX-6 and 15 were shown to synergistically enhance the apoptotic effects of carboplatin. Additionally PBOX-6 was shown to work synergistically with etoposide and doxorubicin. PBOX-6 was shown to inhibit cell clonogenicity in a dose dependent manner.

**Conclusion:** The PBOXs have been shown to induce apoptosis alone and work synergistically in combination with current chemotherapeutic drugs, highlighting their potential as an alternative treatment for neuroblastoma.

**Future work:** We now aim to determine the molecular basis for the PBOX enhancement of the effects of conventional chemotherapy. Furthermore we intend to assess the ability of PBOXs to inhibit tumour growth in an *in vivo* neuroblastoma model.

## NUCLEAR FACTOR KAPPA B: A MECHANISM OF RESISTANCE TO CISPLATIN IN NON SMALL CELL LUNG CANCER

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***Introduction:***
Non-small cell lung cancer (NSCLC) is the leading cause of cancer morbidity and mortality in the Western world with a poor overall 5 year survival of <15%. The most effective systemic chemotherapy for NSCLC is cisplatin-based combination treatment. However, chemoresistance is a major therapeutic problem and understanding the mechanisms involved is critical to the development of new therapeutic intervention strategies. The PI3K/AKT/mTOR pathway plays an important role in NSCLC, representing a novel therapeutic target for overcoming cisplatin resistance.

***Methods:***
A panel of cisplatin resistant NSCLC cell lines including H460 and A549 were developed in our laboratory. H460 parent & resistant cell lines were screened for changes in mRNA expression using the PI3K Profiler array (SABiosciences). Changes in IkBa gene expression were validated by QPCR and protein expression by Western blot and High Content Analysis (HCA). IkBa exons 3-5 were screened for mutations by sequencing. NFkB levels were compared in parent and resistant cells by Western Blot and Immunofluorescence. The effects of DHMEQ (an inhibitor of NFkB translocation to the nucleus) on parent and cisplatin resistant cell lines were assessed by HCA and FACS analysis.

***Results:***
An 11.99 fold increase in IkBa mRNA expression was identified in the cisplatin resistant H460 cells compared to parent cells. QPCR, Western blot and HCA confirmed overexpression of IkBa in resistant cells. No mutations were identified in exons 3-5 of the IkBa gene. NFkB was over-expressed in the resistant cells and inhibition by DHMEQ led to significantly increased apoptosis in cisplatin resistant cells versus parent cells.

***Conclusions:***
The transcription factor NFkB regulates cell survival and the transcription of its own inhibitor, IkBa. No evidence of a truncated or mutated IkBa was detected here. Further work is warranted to elucidate whether mutations or post-translational modifications of NFkB are present here, leading to NFkB-mediated cell survival in cisplatin-resistant cells. Inhibiton of NFkB by DHMEQ resulted in a significant induction of apoptosis in resistant cells. NFkB inhibition may represent a novel therapeutic strategy to overcome resistance to cisplatin in NSCLC.

## TARGETING BREAST CANCER STEM CELLS WITH FKBPL AND ITS THERAPEUTIC PEPTIDE DERIVATIVE, AD-01

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**Aim**: Cancer stem cells (CSCs) are considered one of the main causes of tumour recurrence and resistance to chemotherapy and radiotherapy. The breast CSC (BCSC)-like population has an epithelial specific antigen (ESA) positive (+), $CD44^+$ and $CD24^{low}$ phenotype. Since FKBPL and AD-01 target and signal through the cell surface receptor, $CD44^1$, which is widely expressed on BCSCs, they could be very useful as novel anti-CSC therapies, thereby sensitizing these cells to both radio and chemotherapy.
**Methods:** The mammosphere (MS) assay was utilised in order to establish the effect of FKBPL/AD-01 on the BCSC population in the MCF-7 and MDA-231 cell lines. Small number of primary breast cancer samples was also analyzed. Flow cytometry was carried out to confirm the effect of AD-01 on $ESA^+/CD44^+/CD24^{low}$ subpopulation.
**Results:** AD-01 was highly effective, even at picomolar doses, at inhibiting mammosphere forming efficiency (MFE). The most significant reduction in MFE of 40% was achieved with 100 nM AD-01 in MDA-231, MCF-7 and ZR75 cells. Furthermore, cells stably overexpressing FKBPL also showed a similar reduction in MFE. Surprisingly, even highly chemo- and radiotherapy resistant metastatic primary breast cancer cells treated with 50 pM and 5 nM AD-01 showed a modest reduction in MFE. Flow cytometry confirmed the MFE results where the $ESA^+/CD44^+/CD24^{low}$ cell population was reduced by over 50% after AD-01 treatment. Pre-treatment of MDA-231 and MCF-7 cells with radiation and chemotherapy increases the MFE significantly. However, this increase in MFE was abrogated when AD-01 was added to the treatment. Furthermore, gamma secretase inhibitors in combination with AD-01 appear to have a synergistic effect leading to a significant reduction in MFE.
**Conclusion:** The results obtained so far are very promising. FKBPL and AD-01 already demonstrate well established growth inhibitory[2] and anti-angiogenic activity[1], therefore with AD-01's anti-CSC role; it could also prevent both tumour metastasis and recurrence, both features of resistant CSCs.
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## TARGETING ADAM17 AND NOTCH SIGNALLING AS A POSSIBLE THERAPEUTIC STRATEGY IN BREAST CANCER

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Introduction: The ADAMs are a family of multidomain, transmembrane proteins involved in multiple functions including activation of cell signaling. One of the signaling systems activated by ADAM17 is the NOTCH system. Notch activation has been found to contribute to the development and progression of breast cancer. In this study, we tested the hypothesis that inhibition of ADAM-17 and Notch signaling was a new therapeutic strategy for breast cancer.
Methods: We examined potential relationships between ADAM17 and Notch using a published microarray dataset. We used the ADAM17 specific inhibitor, PF-5480090 (Pfizer) and DAPT (N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester; Sigma) to determine their effects on breast cancer cell lines. Growth inhibitory effects were determined by MTT cell viability assay (Roche) 5 days post treatment. Effects on cell migration, Matrigel™ invasion and colony formation were also assessed.
Results: Using a published microarray dataset, ADAM-17 mRNA levels correlated positively with levels of Notch1 and Notch 3 ($p < 0.0001$, $p = 0.017$ respectively, Spearman Rank test). Patients with 'high' levels of ADAM-17 and Notch 1 (both transcript levels > 75th percentile) had a significantly shorter disease-free and overall survival compared to those with 'low' levels of both (DFS: $p = 0.006$, RR 2.3; OS: $p < 0.0001$, RR 3.4, Wald test). We assessed the effects of combining PF-548 with DAPT in a panel of breast cancer cell lines and found that the combination of the 2 compounds was significantly more effective at reducing cell viability in 4 breast cancer cell lines than either alone (for SKBR3 cells: $p = 0.0005$, for SUM159PT cells: $p = 0.05$, for CAMA1 cells: $p = 0.011$, for MDA-MB-231 cells: $p = 0.028$). In addition, using Hs578i8 cells, combined inhibition of ADAM17 and Notch resulted in a significantly greater reduction in invasion $p = 0.003$ vs PF-548, $p = 0.045$ vs DAPT) and clonogenic survival ($p < 0.001$ vs PF-548, $p = 0.002$ vs DAPT) compared to either alone.
Conclusion: The combination of PF-548 and DAPT resulted in significantly greater growth inhibitory effects in a panel of breast cancer cell lines compared to either of these agents alone. We therefore propose that ADAM17 and Notch may be targets for dual therapeutic intervention in breast cancer.

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## LUMINESCENT RUTHENIUM (II) POLYPYRIDYL FUNCTIONALIZED GOLD NANOPARTICLES AS CELLULAR IMAGING AGENTS

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Gold nanoparticles (AuNPs) are some of the most extensively studied nanomaterials. Because of their biocompatibility, unique size- and shape- dependence, optoelectronic and catalytic properties, AuNPs have attracted enormous amount of interest for applications in biological and chemical detection and analysis. Similarly, Ru (II)-polypyridyl complexes have been intensively studied due to their photophysical properties, where they have been employed for example in luminescent recognition and sensing, as sensitive and structure specific DNA probes. Ru (II)-polypyridyl complexes are known to bind to DNA and cause photosensitized DNA damage suggesting they may be suitable agents for cancer therapy. The combinations of Ru (II)-polypyridyl complexes, spatially separated from the surface of AuNps, by a covalent spacer, were investigated as luminescent probes/imaging agents and the results discussed herein. The AuNp-Ru (II) complexes were all shown to interact with DNA in a strong manner, using ethidium bromide displacement assays and measuring changes in the emission spectrum of the complexes once bound to DNA. Biocompatibility and intracellular fate of these AuNp complexes were studied in the human cervical cancer cell line (HeLa) and the Mesothelioma cell lines (CRL5195 and ONE58). Cell viability tests showed that there was no significant difference between light and dark toxicity of the AuNp-Ru (II) complexes, however differences in toxicity were seen between the three different cell lines, with the ONE58 Mesothelioma cell line being the most sensitive and little or no obvious cytotoxicity in the HeLa and CRL 5195 cell lines. Cellular uptake and localisation studies were then carried out using confocal fluorescence microscopy and transmission electron microscopy (TEM). As anticipated the AuNp complexes undergo rapid cellular uptake and can be seen localised within the cytoplasm and nucleus after 4h. This rapid uptake was further confirmed by fluorescence-activated cell sorting (FACS). These results suggest the AuNp complexes uptake may be due to either receptor mediated transport, or by plasma membrane driven transport due to their cationic nature. In summary these results confirm the potential use of such Ru (II) polypyridyl functionalised AuNps as highly sensitive cellular imaging agents.

Robert B.P. Elmes, Kim N. Orange, Suzanne M. Cloonan, D. Clive Williams, and Thorfinnur Gunnlaugsson. J. Am. Chem. Soc., 2011, 133 (40), pp 15862–15865

## Investigations into the potential of SMAC mimetic therapy in the treatment of Malignant Pleural Mesothelioma

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Malignant Pleural Mesothelioma (MPM) is a fatal, chemoresistant neoplasm arising from cells of the mesothelial lining of the pleural cavity and associated with prolonged asbestos exposure. MPM is extremely unresponsive to conventional chemotherapy and current research has been unsuccessful in developing effective clinical solutions[1]. MPM resistance is associated with elevated expression of Inhibitor of Apoptosis Proteins (IAPs), a protein family inhibiting caspase activation in response to apoptotic stimului[2-3]. XIAP is a central regulator of the caspase cascade, directly sequestering active caspase (-3/-7/-9), while cIAP-1/2 regulate TNFR/NFkB signalling and Caspase-8 activation[4]. IAP inhibitor protein SMAC and synthetic SMAC mimetics directly inhibit XIAP and induce degradation of cIAP-1/2, relieving apoptotic resistance and sensitising cells to chemotherapy in IAP resistant cancer models[5-7].

The potential for SMAC mimetic therapy for treatment of MPM was investigated. Results revealed a correlation between high IAP expressing MPM cells and resistance to chemotherapeutic challenge. Co-treatment with Embelin, a small molecule SMAC mimetic inhibitor of XIAP-BIR3, was found to sensitise IAP resistant MPM cells to cisplatin induced apoptosis (3.7% to 54.8% apoptosis). Embelin synergistically increased apoptosis in all XIAP expressing cells, confirming XIAPs role in MPM apoptotic resistance. Novel computationally designed SMAC mimetic compounds were developed based on a SMAC-AVPI pharmacophore. XIAP-BIR3 fluorescence polarisation and cIAP-1/2 degradation assays revealed that compounds did not bind their computationally predicted targets, but were able to synergistically increase cisplatin induced apoptosis in select MPM cell lines. Lead compouns induced apoptotic PCD, confirmed by caspase dependence, PARP cleavage and apoptotic morphology. Mechanistic analysis revealed MDG 44 induced G1 arrest, upregulation of proteosome regulated proteins (cIAP-1) and increase in cytoplasmic vesicles.

These compounds show potential as novel MPM targeting agents and future work will attempt to elucidate their mechanism/target of action and improve the in silico pharmacophore to develop novel SMAC mimetics for the treatment of IAP resistant MPM.

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## TARGETING uPA AND NOTCH SIGNALLING AS A POSSIBLE THERAPEUTIC AVENUE FOR BREAST CANCER

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Introduction: The Notch signalling pathway plays a key role in cell differentiation, survival, and proliferation and is frequently activated in breast cancer. The effects of NOTCH, appear at least in part, to be mediated by the serine protease urokinase plasminogen activator (uPA). Due to the role played by Notch and uPA in cancer progression, we hypothesised that targeted blockade of these 2 proteins may result in synergistic inhibition of breast cancer cell growth.

Methods: We first examined the correlation between the Notch signalling pathway and the uPA system in a publically available microarray dataset. In addition, we used the γ-secretase inhibitor DAPT (Sigma) alone and in combination with a uPA inhibitor, Pefabloc (Loxo) on breast cancer cell lines in vitro. We assessed their effects on cell viability (MTT: Roche), cell migration and colony formation.

Results: In the breast cancer extracts, Notch1 mRNA levels correlated positively with levels of uPA, plasminogen activator inhibitor 1 (PAI1) and uPA receptor (uPAR) ($p < 0.0001$, $p< 0.0001$, $p < 0.0001$ respectively, Spearman Rank test). Notch3 RNA levels correlated positively with levels of uPA, PAI1 and uPAR ($p < 0.0001$, $p < 0.0001$, $p = 0.012$ respectively, Spearman Rank test). In addition, levels of Notch4 mRNA correlated with uPAR mRNA levels ($p < 0.0001$, Spearman Rank test). Patients with 'high' levels of uPA and Notch 1 (i.e. both transcript levels > 75th percentile) had a significantly shorter disease-free and overall survival compared to those with 'low' levels of both (DFS: $p = 0.0001$, RR 9.2; OS: $p = 0.01$, RR 10.1, Wald test). Similar effects on patient outcome were observed for high uPA/high Notch3 and high uPA/high Notch4. In HCC1143 breast cancer cells in culture, we found that the combination of DAPT plus Pefabloc resulted in a greater inhibition on cellular migration compared to either inhibitor alone ($p = 0.035$ vs DAPT, $p = 0.05$ vs Pefabloc; Student's t-test).

Conclusion: Our results suggest that there is a relationship between the uPA system and the Notch signalling pathway which may be exploited as a therapeutic strategy for breast cancer.
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## AUTOPHAGY IN EPITHELIAL OVARIAN CANCER: TUMOUR PROMOTIVE OR TUMOUR SUPPRESSIVE?

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Macro-autophagy is a form of cellular cannibalism in which a double-membrane vesicle, the autophagosome, encapsulates cellular proteins and organelles. Autophagosomes fuse with lysosomes and degradation of the vesicle contents by lysosomal enzymes facilitates recycling of the damaged/aged proteins[1]. Autophagy can promote cancer cell survival induced by cytotoxic stresses characteristic of tumour microenvironments (e.g. hypoxia) and in response to chemotherapeutics[2]. Autophagy can also initiate cell death (TypeII programmed cell death/terminal autophagy) caused by excessive self-eating of the cell[3] or by inducing apoptosis[4]. Our research has investigated the role of autophagy in mediating cell survival and chemo-resistance to paclitaxel in ovarian cancer cell lines.
Resveratrol is a naturally occurring agent found in red wine which promotes autophagy[5]. Resveratrol (200 uM) induced a decrease in MTT cellular viability in the A2780, UPN251 and Ovcar7 at 24 and 48 h. Flow cytometry analysis (FACs) revealed that loss of viability in Resveratrol treated A2780 cell lines resulted from the activation of apoptosis. Conversely, the decrease in viability measured in the UPN251 and Ovcar7 cell lines in response to Resveratrol resulted from a G1/S phase cell cycle arrest. Resveratrol failed to activate autophagy-induced cell death in these cell lines. In addition the activation of Autophagy in the ovarian cancer cell lines treated with 100nM paclitaxel for 24 h was measured by immunoblotting for LC3. This data revealed that Paclitaxel induced a variable autophagic response in the chemo-sensitive A2780 and the paclitaxel-resistant UPN251 cells. Paclitaxel did not activate autophagy in the paclitaxel-resistant Ovcar7 cell line. FACs revealed that the inhibition of Autophagy with Bafilomycin activated apoptosis in paclitaxel treated UPN251. Therefore, the UPN251 cell lines may escape paclitaxel induced cell death through activation of autophagy to promote cell survival. This effect was not measured in the A2780 or Ovcar7 cell lines.
In conclusion, this data demonstrates that ovarian cancer cell lines have a varying dependence on autophagy to promote cell survival as a mechanism of chemo-resistance to paclitaxel. Further research into the variety of cellular response associated with chemo-resistance will lead to development of personalised chemotherapy for patients.

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## WHOLE GENOME SEQUENCING OF MATCHED PRIMARY AND METASTATIC ACRAL MELANOMAS

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Next generation sequencing has enabled the systematic discovery of somatic alterations in cancer samples. Here we used whole-genome sequencing, targeted whole-exome sequencing and RNA-Seq to characterise somatic mutations, structural variation and loss of heterozygosity in a primary acral melanoma and its lymph node metastasis. Our data show that the somatic mutational rates in this acral melanoma sample pair were more comparable to the rates reported in cancer genomes not associated with mutagenic exposure than in the genome of a melanoma cell line or the transcriptome of melanoma short-term cultures. Despite the perception that acral skin is sun-protected, the dominant mutational signature in these samples is compatible with damage due to ultraviolet light exposure. A nonsense mutation in *ERCC5* discovered in both the primary and metastatic tumours could also have contributed to the mutational signature through accumulation of unrepaired dipyrimidine lesions. However, evidence of transcription-coupled repair was suggested by the lower mutational rate in transcribed regions and expressed genes. The primary and the metastatic tumour genomes are highly similar at the level of global copy number alterations, loss of heterozygosity and single nucleotide variation. Furthermore, the majority of the single nucleotide variants (SNVs) in the primary tumour were propagated in the metastasis and one non-synonymous coding SNV and one splice site mutation appeared to arise de novo in the metastatic lesion.

**EXPRESSION OF TISSUE FACTOR, TISSUE FACTOR PATHWAY INHIBITOR AND VEGF IN OVARIAN CANCER; CORRELATION WITH VENOUS THROMBOEMBOLISM.**

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Introduction: Ovarian cancer is the leading cause of death from gynaecological malignancy and is known to display a particular association with venous thromboembolism (VTE) with reports up to 14% of patients with clear cell carcinoma of the ovary developing thromboembolic complication. Tissue Factor (TF) is a potent trigger for the coagulation cascade, which is regulated by Tissue Factor Pathway Inhibitor (TFPI). VEGF is a marker for angiogenesis. The aim of this study is to measure tumor derived TF and TFPI mRNA and protein and to investigate their potential role in ovarian cancer patient who develop VTE.

Methods: RNA and protein was extracted from 100 stored fresh frozen ovarian tumour sample (19 benign serous cystadenoma, 34 papillary serous cystadenocarcinoma, 16 clear cell carcinoma, 16 borderline ovarian tumours and 15 endometroid carcinoma). 20 cases complicated by VTE were identified and matched to 20 cases without VTE complication. Following reverse transcription; TF, TFPI and VEGF expression was measured using TaqMan real time PCR. TF and TFPI protein level were measured using ELISA assays.

Results: TF mRNA and protein expression was increased in tumours from patient with clear cell carcinoma ($p<0.0001$) and endometroid carcinoma ($p<0.008$) compared with benign tumours. TFPI expression was increased in clear cell carcinoma ($p<0.003$) and a slight increase was observed in endometroid carcinomas. The increase in TF demonstrated no correlation with VEGF expression. TF mRNA and antigen level was increased in malignant tumours of patients who developed VTE compared with malignant tumours of patients who did not develop VTE ($p <0.01$). There was no difference in TFPI or VEGF expression between the two groups.

Conclusion: The increased TF expression in ovarian cancer is associated with a higher risk of VTE. The increase in TF expression is not explained by increased angiogenesis. We consider TF derived from tumour itself as a likely trigger of VTE in ovarian cancer. TF expression was increased in clear cell ovarian cancer and endometroid cancer and this may explain the higher risk of VTE in this subgroup.

**ENDOGENOUS THROMBIN POTENTIAL AND MICROPARTICLES AS PROCOAGULANT MARKERS IN GYNAECOLOGICAL CANCERAbstract Title**

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INTRODUCTION: Gynaecological cancers are associated with an increased risk of venous thromboembolism (VTE), this is particularly prevalent in ovarian cancer and in patients undergoing chemotherapy.

AIM: To compare the procoagulant activity in plasma of patients with benign and malignant lesions of ovary and endometrium using the Endogenous Thrombin Potential (ETP) assay and Microparticle procoagulant (MP) assay.

PATIENTS AND METHODS: Blood samples were obtained following careful venepuncture from 89 patients with ovarian and endometrial cancers preoperatively . Thrombin generation in platelet-poor plasma was measured using the Calibrated Automated Thrombography. Lag time, peak thrombin production, area under the thrombin generation curve (ETP) and time to peak (TTP) were determined. MP procoagulant activity was measured using a functional assay.

RESULTS: Patients with ovarian clear cell carcinoma had higher peak thrombin production ($p<0.03$) and greater ETP( $p<0.003$)compared with benign ovarian tumours. Peak thrombin production and ETP was slightly higher in the neoadjuvant group compared with primary ovarian tumours who did not receive neoadjuvant chemotherapy. In addition the neoadjuvant group had shorter lag times ($p<0.004$) and shorter time to peak ($p<0.03$). Peak thrombin was slightly higher in the endometrial cancer group compared with the benign endometrial group. Addition of thrombomodulin prior to assay produced significant inhibition of ETP and peak thrombin production in all groups ($p<0.01$). In the neoadjuvant group peak thrombin production following thrombomodulin incubation was significantly higher than in benign ovarian group ($p<0.01$). There was no difference in MP procoagulant activity between different cancer groups.

CONCLUSIONS: Patients with clear cell cancer have increased procoagulant activity as measured by ETP assay. This procoagulant activity may be caused by the increased tissue factor expression by tumour tissue which we have reported. The increased procoagulant activity may explain the higher incidence of VTE in these patients. Following chemotherapy, patients have a slight increase in procoagulant activity compared to the chemotherapy naïve cohort. The increase in procoagulant activity was less marked in endometrial cancer.

**PROCOAGULANT ACTIVITY PROFILE IN GYNAECOLOGICAL CANCER PATIENTS POST SURGERY**

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INTRODUCTION: Gynaecological cancer is known to increase the risk of venous thromboembolism (VTE). Women having pelvic surgery are routinely given prophylactic low molecular weight heparin (LMWH) for 5 days post surgery to decrease the VTE risk. Despite this a significant number of women will develop VTE post discharge from hospital.
AIMS: The aim of this study was to evaluate the changes in procoagulant activity in women before and after surgery in patients with malignant versus benign endometrial and ovarian disease.

PATIENT AND METHODS: Blood samples were obtained following careful venepuncture in patients with malignant and benign ovarian and endometrial disease; preoperatively, on day 5 post surgery (4 hours post prophylactic LMWH), two weeks and 6 weeks post discharge from hospital. Thrombin generation in platelet-poor plasma was measured using the Calibrated Automated Thrombography. Lag time, peak thrombin production, area under the thrombin generation curve (ETP), time to peak (TTP) were determined. Microparticle (MP) procoagulant activity was measured using a functional assay..
Blood samples were obtained following careful venepuncture from 89 patients with ovarian and endometrial cancers preoperatively . Thrombin generation in platelet-poor plasma was measured using the Calibrated Automated Thrombography.

RESULT: During prophylctic LMWH therapy, procoagulant markers on day 5 post surgery were decreased in both groups. Lagtime was longer ($p<0.001$), ETP was lower ($p<0.001$), peak thrombin production lower ($p<0.002$), and time to peak longer ($p<0.00$) compared to pre-operative samples. Two weeks post surgery (when LMWH therapy had ceased), ETP was higher ($p<0.05$) in the malignant group compared to the benign group ($p<0.05$) and a slight increase was also found in peak thrombin. There was no significant difference between benign and malignant groups in preoperative, day 5 and week 6 samples. MP procoagulant activity was decreased post operatively but there was no significant difference between the groups.

CONCLUSION: ETP and MP-procoagulant activity were decreased while patients were on LMWH post operatively, however following cessation of LMWH, patients with gynaecological cancer had increased procoagulant activity compared with those with benign disease. This may be related to the cancer itself but may also be explained by a more prolonged and extensive surgery needed to treat the cancer patient compared to benign lesions.

**ROLE OF HMGA2 BINDING TO CHROMATIN DURING CELLULAR SENESCENCE**

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HMGA2 is a non-histone chromatin associated protein involved in growth regulation and pluripotency. HMGA2 can regulate transcription in two ways: By interacting with transcription factors to enhance their affinity for DNA, or by binding to DNA and affecting chromatin structure (1).

HMGA2 is abundantly expressed in early embryos and pluripotent stem cells but not differentiated cells. HGMA2 has been linked with many pleiotropic phenotypes and high levels of expression are frequently reported in benign and malignant tumours. Expression of a truncated form of HMGA2 is also shown to induce transformation (1). Paradoxically, HMGA proteins are shown to localise to the compacted chromatin of inactive X chromosomes and of Senescence Associated Heterochromatin Foci (SAHFs) where they act as structural components and contribute to the stable repression of genes associated with proliferation (2). The increase of chromatin bound HMGA2 in SAHFs coincides with a loss of linker histone H1 (3).
Despite its importance as a regulator of gene expression very little is known about the effect that HMGA2 binding to nucleosomes has at the level of chromatin structure. This study aims to investigate the hypothesis that HMGA2 binds specific nucleosome structures dependent on their DNA and histone variant composition to create SAHFs. HMGA2 interaction with nucleosomes is being characterised by binding, DNA footprinting and mutagenesis analyses.
Due to its toxicity to host cells recombinant expression of HMGA2 has proved to be challenging. Different expression vectors and purification strategies have been used to achieve optimal yield of HMGA2. Defined nucleosomes were produced *in vitro* for use in HMGA2 binding assays. High throughput PCR mutagenesis protocols have been optimised in the lab and will be used to carry out alanine scanning mutagenesis to uncover HMGA2 residues that are crucial for chromatin binding and compaction.

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**THE ROLE OF MYD88 IN CHEMORESISTANCE, DIFFERENTIATION AND HYPOXIA IN CANCER STEM CELLS.**

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Ovarian cancer is the leading cause of gynaecological cancer death worldwide. This high mortality is caused in large part by the development of chemoresistant recurrent disease. The origins of recurrent disease may be explained by the cancer stem cell (CSC) theory. CSCs are a minority population of cancer cells with stem like properties including enhanced proliferation. The adaptor molecule myeloid differentiation-primary response gene (88) (MyD88), is a key constituent of several toll like receptor (TLR) pathways. In normal circumstances TLR pathways mediate the body's response to pathogens; however MyD88 has been recently suggested as a possible marker of cancer stemness in ovarian cancer. Previous data from our lab has shown a negative correlation between MyD88 expression and patient survival. The work presented here investigates the involvement of MyD88 in the CSC response to chemotherapy, differentiation stimulus and hypoxia treatment.

Initial experiments assessing mRNA expression via qPCR analysis showed that MyD88 was involved in the CSC response to all three treatments in both a pluripotent and nullipotent cell line. Functional analysis was then carried out in both cell lines using siRNA gene knockdown and/or gene overexpression via plasmid insertion in each cell line as appropriate. To date this work has shown that knockdown of MyD88 expression in a nullipotent cell line causes them to differentiate in response to stimulus, concurrently overexpression of MyD88 in a pluripotent cell line removes their ability to differentiate in response to stimulus. Current work is assessing whether alterations in gene expression affect the characteristic response of both cell types to hypoxia and chemotherapy. An Affymetrix Gene Array has also been performed to assess how ablation of MyD88 expression affects downstream gene expression. This data is currently being analysed.

So far our data demonstrate the previously unrecognised importance of MyD88 expression to the characteristic responses of both pluripotent and nullipotent CSCs. We suggest that MyD88 may be a valuable prognostic indicator in ovarian cancer patients. Furthermore, blocking of MyD88 expression within the CSC population of a tumour may present a potential therapeutic target.

**OVARIAN CANCER STEM CELLS - IDENTIFICATION, ISOLATION and CHARACTERISATION.**

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Tumourgenicity studies in mice identify cancer stem cells (CSCs) as the founding cells of tumours. CSCs have been linked to chemoresistance, metastasis and relapse across a range of malignancies. Therapeutically targeting CSCs could remove the tumours malignant potential and may circumvent these negative outcomes. Currently, there is no standardised way to isolate CSCs. Ovarian cancer disease progression correlates with the predictions of the CSC hypothesis. In this work ovarian malignancy was chosen as a system in which to screen for CSCs. A range of commonly used techniques were utilised in order to understand the variation of CSC markers reported in the literature and to further understand the various aspects of ovarian malignancy. Six models representing various stages of ovarian malignancy and one model of non malignant ovarian surface epithelium were screened for the presence of CSCs and somatic stem cells respectively. Three flow cytometry based, CSC screens were implemented; ALDEFLUOR, Hoechst Side-Population and Cell Surface Protein Assays. Cells of interest were isolated via Fluoresence-Assisted Cell Sorting. Chemoresistance assays were carried out over 48 – 60 h.
Each screening technique identified putative CSCs (pCSCs) in one or more model systems. To date two of these cell lines have been sorted into their pCSC and non-pCSC sub-populations. In both cases the pCSCs were able to regenerate the non-pCSC phenotype. There was no difference in chemoresistance between pCSCs and non-pCSCs in either cell line. Both sub populations were isolated from chemoresistant cell lines (A2780cis and SK-OV-3). These findings fit with the phenotype seen in the clinic - once a tumour has responded to chemotherapy and relapsed both the differentiated and undifferentiated components exhibit chemoresistance.
To date we have shown that the various techniques for isolation of CSCs do not mark the same cells within an ovarian cancer context. Each 'type' of ovarian cancer stem cells appears to be mutually exclusive. This may reflect different stages of ovarian disease. pCSCs have been identified within chemosensitive cell lines, and work is in progress to isolate these sub-populations. Investigation of the differences between these chemoresistance and chemosensitive cancer stem cells could elucidate the mechanisms behind chemoresistant relapse.

**A ROLE FOR BRCA1 IN THE REGULATION OF RIBOSOME BIOGENESIS**

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BRCA1 is a well characterised tumour suppressor gene which has been found to have roles in DNA damage repair. Preliminary data suggests that it also plays a role in ribosomal biogenesis which enables cell growth and proliferation. This data carried out in a breast cancer cell line (T47D) shows a decrease in the rate of processing of the 47S subunit to the smaller subunits when BRCA1 is knocked out or the overall level reduced.

Preliminary results of co-localisation studies show that a fraction of BRCA1 may be co-localised with factors involved in Pol I transcription i.e. A135 (Pol I), UBF TAF63 and TBP (SL1). However it is unclear whether there is any interaction between the proteins or if they are just present in the same locations. To address this we performed BRCA1 immunoprecipitation from nuclear extracts of various cell lines (including MCF10A and MCF7). The results suggest that BRCA1 potentially can directly interact with some components of Pol-I transcription machinery.

Preliminary data carried out using ChIP assay on breast cancer cell lines shows that BRCA1 is present at the promoter and throughout the rDNA repeat but when DNA damage was induced a significantly higher level was observed overall.

**PROFILING OF MICRORNA IN SERUM AND BLOOD OF OVARIAN CANCER PATIENTS**

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Introduction: Ovarian cancer is the fifth most common cancer in women. In Ireland there are approximately 350 new cases per year and 255 deaths. The vast majority present in advanced stages and this is due to lack of a reliable screening test and the absence of symptoms. Despite an initial response rate of 65%–80% to first-line chemotherapy, most ovarian carcinomas relapse. Acquired resistance to further chemotherapy is generally responsible for treatment failure. There is a need for reliable diagnostic and prognostic markers for this disease. MicroRNAs are small, on average 22 nucleotide non-coding sequences of RNA, which control gene expression either by translational repression or degradation of the messenger RNA transcript. MicroRNA profiling has allowed the identification of signatures associated with diagnosis, prognosis and response to treatment of human tumours. The aim of this study was to profile miRNAs from serum/blood of patients undergoing surgery for ovarian disease to determine if miRNAs have the potential to be used as diagnostic or prognostic markers in ovarian cancer. In addition, the utility of miRNAs to be used as markers of chemotherapy response will be investigated.

Methods: Blood and serum collection is ongoing from all patients undergoing surgery for ovarian cancer and benign ovarian disease. Additional samples are being taken from patients undergoing chemotherapy for ovarian cancer. RNA extraction from serum/ blood was optimised using a modified TRI Reagent ® RT-Blood protocol. Profiling is being carried out using the TaqMan® array MicroRNA cards. A training set of 10 serous papillary ovarian adenocarcinomas and 10 benign serous cystadenomas have been analysed.

Results: Initial analysis has yielded a panel of miRNAs expressed in a percentage of the malignant cases and not in any of benign cases. These include let-7a, miR 7, 130b, 142-5p, 324-5p, 190, RNU44 and 603. miR-130b, 142-5p, 190 and 324-5p have not been previously described in ovarian cancer. Validation is ongoing on a larger sample population.

Conclusion: miRNA signatures may improve detection and treatment of ovarian cancer.

**AUDIT OF USE OF GEFITINIB IN FIRST-LINE TREATMENT OF EGFR MUTATION POSITIVE NON-SMALL-CELL LUNG CANCER (NSCLC) IN NORTHERN IRELAND**

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Background

• The IPASS study reported that patients with EGFR mutation positive locally advanced or metastatic NSCLC had significantly improved progression-free survival, objective response rate and quality of life when treated with gefitinib compared to those treated with paclitaxel and carboplatin

• Gefitinib has been available for use on single patient funding request basis in since March 2010

• The aim of the audit was to assess compliance with NICE guidance and with the business case & assess outcomes experienced in clinical practice compared with trial evidence

Methods

• A retrospective regional audit was performed for all patients who received gefitinib from March 2010 until December 2011

Results

• 18 patients were treated with gefitinib during the period March 2010-December 2011 (average of 9.8/12 months)

• There was 100% compliance with the business case and NICE guidance

• Response rate was comparable to IPASS study but numbers are small

• Data not mature enough for PFS or OS calculations

Conclusions

• All patients who received gefitinib were treated in line with NICE guidance and the business case

• The tolerability of gefitinib, as reported in the clinical trial is reasonably translated into actual clinical practice

Mok, T.S., et al., Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med, 2009. 361(10): p. 947-57.

**THE CLINICAL UTILITY OF HUMAN PAPILLOMA VIRUS STATUS AND P16INK4A/KI-67 AS A TRIAGE TOOL FOR LOW GRADE CERVICAL DISEASE**

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**Introduction:** There are considerable challenges over the appropriate triage of low-grade cervical neoplasia. The association of the Human papilloma Virus (HPV) in the development of cervical cancer has lead to the introduction of HPV DNA testing in many cervical screening programs. However, due to the high prevalence of transient HPV infections in low-grade disease HPV DNA triage in low-grade abnormalities is limited. The use of HPV E6/E7 mRNA detection and biomarkers such as p16INK4A/Ki-67 has the potential to identify clinically significant infections improving diagnostic specificity. The aim of this study is to investigate the utility of these markers for detecting women at risk of developing high grade cervical intraepithelial neoplasia (CIN2+).

**Methods:** Women presenting with persistent low-grade abnormalities LSIL (low grade intraepithelial lesion) and ASCUS (atypical squamous cells of undermined significance) were enrolled from the colposcopy clinic at the National Maternity Hospital, Dublin. Participants provided a cervical smear for HPV testing and immunocytochemical analysis for p16INK4A/Ki-67 dual expression. A biopsy was taken for histological diagnosis. HPV DNA testing was performed using Hybrid Capture 2 (Qiagen), HPV mRNA status was assessed using PreTect HPV Proofer (Norchip), p16INK4A/Ki-67 expression was determined using CINtec PLUS (Roche).

**Results:** To date 1029 patient samples referred with LSIL/ASCUS have been HPV tested from patients with a mean age of 33 years (range 18-65). A total of 240 (23%) had CIN2+ at first visit to colposcopy. Detection of HPV E6/E7 mRNA appears to be more specific 70% (95% CI 0.663-0.730) than HPV DNA testing 45% (95% CI 0.415-0.488) for detection of CIN2+. To date 160 cases have been tested for p16INK4A/Ki-67 dual expression, analysis is currently underway to investigate combined HPV and immunocytochemical testing for optimal clinical sensitivity and specificity.

**Conclusion:** This offers prospective evidence that HPV testing in the management of women presenting with low grade abnormalities could be useful in detecting those at risk of developing high grade disease.

This study is carried out under the umbrella of CERVIVA the Irish Cervical Screening Research Consortium funded by the Health Research Board and The Irish Cancer Society.

## GENERATION OF A NEW TRANSGENIC MOUSE STRAIN AS A MODEL FOR NON-INVASIVE IMAGING AND QUANTIFICATION OF MAMMARY TUMOUR DEVELOPMENT IN GENETICALLY MODIFIED MICE

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Background: Genetically modified animals are important research tools to study cancer because they reiterate the entire process from the initial genetic events in normal cells to metastatic disease. Numerous transgenic models have been created up to date to study breast cancer. Nevertheless, in spite of the advantages, many of the existing transgenic models for breast cancer are also burdened with difficulties in quantification of the transgene-induced tumours. There is a crucial need for making these previously generated models suitable for modern methods of tumour visualisation and quantification, e.g. by bioluminescence-based techniques. This approach was successfully used in the current study.

Results: A new transgenic mouse strain (MMTV-Luc2 mice) expressing Luc2 luciferase primarily in mammary tissue in females, with low-level background expression in internal organs, was generated and bred to homozygosity. After these mice were interbred with MMTV-PyVT mice, all bitransgenic females developed mammary tumours by the age of 10 weeks, the localisation and progression of which could be effectively quantified using the luminescence-based in vivo imaging. Luminescence-based readout allowed for early detection of the locally overgrown mammary tissue and for longitudinal evaluation of local progression of the tumours.

Conclusions: We have created a novel transgenic strain for early detection and quantitative assessment of mammary tumour development in genetically modified mice as an addition and/or a new and more advanced alternative to manual methods. This model, coupled with the most recent advances in in vivo bioluminescent imaging systems, provides for a powerful, yet very feasible, tool in studies utilising genetically modified animals. Specifically, generation of this mouse strain is vital for making many of the existing mammary tumour transgenic models applicable for advanced in vivo imaging techniques. It can also serve as a technology platform and a background strain for further development of new models.

**REAL TIME EXPRESSION PROFILING OF RNA BASED MARKERS OF PROSTATE CANCER IN HUMAN PROSTATE TISSUE AND URINE SAMPLES**

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Introduction and objective:

A fundamental challenge in prostate cancer (CaP) research is the integration of molecular markers into routine clinical use. Cumulative evidence supports a critical role for small regulatory microRNA molecules (miRs) in carcinogenesis. Differential miR expression is firmly established in differentiating CaP from normal tissue, and therefore miRs offer significant potential as an exciting new class of CaP biomarker. Similarly, huge diagnostic potential also rests in the non-invasive measurement of other RNA species, particularly detection of noncoding PCA3 transcripts and the TMPRSS2:ERG fusion gene mRNA in urine, both of which are highly specific to CaP. To date, the expression of CaP related miRs has not been investigated in urine. The aims of this study are to (i) profile urinary expression of 13 miRs , whose up-regulation in CaP has been definitively shown and (ii) to determine their performance in detecting prostate cancer in conjunction with and compared to gold-standard urinary markers PCA3 and TMPRSS2:ERG.

Materials and Methods:

A custom miR microarray compared miRNA expression profiles of 24 human prostate cell line samples (BPH1, PWRE1, DU145, PC3). Significance analysis of microarray data yielded miR's associated with dysregulation. First-catch urine samples were collected from 173 patients with suspected CaP, based on elevated PSA and/or abnormal DRE. Cellular and cell-free total RNA were isolated using Tri reagent and a Norgen RNA purification kit, respectively and reverse transcribed using a high capacity cDNA synthesis kit. PCA3 and TMPRSS2:ERG expression were quantified relative to PGK1 by qRT-PCR on a TaqMan 7900. miR cDNA synthesis was performed using a universal reverse transcription kit and expression calculated relative to let-7e and miR429 using locked nucleic acid primers and sybr green.

Results:

miRNA expression levels were measured by relative quantification (RT-qPCR). MiR-100 shows 7.9-13.25 fold upregulation in cancer cell lines relative to benign. miR-125 is increased 1.7-19.5 fold, miR-24 5.1-4.1 fold, miR-99a 1.5-2.3 fold, and miR-99b 2.4-4.5 fold in cancer cell lines relative to benign. Similar expression levels are observed in85 FFPE tissue samples (32 tumours, 47 benign and 7 HPIN).

Expression of these miRs is currently under investigation using custom TLDAs in the urinary cohort on the basis of these results.

## DEVELOPMENT AND COMPARISON OF PREDICTIVE MODELS TO PREDICT PROSTATE CANCER STAGE

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Prediction tools play a vital role in prostate cancer. Accurate prediction of cancer stage is essential for choosing the appropriate treatment strategy. Using multivariate logistic regression, we previously developed a prediction tool to predict prostate cancer stage at radical prostatectomy, where stage is defined as one of organ confined (OC), extracapsular extension (ECE), seminal vesicle invasion (SVI) or lymph node involvement (SVI). We analyzed data from 341 patients collected through the Irish Prostate Cancer Research Consortium database. Results showed that maximal predictive accuracy was seen for lymph node involvement (area under the curve = 90.8%). Prediction accuracy for seminal vesicle invasion and organ confined disease was 76% and 65.9% respectively. Poorest prediction accuracy was seen for extracapsular extension (area under the curve = 57.7%). The results clearly show that OC and ECE cancer cannot be accurately predicted using this model. This has also been illustrated by other researchers [1]. Following this we collected data from an additional 300 patients through the Irish Prostate Cancer Research Consortium database, with the aim to address the issue of predicting OC and ECE disease more accurately. Using the larger cohort we are currently investigating multiple statistical classification techniques which can be used to develop a model to predict organ confined (OC) or non-organ confined (NOC) cancer. So far we have developed a logistic regression-based model, a classification tree-based model and a support vector machine-based model. Results indicate that the logistic regression model has the best discriminate ability (area under the curve = 83.5%), followed by the support vector machine model (area under the curve = 73.4%). The classification tree model achieves a poor discriminate ability (area under the curve = 55.8%). The next step will involve developing models using further statistical classification techniques, such as K nearest neighbours, artificial neural networks, linear discriminant analysis and random forests.

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**THE EFFECTS OF NITRIC OXIDE AND NITROXYL COMPOUNDS ON CELLULAR PROLIFERATION IN PROSTATE CANCER.**

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**Introduction:** Nitric Oxide (NO) is a free radical produced by upregulation of inducible nitrogen oxide synthase (NOS2) during chronic inflammation. Nitroxyl (HNO) is the reduced form of NO which possesses distinct biological properties separate to NO. The mechanism of HNO synthesis remains unclear but it is known to be a downstream intermediary of NO. We hypothesize that chronic inflammation is instrumental in prostate cancer development and that NO plays a role in the transformation of normal cells. In addition, HNO and NO donors in the form of novel drugs can be effectively used in prostate cancer treatment. Using a selection of NO and HNO donors we are investigating their effects on prostate cell proliferation.

**Experimental Procedures:** To assess the potential transforming ability of NO, RWPE-1 normal prostate cells were exposed to 100, 300, 500µM of the NO donor, DETA/NO. In addition, the effects of IPA/NO (HNO donor) and DEA/NO (NO donor,) both separately and in combination with NSAIDs (IPA/NO-aspirin, DEA/NO-aspirin) were examined on androgen-dependent (CWR22), independent (22Rv1) and metastatic (PC3, DU145) prostate cancer cell survival at various concentrations using toxicity assays.

**Results:** RWPE1 normal prostate cells exposed to 100µM DETA/NO exhibited increased cell proliferation compared to non-treated controls. Exposure of RWPE1 cells to 300µM DETA/NO caused decreased cell proliferation, while 500µM had a cytotoxic effect on the cells. Cells exposed to 300 and 500µM DETA/NO exhibited significant changes in morphology, selecting for a fibroblastic-like phenotype. 250µM IPA/NO-aspirin induced cell death in the prostate cancer cell lines while increasing concentrations of IPA/NO alone did not. At lower concentrations, IPA/NO increased cell proliferation in all cell lines indicating concentration specific effects.

**Conclusions:** Normal prostate cancer cells treated with a NO donor showed altered morphology, suggestive of a more mesenchymal phenotype. Colony forming capacity, cell invasion, migration assays will be performed to assess the transformation ability of this compound. Prostate cancer cell lines treated with a HNO donor alone (IPA/NO) showed improved cell survival at increased concentrations compared to cell lines treated with the HNO donor coupled with the NSAID (IPA/NO-aspirin).

**A PROTEOMIC AND BIOINFORMATIC APPROACH TOWARDS BIOMARKER PANEL IDENTIFICATION IN PROSTATE CANCER: FROM DISCOVERY TO TARGETED VERIFICATION**

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**Background:** Prostate cancer (PCa) is the most common solid cancer diagnosed in men. Currently clinical widely used PSA test cannot distinguish indolent from aggressive PCa or different stages of the disease. Here, we used a label-free LC-MS/MS and multiple reaction monitoring (MRM) based proteomics workflow combined with bioinformatics and statistical approach for the discovery, prioritisation and verification of serum protein biomarker signatures for PCa.

**Methods:** Preclinical serum samples were collected from men with benign prostate hyperplasia, different Gleason score (GS 5 and 7) and stages (organ confined and non-organ confined) of PCa as part of the Irish prostate cancer research consortium bioresource. After removing the 14 most abundant serum proteins, proteomics profiling of these serum samples was carried out using label-free LC-MS/MS (n=30, pooled samples). MRM methods were then developed for candidate serum protein biomarkers and applied to independent clinical samples (n=63).

**Results:** Differentially expressed proteins were prioritized using feature selection and classification methods. Of, 64 candidate protein biomarkers included in the MRM development programme, high quality MRM assays have been developed for 31 proteins. In a refined and optimized assay for all 31 proteins, the mean CV (%) of their measurement using152 transitions was less than 7.0%. With this multiplexed MRM assay, 31 proteins were measured in 63 independent clinical serum samples from patients with different Gleason scores and stages. The 31 protein signature was tested using partlial least squares-discriminant analysis with 200 times bootstrapping and receiver operator curves were generated based on prediction results, which gave AUC values of 0.789 and 0.824 in classifying different Gleason scores and stages, respectively.

**Conclusions:** We are currently undertaking large scale validation study of this 31 protein signature on independent serum samples from the consortium and our international collaborators. With careful validation, these panels in combination with current clinical tools could improve diagnosis and thus patient outcome through the selection of appropriate treatment options.

**PTEN LOSS INDUCES SELECTIVE UP-REGULATION OF CXCL8 SIGNALING TO MODULATE SURVIVAL AND RESISTANCE TO RADIATION**

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PTEN is a haplo-insufficient tumour suppressor and the most frequently mutated gene in prostate cancer (CaP). Moreover, loss of PTEN expression arising from allele deletion or promoter methylation is prevalent early disease. The objective of our study was to characterise how the genetic instability and/or stress-induced signaling arising from PTEN loss modulated the expression of clinically-relevant pro-inflammatory chemokines. Transient (siRNA) or prolonged (shRNA) repression of PTEN resulted in increased transcription-mediated increases in expression of CXCL8 and its receptors, CXCR1 and CXCR1 in DU145 and 22Rv1 cells. Stress-induced expression of CXCL8, CXCR1 and CXCR2 was greater in magnitude and duration in PTEN-null PC3 and LNCaP cells, in contrast to the dampened response observed in PTEN-expressing cells. PTEN had no effect on basal or stress-induced expression of CCL2, CXCL12 or their receptors in prostate cancer cells. Moreover, the efficacy of autocrine CXCL8 signalling to induce HIF-1 and NF-κB transcription was also greater in magnitude and duration in PTEN-null prostate cancer cells or following siRNA-mediated PTEN knockdown in DU145 and 22Rv1 cells. Consistent with *in vitro* data, we observed increased expression of the orthologous murine chemokine KC in atypical cytological features of PTEN+/- prostate tissue relative to normal epithelium in PTEN WT glands. Finally, siRNA or shRNA-mediated attenuation of CXCL8 signaling selectively decreased the viability of CaP cells harbouring partial or complete PTEN-loss. Furthermore, the knockdown of CXCL8 signaling potentiated the sensitivity of PTEN-depleted prostate cancer cells to clinically-relevant doses of ionizing radiation. We conclude that there is a selective upregulation of CXCL8 signalling in PTEN-deficient prostate epithelium, which contributes to the growth, survival and reduced therapeutic sensitivity of these cells.

**Category:** Tumour Microenvironment

**Keywords:** Pten; CXC-chemokines; CXCL8

## HUMAN ENDOGENOUS RETROVIRUS ACTIVATION IN PROSTATE CANCERS: ASSOCIATION WITH DISEASE PROGRESSION

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Introduction: In recent years scientists have observed that several tumour types, including prostate cancer, show increased expression of human endogenous retrovirus (HERV) when compared to normal tissue. The HERVs originated from germ cell infections by exogenous retroviruses during the course of evolution and became incorporated into the human genome. These elements are widely dispersed throughout the genome and are estimated to comprise of greater than >8% of genomic material. The HERV-K family, evolutionarily the youngest HERV, is the only HERV family with complete open reading frames for all viral genes, and thus are the most likely to be biologically active and potentially pathogenic..
Hypothesis: We hypothesize that HERV-K expression is activated with advancing prostate cancer progression and that as a result of this, HERV-K leads to a sustained inflammatory response and subsequent promotion of tumour progression. We believe that HERV-K is suitable target of therapeutic intervention.

Experimental Procedures: For the detection of HERV-K gag, pol and env mRNA proteins were designed in Primer3, and verified for specificity in Primer-BLAST. Levels of HERV-K gag, pol and env type I or II mRNA were quantified in RWPE1 normal immortalized prostate cells, and CRW22 androgen dependent prostate cancer cell lines, and 22Rv1, PC-3 and DU145 androgen independent prostate cancer cell lines. HERV-K gag and env protein expression was detected in cell lines by western blotting and in prostate cancer tissue microarrays of hyperplasia and adenocarcinoma using anti-HERV-K gag monoclonal antibody (MAb) and anti-HERV-K env MAb.
Results: We found that HERV-K is activated in CRW22, 22Rv1, PC3 and DU145 prostate cancer cell lines, but not in RWPE1 normal prostate cells. Additionally, we observed the HERV-K levels were highest in the bone metastasis derived PC3 and brain metastasis derived DU145 cell lines. We also found that HERV-K was differentially expressed between prostate hyperplasia and adenocarcinoma.

Conclusions: HERV-K is activated in prostate cancer and represents a potential therapeutic target for metastatic prostate cancer. Future work will include the effects of HERV-K env inhibition in prostate cancer cells on cell proliferation and cell invasion.

**ASSESSING STATISTICAL ISSUES ASSOCIATED WITH PROTEOMIC BIOMARKER DISCOVERY IN PROSTATE CANCER**

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Background:
Prostate specific antigen (PSA) is the only FDA approved proteomic biomarker used for the diagnosis and prognosis of prostate cancer. However, its use has been linked to over-diagnosis and over-treatment [1,2,3,4]. Because of this and other limitations, it is clear that more specific and sensitive biomarkers are needed to support clinical decision making in prostate cancer [1].

In biomarker selection a statistical technique is used to choose a subset of proteins/protein features that accurately predict the disease state. However, as LC-MS proteomic discovery is generally undertaken on a large number of protein features (peptide m/z values) over a relatively small sample size, it is possible that by random chance alone a number of panels will perfectly predict disease status and lead to misleadingly high AUC values being reported.

Here, we have assessed different statistical packages including Random Forest, Conditional Inference Forest, Deterministic Forest and a range of filtering techniques in a simulation study and on an authentic prostate cancer serum peptide LC-MS discovery dataset to assess the impact varying (i) sample size and (ii) number of features has on biomarker panel selection.

Aims:
1. To investigate through simulation how increasing sample size affects the error rate and consistency of biomarker panel selection.
2. To examine the sensitivity of different statistical methods to varying levels of random noise

Results:
We simulated 12 proteomic datasets of varying sample sizes and varying numbers of non-important features to investigate how the accuracy and consistency of biomarker panels were affected. Each dataset was seeded with a limited number of 'authentic features'. Here we show that when the sample size is small (n=10) it appears that a very 'accurate' biomarker panel has been discovered. However, on further inspection it was found that these panels are random artefacts of the dataset and hence not real indicators of disease. We also found that increasing the sample size rectifies this problem and the chosen biomarker panels reflect the 'true panel'. We also show that filtering techniques over estimate the AUC and give an over inflated sense (impression) of how well the biomarker panel has performed.

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## ACTIVATION OF PP2A TO INHIBIT PROSTATE CANCER PROGRESSION THROUGH INHIBITION OF SET

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

**Aims:** This study aims to manipulate the activity of SET by treating both androgen dependant and independent human prostate cancer cell lines with an apoE mimetic peptide and investigate alterations in the expression of SET and PP2A in prostate cancer. Selected cell lines with resistance to finasteride, a 5-α reductase inhibitor, are also probed for SET and PP2A expression, giving insight into tumours suppressor and activator expression in drug resistant variants.

**Material & Methods:** In this study, the prostate cell lines used were RWPE1 (immortalised normal epithelial), CWR22 (Androgen dependent), 22RV1 (Androgen Independent), DU145 (Androgen Independent brain metastasis) and PC3 (Androgen independent bone metastasis). MDA-MB-231 and MDA-MB-468 were used as positive controls. Toxicity assays were performed using Cognosci compounds 112 and 449. COG449 proved more potent than COG112, and so was used for further experimentation. The cell lines were grown in petri-dishes and dosed with 0nM, 10nM, 100nm and 1000nM of COG449 over 72 hours. Protein and RNA were extracted and PP2A and SET protein and RNA expression detected.

**Results & Ongoing Research:** Toxicity assays revealed $IC_{50}$ of approximately 1000nm for CWR22 and 22RV1, and 1500nM for DU145 and PC3. RWPE1 has an $IC_{50}$ of 500nM. SET is detectable in prostate cancer cell lines. After COG449 treatment we expect to see downregulation of SET, upregulation of PP2A and increased PP2A mediated phosphatase activity.

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**IMPORTANCE OF DIFFERENTIAL STRESS-INDUCED IL-8 EXPRESSION AND SIGNALLING IN REGULATING CANCER CELL FUNCTION IN PTEN WILD-TYPE AND PTEN-DEFICIENT PROSTATE CANCER**

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We have previously shown that expression of the proinflammatory CXC chemokine, interleukin-8 (IL-8, CXCL8) and its receptors CXCR1 and CXCR2 is elevated in malignant prostate cancer epithelium. Studies from our lab and others confirm that hypoxia and/or chemotherapy-induced stresses underpin AP-1, HIF-1 and NFκB-mediated increases in IL-8, CXCR1 and CXCR2 expression in prostate cancer cells.

Another genetic alteration that occurs early in the development of prostate cancer is the loss of the haploinsufficient tumour suppressor gene, PTEN, through mutation or deletion. The aim of the current study is to determine the relevance of PTEN in regulating the induction of IL-8 signaling and the cellular response of stressed prostate cancer cells.
Time-dependent increases in IL-8, CXCR1 and CXCR2 mRNA expression were observed following exposure to hypoxia in PTEN-deficient LNCaP and PC3 prostate cancer cells. In contrast, IL-8, CXCR1 and CXCR2 expression was only marginally up-regulated in PTEN wild-type DU145 and 22Rv1 cells under hypoxic conditions. However, induction of IL-8, CXCR1 and CXCR2 expression was observed when PTEN expression was attenuated in these cell lines. Subsequently, PTEN status was shown to regulate the magnitude and duration of IL-8-promoted signaling and altered gene expression profiles. For example, IL-8 administration increased expression of HIF-1α and increased the activity of this transcription factor in PTEN-deficient LNCaP and PC3 cells but not in PTEN wild-type cells. Furthermore, expression of HIF-1 target genes (VEGF, CA IX) was also induced following IL-8 stimulation in PTEN deficient but not PTEN wild-type cells. Similar responses were observed for NF□B. Furthermore, these IL-8-stimulated responses were revealed following attenuation of PTEN in the DU145 and 22Rv1 cells using siRNA. Functionally, the stress-induced up-regulation in IL-8 signalling underpins an increased survival of hypoxic prostate cancer cells to DNA-damage-based chemotherapy.

In summary, our studies suggest that the magnitude of CXC-chemokine signaling and it subsequent effects is functionally important in PTEN-deficient prostate tumours, promoting proliferation, survival, chemoresistance

**RELEVANCE OF PRO-ANGIOGENIC SIGNALLING AS A MODE OF RELAPSE TO ANTI-ANDROGEN THERAPY IN PROSTATE CANCER.**

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Bicalutamide, a non-steroidal anti-androgen is commonly used in the treatment of both locally advanced and metastatic prostate cancer. However, administration of bicalutamide to LNCaP tumours induces a rapid and sustained development of hypoxia. Sustained treatment-induced hypoxia, has been shown to increase synthesis and secretion of the CXC-chemokine Interleukin-8(CXCL8) and increase androgen receptor (AR) activity in prostate tumours. This was co-incident with increasing oxygen tension and the restoration of tumour vascularization (1). We have previously shown that CXCL8 is a key player in the promotion of androgen-independent growth due to its ability to increase AR expression and activity, and to attenuate the growth inhibitory affects of AR-targeted agents including bicalutamide (2). The objective of this study was (i) to investigate whether the environmental stress of hypoxia can induce changes in the expression and/or activity of the AR and (ii) determine the relevance of hypoxia-induced chemokine signalling and the mechanism by which it may sustain increased AR signalling.

Experiments were conducted on two androgen-dependent cell lines, LNCaP (PTEN-null) and 22Rv1 (PTEN w/t). Hypoxia increased AR expression and potentiated AR transcriptional activity particularly in the PTEN-null LNCaP cell line. Administration of an AR targeted siRNA induced Prostate Specific Antigen (PSA) expression to a much greater extent in the PTEN-null LNCaP cell line when exposed to hypoxia. Hypoxia induced the transcriptional activity of Hypoxia Inducible Factor (HIF) and up-regulated the expression of the HIF target genes, Vascular Endothelial Growth Factor (VEGF) and Carbonic Anhydrase 9 (CA IX). This effect was not attenuated in the presence of an AR siRNA. Furthermore, hypoxia-induced NF-kB transcriptional activity. The magnitude of hypoxia-induced HIF-1 and NF-kB transcription was greater in PTEN-null LNCaP cells. Hypoxia induced the expression of CXCL8 and its receptors, CXCR1 and CXCR2, in each of the cell lines. The magnitude was greater in the PTEN-null cell line LNCaP and was not inhibited in the presence of an AR siRNA. CXCL8 signalling increases and sustains AR, HIF-1 and NF-kB transcription in hypoxic LNCaP cells.

In conclusion, treatment-induced hypoxia increases AR activity while up-regulating transcription programmes that promote castrate-resistant and metastatic disease. Ongoing experiments are characterizing the importance of PTEN status in dictating the cellular response to treatment-induced hypoxia and the role of CXCL8 signalling in sustaining the transcriptional responses observed in hypoxic prostate cancer cells.

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## IGFBP3 AND CD14: A NOVEL PREOPERATIVE SERUM BIOMARKER PANEL TO IDENTIFY EXTRACAPSULAR EXTENSION IN PATIENTS WITH PROSTATE CANCER

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Introduction: The decision for active surveillance or radical prostatectomy versus combined radiotherapy and hormonal therapy is based on several factors, including organ-confined disease status. The Partin Tables (1) are a commonly used tool to estimate the risk of encountering non-organ confined disease during prostatectomy. Large-scale internal validations show only 62% accuracy in determining for extracapsular extension (ECE) using this nomogram. In Irish patients, we have shown that the Partin Tables have a predictive accuracy of 58.1% for ECE. (2)
Aim: To identify and validate a preoperative serum biomarker panel to determine for extracapsular extension.
Methods: Our 2D-DIGE studies identified IGFBP3, ZAG, PEDF, VEGFD, CD14 and IGF-1 as biomarkers for determining various pathological parameters in prostate cancer. We used backwards feature selection techniques to identify an optimum panel for validation with 200 times bootstrapping. Receiver operating curves were generated and the area under the curve (AUC) was used to estimate prediction accuracy. We tested these biomarkers on 20 patients with ECE against 37 patients with no ECE (NECE) and validated this on an independent cohort with ECE (n=19) versus NECE (n=20).
Results: IGFBP3 and CD14 was identified as the optimum panel. When this panel was tested on the validation dataset, the established AUC was 71.7%. This is superior to the majority of validation articles on the Partin Tables to date, and when compared against other routine staging techniques including conventional magnetic resonance imaging, computed topography and transrectal ultrasound.

Conclusion: We demonstrate the first preoperative serum biomarker panel to determine for ECE in prostate cancer patients. This non-invasive technique of testing will help inform treatment choices including active surveillance options in prostate cancer. Larger-scale validation studies are underway to further validate this panel in international cohorts. This panel could also be combined with preoperative clinical parameters to better improve the AUC.

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## PROTEOMIC ANALYSIS OF COMBINED HORMONE AND RADIATION THERAPY FOR LOCALISED PROSTATE CANCER

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Prostate Cancer (PCa) is the most common non-cutaneous cancer among European men with an estimated 89,000 deaths in 2008. Current tests which include: Prostate Specific Antigen (a serum biomarker), digital rectal examination and Gleason grading of needle biopsies remain insensitive and unfit for the purpose of diagnosis and monitoring disease progression. It is therefore evident that more effective tests are urgently needed with a serum biomarker being the most desirable form of test. A particular issue arises among PCa patients who undergo combined hormonal and radiation therapy (CHRT); whilst this treatment is successful for some patients many others fail treatment after some period of time. Supported by the All Ireland Clinical Oncology Research Group we have aligned a cohort of patients in a clinical trial who received CHRT with the acquisition of sequential serum samples on-going for over 5 years. It is proposed that proteomic analysis of these patients may unveil changes in protein expression that indicates failure of treatment. From this trial, patient No. 2 who failed treatment following biochemical recurrence was time matched against patient No. 8 who as of yet shows no signs of failure. Pre-treatment, post-treatment and failure time point samples from patient No. 2 and time matched controls from patient No. 8 were depleted of high abundant proteins using a MARS Hu-14 column. Following trypsin digestion all samples were subjected to label free LC-MS/MS analysis on an Agilent 6520 QTOF mass spectrometer connected online to a 1200 Series nanoflow HPLC. Subsequent retention time alignment and feature filtration was performed with Progenesis LC-MS and statistical analysis using R. From the Mascot search engine and Swiss-Prot database (v57.1) 287 proteins were identified with a false discovery rate of 3.31%. By heat map generation a number of up-regulated and down-regulated proteins were then identified. As such this experiment emphasises the potential of label-free LC-MS/MS analysis to identify protein biomarkers with diagnostic potential and aid with clinical decision making.

**MINING THE METHYLOME OF PROSTATE CANCER: HIGHS AND LOWS OF USING ONLINE DATABASES**

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DNA hypermethylation is well recognised as a major driving force in most human cancers. Over the past decade, there has been a surge in the number of publically available online datasets, largely coinciding with the advent of high-throughput capabilities and novel epigenomic technologies. Such freely-available datasets offer a wealth of opportunities to characterise molecular aberrations in cancer cells and to cross-reference in-house data. The objective of this study was to test the performance of epigenetic datasets for prostate cancer.

Capacity to identify methylated genes in prostate cancer was carried out across 3 freely available text-mining databases: PubMeth (1), MethyCancer (2) and Human Prostate Gene Database (HPGD) (3).

In total, the 3 databases identified 128 genes as hypermethylated in prostate cancer with only 1 gene overlapping all 3 databases (*APC*) and only 5 overlapping >1 database (*CDKN2a, ESR2, GSTP1, RARB, RASSF1*). Analysis revealed the results to be in general alignment with previous reports with respect to the most prevalent functional groups involved. However, shortcomings in sensitivity as well as lack of redundancy across databases undermine the reliability of this approach to extrapolate genes with the greatest association with clinically significant disease. Intriguingly, the most prevalent methylation was reported at 100% for *HIC1* and *TGFBR2*. Yet these genes are only cited in 4 and 8 papers respectively while stalwarts such as *GSTP1* and *RARB* which are definitively shown to be methylated in over 90% and 60% in the literature were not easily apparent in any of the webtools, unless stringent criteria were relaxed.

This excercise clearly demonstrates the power of these large datasets in identifying large amounts of data in very short time. However, their drawback is that they greatly underestimate the complexity of the information generated in each individual study, which all employ different analytical techniques (i.e. PCR versus sequencing versus arrays), across different sample types (cell lines versus tissue, microdissected or not, different pathological grades). Their greatest use is as a starting point for an investigator to highlight potential genes of interest (from a gene-centric point of view) or as a cross-reference for any genome-wide in-house data.

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**INVESTIGATING THE INFLUENCE OF EL102 ON TAXOTERE SENSITIVITY IN PROSTATE CANCER BOTH *IN VITRO* AND *IN VIVO***

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**Introduction:** Taxanes are a family of plant –derived chemotherapeutics which has gathered a great deal of research focus with regard to prostate cancer treatment, in relatively recent years. Taxanes have proven to be potent anti-cancer drugs, with Taxotere (Docetaxel) being administered to patients in the metastatic phase of the disease. Taxotere acts as an anti-mitotic and anti-neoplastic compound. There is mounting evidence to suggest Taxotere's activity may be potentiated when given in parallel with alternate therapeutic agents.
EL102, developed by Elara Pharmaceuticals, is a small molecule inhibitor which carries out a dual role not only as an inducer of apoptosis but also as an inhibitor of angiogenesis.

**Methodology & Results:** We have demonstrated in vitro using cell lines PC3, DU145, 22RV1 and CWR22 that treatment with a combination of EL102 and Taxotere can greatly reduce cancer cell proliferation and migration. Of particular note, both androgen-responsive CWR22 and its androgen-unresponsive daughter cell line, 22RV1, observe an identical sensitivity to EL102. This suggests an ability to circumvent the androgen-refractory. In addition, our in vivo CWR22 mouse models have, upon combination treatment with both Taxotere and EL102, shown drastic reductions in tumour mass when compared to those treated with either drug administered singularly. Furthermore, we have shown that DLKP lung cancer cell lines and its drug resistant variant DLKPA which is 300-fold resistant to taxol, taxotrere and adriamycin, are equally sensitive to EL102. This leads us to believe that EL102 is active against MDR1-driven drug-resistant variants and also that EL102 holds a mechanism of action that is distinct from that of Taxotere or adriamycin.

**Future Studies:** Studies will involve taking a closer look at the characteristics of EL102 which enable the compound to elude drug resistance.

**MODELS OF BYSTANDER SIGNALLING BETWEEN PRIMARY ASTROCYTES AND GLIOMA CELLS: EFFECTS ON SURVIVAL AND MIGRATION/INVASION**

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Malignant gliomas are aggressive brain tumours that grow rapidly, infiltrate the surrounding tissue, and tend to be resistant to chemo and radiation therapy. As a result, there is a high recurrence rate within adjacent and distal sites of the primary cancer, with <10% of patients surviving beyond 5 years. This underscores the urgency of developing novel therapies to target tumour progression, growth, invasion, and prevent recurrence. Radiotherapy causes cell death in the targeted tumour field, while minimizing exposure to the surrounding normal tissue. However, increasing data suggest that glioma migration/invasion may be up-regulated as a counter-productive effect of ionizing radiation. In addition to direct cell killing, there is non-targeted radiation-induced bystander effects (RIBE) that leads to significant cell death within the tumour and surrounding normal tissues. It is unknown whether the RIBE modulates glioma migration/invasion.

The RIBE of primary astrocytes and glioma cells grown as single and co-cultures in 2D and 3D (to mimic tumour conditions *in vivo*) matrices was investigated. Bystander signalling was examined using media transferred from irradiated cultures and in half-exposed/shielded cultures. Bystander signals from irradiated and non-irradiated glioma cells both decreased clonogenic survival of astrocytes in 2D cultures. Nuclear 53BP1 foci formation, a surrogate marker of DNA damage, was increased in astrocyte and glioma bystander cells in 2D and 3D cultures. Increased expression of p38-MAPK, Connexin 43, and COX-2 signalling was observed in 2D bystander cultures. Transwell assays revealed a direct radiation-induced increase in migration, in contrast to a RIBE-induced decrease. Two 3D models have been developed for gliospheres: (i) in collagen matrices with and without astrocytes and (ii) in mouse brain slice cultures. Initial qualitative observation of migration and invasion of gliospheres in both models has been successful and experiments are underway to delineate the signalling pathways. These results may have implications for peripheral tumour margin interactions and normal tissue-mediated toxicity. By comparing the differential molecular factors involved in mediating bystander effects, we aim to find molecular targets that will enhance tumour killing while preserving normal tissue.

**DOSE, DOSE-RATE AND FIELD SIZE EFFECTS ON OUT-OF-FIELD CELL SURVIVAL FOLLOWING EXPOSURE TO INTENSITY-MODULATED RADIATION FIELDS**

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Intensity-modulated radiation therapy (IMRT) is an advanced radiotherapy approach in which highly modulated fields are used to achieve dose conformity across a target tumor volume. Recent data from our laboratory has shown significant alterations in cell survival occurring out-of-field which cannot be accounted for on the basis of scattered dose. This study aimed to determine the impact of area, dose and dose-rate on out-of-field cell survival responses following exposure to intensity-modulated radiation fields.

Cell survival was determined by clonogenic assay in human prostate cancer (DU145) and transformed fibroblast (AG0-1522) cells following exposure to different field configurations delivered using a X-Rad 225 kV X-ray generator. Uniform survival responses were compared to in- and out-of-field responses in which 25 – 75% of the cell population was shielded. Dose delivered to the out-of-field region was varied from 1.6 – 37.2 % of that delivered to the in-field region using different levels of brass shielding. The impact of dose rate on response was determined for 0.2 - 4 Gy / $min^{-1}$ for uniform and modulated exposures.

Survival responses showed little dependence on dose rate and area in- and out-of-field with a trend towards increased survival with decreased in-field area and decreased survival with decreased out-of-field area. Out-of-field survival responses were shown to scale in proportion to dose delivered to the in-field region and also local dose delivered out-of-field. Mathematical modeling of these findings has shown survival response to be highly dependent on dose delivered in- and out-of-field but not on area or dose rate.

These data provide further insight into the radiobiological parameters impacting on cell survival following exposure to modulated irradiation fields highlighting the need for refinement of existing radiobiological models to incorporate non-targeted effects and modulated dose distributions. This work is supported by Cancer Research UK (Grant C1513 / A7047).

**PREDICTORS OF STEM CELL RADIATION RESISTANCE IN HUMAN GLIOBLASTOMAS – AN IMMUNOHISTOCHEMICAL STUDY.**

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Introduction:
Glioblastomas (GBMs) are the most common and aggressive primary malignant brain tumour (median survival of 12-14 months). Although GBMs respond to radiotherapy, subsequent recurrence is inevitable, suggesting suboptimal killing of tumorigenic cells – likely to be mediated by glioma stem-like cells. These cells have been shown to be more radiation-resistant compared with matched non-stem glioma cells. A number of glioma stem cell markers have been reported including CD133, Nestin, Notch-1, A2B5. The functional significance of these different stem cell populations, with particular emphasis on their tumorigenic properties post-irradiation remains to be determined.
Aims of study:
Our hypothesis is that stem cell population(s) with highest expression profile in multiply-irradiated GBM specimens are likely to mediate radiation resistance and this study aims to quantify their expression using immunohistochemical techniques.
Methods:
In collaboration with the University of Pittsburgh, formalin-fixed paraffin-embedded (FFPE) GBM specimens obtained from cases subjected to prior multiple radiation treatments (both fractionated radiotherapy and stereotactic radiosurgery) were used for analysis.
FFPE-sections were immunostained for a panel of stem cell markers (CD133, A2B5, Nestin, Notch) in addition to other biomarkers of potential interest to glioma tumorigenesis and radiation response (CD34 and Mcl-1 as markers of angiogenesis and apoptosis regulation respectively). Fields of view were image-captured and quantified using a 0/+/++/+++ scoring system by two independent reviewers.
Results:
Irradiated specimens indicated hallmarks of radiation effect including radiation necrosis and hyalinization of blood vessels (particularly in the re-irradiated Pittsburgh series). Furthermore, CD34-immunopositivity, indicative of angiogenesis, was evident in all specimens although expression was noticeably less in re-irradiated cases. Nestin and Notch-1 expression was evident in the majority of cases (both pre- and post-radiation) and correlated with Mcl-1 (a marker of anti-apoptosis activation) expression. In contrast to other reports, A2B5 and CD133 immunostaining was absent in the majority of cases.
Discussion:
This study suggests that Nestin/Notch expression correlated with Mcl-1 expression implicating these factors in radiation resistance. Interestingly, the reduced vascularity of re-irradiated tumours from the Pittsburgh series was particularly evident. This is in keeping with a recent report indicating a synergistic effect of SRS/anti-VEGF treatment resulting in improved survival of recurrent GBM cases. Based on the results of this study, we hope to "FACS-sort" the most radiation-resistant stem cell population from tumour specimens for further in vitro characterization.

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## BIOLOGICAL EFFECTIVENESS OF ANTIPROTONS: INVESTIGATING A NOVEL RADIOTHERAPY APPROACH

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Due to their pattern of energy deposition, a better therapeutic ratio for cancer treatment can be achieved by ion therapy compared to conventional photon therapy. In addition, Antiprotons have been proposed to offer an extra biological enhancement over protons due to the fact that they annihilate when they come to rest, thereby causing more damage to targeted cells.

We aim to quantify the DNA damage induced in human fibroblast cells using a 126 MeV antiproton beam at CERN. Specifically we are interested in the contributions that annihilation secondary particles (e.g. neutrons) and bystander signaling may have on the dose, and cellular response in regions outside the target volume. To test this we assessed cellular DNA damage, long-term sub-lethal damage and survival using the phospho-H2AX foci, micronucleus formation and clonogenic survival assays, respectively. Additionally, these experiments have also been conducted using X-rays; carbon-ion and proton beams that are already used in radiotherapy, in order to directly compare the radiobiological effectiveness of anti-protons.

**MITOCHONDRIAL DYSFUNCTION AND RADIORESISTANCE IN OESOPHAGEAL CANCER**

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**Introduction:** Radiation therapy is fundamental to the treatment of oesophageal cancer. However, radioresistance is a significant clinical problem. The elucidation of molecular mechanisms underlying radioresistance would be of substantial clinical benefit. Mitochondria are a critical cellular target of reactive oxygen species (ROS), the generation of which accounts for ~65% of radiation-induced damage. We hypothesise that mitochondrial dysfunction may play a significant role in driving radioresistance in oesophageal cancer.
**Materials and Methods:** An isogenic model of radioresistance in oesophageal adenocarcinoma (OAC) was established by chronically irradiating OE33 cells with fractionated, clinically-relevant doses of 2 Gy X-ray radiation (cumulative dose, 50 Gy). Radiosensitivity was assessed by clonogenic assay. Mitochondrial mutation frequency was determined using the qPCR-based random mutation capture assay. Mitochondrial function was assessed by the measurement of mitochondrial mass, mitochondrial membrane potential and ROS by fluorimetry and high content screening analysis. Expression of 84 mitochondrial-associated genes was assessed using qPCR-based arrays. Validation of gene expression was performed by qPCR.
**Results:** Chronic exposure of OE33 cells to fractionated doses of radiation resulted in a radioresistant subline, OE33 R. Characterisation of this model revealed that, relative to the age- and passage-matched parent control (OE33 P), the radioresistant cells had a significantly increased basal frequency of mitochondrial mutations ($p$ = 0.01). This was coupled with alterations in mitochondrial function and the altered expression of 10 genes (*MIPEP*, *IMMP1L*, *SLC25A22*, *SLC25A30*, *MSTO1*, *SFN*, *p16*, *FIS1* and *STARD3*) involved in regulating mitochondrial function. The mitochondrial fission gene *FIS1* was significantly downregulated ($p$ = 0.04) in OE33 R cells, when compared to OE33 P.
**Conclusion:** We have generated a novel radioresistant subline that differs from its parent only in terms of radiosensitivity. We have demonstrated alterations in mitochondrial mutagenesis, mitochondrial function and gene expression in these radioresistant cells, suggesting that mitochondrial dysfunction may play a role in modulating radioresistance in OAC.

## RESPONSES OF BLADDER TRANSITIONAL CELL CARCINOMA CELL LINES TO MODULATED RADIATION FIELDS

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### Introduction

Bladder cancer is the second most common urological malignancy. Conventional treatment includes surgical resection and radiation therapy. Cell signalling between differentially irradiated cell populations within the target tumour volume is likely to influence both therapeutic outcome and adverse effects.

### Purpose

"To investigate responses of bladder cancer cell lines to uniform and non-uniform radiation fields"

### Study design, materials and methods

Bladder urothelial cancer cell lines, T24 and HT1376 were investigated. Clonogenic cell survival assays were performed to quantify survival responses to uniform and non-uniform radiation field using 225 kVp X-rays. Non-uniform fields were produced by shielding 50% of the cell culture flask. DNA double-stranded breaks were analysed by 53BP1 immunohistochemistry.

### Results

HT1376 cells were less sensitive to radiation induced cell killing in comparison to T24 cells during uniform exposures. Cell survival was determined for the exposed and shielded regions of the flask in non-uniform fields and compared with the survival response in uniform field exposures. In T24 cells (n=3), survival in the shielded region was lower than predicted from the scattered radiation dose and saturated at 30% at a scattered dose of 0.06 Gy. These responses were prevented by physical inhibition of cell-cell communication and indicate a role for bystander signalling. HT1376 cells (n=3) showed no significant changes in cell survival within the shielded region.

53BP1 foci were used as a biomarker for double strand DNA breaks after non-uniform radiation. Changes in foci formation were analysed quantitatively and indicated increased foci number after radiation (1 Gy) in T24 cells indicative of increased DNA damage within the shielded region. Foci volume analysis showed changes within uniform and non-uniform radiation fields.

### Conclusion

The data shows that modulated radiation exposures impact on survival and DNA damage responses. This was related to the underlying radio-sensitivity of the cells to direct irradiation with the most radio-resistant cell type showing minimal impact from a modulated beam.

**THE ROLE OF THE FA/BRCA PATHWAY IN THE RADIATION-INDUCED BYSTANDER EFFECT**

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**Background**

BRCA1/2 and members of the Fanconi Anemia pathway are frequently mutated in cancer, including breast cancer. These proteins are known to be important in DNA detection and repair, making them important in cellular responses to DNA damage, including double strand breaks induced by ionising radiation. The aim of this project is to determine the role of key members of the FA/BRCA pathway in directly irradiated and in non-irradiated bystander cells. A bystander response is the ability of irradiated cells to evoke DNA damage in neighbouring, non-irradiated cells.

**Method**

BRCA1/FANCD2 protein expression was downregulated using siRNA. Cell survival was determined using clonogenic survival assays, and DNA damage was quantified using immunoflourescence staining for DNA damage markers γH2AX and 53BP1. Western blotting was used to confirm knockdown of BRCA1/FANCD2.

**Results**

We have shown that breast cancer cell lines MDA231, MCF7, HCC1937 and the non-tumourigenic cell line MCF10A show varying degrees of sensitivity to radiation, with the BRCA1 mutant cell line HCC1937 being the most sensitive. These cells can be sensitised to direct radiation through silencing of BRCA1 or FANCD2 proteins. All four cell lines also demonstrated a bystander effect whereby directly irradiated cells had the ability to induce DNA damage to non-irradiated cells through the release of soluble factors, as shown by media transfer experiments resulting in decreased clonogenic survival and increased DNA damage. Again, silencing of BRCA1 or FANCD2 sensitised breast cancer cells to the bystander effect. Co-silencing of both BRCA1 and FANCD2 in breast cancer cells resulted in a further sensitisation to both direct radiation and the bystander effect.

**Conclusion**

The FA/BRCA DNA damage response pathway may be important not only in direct radiation effects but also in the radiation-induced bystander effect. This may have implications for localised radiotherapies used in combinations with molecularly targeted agents impacting on DNA damage and repair.

**CELL SURVIVAL AND DNA DAMAGE RESPONSES FOLLOWING EXPOSURE TO MODULATED RADIATION FIELDS.**

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The purpose of this investigation was to determine cell survival and DNA damage responses following exposure to modulated radiation fields. Cell survival was determined in primary human fibroblast (AG0-1522B), human breast cancer (MDA-MB-231), prostate cancer (DU-145) and glioma (T98G) cells following exposure to either uniform or modulated radiation fields delivered using a kV X-ray source. Modulated exposures were delivered by shielding 50% of the flask during irradiation. The DNA damage response was determined within AG0-1522B cells following exposure to a modulated field. Cells were fixed at a range of time points following a modulated exposure of 1 Gy delivered using a kV X-ray source and the levels of DNA damage marker 53BP1 were measured.

Following modulated exposure a significant loss of survival ($p < 0.05$) was observed within the out-of-field areas lower than predicted from the linear quadratic model. The response was cell type dependent and positively correlated with radiosensitivity. Physical inhibition of cellular communication between the in- and out-of-field regions abrogated the out-of-field response. Cell survival out-of-field was shown to increase following pre-treatment of cells with Aminoguanidine, DMSO or cPTIO. Increased levels of 53BP1 were observed out-of-field up to 1 cm from the centre of the flask 30 minutes following exposure to a modulated field. Differential DNA damage responses were observed in-field when compared with a uniform exposure. The observed DNA damage responses were shown to be dependent on intercellular communication. These data indicate that cellular communication between the in and out-of-field regions has an important role on the cellular response following exposure to a modulated radiation field.

**ACTIVATION OF STAT3 FOLLOWING HDAC INHIBITOR TREATMENT AS A RESISTANCE MECHANISM IN COLORECTAL CANCER CELLS**

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Histone deacetylases (HDACs) can regulate both the expression and activity of a diverse range of proteins involved in tumour initiation and progression. The primary role for HDACs is in epigenetic regulation however they have also been shown to alter the activity of a range of proteins, such as transcription factors and proteins involved in cell proliferation through deacetylation. HDAC inhibitors are a new class of anti-cancer drug and are the subject of over 190 clinical trials at present. Pan-HDAC inhibitors, such as Vorinostat (Saha), have recently been approved for use in the treatment of cutaneous T-cell lymphoma.

Here we show that HDAC inhibitor (HDACi) treatment in colorectal cancer (CRC) cells leads to enhanced activation of the STAT3 signalling pathway while demonstrating no activation of the MAPK or Akt pathways. HDACi treatment increases STAT3 and NF-kB acetylation which correlates with significantly increased STAT3 and NF-kB transcriptional activity. Recently our group has identified an acetylation-dependent interaction between Ku70 and FLIP. Acetlyation of Ku70 by HDACi disrupts the complex leading to FLIP degradation and apoptosis.

We postulate that the activation of STAT3 and NF-κB pathways may be a potential survival mechanism following FLIP degradation.

We have also found through the use of siRNA that STAT3 signalling reduces the sensitivity of CRC cells to HDACi treatment. This is likely due to the upregulation of pro-survival STAT3 and/or NF-κB target genes. As STAT3 and NF-κB have been previously shown to interact we are investigating if the interaction of STAT3 and NF-κB as a resistance response to HDACi treatment and FLIP degradation in CRC is acetylation-dependent.

**TREATMENT WITH MEK INHIBITORS PROMOTES EMERGENCE OF A STEM-LIKE POPULATION IN RESISTANT CELLS**

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The MEK/ERK pathway is mutated in approximately 30% of human cancers and thus it represents a promising target for drug development. Extracellular ligands signal via RAS to recruit RAF kinase and activate MEK and its downstream effectors. Mutations within this pathway, such as activating mutations in the *BRAF* gene, can lead to its constitutive activation and result in a signalling addiction to this pathway.
Cancer Stem Cells are reputed to exist in many human cancers and are believed to confer resistance to chemotherapy and be associated with more aggressive phenotypes.
The objective of this study was to investigate the effect of MEK inhibition on a panel of cell lines, to monitor the efficacy of treatment, to assess any cell survival and characterise the differences displayed by cells surviving MEK inhibition compared with untreated counterparts.
The optimal $IC_{50}$ and length of treatment for the MEK inhibitor PD0325901 for each cell line (N-Thy-Ori ($BRAF^{WT}$), 8505c (anaplastic $BRAF^{MUT}$) and SK-Mel-28 ($BRAF^{MUT}$)) was determined using MTT assays. Cell lines were subjected to PD0325901 ($IC_{50}$) or media-only treatment and surviving cells were harvested for mRNA extraction. A relative expression study of MEK pathway genes *MAP2K1*, *MAP2K2* , *MAPK3*, *MAPK1*, *ELK1* and stem cell markers *NANOG* and *POU5F1* was performed using TaqMan assays.
In the anaplastic thyroid cell line 8505c all 5 MEK pathway genes were down-regulated in surviving PD0325901 treated cells. This pattern (of MEK gene expression inhibition) was not seen with the N-Thy-Ori thyroid cell line or the Sk-Mel-28 melanoma cell line.
In N-Thy-Ori and SK-Mel-28 cell lines the stem cell markers (*NANOG* and *POU5F1*) displayed significant upregulation in surviving PD0325901 treated cells. A statistical difference in expression was not seen in the 8505c cells.
The data infer that sub-populations within cell lines display chemotherapeutic resistance. Moreover, we show that upregulation of stemness factors NANOG and OCT4 is associated with resistance to PD0325901 MEK inhibition. The lower comparative difference in expression of stem cell markers observed in the anaplastic cell line most likely reflects a higher *ab initio* stem cell component in this cell line. Whether the upregulation observed in N-thy-ori and SK-mel-28 is an adaptive response to MEK inhibition or due to an inherent stem cell sub-population remains to be determined.

## A NOVEL DIAGNOSTIC ASSAY FOR QUANTIFYING PHOSPHORYLATED PROTEINS

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**Background**

The human epidermal growth factor receptor (HER) pathway plays a critical role in human malignancy including breast and non-small cell lung cancer (NSCLC). Novel therapeutic agents that specifically target members of the HER pathway have shown promising therapeutic efficacy. Initially Trastuzumab, a humanised monoclonal antibody, was approved for the treatment of HER-2 (ErbB-2) positive breast cancer. Several tyrosine kinase inhibitors (TKIs) are currently used in the clinic, including lapatinib a dual inhibitor (EGFR and HER2) and erlotinib (EGFR). Lapatinib has been shown to have anti-tumour activity in HER2 positive breast cancer. NSCLC patients with amplified EGFR or mutations in the EGFR tyrosine kinase domain have shown significant survival benefit with EGFR TKIs. We believe that the phosphorylation status of these receptors may be a strong predictor of response to TKI therapy. However to date the robust quantification of phosphorylated proteins in diagnostic biopsies has proven unreliable. We have developed and are currently validating a novel diagnostic assay for the quantification of phosphorylated proteins in cancer patient biopsies.

**Methods**

A panel of breast cancer cell lines with elevated expression of EGFR and/or HER2 were used to develop this assay. Assay development considerations in particular: cell fixation reagents, and primary/secondary antibody concentration were optimised. Image acquisition and analysis algorithms using a High Content Analysis (HCA) platform were developed. Validation of the assay was performed utilizing HCA and Western blotting. Validation of the assay in tissue biopsies and fine needled aspirates (FNAs) are ongoing.

**Results**

A novel HCA assay was developed that accurately quantifies the activation of EGFR and HER2 and tracks changes in the cellular localisation of these receptors, in a panel of breast and NSCLC cell lines. The assay has been validated for specificity using HCA and Western blotting. Early results from biopsy/FNAs are promising and are currently being validated.

**Conclusion**

This novel assay offers many advantages over the more conventional approaches such as proteomics as we can correlate activities of target molecules with a multitude of cellular physiological parameters within the context of intact cells and tissues, offering the potential of a highly effective research tool and clinical diagnostic.

**OPEN INNOVATION: DRIVER OF GLOBAL HARMONISATION IN THE IRISH BIOBANK AND INFORMATICS NETWORK FOR PATIENT-FOCUSED RESEARCH**

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**Background:** Biobank Ireland Trust's concept of an Irish cancer biobank and informatics network for translational research was described in 2005. In August 2008, the biobank network began, linking a new biobank (SJH) with an established pathology-based biobank (Beaumont/RCSI). We examined how open innovation from international networks has harmonised the Irish network's biobanking with global biobanking practice.

**Methods:** Funding mechanisms, purpose and main focus of the biobank network, buy-in strategy, international involvement, patient consent, patient information, extent of hospital staff involvement, biobank and network management, data handling, SOPs, Quality Control, policy-making, researcher access, attitudes, communication - were contrasted with prevailing practice.

**Results:** A 4-hospital Irish biobank network (SJH, Galway, Beaumont, Cork) has developed "bottom-up", without government funding. Funding sources are diverse, but 11 pharma/biotech companies have provided modest unrestricted grants. Innovations stimulated by discussions with MAWG members have transformed Irish biobanking. Standardised SOPs and QC, and approval by the Research and Ethics Committees of a generic Patient Consent Form, Consent Policy, Information Leaflet, and Sample/Data Release Policy for use at each participating institution, were helped by input from Patient Groups and the Assistant Data Protection Commissioner. Pathologists/SpRs, nurses, surgeons, IT personnel, and hospital management play a central role. The open source database CAISIS - for sample inventory and pathology data - will be developed for online display of restricted data. Coded clinical data is obtainable with the cooperation of the National Cancer Registry. At SJH, researchers biobanking prospectively have begun to share samples and data with the network: this establishes *quid pro quo*, and recognition of a bigger picture than "*my project*". Thousands of high quality breast and colon cancer samples and matching normal tissue - with pathology data from over 1100 patients - are available. Events and media coverage increase public awareness of biobanking.

**Comments:** The network's strength is that people (patients, hospital staff, biobank personnel, researchers, pharma/biotech, patient groups, the public, and government agencies) work together. Each hospital biobank is at a different developmental stage, and Government funding through The Health Research Group, must be lightly administered and carefully allocated in order to leverage Irish translational research.

**C-MET/HGF IN TRIPLE NEGATIVE BREAST CANCER**

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**Introduction:** Triple negative breast cancers (TNBC) lack the expression of oestrogen receptor (ER), progesterone receptor (PR) and the over-expression of HER2. While the mortality rate of breast cancer in general is falling, TNBC remains a disease with a poorer prognosis than other subtypes. Currently there is no recommended systemic treatment regimen for the treatment of TNBC due to the lack of a molecular target. We are currently investigating the role of c-Met and its' ligand, Hepatocyte Growth Factor (HGF), in TNBC as potential molecular targets. c-Met/HGF signalling mediates a diverse range of biological events and has been previously implicated in the development of basal mammary tumours in mice.
**Methods:** Western blotting was used to determine the expression of c-Met and p-Met (Y1234/Y1235) in a panel of TNBC and non-TNBC cell lines. Dependence on HGF for proliferation was examined by cell viability counting using Guava ViaCount.
**Results:** c-Met was detected in all of the seven TNBC cell lines and in three of the seven non-TNBC cell lines tested. p-Met was detected in four of the seven TNBC cell lines and was not detected in any of the non-TNBC cell lines. In the four TNBC cell lines tested (HCC1937, HCC1143, MDA-MB-231 and MDA-MB-468) an increase in proliferation was observed in response to treatment with HGF (50 ng/ml).
**Conclusion:** Expression of c-Met and p-Met is increased in TNBC cell lines. TNBC cell lines demonstrate a dependence on HGF for proliferation. These results suggest that c-Met/HGF may be a viable candidate for targeted therapy in TNBC. We will test this hypothesis further by examining expression and phosphorylation of c-Met in breast tumours from patients with triple negative breast cancer.

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**EXPRESSION OF MICRORNA-9 AND -224 IN TRASTUZUMAB RESISTANT HER2 POSITIVE BREAST CANCER CELL LINES.**

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**Background**: HER2 positive breast cancer accounts for approximately 25 % of all breast cancer cases. Trastuzumab, a humanised monoclonal antibody, is an approved established treatment for HER2 positive breast cancer; however, patients that initially respond frequently develop resistance. The aim of this study is to elucidate the role of microRNAs in acquired trastuzumab resistance, by profiling microRNAs in trastuzumab-resistant cell lines.
**Methods**: MicroRNA was extracted from the HER2 positive trastuzumab resistant cell line (SKBR3-T) and SKBR3 parental cells, in triplicate. MicroRNA profiling was performed using Taqman Low Density Arrays (TLDA). Differentially regulated miRNAs with a cut-off P-value of <0.05 and > 2-fold change were selected for further analysis. Quantitative RT-PCR (qRT-PCR) was performed to confirm alterations in novel microRNAs. MicroRNA was also extracted BT474-T cells, another cell line model of acquired trastuzumab resistance, to further validate the novel targets.
**Results**: Nine differentially regulated microRNAs were identified in the SKBR3-T cells, and were selected for further analysis. qRT-PCR confirmed that 5 miRNAs were up-regulated and 4 were down-regulated in SKBR3-T cells. MiR-9 and miR-224 were identified as novel targets with regards to statistical significance ($P < 0.05$), fold change (> 1.5) and no previous association with trastuzumab resistance in the literature. MiR-9 was 2.2-fold up-regulated ($p=0.04$), while miR-224 was 1.6-fold down-regulated ($p=0.01$) in the SKBR3-T compared to the parental control SKBR3. Furthermore, measurement of these two targets in BT474-T cells showed that miR-9 is 1.14-fold up-regulated ($p=0.68$) and miR-224 is 1340-fold down-regulated ($p=0.003$), compared to the parental BT474 cells, confirming the same trend as the SKBR3-T cells.
**Conclusions**: This is the first report of the involvement of miR-9 and miR-224 in trastuzumab resistance in HER2 positive breast cancer. Future work will include functional studies of miR-9 and miR-224 to examine their effects on cell proliferation and response to trastuzumab in the SKBR3 and SKBR3-T cell lines.

**THE SRC-1/HOXC11 REGULATED S100B IS A BIOMARKER FOR RESPONSE TO TYROSINE KINASE INHIBITOR DASATINIB IN ENDOCRINE RESISTANT BREAST CANCER.**

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Endocrine therapies such as aromatase inhibitors and tamoxifen are standard of care for estrogen receptor positive breast cancer. As resistance to these drugs is common, early detection and effective treatment for metastatic disease are key clinical issues. Resistance can occur at least in part through p-src signalling to steroid receptor coactivator SRC-1. This signalling network results in the production of the biomarker S100Beta. The p-src inhibitor dasatinib is currently in trial for the treatment of breast cancer, though initial results suggest that patient selection with biomarkers may improve efficacy.

SRC-1 has previously been associated with endocrine resistance and is an independent predictor of disease free survival in patients with breast cancer. In this study, we used two cell line models of endocrine resistance to investigate the molecular signalling pathways of SRC-1. Immunoprecipitation and ChIP assays demonstrated that SRC-1 can interact with the developmental protein HOXC11 to regulate expression of the target gene S100Beta. Endocrine resistant cells expressed higher levels of SRC-1, HOXC11, S100Beta and src tyrosine kinase compared to endocrine sensitive cells. Treatment with the p-src inhibitor dasatinib reduced the expression of SRC-1, reduced the interaction between SRC-1 and HOXC11, and reduced the expression of S100Beta. At a functional level, treatment with dasatinib reduced the migratory capacity of endocrine resistant cells to a level comparable with that of endocrine sensitive cells.

The S100Beta protein is secreted into the blood stream and as such, is easy to detect. Analysis of patient blood samples revealed that serum S100Beta levels correlated significantly with tumour SRC-1 levels. Combined, these results suggest that S100Beta may be a useful clinical marker, not only to detect the development of endocrine resistance, but to select patients who would be suitable to dasatinib treatment and to monitor the effectiveness of that treatment.

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***IN VIVO* VALIDATION OF A NOVEL OPTICALLY ACTIVE, CLINICALLY RELEVANT ORTHOTOPIC MOUSE MODEL OF GLIOBLASTOMA MULTIFORME (GBM).**

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Glioblastoma multiforme (GBM), the most aggressive brain tumour is characterized by extensive cell proliferation, angiogenesis and single-cell infiltration into the normal brain.
Recently a human biopsy xenograft model was developed that reflects the genotype and phenotype of the corresponding human tumours [1,2].
The present work was aimed at a further development of this model for the assessment of tumour growth and response to therapy using bioluminescence imaging (BLI). A method for successful lentiviral transduction of the firefly luciferase gene into multicellular spheroids was developed. After in vitro evaluation and selection, luciferase expressing spheroids were injected into the brains of immunodeficient mice. Tumour growth was monitored by BLI and tumour volumes calculated using magnetic resonance imaging (MRI), T1- and T2 sequences. Growing intracerebral lesions could be detected using BLI post-implantation, before any MRI identified lesions were evident. Histologically firefly luciferase-expressing tumours were similar to non-transduced lesions and recapitulated the hallmarks of human GBMs. Luciferase-expressing xenografts showed similar proliferation indices and micro-vessel density counts as wild-type xenografts. By immunohistochemistry, we validated the widespread invasion of luciferase transgene positive tumour cells in the mouse brains.
In conclusion, our study describes a novel optically active model of glioblastoma multiforme (GBM), which closely mimics human pathology with respect to invasion, angiogenesis and proliferation indices. The model may thus be routinely used for the assessment of novel anti-GBM therapeutic approaches implementing well established and cost effective optical imaging strategies.

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**NOVEL HYPOXIC BIOMARKERS OF CHEMORESISTANT OVARIAN CANCER**

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Ovarian cancer is the fourth most common cause of cancer in women. Five year survival rates for patients diagnosed with advanced ovarian cancer are <30% due to chemoresistance within ovarian tumours. Biomarkers which distinguish patients who are likely to become chemoresistant may identify patients who are unlikely to benefit from a particular form of therapy, or those who may be more likely to benefit from one drug type over another. Also, they may represent novel therapeutic targets. Hypoxia has been implicated in the development of chemoresistance in many tumour types. This study examined the effects of hypoxia on chemoresistance in an ovarian cancer model.

Hypoxia was shown to induce resistance to cisplatin in an ovarian cancer cell line model using MTT assays. RNA was extracted from cells in hypoxia and normoxia and interrogated using Affymetrix Human Gene arrays. Array analysis identified numerous genes induced by hypoxia which may be linked to the increased cisplatin resistance. Four genes were selected for analysis in a cohort of patient tumour samples. The tumour samples were grouped according to their clinical response to chemotherapy (carboplatin/paclitaxel). RNA was extracted from macrodissected sections from 40 papillary serous ovarian adenocarcinomas. Taqman PCR was carried out for HIF1A, ANGPTL4, HER3 and BDNF.

Patients who did not respond well to chemotherapy (<6 months progression free survival, PFS) displayed higher levels of HIF1A, ANGPTL4 and HER3 than patients who did respond (at least 12 months PFS). BDNF was only expressed in 6 of the 40 samples, however it is likely that a larger cohort may provide more data on this marker. ANGPTL4 and HER3 have previously been identified as having clinical utility in other cancer types, however this is the first study to link them to hypoxia and chemoresistance in ovarian cancer.

**THE HDAC INHIBITOR VORINOSTAT (SAHA) DOWN-REGULATES C-FLIP AND SENSITIZES HUMAN NON SMALL CELL LUNG CARCINOMA CELL LINES TO IONISING RADIATION.**

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**Introduction**: c-FLIP is an anti-apoptotic protein that blocks death receptor-mediated apoptosis by preventing caspase 8 activation at the death inducing signalling complex (DISC). We have previously shown that c-FLIP is an inhibitor of apoptosis induced by DNA damaging agents and that vorinostat down-regulates c-FLIP protein in various cancer types. The role of c-FLIP in mediating resistance to radiotherapy has not been previously determined.
**Methods**: The NSCLC cell lines used in this study were A549 and H460. c-FLIP protein was silenced using siRNA transfection. Protein levels were determined by Western blotting. To assess apoptosis PARP cleavage was detected by Western blotting and analysis of the sub-G1 population by flow cytometry. Clonogenic assays were used to assess long term survival. Caspase activation was determined using caspase activity assays and Western blotting.
**Results**: Combination treatment of vorinostat and ionising radiation results in increased levels of apoptosis when compared to either treatment alone. siRNA silencing of c-FLIP protein phenocopied the effect of vorinostat by enhancing apoptosis induced by ionising radiation.
**Conclusion**: Radiation therapy makes a significant clinical contribution to the treatment of NSCLC. Strategies to increase the therapeutic index of radiation are sought to improve treatment outcomes. Down-regulation of c-FLIP sensitises NSCLC cell lines to ionising radiation, thus pharmacological inhibition of c-FLIP, using the HDAC inhibitor vorinostat, is a potential combination treatment strategy.

**TARGETING ADAM10 AS A NOVEL MECHANISM FOR THE TREATMENT OF BREAST CANCER**

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Background: The ADAMs are a family of proteases, best known for their role in releasing the extracellular domain of transmembrane proteins. One of the ADAMs, i.e., ADAM10, is involved in the activation of 2 important signalling systems controlling cell growth, invasion and metastasis, i.e. the EGFR and NOTCH signalling systems. Because of its ability to activate both EGFR and NOTCH, ADAM10 is likely to play a role in cancer formation or progression. The aim of this study was therefore to investigate the role of ADAM10 in breast cancer and test its therapeutic potential.

Materials and Methods: ADAM10 mRNA expression was examined in a pooled collection of publically available databases (n ~ 4000). ADAM10 protein was examined in a cohort of 120 breast tumour extracts by ELISA. In addition, ADAM10 expression was decreased by RNAi and the effects of this on cell proliferation, invasion and migration were determined. Using the novel ADAM10 inhibitor GI254023X (GSK), we examined the effect of ADAM10 inhibition on a panel of 13 breast cancer cell lines.

Results: ADAM10 mRNA was found to be significantly elevated in HER2+ breast cancer compared to other subtypes ($p < 0.0001$). High ADAM10 mRNA expression was also observed in basal like breast cancer and was associated with poorer overall survival in this subgroup ($p = 0.0196$). In a cohort of 120 breast tumour extracts, ADAM10 protein was found to be significantly higher in ER-negative compared to ER-positive tumours ($p = 0.005$), in high grade versus low grade tumours ($p < 0.0001$), and in younger than older women ($p = 0.018$). Downregulation of ADAM10 in MDA-MB-231 breast cancer cells resulted in a significant reduction in invasion ($p = 0.0006$) and cellular migration ($p = 0.0002$). Treatment of 13 breast cancer cell lines with GI254023X resulted in variable growth inhibition (from 0 to 52%). Furthermore, GI254023X significantly decreased invasion in the MDA-MB-231 cell line ($p = 0.001$).

Conclusions: Our findings of a correlation between ADAM10 and features of aggressive disease and the fact that its downregulation/inhibition decreased growth, invasion and migration suggests that this ADAM10 protease is involved in the progression of breast cancer. Inhibition of ADAM10 with GI254023X may be a new treatment for breast cancer.

**IDENTIFICATION OF AUTOANTIBODIES AS NOVEL OVARIAN CANCER BIOMARKERS**

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Early diagnosis of ovarian cancer (OC) is the most important determinant of survival. However, symptoms of ovarian cancer are non-specific and biomarkers are unreliable resulting in the majority of ovarian cancers diagnosed at late stage. This research aims to determine the utility of autoantibodies as biomarkers of ovarian cancer, including early stage disease. Autoantibodies are an extremely attractive biomarker entity as they are present in blood and easily adapted into current diagnostic platforms. (E.L.I.S.A.)(1). This research aims to determine the utility of autoantibodies as novel OC biomarkers.

Study approval was obtained from SJH/AMNCH research ethics committee. To profile the autoantibody response two different array platforms were used; the Human Expression Library (hEx1) and the Invitrogen Protoarray. Benign ovarian disease, early stage ovarian cancer and late stage ovarian cancer patient sera, in addition to Non-Remarkable (Healthy) control subjects were screened. Antigens identified to be associated with these cohorts were interrogated by Western immunoblotting and E.L.I.S.A. Pathway analysis was also performed to identify pathway deregulation associated with malignancy.

Autoantibodies to p53 were identified in patient sera. Western blotting confirmed highly specific OC association, present in 25% (n= 5/25) of late stage OC sera and not present in healthy control sera (n=15). In addition novel autoantigens were identified by both array screening platforms, including proteins involved in cell adhesion and migration, proteins associated with endo and exocytic machinery and a transcriptional repressor. Over-represented pathways associated with late stage ovarian cancer were the VEGF signalling pathway and the EGFR1 signalling pathway. Protein array platforms were used to identify autoantibody profiles in OC sera. Autoantibodies to p53 were confirmed in 25% of late stage OC patients in line with published data(2). Pathway analysis performed on antigen/autoantibody profiles indicated that classical cancer pathways (VEGF, EGFR1) may be deregulated. This indicates that autoantibody profiles detect malignancy associated protein pathway deregulation.

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**TARGETING THE PROLIFERATIVE AND QUIESCENT COMPARTMENTS IN CLL**

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Chronic Lymphocytic Leukaemia (CLL) represents the most common adult leukaemia in the Western world. Accumulation of quiescent tumour cells in the blood stream is the main feature of this disease. This quiescent compartment is fuelled by the proliferation of tumour cells that reside in secondary lymphoid organs and bone marrow. In the lymph nodes, antigenic stimulation and signals from the surrounding microenvironment provide an optimal niche where tumour cells proliferate and become highly resistant to chemotherapy. Thus, targeting this proliferative compartment represents a challenge to cure this disease.
Cell division cycle 7 (Cdc7) is a serine threonine kinase that is required for the initiation of DNA replication. PHA-767491, the first small molecule Cdc7 inhibitor induces cell death independently of p53 status in many cancer cell lines and also displays potent antitumour activity *in vivo*. This compound and its more advanced derivatives also inhibit cycling dependent kinase 9 (CDK9), a kinase involved in RNA polymerase II dependent transcription. Inhibition of CDK9 may contribute to the cell death-inducing activity of PHA-767491 through downregulation of myeloid cell leukemia 1 (Mcl-1), an anti-apoptotic protein of the B cell lymphoma 2 (Bcl-2) family.
In this study, we show that PHA-767491 displays a rapid and strong apoptotic-inducing activity in quiescent CLL cells. Apoptosis is independent of the prognostic parameters used to assess CLL and is also accompanied by downregulation of Mcl-1. In an *in vitro* model that partially recapitulates the lymph nodes microenvironment, PHA-767491 inhibits Cdc7 kinase activity and DNA replication, suppresses CDK9 activity and induces Mcl-1 downregulation, however does not promote apoptosis of stimulated CLL cells. This is possibly explained by the upregulation of other members of the Bcl-2 family such as B cell leukemia X long (Bcl-XL) observed in this system.

## PEROXIREDOXIN-1 PROTECTS AGAINST ONCOGENE-INDUCED SUPPRESSION OF THE ESTROGEN RECEPTOR AND IS A BIOMARKER OF FAVOURABLE PROGNOSIS IN ESTROGEN RECEPTOR-POSITIVE BREAST CANCER

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**Background:** Peroxiredoxin-1 (PRDX1) is a multifunctional protein, acting as a hydrogen peroxide ($H_2O_2$) scavenger, molecular chaperone and immune mediator. Differential expression of PRDX1 has been described in many tumour types, including lung and ovarian carcinoma. Despite the wealth of knowledge about PRDX1 functionality, its role in human breast cancer has not been fully elucidated. Preclinical studies suggest that PRDX1 may be protective against oncogene-induced mammary carcinogenesis, indicating that it may be an important biomarker. In this study, we describe PRDX1 as a robust prognostic biomarker in estrogen receptor α (ER)-positive breast cancer and propose a molecular mechanism which explains this observation.
**Experimental design:** The anti-PRDX1 antibody was validated in breast cancer cell lines using Western blotting, immunohistochemistry and reverse phase protein array (RPPA) technology following exogenous overexpression or shRNA-mediated knockdown of PRDX1. PRDX1 protein expression was evaluated using tissue microarray (TMA) or RPPA technology in two independent breast cancer cohorts (n=1224 patients in total). Increase in cellular content of $H_2O_2$ was accomplished via treatment with exogenous $H_2O_2$ or activation of oncogenic pathways. Western blotting or reverse transcription-PCR was used to assess changes in ER expression.
**Results:** High expression of PRDX1 protein was associated with a favourable outcome in ER-positive, but not ER-negative breast cancer cases across both cohorts (log-rank p-value: TMA=0.022; RPPA=0.002). Exogenous treatment with $H_2O_2$ induced ER protein suppression in ER-positive cell lines, which corresponds with previously published results. Knockdown of PRDX1 further sensitised the cells to this effect, suggesting that PRDX1 acts as a protector of ER expression in cells undergoing oxidative stress.
**Conclusion:** Our findings provide robust evidence of the importance of PRDX1 as a biomarker of favourable prognosis in ER-positive breast cancer. This data suggests that PRDX1 may act as a shield against oncogene-induced and $H_2O_2$-mediated ER suppression, and thus, protecting the ER-positive phenotype of the tumour. These results strongly imply a close connection between biological activity of PRDX1 and regulation of the estrogen-mediated signalling in breast cancer.

## NON-APOPTOTIC CELL DEATH MECHANISMS SEAL THE FATE FOR CHEMORESISTANCE FOR WOMEN WITH TRIPLE NEGATIVE BREAST CANCER

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Triple negative breast cancer (TNBC) refers to tumours that are ER, PR and HER2 negative on immunohistochemical (IHC) analysis. Treatment options for TNBC are limited due to lack of a therapeutic target and as a result is managed with standard chemotherapy such as paclitaxel (Taxol®).
Following chemotherapy, the ideal tumour response is apoptotic cell death. However, post-chemotherapy, cells can also maintain viability undergoing **cellular senescence** and **viable autophagy**, two forms of cellular fate. These cells represent highly chemo-refractory cellular populations generating *secretomes* which can directly impact on their surrounding tumour microenvironment (TME) enhancing the malignant phenotype. Moreover, we have demonstrated that downregulation of the mitotic assembly deficient protein (MAD2) through which paclitaxel exerts its apoptoic affect results in the induction of cellular senescence, demonstrable by increased p16 and beta galctosidase staining in epithelial ovarian cancer (EOC). The purpose of this study is to determine the immunohistochemical levels of p16 and MAD2 in TNBC and associate the IHC outputs with grade, stage and subsequent chemoresponse. Formalin-fixed-paraffin-embedded sections from 40 TNBC have been staged, graded, sectioned and stained for p16 and MAD2 and IHC outputs will be presented in relation to subsequent chemoresponse.
The ability to circumvent the induction of viable cellular fates and dysregulated apoptotic pathways post-chemotherapy is central to the design of novel therapies for TNBC, given that differential cellular fates limit the successfulness of therapy for this orphan cancer.

**PRECLINICAL STUDIES WITH PARP INHIBITORS IN BREAST CANCER**

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PARP1 is an enzyme, best known for its repair of single-strand DNA. Inhibition of PARP1 is currently undergoing intensive investigation for the treatment of several different types of malignancy. To date however, efficacy of PARP inhibitors as monotherapy, has been mostly confined to patients with BRCA1/2-related breast and ovarian cancer. Because of the multiple biological similarities between BRCA1/2-associated and triple-negative breast cancer (TNBC), we hypothesise that the latter mentioned subgroup of breast cancer patients may also be sensitive to PARP inhibitors.

The aim of this investigation was therefore to evaluate the anti-proliferative effects of 2 PARP or PARP-like inhibitors, i.e., olaparib and inaparib on a panel of TN (n = 7) and non-TN (n = 5) breast cancer cell lines.

Using monolayer cultures and the MTT assay to assess cytotoxicity, $IC_{50}$ concentrations for olaparib across the 12 cell lines ranged from 3.77-31 μM while $IC_{50}$ concentrations for inaparib varied from 13-70 μM. No difference in sensitivity was seen between the TN and non-TN cell lines. These results with olaparib in monolayes cultures were confirmed using clonogenic growth assays. However, with the latter, lower $IC_{50}$ values were found, i.e., ranging from <0.01-2.5 μM. Using Western blotting, 4 forms of PARP1 were detected in extracts of breast cancer, migrating at 116 kDa (main form), 89 kDa (apoptotic form), 50 kDa (necrotic form and 27 kDa (apoptotic form). The parental form of PARP, i.e., the 116 kDa form was in almost all TN (12/12) and non-TN (73/78, 94%) samples.

We conclude that olaparib is a more potent inhibitor of the in vitro growth of breast cancer cells, that TN and non-TN exhibit similar sensitivity of olaparib and inaparib and that PARP is widely expressed in breast cancer.

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**NEUROMEDIN U EXPRESSION IS ASSOCIATED WITH RESISTANCE TO HER2-TARGETED THERAPIES AND POOR PROGNOSIS IN BREAST CANCER PATIENTS**

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Not all HER2-positive breast cancer patients respond to HER2-targeted agents, unfortunately, and others –who initially benefit– relapse due to development of resistance. Therefore, there is a need to identify biomarkers for improved patient selection with potentially also as co-targets to help overcome resistance in HER2-positive cancers.

Here, through studies of cell line models (SKBR3, HCC1954) and their variants developed by lapatinib exposure for 6 months (SKBR3-$L^R$, HCC1954-$L^R$), we identified NmU mRNA and protein to be at higher levels in resistant cells. Interesting, we found the same trend when studying medium conditioned by these cells i.e. higher levels of NmU external to SKBR3-$L^R$ and HCC1954-$L^R$ cells compared to SKBR3 and HCC1954 parent cells; suggesting potential also as an extracellular predictive biomarker. Through analysis of 3489 breast tumours, we found NmU to be particularly associated with HER2-postive cancers and to be associated with poorer outcome for those patients (p=0.000005). To establish if increased NmU levels might occur early in resistance development (i.e. an early indication of whether or not the tumour cells would respond to HER2-targeting) we exposed cells to lapatinib for 48hrs. and found increased NmU levels internal and external to exposed cells, supporting it as an early event. To establish if changes in NmU levels may also be predictive of response to other HER2-targeted agents, we cloned NmU cDNA, stably transfected it into SKBR3 and HCC1954 cells. NmU over-expression increased resistance not only to lapatinib, but also Trastuzumab and neratinib. Further supporting a functional role for NmU in resistance to HER2-targeted agents, knocked-down of NmU endogenous levels in 4 cell lines (SKBR3-$L^R$, HCC1954-$L^R$ and innately resistant MDA-MB-361, T47D) –using 2 siRNAs– sensitised the cells to lapatinib, Trastuzumab and neratinib. More extensive analysis of our NmU over-expressing and knock-down cell lines also indicated NmU to be associated with increased motility, invasion through extracellular matrix; and *anoikis* resistance.

This is the first study reporting NmU to be associated with breast cancer. Here we have established it to correlate with poor outcome particularly from HER2-positive cancers and to be associated –and, apparently, causally involved- in a mechanism of resistance to HER2-tageted agents.

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**FLIP AS A CRITICAL TARGET FOR SAHA IN MALIGNANT PLEURAL MESOTHELIOMA**

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**Background:** Malignant pleural mesothelioma (MPM) is a rapidly fatal malignancy that is increasing in incidence. One of the main reasons for the failure of anti-cancer therapies is resistance to apoptosis. The caspase 8 inhibitor c-FLIP is an anti-apoptotic protein over-expressed in several cancer types, including MPM. The histone deacetylase (HDAC) inhibitor SAHA (Vorinostat) is currently being evaluated in relapsed mesothelioma. In order to identify clinically relevant predictive biomarkers of response, understanding the mechanisms involved in SAHA-induced apoptosis in MPM is essential. We examined the roles of c-FLIP and caspase 8 in this process.
**Methods:** The mechanism of SAHA-induced apoptosis was assessed in 7 MPM cell lines. RNA interference and overexpression approaches were used, and cell death was assessed by flow cytometry, Western blotting and clonogenic assays.
**Results:** RNAi-mediated FLIP silencing resulted in caspase 8-dependent apoptosis in MPM cell line models. SAHA potently down-regulated c-FLIP protein expression in all 7 MPM cell lines. In 6/7 MPM cell lines, SAHA treatment resulted in significant levels of apoptosis induction. Moreover, this apoptosis was caspase 8-dependent in all 6 sensitive cell lines. SAHA-induced apoptosis was also inhibited by stable c-FLIP overexpression. In contrast, down-regulation of HR23B, a candidate predictive biomarker for HDAC inhibitors, significantly inhibited SAHA-induced apoptosis in only 1/6 SAHA-sensitive MPM cell lines. In addition, SAHA enhanced cisplatin-induced and TRAIL-induced apoptosis in a c-FLIP-dependent manner. Analysis of MPM patient samples demonstrated significant inter-patient variations in c-FLIP and caspase 8 expression.
**Conclusions:** These results suggest that c-FLIP is a major target for SAHA in MPM and identify c-FLIP, caspase 8 and associated signaling molecules as candidate biomarkers for response to SAHA in this disease.

**INHIBITION OF FGFR4 INCREASES OXALIPLATIN AND 5-FLUOROURACIL SENSITIVITY IN KRAS WILD TYPE AND MUTANT COLORECTAL CANCER CELLS**

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Background.

The discovery of underlying mechanisms of drug resistance and the development of novel agents to target these pathways is a priority for patients with advanced colorectal cancer (CRC). The aim of this study was to identify novel targets whose knock-down is important in mediating sensitivity to 5-FU and oxaliplatin in Kras wild type (WT) and mutant (MT) CRC models. One such target is Fibroblast Growth Factor Receptor 4 (FGFR4) which is frequently overexpressed in solid tumours, such as CRC, and has been shown to be an important regulator of cancer cell growth and motility.

Materials and Methods.

Transcriptional profiling (Almac Diagnostics CRC Disease Specific Array) of pre-treatment metastatic CRC liver biopsies and oxaliplatin/5-FU parental and resistant HCT116 cell lines followed by Pathway Analysis and Gene Set Enrichment Analysis (GSEA) were used to identify individual genes from novel drug-sensitivity pathways for incorporation into a RNAi screen.

Results.

We identified panels of in vitro and clinical genes whose expression is altered (acutely and basally) between sensitive and 5-FU- or oxaliplatin- resistant models. The significant pathways involved in 5-FU/oxaliplatin resistance included Cell Cycle, Focal Adhesion, Insulin and MAPK signalling. In the MAPK pathway, we found that FGFR4 silencing potently increased apoptosis in KrasWT and MT CRC cells, and this was further enhanced when FGFR4 siRNA was combined with 5-FU or oxaliplatin. Mechanistically, we found that FGFR4, silencing resulted in strong inhibition of STAT3 activity in both KrasWT and MT CRC cells.

Conclusions.

This study demonstrates the utility of microarray expression data, obtained from pre-clinical and clinical samples, and analyzed by pathway and Gene Set Enrichment Analysis to identify pathways of oxaliplatin/5-FU sensitivity in CRC. In addition, FGFR4 inhibition in combination with 5-FU or oxaliplatin could represent a novel treatment strategy for KrasMT and WT CRC tumours. We are currently investigating FGFR4 small molecule inhibitors in preclinical in vitro and in vivo models.

**AIB1:ER-ALPHA TRANSCRIPTIONAL ACTIVITY IS SELECTIVELY ENHANCED IN AI-RESISTANT BREAST CANCER CELLS**

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Aromatase Inhibitors (AIs) as adjuvant endocrine therapy have shown a clear supremacy over other anti-oestrogens through their clinical success [1-3]. However, as is the case with selective oestrogen receptor modulators (SERMs), prolonged exposure to AIs eventually leads to resistance [5-7]. In this study we investigated the role of AIB1 in the deregulation of ER target genes occurring as a consequence of AI resistance using tissue microarrays of breast cancer patient tissue and cell line models of resistance to the AI letrozole. Expression of AIB1 associated with disease recurrence (p=0.025) and reduced disease free survival time (p=0.0471) in patients treated with an AI as first-line therapy. In a cell line model of resistance to the AI, letrozole (LetR), we found ERalpha/AIB1 promoter recruitment and subsequent expression of, the classic ER target genes pS2 and Myc to be constitutively upregualted in the presence of both androstenedione and the AI letrozole. Moreover, transcription factor recruitment to the cyclin D1 promoter and expression remained sensitive to treatment with steroids which was successfully inhibited by AI treatment. As cyclinD1 expression remains oestrogen regulated in AI resistant cells, it is thought that cyclinD1 might not be solely regulating through ER but through oestrogen signalling to c-jun N-terminal kinase (JNK). Here, recruitment of c-jun and c-fos onto AP1 sites on the cyclinD1 promoter was mediated by oestrogen signalling through JNK. This study establishes a role for AIB1 in AI resistant breast cancer and describes a new mechanism of ERalpha/AIB1 mediated gene regulation which could contribute to the development of a more aggressive tumour phenotype.

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**MITOTIC ARREST DEFICIENCY PROTEIN 2 (MAD2) AND HISTONE DEACETYLASE 6 (HDAC6) PRESENT A COMPLEX RELATIONSHIP IN THEIR REGULATION AND EXPRESSION AND SUBSEQUENT IMPACT ON CHEMORESPONSIVENESS.**

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Ovarian cancer patients presenting with advanced stage (III/IV) cancer are treated with carboplatinum in combination with paclitaxel. Despite a significant initial response rate, fewer than 20% of patients become long-term survivors.

We have published [1] that low MAD2 expression levels associate with reduced progression free survival (PFS) in chemotherapeutically treated patients with high-grade serous ovarian cancer. Moreover, we have demonstrated that MAD2 expression is down-regulated by the microRNA miR-433[1]. Interestingly, miR-433 also down-regulates HDAC6 [2], which uniquely deacetylates α-tubulin prior to HDAC6s binding to β-tubulin; a prerequisite for Ras-induced oncogenic transformation and invasion. Importantly, *in vitro* studies have shown that HDAC6 inhibition in combination with paclitaxel treatment enhances chemoresistant cancer cell death. To date, an interaction between MAD2 and HDAC6 has not been reported.

We have profiled a cohort of ovarian cancer cell lines of varying sensitivity to paclitaxel. The paclitaxel-sensitive A2780 cell line expresses high MAD2 and low HDAC6. Conversely, low MAD2 in the paclitaxel resistant UPN251 and ovcar7 cell lines is associated with high HDAC6 expression. Paclitaxel resistance is also associated with low MAD2 and low HDAC6 expression levels in ovca432 and ovca433 cell lines. Intriguingly, siRNA knockdown of HDAC6 in the UPN251 cell line resulted in reduced protein expression of MAD2, indicating a potential interaction between MAD2 and HDAC6.
In summary, we have identified a novel relationship between MAD2, HDAC6 and chemo-resistance to paclitaxel in epithelial ovarian cancer cells. Profiling the co-expression of MAD2 and HDAC6 in tumour samples may facilitate the sub-typing of ovarian cancer based on response to chemotherapy.

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