



MMI EDUCATION & TRAINING ANNUAL SCIENTIFIC MEETING 2015



Molecular
Medicine
Ireland





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Molecular
Medicine
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Welcome to our 2015 update from current Molecular Medicine Ireland PhD Scholars and from the wider clinical and translational research community. All those now in the midst of PhD research will, I'm sure, turn an interested eye to the future during talks from two MMI Clinician Scientist PhD programme alumni. Dr Fergus McCarthy and Dr Sanjay Chotirmall will describe the post-PhD journey of early-career-building in academic medicine.

Looking to the wider environment of future precision medicine, I am delighted to welcome our keynote speakers. Professor Chris Chamberlain and Professor Sir Gordon Duff will give us unique insights respectively into industry and academic medical research and the landscape for future careers.

As well as supporting graduate students in premier research environments of Ireland in the context of national collaborative structured PhD programmes developed through HEA PRTL funding, with a patient-focus and strong input from industry, MMI provides a central resource to link supporting infrastructures for clinical and translational research.

Thus today, among the presentations in oral and poster sessions and through taking the opportunity to talk with MMI staff, you will see the benefits of collaborative enterprises. These include the Wellcome Trust/HRB Dublin Centre for Clinical Research and the new HRB Clinical Research Coordination Ireland; including focused support for industry in navigating the clinical research environment sponsored by Enterprise Ireland. MMI has key roles in the new SFI Centre for research in medical devices, CÚRAM. Also, disease-focused research consortia bringing together multidisciplinary skill sets, and patient registries gathering data across the Irish hospital system, with strong support from funders including the Irish Cancer Society and the Movember Foundation.

I hope that you find today's MMI Annual Scientific Meeting programme enjoyable, stimulating and that you make the most of talking with speakers, poster presenters and audience members, who may include future collaborators in your research.

Dr Mark Watson

Head of Programmes
Molecular Medicine Ireland



MOLECULAR MEDICINE IRELAND EDUCATION AND TRAINING

MMI is contributing to an optimal environment for successful clinical and translational research by training key groups and individuals through cost-effective collaboration in the development and delivery of structured PhD programmes and widely-available continuing professional development training.

Structured PhD Programmes

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.

The MMI Clinician Scientist Fellowship Programme (CSFP) funded through PRTLI Cycle 4, 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.

The MMI Clinical & Translational Research Scholars Programme (CTRSP), funded through PRTLI Cycle 5, trains science graduates in clinical and translational research through an innovative 4-year structured PhD programme with HRA and industry involvement in governance, courses, placements and mentoring.

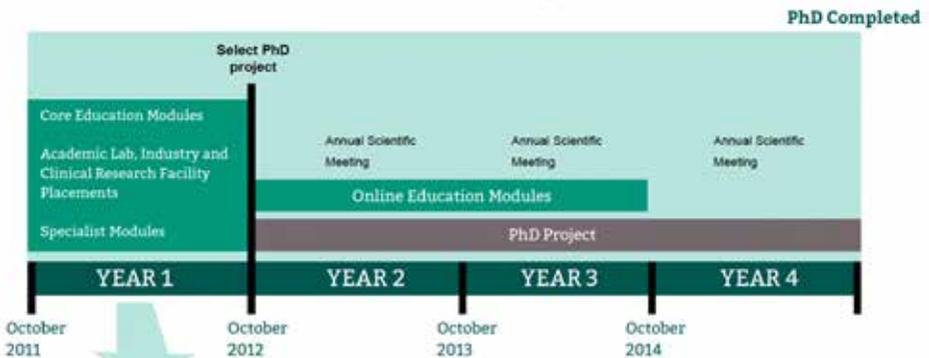
Short courses and workshops

MMI Courses & Workshops build on the research and teaching strengths of the partner institutions, the clinical expertise in the affiliated teaching hospitals and industry strengths in R&D and commercialisation to deliver widely-available continuing professional development opportunities.

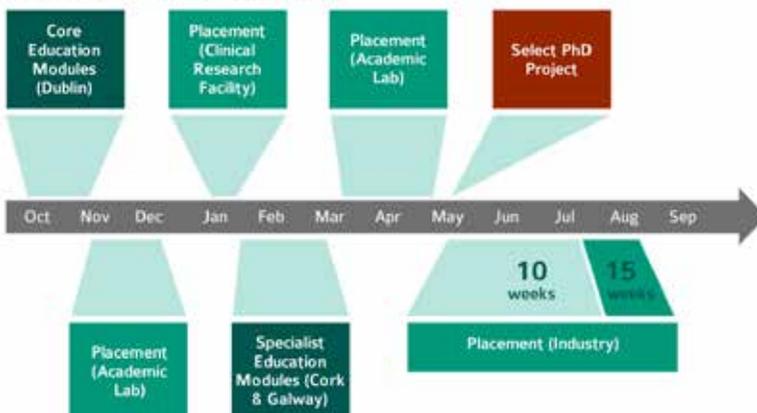
Clinical & Translational Research Scholars Programme

- 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

MMI Clinical & Translational Research Scholars Programme - Timeline



CTRSP Year 1 Timeline





MMI E-Learning: A fresh approach to eLearning development

MMI is coordinating expertise across our partner academic institutions and beyond to develop and deliver resources for research training. These new resources 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'.

Development of one such resource, E-learning, is a current priority area for MMI. We are working with our academic research community, pharmaceutical and medical device companies, the Health Products Regulatory Authority (formerly the Irish Medicines Board) and patients to develop online/blended education modules and information resources. A multimedia approach incorporates filmed presenters with dynamic graphics, case studies, interviews, resources and quizzes.

See more at: <http://www.molecularmedicineireland.ie/elearning>

MMI SkillsLog: A Manage transferable skills online

This is a facility for our PhD students to track and record the transferable skills that they acquire over the course of their PhD. A definition of a transferable skill is an ability developed in one environment that can be applied in other environments. Here we are focusing on skills that are valued across employment sectors. The aim is to help develop tools to succeed, whether in a key position in academia working with industry, or through a career path that takes the student outside academia.

Transferable skills go beyond what is learnt in a formal context (e.g. as expressed in the learning outcomes of an education module). Much of the work done during a PhD has the potential to help students gain and develop transferable skills.

See more at: <http://www.molecularmedicineireland.ie/skillslog-intro>

MMI Online Functionality: Training selection tool

Molecular Medicine Ireland uses online infrastructure to coordinate major collaborative research and education programmes across multiple institutions including universities, principal investigators and industry partners. MMI Clinical and Translational Research Scholars build their own PhD with strong mentor support and use a secure online infrastructure to select research placements, short courses and their PhD projects.

See more at: http://www.molecularmedicineireland.ie/training_selection

MMI PROGRAMMES

Dublin Centre for Clinical Research (DCCR)

The Wellcome Trust – HRB Dublin Centre for Clinical Research (DCCR) is a major investment in clinical research infrastructure being made by the Wellcome Trust and HRB. The aim of the DCCR is to provide the infrastructure – the physical space, facilities and trained staff – needed to support collaborative clinical research studies across Dublin involving the TCD, UCD and RCSI Medical Schools and their associated teaching hospitals. The DCCR network includes research activities taking place at RCSI Clinical Research Centre at Beaumont Hospital and the UCD Clinical Research Centre at Mater Misericordiae University Hospital and St. Vincent's University Hospital and the new clinical research centre at St. James's Hospital.

Further details at: <http://www.molecularmedicineireland.ie/dccr>

Clinical Industry Liaison

In 2009 MMI appointed Fionnuala Gibbons as Clinical Industry Liaison Officer. This role is funded by Enterprise Ireland and was established to provide clinical and regulatory advice to indigenous and academic innovators, SME's and multinationals enabling them to bring their products to market. To date Fionnuala has engaged with over 350 researchers and companies and facilitated over 500 introductions to the clinical community. Development and delivery of training and education workshops has included the first Good Clinical Practice workshop for Medical Devices in Ireland and Health Technology Assessments for Medical Devices.

HRB-CRCI (Clinical Research Coordination Ireland)

Clinical Research Coordination Ireland - with funding from the Health Research Board, Enterprise Ireland and MMI - will develop, in partnership with the five Clinical Research Facilities/ Centres in the Republic of Ireland, an integrated clinical trials network. The aim of the Network is to enhance Ireland's capacity for conducting innovative high quality clinical research for the benefit of people's health and the economy. It will advance the care of patients by enabling a connected and coordinated network for clinical trials. This network will provide the skills, expertise and infrastructure to design, conduct and analyse multi-centre clinical trials. CRCI will support both academic or industry initiated clinical trials involving pharmaceuticals, nutraceuticals or clinical care pathways as well as clinical investigation of medical devices.

CÚRAM

CÚRAM, a new SFI research centre based in NUI Galway, is being established with the prime objective of improving health outcomes for patients by developing innovative implantable medical devices to treat major unmet medical needs. CÚRAM will create implantable 'smart' medical devices, designed and manufactured to respond to the body's environment and to deliver therapeutic agents, such as drugs, exactly where needed. CÚRAM will develop devices using the very latest research from biomaterials, stem cells and drug delivery, working with industry partners and hospital groups to enable rapid translation to the clinic. Molecular Medicine Ireland will support CÚRAM in its mission to translate research to the clinic, and will play a key role in development and delivery of cross-institutional education and training, including online learning and continuing professional development programmes in partnership with industry.

Irish PROgramme for Stratified ProstatE Cancer Therapy (iPROSPECT)

The aim of iPROSPECT is to find markers and indicators present in blood and tissue which correlate with treatment response and which will aid future personalised treatment decisions for patients with metastatic prostate cancer. The clinical study, which is sponsored by ICORG, began recruiting patients in February 2015 and will be carried out in 10 hospital sites across Ireland.

iPROSPECT comprises a series of connected projects, each of which will analyse the patients' samples throughout the course of the disease and integrate their results with the clinical information and patient-reported outcomes in order to understand and predict response to treatments. The programme is funded by the Irish Cancer Society in partnership with Movember. The iPROSPECT team are all members of the National Prostate Cancer Research Consortium (NPCRC), established in 2011.

Further details at: <http://www.molecularmedicineireland.ie/page/g/s/4>

IPCOR

The Irish Prostate Cancer Outcomes Research (IPCOR) is establishing a registry of all newly diagnosed prostate cancer patients in the Republic of Ireland. This research is being carried out by a collaborative partnership involving the National Cancer Registry Ireland, the HRB Clinical Research Facility in Galway, the National Cancer Control Program and the nation's major academic institutions represented by Molecular Medicine Ireland. The study began in February 2014 and is funded by Movember in partnership with the Irish Cancer Society.

IPCOR will collect clinical data and quality of life information from men who have been newly diagnosed with prostate cancer and is developing, for the first time, a national prostate cancer registry. IPCOR will publish annual reports on the outcomes of prostate cancer treatment and care. These reports will provide evidence-based data to doctors, hospitals and policy makers to inform future healthcare decisions that affect prostate cancer care. The ultimate goal of IPCOR is to enhance prostate cancer care, improve patient experiences and maximise quality of life for men diagnosed with prostate cancer in Ireland.

Further details at: <http://www.ipcor.ie/>

Biobanking

Following on from the establishment of a new NSAI National Biotechnology Standards Committee, created to monitor and participate in the work of ISO TC 276, a new association for Irish biobanking stakeholders is being set up. Provisionally titled 'Biobanking Ireland', this initiative is supported by Molecular Medicine Ireland. The proposed association aims to facilitate communication and networking on an all-Ireland basis by drawing on the extensive experience of individuals, institutions, businesses, and agencies active in the field of biobanking. The association would also provide a focus for the island of Ireland to coordinate with established international efforts in all themes of biological sample management.

Further details at: <http://www.molecularmedicineireland.ie/page/g/t/59>

Irish Biomarker Network

The Irish Biomarker Network was established to be a forum for discussion on the issues encountered in biomarker research. It aims to connect scientists, clinicians and industry involved in biomarker research. The network can also help with grant-writing for collaborative funding for biomarker research, validation etc; and with coordination of activities, consortia or programmes. A directory of biomarkers and biobanks is being created to display details of research groups engaged in biomarker research, to share knowledge and best practices in biomarker discovery and development; and to encourage collaborations.

Further details at: http://www.molecularmedicineireland.ie/biomarker_research_platforms

Keynote Speaker: Professor Sir Gordon Duff

Biology and Future Medicine

Professor Sir Gordon Duff

BA MA BM BCh PhD MD(Hon) FFPM(Hon) FRCP(Lon)
FRCP(Edin) FMedSci FRSE

(Principal of St Hilda's College, Oxford)



Gordon Duff trained in Medicine at the Universities of Oxford and London, where he also gained a PhD in Neuropharmacology. Following postgraduate medical posts in London, and junior faculty posts at Yale University, he joined the Edinburgh Medical School in 1984, and was Director of the Molecular Immunology Group where cytokines were identified as therapeutic targets in inflammatory joint diseases.

In 1990 he moved to Sheffield University as Florey Professor of Molecular Medicine, where he was also a member of University Council, Faculty Research Dean and Director of the Division of Genomic Medicine. In January 2013 he was appointed Chairman of the expanded Medicines and Healthcare Products Regulatory Agency, the UK national regulator (MHRA). In 2014 he was appointed Principal of St Hilda's College, Oxford.

He also currently chairs the Academic Health Sciences Centre (AHSC) of Imperial College, London, the MRC-NIHR Phenome Centre at Imperial College, and the AHSC of Trinity College Dublin. Previously Chairman of the Committee on Safety of Medicines (CSM), he was inaugural Chairman of the Commission on Human Medicines (2003 to 2013). In 2006 he chaired the Secretary-of-State's Expert Scientific Group on Phase One Clinical Trials. From 2002 to 2009 he was Chairman of the UK's National Institute for Biological Standards and Control, including the National Stem Cell Bank. He is an advisor on Biological Medicines to the EU, and Chairman of the UK's Scientific Pandemic Influenza Advisory Committee. In 2009-10, he co-chaired, with the Govt Chief Scientist, the Cabinet Office's Scientific Advisory Group for Emergencies (SAGE).

In 2010, at the request of Secretary-of-State, he reviewed and made recommendations on the UK's Organ Donor Register. His research interest is in common inflammatory diseases (HL=71) and he has given many international named lectures, receiving several research awards and medals. He is past-President of the International Cytokine Society and founding editor of the research journal CYTOKINE (Elsevier) and an editorial advisor for the Journal of the Human Genome Organization (HUGO Journal).

He has participated in the launch of several companies in the UK and USA, taking roles as Board Chairman, Director, and Chair of Scientific Advisory Boards. He is an Honorary Fellow of St Peter's College, Oxford, Fellow of the Academy of Medical Sciences, Fellow of the Royal Colleges of Physicians of Edinburgh and London (Croonian Lecturer), and Fellow of the Royal Society of Edinburgh. He received a Knighthood in the Queen's 2007 New Year's List for services to Public Health.

Keynote Speaker: Professor Chris Chamberlain

Informing drug development through diagnostic practice... moving beyond biomarkers

Professor Chris Chamberlain

MD PhD

(Vice President & Head, Experimental Medicine and Diagnostics, UCB Pharma)



Chris Chamberlain has been head of Experimental Medicine and Diagnostics at UCB since 2013, with responsibility for ensuring the effective use of biomarkers and experimental medical approaches across the UCB portfolio. These efforts look to deliver both earliest proof of concept for investigational therapies and the appropriate use of molecular taxonomy and related mechanistic insights best to target such therapies in later development and clinical use.

Chris is a specialist registered physician and a chartered scientist within the UK and has extensive experience across the pharmaceutical industry; he has previously worked at SmithKline Beecham, Roche and AstraZeneca. Chris is Project co-coordinator for PRECISESADS, a large collaborative European study for the discovery of new molecular taxonomy in systemic auto-immune disease (www.precisesads.eu) and chairs the ABPI working group on stratified medicine.



MMI CLINICIAN SCIENTIST FELLOWSHIP PROGRAMME ALUMNI SPEAKERS

Two of our excellent CSFP alumni will provide their perspective of having completed a PhD and of pursuing a career in academic medicine.

MMI CSFP Alumni Talk 1

The next step - Post PhD

Dr Fergus McCarthy

PhD MSc Dip MRCOG MRCP

(NIHR Academic Clinical Lecturer/ Maternal & Fetal Medicine Subspecialist Trainee, Division of Women's Health, Women's Health Academic Centre KHP, St Thomas' Hospital)

Dr McCarthy graduated from University College Cork in 2003 and currently works as an Academic Clinical Lecturer in King's College/ St Thomas' Hospital in London. Dr McCarthy received his PhD in 2012 for his work investigating the aetiology and potential treatments for pre-eclampsia.

Dr McCarthy has a Masters in Epidemiology from the University of London and a Diploma in Clinical Education from the National University of Ireland, Galway. Dr McCarthy has over 50 publications. Awards received include the Royal Academy of Medicine of Ireland Research award in 2012 and 2014, the William Stokes Award from the Royal College of Physicians of Ireland and recently the Harold Malkins award from the Royal College of Obstetricians and Gynaecologists. In 2014, Fergus received a Scholarship to attend the 64th Lindau Nobel Laureate Meeting as one of "600 most qualified young researchers" chosen internationally.

MMI CSFP Alumni Talk 2

Estrogen, Cystic Fibrosis and the 'yellow brick road' from PhD to Academic Trilogy

Dr Sanjay Haresh Chotirmall

MB BCh BAO (NUI) (Hons) MRCPi MRCP (UK) PhD

(Honorary Senior Clinical Lecturer, Imperial College, London & Visiting Assistant Professor, Lee Kong Chian School of Medicine, Singapore)

Sanjay H. Chotirmall graduated from the Royal College of Surgeons in Ireland (RCSI) in 2005. Following the award of a prestigious 'Molecular Medicine Ireland Clinician Scientist Fellowship' (MMI-CSF), he completed a PhD 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 (AJRCCM), as well as the New England Journal of Medicine (NEJM).

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 Center 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 Award (2009). Additionally, his work has been recognised by the Faculty of 1000 Biology and Medicine, an online research service that highlights critical papers published in the biological sciences as recommended by distinguished faculty.

Having published over 50 peer-reviewed papers, and 8 book chapters to date, he is an active member of the ISHAM working group for fungal infections, and the LRPC of Assembly 3 of the European Respiratory Society (ERS). Dr Chotirmall is also an Associate Editor at the journal BMC Pulmonary Medicine.

He is joining the Lee Kong Chian School of Medicine (LKCSOM) in Singapore this summer upon completing his Specialist Training programme in Ireland to establish a Translational Respiratory Research Group, whose focus will be on infection, inflammation, and immunity, in the context of chronic inflammatory respiratory diseases affecting Asian populations.

ORAL PRESENTATIONS

MMI CTRSP Scholars

At the occasion of the MMI Education & Training Annual Meeting, the 20 MMI CTRSP Scholars submitted a poster abstract outlining their PhD research topic. A judging panel selected the 8 poster abstracts to be presented as oral presentations over 3 sessions.

OP 1 Ayokunmi Ajetunmobi (TCD)

Modelling central nervous system development on an in vitro biochip platform

OP 2 Trudy McGarry (UCD)

The effect of novel compound MCC950 in the NLRP3 inflammasome in the RA joint

OP 3 Amruta Naik (UCC)

Investigation of antibody associated hepatitis C virus in the quasispecies pool

OP 4 Serika Naicker (NUI Galway)

Peripheral Blood Monocyte Profiles in Chronic Kidney Disease and End-Stage Renal Disease of Patients Receiving Chronic Hemodialysis

OP 5 Mark Jackson (UCD)

The palladium-catalysed decarboxylative asymmetric protonation and allylic alkylation

OP 6 Michael Healy (UCC)

Assessment of Autophagy Inducers and Differentially Expressed Genes as Modulators of Chemo-sensitivity in Oesophageal Cancer

OP 7 Edel McGarry (NUI Galway)

Identification of a novel ubiquitin specific peptidase involved in maintaining genomic stability

OP 8 Éilis Dockry (TCD)

Epigenetic Targeting of CD1d Increases Cytolytic Activity of Invariant Natural Killer T (iNKT) cells against Non-Small Cell Lung Cancer (NSCLC)

POSTER PRESENTATIONS

MMI CTRSP Scholars

PP 1 Rachel Bermingham (TCD)

Exploring the function of Rora in immunity and inflammation

PP 2 Eanna Connaughton (NUI Galway)

Phenotypic, Functional and Molecular Analysis of Novel Human Monocyte Subpopulations

PP 3 Niamh Denihan (UCC)

The Effect of Haemolysis on the Metabolomic Profile of Umbilical Cord Blood

PP 4 Erin Dolan (UCC)

Characteristics of cognitive deficits in an alpha-synuclein model of Parkinson's disease

PP 5 Róisín Dunne (TCD)

Novel small molecule inhibitors that alter energy metabolism and DNA repair and improve radioresponse in oesophageal and Colorectal Adenocarcinoma

PP 6 Marie Fitzgibbon (NUI Galway)

Characterization of the endocannabinoid system in the descending pain pathway in a mouse model of IFN- α -induced hyperalgesia

PP 7 Oisín Gough (UCD)

FRMD3: A Novel Modulator of Diabetic Nephropathy?

PP 8 Karen Hanrahan (UCD)

The role of ZEB1/ZEB2 and β III-tubulin in mediating docetaxel-resistant prostate cancer

PP 9 Ciara Harty (UCC)

Hepatitis C Proteins Interact Directly with Early Stage Autophagy Proteins

PP 10 Jennifer Hillis (NUI Galway)

Neurotrophin signaling protects CLL cells from death

PP 11 Lauren MacDonagh (TCD)

Inhibition of the cancer stem cell marker, ALDH1, re-sensitizes cisplatin resistant NSCLC cells to cisplatin-induced apoptosis

PP 12 Wesley van Oeffelen (UCC)

Investigating downstream signalling bias of commercial ligands of the growth hormone secretagogue receptor 1a (GHS-R1A)

PP 13 Huong Quach Thi Thu (NUI Galway)

DNA replication in the presence of Cdc7 inhibition in human breast cancer cells

PP 14 Gerard Roche (TCD)

Liver Derived Immune Cells have Increased Expression of Interferon Lambda Receptor

PP 15 Niall Savage (UCC)

Development of a smart needle integrated with a micro-structured impedance sensor for the detection of breast cancer

PP 16 Stephanie Slevin (NUI Galway)

Anti-TNF therapy is associated with rapid reduction of circulating monocyte numbers and blunted monocyte pro-inflammatory response in patients with inflammatory bowel disease

PP 17 Julie Worrell (UCD)

The Regulation of CXCR3 and IL-13R α 2 by IL-13 in Pulmonary Fibrosis

Wider Research Community

PP 18 Mark Bates (TCD)

The role of the TLR4 pathway and the spindle assembly checkpoint in ovarian cancer prognosis

PP 19 Sarah Brophy (TCD)

Investigating the tumour microenvironment in chronic lymphocytic leukaemia: A role for STAT3

PP 20 Ann Byrne (TCD)

Liver Perfusion Fluid: A Source of Adult Stem Cells

PP 21 Oyinlola Dada (UCD)

Bioconjugated NHC-Au-thiosugar complexes as targeted anticancer drugs

PP 22 Laura Gleeson (TCD)

Human macrophages adopt aerobic glycolysis to control mycobacterium tuberculosis infection

PP 23 Maria Hughes (QUB)

Long term changes in high sensitivity measured Troponin I and its association with incident cardiovascular disease in the MORGAM/BiomarCaRE cohort from Denmark

PP 24 Katrina Hutchinson (Biomnis)

The effects of vitamin D supplementation on childhood asthma: a randomized, double-blind, placebo-controlled trial.

PP 25 Conor Kerley (UCD)

Novel clinical aspects of dietary nitrate: potential effects on human cardiorespiratory disorders

PP 26 Aleksandar Krstic (UCD)

MYCN in neuroblastoma: a transcriptional and epigenetic regulator

PP 27 Mark Little (TCD)

Soluble CD163 level as a biomarker of active renal vasculitis

PP 28 Valerie Logan (TCD)

The Irish National Rare Kidney Disease Registry and Biobank: update on recruitment and activity

PP 29 Eoin Mac Réamoinn (TCD)

Innate Immune Gene Expression During Early Murine Embryonic Development: Implications for a Role Outside of Defence?

PP 30 Barbara McGrogan (NCCP)

Towards a safer patient pathway for melanoma: review of referral, primary treatment and multidisciplinary management of suspicious pigmented lesions

PP 31 Gemma O'Connor (RCSI)

Bioengineered inhalable microparticles for the treatment of mycobacterium tuberculosis (MTB) infection

PP 32 Christina Payne (RCSI)

Development of hydrogels for drug and cell delivery to the distal airways

PP 33 Andrew Selfridge (UCD)

Suppression of hypoxia-induced HIF stabilisation by hypercapnia

PP 34 David Walsh (RCSI)

Star shaped polypeptides as non-viral vectors to produce gene activated matrices for bone tissue engineering

PP 35 Sarah Whelan (TCD)

Soluble CD10 and iNKT cells: an inverse relationship on hepatic malignancy

OP1 Ayokunmi Ajetunmobi (TCD)

Modelling central nervous system development on an in vitro biochip platform

Ajetunmobi, A.¹, Tropea, D.², Corvin, A.², Volkov, Y.^{1,3} & Prina-Mello, A.^{1,3}

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³Centre 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. In recent years, in vitro microelectrode 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 [2].

Objectives:

This project seeks to develop microelectrode devices combining electrophysiological and molecular imaging modalities for multi-parametric analysis of cellular and molecular mechanisms of neurodevelopment.

Results:

We have developed a functional biochip devices and tested electrical impedance characteristics. We have also demonstrated biocompatibility of the neuronal model SH-SY5Y human neuroblastoma cells on the biochip surface based on morphological and functional characteristics.

Conclusions:

We have completed the development stage of the platform, showing sensor functionality and biocompatibility. Our next step is to optimise a bioassay for our sensor to determine its potential as an in vitro biomedical tool for studying central nervous system disorders.

References:

1. Gustavsson, A., et al., 2011. doi: 10.1016/j.euroneuro.2011.08.008
2. Ajetunmobi, A., et al., 2014. doi:10.1016/j.pneurobio.2014.09.004

Acknowledgements:

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OP 2 Trudy McGarry (UCD)

The effect of novel compound MCC950 in the NLRP3 inflammasome in the RA joint

McGarry T¹, Robertson AA², Orr C¹, Coll RC³, Cooper MA², O'Neill LA³, Veale, DJ¹ & Fearon U¹.

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Introduction: The NLRP3 inflammasome is a multi-protein complex activated in response to environmental pathogens. These pathogens activate toll-like receptors, initiating a cascade leading to the activation of this inflammasome resulting in caspase-1-dependant cleavage of pro-IL-1 β and IL-18 to their active and mature form. Recent studies have implicated the NLRP3 inflammasome in the pathogenesis of Rheumatoid Arthritis.

Objective: To assess the activity of the NLRP3 inflammasome in the RA joint. Additionally, this study is the first to determine the effects of novel compound MCC950 in the RA joint.

Methods: Initially, to assess if the NLRP3 inflammasome is active in RA, the expression of inflammasome components NLRP3, IL-1 β , IL-18 and Caspase-1 were measured in Rheumatoid Arthritis (RA), Osteoarthritis (OA) and healthy control (HC) ex vivo synovial tissue biopsies by RT-PCR, ELISA, immunohistochemistry and Western blotting. The effects of MCC950, a novel compound targeting NLRP3, was assessed in macrophages derived from both HC peripheral blood mononuclear cells (PBMC) and THP1 cell line. The NLRP3 inflammasome was activated in these cells by incubation with LPS (1 μ g/ml) and ATP (5 mM) in the presence or absence of MCC950 (10nM - 10 μ M), and secretion of NLRP3 inflammasome component IL-1 β was measured by ELISA. RA synovial biopsies were also incubated with MCC950 (100 nM) or basal control for 24 hr and expression of NLRP3, IL-1 β , IL-18 and Caspase-1 were analysed by Taqman PCR, ELISA and Western blotting. The effect of MCC950 on ex vivo pro-inflammatory and pro-angiogenic cytokine secretion (IL-6, IL-8, TNF α , VEGF, Tie-2, bFGF, MMP-3, IL-10) was also determined by multiplex ELISA.

Results: Transcripts of inflammasome components NLRP3, pro-IL-1 β and pro-IL-18 were significantly higher in RA versus OA synovial biopsies. Expression of Caspase-1 protein is also higher in RA versus OA synovial tissue as assessed by Western blotting. In addition, Caspase-1 is highly expressed in RA synovial tissue compared to OA or HC synovium, localised to the lining and sub-lining layers. MCC950 inhibits LPS and ATP induced secretion of inflammasome component IL-1 β in macrophages derived from both HC PBMC and THP1 cells in a dose dependant manner. Furthermore, incubation of synovial ex vivo biopsies with MCC950 results in a decrease of NLRP3 transcripts, pro-IL1 β and pro-IL-18, which was mirrored by a decrease in active IL-1 β and IL-18 secreted from ex vivo RA biopsies ($p < 0.05$). Caspase-1 expression was also inhibited in MCC950-treated ex vivo RA biopsies. Pro-inflammatory cytokines IL-6 and IL-8 were also significantly inhibited following incubation with MCC950. In contrast, MCC950 had no effect on other pro-inflammatory and pro-angiogenic factors TNF α , MMP-3, VEGF, Tie-2, bFGF and IL-10.

Conclusion: MCC950, a novel compound thought to interact with the NLRP3 inflammasome, inhibits NLRP3 inflammasome components IL-1 β , IL-18 and Caspase-1, in addition to other pro-inflammatory cytokines in the joint, but is independent of TNF α . This is the first study to demonstrate the effects of MCC950 in RA patient tissue and may represent a potential novel therapeutic strategy.

OP3 Amruta Naik (UCC)

Investigation of antibody associated hepatitis C virus in the quasispecies pool

Naik¹ A.S*, Harty¹ C., Palmer¹ B., Crosbie² O., Kenny-Walsh² E., Fanning¹ L.J.

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Background: The host immune system responds to viral infection by producing neutralising antibodies (NAbs). However, mutations in the epitopes against which the NAbs were previously produced facilitate viral immune escape(1). Fractionation of virus into antibody (Ab) associated and an Ab free population, followed by analysis at the molecular signature level presents an opportunity to enhance our understanding of evolution of viral envelope proteins and humoral immune escape(2). The objective of the current study was to analyse hypervariable region 1 (HVR1) of glycoprotein of quasispecies in Ab associated and Ab free fractions from sera from chronically infected hepatitis C patients. The present study has made use of sdHCV to investigate interaction of HCV sera with antibodies purified from homologous sera.

Methods: Briefly, sixteen serum samples from a panel of viraemic sera positive for different HCV genotypes were randomly selected. Ab associated virus was purified using Ab spin trap columns. Antibodies purified from patients infected with genotype 1b and 3a were used to determine the permissiveness of homologous serum derived virus infectivity in-vitro in Huh7. Interference in virus infectivity was evaluated by HCV 5'-UTR qRT-PCR assay.

Results: We report the heterogeneity of the E1E2 glycoprotein (H77 nucleotide - 1296 to 1615) from chronically infected patients. Out of 16 samples used in this study; three genotype 1b out of nine, three genotype 1a, two genotype 3a out of three and one genotype 4a were positive for a 319bp region encompassing HVR1 in Ab associated fraction. 5'UTR assay showed reduction in infectivity of homologous virus mixed with antibodies.

Conclusion: We observed differential quasispecies segregation when antibody associated hepatitis C virus is compared to the quasispecies present in the Ab free viral populations from sdHCV. Our data supports the principle that serum derived antibodies that target HCV reduces the infectivity quotient.

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OP 4 Serika Naicker (NUI Galway)

Peripheral Blood Monocyte Profiles in Chronic Kidney Disease and End-Stage Renal Disease of Patients Receiving Chronic Hemodialysis

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Introduction: An acute and chronic pro-inflammatory state exists in patients with chronic kidney disease (CKD) and end-stage renal disease (ESRD), contributing substantially to morbidity and mortality. Persistent inflammation along with the toxic uremic milieu seen in CKD may modulate both the pro- and anti-inflammatory homeostatic role monocytes, a subset of circulating white blood cells involved in innate immunity have in CKD and ESRD. The aim of this study was to investigate alterations in the number of circulating peripheral blood monocyte (PBMCs) subsets in patients with various stages of the disease. We also aimed to profile Scavenger receptor (SR) expression in the major monocyte subsets of these patients and determine if SR expression is altered in this disease state.

Method: Freshly isolated PBMCs from healthy adults (CTRL, n=25), CKD 1-5 (100) and ESKD patients immediately prior to and after HD (n=32), were analyzed using an 8-colour flow cytometry protocol to accurately identify monocyte subsets [Classicals (CD14++CD16-), Intermediates (CD14++CD16+), Non-classicals (CD14+CD16++)] and quantify surface expression of the following SRs; SCARF-1 and P2X7.

Results/Discussion: We show that the number of circulating monocytes progressively increase over the stages of CKD. More specifically, we show that non-classical monocytes ($P < 0.001$) are significantly higher while classical monocytes appear unchanged. Following HD, monocyte numbers significantly decreased ($P < 0.001$), predominately the non-classical ($P < 0.001$) and intermediate subsets ($P < 0.01$).

Surface expression of the SR SCARF1, involved in modified LDL uptake and apoptotic clearance was shown to be lower across CKD stages when compared to healthy controls in all monocyte subsets. The prevalent difference can be seen in patients with ESRD ($P < 0.001$) with HD having no apparent effect on receptor expression. We hypothesize that the phagocytic capability of monocytes are impaired in patients with CKD and ESRD.

P2X7, a purinoceptor for ATP involved in ATP-Mediated cell death, receptor trafficking and inflammation showed no significant changes between the stages of CKD or ESRD and controls. However, we are the first to show that P2X7 is differentially expressed on monocyte subpopulations. Its highest expression can be seen on non-classical monocytes and therefore may implicate an interesting pro-inflammatory role for this receptor in CKD.

OP5 Mark Jackson (UCD)

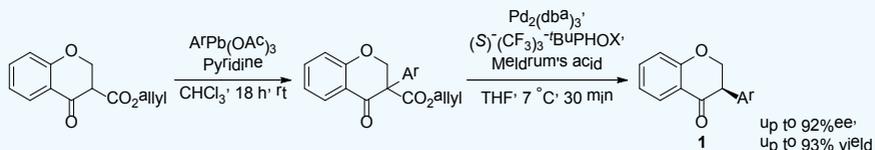
The palladium-catalysed decarboxylative asymmetric protonation and allylic alkylation

Patrick J. Guiry and Mark P. Jackson

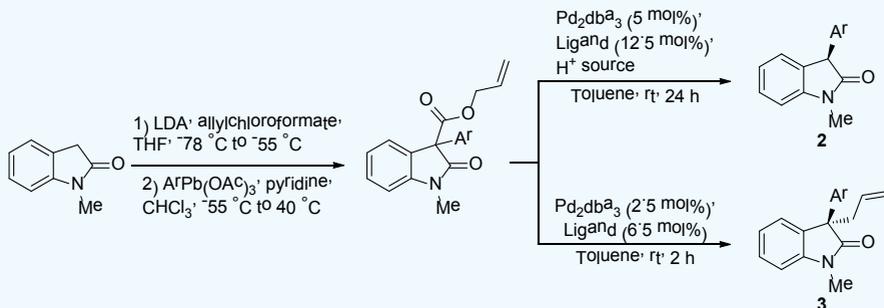
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Asymmetric transition metal-catalysed transformations have emerged as a powerful tool for the generation of stereocentres on the α -position of carbonyls.¹ However, one area in which this has proved more challenging is the formation of a tertiary centre 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



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.



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OP 6 Michael Healy (UCC)

Assessment of Autophagy Inducers and Differentially Expressed Genes as Modulators of Chemo-sensitivity in Oesophageal Cancer

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Background:

Autophagy is a highly conserved cellular process, whereby components of the cytoplasm, such as protein aggregates, organelles and other macromolecules are digested. Our laboratory has previously shown that while chemo-sensitive cell lines (OE21 & OE33) display apoptotic cell death in response to treatment with 5-fluorouracil (5-FU), chemo-resistant oesophageal cancer cell lines (OE19 & KYSE450) only induce autophagy [1].

Potential inducers of autophagy have been screened and tested to see if disruption of this process can chemo-sensitise resistant cells to 5-FU. Affymetrix GeneChip® array data was also examined to assess differential gene expression between the chemosensitive and resistant cell lines.

Results:

(i) A number of compounds including amiodarone, trehalose, carbamazepine and valproic acid (VPA) were analysed for their ability to induce autophagy and the effect on chemo-sensitising cells to 5FU. VPA (2.5mM & 5mM) as a single agent, negatively impacted clonogenic survival of KYSE450 cells. When tested in combination with 5-FU, valproic acid displayed a synergistic effect in decreasing in clonogenic survival. The contribution of autophagy to this enhancement of cytotoxicity with these agents is currently under evaluation.

(ii) Using various inclusion criteria (functional role in apoptosis/autophagy; vesicular trafficking; scientific novelty) and gene databases assembled in our lab, we selected and confirmed differential expression of several genes of interest including; SYT1, TNFAIP3, PRKCA, Trim24 & NT5E. The importance of Trim24 and NT5E in autophagy-associated chemoresistance is currently being investigated.

Conclusion:

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

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OP7 Edel McGarry (NUI Galway)

Identification of a novel ubiquitin specific peptidase involved in maintaining genomic stability

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

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

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

In this study we have tested the hypothesis that CDC7 dependent phosphorylation of Claspin is important to coordinate Claspin interaction with other cellular proteins.

Using a quantitative proteomic approach, we have identified several proteins that differentially co-purify with Claspin when this is extracted from cells treated with a CDC7 kinase inhibitor or from control cells. Among these we find a novel DUB and we have confirmed by reciprocal immunoprecipitation experiments that it binds to Claspin in a CDC7 dependent manner.

Using siRNA knock-down and pharmacological inhibition strategies, we find that this DUB controls Claspin stability in a phase specific manner, it affects DNA replication fork stability and checkpoint responses after replication stress.

Therefore we propose that this DUB is a novel player involved in the maintenance of genomic stability and in the DNA replication stress response pathway.

OP 8 Éilis Dockry (TCD)

Epigenetic Targeting of CD1d Increases Cytolytic Activity of Invariant Natural Killer T (iNKT) cells against Non-Small Cell Lung Cancer (NSCLC)

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Background:

Lung cancer is currently the most common form of cancer related death in the world, causing greater than 1 million deaths each year. Immunotherapy is the fourth most important modality for malignant tumours, and is used in the treatment of NSCLC. CD1d, an MHC class 1-like molecule presents glycolipid antigens to iNKT cells, thereby stimulating their anti-tumour activity. CD1d acts as a target for NKT-mediated killing, however most human and solid mouse tumours are CD1d-negative. Recent evidence has indicated that CD1d expression can be epigenetically regulated.

Objectives:

To determine if epigenetic targeting can increase levels of CD1d at the mRNA level by RT-PCR and at the protein level by flow cytometry.

To establish NSCLC cell lines that stably overexpress CD1d

To confirm that higher levels of CD1d in NSCLC cell lines lead to increases cytolytic activity by iNKT cell lines

To phenotype bronchial lavage (BAL) samples from healthy controls and lung cancer patients to determine if iNKT cells or other CD1d-restricted T cells are numerically altered.

Results and Discussion:

Using DNA methyltransferase (DNMT) and histone deacetylase (HDAC) inhibitors, we found that there was a statistically significant up-regulation of CD1d in both A549 and SK-MES-1 cell lines ($p \leq 0.01$). Using the Fugene protocol, a stably transfected SK-MES-1 cell line that over-expresses CD1d was established. Co-culture of iNKT cells with NSCLC cell lines show a statistically significant induction of CD107a, a marker of cytolytic ability, when CD1d levels are increased ($p \leq 0.05$). BAL samples from non-cancer controls were found using flow cytometry to contain significant numbers of CD1d-restricted cells, including iNKT cells and the V δ 1 and V δ 3 subsets of $\gamma\delta$ T cells. BAL samples from NSCLC patients have yet to be studied.

Conclusion:

CD1d expression in NSCLC cell lines is significantly induced by epigenetic modifying agents, which in turn increases the anti-tumourigenic activity of iNKT cells. These results may have important consequences for treating patients with combined epigenetic targeting agents and immunotherapy.

PP 1 Rachel Bermingham (TCD)

Exploring the function of Rora in immunity and inflammation

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Background: Retinoid-related orphan receptor alpha (Rora) is a transcription factor that is involved in neural cell development, immunity and metabolism. In terms of immunity, we have shown that Rora is required for the development of type 2 innate lymphoid cells (ILC2s), while others have shown roles in Th17 cells.

Objectives: The objective of this study was to explore the function Rora expressing macrophages have in immunity and inflammation. To achieve this Rorasg/sg mice were used which contain a natural mutation in Rora resulting in a non-functioning protein.

Results and Discussion: We show that in the absence of Rora the basal peritoneal macrophage phenotype is altered. Two M2 like peritoneal macrophage subsets were identified by surface marker expression of CD11b F4/80, MHCII and TIM-4. Rorasg/sg mice have significantly increased levels of the CD11b+ F4/80+ MHCII- TIM-4+ peritoneal macrophage population compared to WT mice. Correspondingly Rorasg/sg mice have significantly decreased levels of the CD11b+ F4/80+ MHCII- TIM-4- macrophage population.

TIM-4 is a marker for M2 tissue resident macrophages, which have immuno-suppressive/regulatory functions. We hypothesised that an increased tissue-resident macrophage phenotype in the absence of Rora could alter responses to inflammation. Indeed we show that Rorasg/sg mice have attenuated in vivo responses to LPS compared to WT mice. In-vitro LPS stimulated macrophages from Rorasg/sg mice have decreased inflammatory responses compared to WT mice.

Conclusions: The peritoneal macrophage phenotype is altered in the absence of Rora which in turn alters responses to inflammation. Future studies will use conditional deleter Roraf1/sg LysMCre mice to explore Rora specifically in macrophages.

PP 2 Eanna Connaughton (NUI Galway)

Phenotypic, Functional and Molecular Analysis of Novel Human Monocyte Subpopulations

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Introduction

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. The intermediate subset is of particular interest in inflammatory diseases, for example atherosclerosis, where the subset is expanded. We have been able to subdivide the intermediate population into 2 further subsets based on CD16 and HLA.DR Cell surface expression, termed 'DRmid' and 'DRhi' monocytes.

Methods and Results

Using a transwell assay system, we found that despite Classical and DRmid monocytes expressing similar amounts of the CCR2 receptor, DRmid monocytes exhibit reduced transmigration in response to MCP-1 (chemokine specific to CCR2) ($p \leq 0.05^*$). CCR2 is a G-protein coupled receptor and engagement by MCP-1 causes dissociation of the G-protein subunits, starting a signaling cascade resulting in release of intracellular calcium ($[Ca^{2+}]_i$) from the endoplasmic reticulum (ER). This $[Ca^{2+}]_i$ flux is required for effective actin polymerization and cytoskeletal remodeling involved in transmigration. Using Fluo-4 reagent to quantify $[Ca^{2+}]_i$ release by flow cytometry, results show decreased calcium flux in DRmid monocytes compared to Classical monocytes upon MCP-1 stimulation ($p \leq 0.05^*$).

Conclusions

The response of DRmid monocytes toward MCP-1 induced transmigration and $[Ca^{2+}]_i$ release indicates a less responsive phenotype in comparison to Classical monocytes. Current work focuses on expression of Regulator of G Protein Signaling (RGS) proteins in monocyte subsets. RGS proteins enhance GTPase activity, reducing the time of the GPCR signal by re-association of the G-protein subunits. Differential expression of RGS proteins may account for attenuated MCP-1 signaling in DRmid Intermediate monocytes.

PP 3 Niamh Denihan (UCC)

The effect of haemolysis on the metabolomic profile of umbilical cord blood

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Background:

Metabolomics is defined as the comprehensive study of all low molecular weight biochemicals, (metabolites) present in an organism. Using a systems biology approach, metabolomics in umbilical cord blood (UCB) may offer insight into many perinatal disease processes by uniquely detecting rapid biochemical pathway alterations [1]. In vitro haemolysis is a common technical problem affecting UCB sampling in the delivery room, and can hamper metabolomic analysis. The extent of metabolomic alteration which occurs in haemolysed samples is unknown [2].

Objectives:

The aim is to examine metabolomic differences between haemolysed and non-haemolysed UCB samples, and define a list of mass features or metabolites that are unreliable to measure in haemolysed UCB.

Results and discussion:

Two samples of cord blood were drawn and bio-banked at -80 °C within 3 hours of birth for each infant (n=8). Metabolomic analysis was conducted using FTICR direct infusion mass spectrometer. All spectra were normalised, batch corrected and cleaned. Wilcoxon paired rank sum test found on average 3.5% of mass features were significantly altered between the haemolysed and clean samples. However multivariate analysis illustrated the biological variance between infant samples remained greater than the technical variance introduced by haemolysis.

Conclusion:

This information will be useful for researchers in the field of neonatal metabolomics to avoid false findings in the face of haemolysis, to ensure reproducible and credible results.

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PP 4 Erin Dolan (UCC)

Characteristics of cognitive deficits in an alpha-synuclein model of Parkinson's disease

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Background:

Parkinson's disease (PD) is a neurodegenerative disorder characterised by the loss of dopaminergic neurons in the substantia nigra of the midbrain as well as the formation of Lewy Bodies. These are aggregates of α -synuclein which form proteinaceous inclusions in the neurons. PD is primarily classified as a motor disorder, whereby patients display 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 rodent brains *in vivo*. It has been shown to replicate many of the clinical features of PD, including nigral dopaminergic neuron degeneration, decreased striatal dopamine levels and significant motor impairment (1).

Objectives:

Our study aims to investigate and characterise late-stage cognitive and motor dysfunction using the α -synuclein rat model of PD.

Results:

Early results indicate significant motor dysfunction in α -synuclein-lesioned animals in the stepping ($p=0.001$) and corridor ($p<0.05$) tests 20 weeks post-surgery.

Discussion:

Although we report similar levels of motor impairment to previously published data (1,2), these deficits in motor performance became apparent at a later time-point. Further immunohistochemical and HPLC analysis will be carried out to examine the progression of the neurodegeneration and pathology in various brain regions. In addition, a battery of cognitive tests to assess deficits in working and associative memory and olfactory discrimination will be carried out.

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PP 5 Róisín Dunne (TCD)

Novel small molecule inhibitors that alter energy metabolism and DNA repair and improve radioresponse in oesophageal and Colorectal Adenocarcinoma

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Background

Tumours display high levels of angiogenesis leading to leaky blood vessels and hypoxia, mechanisms of radioresistance. Altered energy metabolism and DNA repair in tumours can also result in radioresistance. Targeting tumour angiogenesis, metabolism and DNA repair may therefore increase radiosensitivity.

Zebrafish intersegmental blood vessel screening identified a novel small molecule inhibitor, RUD1, with strong anti-angiogenic properties. Structural analogues of this compound (RUD2-4) were created. We examined the ability of these inhibitors to alter metabolism, DNA repair gene expression and radioresponse in oesophageal and colorectal cancer cell lines.

Results

An isogenic model of oesophageal radioresistance, OE33P (sensitive) and OE33R (resistant), in addition to the radioresistant colorectal cancer cells, HT29-LUC, were treated with the inhibitors.

Metabolism profiles were generated using Seahorse technology. Oxidative phosphorylation was reduced in OE33P treated with RUD2 and RUD3 ($p < 0.05$), OE33R treated with RUD1-3 ($p < 0.0001$) and HT29-LUC treated with RUD1,3,4 ($p < 0.05$).

Expression of DNA repair genes was assessed using qPCR. Expression of several DNA repair genes was reduced following treatment with the inhibitors. MLH1 expression was reduced in OE33P and OE33R treated with RUD2-4 ($p < 0.0001$). PARP1 expression was reduced in OE33P and OE33R treated with RUD1 ($p < 0.01$). RUD1 also reduced expression of MMS19 in OE33R ($p < 0.01$). MLH1 expression was also reduced in HT29-LUC treated with RUD4 ($p < 0.01$).

Radiosensitivity was assessed by clonogenic assay. RUD4 increased radiosensitivity of OE33P and OE33R ($p < 0.0001$) and HT29-LUC ($p < 0.05$). RUD3 also increased radiosensitivity in OE33R ($p < 0.05$).

Conclusion

We have identified a number of novel anti-angiogenic small molecules that alter energy metabolism and DNA repair gene expression, processes linked to radiation response, in both oesophageal and colorectal cancer cell lines. Two of these inhibitors also improve radiation response.

PP 6 Marie Fitzgibbon (NUI Galway)

Characterization of the endocannabinoid system in the descending pain pathway in a mouse model of IFN- α -induced hyperalgesia

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Interferon-alpha (IFN- α) is a pro-inflammatory cytokine, used in the treatment of various cancers and infections. However, this treatment strategy is associated with a high incidence of depression and painful symptoms [1, 2]. We have recently demonstrated that repeated administration of IFN- α to mice induces depressive-like behaviour and enhanced nociceptive responding [3]. The endocannabinoid system is involved in emotional and nociceptive processing, however it is unknown whether repeated IFN- α alters the endocannabinoid system, provoking these behavioural changes.

Thus, this study investigated 1) the effect of repeated IFN- α administration on endocannabinoid levels and CB1 receptor expression in the descending pain pathway in mice and 2) if endocannabinoid levels differ between saline- and IFN- α treated animals during expression of nociceptive behaviour in the formalin test

IFN- α -treated animals exhibited enhanced nociceptive responding throughout the late phase of the formalin test when compared with saline-treated counterparts. LC-MS-MS analysis revealed that concentrations of the endocannabinoids anandamide (AEA) and 2-arachidonylglycerol (2-AG) did not differ between saline and IFN- α -treated animals in any of the tissues examined. However, in IFN- α -treated animals that received formalin, 2-AG levels in the periaqueductal gray and rostral ventromedial medulla (RVM); and AEA levels in the RVM; were increased when compared to non-formalin-treated counterparts, an effect not observed in saline-treated animals. There was no change in CB1 receptor mRNA expression between IFN- α - and saline-treated animals

In conclusion, repeated IFN- α administration enhances nociceptive responding and increases endocannabinoid levels in key regions of the descending pain pathway in response to formalin, indicating that alterations in the endocannabinoid system may underlie IFN- α -induced hyperalgesia.

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

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PP 7 Oisín Gough (UCD)

FRMD3: A Novel Modulator of Diabetic Nephropathy?

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Objectives:

Genome-wide association studies have identified a single nucleotide polymorphism (SNP, rs1888747 C>G) 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. Renal expression of FRMD3 decreases significantly with severity of DN. In silico analysis of the FRMD3 extended promoter sequence predicts that FRMD3 may be co-regulated with bone morphogenetic protein (BMP) pathway family members. Given the evidence for BMP agonists and antagonists in DN we propose that altered FRMD3 expression may be implicated in the pathophysiology of DN.

Methods:

Expression of FRMD3 and fibrotic markers were measured in primary human mesangial cells (hMCs) and podocytes by quantitative Taqman PCR and western blot following: (i) transfection with FRMD3-targeting siRNA and (ii) stimulation with BMPs (BMP-2/4/6/7; 10ng/ml) or TGF- β 1 (5ng/ml).

Results:

Here we report that reduced FRMD3 expression in primary human mesangial cells (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 by stimulation with TGF- β 1 (5ng/ml). Inhibition of podocyte FRMD3 expression renders podocytes more vulnerable to TGF- β 1-induced injury. We provide evidence for FRMD3-BMP co-regulation by demonstrating that FRMD3 gene expression is significantly up-regulated in hMCs following stimulation with BMPs (BMP-2/4/6 and 7). Additionally, we have found a correlation between serum creatinine and FRMD3 expression in renal biopsy material affected by disease of diverse aetiologies.

Conclusion:

Taken together our findings suggest that altered FRMD3 expression may play a role in the pathogenesis of DN.

PP 8 Karen Hanrahan (UCD)

The role of ZEB1/ZEB2 and β III-tubulin in mediating docetaxel-resistant prostate cancer

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Docetaxel is the gold standard treatment for advanced castrate-resistant prostate cancer (CRPC). However, patients either do not respond or develop resistance. Proteomic analysis of docetaxel-resistant prostate cancer sub-lines developed by our group revealed mechanisms of resistance associated with advanced disease. The sub-lines also demonstrated a coordinated loss and gain of epithelial and mesenchymal markers respectively; characteristic of Epithelial-Mesenchymal Transition (EMT). Studies have demonstrated EMT in prostate cancer progression, metastasis and docetaxel resistance. However, the role of EMT drivers in mediating resistance is not defined. We hypothesise EMT to be a central mechanism of resistance in advanced docetaxel-resistant CRPC, representing a target for therapeutic manipulation.

EMT was characterised functionally (increased invasive capacity, MMP-1 secretion) and mechanically (increased ZEB1, ZEB2 expression) in the PC-3 D12 and DU145 R docetaxel-resistant sub-lines, which was associated with increased β III-tubulin expression. Upon treatment with docetaxel (20nM), the docetaxel-resistant sub-lines demonstrated significant resistance compared to parental controls. Simultaneous siRNA knockdown of ZEB1 and ZEB2 resulted in an increased sensitivity of the docetaxel-resistant cells to docetaxel, which was associated with a down-regulation of β III-tubulin and a re-expression of E-cadherin.

Our results provide evidence of EMT in in vitro models of docetaxel-resistant CRPC, which is associated with differential susceptibility to docetaxel and is partially reversed through knockdown of ZEB1 and ZEB2. We have also identified a link between EMT and β III-tubulin in our models of docetaxel resistance. Current experiments are investigating the tissue expression of ZEB1 and β III-tubulin in prostate cancer metastases following docetaxel therapy, which will determine their clinical relevance as mediators of docetaxel resistance in CRPC.

PP 9 Ciara Harty (UCC)

Hepatitis C Proteins Interact Directly with Early Stage Autophagy Proteins

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Background and Aims:

Autophagy is a complex intracellular pathway involved in recycling cellular components to maintain homeostasis. It is used as a cellular response against bacterial/viral infections. The autophagy pathway is needed for efficient Hepatitis C virus (HCV) replication. Different groups have reported that autophagy inhibitors down regulate HCV replication. ATG5 is an autophagy protein involved in the early stages of the autophagy pathway. It is needed for the development of the early phagophore membrane before the sequestration of cellular components marked for degradation. Carl Guévin (Virology 2010) reported that ATG5 is needed for HCV replication and suggests that HCV utilises ATG5 as a proviral factor at the onset of viral infection. We sought to clarify the role of ATG5 in the life cycle of HCV using a human serum-derived HCV.

Materials and Methods:

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. For this reason, there has been limited use of sdHCV in cell culture. However, we have shown the presence of intracellular HCV proteins, core and NS3, up to 72hrs post-infection. Three colour immunofluorescence experiments were used to validate HCV infection staining three proteins HCV core, HCV NS3 and cellular protein ADRP (Adipose differentiated-related protein). Quantitative real-time PCR (qRT-PCR) was used to determine the levels of intracellular HCV RNA. We examined the interaction of HCV proteins with ATG5 at different timepoints post infection using both immunofluorescence and qRT-PCR.

Result and Conclusions:

HCV proteins, core and NS3, were expressed in the cells from two hours post-infection to seventy-two hours post-infection. We noted HCV RNA was stable. This suggests that the serum derived HCV has the ability to gain entry into the cells, the incoming RNA is translated into protein but this RNA is not replicated within the cell. This allows us to examine the initial factors needed for HCV infection. We show a definite change in the distribution of ATG5 in HCV infected cells, from a scattered cytoplasm pattern to a punctate appearance. Also, there is co-localisation between HCV NS3 and ATG5 suggesting interaction between these proteins. We have also shown direct interaction between HCV core and ATG5. In conclusion, we have shown that two HCV proteins transcribed from the initial incoming RNA strand interact with ATG5. Further studies are on-going to clarify the role of this interaction in the life cycle of HCV.

PP 10 Jennifer Hillis (NUI Galway)

Neurotrophin signaling protects CLL cells from death

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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- κ B activity also contributes to pro-survival signaling [2].

Neurotrophins are a class of growth factors found in many cell types. They signal through the Trk tyrosine kinase receptors and the p75NTR receptor, leading to cell proliferation, survival or death [3].

B cells are known to express these receptors and can be rescued from apoptosis by exposure to neurotrophins [4].

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

Results & Discussion:

Use of neurotrophin or neurotrophin receptor inhibitors induces cell death in Mec1 cells. These cells exhibit a variety of morphologies following this treatment, suggesting induction of multiple modes of cell death.

Use of these compounds induced very low levels of DEVDase activity, along with a lack of PARP cleavage or processing of caspase-3. These findings suggest that classical apoptosis is not induced, supporting the evidence that CLL cells have dysregulated apoptosis [1]. Inhibition of RIP1 partly inhibited this cell death, suggesting that necroptosis may be involved.

We hypothesize that constitutive neurotrophic signaling protects these cells from death by constitutive NF- κ B activation, with the use of neurotrophin or neurotrophin receptor inhibitors abrogating this pro-survival signal.

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

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PP 11 Lauren MacDonagh (TCD)

Inhibition of the cancer stem cell marker, ALDH1, re-sensitizes cisplatin resistant NS-CLC cells to cisplatin-induced apoptosis

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Background:

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

Objectives:

An isogenic model of cisplatin resistance was established by chronically exposing a panel of NSCLC cell lines to cisplatin for 12 months, thereby creating cisplatin resistant (CisR) sublines and their corresponding age-matched parental (PT) cells. There are three aims in this study. Aim 1; identify a CSC population within the resistant sublines. Aim 2; isolate the CSC population, elucidate the functional role of these CSC cells in response to cisplatin and confirm the expression of stemness markers. Aim 3; to re-sensitize the cytotoxic effects of cisplatin via inhibition of CSC-specific markers.

Results:

A significant ALDH1+ve population was isolated from CisR sublines, not found in their PT counterparts. Characterisation of the ALDH1+ve subpopulation confirmed enhanced stem-like properties and resistance to cisplatin compared to their ALDH1-ve counterparts. Importantly, inhibition of ALDH1 activity, using a specific inhibitor, re-sensitized ALDH1+ve CisR cells to cisplatin.

Conclusion:

ALDH1+ve cells represent a CSC population in cisplatin resistant NSCLC. In combination with cisplatin, ALDH1 inhibition may hold promise as a therapeutic strategy in cisplatin resistant lung cancer.

PP 12 Wesley van Oeffelen (UCC)

Investigating downstream signalling bias of commercial ligands of the growth hormone secretagogue receptor 1a (GHS-R1A)

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The hunger-stimulating neuropeptide ghrelin activates the centrally expressed growth hormone secretagogue receptor 1a (GHS-R1A). The GHS-R1A receptor pharmacology is however not as straightforward as it seems: it is known, for example, to dimerize with other receptors in the brain [1, 2] or be functionally biased for its ligands, meaning it prefers one downstream signalling pathway over another [3]. Therefore, the aim was to increase understanding of the pharmacological properties of commercially available GHS-R1A receptor ligands on in vitro calcium mobilization, β -arrestin recruitment and receptor internalization, and their effect on food intake in vivo.

None of the GHS-R1A receptor ligands, including antagonists [D-lys3]-GHRP-6 and JMV2959, or inverse agonist [D-Arg(1), D-Phe(5), D-Trp(7,9), Leu(11)]-Substance P (SP-analog), changed calcium mobilization, but all decreased β -arrestin recruitment leading to a subsequent reduction in receptor internalization (Table 1). This indicates a potential functional bias of these ligands to manipulate the constitutive activity of the GHS-R1A. Furthermore, we found that the [D-lys3]-GHRP-6 and SP-analog significantly potentiated, whereas JMV2959 inhibited, ghrelin-induced calcium mobilization. This suggests that the antagonist [D-lys3]-GHRP-6 may have inverse agonistic properties. In addition, we showed that [D-lys3]-GHRP-6 did not affect food intake stimulated by an injection of ghrelin alone, even though it was previously shown that [D-lys3]-GHRP-6 reduces food intake in mice after an overnight fast [4].

Considering the limited success in the clinical development of ghrelin-targeted drugs [5], these data provide a novel insight on the pharmacological characteristics of the GHS-R1a receptor, which may aid in further developing drugs targeting the GHS-R1a receptor.

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Table 1 Results of the GHS-R1A ligands on calcium mobilization, β -arrestin recruitment and receptor internalization of human embryonic kidney cells overexpressing the GHS-R1A in vitro, and food intake in mice in vivo. Changes are indicated as a significant increase (\uparrow), significant decrease (\downarrow), no significant difference ($=$), and data not available (n/a).

	Calcium mobilization	β -arrestin recruitment	Internalization	Food intake
Agonists:				
Ghrelin	\uparrow	\uparrow	\uparrow	\uparrow
MK-0677	\uparrow	\uparrow	\uparrow	
Antagonists:				
[D-lys ³]-GHRP-6	=	\downarrow	\downarrow	=
JMV2959	=	\downarrow	\downarrow	=
Inverse agonist:				
[D-Arg 1 ,D-Phe 5 ,D-Trp 7,9 ,Leu 11]-Substance P	=	\downarrow	\downarrow	n/a
Pre-treatment ghrelin:				
D-lys ³ -GHRP-6	\uparrow	=	=	=
JMV2959	\downarrow	=	\downarrow	=
[D-Arg 1 ,D-Phe 5 ,D-Trp 7,9 ,Leu 11]-Substance P	\uparrow	\downarrow	=	n/a
Pre-treatment MK-0677:				
D-lys ³ -GHRP-6	\uparrow	=	n/a	n/a
JMV2959	\downarrow	=	n/a	n/a
[D-Arg 1 ,D-Phe 5 ,D-Trp 7,9 ,Leu 11]-Substance P	\uparrow	\downarrow	n/a	n/a

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PP 13 Huong Quach Thi Thu (NUI Galway)

DNA replication in the presence of Cdc7 inhibition in human breast cancer cells

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Background:

Breast cancer is one of the most popular malignant solid tumours in Western women and considered as the second leading cause of cancer death in Ireland [1]. Effects of hormonal and chemical therapies which focus on ER/PR/HER2 receptors were proved, but treatment options for triple-receptor negative (ER-/PR-/HER2-) breast cancer are limited. Recently, inhibition of cell growth by targeting to DNA replication becomes one of the potential targets of chemotherapy for breast cancer treatment.

DNA replication is a critical event by which an original DNA duplicates to produce two identical copies. It occurs in S-phase of cell cycle and requires a cooperation of many enzymatic proteins, including CDC7 and CDK kinases. CDC7 is a Ser/Thr kinase enzyme which involves in many cellular processes, especially in DNA replication [2]. Depletion of CDC7 causes a blockade of S-phase progress and leads to apoptotic cell death without inducing any DNA damage response [3]–[5]. Based on this fact, many CDC7 inhibitors were generated, including a selective CDC7 inhibitor XL413 and a dual-effect CDC7/CDK9 inhibitor PHA767491 [3], [6]. We hypothesise that inhibition of CDC7 kinase by XL413 and PHA767491 restrains breast cancer cell growth by particular effects on initiation and licensing of DNA replication.

Methods:

To examine the effects of Cdc7 inhibition, a non-malignant breast cell line, MCF10A (p53-wild type) and a breast cancer cell line, MDA-MB-231 (p53-mutant) were employed. Both cell lines were treated with drugs (XL413/PHA767491) for a short-term (24 hours) and long-term (24-48-72 hours) treatments and harvested for biochemical assays, including FACS (flow cytometry), WB (Western Blotting) and DNA fibers.

Results:

After 24h treatment with XL413, apoptotic cell death is undetectable. Both cell lines continue to grow while CDC7 is inhibited in a long-term treatment (24-48-72hr). These events are confirmed by the incorporation of EdU (5-ethynyl-2'-deoxyuridine, a nucleoside analogue of Thymidine) to active unwound DNA during S-phase, even with high doses (25 and 50µM XL413). The failure of blocking DNA replication is also demonstrated by observing replication fork patterns after the short-term treatment. Investigating effects of CDC7 inhibition on synchronised G1/S phase cells revealed a delay of DNA synthesis at S phase while cells are treated with XL413. However, cells are still able to go through G2 and enter M phase.

In contrast, with PHA767491 treatment, these effects were not observed. No S-phase cells are recorded after 24h treatment with this drug. Treated cells accumulate at G2 phase and only a minor proportion of mitotic MDA-MB-231 cells can enter S phase in the presence of PHA767491.

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Discussion:

CDC7 inhibition by XL413 declines the S-phase progress of breast cell lines. However, this drug has limited effects on cell growth as well as cell survival. This result agrees with a recent study of Sasi [7]. In contrast, PHA767491 totally blocks DNA synthesis after 24h treatment as well as prevents mitotic cells entering S-phase. Further studies are required to elucidate the mechanism of CDC7 inhibition by both drugs in DNA replication initiation and licensing.

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PP 14 Gerard Roche (TCD)

Liver Derived Immune Cells have Increased Expression of Interferon Lambda Receptor

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Background

Interferon lambda (IFN- λ) is an anti-viral cytokine family important in the resolution of Hepatitis C Virus (HCV) infection. The three subtypes of IFN- λ mediate their effects via the IL-28R1/IL-10R2 receptor complex (IFNLR). IFNLR1 is expressed primarily on epithelial cells such as hepatocytes and specific immune cell populations including dendritic cells. Stimulation of IFNLR results in activation of the JAK-STAT pathway.

Objectives

As HCV is a hepatotropic virus, we hypothesized that immune cell populations within the hepatic environment express IFNLR differentially compared to cells in peripheral blood. The aim of this project was to compare IFNLR1 expression in healthy donor peripheral blood mononuclear cells (PBMCs) with hepatic mononuclear cells (HMNCs) which were isolated from Wisconsin preservative-perfused donor livers during transplantation.

Results and Discussion

Flow cytometric analysis of healthy PBMCs (n=4) and HMNCs (n=4) was performed. For PBMCs, IFNLR1 was expressed on CD19+ B cells (10% positive), CD14+ monocytes (40% positive) and HLA-DR+, Lin-1- dendritic cell (DC) populations (33% positive) but not on CD3+ T cells or CD56+ NK cells. All HMNCs immune subpopulations were positive for IFNLR1 ranging from 50-70% positive.

Conclusions

Increased tissue specific expression of IFNLR1 by hepatic immune cell populations at the primary site of HCV infection suggests a mechanism for HCV infection resolution by the inducing upregulation of IFNLR1 expression and subsequent downstream IFN- λ signalling.

PP 15 Niall Savage (UCC)

Development of a smart needle integrated with a micro-structured impedance sensor for the detection of breast cancer

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

Background:

The aim of this research is to develop a novel medical device for the detection of breast cancer within the clinical setting. Breast cancer is the second most common cancer in women worldwide [1] and the use of population-based screening programmes has increased the demand for more sensitive and specific detection tools to limit the number of patients being misdiagnosed or over-treated.

This research will focus on the development of a minimally invasive diagnostic probe for the determination and localisation of cancerous tissue within the structure of the breast. Gold microelectrodes fabricated on a silicon substrate were developed in Tyndall National Institute and used to obtain electrical impedance recordings from ex vivo tissue samples of both animal and human origin. Functional prototype devices have been produced using both photolithography and metal deposition processes to pattern the dual-electrode structures. The probes have been characterised using a series of techniques including CV and EIS.

The prototype probes have been shown to be reproducibly manufactured and the electrochemical response of the electrodes has been very positive to date. A study of the electrical impedance response of animal tissues (beef, lamb and pork) has shown that a variety of tissues (fat, muscle and liver) can be discriminated using the prototype gold electrodes for the detection of discrete electrical responses. There are a number of potential uses for this device including improved biopsy localisation, cancer-free border determination during lumpectomy and the possibility of DCIS determination without invasive surgery. It is envisaged that this novel device would be used primarily as an adjunct to the gold-standard of x-ray mammography detection of breast cancer tumours during the routine screening process.

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PP 16 Stephanie Slevin (NUI Galway)

Anti-TNF therapy is associated with rapid reduction of circulating monocyte numbers and blunted monocyte pro-inflammatory response in patients with inflammatory bowel disease

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Background:

Monocytes are considered to play a role in the pathogenesis of inflammatory bowel disease (IBD) and may be a specific target of anti-TNF therapies. We studied the acute effects of the anti-TNF mAb infliximab (IFX) on blood monocytes and their subsets in a cohort of IBD patients.

Methods:

(a) Multi-colour flow cytometry of freshly-isolated PBMCs from Healthy Adults (Ctrl, n=21) and Adults with IBD (n=32) prior to (IBD-pre) and immediately following (IBD-post) IFX infusion. (b) Monocyte apoptosis (cleaved caspase 3) and LPS-stimulated TNF and IL-12 production by intracellular staining of IBD-pre and IBD-post samples.

Results:

Total blood monocytes were not statistically different for Ctrl compared to IBD-pre samples (18.5 ± 7.0 vs $10.6 \pm 9.5 \times 10^4/\text{ml}$) but were strikingly reduced (to $4.6 \pm 4.4 \times 10^4/\text{ml}$) in IBD-post samples ($p < 0.0001$ vs IBD-pre). All monocyte subsets were reduced in number following IFX in the IBD cohort but the magnitude of reduction was greater for classical (pre vs post; 7.5 ± 6.3 and $3.4 \pm 3.4 \times 10^4/\text{ml}$, $p < 0.0001$) and intermediate (pre vs post; 3.5 ± 2.7 and $1.9 \pm 1.9 \times 10^4/\text{ml}$, $p < 0.0001$) compared to non-classical (pre vs post; 9.9 ± 6.8 and $7.1 \pm 6.0 \times 10^3/\text{ml}$, $p = 0.01$). Monocyte apoptosis (% cleaved caspase 3+) was low in IBD-pre samples ($0.59 \pm 0.62\%$) and was not increased in IBD-post samples ($0.43 \pm 0.33\%$). Following LPS stimulation, the proportions and total numbers of TNF+ and IL-12+ monocytes were significantly higher in IBD-pre compared to IBD-post samples.

Conclusions:

IFX infusion is associated with striking reduction in circulating monocytes following IFX infusion in IBD patients with greatest effect on classical and intermediate subsets. This is not associated with induction of apoptosis but is accompanied by blunting of monocyte pro-inflammatory response to TLR4 ligation.

PP 17 Julie Worrell (UCD)

The Regulation of CXCR3 and IL-13R α 2 by IL-13 in Pulmonary Fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a chronic progressive form of idiopathic interstitial pneumonia, characterized by fibrosis. The chemokine receptor CXCR3 has a role in limiting fibrosis following lung injury. IL-13 is a pro-inflammatory cytokine that mediates the development of fibrosis and signals via STAT6; it can induce expression of and bind to its own receptor IL-13R α 2. This study hypothesises that CXCR3 is an anti-fibrotic receptor that is negatively regulated by IL-13 [1] and may be positively regulated by PPAR γ .

Primary human lung fibroblasts were obtained via explant culture from both control and IPF patient tissue. Fibroblasts were validated using immunofluorescence or treated with IL-13, CXCL9, Leflunomide and the PPAR γ agonist Rosiglitazone, gene and protein expression was analysed by qPCR and Western Blotting. Immunohistochemistry was carried out on FFPE tissue sections from IPF patients (n=6) and control patients (n=3) for IL-13R α 2, CXCR3 and PPAR γ .

IPF and normal human primary human fibroblasts express CXCR3 at both a gene and protein level. CXCR3 expression is significantly increased by Rosiglitazone at 48 hours and IL-13 causes a significant increase in IL-13R α 2 gene expression at 48 hours in normal fibroblasts. IL-13R α 2 is expressed by fibroblasts within the fibroblastic focus, the overlying type II pneumocytes and some inflammatory cells and CXCR3 is expressed in inflammatory cells; type II hyperplastic pneumocytes, and fibroblasts. The tissue staining pattern suggests that fibroblasts express CXCR3>PPAR γ >IL13R α 2.

Identification of the pathways involved in CXCR3 regulation may lead to the development of novel therapeutic targets in the area of pulmonary fibrosis.

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PP 18 Mark Bates (TCD)

The role of the TLR4 pathway and the spindle assembly checkpoint in ovarian cancer prognosis

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Background:

MyD88 and MAD2 are two potential prognostic biomarkers that have been investigated in ovarian cancer. High MyD88 and Low MAD2 IHC staining is associated with reduced PFS, both markers are also linked to paclitaxel chemoresistance [1][2][3].

Objectives:

The main objective of this study was to assess the in vitro relationship between MAD2 and MyD88, through alteration of MAD2, MyD88 or its receptor TLR4 in two ovarian cancer cell lines using siRNA targeting MAD2, TLR4 or MyD88 and a MyD88 overexpression plasmid vector. Following overexpression/siRNA knockdown procedures, MyD88, TLR4 and MAD2 expression was assessed through qPCR and Western Blot analysis. Mir-433, Mir-21 and Mir-146a gene expression was also assessed by qPCR. Furthermore the effect of TLR4/MyD88 knockdown on chemoresponse was assessed in SKOV-3 cells using the CCK-8 assay.

Results/Discussion:

It was found that knockdown or overexpression of MyD88 in SKOV-3 or A2780 cells respectively or knockdown of TLR4 in SKOV-3 cells had no effect on MAD2 expression or the expression of Mir-21, Mir-433 and Mir-146a. Interestingly however knockdown of MAD2 in both cell lines induced a 3 fold increase in TLR4 expression, furthermore knockdown of TLR4 in SKOV-3 cells was shown to restore chemosensitivity to paclitaxel.

Conclusion:

The results demonstrate a potential in vitro link between TLR4 and MAD2 and support a role for TLR4 in paclitaxel chemoresistance.

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PP 19 Sarah Brophy (TCD)

Investigating the tumour microenvironment in chronic lymphocytic leukaemia: A role for STAT3

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Background:

Chronic Lymphocytic Leukaemia (CLL) is the most prevalent form of leukaemia in adults. CLL has no curative treatment and its pathogenesis is yet to be fully elucidated. The tumour microenvironment is important in CLL, promoting cell proliferation, survival and protection from drug induced apoptosis. Selectins, integrins and chemokines mediate the trafficking of CLL cells to these protective niches. Signal transducer and activator of transcription 3 (STAT3) is constitutively phosphorylated on serine residue 727 in CLL cells.

Objectives:

The objective of this study is to investigate the role of the JAK-STAT3 signalling pathway in mediating microenvironmental interactions and cell survival in CLL.

Results & Discussion:

A panel of JAK-STAT3 pathway inhibitors and STAT3 knockdown were assessed for effects on cell cycle, apoptosis and the protein expression of key cell surface adhesion molecules in CLL cells. The inhibitors S3I-201 and cucurbitacin downregulated the expression of CD62L, a selectin involved in the trafficking of CLL cells to the lymph node microenvironment. In addition, these agents inhibited CLL cell chemotaxis and adhesion to bone marrow stromal cells and endothelial cells under static and shear flow conditions. Microenvironmental factors were seen to regulate the serine phosphorylation STAT3: The pro-survival chemokine, CXCL12, and coculture with stromal cells caused an increase in STAT3 phosphorylation which was downregulated by S3I-201.

Conclusions:

In summary, this study shows a role for STAT3 in CLL cell survival by mediating microenvironmental pro-survival signaling and suggests novel therapeutic strategies for the treatment of this disease.

PP 20 Ann Byrne (TCD)

Liver Perfusion Fluid: A Source of Adult Stem Cells

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Background:

Previous work from our group has verified the presence of hematopoietic stem cells (HSCs) in the adult liver [1]. These CD34+ HSCs isolated from healthy liver biopsy samples demonstrate erythroid, monocytic/granulocytic and biliary epithelial cell differentiation in vitro. More recent work in our group has verified that the washout liver preservation fluid (perfusate) collected at the time of liver transplantation is a rich source of hepatic mononuclear cells (HMNCs). In 2011, Pan et al. investigated the presence of mesenchymal stem cells (MSCs) in liver perfusate and concluded that liver-MSCs represent a bona fide stem cell population [2]. These L-MSCs may contribute to tissue repair and immunomodulation after liver transplantation.

Objectives:

Identify the stem cell populations in liver perfusate. Characterise these populations using flow cytometry and expand L-MSCs with the aim to differentiate these cells to a hepatocyte-like state.

Results & Discussion:

HMNCs isolated from perfusate contain a clear population of HSCs (CD45/34/90/c-Kit+) and CLPs (CD45/34/38+) (n=4). There is also evidence of the presence of a CD105/166+ double positive MSC population (n=4). Upon expansion for 15-18 days, this MSC population shows an up regulation in the co-expression of stem cell markers CD90/166/105 (n=2). This expanded CD45/34- population may possess multi-lineage differentiation potential

Conclusions:

In cases of end stage liver disease, the liver loses this ability to repair itself. Currently, optimal treatment is liver transplantation. This treatment comes with major obstacles including immunological incompatibility. There is a clear need to explore new treatment strategies. We have shown that liver perfusate is a valuable source of HMNCs including HSC and MSCs. These L-MSCs may offer a scalable alternative to allotransplantation, providing cells for transplantation and a cell source for studying disorders of the liver in the future.

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PP 21 Oyinlola Dada (UCD)

Bioconjugated NHC-Au-thiosugar complexes as targeted anticancer drugs

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Background:

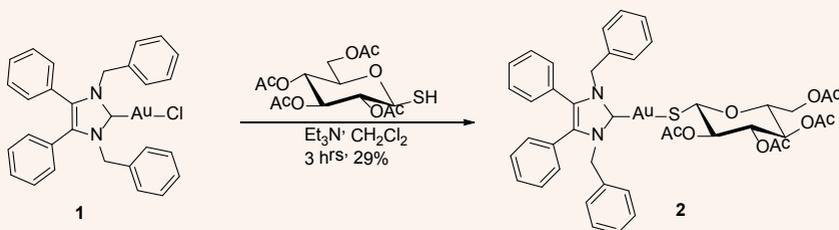
N-Heterocyclic carbenes (NHCs) are stable singlet carbenes that can act as excellent two electron donor ligands towards most elements in the periodic table.¹ The first stable carbenes to be isolated were based on an imidazole ring and this family of imidazol-2-ylidenes are the most studied carbenes. Since the role of disaccharides in cell recognition, metabolism and cell labeling is a well-known concept, the conjugation of saccharides to drugs has become an active area of research in chemistry. The largest contribution to metal-based NHC anticancer drugs is in the field of Au(I/III) complex.² Auranofin is a well studied gold containing compound used in the treatment of rheumatoid arthritis, and in preclinical study has shown efficiency in CLL cell.³

Objectives:

This project focuses on the synthesis of six structurally different glycosyl thiols and their conjugation to NHC-Au(I)chloride **1** for in vitro biological evaluation against the human cancer cell lines Caki-1 (renal) and MCF-7 (breast) for varying degrees of enhanced cytotoxicity.

Results & Discussion:

Compound **2** has been successfully synthesized with the expectation of optimizing the yield and in its purest form in vitro cell testing can be carried out. In previous work carried out in the group, NHC-Au(I)-SR has shown overall better activity against Caki-1 and MCF-7 cell lines superior to that obtained for the NHC-Au(I)-Cl and NHC-Au(I)-OAc.



Conclusions:

Preliminary in vitro cell test of the NHC-Au conjugated glycosyl thiol has shown better activity than its NHC-Au-Cl precursor and NHC-Au-OAc. The significance of this substitution will be further investigated by varying the glycosyl thiol to be conjugated.

References:

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PP 22 Laura Gleeson (TCD)

Human macrophages adopt aerobic glycolysis to control mycobacterium tuberculosis infection

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Background:

Mycobacterium tuberculosis (Mtb) infection represents a major global health threat requiring novel therapeutic and vaccination targets. Recent advances in immunometabolism link metabolic changes in stimulated macrophages to pro-inflammatory function [1]. Alveolar macrophages (AMs) represent the first line of host defence against Mtb in humans, thus defining the role of host macrophage glucose metabolism in the innate immune response to Mtb infection may identify novel targets for treatments and vaccines.

Objectives:

- Define the impact of Mtb infection on human macrophage glucose metabolism.
- Investigate the impact of host glucose metabolism on the cytokine profile of Mtb-infected macrophages.
- Establish the role of host macrophage glucose metabolism on bacillary survival.

Results & Discussion:

Mtb infection induced aerobic glycolysis in human AMs and MDMs, as measured by lactate assay and extracellular flux analyses. Inhibition of Mtb-induced glycolysis decreased production of pro-inflammatory interleukin-1beta (IL-1 β) and increased production of anti-inflammatory interleukin-10, both at mRNA and protein level. Inhibiting glycolysis also increased bacillary intracellular survival. The effect of inhibiting glycolysis on bacillary survival was blocked by anti-IL-1 β antibody, demonstrating that the impact of aerobic glycolysis on bacillary survival is mediated through its induction of IL-1 β .

Conclusions:

We demonstrate that glycolysis-induced IL-1 β plays a role in limiting intracellular bacillary survival in the human macrophage. Our findings suggest that manipulation of host immune metabolism could offer novel therapeutic approaches to Mtb infection.

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PP 23 Maria Hughes (QUB)

Long term changes in high sensitivity measured Troponin I and its association with incident cardiovascular disease in the MORGAM/BiomarCaRE cohort from Denmark

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Background:

Troponin I is a biomarker of cardiac necrosis useful for diagnosing acute myocardial infarction in emergency situations and also for predicting long term risk of cardiovascular disease (CVD) in general populations. High sensitive troponin I (hsTnI) can be detected in 80-90% of the general population aged 30-65 years [1]. Compared to a single hsTnI measure at one time point, repeated measures can detect changing hsTnI levels which may reflect subclinical disease and more optimally stratify risk of cardiovascular outcomes. Investigating this, is an important step in validating hsTnI for prognostic models [2].

Objectives:

To assess the prognostic value of using the trend in hsTnI, measured at 3 timepoints 5 years apart, on future CVD events in a general population compared to a single measure of hsTnI.

Results & Discussion:

Using a prospective Danish study (3875 participants, age range 30-60, 51% female, 581 CVD events), the trend in hsTnI and other risk factors was modelled using a joint (longitudinal and survival) model. Median hsTnI levels changed from 2.6ng/L to 3.4ng/L over 10 years. The joint model performed only marginally better (c-index improvement 0.0094, $p < 0.001$) than using a single measure of hsTnI (c-index improvement 0.0052, $p = 0.043$) for prediction of CVD compared to a model incorporating CVD risk factors only (c-index 0.744 95% C.I. 0.717, 0.772).

Conclusions:

Longitudinal hsTnI measures can improve prediction of 10-year risk of CVD but a single most recent measure is just as effective. Requiring only a single measure of hsTnI simplifies its use as a prognostic marker for primary prevention of CVD. This brings us one step closer to utilising it for prediction in clinical practice.

References:

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PP 24 Katrina Hutchinson (Biomnis)

The effects of vitamin D supplementation on childhood asthma: a randomized, double-blind, placebo-controlled trial.

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Background:

Vitamin D deficiency (VDD) and asthma-incidence/severity share many common risk factors. Vitamin D has a number of biological effects that are likely important in regulating key mechanisms in asthma. Thus, VDD may result in increased prevalence and severity of childhood asthma.

Objectives:

We recruited 43 children, all previously diagnosed with asthma. At baseline and after 15 weeks of daily supplementation with 2,000 IU vitamin D3 or matching placebo, we assessed: pulmonary function, asthma control, quality of life. Vitamin D (25(OH) D), calcium, PTH, phosphate, IgE and hsCRP were measured.

Results & Discussion:

At baseline: mean 25(OH) D was 51nmol/L (24-80). According to the Institute of Medicine guidelines, 21 children had deficient levels (<50nmol/L), while 22 had sufficient 25(OH) D levels (>50nmol/L). There was no significant difference in demographics, serum markers or self-reported measures of asthma control between the two groups. However, pulmonary function was significantly higher in the vitamin D sufficient group, including forced vital capacity FVC% (66 vs. 96%; $p = 0.03$) and forced expiratory volume FEV1% (93 vs. 102%; $p = 0.03$). After the supplementation, compliance was high in both groups (>85%). There were no adverse effects. Despite a significant increase in serum 25(OH) D, there was no significant difference between vitamin D supplements and placebo in terms of pulmonary function, subjective asthma measures and serum biomarkers.

Conclusions:

Our results agree with a recent, randomized controlled trial of vitamin D replenishment in paediatric asthma.1 Vitamin D supplementation warrants further investigation in asthmatic children.

References:

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PP 25 Conor Kerley (UCD)

Novel clinical aspects of dietary nitrate: potential effects on human cardiorespiratory disorders

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Background:

Nitric oxide (NO) is an important cellular signaling molecule. Multiple cardiorespiratory pathologies are associated with perturbations in NO. Dietary nitrate acts as a precursor to NO with potential effects on severe processes including blood pressure (BP) and exercise tolerance (ET).

Objectives:

We hypothesized that dietary nitrate may have utility in cardiorespiratory disorders associated with impaired ET: COPD and dilated cardiomyopathy (DCM) and elevated BP: obstructive sleep apnoea (OSAS).

Results & Discussion:

We used identical protocols to assess the acute effect of dietary nitrate in COPD (n=11) and DCM (n=11). Incremental shuttle walk test, BP and blood testing was performed before and 3h after both a nitrate-rich beverage (NO₃⁻) and matching placebo (PL) in a randomized, crossover fashion. Compared to PL, NO₃⁻ led to increased NO metabolites (p<0.000005), increased ET in COPD (+39m; p<0.01) and DCM (+65m; p=0.06) and decreased BP in COPD only (mmHg; p<0.05) [1]. Further, we demonstrated benefit of 7d dietary nitrate in COPD in terms of ET (+70m; p<0.05) in a pilot study.

We recently completed a randomized, feasibility study in OSAS. We assessed ambulatory BP and several key OSAS symptoms among 3 obese subjects with severe OSAS at baseline, post 14d NO₃⁻ and post 14d PL in a randomized, crossover fashion. Compared to PL, nocturnal dietary nitrate improved subjective OSAS symptoms, improved objective vigilance and decreased nocturnal BP by 17mmHg [2].

Conclusions:

Dietary nitrate has potential as a novel therapeutic, adjunct strategy for diverse cardiorespiratory disorders. However, our preliminary results require confirmation in larger, randomized, controlled trials.

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PP 26 Aleksandar Krstic (UCD)

MYCN in neuroblastoma: a transcriptional and epigenetic regulator

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Background:

Neuroblastoma (NB) is a type of embryonal tumours that arises from precursor cells of the sympathetic nervous system [1]. The MYC gene family members are crucial for understanding the biology of NB, since they affect almost every aspect of their behaviour, driving poor outcome.

Objectives:

Our initial experiments were aimed at the identification of genomic regions bound by MYCN. We addressed this by a ChIP-seq in the human NB SH-SY5Y cells with inducible MYCN expression [2]. In silico analysis prompted us to further investigate the interplay of MYCN and epigenetic regulation.

Results & Discussion:

Bioinformatics analysis revealed that MYCN binds numerous targets mostly in intergenic regions and introns. Pathway analysis showed that a number of MYCN targets are part of the neuronal regulatory networks involved in signalling, survival, drug resistance and differentiation. Testing a panel of six NB cell lines, both MYCN amplified and non-amplified, with a demethylating agent revealed strong response. Also, treatment with the panel of forty five epigenetic leads, particularly upon treatment with bromodomain inhibitors, showed strongly reduced viability of NB cells and dramatic changes in cell morphology.

Conclusions:

Specific epigenetic profile of neuroblastoma cells affects the MYCN transcriptional network and probably the progression of NB. By integrating multiple omics datasets and in vitro analyses our work provides a better insight and potential novel routes for therapeutic interventions in NB.

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PP 27 Mark Little (TCD)

Soluble CD163 level as a biomarker of active renal vasculitis

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Introduction:

A specific biomarker which can separate active renal vasculitis from other causes of renal dysfunction is lacking, with a kidney biopsy often being required to resolve the issue. We hypothesised that shedding into the urine of sCD163, expressed on crescent macrophages, would provide an estimate of active glomerular inflammation.

Methods:

Immunohistochemistry and analysis of mRNA expression in glomeruli was performed on renal tissue for patients with MPO-ANCA vasculitis. Using the Rare Kidney Diseases (RKD) Biobank, urine and serum samples from 430 patients with systemic small vessel vasculitis (SVV), disease controls and healthy controls were then assayed for sCD163 by ELISA. Clinical data were derived from the linked RKD registry. The gold standard for active vasculitis was the clinician assessment >1 month after sampling, with active disease in most cases being supported by biopsy and invariably followed by an escalation in immunosuppression.

Results:

In human renal tissue, the degree of glomerular CD163 expression was tightly correlated with the level observed in urine but not serum. CD163 mRNA levels in microdissected glomeruli were significantly higher in patients with SVV than those with lupus nephritis, diabetic nephropathy and nephrotic syndrome. Serum sCD163 levels were not significantly different in patients with SVV and disease or healthy controls. There was no correlation between serum and urinary sCD163. In an inception cohort which included 176 patients with SVV, urinary sCD163 levels in patients with active renal vasculitis were markedly elevated compared to both healthy and disease controls, and to patients in remission. Of note, urinary sCD163 was undetectable in those with active non-renal vasculitis. ROC curve analysis disclosed an AUC of 0.95 (95% CI 0.9-0.99) for differentiation of active renal vasculitis from remission, and defined an optimum cutoff of 0.33ng/mL/mmol creatinine. Applying this to a validation cohort which included 155 patients with SVV allowed differentiation of active renal vasculitis with a sensitivity of 83%, specificity of 98% and positive likelihood ratio of 21.9.

Conclusions:

Urinary sCD163 associates very tightly with active renal vasculitis. A positive result can therefore be used to guide treatment for renal flare in place of renal biopsy.

PP 28 Valerie Logan (TCD)

The Irish National Rare Kidney Disease Registry and Biobank: update on recruitment and activity

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Introduction:

The RKD Registry and Biobank is a national registry of vasculitis and other rare kidney disease patients, with recording of detailed longitudinal clinical data linked to biological samples. We aim to compile sufficiently large cohorts of patients to address fundamental questions relating to the epidemiology, pathogenesis and pathophysiology of disease along with facilitating phase II/III interventional studies.

Methods:

Blood and urine are collected from patients and healthy controls. Blood samples are processed for plasma, serum, DNA and peripheral blood mononuclear cells (PBMCs) and stabilised RNA. Urine is processed to be suitable for a variety of end-point experiments including metabolomic and proteomic analyses and the extraction of exosomes. Samples are inventoried using Freezerworks software.

Results:

Since September 2012, 760 individuals have been recruited from 6 centres across Ireland (Beaumont, St James's, Tallaght, St Vincent's, Limerick and Cork University Hospitals). Most have contributed biological samples on more than one occasion. In particular, samples have been collected from many patients both at times when they had active disease and when they were in remission.

The RKD Biobank has acted as a platform for collaboration and provided biological samples to both national and international partners, from academic institutions and industry. Collaborators include Agilent Technologies, the European Vasculitis Genetics Consortium, Imperial College London, Duke University, McMaster University, Harvard Medical School and Boston Children's Hospital. These studies have used a range of biological samples to investigate biomarkers of disease, urine metabolomic signatures and genetic linkage of disease.

Conclusions:

The Irish National RKD Registry and Biobank has recruited well beyond its targets. Each sample set is linked to a detailed clinical record, with recorded sample provenance, in line with the upcoming ISO/TC 276 standard. We have supported numerous national and international studies. Efforts now focus on sustainability and alignment with the Irish national biobank strategy.

PP 29 Eoin Mac Réamoinn (TCD)

Innate Immune Gene Expression During Early Murine Embryonic Development: Implications for a Role Outside of Defence?

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Background:

Toll-like receptors (TLRs) demonstrate spatially and temporally restricted expression patterns during embryonic development [1-2], which is striking as restricted expression profiles suggest an active role in embryogenesis. This argument has been strengthened by experimental data implicating TLRs as regulators of progenitor cell activity [3-5].

Objectives:

This project aims to examine the expression of TLRs and downstream effector molecules of the TLR pathway during early murine embryogenesis. These analyses will identify genes for functional testing using CRISPR/Cas9.

Results & Discussion:

The expression profiles of TLR2 and TLR4 vary in an age-dependent manner during key stages of embryonic development when the body plan is being elaborated (Embryonic (E)Day 9.5 – 11.5). Both TLRs are prominently expressed in the developing alimentary system, with expression initially being localized to the hindgut at E9.5. As morphogenesis advances, the expression of both genes broaden and expression profiles were found to overlap during this period. By E11.5 both genes were expressed in the developing nervous system, in neuroanatomical regions such as the midbrain and midbrain-hindbrain boundary. Interestingly, a subunit of the Type 1 Interferon receptor, *lfnar1*, is also highly expressed during this period and was detected in the ectoderm and extra-embryonic membranes. This may indicate a role for TLR-driven interferons in regulating trophoblast proliferation, as has been demonstrated in bovine models.

Conclusions:

These data illustrate that innate immune genes are highly active in embryonic tissues and may have, as of yet, undescribed roles in mediating embryonic stem cell proliferation and tissue patterning that are independent of defence.

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PP 30 Barbara McGrogan (NCCP)

Towards a safer patient pathway for melanoma: review of referral, primary treatment and multidisciplinary management of suspicious pigmented lesions

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Background:

Melanoma is an aggressive cancer. Ireland's incidence and mortality are steadily increasing; 900 cases are diagnosed each year. Incidence will escalate; by 2040 it will increase by up to 327% in males and 175% in females (NCRI, 2014).

In 2010 the NCCP developed a GP guideline to assist GPs in the early recognition of suspicious pigmented lesions and to advise on evidence-based referral. Suspicious pigmented lesions should not be removed in the Primary Care setting. Patients should be referred, with the lesion intact for specialist management. The National GP referral form facilitates referral and is now developed electronically.

Objectives:

The NCCP conducted a survey of sixteen hospitals with dermatology/plastic surgery services to assess adequacy of the patient pathway and make recommendations to align care with evidence based practice.

Results & Discussion:

All hospitals surveyed have 'rapid access' systems for assessing suspicious pigmented lesions. Triage was influenced by the quality of GP referral information provided. The NCCP referral form assists GPs in documenting relevant clinical information.

Urgent cases are usually seen within two weeks. Lesions are removed at the first OPD visit. Overall 94% of hospitals remove urgent lesions within two weeks. MDM approach to diagnosis is well organised in some hospitals. Cancer centres and smaller hospitals collaborate to some extent.

Conclusions:

GPs should be encouraged to use the referral form and guidelines for appropriate triage and prioritisation of patients. National standardisation of the MDM process including central role for pathology departments and implementation of key performance indicators are needed.

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1. Cancer Registry of Ireland Cancer Projections for Ireland 2015-2040.
2. National Institute for Health and Clinical Excellence- Improving Outcomes for people with Skin Tumours including Melanoma, 2006.

PP 31 Gemma O'Connor (RCSI)

Bioengineered inhalable microparticles for the treatment of mycobacterium tuberculosis (MTB) infection

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Background:

Tuberculosis is a global health issue that urgently requires the development of new treatment modalities 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 micronutrients, for Mtb infection. In this study the immunomodulator, all-trans-retinoic acid (atRA) [1,2,3] is studied in comparison to the established anti-tubercular drug rifampicin as a possible inhalable treatment for Mtb infection.

Objectives:

Building on methods previously developed by our group whereby inhalable microparticles (MPs) were successfully engineered to target the alveolar macrophage and hence the site of Mtb infection [4,5], the aim of this study is to encapsulate rifampicin and atRA within biodegradable poly (lactide-co-glycolic acid) (PLGA 503H) microparticles. Once optimized, in vitro testing was carried out to assess the efficacy of the formulations as potential adjunctive treatments for Mtb infection.

Results & Discussion:

Drug-loaded MPs ranging from 2-3µm in size were successfully prepared with SEM images confirming size and morphology. Minimum drug loading of 62.25% ±5.8 (74.6±6.7µg/mg of MPs) and 11.13%±0.01 (16.7±2.04µg/mg MPs) was achieved in atRA- and rifampicin-MPs respectively. Preliminary in vitro efficacy testing showed a decrease in bacillary viability (CFU/ml) when THP-1 derived macrophages infected with Mtb (H37Ra) were treated with rifampicin and atRA at varying concentrations (2.5µg/ml - 20µg/ml).

Conclusions:

The results achieved to date, in addition to in vitro studies carried out previously by the group [4,5], warrants further exploration of inhalable drug-loaded microparticles as a potential adjunctive treatment for Mtb infection.

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PP 32 Christina Payne (RCSI)

Development of hydrogels for drug and cell delivery to the distal airways

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Background:

Current increases in the number of biomolecule and cell-based therapies has resulted in the need for novel delivery vectors to enable therapies to remain in damaged tissue and exert therapeutic benefit for extended periods. To address this need, a novel methylcellulose (MC), collagen and beta-glycerophosphate thermo-responsive hydrogel was formulated. The application of this hydrogel currently being explored is its utility in delivering therapeutic molecules to the distal airways, and all-trans Retinoic Acid is the signalling molecule which is being investigated [1]. Due to poor water solubility of atRA, solid lipid nanoparticles (SLNs) were prepared for encapsulation of the molecule, for subsequent loading into the hydrogel.

Objectives:

To formulate and characterise a thermoresponsive methylcellulose and collagen hydrogel; to assess the ability of the gel to sustain cell viability and act as a controlled-release drug depot; and to assess the efficacy of SLNs as the drug delivery system.

Results & Discussion:

Methylcellulose 2%w/v forms a thermoresponsive hydrogel in combination with collagen 0.1%w/v and beta-glycerophosphate 5.6%w/v, which gels at approx. 37°C, as determined by rheological analysis. Cell viability and proliferation studies showed the gel is capable of supporting rMSC survival and growth over 14 days. atRA-loaded SLNs were assessed for appropriate size and polydispersity index. Optimisation of this formulation resulted in Z-ave 352.24 nm, PDI 0.341, and ZP -4.15 mV.

Conclusions:

Results to date show that this gel has the potential to act as a delivery vector for drugs and cells, which could be delivered to the distal airways for therapeutic benefit.

References:

[1] McGowan S.E. Drug Discovery Today – Disease Mechanisms. 2006, 3(1): 77-84

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PP 33 Andrew Selfridge (UCD)

Suppression of hypoxia-induced HIF stabilisation by hypercapnia

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Introduction:

Hypoxia (low O₂) and hypercapnia (high CO₂) co-exist in a diverse range of pathophysiological states including cancers, respiratory conditions and during inflammation. Hypoxia inducible factor (HIF) is the master transcriptional regulator of the response to low oxygen. Despite the close cellular association between CO₂ and O₂, the potential impact of carbon dioxide on HIF is unclear. Recent studies have demonstrated the ability of carbon dioxide to regulate gene expression. The purpose of this work was to determine the effect of hypercapnia on the HIF pathway.

Methods:

Cells were exposed to normocapnic normoxia (5% CO₂; 21% O₂), normocapnic hypoxia (5% CO₂; 1% O₂) hypercapnic hypoxia (10 CO₂; 1% O₂) and treated with the pharmacological hypoxic mimetic dimethoxallyl glycine (DMOG; 1µM). Experiments were re-capitulated in a mouse model using normocapnia (0.03% CO₂; 21% O₂) and hypercapnia (10% CO₂; 21% O₂) with or without DMOG (8mg per mouse).

Results & Discussion:

Hypercapnia caused a suppression of both hypoxia- and DMOG-induced HIF-1α and HIF target proteins. However, increased CO₂ did not induce changes in HIF-1α mRNA expression. These results suggest that hypercapnia modulates HIF-1α at a post-translational level. HIF-dependent reporter activity was also reduced by increased CO₂ thus demonstrating a functional consequence for the suppression of HIF-1α protein. In vivo, hypercapnia was found to repress levels of the HIF target protein EPO.

Future directions and translation:

Upcoming studies will be aimed at determining the exact mechanism by which CO₂ mediates the hypercapnic suppression of HIF. HIF regulates the transcription of hundreds of genes which have angiogenic, glycolytic and inflammatory functions. Manipulating HIF with CO₂ presents a novel window of opportunity in exploiting the adaptive hypoxic response for therapeutic gain.

PP 34 David Walsh (RCSI)

Star shaped polypeptides as non-viral vectors to produce gene activated matrices for bone tissue engineering

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Background:

Approximately 2.2 million bone graft surgeries are performed each year making bone the 2nd most commonly transplanted tissue worldwide [1]. Collagen-based scaffolds have been shown to be a viable alternative [2, 3]. The aim of this project is to incorporate bio-inspired star shaped poly(lysine) (PLL) polypeptides [4] loaded with pro-osteogenic genes into these scaffolds to enhance regeneration at bone defect sites.

Objectives:

The main objective was to test the hypothesis that novel, star shaped PLL-polypeptides can condense and protect osteoinductive growth factors to facilitate the transfection of rat mesenchymal stem cells (rMSCs). Specifically, the aims included the determination of an optimal complexation method of PLL-polypeptides and pDNA followed by assessment of transfection ability. Materials and Methods: PLL-polypeptides were complexed with luciferase plasmid (pLuc) and characterised for polyplex size and zeta potential. rMSCs were transfected with PLL-pLuc polyplexes of varying N/P ratios to determine optimal in vitro transfection parameters.

Results & Discussion:

Optimal complexation conditions were found to be a time of five minutes, at room temperature with no agitation. PLL-polypeptides interact with pLuc to produce nanoparticles with a suitable size (~200nm) and charge to facilitate cellular transfection. Notably, transgene expression was obtained up to day 14, with luciferin expression of PLL01 at N/P ratios 5 & 2 surpassing that of the positive controls Polyethyleneimine and Superfect. PLL-polypeptides therefore represent a promising delivery vector for incorporation of osteogenic genes into a collagen scaffold to produce a gene activated matrix for enhanced healing at bone defect sites.

References:

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PP 35 Sarah Whelan (TCD)

Soluble CD1D and iNKT cells: an inverse relationship on hepatic malignancy

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Background:

Human invariant natural killer T (iNKT) lymphocytes are important anti-tumour cells characterised by the expression of an invariant T-cell receptor. iNKT cell numbers and their activity are decreased in livers from patients with hepatic malignancy [1]. iNKT cell function is restricted by the non-classical MHC-like molecule CD1D but little is known about CD1D in human liver.

Objectives:

We aimed to examine CD1D and iNKT cell numbers in liver tissue from transplant donors and patients undergoing resection for liver metastases.

Results & Discussion:

Bioinformatics analysis identified and mapped putative splicing events in the CD1D genomic region. RT-PCR was used to detect CD1D in human liver; we found evidence of several splice variants in metastatic liver leading us to hypothesise that a predicted soluble form could inhibit iNKT cells (n=15). qPCR revealed high levels of the novel soluble splice variant of CD1D in human metastatic liver in comparison to relatively low levels in donor liver tissue (Geometric mean relative expression in donor = 0.0003 and metastatic liver = 0.002313, $p < 0.0001$) (n=30). High levels of soluble CD1d was detected in serum from patients with colorectal cancer in comparison to low levels found in donor serum, (n=10). A qPCR method for detecting iNKT cells was developed, which showed that iNKT cells were significantly depleted in tumour-bearing liver in comparison to normal donor liver, (Geometric mean relative expression in donor = 0.0003 and metastatic liver = 0.001725, $p < 0.0001$) (n=10).

Conclusions:

We propose that soluble CD1d directly causes iNKT cell depletion, thus inhibiting their important anti-tumour activity.

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