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Clinician Scientist Fellowship Programme

Annual Meeting - Friday 25 June 2010



MMI Clinician Scientist Fellowship Programme: Annual Meeting

Date: Friday 25 June 2010

Venue: G04, Brookfield Health Sciences Complex, University College Cork

0845	Tea / Coffee
0915	Welcome and Opening Remarks Dr Michael Murphy, President, UCC
Session 1	
Chair: Dr Damian McCartan (RCSI)	
0930	Dr John O'Sullivan (UCC) Low Dose Insulin-like Growth Factor-1: Potent Early and Late Benefits post MI
0945	Dr Sanjay Chotirmall (RCSI) 17 β -Estradiol Inhibits IL-8 In Cystic Fibrosis By Up-Regulating Secretory Leucoprotease Inhibitor
1000	Dr Jane McGrath (TCD) Neuroimaging in Autism Spectrum Disorders
1015	Dr Aonghus O'Loughlin (NUI Galway) Cell Therapy for Diabetic Foot Ulcers
1030	Dr Eoin Feeney (UCD) Mitochondrial Dysfunction in HIV Lipodystrophy – beyond Mitochondrial DNA?
1045	Dr Fergus McCarthy (UCC) The Role of Peroxisome Proliferator Activated Receptor Gamma in Normal Pregnancy and an Animal Model of Pre-Eclampsia
1100	Tea / Coffee
Session 2	
Chair: Dr Fergus McCarthy (UCC)	
1115	Dr Fionnuala Ní Áinle (TCD) Activated Protein C N-Linked Glycans Regulate Cytoprotective Signaling Function on Endothelial Cells
1130	Dr Mark Coyne (NUI Galway) Targeting Cdc7 kinase in Multiple Myeloma
1145	Dr Finian O'Brien (RCSI) The Neurobiology of Psychogenic Non-epileptic Seizures
1200	Dr Brian Walsh (UCC) Biomarkers of Neonatal Hypoxic Ischaemic Encephalopathy
1215	Lunch
1315	Group photographs
1330	Clinician Scientist Keynote Lecture Professor David H. Dockrell (Wellcome Senior Clinical Fellow, University of Sheffield) Macrophage Cell Death is Tightly Coupled with the Performance of Microbicidal Function during Innate Immune Responses

Session 3	
Chair: Dr Fionnuala Ní Áinle (TCD)	
1430	Dr Gerard Curley (NUI Galway) Evaluating Strategies for Repair from Ventilator Induced Lung Injury
1445	Dr Damian McCartan (RCSI) Identifying New Transcriptional Targets for SRC-1 in Breast Cancer
1500	Dr Niall Conlon (TCD) Fcyrii Polymorphisms in Bronchiectasis: Functional Consequences
1515	Dr Ruth Morrell (NUI Galway) The Potential Therapeutic Role of BH3 Mimetics in Overcoming Bone Marrow Microenvironment-Mediated Resistance of Acute Myeloid Leukaemia to Chemotherapy
1530	Dr Daniel Schmidt (UCC) Do the Dynamics of Quasispecies Complexity and Ip-10 Concentration in Chronic Hepatitis C Provide an Opportunity to Individualise Treatment Strategies?
1545	Tea / Coffee
Session 4	
Chair: Dr Gerard Curley (NUI Galway)	
1600	Dr Mazen Al-Alawi (RCSI) Lipoxin A ₄ Increases Airway Surface Liquid height in Cystic Fibrosis Bronchial Epithelia by Stimulating ATP Release, Increasing Cl ⁻ Secretion and Inhibiting Na ⁺ Absorption
1615	Dr Patrick Collier (UCD) Getting to The Heart of Cardiac Remodelling; How Collagen Subtypes may contribute to Phenotype
1630	Dr David Prichard (TCD) Ursodeoxycholic Acid – A Molecular Modulator of the Inflammation Carcinoma Sequence in the Oesophagus?
1645	Dr James Ryan (UCC) Cellular Mechanisms underlying Familial Partial Lipodystrophy, Dunnigan Variety (FPLD)
1700	Dr Nuala Healy (NUI Galway) Detection and Quantification of Systemically Expressed Cancer-Specific miRNAs
1715	Closing Remarks Dr Ruth Barrington, Chief Executive, Molecular Medicine Ireland
1730	Presentation of prizes



Abstracts

Dr John O'Sullivan (UCC)

Low dose insulin-like growth factor-1: potent early and late benefits post MI

Using low-dose insulin-like growth factor-1 (600 pg in total), we demonstrate a significant increase in IGF-1 receptor phosphorylation, pro-survival AKT phosphorylation, without a significant increase in insulin receptor phosphorylation, and significant reduction of apoptosis by TUNEL and Caspase 9 activity in the infarct border zone at 24 hours compared to control. At 8 weeks, we show significant improvement in LV function, infarct size reduction, indices of infarct remodelling, indices of global remodelling, ejection fraction, and regional wall motion and thickening after early single-dose administration of low dose IGF-1.

We conclude that LD-IGF-1 is a safe, easily-administered, potent cardiac regenerative agent.

Dr Sanjay Chotirmall (RCSI)

17 β -Estradiol Inhibits IL-8 In Cystic Fibrosis By Up-Regulating Secretory Leucoprotease Inhibitor

Sanjay H. Chotirmall¹, Catherine M. Greene¹, Irene K. Oglesby¹, Warren Thomas², Shane J. O'Neill¹, Brian J. Harvey² & Noel G. McElvaney¹

¹Respiratory Research Division, Department of Medicine & ²Department of Molecular Medicine, Royal College of Surgeons in Ireland

An unexplained 'gender gap' is observed in cystic fibrosis (CF). We evaluated the effect of 17 β -estradiol (E2) on CF bronchial epithelial cells in vitro and vivo. We found that at physiological concentrations E2 inhibits IL-8 release in response to toll-like receptor (TLR) agonists via ER β in CF bronchial epithelial cells through an up-regulation and nuclear localization of secretory leucoprotease inhibitor (SLPI). This causes an inhibition of NF- κ B activity and subsequent IL-8 release. These data implicate a novel anti-inflammatory mechanism for E2 in females with CF, which predisposes to infection and colonization. This could in part, account for the observed gender dichotomy in CF.

Reference

Chotirmall SH, Greene CM, Oglesby IK, Thomas W, O'Neill SJ, Harvey BJ, McElvaney NG. 17 β -Estradiol Inhibits IL-8 in Cystic Fibrosis by Up-Regulating Secretory Leucoprotease Inhibitor. *Am J Respir Crit Care Med*. 2010 Epub ahead of print 8 Apr.

Dr Jane McGrath (TCD)

Neuroimaging in Autism Spectrum Disorders

Autism and autistic spectrum disorders (ASDs) are devastating neurodevelopmental disorders of childhood, of unknown aetiology, characterised by deficits in social interaction and communication and restricted, repetitive patterns of behaviours, interest and activities.

Neuroimaging research has demonstrated numerous abnormalities in brain structure and function in autism, from which a theory of altered cortical connectivity in autism has emerged. This holds that core social and cognitive deficits in autism are underpinned by abnormal interregional brain connectivity. Functional and structural evidence for this theory is accumulating.

Diffusion Tensor Imaging (DTI) is a non-invasive magnetic resonance imaging (MRI) method for assessing the characteristics of tissue microstructure. DTI allows the measurement of the restricted diffusion of water molecules in tissue in order to produce neural tract images. DTI "tractography" is an analytic process using fibre-tracking software on DTI data that permits investigators to isolate, visualise, and measure volume and microstructural integrity of specific neural tracts. Using these advanced neuroimaging techniques, we can investigate structural connectivity in the brains of individuals with ASD.



Dr Aonghus O'Loughlin (NUI Galway)

Cell Therapy for Diabetic Foot Ulcers

In humans, diabetic foot ulceration can result in amputation. Peripheral vascular disease contributes to non-healing of diabetic foot ulcers. Endothelial progenitor cells (EPCs) promote angiogenesis. Type 1 collagen is a functional biomaterial used in wound repair. A cell-seeded collagen scaffold treatment was developed. A collagen scaffold is effective for delivery of EPCs to wounds with cells demonstrating metabolic activity and interactions with the scaffold. Autologous topical treatment with EPCs seeded in a collagen scaffold was assessed in an animal model of diabetic wound healing. Early results show treatment efficacy in diabetic wound healing.

Dr Eoin Feeney (UCD)

Mitochondrial Dysfunction in HIV Lipodystrophy – beyond Mitochondrial DNA?

HIV lipodystrophy (HIVLD) is associated with exposure to thymidine nucleoside reverse transcriptase inhibitors (tNRTIs) and is manifested by subcutaneous fat loss, visceral fat gain, dyslipidaemia and insulin resistance. The most widely accepted theory is that tNRTIs inhibit DNA polymerase gamma leading to depletion of mitochondrial DNA (mtDNA) and mitochondrial dysfunction. Through clinical samples from 3 international trials of antiretroviral agents, along with exposure experiments in primary adipose tissue culture, we are examining the dynamics of mitochondrial dysfunction in adipose tissue and how other effects of tNRTIs may play a role beyond that of DNA polymerase gamma inhibition.

Dr Fergus McCarthy (UCC)

The Role of Peroxisome Proliferator Activated Receptor Gamma in Normal Pregnancy and an Animal Model of Pre-Eclampsia

F.P. McCarthy¹, S.K. Walsh², E.J. Johns³, L.C. Kenny¹

¹ Anu Research Centre, Department of Obstetrics & Gynaecology, University College Cork, Cork, Ireland.

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³ Department of Physiology, University College Cork, Cork, Ireland

Peroxisome proliferator-activated receptor- γ (PPAR- γ), a nuclear receptor expressed in placental tissue plays a seminal role in pregnancy. We aimed to 1) investigate the effect of PPAR- γ antagonism during uncomplicated pregnancy in rats 2) investigate the role of PPAR- γ activation during complicated pregnancy using the reduced uterine perfusion pressure (RUPP) rat model of pre-eclampsia (PE).

We demonstrated that PPAR- γ antagonism results in a 'pre-eclamptic' type model characterised by hypertension, endothelial dysfunction and restricted fetal growth. PPAR- γ activation in the RUPP rat reverses hypertension and endothelial dysfunction. PPAR- γ agonists may provide a potential therapeutic intervention in the treatment of PE.

Dr Fionnuala Ní Áinle (TCD)

Activated Protein C N-Linked Glycans Regulate Cytoprotective Signaling Function on Endothelial Cells

Activated protein C (APC) has potent anticoagulant and anti-inflammatory properties that limit clot formation, inhibit apoptosis and protect vascular endothelial cell barrier integrity. Administration of APC improves survival in severe sepsis however its use may be complicated by bleeding. In this study, the role of N-linked glycans in modulating APC endothelial cytoprotective signaling via endothelial cell protein C receptor (EPCR)/protease activated receptor 1 (PAR1) was investigated. Enzymatic digestion of APC N-linked glycans (PNG-APC) decreased the APC concentration required to achieve half-maximal inhibition of thrombin-induced endothelial cell barrier permeability by 5-fold. Furthermore, PNG-APC exhibited increased protection against staurosporine-induced endothelial cell apoptosis compared to untreated APC. To investigate the specific N-linked glycans



responsible, recombinant APC variants were generated in which each N-linked glycan attachment site was substituted. Of these, *APC-N329Q* was found to be up to 5-fold more efficient in protecting the endothelial barrier function. Based on these findings, it was possible to generate an APC variant (*APC-L38D/N329Q*) with minimal anticoagulant activity, but 5-fold enhanced endothelial barrier protective function compared to wild type APC. These data provide novel insight into the critical role of APC N-linked glycosylation in modulating EPCR-dependent cytoprotective signaling via PAR1 and suggest that plasma β -protein C, characterized by aberrant N-linked glycosylation at Asn-329, may be particularly important for maintenance of APC cytoprotective functions *in vivo*. These findings are of also of direct clinical relevance as a potential means of enhancing the therapeutic benefit of APC.

Dr Mark Coyne (NUI Galway)

Targeting Cdc7 kinase in Multiple Myeloma

Coyne MRE 1,2, Naughton S2, Hayden PJ2, O'Dwyer ME2 and Santocanale C1 –
1Department of Molecular Medicine, National University of Ireland, Galway, Ireland
2Department of Haematology, Galway University Hospitals, Galway, Ireland.

Myeloma remains an incurable disease. A key feature of progressive myeloma is deregulation of the cell cycle.

Cell division cycle 7 kinase (Cdc7) is an essential protein for the initiation of DNA replication and for cell cycle progression.

This work studies the effects of the prototype Cdc7 protein kinase inhibitor, PHA-767491, in myeloma cells.

Moreover, this presentation will focus on examining the effects of the inhibitor in combination with standards of care and in different microenvironment models. In addition it will expand on new novel roles of Cdc7 evolving directly out of this work currently under investigation.

Dr Finian O'Brien (RCSI)

The Neurobiology of Psychogenic Non-epileptic Seizures

Psychogenic non-epileptic seizures (PNES) are episodes of altered movement, sensation or experience resembling epileptic seizures, but associated with patho-physiological processes and not with ictal discharges in the brain. This condition is classified as a conversion disorder and is present in 20-25% of patients who present to neurology services with epilepsy. People with PNES have a history of high rates of psychopathology, emotional dysregulation and neurocognitive impairment. Although such impairments are associated with brain structural and functional abnormalities, no previous study has examined quantitative brain structure or function in this patient group.

We recruited 20 patients with PNES without co-morbid epilepsy or other neurological condition and 20 healthy controls matched for age, IQ, gender and handedness.

Each participant underwent psychometric & neuropsychological assessment and brain scanning using structural MRI, diffusion tensor imaging and functional MRI. The results of these investigations will be presented and implications for future research and treatment discussed.

Dr Brian Walsh (UCC)

Biomarkers of Neonatal Hypoxic Ischaemic Encephalopathy

Hypoxic ischaemic encephalopathy (HIE) is among the commonest causes of neonatal mortality and morbidity. Until recently no treatment was available, but with the advent of therapeutic hypothermia this is changing. Hypothermia is used for those with moderate to severe HIE, and to be effective must be initiated in the first 5.5 hours of life. Clinically it is difficult to grade HIE within this period, and clinicians are dependent on EEG to identify infants. EEG is sensitive in experienced hands, but is user dependent, and rarely available. This PhD aims to develop a serum biomarker, to predict HIE severity.

Clinician Scientist Keynote Lecture

Professor David H. Dockrell (Wellcome Senior Clinical Fellow, University of Sheffield)

Macrophage Cell Death is Tightly Coupled with the Performance of Microbicidal Function during Innate Immune Responses

Macrophages are critical effectors of innate immunity. They possess a significant but finite capacity to kill ingested microorganisms. The microbicidal capacity of macrophages is dependent on the differentiation dependent competence of the phagolysosomal system. When the intrinsic capacity of macrophages for microbial killing is reached the phagolysosomes trigger a mitochondrial death pathway which aids microbial killing. Analysis of the macrophage proteome and the investigation of genetically modified mice has informed our understanding of this novel death pathway which couples microbial killing to macrophage cell death to maximise host defense and minimise the associated inflammatory cost.

Dr Gerard Curley (NUI Galway)

Evaluating Strategies for Repair from Ventilator Induced Lung Injury

G Curley, B Higgins, D O'Toole, L Kevin, JG Laffey

Mechanical ventilation, a supportive therapy necessary to sustain life in many cases of Acute Lung Injury (ALI), may contribute to and worsen ALI, termed Ventilator Induced Lung Injury (VILI). Strategies to lessen VILI improve mortality in patients with ALI.

Bone-marrow derived mesenchymal stem cells (MSCs) have demonstrated promise for the treatment of (ALI). MSCs reduced pulmonary oedema and pro-inflammatory cytokines, and improved survival in murine E. coli induced ALI (1) and systemic sepsis (2).

We wish to evaluate the potential for MSCs to modulate inflammation and enhance repair after Ventilator Induced Lung Injury (VILI).

1. Gupta, *J Immunol*, 2007, 2. Jianguo Xu *Am J Physiol Lung Cell Mol Physiol*, 2007.

Dr Damian McCartan (RCSI)

Identifying New Transcriptional Targets for SRC-1 in Breast Cancer

Endocrine Oncology Research Group, RCSI

SRC-1 is an important mediator of breast cancer metastasis as a transcription factor coactivator. This work adopted a genome-wide approach to identify SRC-1 regulated target genes.

SRC-1 ChIP sequencing and RNAi knockdown of SRC-1 with subsequent whole genome cDNA expression array analysis were used to identify novel SRC-1 target genes.

186 direct SRC-1 target genes were identified with peaks in the promoter from ChIP sequencing and significant down regulation in SRC-1 knockout cells.

This genome-wide approach has identified a variety of direct SRC-1 target genes. Molecular and translational studies will elucidate their putative role in SRC-1 mediated breast cancer metastasis.

Dr Niall Conlon (TCD)

FcγRIIA Polymorphisms in Bronchiectasis: Functional Consequences

FcγRIIA is an important receptor for IgG2, a key receptor in the immune response against encapsulated bacteria. A common single nucleotide polymorphism of FcγRIIA, H131R, has an impact on the ability of the receptor to bind IgG2. We have identified an increased prevalence of the functionally defective R131 variant in a population of patients with bronchiectasis. We used flow cytometric analysis of phagocytosis of pneumococcal polysaccharide coated beads and labeled pneumococci to demonstrate the functional effects of this polymorphism in freshly isolated neutrophils from healthy donors. Results indicate that this polymorphism affects the ability of cells to phagocytose efficiently and to generate an oxidative burst. These data offer a possible functional explanation for the increase in FcγRIIA R131 observed in our bronchiectasis cohort.



Dr Ruth Morrell (NUI Galway)

The Potential Therapeutic Role of BH3 Mimetics in Overcoming Bone Marrow Microenvironment-Mediated Resistance of Acute Myeloid Leukaemia to Chemotherapy

R Morrell, M O'Dwyer, A Samali, E Szegezdi .

Acute myeloid leukemia (AML) is an aggressive malignancy characterized by differentiation arrest and cell signaling dysregulation which lead to the accumulation of immature myeloid precursors in the bone marrow .A persistent concern , despite advances in treatment over the last forty years, is the high relapse rate despite intensive chemotherapy due to the persistence of resistant leukemic cells localized within the bone marrow . This resistance can be attributed to intrinsic properties of these leukemic stem or progenitor cells, as well as to their interaction with the bone marrow microenvironment.

As currently used standard combination chemotherapies may not effectively target the leukaemic stem cell or the microenvironmental niche, new targeted therapeutic strategies are warranted .

Using an in-vitro human stromal co-culture model to more closely approximate in vivo microenvironment conditions and provide a more relevant model for drug sensitivity testing ,we have demonstrated microenvironmental/stromal protection of AML cell lines and primary patient samples from both spontaneous and chemotherapy-induced apoptosis (using standard chemotherapeutic agents in current clinical use).

We have also used this model system to test the therapeutic efficacy of more novel targeted agents such as the BH3 mimetic , ABT737 , which induces apoptosis by direct inhibition of anti apoptotic molecules BCL2 and BCLxL. . Importantly , preliminary results suggest that ABT737 overcomes stromal mediated protection of AML cells . This effect was observed in a number of cell lines including a subtype of AML known to carry a mutation of FLT3, a receptor tyrosine kinase with important roles in stem cell survival and proliferation , and which is associated clinically with a high relapse rate and poor prognosis.

Data exploring possible mechanisms of stroma mediated protection , in particular , focusing on apoptosis molecules ,will also be presented.

Dr Daniel Schmidt (UCC)

Do the Dynamics of Quasispecies Complexity and Ip-10 Concentration in Chronic Hepatitis C Provide an Opportunity to Individualise Treatment Strategies?

Hepatitis C virus is prone to mutation resulting in the development of numerous slightly different viruses known as quasispecies. Quasispecies complexity [QSC] may be associated with likely response to treatment. Little is known of variations in QSC over time. We plan to measure this and to use the data to create a model using QSC and IP 10 [a pro-inflammatory chemokine] to facilitate earlier identification of treatment responders.

Preliminary data indicating the most prominent genetic sequences have been generated and analysed but, because the sampling and treatment phases are lengthy, definitive findings remain remote.

Dr Mazen Al-Alawi (RCSI)

Lipoxin A4 Increases Airway Surface Liquid height in Cystic Fibrosis Bronchial Epithelia by Stimulating ATP Release, Increasing Cl- Secretion and Inhibiting Na+ Absorption

Mazen Al-Alawi¹, Valia Verriere¹, Olive Mc Cabe¹, Raphael Chiron², Richard W. Costello³, Valerie Urbach⁴ and Brian J. Harvey¹

¹ Departments of Molecular Medicine and ³Respiratory Medicine, RCSI Education and Research Centre, Beaumont Hospital, Dublin 9, Ireland. ² CRCM, Montpellier, France. ⁴ INSERM U661, Montpellier, France.

Lipoxin A₄ (LXA₄) is an endogenous anti-inflammatory lipid mediator which is reduced in Cystic Fibrosis (CF) airways¹. The altered Cl⁻ secretion and Na⁺ hyperabsorption in CF affects the Airway Surface Liquid (ASL) homeostasis and leads to a defective mucociliary clearance, chronic infection, and progressive lung destruction. In this study, the role of LXA₄ in modulating ion transport and ASL height in CF and non-CF airway epithelia was investigated. This study provides evidence for a novel



effect of LXA₄, through purinoreceptor activation, inhibition of Na⁺ absorption and stimulation of Cl⁻ secretion in CF and non-CF epithelia to finally increase ASL height. These novel effects of LXA₄ open up a new therapeutic avenue in the treatment of Cystic fibrosis.

1: Karp CL et al, 2004. *Nat Immunol.* 2004 Apr;5(4):388-92.

Dr Patrick Collier (UCD)

Getting to The Heart of Cardiac Remodelling; How Collagen Subtypes may contribute to Phenotype
P. Collier¹, C. Watson¹, M. Van Es¹, D. Phelan¹, O. Sharif¹, E. Rexhepaj¹, M. Tolan¹, M. Ledwidge², K. McDonald², J. Baugh¹. (1) UCD Conway Institute of Biomolecular and Biomedical Research, Belfield, Dublin 4, Dublin, Ireland (2) Heart Failure Unit, St Vincent's University Hospital Healthcare Group, Elm Park., Dublin, Ireland

Our lab has previously shown that selective modification of collagen subtype III is achievable via cardioprotective aldosterone inhibition. This current work investigates the distribution and biomechanical properties of the two fibrillar cardiac collagen subtypes I&III using immunofluorescent co-staining and confocal laser scanning with atomic force microscopy.

We demonstrate that collagen III fibers exist as discrete entities, are thinner and have intrinsically lower stiffness than collagen I fibers. A more complete understanding of the pathophysiology of the human cardiac collagen network may ultimately allow fibrosis to become a generalised modifiable risk factor that can be targeted in order to improve prognosis.

Dr David Prichard (TCD)

Ursodeoxycholic Acid – A Molecular Modulator of the Inflammation Carcinoma Sequence in the Oesophagus?

Hydrophobic bile acids are aetiological agents in oesophageal inflammation and carcinogenesis. The hydrophilic bile acid ursodeoxycholic acid (UDCA) has proven efficacy in treating cholestatic diseases of the liver partly through anti-inflammatory and anti-apoptotic mechanisms. It may be that UDCA can exert similar protective effects in oesophageal cells. Using high content analysis, cell viability assays, cell cycle analysis and apoptosis assays, our research investigated whether UDCA protects against deoxycholic acid (DCA) induced cell death in the HET1A cell line representing normal oesophageal mucosa. Using time-points identified in these studies, we are now investigating the role of UDCA in modulating genes regulated by exposure to DCA.

Dr James Ryan (UCC)

Cellular Mechanisms underlying Familial Partial Lipodystrophy, Dunnigan Variety (FPLD)

FPLD is a rare autosomal dominant disease that causes abnormal fat (adipose) tissue distribution, insulin resistance, hypertension and ultimately premature cardiovascular death. Our patients were treated with rosiglitazone, a drug used to treat diabetes, and showed a change in fat tissue distribution and a decrease in insulin resistance. We used established adipose cell-line models to show alterations in adipocyte differentiation as a result of over-expressing the mutant gene responsible for FPLD in these cells. B-lymphocytes from patients were shown to carry the abnormal gene. We are currently studying adipose tissue biopsies from patients to identify RNA and protein products associated with the abnormal gene. These studies have shed light on the previously obscure pathogenesis of FPLD.

Dr Nuala Healy (NUI Galway)

Detection and Quantification of Systemically Expressed Cancer-Specific miRNAs

Healy NA, Miller N, Kerin MJ

Department of Surgery, National University of Ireland, Galway

The recent detection and associated dysregulation of mi(cro)RNAs in the circulation of breast cancer patients has generated much interest in their potential to act as novel non-invasive biomarkers. The objective of this study was to evaluate the expression levels of *miR-195* and *Let7-a*



in the circulation of breast cancer patients and to determine suitable endogenous controls for normalisation of RQ-PCR data.

A significant difference in expression levels between cancers and controls was demonstrated for *miR-195* ($p < 0.0005$) and *Let7-a* ($p < 0.005$). Two miRNAs were also identified, whose combined expression values optimally normalised RQ-PCR data using statistical analysis and impact on miRNA expression levels.