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WELLCOME-WOLFSON INSTITUTE FOR EXPERIMENTAL MEDICINE

Postdoctoral Research Symposium 2023

Friday 27th January 2023

Sponsors

We would like to thank the following sponsors for their support in helping deliver this symposium.

Symposium Friday 27th Jan

08:30 – 09:00	**Registration**	
09:00– 09:15	**Welcome and Introduction** **Prof Jose Bengoechea**, Centre Director, Wellcome-Wolfson Institute for Experimental Medicine	
Session 1: Selected Oral Presentations **Chair: Dr Orla Dunne**		
09:15 – 09:30	Dr Michael C. McKelvey	Understanding the extracellular immunoproteasome in acute respiratory distress syndrome
09:30 – 09:45	Dr Evan Troendle	SARS-CoV-2 introductions to the island of Ireland
09:45 – 10:00	Dr Karis Little	Neurovascular unit breakdown in a mouse model of T2D occurs in parallel in the retina and brain
10:00 – 10:15	Dr Michelle Naughton	Spatial transcriptomics of compartmentalised inflammation in Multiple Sclerosis
10:15 – 10:45	**Poster Session 1 (odd numbers) & Coffee**	
10:45 – 11:45	**KEYNOTE SPEAKER SESSION 1** **Dr Jose Romero** Title: Developing novel therapeutics for diabetic eye disease through targeting mitochondrial quality control **Chair: Dr Judith Lechner**	
11:45 – 12:00	**Vendor mini-talk: Leica** *"Coral Life – Capture Life the Moment it Happens'*	

Symposium Friday 27th Jan

12:00 – 13:00	**LUNCH**	
Session 2: Selected Oral Presentations **Chair: Dr Karis Little**		
13:00 – 13:15	Dr Keren Turton	The *Achromobacter* Type 3 secretion system is necessary for NLRC4- and NLRP3-driven pyroptosis
13:15 – 13:30	Dr Pietro Maria Bertelli	Functional and metabolic characterization of human endothelial progenitor cell response to physoxia and hypoxia
13:30 – 13:45	Dr Wei Wang	The NLRP3 inflammasome is regulated by high temperature in an endogenous negative feedback mechanism that limits inflammation
13:45 – 14:00	Dr Ricardo Calderón González	Modelling the gastrointestinal carriage by Klebsiella pneumoniae infections
14:00 – 14:15	Dr Varun Pathak	Human cord blood-derived endothelial colony forming cells for vascular repair
14:15 – 14:45	**Poster Session 2 (even numbers) & Coffee**	
14:45 – 15:45	**KEYNOTE SPEAKER SESSION 2** **Prof. Siobhan McClean** Chronic infection in cystic fibrosis: Adaptation of Gram-negative pathogens to the CF lung British Society for immunology **Chair: Dr Keren Turton**	
Session 3: Postdoc Achievements and Prizes		
15:45 – 16:00	Dr Claire Tonry	Summary of Postdoc Achievements & Oral and poster presentation awards

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 week 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.

Images will also be displayed in Slack and voters can vote by sending a private message to Claire Tonry on Teams.

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 Sam!) for all their help and guidance in organising this Symposium and liaising with sponsors.

Thank you to the PIs, postdocs and students who have volunteered their time to act as the abstract selection panel, poster judges and chairs.

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

Poster Judges

Dr Rebecca Coll, Callum Sloan, Dr Karis Little, Dr Keren Turton

Oral Session Judges

Dr Jose Romero, Dr Judith Lechner, Dr Claire Tonry

Abstract Selection Panel

Dr Dessi Malinova, Dr Rebecca Coll, Dr Eric Campbell

Keynote and Oral Presentation Session Chairs

Dr Keren Turton, Dr Orla Dunne, Dr Judith Lechner, Dr Karis Little

Symposium Organising Committee

Dr Claire Tonry, Dr Keren Turton, Dr Orla Dunne, Dr Judith Lechner, Dr Karis Little

Keynote Speakers

Dr Jose M Romero Hombrebueno PhD
Institute of Inflammation and Ageing
University of Birmingham

After completing his PhD in Visual Neuroscience at the University of Alicante (Spain), Jose came to work at WWIEM in 2012. While here he investigated the mechanisms leading to retinal degeneration in diabetic retinopathy, age-related and inflammatory-related. He was awarded with a Fight for Sight Early Career Investigator Award in 2016, to develop his own independently funded research programme. In 2019, Jose joined the University of Birmingham (Institute of Inflammation and Ageing) as a Hale-Rudd Lecturer in Experimental Ophthalmology.

Jose's research focus is on understanding how mitochondrial dysfunction impacts on eye disease and whether it can be targeted for therapy. Irreversible mitochondrial dysfunction involves a major event in many ocular disorders, including diabetic retinopathy, optic neuropathies, inflammatory and aging-related disorders. The accumulation of broken mitochondria may pose a severe risk for vision, since 1) damaged mitochondria do not produce energy efficiently and disrupts the power system of the retina and 2) they are a source of toxic waste, which may origin retinal disease. In the healthy retina, this problem is prevented by tight Mitochondrial Quality Control (MQC), where faulty mitochondria are simply removed and replaced by newly-synthetized functional units. Using appropriate disease models, Jose pursues to determine how MQC becomes dysregulated in ocular disease and its contribution to neurovascular degeneration. His main goal focuses in applying this knowledge to develop novel interventional strategies for eye therapy.

Keynote Speakers

Prof Siobhán McClean
Associate Professor
School of Biomolecular and Biomedical Science
University College Dublin

Prof McClean completed her PhD at Lincoln's Inn Fields in London. She has since continued her career in Dublin with positions at St. Luke's hospital, Tallaght IT and University College Dublin.

Prof. McClean's research interests are in chronic infection, which is the hallmark of cystic fibrosis lung disease. She has a key interest in respiratory pathogens that cause opportunistic infections in cystic fibrosis patients. In particular, I focus on Burkholderia cepacia complex. This is a highly antimicrobial resistant pathogen and effective treatments are challenging. Prof McClean also has an interest in host responses of epithelial cells, mechanisms of pathogenesis and mechanisms of bacterial adaptation which facilitate chronic colonisation. Her team are currently examining the role of stress proteins in chronic colonization and the switch that controls the transition from acute to chronic infection. Her team also investigate two other pathogens associated with CF-infections, Pseudomonas aeruginosa and genus Pandoraea. In recent years, Prof. McClean has also been focusing her research on vaccine development. As a result of our investigations on mechanisms of attachment of pathogens to lung epithelial cells, Prof. McCleand and her team have identified a number of novel bacterial adhesins, which are potential vaccine candidates. The antigens protect mice against Burkholderia and have potential as novel vaccine antigens. They have identified vaccine candidates against Burkholderia cepacia complex, Burkholderia pseudomallei , the causative agent of the tropical disease, melioidosis and verotoxigenic E. coli.

Session 1 Abstracts – Oral Presentations

Vendor mini-talk: Leica

“Coral Life – Capture Life the Moment it Happens’

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Session 1 Abstracts – Oral Presentations

1. Understanding the extracellular immunoproteasome in acute respiratory distress syndrome

Michael C. McKelvey, Chloe M. McKee, Thea Mawhinney, Rebecca Coll, Cecilia M. O'Kane, Daniel F. McAuley, Sinéad Weldon, 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. However, the IP is increasingly recognised as being present in the extracellular space during pathology, though its mechanisms of release and functions are unknown. We hypothesised that extracellular IP may be a feature of, and play a role in, the acute respiratory distress syndrome (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 intratracheal LPS model. In a series of in vitro experiments, we demonstrate that IP is released constitutively from macrophages, though this release can be exacerbated by activation of the NLRP3 and AIM2 inflammasomes, and that IP release can be abrogated by pharmacological or genetic targeting of the inflammasome pathway. We have found that direct instillation of IP into the lungs of mice does not induce inflammation, suggesting that extracellular IP may play another role in the lung. We plan to explore this possibility further in vivo. Closely linked to this, since the extracellular targets of IP are not known, identifying such targets represents a key aspect of our future work.

In conclusion, extracellular IP is a feature in human ARDS and murine ARDS-like disease. We have identified a novel mechanism of release of IP, which is closely linked to inflammasome activation, and are exploring the potential relevance of extracellular IP.

Session 1 Abstracts – Oral Presentations

2. SARS-CoV-2 introductions to the island of Ireland

Evan P. Troendle 1,†, Alan M. Rice 1,†,‡, Stephen Bridgett 1, Behnam F. Nejad 2, Jennifer M. McKinley 2, The COVID-19 Genomics UK consortium 3, Timofey Skvortsov 4,*, David A. Simpson 1*

1 Wellcome–Wolfson Institute for Experimental Medicine, 2 School of Natural and Built Environment, Queen's University Belfast, Belfast, United Kingdom
3 The COVID-19 Genomics UK consortium
4 School of Pharmacy, Queen's University Belfast, Belfast, United Kingdom

† The authors consider these individuals to be Joint First Authors.
‡ Current address: Milner Centre for Evolution, Department of Life Sciences, University of Bath, Claverton Down, Bath, United Kingdom

As happened throughout the world, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus had a devastating impact as waves of infection spread across the island of Ireland during the coronavirus disease 2019 (COVID-19) pandemic. As SARS-CoV-2 replicates, it slowly accumulates mutations and whole-genome sequencing (WGS) makes it possible to track these genetic changes. Phylogenetic analyses were conducted on 7,603,547 GISAID WGS sequences from across the globe to determine the importation of major viral lineages to the island of Ireland. Using geospatial timeseries analyses of the phylogenies and associated sample metadata across the pandemic, we describe in detail the importation and spread of multiple infection clusters to the island of Ireland for the first time. As expected, introductions spread primarily to adjacent districts, but with some longer distance hops, potentially associated with transport corridors. A better understanding of how these successive introductions have spread could guide future public health responses. The methodological approach employed in this study can be applied broadly to study the spatiotemporal kinetics of viral importations to any geographic region of interest where sufficient WGS sequencing data is available. The high level of SARS-CoV-2 WGS, particularly in the UK, facilitated this analysis. Although our conclusions are nonetheless tempered by relatively sparse and variable sampling, this analysis demonstrates the additional value of WGS beyond surveillance of emerging variants. The opportunity provided by the continually decreasing cost of WGS should be used to adopt WGS more widely to monitor and geo-spatiotemporally investigate future infectious conditions.

3. Neurovascular unit breakdown in a mouse model of T2D occurs in parallel in the retina and brain

Karis Little, Aditi Singh, Angel del Marco, María Llorián-Salvador, Maria Vargas-Soria, Edoardo Pedrini, Reinhold Medina, Rafael Simo, Monica Garcia-Alloza, Vijay Tiwari and Alan W. Stitt

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

Purpose: Type-2 diabetes (T2D) is associated with increased risk of cognitive impairment. The retina has been suggested as a 'window' to the brain. From the perspective of neuronal, glial and vascular degenerative changes in T2D, we have compared the retinal and cortical NVU in a model of T2D (db/db mouse).

Methods: Single-cell sequencing (scRNA-seq) datasets from db/db cortex and retina were assessed for gene expression changes in component cells of the NVU. Neuronal loss was measured by immunohistochemistry (IHC) staining of key markers such as Brn3a and NeuN. Assessment of gliosis was carried out via GFAP, microglia via Iba-1 and vascular integrity via Laminin, Isolectin B4/Collagen IV and albumin.

Results: scRNA-seq analysis revealed that mature neurons degenerate in the db/db cortex, and cone cells are depleted in the db/db retina. Validation via IHC staining confirmed reduced neuronal cell populations in both db/db retina and cortex. (Retina - Cone photoreceptors reduced ($p<0.05$), retinal ganglion cells reduced ($p<0.05$), Cortex - NeuN/DAPI ratio reduced ($p<0.01$)). Retinal gliosis was observed in db/db mice ($p<0.01$), however the levels of cortical GFAP were not significantly increased. Microglia burden was significantly increased in both the retina and cortex ($p<0.05$), and microglia-specific transcripts were increased in the scRNA-seq analysis of db/db cortex.. A significant increase in acellular capillaries ($p<0.05$) and albumin leakage were observed in both db/db retina and cortex.

Conclusions: Similar NVU damage occurs in the retina and cortex of db/db mice. In this model, the retina may serve as a surrogate to brain pathology in T2D.

4. Spatial transcriptomics of compartmentalised inflammation in Multiple Sclerosis

Michelle Naughton 1, Rachael Kee 1, Owain Howell 2, Denise C Fitzgerald 1

1 Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK
2 Institute of Life Science, School of Medicine, Swansea University

Multiple Sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). Inflammation can become compartmentalised in the connective tissue spaces of the vasculature and leptomeninges behind an intact blood-brain-barrier. The relative grade of meningeal inflammation and the presence of tertiary-lymphoid structures (TLS) is associated with an accelerated and more severe disease course. The immune cell composition of TLS in MS is unclear and it is not known whether the cellular profiles of TLS are distinct in different CNS compartments that harbour inflammatory cells. This study aims to characterise the molecular profiles of TLS and other CNS inflammatory areas.

Using FFPE MS tissue from the Dame Ingrid Allen Tissue Collection at Queen's University Belfast, in-depth molecular profiling of TLS and other CNS inflammatory compartments was undertaken. Nanostring digital spatial profiling of the whole transcriptome was performed on TLS, meningeal and perivascular regions of two MS cases. Using this dataset, we identified the highest and most differentially expressed genes in TLS and other CNS inflammatory compartments. A subset of these targets were selected for validation with the RNAscope HiPlex assay. This assay allows detection of up to 12 targets simultaneously at the cellular level. Preliminary findings confirm detection of immune-related genes associated with the TLS transcriptomic profile. Further, it indicates a possible association between cellular phenotypes occurring in TLS and in perivasculature adjacent, but not distal, to TLS. These findings demonstrate that archival FFPE CNS tissue can be used for spatial transcriptomic profiling and this unbiased approach can identify novel molecular signatures.

5. The *Achromobacter* Type 3 secretion system is necessary for NLRC4- and NLRP3-driven pyroptosis

Keren Turton 1, Rebecca Coll 1, Clare Bryant 2 & Miguel Valvano 1

1 Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK
2 Department of Veterinary Medicine, University of Cambridge, Cambridge, UK

Opportunistic bacterial respiratory pathogens are problematic for people with immune deficiencies. *Achromobacter* species typify the challenge: they are multi-drug resistant and poorly-characterised. Our group has investigated the interaction of these Gram-negative bacteria with macrophages, the first-line defenders of the immune system. We mutated key components of the Type 3 Secretion System (T3SS) in several *Achromobacter* clinical isolates and infected THP-1s (a macrophage-like cell line) as well as primary blood monocyte-derived macrophages. *Achromobacter* species with functional T3SSs elicit pyroptosis, a pro-inflammatory form of cell death in macrophages. Pyroptosis can be induced via several different sensor pathways and using knockout THP-1 cells, we showed that either the NLRC4 or NLRP3 inflammasome pathways are sufficient in *Achromobacter* infection. We have established that direct macrophage-bacteria contact is a pre-requisite for pyroptosis, and that phagocytosis of the bacteria enhances it. However, the clinical isolates in our collection differ in the rapidity of cytotoxicity induction. Intriguingly, species was not predictive of cytotoxicity. AC055 infection led to acute cytotoxicity within 1 hour post-infection, while QV306 took about 8 hours to reach the same level even though both are *A. xylosoxidans* strains with nearly identical T3SS structural operons. By mutating a putative regulatory gene immediately upstream of the sigma transcription factor in the T3SS regulatory operon, we were able to make QV306 as cytotoxic as AC055. These data suggest that while *Achromobacter* need a T3SS to elicit pyroptosis in macrophages, other regulatory genes influence the rate of induction.

Session 2 Abstracts – Oral Presentations

6. Functional and metabolic characterization of human endothelial progenitor cell response to physoxia and hypoxia

Pietro Maria Bertelli 1, Jasenka Guduric-Fuchs 1, Varun Pathak 1, Edoardo Pedrini 1, David Hughes 1, Cristina Branco 2, Reinhold J. Medina 1, Alan W. Stitt 1

1. Wellcome-Wolfson Institute for Experimental Medicine, 2. The Patrick G Johnston Centre for Cancer Research, Queen's University Belfast, Belfast, United Kingdom

Progressive ischemia is central to many diseases including heart disease, stroke, peripheral artery disease and retinopathy. Regeneration of degenerative vascular beds using vascular stem cell therapy is a worthwhile therapeutic approach to restore flow and repair damaged tissues. The aim of this project is to study alterations in the metabolic profile of endothelial progenitors during active angiogenesis and identify oxygen levels that facilitate vasoregeneration.

Endothelial colony forming cells (ECFCs) were cultured in standard oxygen levels (normoxia, 21%O2), physoxia (10%O2, 5%O2) and hypoxia (1%O2). RNA-seq analysis, as bulk (GSE142123) and single cell, were used to assess transcriptional changes in ECFCs after exposure to hypoxic conditions. Gene and protein expression were investigated using q-PCR and Western-Blot. Cell functionality was tested using tube formation assays and endothelial barrier capacity was measured using the MaestroPro system. The Seahorse XFe96 Bioanalyzer allowed the investigation of the metabolic profile in ECFCs.

RNA-seq highlighted upregulation of glycolysis-related genes in hypoxia-treated ECFCs, compared to normoxic controls. Physoxia-cultured ECFCs show enhanced tube formation, glycolytic and barrier capacity, compared to ECFCs grown at normoxic conditions. Importantly, hypoxia consistently diminished ECFC function. Physoxia "priming" induced changes in the mitochondrial oxidation of glucose, glutamine, and fatty acids, as well as an increase in glucose uptake and glycogen content.

This study suggests that physoxia "priming" induces metabolic changes in ECFCs, shifts their glycolytic profile and, enhances their vasoreparative capacity.

7. The NLRP3 inflammasome is regulated by high temperature in an endogenous negative feedback mechanism that limits inflammation

Wei Wang and Rebecca Coll

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

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.

Session 2 Abstracts – Oral Presentations

8. Modelling the gastrointestinal carriage by *Klebsiella pneumoniae* infections

Ricardo Calderón González, Alix Lee, Guillermo López Campos, Steven J. Hancock, Joana Sá Pessoa, Amy Dumigan, Ronan McMullan, Eric L. Campbell and José A. Bengoechea

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

Klebsiella pneumoniae is a leading cause of nosocomial and community acquired infections, making *K. pneumoniae* the second pathogen associated with the most deaths attributed to any antibiotic resistant infection. *K. pneumoniae* colonises the nasopharynx and the gastrointestinal tract in an asymptomatic manner without dissemination to other tissues; importantly gastrointestinal colonisation is a requisite for infection. Our understanding of *K. pneumoniae* colonisation is still based on interrogating mouse models in which animals are pre-treated with antibiotics to disturb the colonisation resistance imposed by the gut microbiome. In these models, infection disseminates to other tissues. Here, we report a murine model to allow for the study of the gastrointestinal colonisation of *K. pneumoniae* without tissue dissemination. Hypervirulent and antibiotic resistant strains stably colonise the gastrointestinal tract of in an inbred mouse population without antibiotic treatment. The small intestine is the primary site of colonisation followed by a transition to the colon over time without dissemination to other tissues. Our model recapitulates the disease dynamics of metastatic *K. pneumoniae* strains able to disseminate from the gastrointestinal tract to other sterile sites. Colonisation is associated with mild to moderate histopathology, no significant inflammation, and no effect on the richness of the microbiome. Our model sums up the clinical scenario in which antibiotic treatment disturbs the colonisation of *K. pneumoniae* resulting in dissemination to other tissues. Finally, we establish that the capsule polysaccharide is necessary for the colonisation of the large intestine whereas the type VI secretion system contributes to colonisation across the gastrointestinal tract.

Session 2 Abstracts – Oral Presentations

9. Human cord blood-derived endothelial colony forming cells for vascular repair

Varun Pathak, Kiran Mcloughlin, James Bojdo, Jessica Eyre, Pietro Bertelli, Edoardo Pedrini, Yuxin Wu, Jasenka Guduric-Fuchs, Karla O'Neill, Sandra McAllister, Alan Stitt, David Grieve, Reinhold Medina

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

Vascular diseases like stroke, myocardial infraction and peripheral artery disease are characterised by poor vascular perfusion and are major cause of death and morbidity.. Emerging evidence suggests cell therapy to promote therapeutic angiogenesis and tissue repair. Human cord blood-derived endothelial colony forming cells (ECFCs) have been characterised as 'bona fide' endothelial progenitors. Here, we describe essential preclinical data, as a scientific milestone towards their translation into cytotherapy. A reproducible detailed protocol for ECFC isolation from fresh/frozen human cord blood have been optimised and validated. ECFC identity was confirmed by morphology, transcriptomics, and fluorescence-immunostaining. ECFCs exhibit a cobblestoned-shaped monolayer, an unequivocal endothelial gene signature, and expression of endothelial proteins. ECFCs have high proliferative capacity and can be expanded for up to 82 population doublings in 3 months, before reaching replicative senescence. From a single ECFC isolate, we can generate close to billion cells in less than two weeks. Purity of the ECFCs was confirmed by flow cytometry, showing the lack of hematopoietic markers(<1%) and expression of endothelial markers (>95%). ECFC potency was evaluated by functional assays including Matrigel 3D tubulogenesis, bead sprouting assay in Fibrin, and barrier properties.. We used microfluidic devices to demonstrate that ECFCs form a perfusable vascular network. The safety of ECFCs as a cell therapy product is implied by their normal karyotype, and intrinsic replicative senescence programme. ECFCs can be effectively cryopreserved for 7 years, and we are exploring novel approaches for their ambient transport. As proof-of-concept in vivo, we delivered ECFCs into different rodent models including Matrigel subcutaneous implant, the oxygen induced retinopathy (OIR) model, hind limb ischaemia (HLI) and wound healing model and demonstrate their vasoreparative potential
In summary, our data showcase ECFCs as a highly pure and potent cytotherapy, with a therapeutic role for vascular regeneration and repair and potential application in ischaemic diseases.

Poster Presentations

Number	Presenting Author	Title
1	Jessica Eyre	BMP-9 impedes 3D sprouting angiogenesis through molecular mechanisms that repress a tip cell phenotype
2	Kiran Mcloughlin	Use of Oxygel for the ambient transportation of endothelial colony forming cells (ECFCs) for cell therapy applications
3	Claire Tonry	Identification and evaluation of novel protein biomarkers for atrial fibrillation
4	Laura Gritti	Pattern recognition receptor NLRP3 regulates CNS progenitor cells after myelin damage
5	Amy Dumigan	In vivo single-cell transcriptomics reveal Klebsiella pneumoniae skews lung macrophages to promote infection
6	Aritra Deb	LINC00607: a potential marker for endothelial cell senescence
7	Peter Barabas	Diabetic retinopathy – targeting the source of acrolein, a reactive aldehyde.
8	David Hughes	Developing novel therapies for macular fibrosis secondary to neovascular age-related macular degeneration
9	Hong Guo-Parke	Cellular senescence in severe COPD bronchial epithelium impairs responses to human rhinovirus infection
10	Clara Radulescu	Comparative analysis of Nanopore and Illumina sequencing platforms for the genetic analysis of SARS-CoV-2 in clinical samples from Northern Ireland
11	Judith Lechner	Exploring the APC/EPCR pathway to protect the endothelial cell barrier in the context of diabetic retinopathy
12	Ryan Brown	Genetic deletion of SLPI promotes inflammatory cell recruitment in a model of chronic lung disease

Poster Presentations

Number	Presenting Author	Title
13	Joana Sa Pessoa	A trans-kingdom T6SS effector targets the mitochondria activating the innate receptor NLRX1 to promote infection
14	Josy Augustine	mRNA localisation: a subcellular mechanistic target to treat pathological angiogenesis
15	Orla Dunne	ATP Mediated Mechanisms Underlying Cough Hypersensitivity Syndrome in a Sensory Nerve Model.
16	Karla M. O'Neill	Defining a key role for dna methylation in determining angiogenic function of cord-blood derived endothelial colony forming cells
17	Steven Hancock	Deciphering the connection between the type VI secretion system and the lipopolysaccharide
18	Jasenka Guduric-Fuchs	Senescence of endothelial cells and their associated proinflammatory secretome promote age-related cardiovascular disease
19	Miriam Sartages	Characterising an uncommon Synaptotagmin protein in the vasculature tree
20	Kevin Edgar	Modulation of Tetranectin expression impacts cardiac fibroblast function and is associated with changes in cardiac fibrosis in-vivo.
21	Magdalini Eleftheriadou	Cardiac Organoids. A model to investigate the effect of Diabetes on cardiac development and function
22	Clare Mills	Investigating the use of novel host biomarkers in children with respiratory infections to create improved point-of-care diagnostic tests
23	Kevin Harkin	Evaluating the retinal neurovascular unit in the murine model of experimental Cerebral Malaria.
24	Guanbo Wang	Dissecting the role of infection-driven protein mono-glycosylation in Legionella-host interaction
25	Ahlam Ali	Repurposing Approved drugs as potent antiviral combinations to treat COVID-19 disease