QUEEN'S UNIVERSITY BELFAST

WELLCOME-WOLFSON INSTITUTE FOR EXPERIMENTAL MEDICINE

# Postdoctoral Research Symposium 2024

## Friday 26th January 2024

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# Symposium Friday 26th Jan

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| **08:30 - 09:00** | **Registration** | |
| **09:00 - 09:15** | **Welcome and Introduction**<br>**Professor Charuhas Thakar,** Wellcome-Wolfson Institute for Experimental Medicine | |
| **Session 1: Selected Oral Presentations (Chair: Dr Karis Little)** | | |
| **09:15 - 09:30** | **Dr Peter Barabas** | Polyamine Oxidase Depletion Attenuates Progressive Visual Acuity Loss in Diabetic Mice |
| **09:30 - 09:45** | **Dr Lauren Kerrigan** | Single-nuclei RNA-sequencing reveals novel role for repurposed drug in heart failure |
| **09:45 - 10:00** | **Dr Michael C. McKelvey** | Understanding the extracellular immunoproteasome in the acute respiratory distress syndrome |
| **10:00 - 10:15** | **Dr Wei Wang** | NLRP3 is a thermosensor that is negatively regulated by high temperature |
| **10:15 - 11:00** | **Poster Session 1 (odd numbers) & Coffee** | |
| **11.00 - 12.00** | **Keynote Speaker**<br>**Professor Samuel Fountain,** University of East Anglia<br>*TP2X receptors as new therapeutic targets in cardiovascular & respiratory systems*<br>(Chair: Dr Orla Dunne) | |
| **12.00 - 13.00** | **Lunch (kindly sponsored by MERCK )** | |

# Symposium Friday 26th Jan

| Session 2: Selected Oral Presentations (Chair: Dr Claire Tonry) | | |
|---|---|---|
| **13:00 - 13:15** | **Dr Ricardo Calderón González** | In vivo unsupervised high dimensional analysis to track the interaction between Klebsiella pneumoniae and myeloid cells |
| **13:15 - 13:30** | **Dr Julia Monjaras-Feria** | Secretion Mechanism of Burkholderia Lethal Factor 1 by Outer Membrane Vesicles |
| **13:30 - 13:45** | **Dr Matthew James** | Two cellular AAA+ ATPases associate with rhinovirus non-structural proteins and are essential for rhinovirus replication |
| **13:45 - 14:00** | **Dr Michelle Naughton** | Building a transparent, systematic approach to identification and evaluation of emerging therapeutics for the treatment of progressive multiple sclerosis: MS-SOLES |
| **14:00 - 14:45** | **Poster Session 2 (even numbers) & Coffee** | |
| **Session 3: Selected Oral Presentations (Chair: Dr Keren Turton)** | | |
| **14:45 - 15:00** | **Dr Kiran J. McLoughlin** | Use of a novel ambient transportation system to facilitate cell therapy applications for Endothelial Colony Forming Cells (ECFCs) |
| **15:00 - 15:15** | **Dr Jessica White** | The role of MHC-II in CNS remyelination |
| **15:15 - 15:30** | **Dr Orla Dunne** | Determination of BLU-5937 P2X3 antagonist activity in PNEs and PBEC models |

# Symposium Friday 26th Jan

| Session 4: Postdoc Achievements & Prizes | | |
|---|---|---|
| **15:30 - 16:00** | **Dr Kiran J. McLoughlin** | Summary of postdoc achievements & oral and poster presentation awards |
| **16:00 – 17:00** | **Drinks Reception (kindly sponsored by MERCK )** | |

# Poster Speed Dating and Imaging Competition

## Poster Sessions

**Odd number** posters will be presented during **Poster Session 1**. **Even numbered** poster presenters are asked to stand by their posters during **Poster Session 2.**

## Poster Speed Dating

Postdocs are encouraged to **visit at least 5 different posters.** They will be awarded with a sticker for every poster presenter they have engaged with during the sessions. All those with 5 stickers will be entered into a prize raffle. **You can collect your stickers and sticker sheet at registration.**

## Imaging Competition

During the day of the symposium, images will be displayed on the screens in the inner atrium. Please vote for your favourite image using one of the voting slips beside the ballot box below the display screen. **The owner of the most popular image will win 2 hours of microscope time on any microscope in the imaging CTU!** Please submit all entries to Karis Little (K.Little@qub.ac.uk) by **Thursday 25th January**.

## Vendor Passport Competition

During the lunch and coffee breaks, postdocs are encouraged to visit as many of our generous sponsors as possible. Those who visit all sponsor stands will be entered into a raffle for prizes. You can **collect your passport at registration.**

# Symposium Information

## Thank you!

We would like to express our gratitude to the WWIEM Clerical Support Team (especially Mie, Debi and Sam!) for all their help and guidance in organising this Symposium and liaising with sponsors.

Thank you to the PIs and postdocs who have volunteered their time to act as the poster judges and chairs.

We would also like to thank the members of the WWIEM Postdoctoral Development Committee for their support.

## Poster Judges

Session 1: Dr Keren Turton, Dr Matthew James and Dr Andrea Puhar
Session 2: Dr Kiran J. McLoughlin, Dr Orla Dunne and Dr David Cisneros

## Oral Session Judges

Session 1: Prof Samuel Fountain, Dr Aoife Rodgers and Prof Ultan Power
Session 2: Prof Samuel Fountain, Dr Rebecca Coll and Dr David Cisneros
Session 3: Prof Samuel Fountain, Dr Aurélie Mousnier and Dr Andrea Puhar

## Keynote and Oral Presentation Session Chairs

Oral Session 1: Dr Karis Little
Keynote Session: Dr Orla Dunne
Oral Session 2: Dr Claire Tonry
Oral Session 3: Dr Keren Turton

## Symposium Organising Committee

Dr Kiran McLoughlin, Dr Keren Turton, Dr Orla Dunne, Dr Clare Houston, Dr Karis Little, Dr Matthew James and Dr Claire Tonry

# Keynote Speaker

**Samuel Fountain**
**Professor of Pharmacology,**
**School of Biological Sciences Member, Cells and Tissues**
**University of East Anglia**

Associate Pro-Vice-Chancellor, UEA Doctoral College (2022 - present)
Associate Dean for Postgraduate Research, Faculty of Science (2019 - 2022)
Director of the Biomedical Research Centre, UEA (2015 - 2021)

**Research**

As an early career scientist, Professor Fountain trained in the laboratories of Professor David Beech FMedSci (Leeds) and Professor R. Alan North FRS (Manchester), and subsequently established an independent research group following the award of a prestigious BBSRC David Phillips Fellowship. He has previously collaborated with clinicians at the Norfolk & Norwich University Hospital, the pharmaceutical industry, and other academic groups tackling pressing and challenging projects. His expertise encompasses pharmacological and physiological aspects of vascular and adipose tissues, as well as the biophysical and pharmacological properties of ligand- and voltage-gated ion channels.

# Session 1 Abstracts – Oral Presentations

## 1. Polyamine Oxidase Depletion Attenuates Progressive Visual Acuity Loss in Diabetic Mice

Peter Barabas[1], Peter Benz[2], Remko Bakker[2], Klaus Brilisauer[2], Besnik Bajrami[2], Heike Neubauer[2], and Tim Curtis[1]

1. Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK

2. Boehringer Ingelheim Pharma GmbH & Co, Biberach, Germany

Our prior research established a connection between the accumulation of acrolein adducts and the advancement of diabetic retinopathy (DR). In addressing this pathway, our objective was to diminish endogenously produced acrolein by targeting the enzymes responsible for its synthesis, specifically focusing on polyamine oxidases as major contributors. We sought to determine the relative roles of spermine oxidase (Smox) and polyamine oxidase (Paox) by knocking out these enzymes in C57Bl6J mice, assessing their influence on diabetes-induced retinal changes.

PCR confirmed the knockout status for both strains, revealing the loss of exon 3 in the Smox strain and exons 2-7 in the Paox strain. Although commercially available antibodies failed to differentiate wild type from knockout samples in Western or IHC studies, functional knockout of these enzymes was biochemically confirmed using microplate assays detecting Smox- and Paox-produced H2O2. Diabetic Smox and Paox knockout mouse retinas exhibited reduced spermine concentrations, while brain concentrations remained unaffected. Visual performance declined progressively in wild-type diabetic mice, whereas the decline was significantly reduced at 3 and 6 months in both knockout strains. Notably, Smox knockouts demonstrated the least impairment at 6 months of diabetes, suggesting Smox as a promising target for the treatment of DR.

Acknowledgements: this study is supported by Boehringer Ingelheim (contract #478291).

## 2. Single-nuclei RNA-sequencing reveals novel role for repurposed drug in heart failure

Lauren Kerrigan, Kevin Edgar, Oisin Kappa, Adam Russell-Hallinan, Narainrit Karuna, David Simpson, David Grieve, and Chris Watson

Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK

Introduction: Heart failure is characterized by structural abnormalities of the myocardium including cardiac hypertrophy and remodelling which result in cardiac dysfunction. Evidence indicates that aberrant DNA methylation plays an important role in the development of heart failure. The DNA methylation inhibitor 5-azacytidine (5-aza), is an approved drug used for treatment of haematological malignancies. Patients receiving 5-aza that also suffer from underlying heart disease exhibited improved cardiac function post-treatment. The aim of our work was to examine the status of DNA methylation in the hearts of angiotensin-II-infused mice, elucidate the effect of 5-aza intervention on cardiac pathological changes, and then use single-nuclei RNA-sequencing to identify cell-specific, DNA sensitive genes that could pose as potential therapeutic targets.

Methods: Eight-week-old male C57Bl/6 mice were infused with 1.5mg/kg/day angiotensin-II (or saline) for 28 days; experimental mice received either 5mg/kg 5-aza or PBS, intraperitoneally, every 3 days. An echocardiogram was conducted, and systolic blood pressure measured throughout the study before mice were euthanised. Excised hearts were snap frozen, fixed or underwent fresh single-nuclei isolation for RNA sequencing. Subsequent in vitro validation was carried out on human cardiac fibroblasts treated with angiotensin-II and 5-aza.

Conclusions: We observed global DNA hypermethylation in the hearts of angiotensin-II-infused mice. Cardiac dysfunction and remodelling were attenuated by 5-aza intervention, associated with significant transcriptional repression of pro-fibrotic and pro-hypertrophic genes. Single-nuclei RNA-sequencing revealed that the greatest change in gene expression profiles were observed in cardiac fibroblasts. Genes identified as differentially expressed and sensitive to 5-aza were validated in human cardiac fibroblasts.

### 3. Understanding the extracellular immunoproteasome in the acute respiratory distress syndrome

Michael C. McKelvey, Thea Mawhinney, Chloe M. McKee, Rebecca C. Coll, Cecilia M. O'Kane, Daniel F. McAuley, Sinéad Weldon, and Clifford C. Taggart

Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK

The constitutive proteasome and its inflammation-driven derivative, the immunoproteasome (IP), perform important intracellular proteolytic functions, including terminal protein degradation and antigen processing. However, the IP is increasingly recognised as being present in the extracellular space during pathology, though its mechanisms of release and functions are unknown. The lungs of patients with acute respiratory distress syndrome (ARDS) are flooded with complex oedema fluid and characterising the pathogenic components within this extracellular environment may help identify therapeutic targets. We hypothesised that extracellular IP is a feature of and plays a role in ARDS.

We show that the levels and activity of IP are elevated in bronchoalveolar lavage fluid from patients with ARDS, the human healthy volunteer LPS model and the murine inhaled LPS model. Furthermore, in a series of in vitro experiments, we demonstrate that IP is released constitutively from macrophage-like cells and primary human macrophages. Importantly, this release can be augmented by activation of the NLRP3 and AIM2 inflammasomes. Using both pharmacological and genetic strategies we show that targeting the inflammasome pathway abrogates IP release from macrophages, confirming the importance of this pathway in IP release. We next sought to identify extracellular substrates of IP, the cleavage of which might contribute to the inflamed environment of the lung in ARDS. We report that IP is able to cleave several anti-inflammatory proteins that are present in the ARDS lung, including antiproteases and the phospholipid-binding protein Annexin A1.

In conclusion, extracellular IP is a feature in human ARDS and models of ARDS. We have identified a potential mechanism of release of IP, which is closely linked to inflammasome activation, and postulate that extracellular IP may play a pro-inflammatory role in the acutely inflamed lung.

### 4. NLRP3 is a thermosensor that is negatively regulated by high temperature

Wei Wang and Rebecca C. Coll

Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK

Inflammation is an essential response to infection and injury, but unregulated inflammation is damaging and must be limited by negative feedback signalling. Inflammasomes are intracellular protein complexes that control the production of the pro-inflammatory cytokines IL-1β and IL-18, and a lytic cell death programme known as pyroptosis. Inflammasome signalling is thus a highly inflammatory process that drives both local inflammation and systemic responses such as fever and acute phase protein production. The inflammasome sensor NLRP3 is activated by a vast number of stimuli and senses perturbations of cytoplasmic homeostasis. As temperature is a fundamental environmental stressor, we hypothesised that NLRP3 inflammasome signalling would be sensitive to changes in temperature.

We investigated the effects of high fever range temperatures (FRT) of 41-42°C on NLRP3 activation in macrophages. Short-term (1 hr) incubation of primary mouse bone marrow derived macrophages (BMDM) at FRT significantly decreases NLRP3 activation. The secretion of IL-1β, caspase-1 activation, and pyroptosis are ablated in response to NLRP3 activation by lipopolysaccharide, nigericin, ATP, and R837. Importantly, TNF whose secretion is NLRP3-independent, is not significantly reduced under the same conditions. We observed similar results in human monocyte-derived macrophages (HMDM), where incubation at FRT attenuates NLRP3 activation, while TNF and IL-6 secretion are not significantly reduced. FRT blocks NLRP3 activation in a transcription-independent manner, and the effects on NLRP3 are specific as NLRC4 and AIM2 inflammasome activation are not blocked by FRT.

Mechanistically, we observed that heat shock protein 70 (HSP70) is induced by FRT in BMDM and HMDM. Indeed, treatment of HMDM with activators of HSP70 inhibits NLRP3 activation at 37°C. While at FRT, HSP70 inhibitors enhance NLRP3 signalling. These data demonstrate that HSP70 is a critical temperature-dependent regulator of NLRP3. Our studies suggest that fever may limit NLRP3 activity in a classical negative feedback mechanism.

## 5. In vivo unsupervised high dimensional analysis to track the interaction between Klebsiella pneumoniae and myeloid cells

Ricardo Calderón González, Amy Dumigan, Joana Sa Pessoa, Adrien Kissenpfennig, and José A. Bengoechea

Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK

With the advent of Cellular Microbiology, pathogens and virulence factors were investigated in the context of cell lines, modelling interactions with epithelial cells lining the mucosae, and monocytes/macrophages. High-throughput screens led to the unbiased identification of bacterial and host factors governing each bacterial-cell interaction whereas dual scRNA approaches have provided an unprecedent molecular resolution of the pathogen-cell interaction. However, it is well appreciated that the pathogen-host interface in vivo cannot be recapitulated only by investigating one pathogen-cell interaction.

CyTOF combines the resolution of mass spectrometry with the ability to conduct multiplexed measurements of cell molecules at the single cell resolution, allowing the detection of up to 60 markers. This work was designed to define at the cellular level the host-pathogen interface in vivo by Bacteria-CyTOF (hereafter Bac-CyTOF), an approach leveraging the power of mass cytometry to immunoprofile cells while tracking their interaction with bacteria. We focused on Klebsiella pneumoniae, a leading cause of hospital-acquired, including ventilator-associated pneumonia, and community-acquired infections.

Here we report an atlas of immune cells upon K. pneumoniae infection over time while tracking simultaneously the interaction of Klebsiella with them. Furthermore, by infecting with bacterial strains cleared by the host, and by challenging different mouse genetic backgrounds which differ in their ability to clear Klebsiella, we identify an immune cell environment associated with clearance of K. pneumoniae. These results allowed us to predict the outcome of a K. pneumoniae infection in a mouse strain for which there was no previous evidence of playing any role in the host-Klebsiella interface, demonstrating the power of Bac-CyTOF to dissect the host-pathogen interface.

## 6. Secretion Mechanism of Burkholderia Lethal Factor 1 by Outer Membrane Vesicles

Julia Monjaras-Feria[1], Nichollas Scott[2], and Miguel A. Valvano[1]

1. Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK

2. Department of Microbiology and Immunology, University of Melbourne, Melbourne, 3000, Australia

Burkholderia lethal factor 1 (BLF1) is an important virulence factor produced by Burkholderia pseudomallei, the causative agent of melioidosis, and the first B. pseudomallei toxin with role in virulence identified. BLF1 is toxic for macrophages and epithelial cells, and kills mice challenged by the intraperitoneal route. BLF1 is a glutamine deamidase that deamidates Glu339 in the eukaryotic initiation factor 4A. This modification inactivates the RNA helicase activity required for melting mRNA secondary structures during initiation of translation, which this leads to inhibition of protein synthesis in human cells. How this toxin is translocated from the bacterial cytoplasm is unknown. We demonstrated that BLF1 is a secreted protein that can be detected in culture supernatants and its secretion and translocation into macrophages is independent to any secretion system (T2SS, T3SS, T4SS or T6SS). BLF1 travels from the cytoplasm to the periplasmic space and the outer membrane to be secreted via extracellular membrane vesicles. We also showed that 20 N-terminal amino acids are needed for BLF1 secretion and stability. We found that the N terminal region consists of an amphipathic alpha helix (AH) that is sufficient to transport mCherry and a lipocalin into membrane vesicles. We demonstrate that the amphipathic property of the helix is a requirement for BLF1 transport. Disturbing the amphipathicity in this region not only led to failure to localise in the membrane, but also caused alterations in protein stability.

# Session 2 Abstracts – Oral Presentations

## 7. Two cellular AAA+ ATPases associate with rhinovirus non-structural proteins and are essential for rhinovirus replication

Matthew James[1], Courtney Dane[1], Karolina Wojtania[1], Andrea Goya Grocin[2], Remigiusz A. Serwa[2], Aidan O'Riain[1], Erin Getty[1], Sheerien Manzoor[1], Ultan F. Power[1], Edward W. Tate[2], and Aurélie Mousnier[1]

1. Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK

2. Department of Chemistry, Molecular Sciences Research Hub, Imperial College London, London, UK

Rhinovirus (RV) infections cause common colds and are a major trigger for acute exacerbations of asthma and chronic obstructive pulmonary disease (COPD). However, there are no approved vaccines or antiviral drugs against RVs. During infection, RV non-structural proteins (NSPs) interact with cellular proteins to subvert host cells and facilitate viral replication. An antiviral strategy, which is less likely to lead to the emergence of resistance than directly targeting viral proteins, is to interfere with these cellular proteins hijacked by RV.

While some of these proviral cellular proteins are known, others likely remain undiscovered. To identify new NSP-interacting cellular targets, we infected HeLa cells with RV-A16 and pulled-down NSPs and their interactors by NSP-specific affinity purification. Analysis of the pulled-down proteins by quantitative mass spectrometry revealed a specific enrichment in known proviral host factors (GBF1, PI4KIIIβ, SETD3…), along with two AAA+ ATPases that were not previously shown to be involved in the replication of RV or any other picornavirus. Remarkably, we found that their knockdown drastically inhibits RV replication, while a small molecule inhibitor of these ATPases efficiently blocks the replication of RV species A and C in cell lines and in well-differentiated primary nasal epithelial cell cultures without cytotoxicity. Together, our data show that these ATPases represent novel host factors that are essential for RV replication, most likely at an early stage of the viral replication cycle and independently of cellular transcription. In turn, this highlights their potential as promising drug targets for the treatment of RV infections.

# Session 2 Abstracts – Oral Presentations

## 8. Building a transparent, systematic approach to identification and evaluation of emerging therapeutics for the treatment of progressive multiple sclerosis: MS-SOLES

Michelle Naughton[1], Charis Wong[2, 3], Kaitlyn Hair[2], Sean Smith[2], Malcolm Macleod[2], Denise Fitzgerald[1], and Anna Williams[4]

1. Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK

2. Centre for Clinical Brain Sciences, University of Edinburgh, UK

3. Anne Rowling Regenerative Neurology Clinic , University of Edinburgh, UK

4. Centre for Regenerative Medicine, Institute for Regeneration and Repair, University of Edinburgh, UK

To address the paucity of therapeutics for progressive multiple sclerosis (PMS), the UK MS Society established an advanced clinical trial platform, OCTOPUS. This multi-arm, multi-stage trial aims to accelerate the discovery of treatments for PMS. Drugs that do not meet continuing criteria can be dropped while promising candidates can be added upon recommendations by the Treatment Advisory Committee (TAC). To date, identification and evaluation of drugs have relied solely on manual human efforts, with notable challenges in producing and maintaining a comprehensive, scalable and continuously-updated evidence-base for future drug selection. CAMARADES have pioneered a systematic approach of continuous evaluation of therapeutics for motor neuron disease through Systematic Online Living Evidence Summaries (SOLES) (Wong, et al., 2023) which could be useful for PMS.

Using SOLES methodology, we aim to identify and assess putative therapeutics for PMS through a systematic and continuous evaluation of the relevant published preclinical literature. The results will guide a rigorous, expert-led, evidence-based approach to evaluate candidates for selection to the OCTOPUS trial.

Automated searches of bibliographical databases were performed through Systematic Review Platform (SyRF, app.syrf.org.uk). Machine learning algorithms are being trained and validated to automate screening of retrieved publications. Text-mining algorithms are used to annotate publications for models and interventions described. We are developing an interactive web application reporting living evidence summaries to inform expert discussions. This study protocol is available on the Open Science Framework.

Previously, TAC members manually identified, screened and long-listed candidate drugs. The committee reviewed and scored these based on manually-compiled evidence to produce a shortlist. MS-SOLES will enable more systematic, comprehensive, efficient and up-to-date evidence synthesis, thus enhancing the treatment selection process by our expert-led committee.

# Session 3 Abstracts – Oral Presentations

## 9. Use of a novel ambient transportation system to facilitate cell therapy applications for Endothelial Colony Forming Cells (ECFCs)

Kiran J. Mcloughlin[1], Daniel A. Domingo-Lopez[2], Jessica J. Eyre[1], Pietro M. Bertelli[1], Shannon McDonnell[1], Garry P. Duffy[2], and Reinhold J. Medina[1]

1. Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK

2. University of Galway

Cryopreservation is the gold standard technique used for preserving cells for transportation between laboratories or cell therapy facilities. This process can lead to the deterioration of the preserved material, through ice crystal formation. The response of living cells to ice formation impacts on cell viability and potency. Recovered cells may be impaired or unsuitable for use.

We have developed a Novel Ambient Transportation System (NATs) which carries oxygen to allow for the ambient transportation of cells. In this study, we aim to optimise NATS for the transport of endothelial colony forming cells (ECFCs), a well-characterised type of endothelial progenitor cell, which are currently being developed as a cell therapy for ischaemic conditions.

Initial optimisation of NATS for ECFCs revealed that rehydration of NATS in EGM-2 maintained ECFC viability in ambient temperatures for 72 hours (with 70% viability) and 120 hours (with 50% viability). ECFCs were transported through Europe using NATS and were successfully recovered and compared to cryopreserved controls. ECFCs transported in NATS had comparable viability and proliferation to cryopreserved controls, whilst exhibiting an increased tubular formation capacity in vitro. A second transportation for in vivo studies showed comparable functionality between transportation groups in perfusion of nude mouse subcutaneous injection models.

In summary, we have shown that for ECFCs can be successfully transported at ambient temperatures using a NATS-based system. Future work will further optimise NATS for increased long-term viability and assess phenotypic changes between ambient and cryopreserved transported ECFCs.

# Session 3 Abstracts – Oral Presentations

## 10. The role of MHC-II in CNS remyelination

Jessica White[1], Alerie Guzman de la Fuente[2,3], Andrew Young[1], Abby McClure[1], Kristina Ulicna[1], Mohammad Moffateh[1], Rebecca Ingram[1], Yvonne Dombrowski[1], and Denise Fitzgerald[1]

1. Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK

2. Institute for Health and Biomedical Sciences of Alicante (ISABIAL), Alicante, Spain

3. Institute of Neurosciences CSIC-UMH, San Juan de Alicante, Alicante Spain

Multiple sclerosis (MS) is an immune-mediated inflammatory disease of the central nervous system (CNS), characterised by the loss of myelin and oligodendroglia (demyelination). Although myelin regeneration (remyelination) can occur in MS, it often fails, leading to disease progression and subsequent disability. While there are no treatments targeting myelin repair, our lab has shown that regulatory T cells (Treg) can drive oligodendrocyte progenitor cell (OPC) differentiation and remyelination. The mechanisms underlying this effect remain mostly unknown but have been linked to the T cell activator MHC-II. In MS, MHC-II is highly expressed in de- and remyelinating lesions and can be upregulated by glial cells important to myelin regeneration (microglia, astrocytes, and oligodendrocytes). To further understand the functional significance of MHC-II in CNS regeneration, we used in vitro OPC-T cell co-cultures and an in vivo model of lysolecithin-induced demyelination in WT and MHC-II-deficient mice.

Surprisingly, we found that Treg cells significantly drive OPC differentiation independent of MHC-II in vitro. Immunofluorescence staining of demyelinated spinal cord sections also revealed the absence of MHC-II does not significantly affect the number of oligodendrocyte lineage cells, proliferating OPCs and differentiated oligodendrocytes, but did impair remyelination and the density of proliferating microglia. For microglia/macrophages, this impairment in proliferation was rescued by adoptively transferred Treg in MHC-II KO mice. Taken together, these data suggest a novel MHC-II-independent mechanism of Treg-driven OPC differentiation, and a possible requirement for Treg in the microglial/macrophage response to demyelination. Ongoing work is investigating the mechanisms by which Treg function beyond what is classically known in MS.

## 11. P2X3 sensitisation in an adult human stem cell derived airway sensory neuronal model

Orla Dunne, Lorcan P. McGarvey, and Fionnuala T. Lundy

Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK

The cough reflex is activated through stimulation of airway vagal sensory nerves which, through a poorly defined mechanism, may become hyperresponsive causing troublesome bouts of coughing triggered by innocuous stimuli. ATP gated P2X3 receptors are thought to contribute to the neuronal sensitization associated with chronic cough. This project aimed to examine the effect of airway stimuli on ATP and cytokine release from primary bronchial epithelial cells (PBECs); and to subsequently examine the effect of these cytokines on P2X3 responses in a stem cell derived airway sensory neuronal model.

PBECs obtained from individuals undergoing bronchoscopy were cultured using submerged culture techniques. ATP release from PBECs treated with microbial mimetics LPS, LTA and poly I:C or cigarette smoke extract (CSE) was examined using luciferase assay. Cytokine release from PBECs treated with LPS, LTA and poly I:C were evaluated using inflammatory microarray. Human dental pulp stem cells were differentiated to peripheral neuronal equivalents (PNEs) in neurogenic media for 14 days. Functional P2X3 responses in PNEs, and the effect of IL-4, IP-10 and IL-16 on P2X3 responses were determined by calcium mobilisation assays.

ATP release from PBECs was increased with CSE and poly I:C ($P<0.001$). The expression of 19 cytokines (including IL-4, IP-10 and IL-16) were increased (>2 fold) with LPS, LTA or poly I:C treatment compared to control PBECs. ATP responses in PNEs were inhibited by P2X3 antagonists. A leftward shift of the ATP dose response curve in PNEs was observed with IL-4 (but not with IP-10 or IL-16) pretreatment.

This study has added to our understanding of ATP release in the airway. PNEs express functional P2X3 receptors. The IL-4 mediated leftward shift of the ATP dose response indicates sensitisation of the PNE airway sensory neuronal model.

## Poster Presentations (odd session 1, even session 2)

| Number | Presenting Author | Title |
|---|---|---|
| 1 | Dr Claire Tonry | Proteomic Characterisation of Hypertrophic Obstructive Cardiomyopathy |
| 2 | Dr David Hughes | Developing novel therapies for macular fibrosis secondary to neovascular age-related macular degeneration |
| 3 | Dr Olivier Touzelet | Repurposing Approved drugs as potent antiviral combinations to treat COVID-19 disease |
| 4 | Dr Evan P. Troendle | SARS-CoV-2 in Ireland: Insights from Phylogenetic Analyses and Wastewater Surveillance |
| 5 | Dr Guan-bo Wang | Dissecting the role of infection-driven protein mono-glycosylation in Legionella-host interaction |
| 6 | Dr Hong Guo-Parke | Cellular senescence in severe COPD bronchial epithelium impairs responses to human rhinovirus infection |
| 7 | Dr Jessica Eyre | BMP-9 impedes 3D sprouting angiogenesis through molecular mechanisms that repress a tip cell phenotype |
| 8 | Dr Karis Little | Characterising retinal neurovascular dysfunction in a mouse model of Alzheimer's disease combined with Type 2 diabetes |
| 9 | Dr Keren Turton | The Achromobacter Type 3 secretion system is necessary for NLRC4- and NLRP3-driven pyroptosis |
| 10 | Dr Kevin Edgar | Cardiac fibroblast function is regulated by modulation of Tetranectin expression with associated impact on hypoxia and cardiac fibrosis in-vivo |
| 11 | Dr Kevin Harkin | Evaluating the retinal neurovascular unit in the murine model of experimental Cerebral Malaria |
| 12 | Dr Miriam Sartages Garcia | USP10 DUB protein is partially responsible for augmenting Notch1 signalling in eNOS expressing cells |

## Poster Presentations (odd session 1, even session 2)

| Number | Presenting Author | Title |
|---|---|---|
| 13 | Dr Matthew Pilgrim | The distribution and phenotype of Glioma associated oncogene 1 (Gli1) expressing cells in ocular tissues |
| 14 | Dr Pietro Maria Bertelli | Physoxia and hypoxia alter the functional and metabolic profile of human endothelial progenitor cells |
| 15 | Dr Rebecca Delaney | A multi-omics evaluation of the effects of valaciclovir on the sputum proteome and microbiome in COPD |
| 16 | Dr Karla O'Neill | Defining a key role for DNA methylation in determining angiogenic function of cord-blood derived endothelial colony forming cells |
| 17 | Dr Jasenka Guduric-Fuchs | RIG-I sensing of self-RNA facilitates senescence in aged endothelial cells |
| 18 | Dr Varun Pathak | Human cord blood-derived endothelial colony forming cells for vascular repair |