QUEEN'S UNIVERSITY BELFAST

Irish Association of Pharmacologists

19th Annual Meeting

Friday, 30th November 2018

Hosted by

The Wellcome-Wolfson Institute for Experimental Medicine

I A P

Irish Association of Pharmacologists

BRITISH PHARMACOLOGICAL SOCIETY

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HSC Public Health Agency

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Welcome to Irish Association of Pharmacologists 2018

Dear Colleagues,

Whether you have travelled from nearby, further reaches of Ireland or even from across the water, we are delighted to welcome you to what promises to be a very stimulating meeting.

The programme contains state of the art lectures in areas considered of great relevance to healthcare. This year the focus is on translational pharmacology - identifying new approaches for the treatment of common conditions, such as those of the cardiovascular and respiratory systems, and cancer.

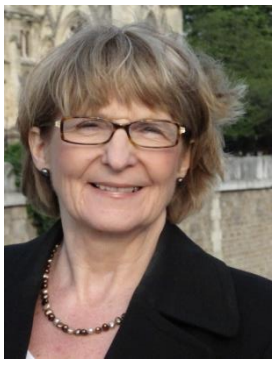
We have received submission of an excellent range of abstracts for free communication and these have been included in two sessions of oral presentations and then a dedicated poster session, to be enjoyed along with probably much needed refreshment at the end of the day. And we hope you also find ample opportunities for making new connections and catching up with colleagues and friends.

As an informal session, we have also scheduled a workshop led by our postgraduate student body and devised to facilitate discussion of the fundamental principles that can be applied to study design and data analysis. This is all so important in striving for that ground breaking paper in a top notch journal.

All IAP members are of course invited to attend the AGM: it is one of the most valuable ways to communicate with you as members and is an opportunity for you to learn about recent Association developments.

With all best wishes for an enjoyable meeting, from the local organisers

Barbara McDermott



Karen McCloskey

Irish Association of Pharmacologists 19th Annual Meeting 30th November 2018

Contributor/Event	Title	Time	Location
Welcome and Opening of Meeting	Welcome to Queen's University and the Wellcome-Wolfson Institute for Experimental Medicine - **Professor Jose Bengoechea (Institute Director)** Opening Address by IAP President & Head of Department Pharmacology & Therapeutics, UCC - **Professor Thomas Walther**	12:45-12.50 12.50-13.00	
Keynote Address One **Professor Jochen Prehn** Royal College of Surgeons in Ireland	**Chair: Professor Dan Longley, QUB** "*Apoptosis sensitisers for the therapy and treatment of colorectal cancer*"	13:00-13:30	
Short Oral Communications Dr Stephanie Annett, RCSI Dr Maria Llorián Salvador, QUB Dr Eoin Brennan, UCD Dr Derek Brazil, QUB	**Chair: Dr David Grieve, QUB** "*The role of FKBPL in LPS induced endothelial barrier dysfunction and macrophage activation*" "*VEGF-B protects in Müller cells under hypoxic and oxidative stress pathological conditions*" "*Novel drugs targeting inflammation in diabetic complications: harnessing the power of pro-resolving lipids*" "*Identification of novel small molecule inhibitors of Gremlin1, a secreted antagonist of bone morphogenetic proteins.*"	13:30-13:42 13:42-13:54 13:54-14:06 14:06-14:18	Basement Seminar Room
Keynote Address Two **Professor Amrita Ahluwalia** William Harvey Research Institute	**Chair: Professor David Williams, RCSI** "*Sodium nitrite as a cardioprotective strategy: Not lost in translation*"	14:20-14:50	
Tea / Coffee served in Foyer			
Postgraduate student-led workshop facilitated by Professor Amrita Ahluwalia, Chief Editor of British Journal of Pharmacology	**Chair: Ms Niamh McKerr, QUB** *Good practices for scientific publication*	14:50-15:20	Atrium

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Contributor/Event	Title	Time	Location
IAP AGM	**Irish Association of Pharmacologists Annual Meeting (Members Only)**	14:50-15:20	Ground Floor Board Room
Short Oral Communications	**Chair: Professor Martina Hennessy, TCD**		Basement Seminar Room
Ms Aisling Heeran, TCD	"*Investigating the effect of radiation on the ex vivo normal rectal and rectal cancer secretome and the effect of the secretome on cellular metabolism*"	15:20-15:32	
Mr Andrew Moore, UCC	"*Determining a fingerprint of peptidase activity to develop medications to counteract resistance to Sunitinib in Renal Cell Carcinoma*"	15:32-15:44	
Dr Patrick Gallagher, QUB	*"Evaluation of intravitreal anti-vascular endothelial growth factor injections on renal function in patients with diabetic macular oedema*"	15:44-15:56	
Dr Cormac Kennedy, TCD	"*A review of oral anticoagulation prescribing in Ireland*"	15:56-16:08	
Keynote Address Three **Professor Danny McAuley** Consultant in Intensive Care Medicine at the Royal Victoria Hospital and Professor at Queen's University Belfast	**Chair: Dr Anne-Marie Liddy, TCD** "*Pharmacological treatment for the acute respiratory distress syndrome: experimental models through to clinical trials*"	16:10-16:40	
Drinks & Snacks served in Atrium			
Poster Session	**Poster Session**	16:40-17:20	Atrium
Prize Giving	**Prize Giving & Close**	17:20-17:30	Atrium

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LIST OF POSTERS

Poster	Abstract Summary
1	Ghrelin enhances GLP-1 induced neuronal activation in the distal colon. Buckley MM, et al. UCC
2	Exendin-4/gastrin/xenin-8-Gln: a novel hybrid peptide that improves diabetic status in high fat fed mice when administered in combination with a stable GIP agonist. Craig SL, et al. QUB
3	Targeting macrophage GLP-1 signalling as an emerging therapy to ameliorate cardiac remodelling associated with diabetes. Abudalo RA, et al. QUB
4	Treatment with fatty acid modified analogues of apelin-13 improves glycaemic control and lipid profiles in diet induced obese diabetic mice. Lo TH, et al. UU
5	C-terminal dipeptide truncation of PYY(1-36) and PYY(3-36) occurs naturally in the circulation and severely diminishes biological activity. Lafferty RA, et al. UU
6	Investigating the therapeutic effects of L-Ergothionine as a treatment for pre-eclampsia. McCarthy C, et al. UCC
7	Prolonging Transplant Survival Through c-FLIP Inhibition and Enhanced allo-T cell Apoptosis. Costello R, et al. QUB
8	The effect of current standards of care on immune checkpoint expression in oesophageal adenocarcinoma. Davern M, et al. TCD (not published in online programme)
9	Unravelling the Role of Cathepsin S in DNA Damage Response Deficient Tumours. Gallagher S, et al. QUB
10	Assessment of Platelet Activation Status and Platelet Reactivity in Multiple Myeloma, Smouldering Myeloma and MGUS Patients Pre-Treatment and During Anti-Myeloma Treatment. O'Sullivan L, et al. UCC
11	Activation and expression of opioid receptors and the glycine beta receptor have opposing effects on the metastatic behaviour of human breast cancer cells. Xue C, et al. UCD
12	Modelling Sub-retinal fibrosis in neovascular Age-related Macular Degeneration. Little K, et al. QUB
13	Developing Molecular Therapies for Primary Open Angle Glaucoma. Lester K, et al. UU
14	Pharmacological characterisation of bradykinin- and thrombin-induced Ca^{2+} signalling in retinal microvascular endothelial cells. Cincola' P, et al. QUB
15	Fingolimod in Experimental Intracerebral Haemorrhage - Are Most Published Research Findings Indeed False? Diaz ACD, et al. UCC
16	Neprilysin degrades murine Aβ more efficiently than human Aβ: Further implication for species-specific amyloid accumulation. Moore A, et al. UCC
17	In vitro characterisation of truncated tau production: Relevance to Alzheimer's disease. Li M, et al, UCC (not published in online programme)
18	Anticholinergic Burden in Acute Coronary Syndrome Elderly Patients – A Retrospective Observational Case Cohort Study. Doyle K, et al. UCC

Keynote Address: Professor Jochen Prehn, Royal College of Surgeon's in Ireland, Dublin, Ireland.

Prof Jochen Prehn, Dr. rer. nat.
Contact Details: Department of Physiology and Centre for Systems Medicine, Royal College of Surgeons (RCSI), 123 St. Stephens Green, Dublin 2, Ireland.
Email: prehn@rcsi.ie

Research Expertise: Apoptosis, Mitochondria, Biomedical and translational systems biology research, Colorectal Cancer

Profile:
Prehn heads up a large, highly skilled and motivated research team at the interface of biomedical and translational systems biology research and focusing on oncology, neuroscience, and metabolic disorders at the CSM (www.systemsmedicineireland.ie) RCSI. He was the first recipient of the Science Foundation Ireland Research Professorship award in 2003 and is considered an international authority on the single-cell analysis and the molecular control of apoptosis, cell death and mitochondrial dysfunction. Prof Prehn has successfully conducted research in these four areas with a particular emphasis on Bcl-2 family proteins and AMPK signalling. A second major research interest lies in real-time imaging of cell death signals in neurons and cancer cells, employing confocal and in vivo imaging techniques. This research includes the development of computational approaches to understand and overcome apoptosis sensitivity and resistance and cellular bioenergetics at a systems rather than single entity level. In collaboration with clinicians (pathology, medicine, and surgery), Prof Prehn translated these approaches into clinically relevant settings, and has initiated multiple large-scale, multi-partner clinical projects such as the APO-COLON and APO-DECIDE clinical studies.

Research Funding History: Excess of €20 Mio in peer-reviewed research funding as PI

Publication Record:
Number of publications: 210
Number of citations: 15,230
H-index 68
Source Google Scholar

"Apoptosis sensitisers for the therapy and treatment of colorectal cancer"

Resistance to apoptosis is a hallmark of cancer. In my presentation I will discuss recent strategies to understand apoptosis resistance holistically, and present novel strategies focusing on BCL-2 antagonists and IAP inhibitors to overcome this resistance in colorectal cancer and breast cancer. I will present system medicine approaches developed by our team that model the biochemical pathways of apoptosis initiation and execution, and discuss their role as stratification tools for apoptosis sensitisers.

Dr Stephanie Annett, Molecular and Cellular Therapeutics, Royal College of Surgeon's in Ireland, Dublin, Ireland.

"The role of FKBPL in LPS induced endothelial barrier dysfunction and macrophage activation"

S. L. Annett[1], S. Spence[2], C. Garciarena[3], S. Kerrigan[3], A. Kissenpfennig[2], T. Robson[1].

[1]Molecular and Cellular Therapeutics, Royal College of Surgeons Ireland, Dublin, Ireland, [2]Centre for Experimental Medicine, Queen's University Belfast, Belfast, United Kingdom, [3]School of Pharmacy, Royal College of Surgeons Ireland, Dublin, Ireland.

Introduction Loss of endothelial barrier function and excessive inflammation contributes to organ failure in sepsis patients. FKBPL is a divergent member of the immunophilin protein family and it has potent anti-angiogenic activity mediated through the cell surface receptor, CD44 (1 – 3). Furthermore, FKBPL $^{+/-}$ mice exhibit enhanced endothelial permeability (4). We investigated the ability of FKBPL to control endothelial barrier function and macrophage activation in LPS-induced sepsis.

Methods *In vitro* endothelial permeability assays and immunofluorescence were used to analyze barrier permeability in siFKBPL transfected human microvascular endothelial cells (HMECs) after LPS stimulation. NFκB signalling in siFKBPL HMECs was investigated by western blot. BMDMs extracted from C57/6N mice were treated with LPS ± rFKBPL and gene expression analyzed using qPCR. *In vivo* LPS survival in Fkbpl$^{+/-}$ and Fkbpl$^{+/+}$ mice was assessed.

Results *In vitro* endothelial permeability assays demonstrate that FKBPL knockdown in HMECs leads to increased barrier permeability; supporting our mouse studies. Real-time impedance measurements and immunofluorescence studies demonstrate that FKBPL knockdown in HMECs results in greater barrier dysfunction in response to LPS and reduced VE-cadherin mediated cell-cell contacts. FKBPL knockdown in HMECs results in increased phosphorylation of p65 in response to LPS stimulation. LPS stimulated BMDMs treated with rFKBPL have reduced expression of the pro-inflammatory cytokines, IL-1β, IL-18, IL-6, COX2, TNFα, NLRP3, NFKB1, STAT3. Finally, *in vivo* studies demonstrate that Fkbpl$^{+/-}$ mice have significantly reduced survival following LPS administration.

Conclusion FKBPL has a potential role in protection against LPS-induced sepsis via modulation of NFκB pathway. This could be partly mediated by controlling endothelial barrier function and macrophage activation.

References:

1. Robson, T., & James, I. F. (2012). *Drug Discovery Today*, *17*(11–12), 544–548.
2. Valentine, A., et al (2011). *Clinical Cancer Research, 17 (5),* 17 (5), 1044-56
3. Yakkundi, A., et al. (2015).. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *35*(4)
4. Yakkundi, A. et al (2013). *PloS One*, *8*(2), e55075.

Dr Maria Llorián-Salvador, Wellcome Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK.

"VEGF-B protects in Müller cells under hypoxic and oxidative stress pathological conditions"

M. Llorián-Salvador[1], J. Lechner[1], J. Augustine[1], M. Chen[1], H. Xu[1].

[1]Welcome-Wolfson Institute of Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast, UK.

i) Müller cells play an important role in retinal pathophysiology. Müller cell-derived vascular endothelial growth factor (VEGF) is critically involved in retinal cell survival and function, although pathological levels of VEGF can induce the breakdown of the blood retinal barrier leading to macular oedema. The role of Müller cell-derived VEGF-A isoform in retinal health and disease has been studied extensively. Whereas, the role of the different VEGF isoforms (A-D) in retinal pathophysiology remains poorly-defined.

ii) VEGF expression profile in primary murine Müller cells and an immortalized cell line QMMuC1 along with the role of VEGF in Müller cell activation and functions have been examined. Glial fibrillary acidic protein (GFAP), water and ion channels AQP4 and Kir4.1 and GLAST and Glutamine Synthetase (GS) expression were measured by RT-PCR and Western Blot.

iii) VEGF-B was the highest expressed VEGF member, along with its receptors VEGFR1 and NRP1 co-receptor. VEGF-B neutralization did not affect the viability, functionality or induce gliosis in Müller cells in normal conditions. Hypoxia and oxidative stress (4HNE) significantly altered the expression of VEGF-B and its receptors in QMMuC1 cells. Blocking VEGF-B and/or its receptors compromise cell survival under hypoxia or oxidative stress. VEGF-B neutralization decreased Kir4.1 and AQP4 expression under these stress conditions, suggesting a role of VEGF-B in water and ion maintenance. Furthermore, the addition of recombinant VEGF-B effectively restored normal expression of GS under hypoxic conditions, indicating a positive effect in glutamate clearance exerted by this growth factor.

iv) VEGF-B is an important neurotrophic growth factor for Müller cells.

Dr Eoin Brennan, UCD Diabetes Complications Research Centre, UCD Conway Institute of Biomolecular and Biomedical Research, UCD School of Medicine, University College Dublin, Dublin, Ireland.

"Novel drugs targeting inflammation in diabetic complications: harnessing the power of pro-resolving lipids."

E.P. Brennan[1,2], M. Mohan[2,3], M. de Gaetano[1], M. Marai[1], A. Cacace[1], D. Andrews[1], D. Crean[4], S. Sheehan[5], J.F. Dowdall[5], M. Barry[5], O. Belton[6], S. Tasadaque Ali-Shah[7], P.J. Guiry[7], K. Jandeleit-Dahm[2,3], M.E. Cooper[2,3], P. Kantharidis[2,3] and C. Godson[1].

[1]UCD Diabetes Complications Research Centre, UCD Conway Institute of Biomolecular and Biomedical Research, UCD School of Medicine, University College Dublin, Dublin, Ireland. [2] JDRF Danielle Alberti Memorial Centre for Diabetes Complications, Diabetes Division, Baker IDI Heart and Diabetes Institute, Melbourne, Australia.[3]Department of Diabetes, Central Clinical School, Monash University, Clayton, Victoria, Australia. [4]School of Veterinary Medicine, University College Dublin, Dublin, Ireland. [5]Department of Vascular Surgery, St. Vincent's University Hospital, Dublin, Ireland. [6]School of Biomolecular and Biomedical Science, University College Dublin, Dublin, Ireland. [7]Centre for Synthesis and Chemical Biology, UCD School of Chemistry and Chemical Biology, University College Dublin, Dublin, Ireland.

Failure in the resolution of inflammation is believed to drive the chronic 'sterile' inflammatory state frequently observed in complex disease, including cardiovascular disease, diabetes, obesity and cancer. It has been proposed that the same mechanisms that induce inflammation also program its resolution, via the generation of endogenous specialized pro-resolving lipid mediators (SPMs) including Lipoxins (LXs). It is now known that LXs signal through a specific receptor (ALX/FPR2), prompting an interest in designing synthetic molecules capable of agonism at this receptor. Here, we investigated whether endogenous LXA4 and a chemically synthesized LX mimic (Benzo-LXA4) can modulate diabetic complications in the streptozotocin-induced diabetic ApoE$^{-/-}$ mice.[1-2]

In mice with established disease, treatment with LXs led to a significant reduction in aortic plaque development, and attenuated inflammatory responses, including the expression of *vcam1*, *mcp-1*, *il-6*, and *il-1b*. Secretome profiling of human carotid plaque explants treated with LXs indicated changes to proinflammatory cytokine release, including tumor necrosis factor-a and interleukin-1b. LXs also preserved kidney function, as evidence by an attenuation in the development of diabetes-induced albuminuria, mesangial expansion, and collagen deposition. Comprehensive analysis of the renal transcriptome by RNAseq identified transcriptional networks modulated by LXs. These data suggest that LXs may have therapeutic potential in the context of diabetes-associated vascular complications.

References:

1. Fullerton, J. Resolution of inflammation: a new therapeutic frontier. *Nature reviews. Drug discovery* **15**, 551-567(2016).
2. Brennan, E. Lipoxins Protect Against Inflammation in Diabetes-Associated Atherosclerosis. *Diabetes*.db17-1317(2018).
3. Brennan, E. Lipoxins Regulate the Early Growth Response-1 Network and Reverse Diabetic Kidney Disease. *JASN*.29;1437-1448(2018).

Keynote Address: Professor Amrita Ahluwalia, William Harvey Research Institute, London, UK.

Amrita Ahluwalia is Professor of Vascular Pharmacology and Co-Director of the William Harvey Research Institute, Bart's and the London School of Medicine and Dentistry at QMUL. Her research focuses on enhancing understanding of the inflammatory processes involved in diseases of the cardiovascular system and in this way identifying novel therapeutic targets. As well as her interests in the cardiovascular system she is committed to establishing gender equality in the work place. Amrita established the first national mentoring scheme for women of a learned society (British Pharmacological Society) in 2005 in addition to a number of other equality initiatives including establishment of the AstraZeneca Prize for women in Pharmacology given by the BPS. She is the current Editor-In-Chief of British Journal of Pharmacology and through this role has led numerous initiatives in improving transparency standards and reproducibility.

"Sodium nitrite as a cardioprotective strategy: Not lost in translation."

A major research focus of her group is the study of the bioactivity of the reductive nitrate-to nitrite to NO pathway, often dubbed the enterosalivary circuit of inorganic nitrate. Prof Ahluwalia's group has made seminal discoveries in the field not least the cardioprotective actions of nitrite and the blood pressure lowering efficacy of dietary nitrate.

Postgraduate student-led workshop facilitated by Professor Amrita Ahluwalia, Chief Editor of the British Journal of Pharmacology

Chair: Ms Niamh McKerr

"*Good Practices in Scientific Publication*"

The British Journal of Pharmacology (BJP) is a broad-based journal giving leading international coverage of all aspects of experimental pharmacology. It publishes high quality original research and authoritative reviews. Each year a range of themed issues are published and a must-read supplement, the Concise Guide to Pharmacology, is published biennially.

The content covers all major organ systems and diseases and is read by pharmacologists, toxicologists, pharmaceutical researchers, medicinal chemists, molecular biologists, physiologists, neuroscientists, target discovery and drug discovery researchers, pharmacists and clinicians.

In 2015, BJP issued new guidelines for authors to raise the transparency and reproducibility of published research and more recently has provided updates on experimental design, analysis and their reporting, goals and practicalities of immunoblotting and immunohistochemistry, and data sharing including the use of scatter plots instead of bar charts.

Full information is available at:
https://bpspubs.onlinelibrary.wiley.com/hub/journal/14765381/journal-resources/policy-editorials.html

The session will be conducted in an informal question/answer format exploring the above and also any general issues brought out by attendees, who should leave with a better understanding of how to go about getting their research published in any highly rated journal.

Ms Aisling Heeran, Department of Surgery, Trinity Translational Medicine Institute, Dublin, Ireland.

"Investigating the effect of radiation on the ex vivo normal rectal and rectal cancer secretome and the effect of the secretome on cellular metabolism."

A. Heeran[1], M. Dunne[1], M. Morrissey[1], H. Berrigan[1], C. Buckley[1], A. Buckley[1], N. Clarke[1], A. Cannon[1], C. Dunne[2], J. Larkin[2], N. Lynam-Lennon[1], J. O'Sullivan[1].

[1]Department of Surgery, Trinity Translational Medicine Institute, Dublin. [2]St. James's Hospital, Dublin 8.

Neoadjuvant-chemoradiotherapy is standard of care for rectal cancer (RC), however 80% of patients either achieve a partial or no response to treatment. Upregulation of inflammation and metabolic reprogramming is associated with a poor response to neoadjuvant-chemoradiotherapy (1) (2) (3). A comprehensive profile of inflammatory mediators released from RC and normal rectal (NR) tissue or the effect of this secretome on cellular metabolism has not previously been investigated, nor do we know the effect of radiation on the inflammatory secretome or cellular metabolism.

Following patient consent, fresh human RC and NR tissue were cultured *ex vivo* and mock-irradiated or irradiated with 1.8Gy radiation. Following 24hours, the tissue conditioned media was collected and screened for the expression of 54 inflammatory mediators using MSD-multiplex system to determine if inflammatory secretions differ between RC and NR tissue. The effect of the tissue secretome on SW837 RC cell metabolism was investigated using the Seahorse XFe24 Analyser (Aligent Technologies).

A significant increase in GM-CSF, MIP1α, MIP1β, IL-1RA, IL-1α, MDC, TSLP, IL-17A, IL-6 and IP-10 ($p<0.05$) was observed in RC tissue compared to NR tissue. Radiation may alter the secretion of bFGF and IL-16 in NR tissue and IL-15 and IL-16 in RC tissue. Both the irradiated NR and mock-irradiated RC secretome induce significant metabolic alterations in SW837 RC cells compared to the mock-irradiated NR secretome.

Several inflammatory pathway components show alterations between NR and RC tissue. Furthermore, the inflammatory secretome may be altered post-irradiation and this may affect cellular metabolism. This may reveal novel therapeutic targets for radio-sensitizing agents.

References:

1. Laine A, Iyengar P, Pandita TK. The Role of Inflammatory Pathways in Cancer-Associated Cachexia and Radiation Resistance. Mol Cancer Res [Internet]. 2013 Sep 20;11(9):967–72. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4133095/
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3. Zannella VE, Pra AD, Muaddi H, McKee TD, Stapleton S, Sykes J, et al. Reprogramming Metabolism with Metformin Improves Tumor Oxygenation and Radiotherapy Response. Clin Cancer Res [Internet]. 2013 Oct 18; Available from: http://clincancerres.aacrjournals.org/content/early/2013/11/22/1078-0432.CCR-13-1787.abstract

Mr Andrew Moore, Department Pharmacology & Therapeutics, University College Cork, Cork, Ireland.

"Determining a fingerprint of peptidase activity to develop medications to counteract resistance to Sunitinib in Renal Cell Carcinoma."

A. Moore[1], P. Khanna[2], R. Bhatt[2], and T. Walther[1].

[1]Department of Pharmacology and Therapeutics, School of Medicine and School of Pharmacy, University College Cork, Cork, Ireland. [2]Division of Hematology-Oncology and Cancer Biology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA.

Renal cell carcinoma is one of the top ten most common cancers in both men and women. Beside programmed death-1 pathway (PD-1) inhibitors, agents targeting vascular endothelial growth factor (VEGF) receptors, such as Sunitinib, are the main treatment options. Unfortunately, about 70% of the patients on VEGF targeted therapies develop hypertension. Furthermore, treatment success is limited, since resistance to VEGF inhibitors ultimately develops at a median within one year of therapy. Thus, the development of novel targets to sustain the efficacy of VEGF inhibitors could improve the treatment.

It is well-known that the renin-angiotensin system (RAS) plays a key role in cardiovascular health including regulating hypertension. In particular, the angiotensin (Ang)-(1-7) /Ang II ratio is one of the most important metrics within the RAS. In this study, we aimed to measure the activity of several peptidases generating or truncating Ang peptides (such as ACE, ACE2, APA, NEP, etc.) in RCC tumours collected from mice with and without Sunitinib.

Sunitinib treatment led to faster degradation of Ang-(1-7) and the increased degradation was related to both an increase in amino peptidase activity and ACE. Analyses of Ang II degradation discovered less ACE2-like activity and confirmed an increase in amino peptidase activity.

Taken together, our experiments show that Sunitinib reduces the amount of Ang-(1-7) present in the tumours by upregulating Ang-(1-7) degrading peptidases and downregulating peptidases generating the heptapeptide. The results open the avenue to specific pharmacological interventions to be used alongside the VEGF receptor inhibitors to develop powerful new treatment options.

Dr Patrick Gallagher, Centre for Public Health, Queen's University Belfast, Belfast, UK.

"Evaluation of intravitreal anti-vascular endothelial growth factor injections on renal function in patients with diabetic macular oedema."

P. Gallagher[1], T. Douglas[2], J.A. Little[2], G. Silvestri[3], G.J. McKay[1].

[1]Centre for Public Health, Queens University Belfast, Belfast. [2]Biomedical Sciences Research Institute, Ulster University, Belfast. [3]Department of Ophthalmology, Belfast Health and Social Care Trust, Belfast.

Administering a small dosage of anti-vascular endothelial growth factor (anti-VEGF) by intraocular injection has given these agents a very safe systemic profile (1). Nevertheless, systemic effects and several incidents of acute kidney injury following anti-VEGF injection have been reported (2-4). We assessed the long-term effect of multiple intravitreal anti-VEGF injections on the rate of change of estimated glomerular filtration rate (eGFR). A total of 92 patients with diabetic macular oedema (57.6% male, 78.3% type 2 diabetes mellitus [T2DM]) were included in a retrospective audit in which electronic healthcare records were assessed. The mean duration of diabetes was 16.1 years and mean HbA1c was 66.9 mmol/mol. A high prevalence of co-morbidities existed with 82.6%, 76.1% and 33.7% of patients having hypertension, hyperlipidaemia and chronic kidney disease, respectively. On average, 26.9 intravitreal anti-VEGF injections were given per patient over a mean duration of 31 months. Renal function declined from a mean baseline eGFR of 75.4 ± 21.0 ml/min/1.73m^2 to a follow up eGFR of 66.6 ± 22.7 ml/min/1.73m^2. However, no association between increasing number of intravitreal anti-VEGF injections and rate of eGFR decline was detected (beta = 0.035; p=0.206, confidence intervals [CI]: -0.2, 0.89), which remained non-significant following adjustment for hypertension, cerebrovascular disease and T2DM (beta = 0.036; p=0.198, CI: -0.019, 0.092). This audit suggests regular long-term intravitreal VEGF inhibition does not significantly alter the rate of eGFR beyond that of natural decline. Further evaluation of renal safety of intravitreal anti-VEGF injections is warranted, particularly in a larger sample of high-risk groups.

References:

1. A Phase II Randomized Clinical Trial of Intravitreal Bevacizumab for Diabetic Macular Edema. Ophthalmology. 2007;114(10):1860-1867.e7.
2. Georgalas I, Papaconstantinou D, Papadopoulos K, Pagoulatos D, Karagiannis D, Koutsandrea C. Renal injury following intravitreal anti-VEGF administration in diabetic patients with proliferative diabetic retinopathy and chronic kidney disease--a possible side effect? Curr Drug Saf. 2014;9(2):156-8.
3. Huang Y, Chen S, Hsu M, Hwang D. Acute renal failure after intravitreal antivascular endothelial growth factor therapy. Journal of the Formosan Medical Association. 2017;116(6):490-492.
4. Cheungpasitporn W, Chebib FT, Cornell LD, Brodin ML, Nasr SH, Schinstock CA et al. Intravitreal Antivascular Endothelial Growth Factor Therapy May Induce Proteinuria and Antibody Mediated Injury in Renal Allografts. Transplantation. 2015; 99(11):2382-6.

Dr Cormac Kennedy, Department of Pharmacology and Therapeutics, Health Sciences Centre, Trinity College Dublin, Ireland.

"A review of oral anticoagulation prescribing in Ireland."

C.A. Kennedy[1], S. Lucey [1,2], M. Barry [1,2].

[1]Department of Pharmacology and Therapeutics, Health Sciences Centre, Trinity College Dublin, Ireland. [2]Medicines Management Program, Health Services Executive, Dr Steevens' Hospital, Dublin 8, Ireland.

Introduction Warfarin has been the mainstay of oral anticoagulant (OAC) therapy for decades. More recently, direct oral anticoagulants (DOACs) have become available. International evidence suggests DOAC are now replacing warfarin as first choice anticoagulant for stroke prevention.[1]

Aim To review the prescribing of DOACs, as well as the associated cost, in the Republic of Ireland.

Methods This study reviewed data from the Health Service Executive-Primary Care Reimbursement Scheme (HSE-PCRS) pharmacy claims database. The reimbursement data for oral anticoagulants was identified using WHO Anatomical Therapeutic Chemical classification code. The patient and expenditure data of warfarin, apixaban, dabigatran, edoxaban and rivaroxaban for 2014 was compared to that for 2017.

Results An average of 48,068 patients prescribed OACs each month in 2014. This increased by 41% to 65,832 per month in 2017. The number of patients prescribed warfarin as a proportion of the total number on OAC decreased from 68% to 32% when 2014 was compared to 2017. Conversely, a three-fold increase in patients prescribed DOACs was evident for this period, with a 36% rise in patients prescribed DOAC as a proportion of all patients prescribed OACs. For the same period the expenditure on DOACs increased by almost three-fold, thus increasing total OAC expenditure by a multiple of 2.25.

Conclusion The period 2014 to 2017 demonstrated an increase in patients prescribed DOACs, with a resulting increase in total patients on OACs. This change in practice, however, involved a large increase in health expenditure which has yet to be justified by real world data.

References:

1. Admassie E, Chalmers L, Bereznicki LR. Changes in Oral Anticoagulant Prescribing for Stroke Prevention in Patients With Atrial Fibrillation. Am J Cardiol. 2017 Oct 1;120(7):1133-1138.

Keynote Address: **Professor Danny McAuley, Royal Victoria Hospital, Belfast and Queen's University Belfast, Belfast, UK.**

Danny McAuley is a Consultant and Professor in Intensive Care Medicine at the Regional Intensive Care Unit at the Royal Victoria Hospital and Queen's University of Belfast. He undertook his training in Belfast, Birmingham, London and San Francisco. He undertook his MD in the Department of Therapeutics and Pharmacology at Queen's University of Belfast. He is Co-Director of Research for the UK Intensive Care Society.

His research strategy has 2 major themes focusing on Acute Respiratory Distress Syndrome (ARDS) and clinical trials. His research strategy in ARDS is to investigate potential novel therapeutic agents in *in vitro*, *in vivo* in clinically relevant models of ALI and in phase 2 clinical trials to inform subsequent phase 3 clinical trials. His other main research interest is phase 2/3 clinical trials in the critically ill.

"Pharmacological treatment for the acute respiratory distress syndrome: experimental models through to clinical trials."

Often phase 3 clinical trials are based on small pilot studies with inadequate phase 2 trial data and limited mechanistic data to provide a sound scientific rationale.

To identify novel treatments for the acute respiratory distress syndrome (ARDS), there is a need to investigate these agents initially in relevant human models of ARDS to inform subsequent clinical trials. A series of models of ARDS will be described including an ex vivo perfused human lung model, inhaled endotoxin-induced ARDS in healthy subjects, one lung ventilation as a model of direct ARDS and ischaemia-reperfusion induced lung injury (in the setting of elective abdominal aortic aneurysm repair) as a model of indirect ARDS.

Finally, on the basis of current data, evidence will be presented for novel therapeutic agents as potential therapeutic agents in ARDS. In vitro data as well as data from observational studies, models of ARDS and early phase clinical trials will be presented.

POSTER PRESENTATIONS

STAND ONE:

Dr Maria Buckley, Department Pharmacology & Therapeutics, Department of Physiology, APC Microbiome Institute, University College Cork, Cork, Ireland.

"Ghrelin enhances GLP-1 induced neuronal activation in the distal colon."

M.M. Buckley[1,2,3], R. O'Brien[2] and D. O'Malley[1,2].

[1]APC Microbiome Institute, [2]Department of Physiology, [3]Department of Pharmacology & Therapeutics, University College Cork, Cork Ireland.

Irritable Bowel Syndrome (IBS), a chronic condition characterised by cramping, abdominal pain, constipation and/or diarrhoea, afflicts 10-20% of the population with symptoms exacerbated following a meal. Both the orexigenic hormone, ghrelin and the incretin hormone, glucagon like peptide-1 (GLP-1) been implicated in gut motility and thus, may be important in post-prandial exacerbation of IBS symptoms.

Colonic myenteric plexi were prepared from male Sprague Dawley (SD) rat controls and Wistar Kyoto (WKY) rats, which are an animal model of IBS. Real-time calcium imaging experiments on myenteric neurons were conducted using a standard epifluorescence imager. Distal colon with intact vagal innervation from SD and WKY rats were placed in a tissue bath and exposed colonic myenteric neurons were stimulated and nerve activity from the vagus was recorded using a bipolar electrode.

GLP-1 induced a small increase in intracellular calcium levels in myenteric neurons of both SD and WKY rats, whereas prior exposure to ghrelin enhanced the GLP-1 evoked response in both SD ($n=24$, $p<0.01$) and WKY ($n=40$, $p<0.001$) rats. Exposure of colonic myenteric neurons to GLP-1 and ghrelin in both SD and WKY also stimulated vagal nerve firing and the GLP-1-evoked neural response was potentiated by prior exposure to ghrelin in both rat strains (SD: $p<0.001$, WKY: $p<0.01$).

Prior exposure to ghrelin, as may occur during the ghrelin peak prior to food ingestion, appears to sensitise colonic myenteric neurons to the neuro-stimulatory effects of GLP-1. Moreover, sensitisation of myenteric neurons by exposure to ghrelin enhances the gut-to-brain signalling via the vagus nerve.

STAND TWO:

Ms Sarah Craig, School of Biomedical Sciences, Ulster University, Coleraine, UK.

"Exendin-4/gastrin/xenin-8-Gln: a novel hybrid peptide that improves diabetic status in high fat fed mice when administered in combination with a stable GIP agonist."

S.L. Craig[1], A. Hasib[1], M.T. Ng[1], P.R. Flatt[1], V.A. Gault[1], N. Irwin[1].
[1]School of Biomedical Sciences, Ulster University, Coleraine, UK.

Background and aims: Enteroendocrine derived hormones such as gastrin, glucagon-like-peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP) and xenin are known to exert complementary beneficial metabolic effects in diabetes. The present study characterised a novel fusion peptide, exendin-4/gastrin/xenin-8-Gln, and evaluated therapeutic utility in combination with the GIP receptor agonist, (DAla^2)GIP, in high fat fed mice. **Materials and methods**: Exendin-4/gastrin/xenin-8-Gln was synthesised by coupling residues 1-28 of exendin-4, with gastrin-6 and xenin-8-Gln, using 8-amino-3,6-dioxaoctanoic acid linker molecules. The peptide was incubated with murine plasma (n=4) to assess enzyme stability. BRIN-BD11 cells were used to evaluate insulinotropic activity of exendin-4/gastrin/xenin-8-Gln (10^{-12}-10^{-6} M), with GLP-1, neurotensin, and CCK-B receptor antagonists employed to ascertain insulin secretory receptor balance. Acute effects of the fusion peptide on food intake, glucose and insulin concentrations were examined in lean mice (n=8). High fat fed (HFF) mice (n=8) were used to assess chronic effects of exendin-4/gastrin/xenin-8-Gln alone, and in combination with (DAla^2)GIP, (each peptide at 25 nmol/kg; *ip*) using a twice-daily injection regimen for 21 days. Body weight, glucose and insulin concentrations were measured every 3 days. Oral glucose tolerance (18 mmol/kg), metabolic response to GIP (25 nmol/kg; *ip*) and insulin sensitivity (10 U/kg; *ip*) were determined at the end of the study. Plasma lipid, glucagon and amylase activity were also assessed on day 21. **Results:** Exendin-4/gastrin/xenin-8-Gln was enzyme resistant and enhanced (P<0.001) insulin secretion from BRIN-BD11 cells, with GLP-1 and neurotensin receptor pathways being important. Acute injection of exendin-4/gastrin/xenin-8-Gln in combination with glucose significantly (P<0.001) lowered glucose and increased insulin concentrations in mice, with antihyperglycaemic effects evident (P<0.001) 8 h post-injection. Exendin-4/gastrin/xenin-8-Gln also induced significant (P<0.001) appetite suppressive effects. Administration of exendin-4/gastrin/xenin-8-Gln alone, or in combination with (DAla^2)GIP, twice daily for 21 days in HFF mice, reduced (P<0.01) percentage body fat compared to saline controls. The treatment regimens significantly (P<0.05-P<0.001) decreased circulating glucose and increased insulin concentrations, with no impact on glucagon levels or amylase activity. Exendin-4/gastrin/xenin-8-Gln in combination with (DAla^2)GIP also reduced (P<0.05) LDL-cholesterol levels. In addition, the combined treatment group presented with clear improvements in glucose tolerance, which was superior to either treatment alone. Similarly, GIP-induced reductions in blood glucose and elevations of insulin were enhanced (P<0.05-P<0.01) by treatment with exendin-4/gastrin/xenin-8-Gln in combination with (DAla^2)GIP. All treatment groups had superior (P<0.05-P<0.001) glucose-lowering actions in response to exogenous insulin administration. **Conclusions**: Exendin-4/gastrin/xenin-8-Gln is a fusion peptide with clear antidiabetic potential. Efficacy was improved through concurrent administration of a stable GIP molecule, adding support to the promise of multi-targeting peptides for the treatment of diabetes.

STAND THREE:

Ms Rawan Abudalo, Wellcome Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK.

"Targeting macrophage GLP-1 signalling as an emerging therapy to ameliorate cardiac remodelling associated with diabetes."

R.A. Abudalo[1], K. S. Edgar[1], K.M. O'Neill[1], A. Moez[1], B.D. Green[2], D.J. Grieve[1].

[1]Wellcome Wolfson Institute for Experimental Medicine, [2]Institute of Global Food Security, Queen's University Belfast, Belfast, UK.

Background: The incidence of cardiovascular complications in diabetes and associated morbidity and mortality are increasing alarmingly. Hyperglycaemia-induced inflammation specifically contributes to extracellular matrix (ECM) changes and diastolic dysfunction which accelerate heart failure progression and are common in diabetic patients. Glucagon-like peptide (GLP-1) is an incretin hormone that plays a crucial role in glucose homeostasis and is targeted therapeutically in hyperglycaemia (e.g. liraglutide), which also demonstrates cardioprotective actions specifically involving modulation of inflammation and ECM remodelling. The aim of this study was to investigate precisely how GLP-1 receptor (GLP-1R) activation affects inflammatory signalling in experimental diabetes.

Methods: Mouse bone marrow-derived macrophages (BMDM) were exposed to normal (5.5mM) or high glucose (25mM) in the presence/absence of the GLP-1R agonist liraglutide (1nM), to assess effects on cellular and secreted cytokine/chemokines (real-time RT-PCR, proteome array). Effects on paracrine signalling were interrogated by incubating conditioned media from glucose/liraglutide-treated BMDM with mouse *NIH/3T3* cardiac fibroblasts prior to analysis of markers of myofibroblast differentiation (real-time RT-PCR, western blot).

Results: BMDM, but not cardiac fibroblasts, were confirmed to express the GLP-1R. Interestingly, liraglutide exerted differential effects on macrophage cytokine expression under both normoglycaemic and hyperglycaemic conditions (e.g. IL-1β, CCL2, NOS2) and altered glucose-induced cytokine/chemokine secretion (e.g. CXCL10, TNF-α, CCL12). Furthermore, liraglutide specifically attenuated myofibroblast differentiation (α-SMA, CTGF) and inflammation (COX-2, IL-6) in high but normal glucose conditions.

Conclusion: Taken together, these results indicate that activation of GLP-1 signalling in vitro promotes specific actions on macrophages, which may represent a novel strategy to improve cardiovascular outcomes in clinical diabetes.

STAND FOUR:

Mr Tak Lo, Diabetes Research Group, School of Biomedical Sciences, Ulster University, Coleraine, UK.

"Treatment with fatty acid modified analogues of apelin-13 improves glycaemic control and lipid profiles in diet induced obese diabetic mice."

T. Lo[1], N. Irwin[1], V. Parthsarathy[1], C. Hogg[1], P.R. Flatt[1] & F. O'Harte[1].
[1]Diabetes Research Group, Ulster University, Coleraine, UK.

Background: Apelin-13 is an adipokine, which has promising metabolic effects but is rapidly degraded in plasma.

The antidiabetic effects of chronic administration (28 days) of stable long acting fatty acid modified apelin analogues, namely, (Lys^{8}GluPAL)apelin-13 amide and pGlu(Lys^{8}GluPAL)apelin-13 amide, were investigated in diet induced obese-diabetic mice.

Methods: Male adult (8 week old) NIH Swiss mice (groups n=8) were maintained either on a high-fat diet (45% fat) for 20 weeks, or control mice were fed a normal rodent chow diet (10% fat). When diet induced obesity-diabetes was established after high-fat feeding, mice were injected i.p. once daily with apelin analogues, liraglutide (25 nmol/kg) or saline (controls).

Results: Administration of (Lys^{8}GluPAL)apelin-13 amide and pGlu(Lys^{8}GluPAL)apelin-13 amide for 28 days significantly reduced food intake and decreased body weight. Non-fasting glucose was reduced ($p<0.01$ to $p<0.001$) and circulating insulin concentrations elevated ($p<0.01$ to $p<0.001$). This was accompanied by enhanced insulin responses ($p<0.01$ to $p<0.001$) and significant reductions in glucose excursion after both oral ($p<0.01$) or i.p. ($p<0.01$) glucose challenges and feeding. Apelin analogues also significantly improved HbA1c ($p<0.01$), enhanced insulin sensitivity ($p<0.01$), reduced triglycerides ($p<0.001$), increased HDL-cholesterol ($p<0.01$) and decreased LDL-cholesterol ($p<0.01$), compared to saline treated diet induced obese mice. Cholesterol levels were decreased ($p<0.01$) by pGlu(Lys^{8}GluPAL)apelin-13 amide and apelin treated mice showed improved bone mineral content, reduced fat deposits and increased plasma GLP-1 concentrations. These data show that long-term treatment with acylated analogues (Lys^{8}GluPAL)apelin-13 amide and particularly pGlu(Lys^{8}GluPAL)apelin-13 amide resulted in similar or enhanced therapeutic responses compared to liraglutide in high-fat fed diet induced obese mice.

Conclusion: Stable fatty acid modified apelin-13 analogues represent a new and exciting therapeutic development, which can ameliorate the effects of diet induced obesity diabetes.

STAND FIVE:

Mr Ryan Lafferty, School of Biomedical Sciences, Ulster University, Coleraine, UK.

"C-terminal dipeptide truncation of PYY(1-36) and PYY(3-36) occurs naturally in the circulation and severely diminishes biological activity."

R.A. Lafferty[1], P.R. Flatt[1], N. Irwin[1].
[1]School of Biomedical Sciences, Ulster University, Coleraine, BT52 1SA, UK

Background and aims: Peptide YY (PYY) exists in two major circulating forms. PYY(3-36), generated by the action of DPP-4, has anorectic actions, with possible therapeutic implications for obesity, whereas the importance of PYY(1-36) for the regulation of pancreatic beta-cell survival has recently been described. Enzymatic C-terminal truncation of PYY related peptides has been reported, but physiological impact of this remains uncertain. The present study has characterised plasma C-terminal degradation products of PYY(1-36) and PYY(3-36), and evaluated effects on function, proliferation and survival of beta-cells as well as feeding behaviour and glucose homeostasis. **Materials and methods**: PYY(1-36) and PYY (3-36) were incubated with murine plasma (4 h, n=4) and evaluated by HPLC/MS. BRIN-BD11 beta-cells (n=8) were used to evaluate acute (20 min) effects on insulin release of PYY(1-36), PYY(3-36) plus their related C-terminal degradation products. Actions of PYY peptides on beta-cell proliferation, by Ki-67 staining, and protection against cytokine-induced apoptosis, by TUNEL assay, were examined in clonal rodent BRIN BD11 and human 1.1B4 cells (n=4). Acute effects of the peptides (25 nmol/kg; i.p.) on food intake, glucose and insulin concentrations were evaluated in overnight fasted (12 h) mice (n=8). The impact of the ACE inhibitor captopril (50 mg/kg, i.p.) on PYY(3-36) induced appetite suppression was also assessed in mice (n=8). **Results:** C-terminal degradation products, PYY(1-34) and PYY(3-34), were detected by HPLC/MS analyses following incubation of PYY(1-36) and PYY(3-36) in murine plasma. PYY(1-36) and PYY(3-36) inhibited ($P<0.05$ - $P<0.001$) insulin secretion (IS) from BRIN-BD11 beta-cells, whereas PYY(1-34) and PYY(3-34) had no effect on IS. All peptides examined lacked effects on glucose tolerance or glucose-induced insulin release. However, both PYY(1-36) and PYY(3-36) significantly ($P<0.05$ - $P<0.001$) enhanced proliferation of BRIN BD11 and 1.1B4 beta-cells, and also fully protected ($P<0.01$ - $P<0.001$) these cells against cytokine-induced apoptosis. The C-terminal degradation products, PYY(1-34) and PYY(3-34), were entirely ineffective in this regard. As expected, PYY(3-36) induced clear reductions ($P<0.05$ - $P<0.01$) of food intake in mice, but these effects were eliminated by removal of the C-terminus from PYY(3-36). Interestingly, captopril significantly ($P<0.05$) augmented the appetite suppressive actions of PYY(3-36). **Conclusions**: PYY is an enteroendocrine derived peptide hormone with an important role in metabolism, energy expenditure and pancreatic beta-cell survival. The impact of C-terminal degradation of both PYY(1-36) and PYY(3-34) on receptor interaction and subsequent bioactive profile at islet and hypothalamic sites of action needs further consideration, as it appears to dramatically diminish biological activity.

STAND SIX:

Dr Cathal McCarthy, Department Pharmacology & Therapeutics, University College Cork, Cork, Ireland.

"Investigating the therapeutic effects of L-Ergothionine as a treatment for pre-eclampsia."

R. Williamson[1], F. McCarthy [1] L.C. Kenny [2] C. McCarthy[3]

[1]Irish Centre for Fetal and Neonatal Translational Research (INFANT), Cork University Maternity Hospital, Cork.
[2]Department of Women's and Children's Health Institute of Translational Medicine, University of Liverpool.
[3] Department of Pharmacology and Therapeutics, Western Gateway Building, University College Cork.

Introduction: Pre-eclampsia is proposed to result from placental ischemia, exposing the placenta to elevated levels of oxidative stress. L-Ergothioneine is a natural water-soluble compound derived from histidine and has been shown to possess antioxidant, cytoprotective and anti-inflammatory effects, establishing its potential as a treatment for pre-eclampsia.

Objectives: To investigate the therapeutic effects of L-ergothionine as a treatment for pre-eclampsia using the *in vivo* reduced uterine perfusion pressure (RUPP) rat model.

Methods: L-ergothionine (25mg/kg/day) was administered on gestational day 11 (GD11). RUPP surgery was performed by placing silver clips on the abdominal aorta and the ovarian arteries on GD14. Mean arterial blood pressure, proteinuria and pup weight were measured in all groups. Data is presented using the mean ± SD.

Results: Mean arterial blood pressure was increased in RUPP group compared to Sham group (130.874 ± 3.54 mmHg v 120.06 ± 5.81 mmHg)**.** Mean arterial blood pressure was significantly decreased in the RUPP treatment group compared to RUPP control group (118.381 ± 4.86 mmHg v 130.87 ± 3.54 mmHg, P= 0.05). Microalbumin:creatine ratio was reduced in the RUPP treatment group compared to RUPP control (10.13 ± 1.87 mg/mmol v 20.18 ± 7.55 mg/mmol, P= 0.25). L-ergothionine rescued fetal growth restriction in the RUPP treatment group when compared with the RUPP control group (1.98g ± 0.03 v 1.80g ± 0.04, P>0.0006).

Conclusion: Pre-treatment with L-ergothionine positively regulated a number of the pathophysiological characteristics of pre-eclampsia in the RUPP rat model therefore highlighting its potential as a treatment strategy for pre-eclampsia.

STAND SEVEN:

Mr Russell Costello, Wellcome Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK.

"Prolonging Transplant Survival Through c-FLIP Inhibition and Enhanced allo-T cell Apoptosis."

R. Costello[1], J. McDaid[1], D. Longley[2], A. Kissenpfennig[1].

[1]Wellcome Wolfson Institute for Experimental Medicine, Queen's University Belfast, [2]Centre for Cancer Research & Cell Biology, Queen's University Belfast, Belfast, UK.

Purpose of project: Organ transplantation is a critical therapeutic option for end stage organ failure. Current median survival of heart, lung, liver and kidney transplants is 10-16 years, before loss of the graft to chronic rejection[1]. Whilst effective treatments exist for cases of acute rejection the same is not true of chronic rejection, limiting graft survival.

We are investigating the potential of pharmacological inhibition of c-FLIP[2], using Histone Deactylase Inhibitors (HDACis)[3] as well as novel small molecule c-FLIP inhibitors, to selectively enhance apoptosis in allo-reactive T cells. If successful this may prolong organ graft survival, whilst potentially having lower occurrence of side effects compared to current immunosuppressant strategies.

Methods: Current work has involved *in vitro* experiments to ascertain the ability of the HDACis, Vorinostat and Entinostat, to induce apoptosis within activated murine T cells from total splenocytes. We have used flow cytometry with markers of cell death and apoptosis, as well as markers of T cell populations, to ascertain the incidence of apoptosis within the T cell populations and lymphocyte population more generally.

Results: Vorinostat and Entinostat induce apoptosis within splenocyte populations, in which T cells have been activated. A dose-response relationship exists, with cells treated with higher doses of Vorinostat or Entinostat having a greater incidence of apoptosis, but at the expense of selectivity for activated T cells. Preliminary data suggests Entinostat may more selectively induce apoptosis in activated T cells than Vorinostat.

Conclusion: Vorinostat and Entinostat may have potential going forward as immunosuppressive therapies for transplantation.

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STAND NINE:

Ms Samantha Gallagher, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast, UK.

"Unravelling the Role of Cathepsin S in DNA Damage Response Deficient Tumours."

S. Gallaher[1], R. Wilkinson[1], R. Burden[2], R. Williams[1], C. Scott[1].

[1]School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast. [2]School of Pharmacy, Queen's University Belfast, Belfast, UK.

Purpose: Metastatic cancers are often incurable and are almost always life-limiting. Cancers expressing deficiencies in BRCA/Fanconi anaemia (FA) pathways have a predisposition to metastasise. The life expectancy for a patient with metastatic cancer is 26 months and it is estimated that <25% survive 5 years post-diagnosis [1]. Therefore, current clinical treatments aim to reduce the rate of metastases and alleviate patient symptoms.

Cathepsin S (CTSS) is a lysosomal protease, normally confined to antigen presenting cells (APCs), has been found to be inappropriately expressed in the tumour microenvironment (TME) [2]. Although the exact role of this protease in cancer is not yet defined, examination of CTSS in clinical samples has identified it as a poor prognostic marker [3]. The aim of this study was to identify CTSS in BRCA/FA deficient tumours and investigate the effect of CTSS inhibition in these tumours.

Methods: Expression of CTSS was measured by western blot and RNA analysis. Invasion assays were performed on BRCA1-, FANC C-, FANC G- and FANC P- cell lines.

Results: Analysis of the cell types found that expression of CTSS is upregulated in the TME in BRCA1 deficient tumours. Treatment with a CTSS selective inhibitor significantly reduced the invasiveness of these cells.

Conclusion: The findings of this study suggest elevated CTSS expression from metastatic tumour cell lines with BRCA/FA pathway deficiencies encourages tumour invasiveness and highlights a potential target for the treatment of metastatic cancers.

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STAND TEN:

Ms Leanne O'Sullivan, School of Biochemistry & Cell Biology, University College Cork, Cork, Ireland.

"Assessment of Platelet Activation Status and Platelet Reactivity in Multiple Myeloma, Smouldering Myeloma and MGUS Patients Pre-Treatment and During Anti-Myeloma Treatment."

L. O'Sullivan[1], G. Meade-Murphy[2], V. Mykytiv[3], O. Gilligan[3], P. Young[1], M.R. Cahill[3,4].

[1]School of Biochemistry & Cell Biology, University College Cork, Cork. [2]School of Pharmacology & Therapeutics, University College Cork, Cork. [3]Haematology Department, Cork University Hospital, Cork. [4]School of Medicine & Health, University College Cork, Cork, Ireland.

Introduction: Thrombotic events are reported in up to 10% of patients with Multiple Myeloma (MM), Smouldering Myeloma (SM) and the premalignant disorder, monoclonal gammopathy of undetermined significance (MGUS). Platelet dysfunction is a feature of this thrombotic risk thought to arise from both the disease state and associated treatment regimens including dexamethasone, Thalidomide, Lenalidomide and proteasome inhibitors [1,2]. Platelet hyperactivity and the influence of circulating paraprotein levels on platelet activity is investigated in MM, SM and MGUS patients and healthy controls pre and post anti-myeloma and anti-coagulant treatment in this study.

Methods: Platelet fibrinogen receptor activation (PAC-1), granule release (CD62P, CD63), phosphatidylserine exposure (Annexin V) and Platelet-Leucocyte Aggregates (CD61/CD45) were assessed by flow cytometry at baseline and following treatment. Platelet reactivity in response to ADP and TRAP-6 were assessed by flow cytometry. Platelet production was examined by quantification of reticulated platelets. The influence of paraprotein levels on platelet activity was investigated by assessing thrombus formation using an in-vitro flow cell assay and by assessing paraprotein coating of platelets.

Results: Results indicate that platelets are hyperactivated in MM patients and in pre-malignant SM and MGUS patients. Hyperactivity appears maintained during treatment. Platelet-Monocyte aggregates are found also to be increased for all patients versus healthy controls and in patients receiving treatment. Platelet reactivity to ADP and TRAP-6 show dysregulation in patient groups.

Conclusion: Characterisation of platelet dysregulation due to the disease state and anti-myeloma therapies may guide management of thrombotic risk and highlight alternative therapeutic targets in the prevention of myeloma associated thrombosis.

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STAND ELEVEN:

Mr Chao Xue, School of Medicine, Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland.

"Activation and expression of opioid receptors and the glycine beta receptor have opposing effects on the metastatic behaviour of human breast cancer cells."

C. Xue[1], P.D. Crowley[1], D.J. Buggy[1,2,3], H.C. Gallagher [1,4].

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Breast cancer-related mortality is due to the burden of metastatic disease. The peri-operative period is a critical time in metastatic development and perioperative factors, including anaesthetic /analgesic drugs, may influence cancer outcome. We previously identified nine anaesthetic/analgesic receptor targets whose expression is associated with clinical metastasis in breast cancer. Here, we aimed to provide a functional basis for these observations by investigating receptor-mediated mechanisms by which analgesic/anaesthetic drugs influence metastasis.

Migration on collagen was determined using the OrisTM Cell Migration Assay and invasion was measured using a three-dimensional, hanging-drop spheroid assay. Protein expression was determined by immunoblotting with densitometry.
The migratory potential of five human breast carcinoma cell lines was: MCF7 > CAL51 > MDA-MB-231 > BT-549 > MDA-MB-468 and their invasive potential was: MDA-MB-231> BT549 ≈ MCF7 > CAL51 >>> MDA-MB-468. Five of the nine receptors displayed differential expression patterns in these cell lines with differing invasive/migratory properties.

Acute exposure to morphine increased subsequent migration/invasion of MCF-7 and MDA-MB-231 breast cancer cells. These effects were reversed by both a OPRμ and a OPRδ receptor antagonist, indicating that consistent with our earlier gene expression study[1], both opioid receptors may be positively associated with metastasis.

Glycine decreased the migration of MCF-7 cells and inhibited invasion of both BT-549 and MDA-MB-231 cells. These effects were only reversed by the specific GLRβ antagonist, strychnine, in cell lines that expressed that receptor prominently. We previously found that expression of the GLRβ receptor is inversely associated with clinical metastasis[1].

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STAND TWELVE:

Ms Karis Little,

"Modelling Sub-retinal fibrosis in neovascular Age-related Macular Degeneration."

K. Little[1], M. Llorian-Salvador[1], Ó. O'Shaughnessy[1], M. Chen, H. Xu[1].

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Neovascular Age Related Macular Degeneration (nAMD) is a sight threatening disease characterised by choroidal neovascularisation (CNV). Approximately one third of patients with nAMD develop sub-retinal fibrosis. An animal model has been developed which models CNV, however a model of sub-retinal fibrosis does not exist. This study aimed to develop a model of sub-retinal fibrosis, which could be used to elucidate the potential of anti-fibrotic therapeutics in nAMD.

Building upon the previously described CNV model (1), 7 days after initial laser burn to Bruch's membrane, a second laser is directed to the newly formed CNV lesion. The aim was to stimulate leakage/ bleeding of the new vessels formed, which mimics the human pathogenesis. At various time points, *in vivo* imaging (OCT, fundus and fluorescence angiography) was performed. Ex-vitro studies were carried out on RPE flatmounts or paraffin wax embedded tissue sections to measure the fibrotic lesion.

Our model produces a lesion (measured by OCT) which is larger, and more stable than the one formed in the CNV model. Fibrosis was measured via Collagen 1 staining, and was seen to be increased in the fibrosis model, compared to CNV model. Maximum level of fibrosis was achieved 30 days after 2nd laser application.

We conclude that our Fibrosis model produces a more stable and reliable situation in which to study the development of fibrotic lesions following CNV in nAMD. This model will be a useful tool to understand the mechanism underlying sub-retinal fibrosis in nAMD and to study therapeutic drugs to combat it.

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STAND THIRTEEN:

Dr Karen Lester, Ulster University, Coleraine, UK.

"Developing Molecular Therapies for Primary Open Angle Glaucoma."

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Primary Open Angle Glaucoma (POAG) is a leading cause of irreversible blindness worldwide, and the only modifiable risk factor is intraocular pressure (IOP). Growth factors present in the aqueous humour regulate the ECM, increasing outflow resistance. TGF-β alters ECM production and turnover in the trabecular meshwork (TM) and has been shown in numerous studies to play a role in the pathogenesis of glaucoma. No current pharmacological interventions target the effects of TGF-β which damages the TM producing elevated IOP. Small, naturally occurring regulatory genes, microRNAs, which target TGF-β, can be manipulated therapeutically.

The aim of this project was to identify dysregulated miRNAs in primary human trabecular meshwork cells following exposure to TGF-β2 and to explore their therapeutic potential.
Normal primary human trabecular meshwork cells isolated from donor tissue (n=5) were manipulated with TGF-β2 and total RNA extracted. RNA-Seq was performed using the Illumina NextSeq500™ platform and genome-wide miRNA micro-arrays (Exiqon, Denmark) used to evaluate over 2000 miRNAs. Functional analyses of identified miRNAs were performed in primary and immortalised human TM cells.

Sequencing data was of high quality with 99% of reads obtaining Q score>30. Differentially expressed genes (DEGs) were ranked by logFC and p values. A number of key regulators of the TGFβ and Wnt signalling pathways were in the significantly altered
DEGs and were validated by qPCR. Micro-array expression data identified 3 miRNAs (miR-145, miR-143, and miR-4328) that showed significant altered expression. Furthermore, two were also identified in glaucomatous TM versus normal treated TM. miR-145 and miR-143 are regulators of components of the RhoA signalling pathway and can be used to manipulate α-SMA expression following TGF-β treatment.

RNA-Seq is a powerful tool to investigate genome-wide alterations in gene expression in TM treated with a known glaucoma stimulus (TGFβ-2) as a hypothesis-independent approach and implicated alterations in RhoA signalling in POAG pathogenesis. RNA-Seq can identify therapeutic targets for future molecular therapies to treat the TM in POAG. Glauco-miR-1 and -2 regulate key downstream targets in the RhoA pathway. Delivery of antagoMirs of these miRNAs inhibited the expression of αSMA and with further work could be used as molecular therapies to prevent TGF-β induced fibrosis in the TM in POAG.

STAND FOURTEEN:

Ms Patrizia Cincola', Wellcome Wolfson Institute for Experimental Medicine, Queen's University Belfast

"Pharmacological characterisation of bradykinin- and thrombin-induced Ca^{2+} signalling in retinal microvascular endothelial cells."

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Diabetic macular oedema (DMO) is a major sight-threatening complication of diabetic retinopathy. Hyperpermeability of retinal vessels due to breakdown of the inner blood-retinal-barrier (iBRB) is a key feature DMO. Two vasopermeability factors that are believed to contribute to the development of DMO are bradykinin (BK) and thrombin (THR). In the present study, we have examined the effects of BK and THR on Ca2+ signalling in retinal microvascular endothelial cells (RMECs). Cultured RMECs were incubated with the Ca2+-sensitive dye, FURA-2AM and Ca2+ responses were measured using the Molecular Devices FlexStation3 system. BK and THR evoked robust intracellular Ca2+ responses that comprised of an initial transient phase, followed by a sustained plateau that remained elevated above the baseline. EC50 values for peak BK and THR-induced Ca2+ responses were 1.63 nM and 0.27 units/ml, respectively. RMECs were pre-incubated with the Ca2+ chelator, BAPTA-AM, or inhibitors of the endoplasmic reticulum SERCA pump, G-α(q/11) and phospholipase C. Each of these treatments blocked the peak and plateau phases of the BK and THR responses. Using selective BK and THR agonists and antagonists, we found that BK acts primarily through the BK2 receptor, while THR-induced Ca2+ responses occurred mainly through Protease Activated Receptor 1 (PAR1). Our data provide a better understanding of BK- and THR-induced Ca2+ signalling in RMECs and forms the basis for investigating the involvement of these factors in mediating iBRB breakdown.

STAND FIFTEEN:

Ms Andrea Diaz Diaz, School of Pharmacy, University College Cork, Cork, Ireland

"Fingolimod in Experimental Intracerebral Haemorrhage - Are Most Published Research Findings Indeed False?"

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Stroke (generally ischemic, haemorrhagic in 15% of cases) is a major cause of morbidity and mortality. Although hundreds of drugs are effective in animal models of either stroke subtypes, translation to the clinic has been poor, and there is currently no specific treatment for intracerebral haemorrhage (ICH). Reasons for this poor translation may include fundamental model limitations, but also deficient study design (e.g. lack of blinding, random allocation, a priori power calculation). We decided to test fingolimod (a multiple sclerosis drug with abundant evidence of efficacy in animal models of ischemic stroke, and more limited evidence in experimental ICH) in a study with a more rigorous design. ICH was induced by intrastriatal collagenase in C57BL/6JOlaHsd male and female mice (n=15 per group per power calculation). Fingolimod (0.5 mg/kg) or saline was administered i.p at 0.5, 24 and 72 hours after surgery, in a randomized and blinded manner. Functional recovery was assessed with cylinder, wire hanging, and foot fault tests. Saline-treated females showed increased mortality compared to saline-treated males. There was no difference associated with treatment or gender in any of the behavioural tests and lesion volumes did not differ in any of the groups. Differences in dosing regimen may explain why fingolimod showed no benefit in this study, but it is also possible that the positive effect in previously published studies was adding weight to the "increasing concern that most current published research findings are false" (1).

References:

1. Ioannidis JPA (2005) Why Most Published Research Findings Are False. PLoS Med 2(8): e124

STAND SIXTEEN:

Mr Andrew Moore, Department Pharmacology & Therapeutics, University College Cork, Cork, Ireland.

"Neprilysin degrades murine Aβ more efficiently than human Aβ: Further implication for species-specific amyloid accumulation."

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For over a century, aggregated forms of amyloid-β protein (Aβ) have been viewed as a key hallmark of brains affected by Alzheimer's disease (AD). Today, it remains unknown whether Aβ aggregates (oligomers, fibrils or plaques) originate from increased production or decreased catabolism of Aβ. Neprilysin (NEP) is a ubiquitously distributed peptidase, known to degrade Aβ, amongst other peptides. In this study, we identified differences in NEP-mediated catabolism of murine and human forms of Aβ, using recombinant NEP, membrane-bound NEP from cells overexpressing the murine peptidase or from human organ preparations with high NEP activity, and purified soluble NEP. NEP degraded murine Aβ (mAβ) faster than human Aβ (hAβ). These findings were observed with full-length Aβ containing 40 or 42 amino acids (Aβ1-40 and Aβ1-42) and a truncated form (Aβ4-15), which (i) contains one of the main NEP cleavage sites for Aβ (between positions 9 and 10), (ii) harbours all three amino acid differences between murine and human Aβ sequences, and (iii) is less prone to aggregation and thus might be a simpler model to investigate Aβ biochemistry. While it has previously been shown that mAβ has a far lower propensity to aggregate than hAβ, evidence from this study suggests that a more favourable NEP-mediated turnover of mAβ may provide additional protection against Aβ aggregation in murine species.

STAND EIGHTEEN:

Dr Kate Doyle, Department of Geriatric Medicine, Cork University Hospital, Wilton, Cork.

"Anticholinergic Burden in Acute Coronary Syndrome Elderly Patients – A Retrospective Observational Case Cohort Study."

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i) Problem being addressed in the study

It is known that higher anticholinergic medication burden is associated with increased cardiovascular disease and mortality (1). In addition, they have recently been shown to have an associated increased risk of dementia in older adults (2). Given the physiological and pharmacological effects of anticholinergics we aimed to assess the anticholinergic burden in elderly patients presenting with acute coronary syndromes (ACS) at time of admission and discharge.

ii) How the study was performed

We performed a retrospective observational case cohort study. Patients aged 65 years and older having experienced a documented ACS were identified via our local cardiac HIPE database. The Drug Burden Index (DBI) was calculated using the Anticholinergic Burden Calculator [www.anticholinergicscales.es] at admission and discharge.

iii) Results

n = 49, female = 21 (43%). The mean DBI on admission was 0.14 (SD 0.44) and at discharge was 0.22 (SD 0.58).

iv) Conclusion

Patients ≥65 years presenting with an ACS have a higher DBI on discharge compared to admission. Medications initiated during acute hospitalisation accounting for this increase could potentially be associated with poorer outcomes.

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2. Richardson K., Fox C., Maidment I., Steel N., Loke Yoon K, Arthur A. et al. Anticholinergic drugs and risk of dementia: case-control study *BMJ* 2018; 361 :k1315