Molecular Medicine Ireland

MMI EDUCATION & TRAINING ANNUAL SCIENTIFIC MEETING 2014

NUI Galway OÉ Gaillimh

UCD DUBLIN

RCSI

ucc
Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

TIMETABLE

0915 **Registration + Tea / Coffee**

0930 **Welcome and Opening Remarks: Dr Graham Love** (CEO, Molecular Medicine Ireland)

Session 1

Chair: **Prof Catherine Godson** (Director of UCD Diabetes Research Centre & Professor of Molecular Medicine, UCD School of Medicine & Medical Science)

MMI Clinical & Translational Research Scholars Talks

0945 **Roisin Dunne (TCD)**
The Effects of Novel Small Molecule Inhibitors on Energy Metabolism, DNA Repair and Radiation Response in an Isogenic Model of Oesophageal Cancer Radioresistance

1000 **Oisin Gough (UCD)**
FRMD3: A Novel Modulator of Diabetic Nephropathy?

1015 **Jennifer Hillis (NUI Galway)**
Neurotrophin Signaling Protects Cll Cells From Death

MMI Clinician Scientist Fellowship Programme Alumni Talk 1

1030 **Dr Sanjay Chotirmall** (Specialist Registrar in Respiratory Medicine, Respiratory Research Division of RCSI, Beaumont Hospital)
Estrogen, Cystic Fibrosis and the "yellow brick road" from PhD to Academic Trilogy

1055 **Poster Viewing + Tea / Coffee**

Session 2

Chair: **Prof Anita Maguire** (Professor of Pharmaceutical Chemistry & Vice President For Research & Innovation, UCC)

MMI Clinical & Translational Research Scholars Talks

1125 **Wesley van Oeffelen (UCC)**
Hypothalamic Growth Hormone Secretagogue Receptor (GHSR1α) Expression Facilitates Food Reward Seeking but is Compensated During Stress

1140 **Lauren McDonagh (TCD)**
Microrna Profiling and Characterisation of Putatative Cancer Stem Cells in Cisplatin Resistant Non-Small Cell Lung Cancer

1155 **Eanna Connaughton (NUI Galway)**
Phenotypic, Functional and Molecular Analysis of Novel Human Monocyte Subpopulations

Keynote Address

1210 Dr Willard Dere (Senior Vice-President and International Chief Medical Officer, Amgen)
The Past, Present, and Future of the Biopharmaceutical industry: Reflections over the past quarter century

1310 **Poster Session (adjudicated) + Lunch**

Session 3

Chair: **Prof Aideen Long** (Associate Professor, Institute of Molecular Medicine & Dean of Graduate Studies, TCD)

MMI Clinical & Translational Research Scholars Talks

1440 **Julie Worrell (UCD)**
Overexpression of CXCR3 in NIH-3T3 Fibroblasts: Functional Effects and Regulation of IL-13Rα2 Expression

1455 **Niall Savage (UCC)**
Micro-structured Electrodes for the in vitro Detection of Impedance in Cancer Cell Lines

MMI Clinician Scientist Fellowship Programme Alumni Talk 2

1510 Dr Jane McGrath (Department of Psychiatry, Trinity Centre for Health Sciences, St James's Hospital)
Understanding Neural Connectivity in Autism Spectrum Disorders

1535 **Tea / Coffee + Poster Viewing**

Session 4

Chair: **Prof Larry Egan** (Chair of Clinical Pharmacology and Head of the Department of Pharmacology and Therapeutics, NUI Galway)

Keynote Address

1605 Professor John Iredale (Professor of Medicine at the University of Edinburgh and Director of the MRC Centre for Inflammation Research) Cirrhosis: Transplanting thoughts into Therapies

Closing Remarks

1705 Prof Larry Egan (Chair of Clinical Pharmacology and Head of the Department of Pharmacology and Therapeutics, NUI Galway)

1720 **Presentation of Prizes & Wine Reception**

Molecular Medicine Ireland

WELCOME

It is a great pleasure to welcome you to this annual opportunity to catch up on the outputs of Molecular Medicine Ireland's Education and Training programmes. A key output, well-trained practitioners of clinical and translational research, will be demonstrated through today's oral and poster presentations from current MMI Clinical & Translational Research Scholars and talks from MMI Clinician Scientist Fellowship Alumni. Panels are on hand to adjudicate for the award of the MMI Medal and other prizes.

Other outputs stem from MMI bringing together expertise across our partner academic institutions and beyond to develop and deliver resources for research training. These are used initially within MMI education & training programmes, but they are very much seen as opportunities to use the momentum (and funding) of a particular programme to develop mainstreamed and sustained 'soft infrastructure'.

Today you will have the opportunity to see the online SkillsLog that captures transferable skills identified by our PhD students and enables them to share their progress with mentors who advise on career development. You will also be able to view some of the E-Learning developed by MMI with our academic research community, pharmaceutical and medical device companies, the Irish Medicines Board and patients.

We also have posters from the wider clinical and translational research community, keynote addresses that provide international perspectives from industry and from academic medicine, and a diverse audience with clinical and basic science backgrounds based in universities, hospitals and industry.

Enjoy!

Dr Mark Watson

Programme Manager -Education & Training
Molecular Medicine Ireland

MMI STRUCTURED PHD PROGRAMMES

Molecular Medicine Ireland

MMI and its partner institutions train individuals in clinical and translational research through collaborative structured PhD programmes that combine access to high-quality investigators, state-of-the-art research infrastructures and opportunities to work with our industry partners.





Clinician Scientist Fellowship Programme

The MMI Clinician Scientist Fellowship Programme (CSFP) trains clinician scientists through a 3-year structured PhD programme. Medical graduates undertake PhD research in any of five academic institutions and come together to participate in structured training and scientific meetings.

- Co-supervision by basic and clinician scientists in a collaborative translational research training environment
- Research areas include cancer, cystic fibrosis, autism, diabetes, heart disease, disorders of pregnancy

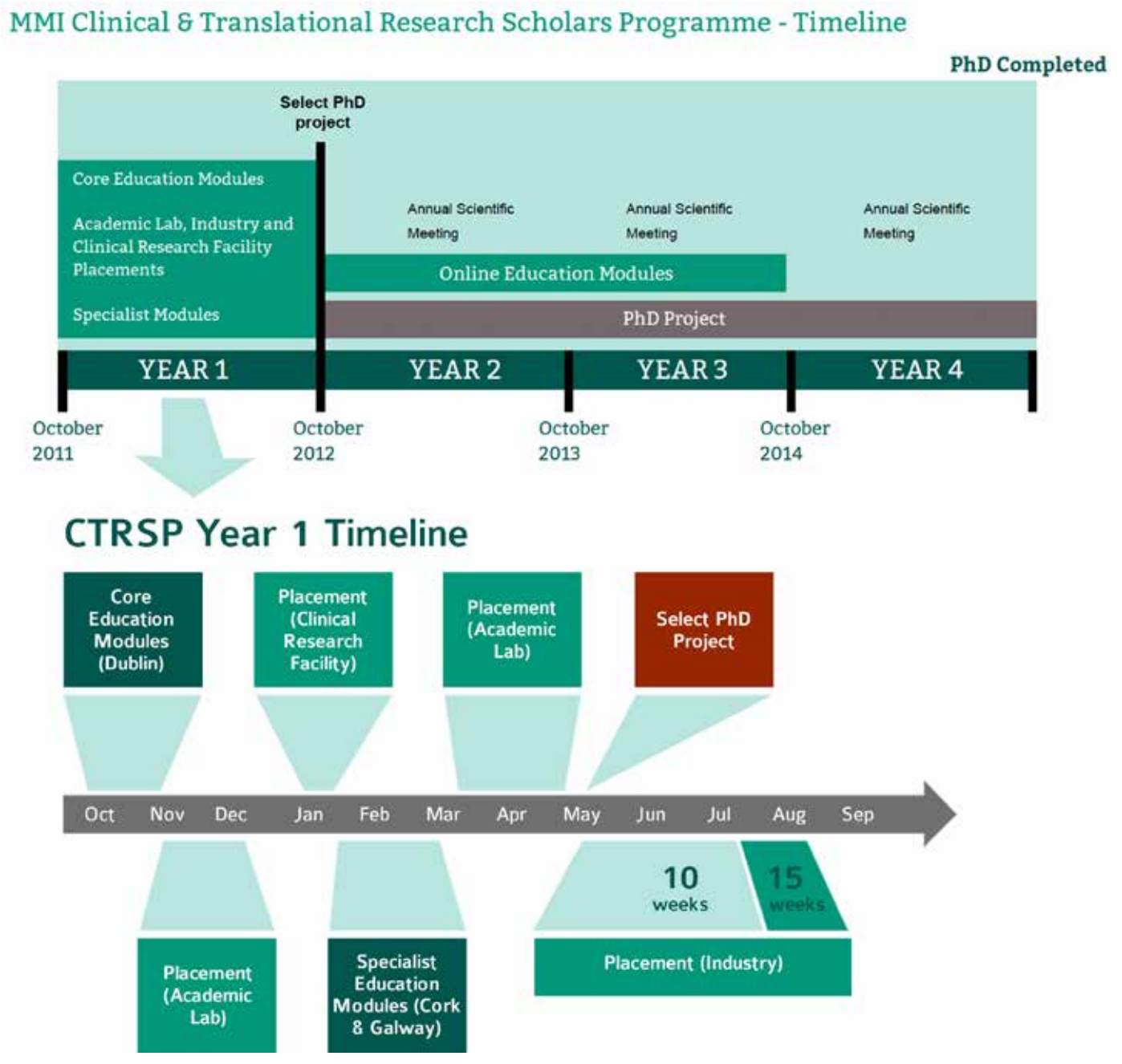
The MMI Clinician Scientist Curriculum was developed from the Clinician Scientist Fellowship Programme and is accessible via the MMI website to all medical graduates undertaking research studies in the MMI partner institutions. Over sixty graduate education modules taking place in the MMI partner institutions have been assembled and aligned to curriculum topics with information on each module. Participants can select modules of interest and register to attend.




Clinical & Translational Research Scholars Programme

The MMI Clinical & Translational Research Scholars Programme (CTRSP) trains science graduates in clinical and translational research through an innovative 4-year structured PhD programme with Irish Medicines Board and industry involvement in governance, courses, placements and mentoring.

- Placements in pharmaceutical and medical device companies; over 90 principal investigators offering academic lab placements and PhD projects; placements in clinical research facilities
- Online coordination hub enables students to select PhD components (education modules, placements and PhD project) to build their own PhD, track progress and participate in e-learning
- Focus on individual transferable skill development, with each student identifying and developing key skills from all aspects of their PhD studies, supported by industry mentors



eLearning Development

As part of the CTRSP, Molecular Medicine Ireland is developing a suite of online education modules. MMI and its academic partners are working with industry and the Irish Medicines Board to produce innovative training resources that will have impacts beyond this programme.

Demonstrations of MMI E-Learning and the online SkillsLog are available during the Annual Meeting. Please access via the iPads provided.




MMI Short Courses & Workshops in Clinical and Translational Research

MMI Courses & Workshops build on the research and teaching strengths of the partner institutions, clinical expertise in the affiliated teaching hospitals and industry strengths in R&D and commercialisation to deliver widely-available continuing professional development opportunities. Training in clinical research, including GCP workshops, is delivered by ICRIN staff.

Acknowledgement of support for MMI Education & Training

MMI Education & Training Programmes would not exist without the large number of scientists and clinicians from our partner academic institutions and affiliated teaching hospitals and further afield who contribute to design and delivery. We are also indebted to contributors from the commercial sectors, who participate as faculty and sponsors.

If you would like to discuss working with us, please contact:
education@molecularmedicineireland.ie

The students participating in MMI structured PhD programmes, the attendees of MMI Courses & Workshops, and the wider research community are the focus of these efforts.

The MMI Clinical & Translational Research Scholars Programme is funded under the Programme for Research in Third Level Institutions (PRTLI) Cycle 5, and co-funded under the European Regional Development Fund (ERDF).

Ireland's EU Structural Funds Programmes 2007 - 2013
Co-funded by the Irish Government and the European Union

EUROPEAN REGIONAL DEVELOPMENT FUND

An Roinn Fiontar, Trádála agus Nuálaíochta
Department of Enterprise, Trade and Innovation

HEA
Higher Education Authority
An tÚdarás um Ard-Oideacha

Find out more

Visit http://www.molecularmedicineireland.ie for more information on MMI Education & Training activities and other MMI focus areas.

During the Annual Meeting there will be opportunities to obtain information on and discuss the activities of the Dublin Centre for Clinical Research (DCCR) and the Irish Clinical Research Infrastructure Network (ICRIN).

KEYNOTE SPEAKERS

Dr Willard Dere M.D.

(Senior Vice-President and International Chief Medical Officer, Amgen)

Willard Dere is senior vice president and International Chief Medical Officer at Amgen. In this role, he oversees the clinical development of Amgen's pipeline candidates and marketed therapies in the international setting.

Dere joined Amgen in July 2003 as vice president of the Inflammation and Bone Therapeutic Area, and then from July to December 2004, he served as vice president and head of the General Medicine Therapeutic Area. Prior to his current position, Dere was senior vice president and corporate chief medical officer from December 2004 to January 2007. Dere joined Amgen after 14 years at Eli Lilly & Company, where he held a number of positions, most recently vice president, Medical; Endocrine, Bone and General Medicine Research and Development. In addition, since 1989 Dere has held an academic appointment at Indiana University School of Medicine, where he is clinical associate professor, and hospital appointments in Indianapolis at Wishard Memorial Hospital and Richard Roudebush Veterans Administration Medical Center, where he has taught physical diagnosis and served as an attending physician in internal medicine.

A California native, Dere received his medical and undergraduate degrees from the University of California at Davis. He pursued clinical training at the University of Utah Affiliated Hospitals and the University of California at San Francisco Affiliated Hospitals. He was an assistant professor at the University of Utah School of Medicine and held hospital appointments at the University of Utah Health Sciences Center and Veterans Administration Medical Center in Salt Lake City.

While in academia, Dere received numerous teaching awards, and more recently has served on committees of several professional organizations, including the Pharmaceutical Research and Manufacturers of America.

(PhRMA), the American Society of Bone and Mineral Research and the Group for Respect, Excellence and Ethics in Science. He has published 70 articles and abstracts, and co-edited a textbook in ambulatory care medicine.

Professor John Iredale

(Professor of Medicine at the University of Edinburgh and Director of the MRC Centre for Inflammation Research)

Professor John P Iredale is Regius Professor of Medical Science at the University of Edinburgh, Director of the MRC Centre for Inflammation Research, University of Edinburgh and Dean of Clinical Medicine, and Co-Director of the Wellcome Trust funded Clinician Scientist Training Programme (ECAT). He combines leading a research group with clinical practice in hepatology.

Previously Professor Iredale held the chairs of Medicine at the University of Edinburgh and of Medicine and Hepatology at the University of Southampton, UK. He has worked in the field of the pathogenesis of liver fibrosis, and latterly the derivation of hepatic endoderm in the application of embryonic stem cells and iPSC to models of liver injury and repair, for 20 years. His work has highlighted the potential reversibility of liver fibrosis and has defined the major mechanisms that mediate this process.

MMI Clinician Scientist Fellowship Programme Alumni Talks

Dr Sanjay Chotirmall

(Specialist Registrar in Respiratory Medicine, Respiratory Research Division of RCSI, Beaumont Hospital)

Dr Sanjay H. Chotirmall graduated from the Royal College of Surgeons (RCSI) in 2005 with an honors degree in Medicine and in the top 5% of his class. He also won the colleges fold medal in Microbiology in 2003. During his early clinical training in Beaumont Hospital, Dublin he attained memberships of the Royal College of Physicians of Ireland (MRCPI) and the United Kingdom (MRCP UK) by examination. In 2007, Dr Chotirmall was awarded a prestigious 'Molecular Medicine Ireland (MMI) Clinician Scientist Fellowship' (MMI-CSFP) where he completed a PhD in Professor Gerry McElvaney's laboratory investigating the role of estrogen in cystic fibrosis. This work led to high impact publications in the American Journal of Respiratory and Critical Care Medicine (AJRC-CM) and the New England Journal of Medicine (NEJM) both of which received national press focus with articles in the Irish Times, Irish Examiner and Irish Independent.

Dr Chotirmall has been awarded the Royal Academy of Medicine of Ireland (RAMI) Doctor award on two occasions (2010 & 2013), the Irish Thoracic Society Award (2011), the Dublin Centre for Clinical Research (DCCR) Young Investigator Award (2011), the MMI-CSFP Medal (2011), the Royal College of Physicians William Stokes Award for Research (2010) and the American Thoracic Societies International Trainee Award (2009) in addition to recognition by the Faculty of 1000 Biology and Medicine, an on;ine research service that highlights critical papers published in the biological sciences as recommended by distinguished faculty. Having published over 35 peer-reviewed papers and 7 book chapters to date, he is regularly and invited speaker at both national and international meetings. He remains an active member of the International Society of Human and Animal Mycology's (ISHAM) working group for fungal infections in cystic fibrosis, a member of the Long Range Planning Committee (LRPC) of Assembly 3 of the European Respiratory Society (ERS) and an Associate Editor at the journal BMC Pulmonary Medicine.

He is currently completing his training on the Respiratory Specialist Registrar (SpR) scheme following which he will be taking up a full-time academic position back home in Singapore at the Lee Kong Chian School of Medicine, a new medical school set up by Imperial College London.

Dr Jane McGrath

(Department of Psychiatry, Trinity Centre for Health Sciences, St James's Hospital)

Dr Jane McGrath has a medical degree from Trinity College, and completed membership teams in psychiatry in 2006. She was awarded a Molecular Medicine Ireland Clinician Scientist Fellowship in 2008 and completed her PhD in 2012. Her work focused on investigating neural connectivity in autism spectrum disorders using a variety of brain imaging techniques.

Jane is now finishing her Higher Specialist Training in Child and Adolescent Psychiatry. She continues to be actively involved in autism research in the Department of Psychiatry Autism Research group and has ongoing studies investigating brain structure and function in autism.

ORAL PRESENTATION ABSTRACTS

OP1

The Effects of Novel Small Molecule Inhibitors on Energy Metabolism, DNA Repair and Radiation Response in an Isogenic Model of Oesophageal Cancer Radioresistance

Róisín Dunne[1], Niamh Lynam-Lennon[1], John V Reynolds[1], Breandán Kennedy[2] and Jacintha O'Sullivan[1]
[1]Department of Surgery, Trinity Centre for Health Sciences, St. James's Hospital, Dublin 8
[2]UCD School of Biomolecular and Biomedical Sciences, University College Dublin, Dublin 4

Background
Tumours display high levels of angiogenesis and radiotherapy can activate angiogenic mediators, leading to leaky blood vessels and hypoxia, mechanisms of radioresistance. Tumours display high rates of aerobic glycolysis which enables adaptation to hypoxia. Our group has shown that altered metabolism and DNA repair is associated with radioresistance in oesophageal cancer. Targeting tumour angiogenesis, metabolism and DNA repair may therefore increase radiosensitivity. Zebrafish screening identified a novel small molecule inhibitor, RUD1, with significant anti-angiogenic properties. Structural analogues of this compound (RUD2-7) were created.

Results
An isogenic model of oesophageal cancer radioresistance, OE33P (parental) and OE33R (radioresistant) was used.
Seahorse Biosciences technology assessed metabolism. Glycolysis was reduced in OE33P treated with RUD1 ($p<0.05$). Oxidative phosphorylation was reduced in OE33P treated with RUD3 ($p<0.05$) and OE33R treated with RUD2 and RUD3 ($p<0.05$).
Expression of several DNA repair genes was reduced following treatment with the inhibitors. MLH1 expression was reduced in OE33P and OE33R treated with RUD2-4 ($p<0.05$). PARP1 expression was reduced in OE33P and OE33R treated with RUD1 ($p<0.01$). RUD1 also reduced expression of MMS19, XPA and RAD23A in OE33R ($p<0.05$).
Radiation response was assessed by clonogenic assay. Radiosensitivity was increased when OE33R was treated with RUD3 ($p<0.05$) and RUD4 ($p<0.001$) for 72 hours prior to irradiation. Pre-treatment with RUD4 for 72 hours also increased the radiosensitivity of OE33P ($p<0.05$).

Conclusions
We have identified a number of novel inhibitors that alter angiogenesis, and reduce energy metabolism, DNA repair gene expression and radioresistance in oesophageal cancer cells.

OP2

FRMD3: A Novel Modulator of Diabetic Nephropathy?

Gough, O.S.[1], Brennan, E.P. [1], Martin, F.[1], Martini, S.[2], Kretzler, M.[2], Godson C. [1]
[1]Diabetes Complications Research Centre, Conway Institute, School of Medicine and Medical Sciences, University College Dublin, Dublin, Ireland;
[2]Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan.

Background
Several genome-wide association studies have identified a single nucleotide polymorphism (SNP, rs1888747) in the extended promoter of the FERM domain containing 3 (FRMD3) gene associated with increased risk of diabetic nephropathy (DN) in type 1 and type 2 diabetes[1–4]. Renal expression of FRMD3 decreases significantly with severity of DN[5]. Transcriptional regulatory pattern analysis of the FRMD3 extended promoter sequence incorporating the SNP suggests that FRMD3 could be co-regulated with bone morphogenetic protein (BMP) pathway family members[5].

Objectives
Given the evidence for BMP agonists and antagonists in DN we propose that altered FRMD3 expression may be implicated in the pathophysiology of DN.

Results & Discussion
Here we report that FRMD3 gene expression is co-regulated with BMPs and is up-regulated in primary human mesangial cells (hMCs) following stimulation with BMPs (BMP-2/4/6/7; 10ng/ml). We show that reduced FRMD3 expression in primary hMCs is associated with increased expression of DN-associated connective tissue growth factor (CTGF), jagged 1 (JAG1) and fibronectin 1 (FN1) genes. This was further exacerbated with TGF-β1 (10ng/ml) stimulation suggesting that a loss of FRMD3 expression may be associated with increased extracellular matrix desposition in DN. Finally, inhibition of podocyte FRMD3 expression renders podocytes more vulnerable to TGF-β1-induced injury. A decrease in podocyte FRMD3 may be implicated in podocyte effacement and onset of microalbuminurea.

Conclusions
Our findings suggest that a loss of FRMD3 expression may be implicated in progressive fibrosis in DN and that reno-protective effects of BMPs may be due, in part, to increased FRMD3 expression.

References
1. Maeda S, Araki S, Babazono T, Toyoda M, Umezono T, Kawai K, et al. Replication Study for the Association Between Four Loci Identified by a Genome-Wide Association Study on European American Subjects With Type 1 Diabetes and Susceptibility to Diabetic Nephropathy in Japanese Subjects With Type 2 Diabetes. Diabetes [Internet]. 2010 Aug 1;59(8):2075–9. Available from: http://diabetes.diabetesjournals.org/content/59/8/2075.abstract
2. Pezzolesi MG, Poznik GD, Mychaleckyj JC, Paterson AD, Barati MT, Klein JB, et al. Genome-wide association scan for diabetic nephropathy susceptibility genes in type 1 diabetes. Diabetes [Internet]. 2009 Jun [cited 2013 Sep 13];58(6):1403–10. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2682673&tool=pmcentrez&rendertype=abstract
3. Mooyaart AL, Valk EJJ, Es LA, Bruijn JA, Heer E, Freedman BI, et al. Genetic associations in diabetic nephropathy: a meta-analysis. Diabetologia [Internet]. Springer-Verlag; 2011;54(3):544–53. Available from: http://dx.doi.org/10.1007/s00125-010-1996-1
4. Williams WW, Salem RM, McKnight AJ, Sandholm N, Forsblom C, Taylor A, et al. Association Testing of Previously Reported Variants in a Large Case-Control Meta-analysis of Diabetic Nephropathy. Diabetes [Internet]. 2012 Aug 1;61(8):2187–94. Available from: http://diabetes.diabetesjournals.org/content/61/8/2187.abstract
5. Martini S, Nair V, Patel SR, Eichinger F, Nelson RG, Weil EJ, et al. From SNP to Transcriptional Mechanism: A Model for FRMD3 in Diabetic Nephropathy. Diabetes [Internet]. 2013;62(7):2605–12. Available from: http://diabetes.diabetesjournals.org/content/62/7/2605.abstract

ORAL PRESENTATION ABSTRACTS

OP3

Neurotrophin Signaling Protects CLL Cells from Death

Hillis, J.[1], Gorman, A.M[1].
[1]Apoptosis Research Centre, School of Natural Sciences, National University of Ireland, Galway

Background
Chronic lymphocytic leukemia (CLL) is a fatal malignancy resulting from dysregulated B-cell death [1]. CLL cells frequently overexpress anti-apoptotic proteins and lose p53 expression. Constitutive NF- κB activity also contributes to pro-survival signaling [2].

Nerve growth factor (NGF) is a neurotrophin which can rescue B lymphocytes from apoptosis [3]. p75NTR is a neurotrophin receptor expressed in many cell types and is dysregulated in several cancers. It can direct cells towards survival or apoptosis, in a cell-type specific manner [4]. TrkA is an NGF-specific receptor tyrosine kinase whose activation causes cell proliferation [5].

Here we describe a role for NGF in Mec-1 CLL cells.

Results & Discussion
We have shown that inhibition of NGF-signaling induces cell death in Mec1 cells. These cells exhibit a variety of nuclear morphologies following NGF inhibition, suggesting induction of multiple modes of cell death. Interestingly, NGF inhibition induced very low levels of DEVDase activity, along with a lack of PARP cleavage or processing of caspase-3. Together, these findings suggest that conventional apoptosis is not induced on NGF-inhibition, supporting the evidence that CLL cells have dysregulated apoptosis [1].

We hypothesize that p75NTR protects these cells from death by constitutive NGF-mediated signaling leading to activation of NF-κB, with inhibition of the receptor/NGF abrogating this pro-survival signal. Initial data also suggest an involvement of TrkA signaling.

Further investigation into the mechanism of cell death induced by inhibition of NGF signaling could contribute to identification of a novel therapeutic strategy for CLL.

References
1. Packham, G. and Stevenson, F. K. (2005). Bodyguards and assassins: Bcl-2 family proteins and apoptosis control in chronic lymphocytic leukaemia. Immunology, 114:441-449.
2. Hertlein, E. and Byrd, J. C. (2010). Signalling to drug resistance in CLL. Best Pract Res Cl Ha, 23:121-131.
3. Kronfeld, I. et al. (2002). NGF rescues human B lymphocytes from anti-IgM induced apoptosis by activation of PKC zeta. Eur J Immunol, 32:136-143.
4. Friedman, W. J. and Greene, L. A. (1999). Neurotrophin signaling via Trks and p75. Exp Cell Res, 253:131-142.
5. Klein, R. et al. (1991). The trk proto-oncogene encodes a receptor for nerve growth factor. Cell 65: 189–197.

OP4

Hypothalmic Growth Hormone Secretagogue Receptor (GHSR1α) Expression Facilities Food Reward Seeking but is Compensated during Stress

Van Oeffelen, W.E.P.A[1], Schellekens, H.[2], Scott, K.A.[1], Dinan, T.G.[3,4], Cryan, J.F[1,3]
[1] Department of Anatomy & Neuroscience, University College Cork, Cork, Ireland
[2] Food for Health Ireland, University College Cork, Cork, Ireland
[4] Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland
[3] Department of Psychiatry, University College Cork, Cork, Ireland

Description
Ghrelin potently increases food intake through binding the growth hormone secretagogue receptor 1α (GHSR1α) in the arcuate nucleus (Arc) of the hypothalamus. In addition, ghrelin has been shown to mediate stress-induced reward seeking in mice (Chuang, Perello et al. 2011). However, it is unknown whether hypothalamic GHSR1α signalling is directly involved in reward seeking during stress, which is investigated in this study.

To this end, 32 male C57BL6/j mice were trained to nosepoke for a sucrose reward in exponential fashion, which indicates food reward seeking (Finger, Dinan et al. 2012). The GHSR1α receptor was knocked down in the Arc, which blocked ghrelin (2nmol/10g IP) induced food intake (control $p<0.05$ vs knockdown $p=0.376$). Mice were subjected to 19 days of social defeat stress or served as control. The GHSR1α knockdown significantly reduced responding in unstressed mice ($p<0.01$), whereas this was not observed in stressed animals ($p=0.152$). The GHSR1α knockdown also blunted the reduction of operant responding after the mice were allowed access to food three hours before the operant task ($F(1, 28)=6.628$; $p<0.05$), which was again not observed in the stressed mice. In contrast, GHSR1α knockdown did not affect non-operant hedonic behaviour in the female urine sniffing test, locomotor activity in the open field test, or depressive-like behaviour in the forced swim test.
These data indicates that hypothalamic GHSR1α signalling is crucial for the motivation to seek food-reward under normal conditions, but not under stress. Thus, food-reward seeking behaviour may be partially compensated by extra-hypothalamic GHSR1α signalling during stress.

References
Chuang, J. C., M. Perello, I. Sakata, S. Osborne-Lawrence, J. M. Savitt, M. Lutter and J. M. Zigman (2011). “Ghrelin mediates stress-induced food-reward behavior in mice.” J Clin Invest 121(7): 2684-2692.
Finger, B. C., T. G. Dinan and J. F. Cryan (2012). “Diet-induced obesity blunts the behavioural effects of ghrelin: studies in a mouse-progressive ratio task.” Psychopharmacology (Berl) 220(1): 173-181.

ORAL PRESENTATION ABSTRACTS

OP5

Microrna Profiling and Characterisation of Putatative Cancer Stem Cells in Cisplatin Resistant Non-Small Cell Lung Cancer

Lauren MacDonagh[1], Steven G. Gray[1], Kenneth J. O'Byrne[1, 2], Martin P. Barr[1].
[1] Thoracic Oncology Research Group, School of Clinical Medicine, Institute of Molecular Medicine, Trinity Centre for Health Sciences, St. James's Hospital and Trinity College Dublin, [2] Cancer & Ageing Research Program, Queensland University of Technology, Brisbane, Australia.

Background
Lung cancer is the leading cause of cancer-related death worldwide, where NSCLC accounts for 85% of cases. While cisplatin-based chemotherapy remains the gold standard treatment for lung cancer, response rates are low due to increasing development of resistance to cisplatin. Circumventing cisplatin resistance is a critical goal in the development of novel strategies for this disease.

Objectives
MicroRNA profiling of a panel of isogenic cisplatin resistant NSCLC cells was carried out using 7th Generation human miRCURY LNA™ microRNA Arrays, consisting of 1,919 miRNAs (Exiqon). Parental (PT) and Cisplatin resistant (CisR) cells were cultured in specific growth media for putative cancer stem cell analysis based on tumour cell expression of a panel of stem cell markers.

Results
A 10-miR signature was validated using miRCURY LNA™ Universal RT technology (Exiqon) in our panel of cell lines. Significant differential expression of microRNA's was found across all chemoresistant cell lines relative to their parental counterparts. Altered expression of the putative CSC markers, Oct-4, Sox-2, Nanog, Klf-4, c-Myc and lung cancer-specific CSC identifiers, CD133 and ALDH1, was observed in CisR-derived tumour spheres compared to cells grown as monolayer cultures.

Conclusion
Of the 10-miR signature validated in five cisplatin resistant NSCLC cells, significant alterations were found in seven of these miRNA's. Antagomirs and pre-miR's will be used to target these miRNA's of interest and identify their role in conferring cisplatin resistance in NSCLC. An in vivo study, examining the tumourigenic potential of CD133+/ALDH1+ lung cancer cells in NOD/SCID mice is currently under investigation.

OP6

Phenotypic, Functional and Molecular Analysis of Novel Human Monocyte Subpopulations

Connaughton, E.P.[1], Dennedy, C.[1], Hanley, S.A[1], Ceredig, R.1, Griffin M.D.[1]
[1]Immunology Group, Regenerative Medicine Institute, National University of Ireland Galway

Background
Based on CD14 and CD16 expression, human blood monocytes can be divided into 3 distinct subpopulations, namely CD14++CD16- "Classical", CD14++CD16+ "Intermediate" and CD14+CD16++ "Non-Classical" subsets. Functional differences between human subsets can be improved however. Furthermore, we have shown that the Intermediate monocyte subset can be further subdivided based HLA.DR (MHC II) and CD16 surface expression, producing 2 additional subsets, DRmid and DRhi subsets. In this study, we have developed an in vitro monocyte transendothelial migration assay using primary human aortic cells (HAECs) seeded onto a fibronectin-coated Transwell® system. The HAEC monolayer is stimulated with TNF-α, and unfractionated human peripheral blood mononuclear cells (PBMCs) are introduced to the upper chamber and cultured for 15 minutes to 4 hours. Following addition of compatible counting beads, monocyte subset analysis and quantification as well as endothelial cell surface analysis are simultaneously performed by multi-colour flow cytometry.

Additionally, we have used centrifugal elutriation (CE) for enrichment of monocytes from unfractionated human PBMCs, consistently enriching the monocyte fraction from 13.1% to 65.2% of total cells. Importantly, CE provided consistent enrichment of all monocyte subsets including both DRmid and DRhi Intermediates and did not activate the cells, as evidenced by lack of pro-inflammatory cytokine production in subsequent culture.

Results
Results to date confirm distinct adhesion and migration characteristics for Classical, Intermediate and Non-Classical monocytes as well as for the DRmid and DRhi Intermediate monocyte subsets. In ongoing work, the system will be adapted to compare monocyte subsets with and without exposure to atherogenic lipids and lipoproteins.

ORAL PRESENTATION ABSTRACTS

OP7

Overexpression of CXCR3 in NIH-3T3 Fibroblasts: Functional Effects and Regulation of IL-13Rα2 Expression

Julie C. Worrell[1], J.C. Barnes[1], R.V. Lumsden[1], S.M. Walsh[2], D.A. Boylan[1], A. Fabre[2], R. Kane[1] and M.P. Keane[2]
[1] UCD Conway Institute of Biomolecular and Biomedical research, University College Dublin, Belfield, Dublin 4, Ireland. [2] Dept. of Respiratory Medicine, St Vincent's University Hospital and School of Medicine and Medical Science, UCD Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland.

Background

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive form of idiopathic interstitial pneumonia, characterized by fibrosis [1]. The chemokine ligands CXCL9, CXCL10 and CXCL11 have been implicated in vascular remodelling and fibroblast motility during the development of pulmonary fibrosis [2] and their receptor CXCR3 (7 trans-membrane domain GPCR) has been shown to have a non-redundant role in limiting fibrosis following lung injury. IL-13 is a pro-inflammatory cytokine that mediates the development of fibrosis and it can induce expression of and bind to its own receptor IL-13Rα2. IL-13Rα2 is proposed to act as a non-signalling decoy receptor [3].

In this study, NIH-3T3 fibroblasts were treated with various cytokines, and the expression of IL-13Rα2, CXCR3 and collagen were measured. The functional impact of overexpressing CXCR3 by transfection in these fibroblasts was also examined using Sircol and BrdU assays.
We have shown that NIH-3T3 fibroblasts express CXCR3. CXCL9, CXCL10 and CXCL11 significantly up-regulate the expression of IL-13Rα2 at 24 hours in NIH-3T3 fibroblasts and IL-13 has been shown to negatively regulate CXCR3 expression. Transfection with CXCR3 caused a significant decrease in Col1A1 gene expression at 48 hours following stimulation with IL-13 and soluble collagen was significantly decreased in transfected cells upon stimulation with CXCL9 and CXCL11.

The 'fibrogenic potential' of NIH-3T3 fibroblasts is affected at both a gene and protein level following overexpression of CXCR3. By exploiting the link between two potentially anti-fibrotic receptors; CXCR3 and IL-13Rα2 it may be possible to unlock their therapeutic potential and development new treatments in the area of pulmonary fibrosis.

References

Raghu G., et al., (2011). An Official ATS/ERS/JRS/ALAT Statement: Idiopathic Pulmonary Fibrosis: Evidence-based Guidelines for Diagnosis and Management. Am J Respir Crit Care Med. 183 788–824
Keane, M.P., et al., (1997). The CXC chemokines, IL-8 and IP-10, regulate angiogenic activity in idiopathic pulmonary fibrosis. J Immunol. 159(3): p.1437-43.
Zhang, J.G., et al., (1997). Identification, purification and characterization of a soluble interleukin (IL)-13-binding protein. J Biol Chem. 272(14): 9474-9480

OP8

Micro-structured Electrodes for the in vitro Detection of Impedance in Cancer Cell Lines

Niall T.P. Savage[1], Brian D. O'Donnell[2], Martin J. O'Sullivan[2], Eric J. Moore[1].
[1]Sensing and Separation Group, Department of Chemistry and Life Science Interface Group, Tyndall National Institute, University College Cork, Cork, Ireland
[2]BreastCheck and Cork University Hospital, Cork, Ireland

Background

Breast cancer is the term used to categorise a diverse range of oncologic conditions which can affect both the ducts and lobules of the breast [1]. The aim of this project is to create a novel electrical impedance device for the detection of invasive breast carcinomas (IBC). Electrical impedance measures the opposition that a circuit presents to a current when a voltage is applied. Each cell type in the human body is composed of different chemical and physical elements which result in characteristic impedance signals generated by each cell variant [2].

Our plan is to use the process of photolithography to pattern dual micro-structured electrodes onto a 10 cm silicon wafer. By applying a minute current across these electrodes it will allow real-time identification of cell populations as either healthy or cancerous. These probes as well as 9-electrode arrays will be used to determine the electrical impedance of a number of cell culture models (HT-27 and MCF-7 immortalised cell lines) and excisional human biopsy samples (that have been pathologically defined). All of the fabrication processes and materials used to create the device will result in it being biocompatible with human tissues.

There are a number of potential uses for this device including improved biopsy localisation, cancer-free border determination during lumpectomy and the possibility of benign tumour determination without the need for invasive surgery.

References

[1] – American Cancer Society (2012) Breast Cancer Facts & Figures 2011-2012. Atlanta: American Cancer Society, Inc.
[2] – Morimoto T, Kimura S, Konishi Y, Komaki K, Uyama T, Monden Y, Kinouchi Y, Iritani T (1993) A study of the electrical bio-impedance of tumors. J Invest Surg 6:25-32

POSTER PRESENTATION ABSTRACTS

PP1

Modelling the Brain: A Nanotechnological Approach

Ajetunmobi, A.[1], Tropea, D.[2], Corvin, A.[2], Volkov, Y.[1,3] & Prina-Mello, A.[1,3]
[1]Department of Clinical Medicine, Institute of Molecular Medicine, St. James' Hospital, Trinity College Dublin. [2]Department of Psychiatry, Institute of Molecular Medicine, St. James' Hospital, Trinity College Dublin. [3]Centre for Research on Adaptive Nanostructures and Nanodevices (CRANN), Trinity College Dublin.

Background
The combined impact of neurodegenerative and neuropsychiatric diseases on the human population is significant, contributing to over 55% of the €800 billion total costs of brain disorders across Europe [1]. Studying the relationship between genes, synapse development and neural circuit function in the pathogenesis of these disorders poses unique technical challenges which are difficult to resolve with current technologies [2]. In recent years, in vitro multielectrode array (MEA) technology has emerged as a novel diagnostic tool, providing a means of studying cultured neuronal networks non-destructively in comparison to more established techniques. An MEA functions by forming a unique electrical interface with neurons that are cultured directly onto electrode micro-devices.

Objectives
This platform seeks to develop a unique MEA device integrating electrophysiological and molecular imaging modalities for the multi-parametric analysis of cellular and molecular mechanisms of neural plasticity [3, 4].

Results
We have tested neuronal cell viability on our device substrate using confocal and atomic force microscopy. Neuronal model shows biocompatibility on array substrate based on morphological and functional characteristics.

Conclusions
We have completed the early steps in biochip development showing neuronal cell viability on biosensor substrate. This indicates the potential use of our platform as a potential early diagnostic tool for personalised therapy of central nervous system disorders.

References
1. Gustavsson, A., et al., 2011. doi: 10.1016/j.euroneuro.2011.08.008
2. Lee, W.C.A. and R. Reid, 2011. doi: 10.1016/j.conb.2011.07.004
3. Balasubramaniam, S., et al., 2011. doi:10.1016/j.nancom.2011.05.004
4. Marconi, E., et al., 2012. doi: 10.1371/journal.pone.0034648

PP2

The Role Of Transcription Factor Rorα in Macrophage Function

Bermingham, R. [1], Hams, E. [1,2] and Fallon, P.G.[1,2]
[1] Trinity Biomedical Sciences Institute, School of Medicine, Trinity College Dublin, Ireland.
[2] National Children's Research Centre, Our Lady's Children's Hospital, Dublin, Ireland.

Background
Retinoid-related orphan receptor alpha (Rorα) is a transcription factor that has a role in neural cell development, metabolism and immunity (1,2,3). In addition, it is known that Rorα plays a role in the circadian clock (4). It is becoming increasingly apparent that circadian rhythm has an important role in immune responses, in particular the response to infection, with macrophages exhibiting diurnal rhythm (5). Rorαsg/sg mice have a natural mutation in RORα and act as a mouse model for exploring Rorα function.

Objectives
Our interest relates to the potential roles of Rorα in immunity and the regulation of macrophage function with a particular focus on the role of macrophages in LPS-induced sepsis. We examined LPS induced sepsis in both wild-type and mutant Rorαsg/sg mice and explored if there was a diurnal rhythm associated with responses.

Results and Discussion
RORαsg/sg mice demonstrate decreased basal accumulation of classically activated macrophages (F4/80int CD11b+; M1) in comparison to wild-type mice, and have an increased susceptibility to LPS-induced sepsis. In addition, both RORαsg/sg mice and WT mice given LPS during active phase (night) show exacerbation of clinical scores compared to mice injected during rest phase (day).

Conclusions
Our data demonstrates an alteration in macrophage polarization in RORαsg/sg mice. We also note a differential response of RORαsg/sg mice to LPS-induced sepsis in the context of circadian rhythm. As peritoneal macrophages play a pivotal role in the response to LPS, the observed exacerbation of sepsis in RORαsg/sg could be related to the altered peritoneal macrophage phenotype in these mice.

References
1. Boukhtouche, F., Janmaat, S., Vodjdani, G., Gautheron, V., Mallet, J., Dusart I., and Mariani, J. (2006) Retinoid-related orphan receptor alpha controls the early steps of Purkinje cell dendritic differentiation. J. Neurosci. 5, 1531-1538.
2. Kang, H. S., Okamoto, K., Takeda, Y., Beak, J. Y., Gerrish, K., Bortner, C. D., et al. (2011) Transcriptional profiling reveals a role for RORa in regulating gene expression in obesity associated inflammation and hepatic steatosis. Physiol. Genomics 43, 818-828.
3 Yang, X.O., Pappu, B.P., Nutieva, R., Akimzhanov, A., Kang, H.S., Chung, Y., Ma, L., Shah, B., Panopoulos, A.D., Schluns, K. S., Watowich, S.S., Tian, Q., Jetten, A.M. and Dong, C. (2008) Immunity, 1, 29-39.
4. Sato, T.K., Panda, S., Miraglia, L.J., Reyes, T.M., Rudic, R.D., McNamara P., Naik, K.A., Fitgerald, G.A, Kay, S.A., Hogenesch, J.B. (2004) Neuron 4, 527-537
5. Nguyen, K.D., Fentress, S.J., Qiu, Y., Yun, K., Coc, J.S. and Chawla, A. (2013) Circadian gene Bmal1 regulates diurnal oscillations of Ly6Chi inflammatory monocytes. 341, 1483-1484.

PP3

Metabolomic Biomarkers in Hypoxic Ischaemic Encephalopathy

Denihan NM, Looney AM, Ahearne C, Walsh BH, Boylan GB, Murray DM
Neonatal Brain Research Group, Department of Paediatrics and Child Health, Cork University Maternity Hospital, Ireland
The Irish Centre for Fetal and Neonatal Translational Research (INFANT), University College Cork and Cork University Maternity Hospital, Ireland

Background
Perinatal asphyxia occurs in 20 per 1000 live births of which approximately 2-3 will go on to develop hypoxic ischaemic encephalopathy (HIE) with subsequent neonatal death or long term disability [1]. The advent of therapeutic hypothermia has heightened the need for early and accurate prediction of HIE severity [2]. Using Nuclear Magnetic Resonance and Mass Spectroscopy platforms we have discovered a number of bio-chemicals in umbilical cord blood (UCB) with the potential to predict moderate/severe HIE [3,4]. These metabolite models can differentiate between HIE and controls with an AUC of 0.93 and have given valuable insight into the alteration of systemic metabolic processes in HIE.

Objectives
Candidate biomarkers will be assessed for ease of measurement, robust nature in UCB and the feasible development of a diagnostic test. An untargeted metabolomic method will complete the final phase of biomarker discovery. Bioinformatics will assess the combined data from all three discovery platforms to build a highly sensitive and specific metabolite model.

Results and Discussion
The effect of serum haemolysis on UCB metabolites was investigated using data from previously analysed control samples. Of the 185 metabolites identified 43 were significantly altered in haemolysed samples. Nine of theses metabolites were potential biomarkers of HIE and may be excluded in future analysis.
We have identified a metabolomic profile combining glycerol and succinate which predicts severe encephalopathy and death in neonatal HIE.

Conclusion
We aim to isolate robust biochemical markers from the metabolomic signature of UCB to develop a point-of-care test to predict HIE severity.

References
1. Volpe JJ (2001) Neurology of the Newborn. Philadelphia: Saunders.
2. Shah PS (2010) Hypothermia: a systematic review and meta-analysis of clinical trials. Seminars in Fetal & Neonatal Medicine 15: 238-246.
3. Walsh BH, Broadhurst DI, Mandal R, Wishart DS, Boylan GB, et al. (2012) The metabolomic profile of umbilical cord blood in neonatal hypoxic ischaemic encephalopathy. Plos One 7: 5.
4. Reinke SN, Walsh BH, Boylan GB, Sykes BD, Kenny LC, et al. (2013) 1H NMR Derived Metabolomic Profile of Neonatal Asphyxia in Umbilical Cord Serum: Implications for Hypoxic Ischemic Encephalopathy. Journal of Proteome Research 12: 4230-4239.

PP4

Epigenetic Targeting of CD1d Increases Anti-Tumor Activity of iNKT Activity in Non-Small Cell Lung Cancer (NSCLC)

Éilis Dockry[1, 2], Yasmeen Giuma[1], Derek G. Doherty[1] and Steven G. Gray[2, 3]
[1] Department of Immunology, Trinity College Dublin, Ireland
[2] Thoracic Oncology Research Group, Institute of Molecular Medicine, St. James's Hospital, Dublin, Ireland
[3] HOPE Directorate, St. James's Hospital, Dublin, Ireland

Background
Immunotherapy is the fourth most important modality for malignant tumours, and is used in treating NSCLC. CD1d, a MHC class 1-like molecule, presents glycolipids to invariant natural killer T (iNKT) cells [1], thereby contributing to their anti-tumour activity. CD1d acts as a target for NKT-mediated killing, however most human and solid mouse tumours are CD1d-negative. Recently evidence has indicated that CD1d expression can be epigenetically regulated [2].

Objectives
To treat NSCLC cell lines with iNKT cells to examine cytolytic degranulation by flow cytometry analysis of cell-surface CD107a expression.
To examine CD1d expression in NSCLC following treatment with various epigenetic modifying agents at the mRNA level by RT-PCR, and at the protein level by flow cytometry analysis.
To confirm that changes in CD1d expression are the result of epigenetic changes using chromatin immunoprecipitation (ChIP) analysis at the promoter level.

Results and Discussion
When NSCLC cell lines were treated with iNKT cells we observed that there was only a slight induction of CD107a expression by iNKT cells. Using RT-PCR we found that both histone deacetylase inhibitors (SAHA) and DNA methyltransferase inhibitors (DNMTi) significantly induced CD1d expression in both A549 and SK-MES-1 cell lines ($p \leq 0.005$). We confirmed, using ChIP analysis that this was due to changes at the promoter level. Using flow cytometry we showed that Decitabine significantly induced CD1d expression in SK-MES-1 cell lines ($p < 0.05$).

Conclusion
CD1d expression is significantly induced using HDi SAHA and DNMTi DAC and GEM, indicating that epigenetic modifying agents can be used to increase the anti-tumourigenic activity of iNKT cells. These results may have important consequences for treating patients with combined epigenetic targeting agents and immunotherapy.

References
Vincent MS, Gumperz JE, Brenner MB (2003) Understanding the function of CD1-restricted T cells. Nat Immunol 4: 517–523.
Yang PM, Lin PJ, Chen CC (2012) CD1d induction in solid tumour cells by histone deacetylase inhibitors through inhibition of HDAC1/2 and activation of Sp1. Epigenetics 7(4): 390 – 399.

PP5

Characteristics of Cognitive Deficits in an Alpha-Synuclein Model of Parkinson's Disease

Dolan EK, Nolan YM and Sullivan AM.
Dept of Anatomy and Neuroscience, University College Cork, Ireland

Background

Parkinson's disease (PD) is a neurodegenerative disorder characterised by the loss of dopaminergic neurons in the substantia nigra of the midbrain and the formation of Lewy Bodies, aggregates of α-synuclein which form proteinaceous inclusions in the neurons. PD is primarily classified as a motor disorder, characterised by bradykinesia, rigidity, tremor and postural instability. As the disease progresses, psychiatric and cognitive symptoms appear, including depression, dementia and dysexecutive syndrome.

A newly-developed animal model of PD involves viral vector-mediated overexpression of α-synuclein in rodents in vivo, which has been shown to replicate many of the clinical features of PD, including nigral dopaminergic neuron degeneration, decreases in striatal dopamine levels and significant motor impairment.

This animal model displays many of the basic pathological, neurochemical and behavioural features of PD. It has an advantage over classic PD animal models as it displays the pathology and progressive neurodegeneration that are characteristic of the disease in humans.

Objectives

Our study aims to characterise late-stage cognitive dysfunction using the α-synuclein model in rodents. We will conduct a series of motor and cognitive tests over an extended time-period to assess deficits in working and associative memory, olfactory discrimination and motor co-ordination. We will perform post-mortem immunohistochemistry and HPLC to examine the progression of the neurodegeneration and pathology in various brain regions over time.

PP6

Transient depressive-like behaviour in response to repeated interferon-alpha administration in mice

M. Fitzgibbon[1, 3], N. O'Reilly[1, 2], D.M. Kerr[1, 2, 3], D.P. Finn[2, 3], M. Roche[1, 3]
[1]Physiology, [2]Pharmacology and Therapeutics, School of Medicine, [3]NCBES Galway Neuroscience Centre and Centre for Pain Research, National University of Ireland Galway, Ireland

Background

Interferon-alpha (IFN-α) is a pro-inflammatory cytokine commonly used to treat various cancers and infections including hepatitis B and C. However, this treatment strategy is associated with depression and a high incidence of painful symptoms, resulting in poor compliance and eventual discontinuation of therapy (1)(2). The present study investigated the effect of repeated IFN-α administration on depressive-like and nociceptive behavior in mice and associated changes in glial activity and neurotrophin expression in discrete brain regions.

Repeated IFN-α administration did not alter body weight or locomotor activity over the course of the study. IFN-α administration for 10, but not 14, days increased immobility in the forced swim test when compared to saline-treated counterparts. IFN-α induced a transient reduction in sucrose preference on day 4 and day 7, which returned to baseline levels by day 10. There was no effect of repeated IFN-α on latency to respond to a noxious thermal stimulus in the hot plate test. The mRNA expression of CD11b (marker of microglial activation), GFAP (marker of astrocyte activation) and brain derived neurotrophic factor in the hippocampus and prefrontal cortex did not differ between saline- and IFN-α treated mice.

Summary

In summary, repeated administration of IFN-α induced transient depressive-like behaviour in mice as assessed using the sucrose preference and forced swim tests, effects not accompanied by alterations in locomotor activity, body weight or thermal nociceptive responding. This model provides a means of investigating the neurobiological mechanisms underlying inflammation-induced transient depressive-like behaviour.

Funding from Molecular Medicine Ireland Clinical & Translational Research Scholars Programme is acknowledged.

References

1. Raison CL, Demetrashvili M, Capuron L, Miller AH. Neuropsychiatric adverse effects of interferon-alpha: recognition and management. CNS Drugs. 2005;19(2):105-23.
2. Nogueira JB, Sena LC, Quintans Jde S, Almeida JR, França AV, Júnior LJ. Side effects of the therapy with peginterferon and ribavirin in chronic hepatitis C: a small audit. J Pharm Practice. 2012;25(1):85-8.

PP7

Investigating the Role of Epithelial-Mesenchymal Transition in Differential Apoptotic Susceptibility in Advanced Docetaxel-Resistant Prostate Cancer

Hanrahan K[1]., Prencipe M[1]., O' Neill A[1]., Lundon D[1]., Watson RW[1].
[1] UCD School of Medicine and Medical Sciences, Conway Institute of Biomolecular and Biomedical Science, University College Dublin.

Background
Docetaxel therapy is the gold standard treatment for advanced castrate-resistant prostate cancer (CRPC). However, patients do not respond or develop resistance over time. Transcriptomic and proteomic analysis of docetaxel-resistant prostate cancer sub-lines developed by our group revealed multiple mechanisms of resistance in line with advanced disease, including over-expression of anti-apoptotic proteins and alterations of NF-κB activation [1]. The sub-lines also demonstrated a coordinated loss and gain of epithelial and mesenchymal markers respectively; a process characteristic of Epithelial-Mesenchymal Transition (EMT) [2]. Studies have highlighted a role of EMT in prostate cancer progression, metastasis [3] and docetaxel resistance [4]. However, the role of EMT drivers in mediating resistance is not defined. We hypothesise EMT to be a central mechanism of apoptotic resistance in advanced docetaxel-resistant prostate cancer, representing a target for therapeutic manipulation.

EMT was characterised functionally (increased invasion, cell-colony scattering, MMP-1 expression) and mechanistically (increased ZEB1, ZEB2 expression) in the PC-3 D12 and DU145 R docetaxel-resistant sub-lines. To investigate the role of EMT in mediating differential apoptotic susceptibility, the in vitro models were treated with various apoptotic triggers. The PC-3 D12 and DU145 R sub-lines demonstrated significant resistance to docetaxel compared to their parental controls. However, the docetaxel resistant cells were not resistant to cisplatin or detachment induced apoptosis, which trigger apoptosis through other pathways.

Results
Our results provide evidence of differential apoptotic susceptibility in in vitro models of docetaxel resistance. This selective resistance to docetaxel may direct the source of inhibition and reveal it as a target for manipulation, which may or may not be EMT dependent.

References
1. O'Neill A, Prencipe M, Dowling C, Fan Y, Muirane L, Gallagher WM, O'Connor D, O'Connor R, Devery A, Corcoran C, Rani S, O'Driscoll L, Fitzpatrick JM, Watson RW. Characterisation and manipulation of docetaxel resistant prostate cancer cell lines. Mol Cancer 2011, 10: 126.
2. O'Connell K, Prencipe M, O'Neill A, Corcoran C, Rani S, Henry M, Dowling P, Meleady P, O'Driscoll L, Watson RW, O'Connor R.The use of LC-MS to identify differentially expressed proteins in docetaxel-resistant prostate cancer cell lines. Proteomics 2012, 12: 2116-2126
3. Lue HW, Yang X, Wang R, Qian W, Xu RZ, Lyles R, Osunkoya AO, Zhou BP, Vessella RL, Zayzafoon M, Liu ZR, Zhau HE, Chung LW. LIV-1 promotes prostate cancer epithelial-to-mesenchymal transition and metastasis through HB-EGF shedding and EGFR-mediated ERK signalling. PLoS One 2011, 6(11) e27720.
4. Puhr, M. et al., 2012. Epithelial-to-Mesenchymal Transition Leads to Docetaxel Resistance in Prostate Cancer and Is Mediated by Reduced Expression of miR-200c and miR-205. The American Journal of Pathology, 181(6), pp.2188–2201.

PP8

Hepatitis C Virus Disruption of Lipid and Autophagy Pathways Provides an Opportunity for the Discovery of New Adjunct Therapies

Harty[1] C.H., McCarthy[3] T., Crosbie[2] O., Kenny-Walsh[2] E., Fanning[1] L.J.
[1] Molecular virology diagnostic and research laboratory, Department of Medicine, University College Cork, Cork, Ireland. [2] Hepatology, Cork University Hospital. [3] Dept. of Biochemistry, University College Cork.

Background
Hepatitis C virus (HCV) infects 2-3% of the world's population [1]. Treatment efficacy for genotypes 2 and 3 approaches 75-80% with dual therapy (pegylated interferon and ribavirin). Patients who fail therapy have no secondary treatment options, may develop steatosis and advance to end stage liver disease. HCV life cycle is dependent on lipid metabolism and usurps the early stages of the autophagy pathway to create an environment suitable for replication [2].

We have developed a reproducible in vitro infection model for serum derived HCV (sdHCV). The inability of HCV to complete a full cycle of replication in vitro is well established[3]. For this reason, there has been limited use of sdHCV in cell culture. However, we have shown the presence intracellular HCV proteins, core and NS3, up to 48hrs post-infection. As expected, we have observed core protein surrounding lipid droplets in hepatocytes. Displacement of the adipose differentiation related protein (ADRP) from the surface of lipid droplets by HCV core protein has been shown [4], we have confirmed this with our chosen in vitro model. It has been shown that autophagy proteins are required for the translation of incoming HCV RNA [5]. We have shown upregulation of early stage autophagy protein, Atg5, in hepatocytes infected with sdHCV. We have observed co-localisation of the proteins NS3 and Atg5 indicating the direct interaction of HCV proteins with autophagy proteins.

Further Investigations
Having identified the new biology of NS3 and ATG5 interaction, I plan to further investigate how viral infection modulates lipid metabolism and autophagy.

References
1.Jones, C.T., et al., Real-time imaging of hepatitis C virus infection using a fluorescent cell-based reporter system. Nat Biotechnol, 2010. 28(2): p. 167-71.
2.Dreux, M. and F.V. Chisari, Impact of the autophagy machinery on hepatitis C virus infection. Viruses, 2011. 3(8): p. 1342-57.
3.Bartenschlager, R. and V. Lohmann, Novel cell culture systems for the hepatitis C virus. Antiviral Res, 2001. 52(1): p. 1-17.
4.Boulant, S., et al., Hepatitis C virus core protein induces lipid droplet redistribution in a microtubule- and dynein-dependent manner. Traffic, 2008. 9(8): p. 1268-82.
5. Dreux, M., et al., The autophagy machinery is required to initiate hepatitis C virus replication. Proc Natl Acad Sci U S A, 2009. 106(33): p. 14046-51.

PP9

Assessment of Autophagy Associated Genes and Inducers as Modulators of Chemo-Toxicity in Oesophageal Cancer

Healy M.K.[1], O'Donovan TR[1],Buckley B.[2], McKenna S.L.[1]
[1] Bon Secours Hospital, College Road, Cork, Ireland.
[2] Cork Cancer Research Centre, Biosciences Institute, Cork, Ireland.

Background
Autophagy is a highly conserved cellular process, whereby components of the cytoplasm, such as protein aggregates, organelles and other macromolecules are digested. Our laboratory has shown that chemo-resistant oesophageal cancer cell lines (OE19 & KYSE450) induce autophagy in response to 5-fluorouracil (5-FU) [1]. In this study we are investigating how autophagy may be modulated for better chemotherapeutic effect.

Methodology
(i) KYSE450 cells were treated with several potential inducers of autophagy to assess their therapeutic potential when combined with 5FU.

(ii) Using apoptotic and autophagic gene databases along with mining of gene array data we have generated a list of genes that may have functional importance in autophagy or apoptosis susceptibility.

Results
(i) Valproic acid (2.5mM, 5mM & 7.5mM) as a single agent, negatively impacted clonogenic survival of KYSE450 cells. When tested in combination with 5-FU, valproic acid displayed a synergistic effect in decreasing clonogenic survival. The contribution of autophagy to this enhancement of cytotoxicity with these agents is not currently known.

(ii) A number of genes of interest are being analysed by rtPCR to confirm differential expression between chemo-sensitive/resistant cell lines. Functional effects on chemo-sensitivity will be evaluated.

Conclusion
Valproic acid in combination with 5-FU may represent a novel treatment strategy for chemo-resistant oesophageal cancer cells.
We are also analysing several differentially expressed genes for functional importance in drug sensitivity/resistance.

References
1. O'Donovan, T.R., O'Sullivan, G.C., and McKenna, S.L. (2011). Induction of autophagy by drug-resistant esophageal cancer cells promotes their survival and recovery following treatment with chemotherapeutics. Autophagy 7, 509-524.

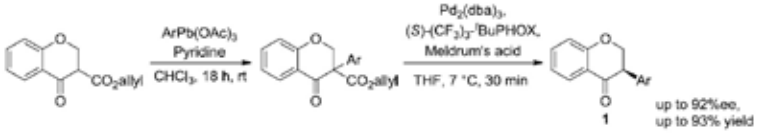
PP10

The Palladium-Catalysed Decarboxylative – Asymmetric Protonation and Allylic Alkylation

Patrick J. Guiry and **Mark P. Jackson**
Centre for Synthesis and Chemical Biology, School of Chemistry and Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland
email: pat.guiry@ucd.ie, mark.jackson@ucd.ie

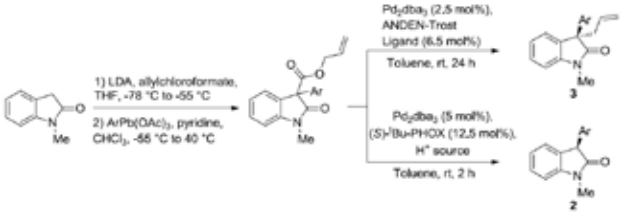
Asymmetric transition metal catalysed transformations have emerged as a powerful tool for the generation of stereocentres on the α-position of carbonyls.[1] However, one area in which this has proved more challenging is the formation of a tertiary centre containing an aryl group, mainly due to lability of the resulting stereocentre. Recently Stoltz reported a Pd-catalysed decarboxylative asymmetric protonation of α-alkyl and α-benzyl ketones.[2,3] Our research group has further developed this methodology to generate sterically hindered tertiary α-aryl ketones in the first catalytic asymmetric synthesis of isoflavanones 1 (Scheme 1).[4]

Scheme 1 The first catalytic asymmetric synthesis of isoflavanones **1.**



Oxindoles are important scaffolds in many biologically active molecules.[5] The vast majority of these include substitution at the 3-position. The focus of the current project is to expand the decarboxylative protonation to the asymmetric synthesis of α-aryl oxindoles of type 2. We aim to investigate the influence of the steric bulk of the aryl group on the enantioselectivity of the related allylation to form oxindoles of type 3. This poster will highlight our recent progress in this area.

Scheme 2 Catalytic asymmetric synthesis of oxindoles of type **2** and **3.**



References
1. Watson, A & MacMillan, D 2011, P. A. Evans (ed.), in:Science of Synthesis: Stereoselective Synthesis 3; Stereoselective Pericyclic Reactions Cross-coupling, and C-H and C-X Activation. Thieme, Stuttgart, pp. 675-745.
2. Marinescu, S. C., Nishimata, T., Mohr, T., Stoltz, B. M. Org. Lett., 2008, 10, 1039-1042.
3. Mohr, T., Nishimata, T., Behenna, D., Stolz, B. M. J. Am. Chem. Soc. 2006, 128, 11348-11349.
4. Carroll, M. P., Müller-Bunz, H., Guiry, P. J. Chem. Commun., 2012, 48, 11142-11144.
5. Trost, B. M., Brennan, M. K. Synthesis 2009, 3003-3025.

PP11

Identifying CDC7-Dependent Claspin Protein Interactors

Edel McGarry[1], Michael D. Rainey[1] and Corrado Santocanale[1]
[1]Centre for Chromosome Biology and National Centre for Biomedical Engineering Science, National University of Ireland, Galway,

Background
Cells possess checkpoint pathways important for genome stability and preventing cancer. These signalling pathways include the ATR and Chk1 kinases which are activated in response to DNA damage or replication block. Activation of Chk1 by ATR requires the mediator protein Claspin.[1]
Claspin also has a role in the unperturbed cell cycle regulating normal rates of fork progression. Claspin interacts with many replisome components including Mcm2 and Cdc7. Cdc7 is an essential kinase which is required for the initiation of DNA replication in eukaryotic cells. Cdc7 is up-regulated in many cancers. Several Cdc7 kinase inhibitors are being explored in clinical trials.Cdc7 kinase inhibition attenuates the Claspin-Mcm2 interaction. [2]

Objectives
We hypothesize that Cdc7 dependent phosphorylation of Claspin is important to coordinate Claspin interaction with other cellular proteins. We will test this hypothesis by identifying proteins that interact with Claspin in a Cdc7-dependent manner. Proteins that differentiallly bind to Claspin will be investigated.

Results, Discussion & Conclusion
Cells conditionally overexpressing Claspin fused to a STREP and FLAG Tag have been characterised. The over-expression of this Claspin fusion protein does not affect cell growth, cell proliferation, or cellular location.

A purification protocol for Claspin has been extensively optimised. Differential banding patterns, as observed by SDS-PAGE, from purified Claspin from either untreated cells or cells treated with a Cdc7 inhibitor support the hypothesis that Claspin-protein interactions can be modulated by Cdc7 inhibition. A SILAC strategy has been employed to allow the quantitative identification of co-purified proteins.
From this work we expect to gain mechanistic insights into an important tumour preventing pathway as well as understanding the mechanism of action of Cdc7 inhibitors currently under investigation as experimental anticancer drugs.

References
1. Petermann E, Helleday T, Caldecott KW. Claspin promotes normal replication fork rates in human cells. Mol Biol Cell 2008; 19:2373-8.
2. Rainey MD, Harhen B, Wang GN, Murphy PV, Santocanale C. Cdc7-dependent and -independent phosphorylation of Claspin in the induction of the DNA replication checkpoint. Cell Cycle 2013; 12:1560-8.

PP12

Toll-Like Receptor 2 Activation Induces Pro-inflammatory, Inflammasome and Notch Signalling Pathways in Rheumatoid Athritis

Trudy McGarry[1], Wei Gao[1], Mary Connolly[1], Gavin Walsh[1], Jennifer McCormick[1], Douglas Veale[1] and Ursula Fearon[1]
[1] St Vincent's University Hospital, Dublin Academic Medical Centre and the Conway Institute of Biomolecular and Biomedical Research, Dublin, Ireland

Introduction
Rheumatoid Athritis (RA) is characterized by inflammation and proliferation of synovial tissue, leading to progressive degradation of articular cartilage and bone. This study examines the effect if Toll-Like Receptor (TLR) 2 activation on the inflammasome, cell migration/invasion and Notch signalling pathways

Methods
RA synovial fibroblast cells (SFC) and RA ex vivo explant cytokine release and NLRP3 expression in response to Pam3CSK4 (TLR2-ligand) (1µg/ml) were measured by ELISA, MSD multiplex assay and Western Blotting. TLR2-induced RASFC invasion, migration/cytoskeletal rearrangement, MMP-3 and TIMP-1 expression were assessed by wound repair assays, F-actin immunofluorescence and ELISA. Notch-1 expression in RASFC and whole RA ex vivo explant in response to Pam3CSK4 (1µg/ml) was determined by Western Blotting and RT-PCR. Expression of Notch signalling components (Notch-1, D11-4, Jagged-1 and HRT-1) were assessed in RA, osteoarthritis (OA) and healthy control synovial tissue by immunohistochemistry. Finally, TLR2-induced cytokine production, cell migration and cytoskeletal rearrangement in the presence or absence of Notch-1 siRNA or a γ-secretase inhibitor N-(N-(3,5-Difluorophenacetyl-L-alanly))-S-phenylglycine-t-Butyl Ester (DAPT) was assessed.

Results
Pam3CSK4 specifically induced IL-6, IL-8, TNFα and IL-1β in RA ex vivo explants and RASFC ($p<0.05$), with no increase in IFNγ. Pam3CSK4 significantly induced NLRP3 expression and downstream inflammasome-related cytokines IL-1β and IL-18 ($p<0.05$). Pam3CSK4 significantly induced cell migration, ECM degradation and increased the MMP-3/TIMP-1 ratio (all $p<0.05$). Pam3CSK4 induced RA synovial explant outgrowth consistent with TLR2-induced RASFC migration and ECM degradation. Notch signalling components were significantly increased in RA synovium compared to OA and control synovium ($p<0.05$). Pam3CSK4 induced Notch-1 and Jagged-1 mRNA expression with differential effects observed for active Notch-1 intracellular (IC) protein expression. Pam3CSK4-induced cytokine production was in part inhibited in the presence of either Notch-1 siRNA or DAPT.

Conclusion
TLR2 activation in RA activates inflammasome, cytokine production and migrational/invasive mechanisms, effects that may in part be mediated by the Notch-1 signalling pathway.

PP13

Investigation of E1E2 Glycoprotein in Hepatitis C Virus

Naik[1] A.S., Crosbie[2] O., Kenny-Walsh[2] E., Fanning[1] L.J.
[1] Molecular virology diagnostic and research laboratory, Department of Medicine, University College Cork, Cork, Ireland. [2] Hepatology, Cork University Hospital.

Hepatitis C virus (HCV) is an enveloped virus which circulates in infected individuals as quasispecies [1]. HCV encodes two highly glycosylated envelope glycoproteins E1E2 [2]. The E1E2 complex is involved in fusion and entry of virus into the hepatocytes. E1E2 is hypervariable in nature and is a target of humoral immune system. The immune system produces antibodies against susceptible virions which are removed from the heterogenous virus population leading to the emergence of virions with modulated surface glycoproteins [3].
The objective of the current study involves separation of IgG enriched and IgG depleted fraction of quasispecies [4] followed by amplification and analysis of E1E2 glycoprotein to identify neutralizing epitopes in E1E2 receptor binding domain.

Serum samples from a panel of viraemic sera positive for different HCV genotypes will be randomly selected. Samples will be separated into IgG enriched and IgG-depleted fractions using Ab Spin Trap TM columns which has Protein G Sepharose TM high Performance medium. The separated fractions will be amplified and sequenced for full length E1E2 glycoprotien. Sequence comparison between the IgG-enriched and depleted fractions will give an insight into mutations, possible glycosylation sites and neutralizing epitopes in E1E2 glycoprotein. For the exploration of E1E2, I have optimised the PCR conditions for full length amplification of E1E2 of genotypes 1a, 1b, 3a, 4a and 4e.

The outcome of the project will be improving our understanding about role of E1E2 glycoproteins in adaptive immunovirology of HCV.

References

Bowen D.G., Walker C.M.(2005). The origin of quasispecies: cause or consequence of chronic hepatitis C viral infection. J Hepatol , 42, 408-417.
Beeck A. , Voisset C, Bartosch B., Ciczora Y., Cocquerel L., Keck Z., Foung S., Cosset F., Dubuisson J. Characterization of Functional Hepatitis C Virus Envelope Glycoproteins(2004). JOURNAL OF VIROLOGY,78(6), 2994–3002.
Hino K., Fujii K., Korenaga M., Murakami C., Okazaki M., Okuda M., Okita K. Correlation between relative number of circulating low-density hepatitis C virus particles and disease activity in patients with chronic hepatitis C(1997). Dig Dis Sci 42,2476-2481.
Moreau I., O'Sullivan H., Murray C., Levis J., Crosbie O., Kenny-Walsh E.,
Fanning L. (2008). Separation of Hepatitis C genotype 4a into IgG-depleted and IgGenriched fractions reveals a unique quasispecies profile. Virology Journal 5, 103.

PP14

Optimisation of a Multi-Colour Flow Cytometry Protocol for Analysis of Human Peripheral Blood Monocyte Profiles in a Cohort of Chronic Kidney Disease Patients

Serika Naicker[1], Deirdre Cotter[2], Dr. Vincent Tormey[2], Prof. Rhodri Ceredig[1] and Prof. Matthew Griffin[1].
[1]Regenerative Medicine Institute, School of Medicine, National University Ireland, Galway.
[2]Clinical Immunology Laboratory, Galway University Hospital.

Abstract

Chronic Kidney Disease (CKD), affecting as much as 10% of the world's population, is characterised by the gradual decline of kidney function eventually leading to End Stage Renal Disease (ESRD) and the requirement for dialysis or kidney transplantation to sustain life. Increasingly, studies indicate that CKD, in particular the later stages, is associated with an accelerated and abnormal activity of elements of the immune system[1].

Monocytes, a subset of circulating white blood cells involved in innate immunity, have been reported to be altered in patients with CKD, although their direct role in CKD progression is not well understood[2]. Furthermore, the influence of this disease on monocyte transmigration to peripheral tissues and activation/differentiation following migration are not well elucidated at present in human subjects[3].

In order to confidently identify and characterise human blood monocytes using flow cytometry, we have initially set out to optimise a multi-colour flow cytometry protocol using the Beckman Coulter Navios® flow cytometer with tri-laser technology and ten colour fluorescence capability. Fluorochrome-coupled monoclonal antibodies directed against several primary monocyte identification and classification markers including CD16, CD14, CD33, CD 56, HLA-DR and CX3CR1 are combined with antibodies against surface or intracellular proteins involved in monocyte inflammatory functions. The optimal staining concentrations for a panel of these antibodies has been determined through a series of titration experiments.

Once optimised, this novel flow cytometry-based protocol for quantifying and functionally phenotyping monocyte subsets in peripheral blood will be applied to prospectively acquired samples from a large cohort of patients with CKD enrolled from the Galway University Hospitals. These profiles will be compared to those of healthy adult volunteers. A specific focus of this project will be to compare the expression of proteins from the scavenger receptor family in monocyte subpopulations. This diverse protein family plays an important role in mediating monocyte responses to a wide variety of biomolecules – some of which we hypothesise to be altered in CKD/ESRD.

Reference

1. The immune system and kidney disease: basic concepts and clinical implications (2013). Kurts C., Panzer U., Anders H.J & Rees A.J. Nature Reviews Immunology 13 738–753.
2. Monocyte subpopulations and cardiovascular risk in chronic kidney disease (2012). Heine G.H., Ortiz A., Massy Z.A., Lindholm B., Wiecek A., Martínez-Castelao A., Covic A., Goldsmith D., Süleymanlar G., London G.M., Parati G., Sicari R., Zoccali C & Fliser D. Nat Rev Nephrol 8(6) 362-9.
3. Atherosclerosis in chronic kidney disease: the role of macrophages(2011). Kon V., Linton M.F, and Fazio S. Nat Rev Nephrol. 7(1): 10.1038.

PP15

Combination of Cdc7 and PARP inhibitors for breast cancer treatment

Quach Thi Thu, H.[1,2], Rainey, MD[1,2]. and Santocanale, C[1,2].
[1]Centre for Chromosome Biology (CCB) and [2]National Centre for Bioengineering and Sciences (NCBES); School of Natural Sciences; National University of Ireland Galway; Galway, Ireland

Background
Cdc7 (cell division cycle 7) is a serine/threonine kinase involved in the regulation of DNA replication by phosphorylating several subunits of MCM (mini-chromosome maintenance) complex, the core component of replicative DNA helicase which is required for the firing of replication origins and the replication fork progression. PARPs, poly(ADP-ribose) polymerases, are a family of enzymes that, by transferring PAR, poly(ADP-ribose) to acceptor proteins, regulate their functions. In particular, PARP1 has an important role in DNA repair. Inhibition of Cdc7 and of PARPs have shown significant efficacy in cancer treatment in preclinical and in clinical studies respectively [1], [2]. Importantly, both Cdc7 and PARP inhibition individually affects the efficiency of S-phase progression [3]–[5].

Objectives
The primary objective of this study is to assess the effects of pharmacological inhibition of Cdc7 and PARP either as single agent or in combination in breast cancer cells. We also aim to assess molecular mechanisms underlying a potential additive anticancer activity of this combination.

Results & Discussion
MCF10A (wild type p53) and MDA-MB-231 (mutant p53) cells were treated with a Cdc7 inhibitor (XL413) and a PARP inhibitor (AZD2281), also known as Olaparib, either as single agent or in combination. Interestingly, we observed that the combination of both inhibitors significantly reduced the survival of both cell types. Preliminary observations suggested that after 24 hours of treatment with both inhibitors, cells encountered a strong delay in S-phase without obvious signs of cell death by apoptosis or necrosis.

Conclusions
The combination of Cdc7 and PARP inhibitors may have a potential therapeutic value in breast cancer. Further studies are needed to evaluate the therapeutic potential of this combination and to understand the molecular mechanisms underlying the observed additive effects.

References
[1] A. Montagnoli, B. Valsasina, V. Croci, M. Menichincheri, S. Rainoldi, V. Marchesi, M. Tibolla, P. Tenca, D. Brotherton, C. Albanese, V. Patton, R. Alzani, A. Ciavolella, F. Sola, A. Molinari, D. Volpi, N. Avanzi, F. Fiorentini, M. Cattoni, S. Healy, D. Ballinari, E. Pesenti, A. Isacchi, J. Moll, A. Bensimon, E. Vanotti, and C. Santocanale, "A Cdc7 kinase inhibitor restricts initiation of DNA replication and has antitumor activity.," Nat. Chem. Biol., vol. 4, no. 6, pp. 357–65, Jun. 2008.
[2] W. D. Joo, I. Visintin, and G. Mor, "Targeted cancer therapy-are the days of systemic chemotherapy numbered?," Maturitas, vol. 76, no. 4, pp. 308–14, Dec. 2013.
[3] A. Montagnoli, P. Tenca, F. Sola, D. Carpani, D. Brotherton, C. Albanese, and C. Santocanale, "Cdc7 Inhibition Reveals a p53-Dependent Replication Checkpoint That Is Defective in Cancer Cells," Cancer Res, vol. 64, pp. 7110–7116, 2004.
[4] P. Tenca, D. Brotherton, A. Montagnoli, S. Rainoldi, C. Albanese, and C. Santocanale, "Cdc7 Is an Active Kinase in Human Cancer Cells Undergoing Replication Stress *," J Biol Chem, vol. 282, no. 1, pp. 208–215, 2007.
[5] H. Farmer, N. McCabe, C. J. Lord, A. N. J. Tutt, D. a Johnson, T. B. Richardson, M. Santarosa, K. J. Dillon, I. Hickson, C. Knights, N. M. B. Martin, S. P. Jackson, G. C. M. Smith, and A. Ashworth, "Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy.," Nature, vol. 434, no. 7035, pp. 917–21, Apr. 2005.

PP16

Cell Specific Expression of Interferon Lambda Receptor

Roche, G.A.[1], Robinson, M.W.1, Kelly, A [2], and O' Farrelly, C[1].
[1]School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin (TCD), Dublin, Ireland.
[2] Manchester Collaborative Centre for Inflammation Research, Faculty of Life Sciences, University of Manchester, Manchester M13 9NT.

Background
Interferon lambda (IFN-λ) or Type III IFN is an anti-viral cytokine family important in the resolution of HCV infection [1]. The three subtypes of IFN-λ: IL-28A, IL-28B and IL-29 mediate their effects via the IL- 28R1/IL-10R2 receptor complex. Unlike the receptor for IFN-α or IFN-β (Type I IFNs), IFN-λ receptor expression appears to be limited to certain populations of cells including epithelial cells and some immune cells.

Objectives
In order to identify immune cell subsets responsive to IFN lambda we examined IL- 28R1 and IL-10R2 receptor expression on NK cells, T cells and monocytes in whole blood using flow cytometry.

Results
Whole blood samples were obtained from 9 healthy donors. Expression of the IL-28R1 and IL-10R2 receptor chains on CD3+ T cells, CD56+ NK cells and CD14+ monocytes was examined in three healthy donors by staining followed by flow cytometric analysis. T cells and NK cells showed no IL-28R1 or IL-10R2 expression. In contrast, a proportion of monocytes were positive for IL- 28R1 and IL-10R2. Analysis of Classical (CD14++, CD16-), Intermediate (CD14++, CD16+), and Non-classical (CD14+, CD16++) monocytes was performed on whole blood from healthy donors (n=6). It was found that each subset monocyte cell population expressed the IFN-λ receptor in equal proportions with an average percent positivity for IL- 28R1 of >50% and IL-10R2 of >80%.

Discussion
The expression of the IFN-λ receptor on each subpopulation of monocytes suggests they are involved in IFN-λ signalling in vivo. Elucidating the exact mechanisms of IFN-λ signalling is relevant in studying the immune response to HCV infection.

References
1. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. (2009) Nature 461, 399–401.

Acknowledgments: This work was funded by Molecular Medicine Ireland as part of the Clinical & Translational Research Scholars Programme.

PP17

The Effects of anti-TNF Antibody Therapy on Blood Monocytes in Patients with Inflammatory Bowel Disease

Stephanie Slevin[1], Conall Dennedy[1], Andreia Ribeiro[1], Rhodri Ceredig[1], Matthew D Griffin[1] and Laurence J Egan[2]
[1]Regenerative Medicine Institute, School of Medicine, National University of Ireland, Galway.
[2]Discipline of Pharmacology and Therapeutics, National University of Ireland, Galway.

Abstract
Monocytes, recently classified as CD14++/CD16- (classical), CD14++/CD16+ (intermediate) and CD14+/CD16++ (non-classical), play an as-yet poorly characterized role in inflammatory bowel disease (IBD) pathogenesis. In this study, proportions and absolute numbers of circulating monocyte subsets were determined by flow cytometry of fresh PBMC samples from 4 groups: A. Healthy controls (n=11); B. Crohn's disease (n=17) and C. Ulcerative colitis patients not receiving infliximab (n=12) and D. IBD patients receiving infliximab (n=29). For Group D, samples were analyzed before and after anti-TNF-α infusions.

Total monocyte numbers were higher in Group B and C compared to groups A and D, (17.7±9.9 and 22.8±8.5; 15.1±6.1 and 19.0±10.8 x104 cell/ml respectively; p<0.001, one way ANOVA). Group D total monocytes were reduced to 7.3±4.9 x104 cells/ml following infliximab (p<0.001, paired t-test). Subset analysis showed increased classical monocytes in ulcerative colitis patients compared to the other groups (Group A 8.6±3.6; Group B 10.2±7.1; Group C 13.7±5.7; Group D 6.7±5.2 pre-infliximab and 2.6±2.4 x104 cells/ml post-infliximab, p<0.001). Similar trends were present for the non-classical monocytes (Group A 10.8±6.2; Group B 12.5±13.0; Group C 15.6±12.8; Group D 10.2±8.5 pre-infliximab and 6.0±6.04x103 post-infliximab, p<0.001). The intermediate subset was increased in crohn's disease patients and reduced following infusion of infliximab (Group A 10.8±5.8; Group B 34.7±25.7; Group C 32.6±43.0; Group D 51.7.0±60.9 pre-infliximab and 17.2±19.0 x103 post-infliximab, p<0.001).

Total lymphocyte numbers were significantly reduced in Group D following infliximab therapy (pre 53.2±38.8 and post 26.2±26.5x104 cells/ml). However, Group D responded to infliximab with a significant increase in their granulocyte numbers (pre therapy 19.3±8.3 and post therapy 28.7±17.2 x105 cells/ml).

Freshly isolated PBMCs from Group A showed preferential binding of Infliximab to the Classical and Intermediate monocyte populations. Following anti-TNF therapy PBMCs from Group D show no increased intracellular cleaved caspase 3. Functional assays carried out on Group D also show blunted IL-12 production when stimulated with LPS following infliximab infusion in comparison to healthy controls.

Thus, inflammatory bowel disease is associated with an increase in total monocytes in the circulation compared to healthy controls and infliximab infusion leads to a significant reduction in total monocyte numbers and subsets but also lymphocyte counts. Granulocyte numbers however are significantly increased following this therapy. Infliximab preferentially binds to particular subsets of PBMCs and following treatment, causes a blunt pro-inflammatory cytokine production by LPS stimulated monocytes

PP18

Pilot Data on Brain-to-Blood Efflux of Beta-Amyloid Peptides in Man

Heverin, M.[1], Religa, D.[1], Wahren, J.[2], Diczfalusy, U.[1], Björkhem, I.[1], **Meaney, S.**[3]
[1] Department of Laboratory Medicine, Karolinska University Hospital - Huddinge, Sweden.
[2] Department of Surgical Sciences, Karolinska University Hospital - Solna, Sweden [3] School of Biological Sciences, College of Sciences and Health, Dublin Institute of Technology – Kevin Street, Dublin 8.

Background
Alzheimer's disease is thought to be caused by an increase in the production or impairment of the clearance of beta amyloid peptide(s). Flux of beta amyloid peptides across the blood-brain barrier is thought to contribute to the elimination of amyloid from the brain, e.g. by immunotherapy. Limited data has been presented about the in-vivo production rates in humans (1). There are no data available on brain-to-blood output of beta amyloid peptides in man or of potential hepatic clearance.

Objectives
To investigate if the concentration beta-amyloid peptides is different in jugular venous plasma and arterial plasma and so determine direct values for brain-to-blood beta-amyloid efflux and hepatic clearance in man.
Results and Discussion
Plasma samples available in connection with (2) were analysed by an ELISA method for Aβx-40 and Aβx-42. Although several participants demonstrated a net brain-to-blood output of beta-amyloid the relatively high variability and the small participant cohort (n=10) complicated statistical analyses. A similar pattern was observed with regards to hepatic uptake, with many, but not all, participants demonstrating uptake of beta-amyloid.

Conclusions
Although preliminary, this work presents some of the only data on inter-organ beta-amyloid dynamics in man. It will facilitate design of larger studies to conclusively establish the fluxes of beta-amyloid in man. Such fluxes may be of relevance for evaluation of amyloid mobilising therapies.

References
1. Mawuenyega K.G., Kasten T., Sigurdson W., Bateman R.J. (2013) Amyloid-beta isoform metabolism quantitation by stable isotope-labeled kinetics. Anal Biochem. 440, 56-62.
2. Meaney S., Heverin M., Panzenboeck U., Ekström L., Axelsson M., Andersson U., Diczfalusy U., Pikuleva I., Wahren J., Sattler W., Björkhem I. (2007) Novel route for elimination of brain oxysterols across the blood-brain barrier: conversion into 7alpha-hydroxy-3-oxo-4-cholestenoic acid. J Lipid Res. 2007 48,944-51.

PP19

Semi-Interpenetrating Polymer Networks (SIPINS) Incorporating Polygalacturonic Acid for Implantable Medical Device Applications

O' Carroll A.N., McCoy C.P., Carson L
Queen's University Belfast, School of Pharmacy, 97 Lisburn Road, Belfast. BT9 7JL. UK

Background
Over 20 million people in the US are living with an implantable medical device [ADDIN RW.CITE{{3114 Higgins,DavidM 2009}}1], with similar figures anticipated for Europe. Complications in the use of medical implants include the Foreign Body Response (FBR) characterised by macrophage adherence and fusion, and device-related infection due to bacterial biofilm formationADDIN RW.CITE{{3124 Harding,JacquelineL 2014}}2. Both can have detrimental consequences on the structural and functional integrity of the medical device [ADDIN RW.CITE{{3101 Anderson,JamesM 2008; 3124 Harding,JacquelineL 2014}}2,3], often necessitating removal; a painful and expensive procedure [ADDIN RW.CITE{{3121 Mah,Thien-FahC 2001}}4]. Materials are sought to attenuate both the FBR and device-related infection, leading to medical devices with improved biocompatibility and performance.

Objectives
The present work involves development of a semi-interpenetrating network (SIPN) hydrogel containing polygalacturonic acid (PGA), a biopolysaccharide similar in structure to hyaluronic acid. We aim to synthesise, characterise and determine the in vitro biocompatibility of the developed SIPN.

Results & Discussion
We have successfully incorporated PGA into a poly(HEMA) based hydrogel, which shows favourable swelling and wettability. The surface topography appears altered in comparison to the control material, with pronounced micrometer-scale features. In terms of in vitro performance, the SIPN showed increased protein adsorption, and biofilm formation (Staphylococcus epidermidis and Escherichia coli, up to 1 Log CFU/sample greater than control). However the SIPN displayed minimal cytotoxicity towards L929 fibroblasts, and was resistant to the adherence of RAW 264.7 macrophages.

Conclusions
The PGA incorporated SIPN lacks cytotoxicity and shows reduced macrophage adherence, however the increased biofilm formation highlights a concern regarding possible device related infection in clinical use. Future work will focus on strategies to reduce bacterial adherence, while maintaining biocompatibility.

References
ADDIN RW.BIB1. Higgins DM, Basaraba RJ, Hohnbaum AC, Lee EJ, Grainger DW, Gonzalez-Juarrero M. (2009). Localized immunosuppressive environment in the foreign body response to implanted biomaterials. The American Journal of Pathology. 175,161-170.
2. Harding JL, Reynolds MM. (2014). Combating medical device fouling. Trends Biotechnol. In press.
3. Anderson JM, Rodriguez A, Chang DT. (2008). Foreign body reaction to biomaterials. Seminars in immunology. 20,86-100.
4. Mah TC, O'Toole GA. (2001). Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol. 9,34-39.

PP20

A Randomised Trial of Placing Preterm Infants on Their Back or Left Side at Birth: The BREL Trial (ISRCTN74486341)

Emily J. Stenke[1], **Emily A. Kieran** [1-3], Lisa K. McCarthy [1-3], Jennifer A. Dawson[4], Jeroen J. Van Vonderen[5], C. Omar F. Kamlin[4], Peter G. Davis[4], Arjan B. Te Pas[5], Colm P.F. O'Donnell[1-3]
[1] National Maternity Hospital, Dublin, Ireland [2] National Children's Research Centre, Dublin, Ireland [3] University College Dublin, Ireland [4] Royal Women's Hospital, Melbourne, Australia [5] Leiden University Medical Centre, Leiden, Netherlands

Background
Basic life support guidelines recommend placing spontaneously breathing children and adults on their side. Newborn infants are placed on their backs after birth. The majority of preterm newborns breathe spontaneously. We hypothesised that preterm infants would breathe more effectively if placed on their left side rather than on their backs immediately after birth.

Methods
In this multicentre randomised trial, infants < 32 weeks without congenital anomalies were randomised before delivery to be placed on their back (B) or on their left side (L). Respiratory support was given in the delivery room (DR) with a T-piece and face mask, and with initial FiO2 30%. The primary outcome was pre-ductal oxygen saturation (SpO2) at 5 minutes of life.

Results
We enrolled 87 infants. The groups were well matched for demographic and clinical variables. The mean (SD) SpO2 was 72 (23) % for B infants and 71 (24) % for L infants (p=0.956). We found no differences in secondary outcomes between the groups.

Conclusions
Preterm newborns placed on their left side did not have higher SpO2 at 5 minutes after birth. Placing infants on their left side may be a reasonable alternative to placing them on their backs.

	BACK (N = 41)	LEFT SIDE (N =46)	P value
SpO_2 at 5 min (%)*	72 (23)	71 (24)	0.956
HR at 5 min (bpm)*	137 (30)	132 (34)	0.523
SpO_2 at 10 min (%)*	89 (8)	87 (16)	0.532
HR at 10 min (bpm)*	153 (18)	153 (22)	0.928
Time to reach SpO_2 90% (s)*	424 (185)	406 (202)	0.702

*Mean (standard deviation), #n (%), ~median (IQR)

PP21

A Randomised Trial of Estimating Umbilical Catheter Insertion Depth in Newborns Using Birth Weight or Surface Measurements: The WorM Trial (ISRCTN17864069)

Emily A. Kieran[1-3], Eoghan E. Laffan[1], Colm P.F. O'Donnell [1-3]
[1] National Maternity Hospital, Dublin, Ireland
[2] National Children's Research Centre, Dublin, Ireland
[3] School of Medicine & Medical Science, University College Dublin, Ireland

Background
Incorrect tip position on X-ray is associated with increased rate of complications with umbilical venous and arterial catheters (UAC and UVCs). Catheter insertion depth (ID) is often estimated using surface measurement (M).

Objective
We hypothesised that using birth-weight (BW) derived formulae rather than M would increase the number of correctly positioned catheters. Newborns without congenital anomalies who had UVC and/or UACs inserted were randomised to have the ID estimated using BW (W) [UVC: ID (cm) = (BW x 1.5) + 5; UAC: ID (cm) = (BW x 3) +9] or using shoulder tip to umbilicus measurement (M). The primary outcome was correct catheter tip position on X-ray (UVC T9–T10; UAC T6–T10).

Results
We enrolled 101 newborns. UVC insertion was successful in 97 (96%). The UVC was secured at less than the estimated ID in 22/97 (23%) infants, most often because the catheter would not advance. There was no difference in correctly placed UVCs between groups (Table). UAC insertion was attempted in 87 infants and was successful in 62 (71%) (Table). More infants in the W group had a UAC tip in the correct position (Table). We found no differences in secondary outcomes.

Conclusions
Estimating UVC ID using BW did not result in more correctly placed UVCs. When UAC insertion is successful, estimating ID using BW results in more correctly placed catheters.

	WEIGHT (N=53)	MEASURE (N=48)	P value
UVC			
Successful #	51/53 (96)	46/48 (96)	NS [b]
T9-T10#	16/51 (31)	13/46 (28)	NS [b]
Too high (<T9) #	11/51 (22)	5/46 (11)	NS [b]
Too low (>T10) #	10/51 (20)	20/46 (43)	**0.015** [b]
Portal venous system#	14/51 (27)	8/46 (17)	NS [b]
UAC	WEIGHT (N=46)	MEASURE (N=41)	
Successful#	32/46 (70)	30/41 (73)	NS [b]
T6-T10 #	29/32 (91)	15/30 (50)	**0.001** [b]
Too high (<T6) #	3/32 (9)	0/30 (0)	NS [b]
Too low (<T10) #	0/32 (0)	15/30 (50)	**0.000** [b]

*Median (IQR), #n(%), NS = not significant
[a]Independent t tests for medians
[b]Fisher's exact test

PP22

Education of Medical Students through the Use of a Multimedia Chest Radiology Application Suitable for the iPad and iPhone

Joyce EA[1,2,3], **McMorrow J.P.**[1,3], Byrne D.[1,3], Walsh J. P.[2], O' Keeffe S.[2,3], Meaney J.F.M.1,2,3.
1Centre for Advanced Medical Imaging, St. James's Hospital, Dublin 8; 2St. James's Hospital Dublin, Dublin 8; 3Trinity College Dublin, Dublin 2.

Background
The ability to interpret plain chest X-rays is a crucial element in every medical student's education. Here we present a comprehensive teaching application suitable for iPads and iPhones, aimed at improving students' knowledge and interpretation of chest radiology. This interactive teaching tool allows students to access a large volume of educational material in an environment of their choice, without the need for Internet availability after the initial download.

Results and Discussion
The application is based on 10,000 images culled from 284 cases covering a wide spectrum of chest diseases, which lays the foundation for the systematic evaluation of chest images. The platform on which this application is built, offers PACS-like functionality such as scroll, zoom and pan, but also provides enhancements such as background clinical information, radiologists' reports and diagnoses and interactive image annotation linking text to images.

Other features such as fusion of cine MR images of valve motion with phonocardiograms simplify the understanding of valve dysfunction and murmurs

Conclusion
The module is a valuable teaching tool for medical students, allowing them to acquire the skills necessary to interpret chest imaging in a mobile format. Utilization of the touch screen functionality of iOS devices enables ease of student interaction with the material provided.

PP23

Inhibition of Glycolysis in H37RA-Infectd Macrophages Reduces Secretion of Pro-inflammatory Interleukin 1 beta and Facilitates Mycobacterial Growth

LE Gleeson[1], F Sheedy[1], M Coleman[1], S O'Leary[1], M O'Sullivan[1], LAJ O'Neill[2], J Keane[1].
[1] Institute of Molecular Medicine, Trinity Centre for Health Sciences, St James's Hospital, Dublin 8
[2] Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin 2

Background
Emerging evidence demonstrates metabolic changes in immune cells are required for pro-inflammatory functions [1,2]. Stimulated macrophages adopt glycolysis as their primary method of glucose metabolism in place of oxidative phosphorylation, used by the quiescent macrophage [3]. Alveolar macrophages represent the first line of host defence to tuberculosis, therefore manipulation of host immune glucose metabolism potentially offers a novel therapeutic target.

Objective
This work elucidates the impact of Mycobacterium tuberculosis-induced changes in macrophage glucose metabolism on innate immune response to infection, in terms of pro-inflammatory cytokine production and bacterial survival.

Results & Discussion
The glycolytic pathway of glucose metabolism was inhibited using 2-deoxyglucose in macrophages (THP-1 cells, mouse BMDMs, human MDMs) infected with Mycobacterium tuberculosis (Mtb) strain H37Ra. Inhibition of glycolysis resulted in a significant decrease in pro-inflammatory cytokine interleukin-1beta (IL1-β) at 24 hours post infection, though levels of tumour necrosis factor-alpha (TNFα) remained unchanged. This suggests a distinct pathway of IL1-β production induced by Mtb infection mediated through infection-induced changes in glucose metabolism.
Inhibition of glycolysis also resulted in a significant increase in H37Ra colony forming units (CFUs) at 20 Days from cells lysed Day 7 post infection, demonstrating a role for glycolysis in mycobacterial killing.

Conclusions
Our data suggest a role for glycolysis in the inflammation seen in active tuberculous disease. Specifically, glycolysis is required for optimal production of IL1-β. Our data further demonstrate that mycobacteria-induced changes in macrophage glucose metabolism facilitate increased killing of Mtb, suggesting a potential therapeutic target to enhance the first line host immune response to tuberculosis.

References
Tannahill GM, Curtis AM, Adamik E et al. Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. Nature, 2013. 496(7444):238-243.
O'Neill LA, Hardie DG. Metabolism of inflammation limited by AMPK and pseudo-starvation. Nature, 2013. 493(7432):346-55.
Rodriguez-Prados JC, Traves PG, Cuenca J et al. Substrate fate in activated macrophages: a comparison between innate, classic and alternative activation. Journal of Immunology, 2010. 185(1):605-14.

PP24

Urine Cotinine Levels as a Measure of Second Hand Smoke (SHS) Exposure in ICU Patients – A Feasibility Study

Murphy O.A , Chong S.G., Fitzpatrick G.J.
Department of Intensive Care, Tallaght Hospital & TCD

Background
Evidence suggests that in trauma patients SHS exposure is associated with increased risk of developing lung injury[1].

Objective
To determine the feasibility of assessing SHS exposure by urinary cotinine (nicotine metabolite) analysis in a general ICU population in Ireland.

Results and Discussion
Following ethical approval we obtained urine samples from patients referred to ICU within 24 hours of hospital admission over an 8 week period. There were 61 new admissions to ICU, 39 of whom (64%) were admitted within 24 hours of hospitalisation. Samples were obtained in 23 of these (59%) and in 20 (87%) consent was obtained for cotinine analysis using HLPCC. Cut off levels of < 5 ug/L, 5-50ug/L and > 50ug/L classified patients as non-smokers, SHS exposure or active smokers[2]. Mean age was 58.3yrs (19-78), 65% were male. By history 11 of the 20 (55%) were smokers and cotinine was > 50ug/L in all these. In 8 of 9 non-smokers cotinine was <5 ug/L. In one non-smoker (11%) the urinary cotinine was >5 ug/L suggesting exposure to SHS.

Conclusions
ICU admissions outside the 24 hours window (36%) and difficulty in obtaining samples in 41% of eligible patients (night admissions etc) significantly reduced potential study population. Obtaining written consent was not a barrier.

The low detection rate of SHS exposure may be due to low level of SHS exposure in this population or to a lack of sensitivity of urinary cotinine when sampled up to 24 hours after admission.

References
Calfee C.S., Matthay M.A., Eisner M.D.,et al (2011) Active and passive cigarette smoking and acute lung injury after severe blunt trauma. Am J Respir Crit Care Med 183, 1660-1665.
2. Moyer T.P., Charlson J.R., Enger R.J. et al (2002) Simultaneous analysis of nicotine, nicotine metabolites and tobacco alkaloids in serum or urine by tandem mass spectrometry with clinically relevant metabolic profiles. Clinical Chemistry 48, 1460-1471

PP25

Inhalable Microparticles Encapsulating the Immunomodulator, All-trans-Retinoic Acid for the Treatment of Mycobacterium Tuberculosis (MTB) Infection

O'Connor, G[1,2], Coleman, M[2], Lawlor, C[1,2], Keane, J[2], O'Sullivan, M[2],Cryan,SA [1,3]
[1] School of Pharmacy, Royal College of Surgeons in Ireland, Dublin 2, Ireland.
[2] Department of Clinical Medicine, Institute of Molecular Medicine, Trinity College Dublin, St James Hospital, Dublin 8, Ireland.
[3]Trinity Centre for Bioengineering, Trinity College Dublin, Dublin 2, Ireland

Background
Tuberculosis is a global health issue that urgently requires the development of new treatments in order to improve compliance, efficiency and, thereby, prognosis for patients. Emergence of multi-drug resistant strains worldwide has led to renewed interest in adjunctive therapies, including micro-nutrients, for Mtb infection. In this study all-trans-retinoic acid (atRA), the active metabolite of vitamin A has been chosen for its immunmodulatory properties[1,2,3].

Objectives
Building on methods previously developed by our group whereby inhalable microparticles were successfully engineered to target the alveolar macrophage and hence the site of Mtb infection[4], the aim of this study is to encapsulate atRA within biodegradable poly(lactide-co-glycolic acid)(PLGA) microparticles. The atRA-loaded microparticles (atRA-MPs) undergo pharmaceutical characterisation as well as in vitro efficacy testing in an in vitro model of Mtb infection.

Results & Discussion
AtRA-MPs ranging from 2-3µm in size were successfully prepared and SEM images confirmed size and morphology. The atRA-MPs had an overall negative zeta potential -24.5 to -28.0mV. Using a loading dose of 6mg atRA the encapsulation efficiency was 17.3% ± 0.4 giving 0.1035mg of atRA/mg of MPs. Release studies were conducted in physiological buffer at 37˚C using a Float-A-Lyzer®G2 dialysis membrane displaying controlled release of atRA from MPs over 5 days.

Conclusions
A method to manufacture atRA-MPs was successfully developed in line with the specifications required for targeted pulmonary delivery of anti-tubercular medication[4,5].The results achieved in addition to in vitro studies carried out previously by the group[4,5] warrants further exploration of atRA-MPs as a potential adjunctive treatment for Mtb infection.

References
Coleman, M.M. Ruane, D. Moran, B. Dunne, P.J. Keane, J. Mills, H.G. (2013). Alveolar Macrophages Contribute to Respiratory Tolerance by Inducing FoxP3 Expression in Naïve T Cells. American Journal of Respiratory Cell and Molecular Biology, 48(6): 773-780.
Rodrigo Mora, J. Iwata, M. and H. Von Andrian, U. (2008). Vitamin Effects on the Immune System: Vitamins A & D take Centre Stage. Nature Reviews Immunology, 8(9): 685-698.
Wheelright, M. Kim, E. W. Inkeles, M.S. De Leon, A. Pellegrini, M. Krutzik, S.R. Liu, P.T. (2014). All-Trans Retinoic Acid-Triggered Antimicrobial Activity against Mycobacterium tuberculosis Is Dependent on NPC2. J Immunol, published online Feb 5 2014.
Lawlor, C. O'Sullivan, M. P. Sivadas, N. O'Leary, S. Gallagher, P.J. Keane. J. Cryan, S.A. (2011). The Application of High-Content Analysis in the Study of Targeted Particulate Delivery Systems for Intracellular Drug Delivery to Alveolar Macrophages. Mol. Pharmaceutics, 8: 1100-1112.
Lawlor, C. O'Sullivan, M.P, Rice, B. Dillon, P. Gallagher, P.J. O'Leary, S. Shoyele, S. Keane, J.(2012). Therapeutic Aerosol Bioengineering of targeted, inhalable microparticle formulations to treat Mycobacterium tuberculosis (Mtb). Journal of materials science. Materials in medicine, 23:89-98.

PP26

Data for Mycobacteria Isolates from the Irish Mycobacteria Reference Laboratory

Roycroft E[1, 2], Fitzgibbon M[2], O'Toole RF[1], Walker TM[3], Crook DW[3], Rogers TR[1, 2]
[1] Dept. Clinical Microbiology, School of Medicine, Trinity College, Dublin (TCD)
[2] Irish Mycobacteria Reference Laboratory (IMRL), Clinical Microbiology Dept., Central Pathology Laboratory, St. James' Hospital, Dublin
[3] Nuffield Department of Medicine, John Radcliffe Hospital, University of Oxford

Background
For optimum recovery of mycobacteria, specimens are cultured for up to six weeks using a liquid-based culture-system. Culture-positive isolates are identified using various molecular tests and, if M. tuberculosis Complex (MTC) is detected, susceptibility-testing is performed for first-line anti-tuberculous agents. If multi-drug resistant (MDR) tuberculosis is suspected, the isolate is sent to an overseas reference laboratory for further susceptibilities. Due to the fastidious nature of MTC, results may not be available for weeks. Molecular technologies are becoming increasingly useful for by-passing these time-intensive processes and for guiding appropriate treatment [1, 2, 3, 4].
The IMRL and Dept. of Clinical Microbiology, TCD, participated in an international collaboration, led and coordinated by Oxford, to validate Next Generation Sequencing (NGS) as a diagnostic technique that could decrease turnaround times to identification and susceptibility-testing of mycobacteria.

Objectives
To sequence (using Illumina® MiSeqTM NGS platform) culture-positive mycobacterial isolates recovered in the IMRL, and analyse them using a DNA sequencing pipeline devised in Oxford. This pipeline should identify isolates to the species level, determine their relatedness to those in an already-established database, and produce a resistance profile based on genomic mutations present.

Results and Discussion
From an analysis of 16 diverse IMRL isolates, there was 100% agreement for species identification. Resistotypes from the NGS were confirmed in the IMRL using molecular and/or phenotypic susceptibility testing. Discrepancies are being investigated [5].

Conclusion
Next Generation sequencing of mycobacteria could be of great benefit to patient treatment, infection control and public health in the future, especially in cases of MDR tuberculosis.

References
1. Cole, S., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., ... & Barrell, B. G. (1998). Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. Nature, 393(6685), 537-544
2. Daum, L. T., Fourie, P. B., Bhattacharyya, S., Ismail, N. A., Gradus, S., Maningi, N. E., ... & Fischer, G. W. (2013). Next-Generation Sequencing for Identifying Pyrazinamide Resistance in Mycobacterium tuberculosis. Clinical Infectious Diseases, cit811.
3. Hillemann, D., Rüsch-Gerdes, S., & Richter, E. (2007). Evaluation of the GenoType MTBDRplus assay for rifampin and isoniazid susceptibility testing of Mycobacterium tuberculosis strains and clinical specimens. Journal of clinical microbiology, 45(8), 2635-2640.
4. Evans, C. A. (2011). GeneXpert—a game-changer for tuberculosis control?.PLoS medicine, 8(7), e1001064.
5. Köser, C. U., Bryant, J. M., Becq, J., Török, M. E., Ellington, M. J., Marti-Renom, M. A., ... & Peacock, S. J. (2013). Whole-genome sequencing for rapid susceptibility testing of M. tuberculosis. New England Journal of Medicine,369(3), 290-292.

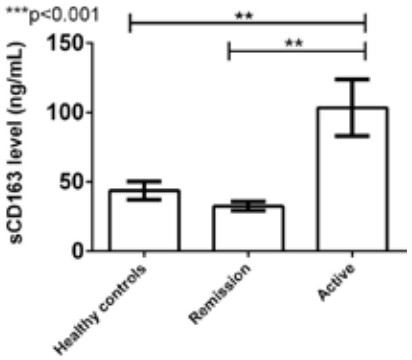
PP27

Soluble CD163 Level as a Biomarker of Active Disease in Systemic Vasculitis

Kennedy C*[1], Wong L*[1], Elliot L*[2], O'Reilly V[1], Coughlan A[1], O'Hara P[1], Feighery C[2], Moran S[3], Mellotte G[1], Clarkson M[3], Little MA[1].
[1]Trinity Health Kidney Centre, Tallaght Hospital, Dublin, [2]Dept of Immunology, Trinity College Dublin, [3]Cork University Hospital, Cork, *Joint first author

Background
Despite advances in the understanding and management of small vessel vasculitis, a sensitive and specific biomarker which can track disease activity, inform treatment decisions and predict outcome is lacking. CD163, a scavenger receptor protein expressed exclusively on myeloid antigen presenting cells, is upregulated in inflammatory states. Its soluble form in plasma - sCD163 - is being investigated as a biomarker in a number of inflammatory diseases. We hypothesised that the serum level of sCD163 in patients with systemic small vessel vasculitis mirrors disease activity.

Results & Discussion
Serum samples from patients with small vessel vasculitis and healthy controls were obtained from the Rare Kidney Diseases (RKD) Biobank and sCD163 levels were assayed by ELISA. Clinical data were derived from the linked RKD registry. Investigators were blinded to the disease activity status and diagnosis. Immunohistochemistry was performed to look for CD163 in the kidney of WKY rats with vasculitis induced by immunisation with myeloperoxidase (MPO). Serum sCD163 level was 43.6±6.7, 32.4±3.2 and 103.4±20.3 ng/mL in healthy controls (n=39), patients in remission (n=89) and patients with active disease (n=46) respectively (Fig 1, p<0.001). The sCD163 level was significantly higher in patients with active disease than those in remission (p<0.005) and was superior to CRP at characterising the patient cohorts. Patients with active PR3 ANCA disease (142.4±34.4 ng/mL) had higher sCD163 levels than those with active MPO ANCA disease (70.8±15.1). Consistent with this finding, rats with MPO-ANCA vasculitis did not have detectable CD163 in their kidneys. The sCD163 level in patients with highly active anti-GBM disease (33.8±12.1, n=5) was not significantly different from remission ANCA vasculitis patients.



Conclusion
sCD163 levels mirror disease activity in systemic vasculitis and may be useful as a potential biomarker to distinguish active vasculitis from infection, particularly in those with PR3-ANCA associated granulomatous disease. This finding appears to be specific to ANCA-mediated vasculitis as it was not observed in anti-GBM disease, despite a more severe clinical phenotype.

PP28

Investigation of MIR-433 Targets in Different Ovarian Cancer Cell Lines

Furlong F, Sharpe D and **Byrne T**.
Queens University Belfast

Background
MicroRNAs (miRNAs) are non-coding endogenous RNA molecules that act as post-transcriptional regulators of gene expression. Previously we demonstrated that high miR-433 expression renders ovarian cancer cells more chemoresistant through the downregulation of the MAD2 and HDAC6 proteins. Additionally, high miR-433 expression is associated with poor survival in patients with ovarian cancer [1,2,3].

Objectives
The objective of this study is to elucidate the role of miR-433 and the miR-433 targets in carboplatin chemoresistance.

Results and Discussion
MiR-433 downregulates the expression of a number of cancer associated proteins (Table 1). In silico analysis of the Cancer Genome Database (TCGA) revealed that lower mRNA expression levels of the miR-433 target, SFRP2 is significantly associated with better overall survival in patients with ovarian cancer. The role of SFRP2 in resistance to carboplatin is unknown. To investigate the role of miR-433 and the miR-433 targets, the expression levels of miR-433 and the miR-433 targets, MAD2, SFRP2 and HDAC6 were profiled in a panel of epithelial ovarian cancer cell lines using real time PCR and western blot analysis. In addition, MTT assays were performed to determine the sensitivity of the cells to carboplatin. This data was then correlated to determine if miR-433 and miR-433 target levels in different ovarian cancer cell lines affects their sensitivity to carboplatin.

Conclusions
This data has shown that higher levels of miR-433 may be associated with carboplatin resistance. However, the regulation of MAD2, HDAC6 and SFRP2 by miR-433 in mediating resistance to carboplatin has not yet been determined.

References
Furlong, F., Fitzpatrick, P., O'Toole, S., Phelan, S., McGrogan, B., Maguire, A, et al. (2012) Low MAD2 expression levels associate with reduced progression-free survival in patients with high-grade serous epithelial ovarian cancer. J Pathol, 226: 746–755.
Prencipe M, Fitzpatrick P, Gorman S, Mosetto M, Klinger R, Furlong F, et al. Cellular senescence induced by aberrant MAD2 levels impacts on paclitaxel responsiveness in vitro. Br J Cancer 2009 NOV 24;101(11):1900-1908.
Prencipe M, McGoldrick A, Perry AS, O'Grady A, Phelan S, McGrogan B, et al. MAD2 downregulation in hypoxia is independent of promoter hypermethylation. Cell Cycle 2010 JUL 15;9(14):2856-2865.

PP29

Targeting Treatment Resistant Ovarian Cancer Stem Cells with the Novel Protein, FKBPL, and its Peptide Derivatives

Annett, S. L.[1], McClements, L. [1], Yakkundi, A. [1] and Robson, T. [1]
[1] School of Pharmacy, The Queen's University of Belfast

Background
Ovarian cancer is the most lethal gynecological cancer and currently 80% of patents will respond to initial therapy but tragically the majority will relapse. A potential mechanism for tumour relapse is a subpopulation of cancer stem cells (CSCs).
FKBPL, a divergent member of the FKBP family, has a well-established anti-angiogenic activity [1][2]. A novel drug (ALM201) derived from the protein is entering Phase I cancer clinical trials within the next few months.
ALM201 binds to the cell surface receptor antigen, CD44, and is thought to regulate the CD44 pathway [3]. Moreover, CD44 has been identified as a CSC marker in a number of tumours including breast and ovarian.

Objectives
The ability of FKBPL therapeutic peptides (AD01/AML201) to selectively target breast CSCs has been well documented and here, for the first time, we investigate its role in targeting ovarian CSCs [4].

Results and Discussion
Tumoursphere assays have demonstrated that ALM201 is effective at reducing ovarian CSCs in vitro and inhibiting self-renewal over multiple generations. Furthermore, clonogenic assays suggested that ALM201 mediates ovarian CSC differentiation, with a significant decrease in the number of holoclones and an associated increase in meroclones/paraclones.
Our data strongly suggest that FKBPL has a role in CSC signaling and ALM201 reduces this resistant cell population by differentiating these cells into a treatment-sensitive phenotype.

Conclusion
ALM201 appears to have dual anti-angiogenic and anti-CSC activity which will be advantageous as this agent enters phase I/II clinical trial, with potential implications for scheduling, in combination with standard therapies, in late stage trials.

References
McKeen H.D., Byrne C., Jithesh P.V., et al (2010). FKBPL regulates estrogen receptor signaling and determines response to endocrine therapy. Cancer Research 70(3):1090-1100
Valentine A, O'Rourke M, Yakkundi A, et al (2011). FKBPL and Peptide Derivatives: Novel Biological Agents That Inhibit Angiogenesis by a CD44-Dependent Mechanism. Clinical Cancer Research 17(5):1044-56
Yakkundi A, McCallum L, O'Kane A, et al (2013) The Anti-Migratory Effects of FKBPL and Its Peptide Derivative, AD-01: Regulation of CD44 and the Cytoskeletal Pathway PLoS ONE 8(2): e55075. doi:10.1371/journal.pone.0055075.
McClements L, Yakkundi A, Papaspyropoulos A, et al (2013). Targeting treatment resistant breast cancer stem cells with FKBPL and its peptide derivative, AD-01, via the CD44 pathway. Clinical Cancer Research 19 (14): 3881 - 3893

PP30

Epigenome-wide Mapping of Prostate Tumorigenesis: A Promise for Prognosis?

Colm J. O'Rourke [1*], Anna L. Walsh[1,2*], Jeff Hansen [3], Darach Golden [4], Mike Emmert-Buck [3], Brian Hayes [5], Colm Morrissey [6], Robert L. Vessella [6], Stephen P. Finn [5], Thomas H. Lynch [2], Donal Hollywood [1], Antoinette S. Perry [1]
[1] Prostate Molecular Oncology Group, Institute of Molecular Medicine, Trinity College Dublin. [2] Department of Urology, St. James's Hospital, Dublin. [3] Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, USA. [4] Trinity Centre for High Performance Computing, Trinity College Dublin. [5]Department of Histopathology, St. James's Hospital, Dublin. [6]Department of Urology, University of Washington, Seattle, USA.

Facilitated by poorly de-lineated pathology and atypically low mutation frequency, prostate cancer (CaP) has continued to evade any molecular taxonomic sub-classification, hindering prognostication and manifesting in significant over-treatment. Analogous to mutations, numerous epi-mutations have been reported by us and others in CaP, which drive prostate tumorigenesis. Herein, we apply an epigenome-wide discovery approach to identify novel DNA methylation and miRNA signatures for translation into clinical use.

Six patient cohorts reflecting the step-wise progression of benign tissue through precursor lesions to CaP were identified. Laser capture microdisscetion (LCM) was performed to enrich for target epithelial cells. Genome-wide methylation analysis was carried out using Infinium 450k beadchip. miRNome-wide profiling was performed with LNA enhanced qPCR miRNome array (Exiqon).

Relative quantification of LCM samples to whole slide scrapes revealed reduced VIM (stromal marker), consistent CK8 (luminal marker) and reduced p63 (basal marker) expression with increasing tumor stage, thereby validating target enrichment. Methylome-wide profiling displayed highly significant reproducibility (R^2=0.98460.9967, $p<0.0001$). Top 1,000 significant probes were manually curated for clusters, termed differentially methylated regions (DMRs). DMRs were evenly distributed across chromosomes, primarily targeted 5'-regulatory regions and typically localised to CpG islands.

Amongst these, 19 significant genomic regions were unmasked, 3 of which have previously been reported as involved in prostate tumorigenesis. By integrating DMRs with miRNomic data, the prognostic power of these findings will be assayed in an independent cohort of radical prostatectomy specimens (n=110) via quantitative methylation-specific PCR (QMSP) and qPCR respectively.

Here, we report the first epigenomic roadmap of prostate tumorigenesis.

PP31

Mutant p53: A Therapeutic Target for Treatment of Triple-Negative Breast Cancer?

Naoise C. Synnott[1,2], Aisling Pierce[1,2], Maeve Mullooly[1,2], Francesco Caiazza[1,2], Patricia M. McGowan[1,2], Norma O'Donovan[3], John Crown[1,3], Michael J. Duffy[1].
[1]St Vincent's University Hospital, [2]UCD School of Medicine and Medical Science, University College Dublin, and [3]Dublin City University, Ireland.

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

Methods
Two compounds, PRIMA-1 and PRIMA-1MET which have previously been shown to reactivate mutant p53 and convert it to a form with wild-type properties were investigated in a panel of 18 p53 mutant breast cancer cell lines (TNBC = 12; non-TNBC = 6). Cytoxicity was determined using the MTT assay, while induction of apoptosis was measured using flow cytometry.

Results
Using the MTT assay, IC50 concentrations across 12 p53 mutant TNBC cell lines ranged from 1.4 to 15.1 μM for PRIMA-1 and from 0.9 to 11.9 μM for PRIMA-1MET. Response to PRIMA-1 correlated significantly with that to PRIMA-1MET ($p<0.0001$). Inhibition of cell growth varied from 4.1 to 90.8% for PRIMA-1 and from 3.1 to 96.6% for PRIMA-1MET, using concentration of inhibitor at 6.25 μM. PRIMA-1 and PRIMA-1MET also reduced the ability of the mutant p53 cells to form colonies with IC50 values ranging from 1.4 to 10.7 μM for PRIMA-1 and from 0.69 to 9.6 μM for PRIMA-1MET. In addition to inhibiting cell proliferation, both PRIMA-1 and PRIMA-1MET also induced apoptosis in MDA-MB-453 cells.

Conclusion
Our preclinical results suggest that targeting mutant p53 with either PRIMA-1 or PRIMA-1MET is a potential new approach for treating p53-mutated breast cancer including the subgroup with triple-negative disease.

References
DC Koboldt et al. Nature 000, 1-10 (2012) doi:10.1038/nature11412

Acknowledgement: This work was funded by the BREAST-PREDICT (CCRC13GAL) programme of the Irish Cancer Society.

PP32

Severe Acute Pancreatitis in ICU – a 5 year audit

R Durrani, O Murphy, G Fitzpatrick
Tallaght Hospital & Trinity College Dublin, Intensive Care, Dublin, Ireland

Introduction
Severe Acute Pancreatitis (SAP) is associated with significant mortality and morbidity. The object of this study is to examine the profile, outcome and resource utilisation for patients with SAP admitted to the ICU in a university teaching hospital over a 5 year period.

Methods
A retrospective observational study was carried out of all patients admitted to the ICU from Jan 1st 2008 to Dec 31st 2012 with SAP. Data was collected from the ICU database (AcuBase), the medical records and the ICU clinical information system. Data collected included patient demographics, etiology of SAP, data for Apache II, Imrie, Ranson and Acute Kidney Injury Network (AKIN) scores, and requirement for organ support. Outcomes recorded were length of stay, ICU mortality and hospital mortality. Cost of ICU care was calculated based on previously reported methodology

Results
Thirty eight eligible patients were identified. Mean age was 51.4 years (range 24-86), 68% were males. Commonest etiologies were alcohol (53%) and gallstone pancreatitis (24%). The mean APACHE II score was 18.5 (IQR 14-23). Twenty eight patients (74%) required mechanical ventilation, 3 of whom required high frequency oscillation (all 3 survived). Twenty two patients (58%) had evidence of an acute kidney injury on admission(AKIN criteria). Eighteen (47%) required Renal Replacement Therapy (RRT) and 60% required inotropes. The ICU mortality and the hospital mortality was 26%. There was no significant difference in age, APACHE II, Imrie,or Ranson scores between survivors and non survivors. The median length of stay in ICU was 11 Days (IQR 5.25– 28.5) and the median hospital stay was 45.4 days (IQR 22.25-104.5). Nine patients (24%) required multiple ICU admissions and the mortality was significantly higher in this group ($p<0.05$, Chi Square test). In total 834 ICU bed days were taken up by 38 patients. Based on a median cost of an ICU bed day of € 2205 [2] the total cost of ICU care for these patients is estimated at €1,838,970 or almost €50,000 per patient

Conclusions
A hospital mortality rate of 26% is similar to that reported recently from a specialist unit the UK [1] but less than the 42% reported in the UK in 2007 [2] suggesting some improvement in recent years. SAP is associated with prolonged ICU and hospital stay and significant resource utilisation.

References
1. Pavlidis et al: Crit Care Res Pract 2013; ID 897107
2. Harrison et al: Crit Care 2007; 11:S1

PP33

Investigating the Individual and Combined Impact of Two Variants in the PCM-1 Gene on Breast Cancer Risk

McVeigh T.P.[1], Paranjape T. [2], Slack F. [2], Weidhaas J.B. [2], Sweeney K.J. [1], Kerin M.J. [1], Miller N.M. [1]
[1] Discipline of Surgery, Clinical Sciences Institute, National University of Ireland, Galway
[2] Yale University, New Haven, Connecticut, USA

Background
Inherited predisposition to breast and ovarian malignancies is largely unexplained, but it is known that these cancers share some common genetic susceptibility loci. High-throughput sequencing of candidate genes has identified two novel variants within the 3' UTR of PCM-1 gene (peri-centriolar material, (8p22-21.3)), that positively associate with ovarian cancer [1]. These include a 4bp insertion (ATTT), and a single nucleotide polymorphism (SNP), G>A, located nine base pairs upstream.

Objectives
The aim of our study was to investigate the role of these two variants in breast cancer.

Methods
DNA was extracted from whole blood of 384 patients with breast cancer and 384 unaffected controls. The genomic DNA fragment containing the two variants was amplified by PCR. The PCR product was verified using agarose gel electrophoresis and cleaned using T-SAP/Exol mix. The digested product was then subjected to bidirectional sequencing for target regions in the PCM1 gene, using two sets of nested primers. Data analysis was performed using Chromas Lite and SPSS software.

Results
Both variants were detected in this cohort, occurring separately and together. Fifty-four (14%) cases and sixty-six (18%) controls were homozygous for insertion ATTT, and the majority of cases (n=242, 63%) and controls (n=227, 60%) expressed one copy of the minor allele (p=0.43, X2). There was no significant difference between cases and controls in minor allele frequency at the locus of the single base change. Paired expression of the minor alleles at the two loci was noted in 14% (n=54) cases, and 18% (n=66) of controls.

Conclusion
Variants within the PCM-1 gene did not positively associate with breast cancer in our cohort. This study illustrates the potential co-existence and interaction of multiple variants within short segments of the 3' UTR of candidate genes.

References
1. Chen, X., Paranjape, T., Stahlhut, C., McVeigh T., et al, (2014) Targeted resequencing of the microRNAome and 3'UTRome reveals functional germline DNA variants with altered prevalence in epithelial ovarian cancer, article under peer review

PP34

The Association between Lipoprotein Associated Phospholipase A2 (Lp-PLA2) and CVD Risk in an Irish Population

E.Dunleavy[1], M.Louw[1], J. Mason[2], Y.Rochev[3], **K.Hutchinson**[1]
[1] Pathology Department, Biomnis Ireland, Dublin
[2] Biochemistry Department, Trinity College Dublin
[3] NCBES, National University of Ireland, Galway, Ireland

Background
Lp-PLA2 plays a role in the pathogenesis of atherosclerotic plaque in cardiovascular disease. New AACE guidelines (1) recommend Lp-PLA2 measurement as part of global risk assessment for patients with dyslipidemia.

Objectives
The purpose of this study was to identify those most at risk of developing cardiovascular disease, through the measurement of Lp-PLA2, and to determine the efficacy of Lp-PLA2 as a predictor of CVD.

Results & Discussion
Participants were classified into low (n=52), intermediate (n=52) and high (n=52) CVD risk categories using the Systematic Coronary Risk Evaluation system. Samples were analysed for Lp-PLA2 to establish whether there was a link between Lp-PLA2 activity and a) participant risk classification and b) traditional risk factors. Mean Lp-PLA2 levels were 200±48, 220±38 and 225±36 nmol/min/ml (p=0.005). Lp-PLA2 was significantly correlated (p< 0.05) and showed a predictive relationship (p<0.01) with age, gender, total and LDL cholesterol, vitamin D, uric acid and risk classification. There was no correlation with insulin, glucose, or the calculated HOMA-IR, suggesting that while Lp-PLA2 is strongly associated with CVD risk factors, it is not linked with metabolic syndrome.

Conclusions
Lp-PLA2 is increased in higher CVD risk groups, associated with traditional risk factors, and appears to have a role in disease prediction but it will take time to accumulate data.

References
Jellinger PS et al . American Association of Clinical Endocrinologists' Guidelines for Management of Dyslipidemia and Prevention of Atherosclerosis. Endocrine Practice 2012; 18(1): 3-27.

PP35

Non-Invasive Epigenetic Profiling for the Early Detection of Aggressive Prostate Cancer

Tuzova A.V[1], De Barra L[1], Manecksha R[2], Lynch T[2], Clark J[3], Perry A.S[1]
[1] Institute of Molecular Medicine, Trinity College Dublin, Ireland, [2] St James's Hospital, [3] University of East Anglia.

Background
Prostate cancer (PCa) is the most common non-cutaneous malignancy and third leading cause of cancer related deaths in men in the Western world. Advent of opportunistic PSA testing in asymptomatic men has resulted in over-treatment of clinically indolent disease. This is associated with significant co-morbidities and economic cost. Our aim is to translate methylation markers developed in our laboratory into a non-invasive urine test for early detection of high-risk PCa and to discriminate from low-risk indolent disease.

Methods
Ethical approval was granted for collection of bio-specimens (blood plasma, serum, post-DRE pre-biopsy urine and FFPE biopsy cores) from patients undergoing TRUS-biopsy at three Dublin hospitals. Clinical, lifestyle and pathological data are also recorded. Quantitative methylation-specific PCR measured hypermethylation of a gene panel (n=6) on a pilot set of urinary cell pellet DNA (n=20) in a blinded manner. Through collaboration with the Movember GAP1 urinary team, patient samples will be shared amongst co-investigators to deliver a highly specific, sensitive and multi-disciplinary biomarker panel.

Results
To date, 225 patients have been recruited across the 3 Dublin hospitals. The majority (73%) of patients have no tumour diagnosed. In the pilot study, 8/9 benign cases, 1 low-risk case and 3/4 intermediate risk cases had no gene methylation. In contrast, 4/6 high-risk cases showed hypermethylation in at least one locus.

Conclusions
Pilot methylation analysis on a small set of patient urines successfully stratified patients into high- and low-risk groups. Further analysis on a larger patient cohort (n=200) will determine the promise of this technique.

Presentation Adjudication Panels

CTRSP Scholar Oral Presentations

Professor Catherine Godson
(Director of UCD Diabetes Research Centre and Professor of Molecular Medicine, UCD School of Medicine and Medical Science)

Professor Josephine Hegarty
(Professor of Nursing and Director of Graduate Studies, Catherine McAuley School of Nursing & Midwifery, UCC)

Professor Laurence Egan
(Chair of Clinical Pharmacology and Head of the Department of Pharmacology and Therapeutics, NUI Galway)

Poster Presentations

Professor John Iredale
(Professor of Medicine at the University of Edinburgh and Director of the MRC Centre for Inflammation Research)

Dr Willard Dere
(Senior Vice-President and International Chief Medical Officer, Amgen)

Professor Anita Maguire
(Professor of Pharmaceutical Chemistry & Vice President For Research & Innovation, UCC)

Professor Mike Clarke
(Chair of Research Methodology and Director of the All-Ireland Hub for Trials Methodology Research at Queen's University, Belfast)

Molecular
Medicine
Ireland

Molecular Medicine Ireland
Newman House
85a St Stephens Green
Dublin 2
Ireland
P + 353 (0)1 4779 820
F + 353 (0)1 4779 823
E info@molecularmedicineireland.ie
www.molecularmedicineireland.ie