



Molecular
Medicine
Ireland



Ireland's EU Structural Funds
Programmes 2007 - 2013

Co-funded by the Irish Government
and the European Union



HEA

Higher Education Authority
An tÚdarás um Ard-Oideachas

Clinician Scientist Fellowship Programme

Annual Meeting

11 July 2009



Clinician Scientist Fellowship Programme Annual Meeting

Date: 11 July 2009

Venue: IT125, 1st Floor, IT Building, NUI Galway

0845	Tea / Coffee
0915	Welcome and Opening Remarks Professor Terry Smith, Vice-President for Research, NUI Galway
Session 1	
Chair: Dr Finian O'Brien (RCSI)	
0930	Dr Gerard Curley (NUIG) <i>Investigation of the effects of Hypercapnic Acidosis, the role of NF-κB, and the therapeutic potential of gene and stem cell strategies to modulate NF-κB, activity during the repair profile following Ventilation induced Lung Injury</i>
0945	Dr Mazen Al-alawi (RCSI) <i>Effect of Lipoxin A₄ in Modifying the Airway Surface Liquid Layer</i>
1000	Dr Eoin Feeney (UCD) <i>Human and in vitro studies examining the early effects of antiretroviral drugs on mitochondrial DNA and genes regulating lipid metabolism</i>
1015	Dr Niall Conlon (TCD) <i>Immune variation in idiopathic bronchiectasis</i>
1030	Dr Aoife Lowery (NUIG) <i>Breast Cancer associated microRNAs - classification using expression profiling and artificial neural networks</i>
1045	Dr John O'Sullivan (UCC) <i>Cytokine Therapy for Myocardial Infarction</i>
1100	Tea / Coffee
Session 2	
Chair: Dr Oliver Schubert (RCSI)	
1115	Dr Jane McGrath (TCD) <i>Brain Function and Connectivity during Attention Orienting in Autism Spectrum Disorder</i>
1130	Dr Ruth Morrell (NUIG) <i>The Potential Therapeutic role of BH3 mimetics in overcoming Bcl2 resistance in Haematological Malignancies</i>
1145	Dr Patrick Collier (UCD) <i>Unravelling Cardiac fibrosis – is it initiated by activated endothelium, perpetuated by serum factors and unmasked by natriuretic peptides?</i>
1200	Dr James Ryan (UCC) <i>Cellular mechanisms of insulin resistance due to the r482w mutation of the Imna gene in familial partial lipodystrophy, dunnigan variety (fpld)</i>
1215	Dr David Prichard (TCD) <i>Ursodeoxycholic Acid – a Molecular Modulator of the Inflammation-Cancer Sequence in the Oesophagus?</i>
1230	Lunch including Poster Viewing
1315	Discussion of Structured Training
1345	Clinician Scientist Keynote Lecture Professor Sherine Gabriel (William J. and Charles H. Mayo Professor of Epidemiology and of Medicine, Mayo Clinic, MN, US) <i>Heart Disease and Premature Death in Rheumatoid Arthritis: Lessons from Epidemiology</i>

Session 3	
Chair: Dr Niall Conlon (TCD)	
1430	Dr Finian O'Brien (RCSI) <i>The Neurobiology of Psychogenic Non-Epileptic Seizures</i>
1445	Dr Fionnuala Ní Ainle (TCD) <i>The Anticoagulant Properties of Protamine are Enhanced by Activated Protein C</i>
1500	Dr Aonghus O'Loughlin (NUIG) <i>Novel Cell Based approaches in the treatment of diabetic foot ulcers</i>
1515	Dr Damian McCartan (RCSI) <i>HOXC11: a non steroidal mediator of endocrine resistance in breast cancer?</i>
1530	Tea / Coffee
Session 4	
Chair: Dr John O'Sullivan (UCC)	
1545	Dr Sanjay Chotirmall (RCSI) <i>Oestrogen in Cystic Fibrosis (CF): An old hormone up to new tricks?</i>
1600	Dr Aidan Ryan (UCD) <i>Novel fibro-suppressant activities of lipoxin A4 in renal cells</i>
1615	Dr Mark Coyne (NUIG) <i>Myeloma: Cell Cycle Dysregulation</i>
1630	Dr Oliver Schubert (RCSI) <i>Neuroproteomic Analysis of Schizophrenia and Disease-Associated Neuronal Signalling Defects</i>
1645	Closing Remarks Dr Ruth Barrington, Chief Executive, Molecular Medicine Ireland
1700	Wine reception and presentation of prizes

Judging Panel for Fellows' presentations

Chair: Professor Matthew Griffin (Professor of Transplant Biology, NUI Galway)

Dr Christine A. Dingivan (Chief Medical Officer, PPD, Inc.)

Professor Sherine Gabriel (William J. and Charles H. Mayo Professor of Medicine and Epidemiology, Mayo Clinic, MN, US)

Posters

Posters will be presented by the second intake of MMI Fellows.

Venue: IT Building Foyer, NUI Galway	
Dr Brian Walsh (UCC)	<i>BiHivE study; Biomarkers in hypoxic-ischaemic encephalopathy</i>
Dr Fergus McCarthy (UCC)	<i>The role of PPAR-γ in the pathogenesis of pre-eclampsia</i>
Dr Daniel Schmidt (UCC)	<i>Do the dynamics of quasispecies complexity and IP-10 concentration in chronic hepatitis C provide an opportunity to individualise treatment strategies?</i>

Clinician Scientist Fellowship Programme Annual Meeting

Date: 11 July 2009; Venue: NUI Galway

Abstracts

Dr Gerard Curley

Investigation of the effects of Hypercapnic Acidosis, the role of NF- κ B, and the therapeutic potential of gene and stem cell strategies to modulate NF- κ B, activity during the repair profile following Ventilation induced Lung Injury

Introduction: Mechanical ventilation may worsen ALI/ARDS, a process termed Ventilator Induced Lung Injury (VILI). Deliberately induced Hypercapnic Acidosis (HA) is protective in multiple lung injury models. However, HA may inhibit the host response to bacterial sepsis, and also retard the repair process and slow recovery following ALI/ARDS.

Aims and Objectives: To determine: (a) the effects of HA on the recovery profile following ventilation induced lung injury; (b) the role of the NF- κ B pathway in mediating these effects of HA, and the potential for gene and stem cell therapies to directly modulate the NF- κ B pathway to enhance recovery following VILI.

Dr Mazen Al-Alawi

Effect of Lipoxin A₄ in Modifying the Airway Surface Liquid Layer

A key aspect of the lung's innate defence system is the ability of the epithelium to regulate the Airway Surface Liquid (ASL) volume. The transporters involved in this ion transport are required for effective ciliary beating and muco-ciliary clearance quality. Lipoxin A₄ (LXA₄) is an endogenous anti-inflammatory molecule produced from arachidonic acid. LXA₄ has been reported to be reduced in inflammatory lung disease such as cystic fibrosis (CF). One of the therapeutic avenues in CF is to explore a way to inhibit the Na⁺ hyper-absorption in the lung. Our aim is to investigate the use of LXA₄ as a complimentary therapy in inflammatory lung disease.

Dr Eoin Feeney

Human and in vitro studies examining the early effects of antiretroviral drugs on mitochondrial DNA and genes regulating lipid metabolism

Nucleoside reverse transcriptase inhibitors (NRTIs) are postulated to cause toxicity through mitochondrial DNA (mtDNA) polymerase- γ inhibition, leading to mtDNA depletion and premature chain termination. We examine the effects of abacavir, a NRTI associated with an excess risk of myocardial infarction and alterations in platelet activity, on platelet mtDNA quantity and quality, compared to platelets from HIV-negative controls and HIV-positive patients on other antiviral therapy. We also examined the effects of ddI and d4T, two NRTIs with known mitochondrial toxicities, on mtDNA in PBMCs from HIV-positive patients with lactic acidosis or symptomatic hyperlactemia on antiretroviral therapy in a nested case-control study.

Dr Niall Conlon

Immune Variation in Idiopathic Bronchiectasis

Niall Conlon, Con Feighery, Mary Keogan, John Jackson

Clinical, serological and genetic findings in an initial cohort of patients with bronchiectasis will be discussed. Evidence of an association between a functional polymorphism in CD32 and bronchiectasis will be presented. Data on the genotype of mannose binding lectin, its correlation with functional and total serum levels and with clinical state will also be presented. We will show preliminary data from a novel flow cytometric opsonophagocytic assay and discuss plans for its use in evaluation of the immune system in bronchiectasis.

Dr Aoife Lowery

Breast Cancer associated microRNAs - classification using expression profiling and artificial neural networks

The search for novel biomarkers to improve prognosis and guide individualised therapy in breast cancer is ongoing, and increasingly complex due to the heterogeneous nature of the disease. MicroRNAs are naturally occurring RNA molecules that play important regulatory roles in plants and animals by targeting mRNAs for cleavage or translational repression. Characteristically, miRNAs are noncoding, single-stranded short (18-22 nucleotides) RNAs.

Accumulating evidence indicates that miRNAs play a pivotal role in many cellular functions via regulation of gene expression. Furthermore, their deregulation has been demonstrated in carcinogenesis, where they have been shown to function as oncogenes or tumour suppressors. The emergence of miRNAs as regulators of gene expression identifies them as obvious candidates for the novel diagnostic and prognostic indicators and therapeutic targets in breast cancer. The primary objectives of this study are to identify miRNAs that are dysregulated in breast cancer, and elucidate their precise function or contribution to the carcinogenic process. These microRNAs will potentially be subsequently developed/utilised as prognostic biomarkers or as therapeutic targets themselves.

Achieving this objective will lead to improved classification of breast tumours, and a more refined set of criteria for prognostication. Improved prognostic tools will enable better selection of patients for adjuvant chemotherapy and thereby reduce the number of patients exposed to short and long term side effects of unnecessary cytotoxic treatments. This positive outcome would also result in a reduced economic burden of breast cancer on society by sparing some patients unnecessary therapy and in selecting others for more appropriate targeted intervention. The identification of specific microRNAs as potential therapeutic targets for breast cancer therapy will also accelerate the use of targeted/tailored therapy for breast cancer patients.

Dr John O'Sullivan

Cytokine Therapy for Myocardial Infarction

There has been much interest lately in the putative benefit of progenitor cells in improving myocardial function post infarction. The degree of benefit deriving from cell therapy cannot be agreed upon, but it has been unanimously small. Even less well established is the mechanism of benefit. There have been a number of cell therapy studies which have indirectly implicated the potential of constituent medium and associated factors. We are taking this one step further; we are delivering these factors in a dose-dependent manner in a large animal model of myocardial infarction to determine the optimal dose, and delivery system.

Dr Jane McGrath

Brain Function and Connectivity during Attention Orienting in Autism Spectrum Disorder

Background: Orienting attention to new and important sources of information is crucial for learning and normal socio-emotional development. Difficulties in attention orienting may contribute to the development of a number of core features of Autism Spectrum Disorder (ASD).

Objectives: To compare brain activation and functional connectivity during attention orienting in individuals with ASD and controls.

Methods: Participants with ASD and controls will perform an attention orienting task during functional MRI. Regions of brain activation and functional connectivity will be compared between cases and controls.

Results: Preliminary results are in keeping with the research that suggests that two frontoparietal networks are involved in attention orienting.

Conclusions: We expect to see differences in brain activation and functional connectivity between cases and controls during fMRI.

Dr Ruth Morrell

The Potential Therapeutic role of BH3 mimetics in overcoming Bcl2 resistance in Haematological Malignancies

In cancer, cell survival is a delicate balance regulated through the complex interplay between different members of the Bcl-2 family of proteins. These include: (1) anti-apoptotic family members (2) multi-domain - and (3) BH3-only proapoptotic family members. Impairment of the cells' ability to undergo apoptosis as determined by the balance of these proteins has been correlated with poor overall survival in a variety of different haematological malignancies and increased expression of anti-apoptotic Bcl-2 family members contributes to resistance to a variety of apoptotic stimuli, including chemotherapy, radiation and death receptor ligands .

Therapeutic strategies targeting Bcl-2 family members represent a promising prospect for treating haematological malignancies. Recently BH3 mimetics such as ABT-737 and Obatoclax have been developed, which induce apoptosis by direct inhibition of anti apoptotic molecules with potent single agent activity in lymphoid and myeloid cell lines.

The principal aim of this project is to explore the relative contribution of the Bcl-2 pathway to drug resistance in haematological malignancies (focusing mainly on myeloid malignancies) and the potential therapeutic role of BH3 mimetics alone and in combination with standard chemotherapeutic agents in both in vitro and in xenograft animal models.

Dr Patrick Collier

Unravelling Cardiac fibrosis – is it initiated by activated endothelium, perpetuated by serum factors and unmasked by natriuretic peptides?

A key goal of secondary preventative strategies within cardiovascular health is the prevention of adverse cardiac remodelling such as the development of *myocardial interstitial fibrosis (MIF)*. This serpiginous accumulation of collagen around cardiomyocytes determines both myocardial stiffness and arrhythmogenic risk and as such, represents an adverse marker of prognosis that correlates with disease progression.

This work examines the hypothesis that endothelial activation underlies the onset of this fibrotic process and that serum factors may be responsible for perpetuating pro-fibrotic pathways. Candidate biomarkers of MIF including natriuretic peptides are investigated while the precise collagen subtypes that predominate in MIF are further characterised.

Dr James Ryan

*Cellular mechanisms of insulin resistance due to the r482w mutation of the *lmna* gene in familial partial lipodystrophy, dunnigan variety (*fpld*)*

FPLD is a rare monogenic cause of insulin resistance arising from mutations of the LMNA or PPAR γ genes.

Two adolescents with a severe phenotype of FPLD presented with secondary amenorrhoea. Initial treatment with metformin was switched to rosiglitazone. Menses returned post treatment change. The improvement in insulin resistance scores on metformin was maintained on rosiglitazone. A large increase in cervical adipose tissue was noted in both patients.

We compared adipocyte differentiation of 3T3-L1 pre-adipocytes over-expressing wild type LMNA and R482W mutated LMNA with controls. Oil-Red-O staining showed less triglyceride accumulation in cells over-expressing mutant LMNA compared with wild-type, which in turn had less triglyceride than controls.

PPAR γ expression is inhibited by over-expression of both wild type LMNA and the R482W mutant. The increase in cervical adipose tissue may be due to rosiglitazone restoring the ability of neck adipocytes to store triglycerides.

Dr David Prichard

Ursodeoxycholic Acid – a Molecular Modulator of the Inflammation-Cancer Sequence in the Oesophagus?

Barrett's Oesophagus arises most frequently in the setting of mixed type gastro-oesophageal reflux disease. This condition is thought to occur as a result of chronic repetitive exposure of the oesophagus to inflammatory and tumour promoting compounds including low pH and bile acids. Ursodeoxycholic acid (UDCA) has been shown to interact with the glucocorticoid receptor (GR) in a fashion that is thought to promote transrepressive genomic effects rather than transactivational ones. It potentially offers an anti-inflammatory molecule with a minimal side effect profile. We are characterising the interaction of UDCA and the GR in oesophageal cells lines using High Content Analysis, RT-PCR and Western blot analysis.

Dr Finian O'Brien

The Neurobiology of Psychogenic Non-Epileptic Seizures

20-25% of people with epilepsy have psychogenic non-epileptic seizures (PNES), seizures that occur without discernibly abnormal electrical activity in the brain.

Several studies have suggested that people with PNES have a history of exposure to adverse environmental stressors and have specific difficulties in recognising and verbalising emotions, both of which can be associated with alterations in brain structure. Despite this, no study has investigated brain structure and function in people with PNES.

I will recruit 30 people with PNES and 30 matched controls and examine their brain structure using structural MRI and their emotional processing function using functional MRI .

Dr Fionnuala Ni Ainle

The Anticoagulant Properties of Protamine are Enhanced by Activated Protein C

Protamine sulphate is a cationic polypeptide used widely in the reversal of systemic heparinization. Paradoxically, protamine also possesses intrinsic anticoagulant properties. The molecular mechanism underlying the adverse anticoagulant activity of protamine has not been characterised.

In this study, protamine exerted anticoagulant properties in both *in vitro* and *ex vivo* coagulation assays and prolonged tail bleeding time following protamine administration to BALB/c mice. The anticoagulant properties of protamine were mediated by inhibition of factor V activation. Consequently, the anticoagulant activity of activated protein C in plasma was also significantly enhanced in the presence of protamine. In conclusion, excess protamine prevents blood clotting by inhibiting the generation of a crucial cofactor for clot formation. These results are of both scientific interest and also of direct clinical significance.

Dr Aonghus O'Loughlin

Novel Cell Based approaches in the treatment of diabetic foot ulcers.

Diabetic foot ulceration is a leading cause of foot amputation, and increased morbidity and mortality in people with diabetes mellitus. A collagen gel is an effective scaffold for localised delivery of peripheral blood mononuclear cells and circulating angiogenic cells. Calcein and resazurin viability assays indicate that peripheral blood mononuclear cells and circulating angiogenic cells are viable at 24hrs on a collagen scaffold. The addition of fibronectin increases metabolic activity. Scanning electron and confocal microscopy indicate cells are distributed throughout, and interact with the collagen scaffold. *In vivo* autologous treatments of the diabetic rabbit ear ulcer model will determine therapeutic efficacy of cell delivery to a diabetic wound with a collagen scaffold.

Dr Damian McCartan

HOXc11: a non steroidal mediator of endocrine resistance in breast cancer?

The nuclear receptor coactivator, SRC-1 is an important mediator of endocrine resistance in breast cancer. We identified the homeobox protein HOXc11 as interacting with SRC-1 in endocrine resistant breast cancer cells. We aimed to identify potential HOXc11/SRC-1 target genes and assess their clinical significance.

Co-immunoprecipitation assays confirmed SRC-1/HOXc11 interaction after tamoxifen treatment in endocrine resistant LY2 cells. ChIP assays showed recruitment of HOXc11, ER α and SRC-1 to the S100 β promoter after treatment with tamoxifen. S100 β is a secreted protein. Using an ELISA we found elevated serum levels in 20% of breast cancer patients, pre-treatment, when compared to age matched controls.

Dr Sanjay Chotirmall

Oestrogen in Cystic Fibrosis (CF): An old hormone up to new tricks?

Chotirmall SH, Greene CM, Harvey BJ, McElvaney NG
Respiratory Research Division, RCSI, Dublin 9

Cystic Fibrosis (CF) results from dysfunctional cystic fibrosis transmembrane conductance regulator (CFTR) function. The Irish population has both the highest incidence (2.98/10,000 individuals) and carrier rate (1 in 19) worldwide. An established gender gap, yet unexplained exists in CF – females have more aggressive disease, worse lung function and earlier colonization with *Pseudomonas spp* all contributing to earlier mortality. Little is known about effects of oestrogen (E2) within the CF lung and this work investigates the inflammatory effects of E2 exposure on CF airway epithelium.

ER- β is the major oestrogen receptor expressed by CF airway epithelial cells and is proportionally higher in tracheal versus bronchial cells. This contrasts non-CF airway epithelial cells (ER-alpha>beta). At physiological concentrations, E2 (1-10nM) can upregulate TLR 1, 3 and 4 surface expression on CF airway epithelial cells but shows a dose-dependent inhibition of IL-8 secretion in both CF and non-CF airway epithelial cells in response to TLR 1-6 & 9 agonists.

Dr Aidan Ryan

Novel fibro-suppressant activities of lipoxin A4 in renal cells

Ryan A Murphy M Borgeson E, Holthofer K, Docherty NG, Sadlier D, Godson C.

UCD Diabetes Research Centre, UCD Conway Institute and School of Medicine and Medical Sciences,
University College Dublin, Belfield, Dublin 4, Ireland

Tubulo-interstitial fibrosis is the final common pathway in chronic kidney disease (CKD) leading to end-stage kidney disease and involves recruitment of circulating fibrocytes, activation and proliferation of resident renal fibroblasts and transition of epithelial cells to a mesenchymal phenotype. Here we have explored whether LXA₄ (1 nM) might impact on TGF- β 1 (10ng/ml) driven renal fibroblast proliferation, activation and extracellular matrix production. LXA₄ (1nM) modulated TGF- β (10ng/ml) driven fibroblast proliferation and post-receptor signaling events including Smad-2 phosphorylation, alpha-smooth muscle actin, CTGF and thrombospondin-1 expression. In aggregate our data suggest that LXA₄ may have potential as novel fibro-suppressants agents in vitro.

This work is supported by Science Foundation Ireland, The Wellcome Trust, Molecular Medicine Ireland, EISOSANOK consortium The Health Research Board.

Dr Mark Coyne

Myeloma: Cell Cycle Dysregulation

Myeloma remains an incurable cancer and the development of new therapeutic approaches is of fundamental importance. Myeloma is characterised by altered cell cycle control. CDC7 is an essential kinase required for initiation of DNA replication and cell cycle progression. PHA767491 is the prototype of Cdc7 kinases inhibitors. A key feature of these compounds is their capability of reducing the expression of an important anti-apoptotic Bcl2 family member protein Mcl1, which has been shown to cause resistance to the drugs currently used to treat myeloma. This presentation will illustrate ongoing work supporting CDC7 as a potential novel target in treating myeloma.

Dr Oliver Schubert

Neuroproteomic Analysis of Schizophrenia and Disease-Associated Neuronal Signalling Defects

Supervisors: Prof. David Cotter, Prof. Jochen Prehn

Structural and functional changes of the hippocampus in psychotic disorders have been demonstrated in numerous studies. However, underlying molecular mechanisms are not well understood.

Using post-mortem brain samples, we applied the Difference In-Gel Electrophoresis method (DIGE) to analyze the expression of basic proteins (PI range pH 6-11) in schizophrenia and bipolar affective disorder. Homogenates of whole hippocampus were derived from a well matched sub-sample of the Stanley Brain Collection which consisted of 10 subjects with schizophrenia, bipolar affective disorder, and control cases, respectively. Analysis revealed differential expression of 23 proteins in schizophrenia and 17 proteins in bipolar affective disorder. 8 of these proteins were altered in both disorders. Proteins are currently identified using mass spectrometry, and subjected to advanced pathway analysis to reveal associated biological networks and global functions.

The approach taken may yield novel biomarker proteins and drug targets for diagnosis and treatment of psychotic disorders.

Poster Abstracts

Dr Brian Walsh

BiHivE study; Biomarkers in hypoxic-ischaemic encephalopathy

Aim: To develop a combined early predictive biomarker based algorithm for Hypoxic Ischaemic Encephalopathy severity, and validate it with early EEG monitoring.

Infants born with signs of asphyxia will be included. A matched control population will be recruited from the BASELINE birth cohort. Umbilical cord blood and urine samples will be collected and stored from these infants, and all test infants will have an EEG. The sample assays will be analysed to measure levels of IL-6, protein S100B, isoprostanes and neuregulin. Univariate tests, followed by linear and non-linear multivariate modelling will be applied to develop a combined predictive algorithm.

Dr Fergus McCarthy

The role of PPAR- γ in the pathogenesis of pre-eclampsia

Pre-eclampsia (PE), a multisystemic disorder of pregnancy remains a leading cause of maternal and perinatal morbidity and mortality. The development of an inadequate uteroplacental perfusion system resulting in placental ischaemia and activation and dysfunction of the maternal vascular endothelium have emerged as key events in the pathogenesis of PE.

Peroxisome proliferator activated receptor- γ (PPAR- γ) plays a seminal role in the development of healthy pregnancies. Activation of this receptor has been shown to antagonize a number of the hallmark pathophysiological events associated with PE. The aim of this project is to investigate PPAR- γ activation as a novel therapeutic intervention for PE.

Dr Daniel Schmidt

Do the dynamics of quasispecies complexity and IP-10 concentration in chronic hepatitis C provide an opportunity to individualise treatment strategies?

Authors: Schmidt D, Crosbie O, Fanning LJ, Kenny-Walsh E

Variations in HCV quasispecies complexity [QSC] may identify windows of improved sensitivity to treatment. Interferon gamma inducible protein [IP-10] levels could help identify likely treatment responders. Changes in early treatment viral load have shown potential in predicting treatment responders. We will measure QSC, IP-10 over four months before treatment and viral kinetics in the first 4 weeks of treatment. We plan to use them to create a mathematical model to identify treatment responders.