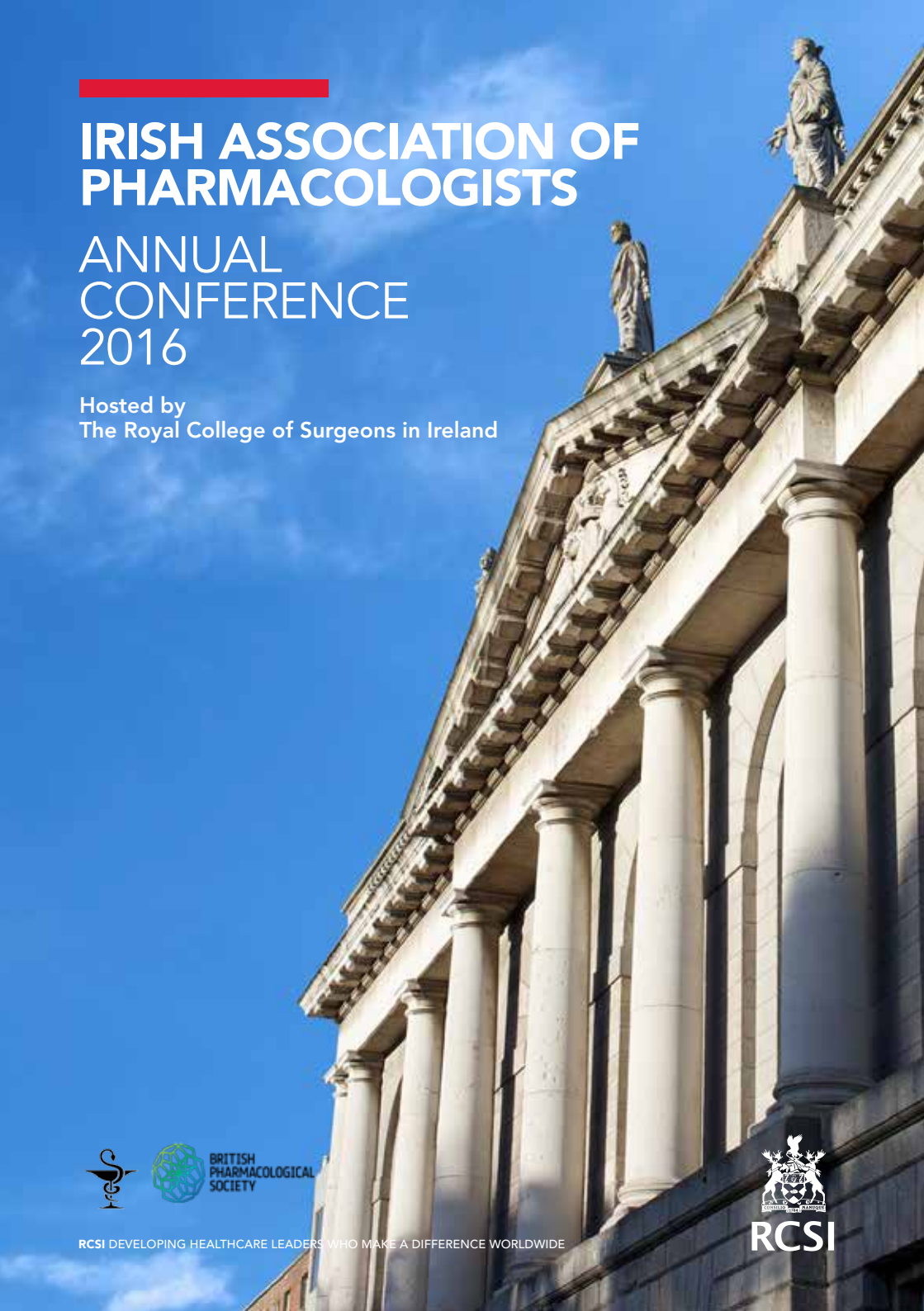
IRISH ASSOCIATION OF PHARMACOLOGISTS

ANNUAL CONFERENCE 2016

Hosted by
The Royal College of Surgeons in Ireland



BRITISH PHARMACOLOGICAL SOCIETY

RCSI

RCSI DEVELOPING HEALTHCARE LEADERS WHO MAKE A DIFFERENCE WORLDWIDE

IRISH ASSOCIATION OF PHARMACOLOGIST ANNUAL CONFERENCE 2016 **PROGRAMME**

Time	Session	Venue
12.30	**WELCOME** Professor Niamh Moran	College Hall
SESSION 1:	PHARMACOLOGY & DRUG DEVELOPMENT Chair: Dr. Steve Kerrigan	
12.40	**Keynote** ***Harnessing the anti-angiogenic and anti-cancer stem cell properties of FKBPL; discovery to clinic*** Professor Tracy Robson, Head & Professor of Molecular & Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin 2.	College Hall
1.10	**Oral Presentation** Structure-activity relationship of a novel family of cysteinyl leukotriene receptor antagonist quinoline compounds with anti-angiogenic activity **Reynolds AL**[1], Ventosa P[2], Granander J[3], Kilty C[1], Young E[1], Butler CT[1], Galvin O[1], Merrigan S[1], Sasore T[1], Fernandez Y[2], Gilheany D[3], Kennedy BN[1]. [1]Conway Institute, University College Dublin. [2]Gadea Pharmaceutical Group, Valladolid, Spain. [3]Centre for Synthesis & Chemical Biology, University College Dublin.	
1.20	**Oral Presentation** Endothelial dysfunction in severe bloodstream infection **McHale TM1**, Garciarena CD[1], Smith S[2], Kerrigan SW[1]. [1]Cardiovascular research infection group, Royal College of Surgeons in Ireland. [2]Department of Clinical Microbiology, School of Medicine, Trinity College Dublin.	
1.30	Question & Answers Session	
1:40	LUNCH & POSTER SESSION	Boardroom
SESSION 2:	PHARMACOLOGY & DRUGS IN PUBLIC HEALTH Chair: Dr. Dermot Cox	
2.30	**Keynote** ***A reflection on the forensic analysis of controlled substances in Ireland, is there a benefit to society?*** Dr. John Power, Forensic Scientist Team Leader, Garda HQ, Dublin 8.	College Hall
3.00	**Oral Presentation** Patterns of statin initiation and continuation in cancer patients towards end-of-life **Smith A**[1], Murphy L[2], Bennett K[2], Barron I[3]. [1]Department of Pharmacology & Therapeutics, Trinity College Dublin. [2]Population Health, Royal College of Surgeons in Ireland. [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA.	
3.10	**Oral Presentation** The Angiotensin-(1-7)/Mas axis improves pancreatic β-cell function in vitro and in vivo Sahr A[1], **Tetzner A[2]**, Wolke C[1], Maczewsky J[3], Krippeit-Drews P[3], Drews G[3], Venz S[1], van den Brandt J[4], Lancaster R[2], Berg S[4], Lendeckel U[1], Walther T[2]. 1Institute of Medical Biochemistry and Molecular Biology, University Medicine Greifswald, Germany. [2]Department of Pharmacology and Therapeutics, University College Cork. [3]Department of Pharmacology, Institute of Pharmacy, University of Tübingen, Germany. [4]Central Core & Research Facility of Laboratory Animals, University Medicine Greifswald, Germany.	

Time	Session	Venue
3.20	Question & Answers Session	College Hall
3.30 **SESSION 3:**	COFFEE BREAK and Poster viewing PERSONALISED MEDICINE & CARDIOVASCULAR DISEASE Chair: Professor David Williams	Boardroom
3.40	**Keynote** ***Precision Cardiovascular Medicine – Progress to date*** Professor Alice Stanton, Professor Cardiovascular Therapeutics, Intermediate Cycle Director, Royal College of Surgeons in Ireland, Dublin 2.	College Hall
4.10	**Oral Presentation** Lipid lowering therapy for secondary stroke prevention in a stroke clinic **Kennedy CA,** O'Brien H, Williams DJ. Departments of Geriatrics & Stroke Medicine, Beaumont Hospital, Dublin.	
4.20	**Oral Presentation** Simvastatin reduces interleukin-1 beta secretion from peripheral blood mononuclear cells when treated with cholesterol crystals Gangadharan N, Kavanagh P, Wash PT, Hemeryck L, Kieran J, Barry M, **Lucitt M**. Department of Pharmacology and Therapeutics, School of Medicine, Trinity College Dublin.	
4.30	Question & Answers Session	
4:40 **SESSION 4:**	COFFEE BREAK and Poster viewing PERSONALISED MEDICINE & CANCER Chair: Dr. Darran O'Connor	Boardroom
4.50	**Oral Presentation** Functional genomic screening identifies USP11 as a novel regulator of ERα transcriptional activity in breast cancer **Dwane L**[1], O'Connor AE[2], Dirac AM[3], Jirstrom K[4], Crown JP[5], Bernards R[3], Gallagher WM[2], Ni Chonghaile T[6] and O'Connor DP[1]. [1]Molecular and Cellular Therapeutics, Royal College of Surgeons Ireland. [2]Cancer Biology and Therapeutics Laboratory, UCD School of Biomolecular and Biomedical Science, University College Dublin. [3]Division of Molecular Carcinogenesis, Netherlands Cancer Institute, Amsterdam, The Netherlands. [4]Department of Laboratory Medicine, Malmö University Hospital, Lund University, Malmö, Sweden. [5] St Vincent's University Hospital. 6Physiology and Medical Physics, Royal College of Surgeons Ireland.	College Hall
5.00	**Oral Presentation** miR-134 in Breast cancer-derived extracellular vesicles **Lowry MC**[1], O'Brien K[1], Corcoran C[1], Martinez VG[1], Daly M[1], Rani S[1], Gallagher WM[2], Radomski MW, MacLeod RAF[3], O'Driscoll L[1]. [1]School of Pharmacy, Trinity College Dublin. [2]Cancer Biology and Therapeutics Laboratory, University College Dublin. [3]Leibniz Institute, Germany.	
5.10	**Keynote** ***Molecular Oncology*** Professor John Crown, Consultant Medical Oncologist, St. Vincent's University Hospital, Dublin 4.	
5.40	Question & Answers Session	
5.50	Close: Professor Niamh Moran	
6.00	Wine Reception	Boardroom

Poster	ABSTRACT SUMMARY
1	Cilengitide: promising new therapy for the treatment of Staphylococcus aureus induced sepsis. Garciarena C, et al. RCSI.
2	The multifaceted activity of curcumin in cancer chemoprevention and anticancer treatment. Obaidi I, et al. UCD.
3	RDS8, a natural compound, changes the expression of tight junction protein JAM-A and induces cell death in breast cancer cells. Sri Vellanki, et al. RCSI & Beaumont Hospital.
4	Development of a novel therapeutic target to Junctional Adhesion Molecule-A (JAM-A) in breast cancer. Smith YE, et al. Education and Research Centre, RCSI
5	Outcomes associated with non-adherence to anti-hypertensives and statins. MacAvin MJ, et al. TCD and RCSI.
6	Predicting responsiveness and sensitization of glioblastoma to current and novel treatment options. Murphy BM, et al. RCSI.
7	Post-mortem examination of an aggressive case of medullary thyroid carcinoma characterized by catastrophic genomic abnormalities. Das S, et al. UCD and RCSI.
8	Activation of apoptosis through targeting VDAC1 with miR-324-5p in neuroblastoma. Piskareva O, et al. RCSI and Our Lady's Children's Hospital, Crumlin.
9	Impact of safety warning on domperidone prescribing in Ireland. Teeling M, et al. TCD.
10	A novel role for JAM-A in resistance to HER2-targeted therapies. Leech AO, et al. RCSI.
11	Natural compound 'C4' as a novel therapeutic in triple-negative breast cancer. Richards CE, et al. RCSI.
12	The virtually mature BNP (BNP1-32) is a precursor for the more potent BNP1-30. Moore A, et al. UCC.
13	An investigation into whether therapeutic control of miRNA's could be beneficial in sepsis. Watkin RL, et al. RCSI.
14	Real-time Imaging of Lysosomal Disruption with a pH Responsive NIR Fluorophore. Cheung S, et al. RCSI
15	Identification of anti-angiogenic and anti-metabolic compounds in-vitro and in-vivo in zebrafish to determine if novel dual action drugs can enhance radiosensitivity in oesophageal adenocarcincoma. Buckley A, et al. TCD.
16	The role of the endothelium and platelets in the development of neonatal sepsis. Emagha UT, et al. RCSI.
17	Cdk7: a prognostic marker of poor outcome and rational therapeutic target in triple-negative breast cancer. Li B, et al. UCD.
18	Vitamin D receptor agonists attenuate ocular developmental angiogenesis and regulate the expression of angiomiR miRNA21 and pro-angiogenic factor VEGF in Zebrafish. Merrigan S, et al. UCD.
19	Bromodomain inhibition as a novel therapeutic for invasive lobular carcinoma. Walsh L, et al. RCSI.
20	The role of the anorectic neuropeptide CART in breast cancer. Mooney B, et al. RCSI.
21	Trends in prescribing of antipsychotics in dementia: a population-based study. Gul Z, et al. TCD.
22	Uncovering a novel upstream regulator of receptor tyrosine kinase signalling in breast cancer. Cruz RGB, et al. Education & Research Centre, RCSI.
23	Protective effect of TRPM7 inhibition in a model of breast tumour calcification. O'Grady S, et al. RCSI.

CILENGITIDE: PROMISING NEW THERAPY FOR THE TREATMENT OF STAPHYLOCOCCUS AUREUS INDUCED SEPSIS

Garciarena C[1], McDonnell C[1], Claes J[2], Verhamme P[2], Kerrigan SW[1]. [1] School of Pharmacy, RCSI; [2]Leuven University, Belgium.

Sepsis, a life threatening condition, is frequently caused by the gram positive bacteria Staphylococcus aureus (S. aureus). Vascular endothelial dysfunction with associated oedema and organ failure is a hallmark of sepsis, yet the effects of direct interaction between S. aureus and endothelial cells (ECs) have been largely overseen. We investigated the signals triggered by S. aureus binding to ECs in order to develop novel strategies for sepsis treatment.

Experiments were performed using S. aureus Newman, sheared human aortic ECs (HAoECs), human plasma (ethical approval RCSI), and anaesthetised C57Bl/6 mice (ethical approval Leuven University). Data are mean±SEM. ANOVA and t-test were used, $P<0.05$ was considered significant.

We found that S. aureus binding to HAoECs mediated clumping factor A (ClfA) and fibrinogen, induced a transient rise of intracellular Ca2+ (25±2%, n=6-8, Fluo-4), followed by vWf strings deposition on HAoECs' surface, (immunofluorescence, +70±20% vs uninfected). The αVβ3 integrin antagonist cilengitide significantly reduced ClfA-induced bacteria binding to HAoECs in vitro under flow (up to 75%). In vivo, cilengitide reduced S. aureus binding to the mouse mesenteric endothelium by 83±6% vs untreated animals (n=10-17). Significantly, cilengitide also attenuated the deleterious effects induced by S. aureus on HAoECs proliferation, apoptosis (25±5%), barrier function (determined by permeability, decreased from 56±5% to 27±8) and VE-cadherin expression.

Our results reveal a key mechanism in the development of endothelial dysfunction occurring in sepsis and identify αVβ3-blockade as a potential new strategy for the treatment of sepsis.

Funding: Science Foundation of Ireland Career Development award 13/SFI/2119.

THE MULTIFACETED ACTIVITY OF CURCUMIN IN CANCER CHEMOPREVENTION AND ANTICANCER TREATMENT

Obaidi I and McMorrow T.
Renal Disease Research Group, UCD School of Biomolecular and Biomedical Science, Conway Institute, University College Dublin, Belfield, Dublin 4.

Despite the obvious developments in treatment modalities over the last era, the incidence and deaths due to cancers have not changed in the last thirty years. Moreover, the current anticancer drugs are of limited efficacy, with long lists of adverse effects and also very expensive. Therefore, identifying a xenobiotic that is free of these disadvantages is the 'Holy Grail' of cancer treatment [1]. Curcumin is a polyphenolic compound derived from the rhizome of curcuma longa and is well known for a diverse number of potential therapeutic actions [2]. It has both cancer chemopreventive and anticancer potential against different kinds of cancers [3]. Our study aimed at investigating the chemopreventive and the anticancer potential of curcumin in renal cell carcinoma, as well as insight into the molecular mechanisms of both.

Results indicate that curcumin protected normal human renal RPTEC/TERT1 cells from the carcinogenic insult of potassium bromate and sensitized cancerous ACHN renal cells to TRAIL induced apoptosis via activation of both the intrinsic and the extrinsic pathways of apoptosis. This included downregulation of cellular FLICE, (FADD-like IL-1β-converting enzyme)-inhibitory protein (c-FLIP), induction of Endoplasmic Reticulum stress, and arrest of ACHN cell cycle. While curcumin treatment also reduced DNA mutations and subsequent activation of oncogenes induced by the carcinogen, potassium bromate, in normal renal RPTEC/TERT1 cells.

Therefore the multi-faceted activity of curcumin as both an anticancer agent and chemopreventive agent may prove to be a very useful cancer therapeutic strategy.
This study was funded by Iraqi government/ Ministry of higher Education and Scientific Affairs

References

1.Shanmugam, M.K., et al., Molecules, 2015. 20(2): p. 2728-2769.
2.Chattopadhyay, I., et al., Curr Sci, 2004. 87(1): p. 44-53.
3.Singh, S. and A. Khar, Anti-Cancer Agents in Medicinal Chemistry, 2006. 6(3): p. 259-270.

RDS8, A NATURAL COMPOUND , CHANGES THE EXPRESSION OF TIGHT JUNCTION PROTEIN JAM-A AND INDUCES CELL DEATH IN BREAST CANCER CELLS

Sri HariKrishna Vellanki, Rodrigo G.B .Cruz, Catherine E. Richards, Lance Hudson, Ann M. Hopkins.
Department of Surgery, Royal College of Surgeons in Ireland, RCSI Smurfit Building, Beaumont Hospital, Dublin 9.

Junctional adhesion molecule-A (JAM-A) is a membranous cell-cell adhesion protein involved in tight junction formation in epithelial and endothelial cells. Its overexpression in breast tumours has recently been linked with increased risk of metastasis. We screened a natural compound chemical library in an attempt to identify compounds that could target JAM-A and have anticancer properties. Using MDA-MB-231 breast cancer cells induced to over-express JAM-A, we have identified a natural compound of interest, RDS8. We have treated MDA-MB-231-, Hs578T- and SKBR3- JAM-A over-expressing cells with RDS8 and observed loss of cell viability in a dose-dependent manner in these cells at 24hrs. Since JAM-A overexpression has been associated with metastasis, we did migration assays in MDA-MB-231 JAM-A overexpressing cells and found that cells treated with RDS8 did not migrate compared to controls. Furthermore, we performed western blot analysis to elucidate the signalling pathways involved in MDA-MB-231 cells overexpressing JAM-A, and found that EpHA2 is cleaved and down-regulated in cells treated with RDS8 compared to control cells. Interestingly we saw upregulation of JAM-A in cells treated with RDS8. Further analysis of the signalling pathway showed that inhibition of survival pathways and down-regulation of Bcl-2 is observed compared to control cells. In addition we investigated the kind of cell death mechanism involved, and observed loss of mitochondrial membrane potential, cleavage of caspase 3 and increased LC3 II cleavage in cells treated with RDS8 compared to control cells. Furthermore we investigated the above mechanism in primary cultures (under passage 10) derived from breast cancer patients, and found loss of cell viability in a dose-dependent manner at 24hrs. Elucidation of signalling pathways in these cells highlighted downregulation of EpHA2 both in full length and cleaved form compared to controls. RDS8 induced down-regulation of JAM-A, Bcl2 and inhibition of survival pathways consistent with its effects in MDA-MB-231 JAM-A overexpressing cells. To conclude, the natural compound RDS8 alters JAM-A expression and induces destructive autophagy in breast cancer cells.

Acknowledgement:
The NCI/DTP Open Chemical Repository https://dtp.cancer.gov
This abstract has emanated from research conducted with the financial support of Science Foundation Ireland (SFI) under grant number 13/IA/1994.

DEVELOPMENT OF A NOVEL THERAPEUTIC TARGET TO JUNCTIONAL ADHESION MOLECULE-A (JAM-A) IN BREAST CANCER

Smith YE[1], Brennan K[1], Hudson L[1] and Hopkins AM[1]. [1]Department of Surgery, Royal College of Surgeons in Ireland, RCSI Education and Research Centre, Smurfit Building, Beaumont Hospital, Dublin 9, Ireland.

Junctional Adhesion Molecule-A (JAM-A) is a cell-cell adhesion protein whose increased expression is associated with poor prognosis in patients with invasive breast cancer. Overexpression of JAM-A has also been correlated with the human epidermal growth factor receptor (HER2) genotype of breast cancer. However, whether JAM-A has potential as a pharmacological target in breast cancer remains unknown. We developed two novel small molecule inhibitors, KB3 and JBS-2, specifically targeting JAM-A and tested these in a HER2/JAM-A positive model of ductal carcinoma in situ (DCIS), and in primary cultures.

KB3 and JBS-2, were tested in a cell line model of DCIS, SUM-225, in addition to primary breast cancer cells obtained with full informed consent and ethical approval from the Beaumont Hospital medical ethics (research) committee. KB3 and JBS-2 significantly inhibited SUM-225 and primary breast cancer cell proliferation. Treatment of SUM-225 cells with the HER2/EGFR inhibitor lapatinib significantly inhibited proliferation in a concentration-dependent manner. This suggests that SUM-225 cells may be a useful model in which to examine pharmacological co-targeting of JAM-A, HER2 and/or EGFR. Interestingly, a tissue microarray (TMA) stained for JAM-A identified patients with DCIS as having increased JAM-A expression.

In conclusion, JAM-A specific inhibitors may have anti-tumorigenic effects alone or in combination with HER-2 specific therapeutics in HER2-positive breast cancer. Increased expression of JAM-A in DCIS coupled with the responsiveness of a DCIS cell line model to a JAM-A inhibitor suggests value in investigating JAM-A inhibitors as preventative agents in this setting.

This research was funded by the Health Research Board HRA-POR-2014-545 (to AMH).

OUTCOMES ASSOCIATED WITH NON-ADHERENCE TO ANTI-HYPERTENSIVES AND STATINS

MacAvin MJ1, Teeling M1, Okoh P1, Connelly M1, Cahir C2, Bennett K2.
Department of Pharmacology and Therapeutics, TCD1 and RCSI2

Non-adherence to medicines is associated with poorer patient outcomes. The objectives of this study were (i) to determine the effect of non-adherence in relation to: blood pressure (BP) in anti-hypertensive use and (ii) LDL and total cholesterol levels in statin use.

A retrospective cohort study with health assessments linked to pharmacy claims data from community dwelling participants aged ≥ 50 years from 2009-2011 was conducted.

Exposure was proportion of days covered (PDC) for 5 classes of antihypertensive medication and all statins over 12 months prior to health assessment. Adherence assumed at PDC≥ 80%.

Main outcome measures were: (i) Mean systolic and diastolic BP measurement (from two recordings) and (ii) total cholesterol (TC) and LDL cholesterol levels.

Statistical analysis included multivariable linear and logistic regression, adjusting for covariates including age, gender, smoking status, chronic disease, educational level and BMI. There were n=998 receiving anti-hypertensive medications, with 56.8% adherent. The adjusted β coefficient for systolic BP for adherent vs non-adherent was non-significant, but was significant for diastolic BP. There were n=771 receiving statins and having blood lipid data, with 62.5% adherent. Adherent subjects were more likely to reach target TC and LDL.

Adherence to anti-hypertensive medication reduced diastolic but not systolic BP and adherence to statins improved targets for TC and LDL cholesterol.

PREDICTING THE RESPONSIVENESS AND SENSITIZATION OF GLIOBLASTOMA TO CURRENT AND NOVEL TREATMENT OPTIONS

Birgit C. Weyhenmeyer[1],, Janis Noonan[1], Maximilian L. Würstle[1], Frank Lincoln[1], Grainne Johnston[1], Markus Rehm[1],[2], Brona M. Murphy[1]
[1]Department of Physiology & Medical Physics, Centre for Systems Medicine, Royal College of Surgeons in Ireland, Dublin 2, Ireland; [2]Institute of Cell Biology and Immunology, Faculty of Energy-, Process- and Biotechnology, Stuttgart Research Center Systems Biology, University of Stuttgart, Germany.

Genotoxic chemotherapy with temozolomide (TMZ) is a mainstay of treatment for glioblastoma (GBM); however, at best, TMZ provides only modest survival benefit to a subset of patients. Recent insight into the heterogeneous nature of GBM suggests a more personalized approach to treatment may be necessary to overcome cancer drug resistance and improve patient care. These include novel therapies that can be used both alone and with TMZ to selectively reactivate apoptosis within malignant cells. For this approach to work, reliable molecular signatures that can accurately predict treatment responsiveness need to be identified first. Here, we describe the first proof-of-principle study that merges quantitative protein-based analysis of apoptosis signaling networks with data- and knowledge-driven mathematical systems modeling to predict treatment responsiveness of GBM cell lines to various apoptosis-inducing stimuli. These include monotherapies with TMZ and TRAIL, which activate the intrinsic and extrinsic apoptosis pathways, respectively, as well as combination therapies of TMZ+TRAIL. We also successfully employed this approach to predict whether individual GBM cell lines could be sensitized to TMZ or TRAIL via the selective targeting of Bcl-2/Bcl-xL proteins with ABT-737. Our findings suggest that systems biology-based approaches could assist in personalizing treatment decisions in GBM to optimize cell death induction.

Funding Acknowledgements:
Irish Health Research Board (HRA_POR/2012/88 and HRA_POR/2013/245), the RCSI Research Committee (GR 08-0155) and the European Union (FP7 IAPP SYS-MEL; FP7 APO-DECIDE; Horizon 2020 MSC ETN MEL-PLEX, Horizon 2020 MSC ETN TRAIN-ERS).

POST-MORTEM EXAMINATION OF AN AGGRESSIVE CASE OF MEDULLARY THYROID CARCINOMA CHARACTERIZED BY CATASTROPHIC GENOMIC ABNORMALITIES

Sudipto Das[3,4*], Deirdre Kelly[1,2*], Bruce Moran[3,4*,] Kathleen Han[2,5], Niall Mulligan[2,5,] Ciara Barrett[2,5], Patrick G. Buckley[6,] Peter Mc Mahon[1,2,] J Mc Caffrey[1,2,] Hendrik F. Van Essen[7], Kate Connor[3,4], Bauke Ylstra7, Diether Lambrechts[8], William M. Gallagher[3], Darran P. O'Connor[3,4§], Catherine M. Kelly[1,2§]

[1]Department of Medical Oncology, Mater Misericordiae University Hospital, Eccles St., Dublin 7. [2]Conway Institute, University Hospital Dublin, Belfield, Dublin 4, Ireland. [3]Cancer Biology and Therapeutics lab, Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland.[4] Department of Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, 123 St. Stephens Green, Dublin 2, Ireland. 7. [5]Department of cellular pathology, Mater Misericordiae University Hospital, Eccles St., Dublin.[6]Molecular Pathology Laboratory, Clinical Directorate of Laboratory Medicine, Beaumont Hospital, Dublin 9, Ireland.[7]Department of Pathology, VU University Medical Center, 1007 MB Amsterdam, The Netherlands. [8]Laboratory of Translational Genetics, Versailus Research Center, VIB, K.U., Leuven, Belgium.

Catastrophic genomic alterations can drive unusually aggressive cancer phenotypes. We describe a diagnostically challenging rapidly fatal case of medullary thyroid carcinoma (MTC) occurring in a young, morbidly obese man presenting with diffuse bone marrow involvement and disseminated intravascular coagulation. Whole-exome sequencing and shallow whole-genome sequencing was carried out for the primary tumour and multiple metastases. We identified three germline SNP's within the RET proto-oncogene which remained undetected using routine hospital genetic testing procedures. Indeed, one of the variants identified (L769L) has been previously reported in literature to be associated aggressive MTC presentation, yet remains untested for in the routine diagnosis of MTC. Supported by findings from shallow whole genome sequencing, we report for the first time in thyroid cancer, the occurrence of a catastrophic "chromothripsis-like pattern" (CTLP) event, which involved shattering of chromosome 4 leading to complete abrogation of normal chromosomal function, in addition to dramatic wide-spread copy number aberrations (CNA), across both primary tumour and bone marrow samples. We further describe the presence of loss-of-heterozygosity (LOH) in key genes involved in DNA repair mechanism pathways such as ATM, which possibly facilitated the CTLP event, in addition to LOH in other disease-associated genes such as ALK and NOTCH1 as key drivers of the aggressive and rapidly fatal clinical course in this patient and unresponsiveness to the standard-of-care targeted agent chosen. Given a possible rapid generation of tumor neo-antigens as a result of the CTLP event, immunotherapy may have been more suitable as a treatment option. Moreover, the presence of disease-associated SNP's within the RET proto-oncogene, support their inclusion as part of routine RET genetic testing for aggressive MTC cases. These results provide a rationale for application of comprehensive genomic analysis of cancers presenting with unusually aggressive behavior to facilitate more appropriate therapeutic options and diagnoses.

Funded by: The Mater Foundation, Irish Cancer Society and Irish Cancer Society collaborative cancer center *"BREAST-PREDICT"*

ACTIVATION OF APOPTOSIS THROUGH TARGETING VDAC1 WITH MIR-324-5P IN NEUROBLASTOMA

Piskareva O[1,2], Nolan J[1,2], Conlon R1, Stallings RL[1,2]
1 Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland& [2]Children's Research Centre Our Lady's Children's Hospital, Crumlin, Dublin.

MiRNAs are post-transcriptional regulators of gene expression and their deregulation can contribute to pathogenesis of many different cancer types, including neuroblastoma. The expression levels of specific miRNAs can be significantly associated with clinical outcome. MiRNA-324-5p is significantly down regulated in MYCN amplified tumours (1) and analysis of 328 tumours in the Neuroblastoma Research Consortium dataset (Amsterdam, Dublin, Essen, Genoa, Ghent) revealed that down-regulation of this miRNA is significantly associated with poor overall patient survival (p =4.3e-4). Ectopic overexpression of miR-324-5p in neuroblastoma cell lines significantly inhibited cell viability and proliferation (p=0.016, Annova) and inversely correlated with the expression of the voltage-dependent anion channel 1 (VDAC1) on mRNA and protein levels. Over-expression of miR-324-5p in Kelly, NB1691, SKNAS cells led to a significant decrease in both VDAC1 mRNA and protein, while the use of luciferase reporter plasmids confirmed that miR-324-5p directly targets the VDAC1 3' UTR (p=0.008). Overexpression of miR-324-5p arrests neuroblastoma cells in S-phase suppressing cell viability and proliferation and leads in activation of early (p=0.0005) and late (p=0.0006) apoptosis. Our findings mark miR-324-5p as a negative regulator of VDAC1 and indicate that miR-324-5p is a potential tumour suppressor miRNA in neuroblastoma and an attractive candidate for miR-based therapeutics.

This work was funded by the National Children's Research Centre.
1. Bray I, et al, PLoS One. 2009 4:e7850

IMPACT OF SAFETY WARNING ON DOMPERIDONE PRESCRIBING IN IRELAND

Teeling M[1], MacAvin MJ[1], Bennett K.[2] [1]Dept. of Pharmacology & Therapeutics, Trinity College Dublin, [2]Population Health Sciences Division, RCSI, Dublin 2.

In 2014, a EU (cardiac) safety review recommended restrictions on use of domperidone as follows: 30mg/d max oral dose for adults, use limited to one week, and use contraindicated in those with cardiac disease or in those taking QT-prolonging drugs/potent CYP3A4 inhibitors. This study investigated the impact of these safety warnings on domperidone prescribing in Ireland.

The HSE–PCRS pharmacy claims database was used to identify the study cohort (aged 18+), prescribed domperidone from Jan 2014 to Oct 2015. The dose was available for each claim, and co-prescription with the following drug classes was identified (by ATC) and calculated as a percentage of the total number of claims: anti-arrhythmics, macrolide antimicrobials and the SSRIs citalopram and escitalopram. Prescribing patterns were investigated before and after the issue of the safety advisory (May 2014). Segmented regression analysis was used to examine change in trend before and after May 2014.

Of 397,572 claims for domperidone identified in the study cohort, there was a significant decline in domperidone prescribing during the study ($p=0.012$), which did not change after the warning. No significant change in co-prescription of SSRIs or anti-arrhythmic agents was observed over time while co-prescription with any macrolide increased after the safety advisory ($p=0.031$). In those aged 60+ years, 10% of claims ($n=1332$) were for doses > 30mg/day at the start of the study; there was no significant change in trend after issue of the safety advisory.

The safety warnings appear to have had little effect on domperidone prescribing patterns in Ireland, including co-prescription with contraindicated drug classes, in any adult age group.

A NOVEL ROLE FOR JAM-A IN RESISTANCE TO HER2-TARGETED THERAPIES

Leech AO[1], Vellanki SH[1], Keogh A[1], Hudson L[1], O'Donovan N[2], Sheehan K[3], Kay EW[3], Hill ADK[1], Hopkins AM[1]. Departments of [1]Surgery & [3]Pathology, Royal College of Surgeons in Ireland, [2]National Institute for Cellular Biotechnology, Dublin City University.

Overexpression of Junctional Adhesion Molecule-A (JAM-A) in breast tumours associates with reduced patient survival and overexpression of HER2. As anti-HER2 treatment efficacy is often limited by drug resistance, new predictive markers and therapeutic targets are needed. Having previously shown that JAM-A expression levels regulate those of HER2, we hypothesised that JAM-A stabilises HER2 expression/signalling and participates in HER2-therapy resistance. In Lapatinib- and Trastuzumab-resistant breast cancer cells, JAM-A gene silencing or pharmacological inhibition combined with anti-HER2 treatment exerted superior anti-proliferative and pro-apoptotic effects than targeting HER2 alone. Combined treatment additively decreased HER2 and EGFR expression and phosphorylation of Akt and ERK, compared to anti-HER2 treatment alone. Drug-resistant cells exhibited JAM-A cleavage from the cell membrane and expressed augmented levels of enzymes known to cleave JAM-A. Exogenous treatment with recombinant soluble JAM-A increased cancer cell invasion in vitro and in a semi-in vivo model monitoring tumour invasion across the chick chorionic allantoic membrane. Finally, a small dataset (n=21) of serum samples from patients treated with HER2-targeted therapies revealed a novel correlation between JAM-A cleavage and the development of clinical drug resistance. In conclusion, our data suggest that

cleaved JAM-A holds potential as a novel blood biomarker of resistance to HER2-targeted therapies, and that co-targeting JAM-A and HER2 merits exploration in patients resistant to HER2-targeted therapies.

BREAST CANCER IRELAND
researching a cure

NATURAL COMPOUND 'C4' AS A NOVEL THERAPEUTIC IN TRIPLE-NEGATIVE BREAST CANCER

Richards CE, Vellanki SH, Smith YE, Leech A, Cruz RGB, Hudson L, Hopkins AM. Department of Surgery, RCSI, Smurfit Building, Beaumont Hospital, Dublin 9.

Triple-negative breast cancers (TNBC) are those in which tumours fail to express three common prognostic and therapeutic markers, namely estrogen receptor, progesterone receptor and human epidermal growth factor receptor-2 (HER2). TNBCs comprise ~15% of breast cancer cases and are associated with poor prognosis and limited treatment options. Screening of a natural compound library identified a compound, C4, with promising bioactivity against TNBC cells in vitro. Specifically, cell viability was decreased in a concentration- and time-dependent fashion following treatment with C4. This coincided with an unexpected increase in pAkt expression. Both cell viability deficits and enhanced pAkt levels downstream of C4 treatment were rescued by the antioxidant N-acetyl-L-cysteine (NAC), suggesting a central role for reactive oxygen species (ROS) in the mechanism of action of C4. Our investigations to date on the putative mechanisms of cell death induced by C4 have provisionally ruled out apoptosis, necroptosis and ferroptosis. This natural compound merits further investigation as a potentially novel therapeutic for TNBCs, particularly in combination with current therapies.

This abstract has emanated from research conducted with the financial support of Science Foundation Ireland (SFI) under grant number 13/IA/1994.
Acknowledgement: The NCI/DTP Open Chemical Repository" https://dtp.cancer.gov

THE VIRTUALLY MATURE BNP (BNP1-32) IS A PRECURSOR FOR THE MORE POTENT BNP1-30

Anja Schwiebs[1], Andrew Moore[2], Yong Wang[1], Xudong Zhu[3], Kristin Pankow[4], Wolf-Eberhard Siems[4], Thomas Walther[2]. [1]Department of Cardiology, Justus-Liebig-Universität Giessen, Germany. [2]Department of Pharmacology and Therapeutics, University College Cork, Ireland. 3Centre for Cardiovascular and Metabolic Research, University of Hull, United Kingdom. [4]Leibniz-Institut für Molekulare Pharmakologie, Berlin, Germany.

The mature B-type natriuretic peptide (BNP1-32) exerts vasorelaxing and cardioprotective activity. BNP is used as a biomarker for the diagnosis of cardiopathological conditions and recombinant BNP1-32 as a drug for the treatment of such. BNP1-32 has a short half-life time and thus, similar to other vasoactive peptides like angiotensin II and bradykinin, can be enzymatically truncated forming bioactive metabolites. We aimed to investigate the metabolism of BNP1-32 in lung, to identify potential new BNP metabolites and to disclose their biological activity compared to the mature BNP1-32, in vitro and in vivo.

Using High Performance Liquid Chromatography and Mass-Spectrometry, we identified a new BNP metabolite, BNP1-30, in the lung being generated by endothelin-converting enzyme-1. BNP1-30 is more efficient in stimulating the natriuretic peptide receptor (NPR) A and, in contrast to BNP1-32, is also able to stimulate the NPRB. In vivo, BNP1-30 reduced the mean arterial blood pressure of normotensive mice after acute infusion significantly more than BNP1-32. In a model of severe hypertension, a 3-day infusion of BNP1-30 was able to reduce systolic blood pressure by 30 mmHg and to improve markers of heart failure, while BNP1-32 was without significant effect. Importantly, BNP1-30 could not only be detected in rodents but is also generated in the human lung.

Our results suggest that BNP1-32 is only the precursor for the biologically more active BNP1-30 leading to a fundamental extension of the natriuretic-peptide system. Due to the expanded activity, BNP1-30 might be a promising treatment option for cardiovascular diseases. Furthermore, its potency as a new diagnostic marker of specific cardiac diseases should be evaluated.

AN INVESTIGATION INTO WHETHER THERAPEUTIC CONTROL OF MIRNA'S COULD BE BENEFICIAL IN SEPSIS

Watkin RL[1], Piskareva O[2], Stallings R[2], Kerrigan SW[1]. [1]Cardiovascular Infection Research Group and [2]Cancer Genetics Research Group, Royal College of Surgeons in Ireland, Dublin 2, Ireland.

Sepsis is a leading killer in ICUs and is often caused by the gram positive bacteria Staphylococcus aureus. Upon entering the bloodstream, S. aureus is capable of binding to the inner lining of the blood vessels, the endothelium, triggering endothelial dysfunction, inflammation and oedema. However, despite high mortality rates, little is known about the signals leading to this response. miRNA are predicted to regulate 60% of the human genome including that of the endothelium. This research aims to ascertain if an alternative endothelial miRNA profile is produced by S. aureus infection and to investigate resulting regulation that may cause endothelial dysfunction. miRNA profiles of infected and uninfected cells were determined by RT-qPCR and miRNA with significant expression changes verified (RQ = $2^{-\Delta\Delta Ct}$). Potential mRNA targets were established using online databases. Proliferation and permeability functional assays investigated the role of detected targets in endothelial dysfunction. 93 miRNA were differentially expressed in infected endothelial cells ($N=4$, $p<0.05$). KEGG pathway analysis demonstrates significant enrichment of targets in Linoleic Acid Metabolism (8 targets, $p=0.0007$) and the MARK Signalling Pathway (3 targets, $p=0.006$), pathways which may affect dysregulated inflammation. Additional targets of interest were linked to vascular permeability and proliferation both of which are worsened by infection. This atypical miRNA profile may explain how S. aureus causes endothelial dysfunction with resulting intrinsic signalling leading to dysregulation of proliferation and permeability.

Funding: Science Foundation Ireland (SFI) under Grant Number 13/CDA/2119

REAL-TIME IMAGING OF LYSOSOMAL DISRUPTION WITH A PH RESPONSIVE NIR FLUOROPHORE

Cheung S, O'Shea DF, Department of Pharmaceutical and Medicinal Chemistry, RCSI, Dublin 2

Near infrared (NIR) fluorescence is a useful tool for probing biological processes as it allows for real time imaging. Our group has developed NIR fluorophores which are fluorescently silent at pH7, but in acidic lysosomes (pH~5) have a strong emission (1). This permits highly selective staining of lysosomes in living cells and in real time imaging of their disruption. Prodigiosin is a bacterial pigment which is a highly potent anion receptor that has been reported to selectively de-acidify lysosomes (2). This process causes apoptosis, which is triggered by a low cytosolic pH. As part of my PhD a new method to 4D-image in real-time lysosome disruption by prodigiosin has been developed which allows visualization of all the key stages of the process from lysosome de-acidification, to cytosol acidification and cell entry into apoptosis.

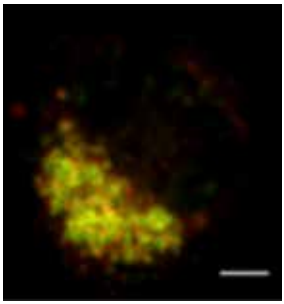


Fig.1 GFP labelled lysosomal membrane (green) surrounding NIR fluorophore "on" at pH5 (red)

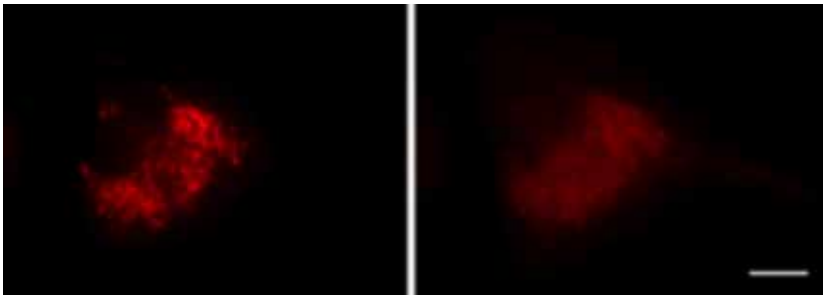


Fig.2 Selectively stained lysosomes (left), and prodigiosin induced deacidification of lysosomes (right).

1. Murtagh J, Frimannsson DO, O'Shea DF. Org. Lett. 2009; 1:5386–5389
2. Gale PA, Pérez-Tomás R, Quesada R. Acc. Chem. Res. 2013; 46: 612-624

IDENTIFICATION OF ANTI-ANGIOGENIC AND ANTI-METABOLIC COMPOUNDS IN-VITRO AND IN-VIVO IN ZEBRAFISH TO DETERMINE IF NOVEL DUAL ACTION DRUGS CAN ENHANCE RADIOSENSITIVITY IN OESOPHAGEAL ADENOCARCINCOMA

Amy Buckley [1] , Niamh Lynam-Lennon [1] , Aoife Cannon [1] , Róisín Byrne [1] , Alison Reynolds [2] , John V. Reynolds, Breandán Kennedy [2] , Jacintha O'Sullivan [1]
[1]Trinity Translational Medicine Institute, Department Of Surgery, St. James's Hospital, Trinity College Dublin

2UCD Conway Institute & UCD School of Biomolecular and Biomedical Science, University College Dublin

Oesophageal Cancer (OAC) is an aggressive disease with a dismal cure rate of approximately 15-20%. Current therapeutic regimes focus on neo-adjuvant treatment with chemo-radiation therapy prior to surgery. Unfortunately, only 20-30% of patients show a beneficial response, with 70-80% of patients receiving treatment with no benefit. An upregulation of angiogenesis, metabolism and DNA repair is correlated with radiation resistance in OAC. This clinical challenge of treatment resistance reinforces the need for novel therapies that can act as radio-sensitisers in the neo-adjuvant setting.

Intersegmental vessel assay (ISV) screens were performed in Tg(fli1:EGFP) zebrafish to identify the anti-angiogenic potential of the CC8 family of analogues (n=23). The most potent anti-angiogenic compounds, were screened to evaluate their anti-metabolic activity in zebrafish and in an isogenic oesophageal in vitro model of radio-resistance using the XFe24 Seahorse Analyser. The effect of our structural analogues on radio-sensitivity was evaluated using the clonogenic assay.

11B_CC8 and 4 analogues produced the greatest anti-angiogenic activity in-vivo in zebrafish. 11_CC8, 11B_CC7_HCl and 11B_CC8_HCl produced a significant reduction in oxygen consumption rate (OCR), a measure of oxidative phosphorylation in zebrafish, with 11B_CC8 AND 11B_CC7_HCl producing a simultaneous reduction in extracellular acidification rate (ECAR), a measure of glycolysis in vivo. 11B_CC8 and 11B_CC7_HCl showed the greatest anti-metabolic activity in vitro. Treatment with novel dual action compound 11B_CC8 radio-sensitised OE33P and OE33R cells to radiation when compared to untreated irradiated cells. Novel dual action small molecule compounds which inhibit both developmental angiogenesis and metabolism can radio-sensitise radiation-resistant OAC cells to radiation in-vitro.

THE ROLE OF THE ENDOTHELIUM AND PLATELETS IN THE DEVELOPMENT OF NEONATAL SEPSIS

Emagha UT1, Hughes L[1], McDonnell C[1], Meehan M[2], Kerrigan SW[1].
1Royal College of Surgeons in Ireland, School of Pharmacy & Molecular and Cellular Therapeutics, 2Temple Street Children's University Hospital, Dublin, Ireland

Sepsis is an expensive disease and a major cause of morbidity and mortality in neonates. It is characterised by microthrombi formation and endothelial dysregulation. The intestinal and vaginal microflora, Streptococcus agalactiae, commonly known as Group B Streptococcus (GBS), is the most common neonatal sepsis pathogen in the western world. The molecular interactions by which GBS induces platelet activation and endothelial dysregulation in sepsis are poorly understood.

The aim of this study was to investigate the molecular interaction between human vascular endothelial cells, platelets and GBS.

The study design was approved by the RCSI Ethics Committee Dublin (REC679b). Human aortic endothelial cells (HAoECs) sheared at 10dynes/cm2 were incubated ± 10ng/ml TNF- with GBS in the presence of human plasma. Adherence of GBS to HAoECs was measured by fluorimetry. Aggregometry was used to assess the ability of 20 GBS clinical isolates to induce platelet aggregation with inhibitors.

Using a clinically relevant model, GBS adhered to sheared HAoECs independent of plasma or its components (P=NS). GBS strains induced platelet aggregation (60.3±17.4%) in a non-serotype dependent manner. Cyclooxygenase and FcγRIIa inhibition but not TLR2 abolished the aggregation ($P<0.01$). IgG was crucial for aggregation using washed platelets ($P<0.01$).

Here, we show that GBS has the ability to directly bind the endothelium with resultant platelet activation. GBS induced platelet aggregation is non-serotype dependent and the Immunoglobulin receptor, FcγRIIa may contribute to GBS induced platelet aggregation. This knowledge will contribute to the discovery of a novel molecular therapy in neonatal sepsis.

CDK7: A PROGNOSTIC MARKER OF POOR OUTCOME AND RATIONAL THERAPEUTIC TARGET IN TRIPLE-NEGATIVE BREAST CANCER

Li B, Ni Chonghaile T[2], Fan Y[1], Madden SF[4], Klinger R[1], O'Connor AE[1], O'Hurley G[5], Udupi GM[5], Joseph J[5], Tarrant F[1], Conroy E[1], Jirstrom K[6], O'Connor DP[3] and Gallagher WM[1, 5]
1UCD Conway Institute, University College Dublin; Dept. of [2]Physiology & Medical Physics, [3]Molecular and Cellular Therapeutics, [4]Population Health Sciences Division, Royal College of Surgeons in Ireland; [5]OncoMark Ltd, Dublin; [6]Lund University, Sweden.

There is an urgent need to identify biomarkers of outcome and targeted therapeutics for triple-negative breast cancer (TNBC) patients. We aimed to identify novel kinase targets that may play a pivotal role in the progression of TNBC and offer new therapeutic prospects.

Survival analysis was performed to investigate the association between CDK7 mRNA expression and clinical outcomes. CDK7 protein expression was evaluated via immunohistochemical staining of TNBC tissue microarray (TMA) cohorts, and survival analysis was performed. To therapeutically target CDK7, BS-181 and THZ1 were tested in vitro. MTT and Annexin V/propidium iodide assays were performed to evaluate cell proliferation and apoptosis following CDK7 inhibitor treatment. Dynamic BH3 profiling technology was utilized to evaluate survival dependency following THZ1 treatment of TNBC cells.

CDK7 mRNA expression was found associated with poor recurrence-free survival in a public TNBC dataset and with poor breast cancer-specific survival (BSSS) in the METABRIC dataset. CDK7 protein expression was associated with BCSS in the RATHER and METABRIC TNBC TMA cohorts. BS-181 and THZ1 led to RNA transcription inhibition, and induced apoptosis in the TNBC cell lines evaluated. THZ1 demonstrated 1000-fold higher potency than BS-181. THZ1 caused an increased survival dependency on BCL-2/BCL-XL. A combination treatment for TNBC involving THZ1 and ABT-263/ABT-199 was identified.

In conclusion, CDK7 is a promising marker of poor prognosis in TNBC. Targeting CDK7 alone or in combination with the BH3 mimetics ABT-263/ABT-199 may be a useful therapeutic strategy for TNBC.

VITAMIN D RECEPTOR AGONISTS ATTENUATE OCULAR DEVELOPMENTAL ANGIOGENESIS AND REGULATE THE EXPRESSION OF ANGIOMIR MIRNA21 AND PRO-ANGIOGENIC FACTO

VEGF in Zebrafish

Merrigan Stephanie[1]

The highlighted section has run onto the line with the name VEGF in Zebrafish is part of the title.

Abnormal ocular vasculature growth (angiogenesis) can underpin age- and diabetes-related blindness. Current treatments have several limitations. Our objective was to discover a novel inhibitor of angiogenesis through phenotype-based screening of a bioactive drug library.

ICCB library compounds were screened for inhibition of ocular vessel development in zebrafish larvae. Hit compounds were tested for inhibition of non-ocular intersegmental vessel development. mRNA/miRNA expression studies were carried out by qRT-PCR. Retinal morphology was examined by light microscopy. ELISA determined ARPE-19 cell angiogenic/ inflammatory factor secretions.

Hit compound calcitriol and 6 additional vitamin D receptor agonists (VDRAs) significantly and specifically inhibited ocular developmental angiogenesis. Safety studies showed calcitriol-treated larvae to have normal retinal lamination/morphology. miR21, VEGFaa and VEGFab expression was significantly upregulated in calcitriol-treated eyes and miR150 expression unchanged. VEGF receptors flt1 and kdrl were not significantly upregulated but a dose dependant increase in expression was seen in calcitriol-treated eyes. In ARPE-19 cells; preliminary data shows TNFα induced IL-8 and GM-CSF expression to be attenuated by VDRA treatment.

VDRAs significantly, specifically and safely inhibit ocular developmental angiogenesis. This activity correlates with increased miR21 and VEGF. Future studies will evaluate the anti-angiogenic mechanism of VDRAs and validate activity in additional models.

BROMODOMAIN INHIBITION AS A NOVEL THERAPEUTIC FOR INVASIVE LOBULAR CARCINOMA

Walsh L, Tarrant F[2], Fan Y[2], Das S[1], Moran B[2], Gallagher WM[2], Ní Chonghaile T[3*], O'Connor D[1*]. Department of Molecular and Cellular Therapeutics[1] & Physiology and Medical Physics[3] RCSI, Dublin 2. School of Biomolecular & Biomedical Science[2], Conway Institute, UCD, Dublin 4.

Invasive lobular carcinoma (ILC) is a breast cancer subtype comprising 10% of breast tumours. The majority (90%) of ILC are oestrogen receptor (ER)-positive and are treated with endocrine therapy. Unfortunately, 33% of women are resistant to endocrine therapy and up to 40% will relapse following treatment. Therefore, novel therapies are required. Deregulated transcription is a recurring theme in cancer. The BET family of proteins (BRD2, BRD3, BRD4, BRDT) function as chromatin readers that bind acetylated lysine residues on histones and regulate transcription.

We performed RNA-Sequencing analysis on 61 primary ILC samples and found that high BRD3 expression is associated with poor survival. We validated this in a second cohort of 99 ILC primary samples. Next, we assessed the sensitivity of ILC cell lines to BET inhibition with the small molecule inhibitor JQ1 using MTT assay and annexin V/propidium iodide staining. JQ1 inhibits cell growth in all cell lines tested, however apoptosis was only induced in two of the cell lines. JQ1 also downregulates growth and survival genes including MYC, ER and BCL-XL as measured by Western blotting. This led us to assess the combination of JQ1 and the BH3 mimetic ABT-263 (BCL-2, BCL-XL, BCL-W inhibitor). This combination was synergistic and enhanced or induced apoptosis in the cell lines. Our work suggests that JQ1 in combination with ABT-263 may be a rational therapeutic combination for ILC.

The authors acknowledge grant support from BREAST-PREDICT as part of the Irish Cancer Society and the EU FP7 project RATHER

THE ROLE OF THE ANORECTIC NEUROPEPTIDE CART IN BREAST CANCER

Brian Mooney[1], Sudipto Das[1], Rut Klinger[2], Eric Conway[3], Ben Doyle[4], Kieran Wynne[2], Adrian Bracken[3], Gerard Cagney[2], David Cotter[4], William M. Gallagher[2], Triona Ni Chonghaile[5], Darran O'Connor[1]

[1] Molecular and Cellular Therapeutics, Royal College of Surgeons Ireland, Dublin 2, [2] UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Dublin 4, [3] Smurfit Institute of Genetics, Trinity College Dublin, Dublin 2, [4] Department of Psychiatry, Beaumont Hospital, Royal College of Surgeons in Ireland, Dublin 9, [5] Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin 2.

The cocaine- and amphetamine-regulated transcript (CART) was first discovered as a peptide up-regulated by the administration of cocaine and amphetamine to rats. CART peptides regulate many physiological processes such as feeding and reward. Previous studies have demonstrated that high expression of CART correlate with poor overall survival in Estrogen Receptor-Positive (ER+), lymph node-negative breast cancer. CART expression also correlated with poor response to tamoxifen. The aim of this study was to elucidate the mechanism(s) by which CART signals in ER+ breast cancer. MAP-Kinase pathway activation and levels of downstream gene-targets of ER-alpha (ERα) were assessed post CART stimulation via Western Blot and qPCR in an effort to understand the influence CART has on ERα activity. Additionally, the ability of CART to activate ERα using three LXD motifs (nuclear receptor co-activator recognition motifs) present within the CART sequence was also assessed. This was achieved through selectively mutating these motifs and testing whether CART still possessed the ability to activate ERα using an ERE-Dual luciferase reporter assay. Treatment of cells with CART demonstrated an increase in MAP-Kinase pathway activity through the detection of increasing phosphorylated ERK levels. CART stimulation also resulted in an increase in Progesterone Receptor and Cyclin D1 levels, both known ERα gene targets. Moreover, mutagenesis of each LXD motif within CART resulted in significant decreases in ERα activity, suggesting a potential structure-function relationship between CART and ERα. In conclusion, we suggest that CART can activate ERα in a ligand-independent manner through the MAP-Kinase pathway, and also potentially through specific LXD motifs within its sequence. Mass Spectrometry based proteomic studies are currently ongoing, with the specific goal of identifying binding partners of ERα which are influenced by CART expression. With these studies, we aim to identify novel therapeutic targets for alleviating tamoxifen resistance in ER+ breast cancer patients.

This research is kindly supported by The Irish Cancer Society.

TRENDS IN PRESCRIBING OF ANTIPSYCHOTICS IN DEMENTIA: A POPULATION-BASED STUDY

Gul Z[1], Teeling M[1], MacAvin MJ[1] , Bennett K[2]. Department of Pharmacology and Therapeutics, TCD[1] and RCSI[2]

Evidence that typical and atypical antipsychotics (APS) increase all-cause mortality and stroke in patients with dementia is growing.

This study examined the trends in co-prescribing of APS in community dwelling individuals aged ≥ 70 years receiving anti-dementia drugs (ADD).

A retrospective cohort study was undertaken in community dwellers aged ≥ 70 years prescribed >1ADD, co-prescribed APS during 2009- 2014, using a pharmacy claims database.

Generalised estimating equations were used to examine trends in percentage use of APS over time (interactions time by gender, age, typical/atypical APS). Predictors of APS use in new initiators of ADD, including age, gender and year of initiation, were determined using logistic regression. Adjusted odds ratios (OR) and 95% confidence intervals (CI) are presented.

There was an increase in the overall prevalence of co-prescribed APS in patients receiving ADD from 2009-2014. The percentage of APS use in males on ADDs increased slightly from 31.5% (Jan 2009) to 33.4% (Dec 2014) but remained static in females. Patients aged ≥85 years had the highest APS use (34.2%), followed by 70-79 (30.6%) and 80-84 years(30.5%; $p<0.001$). There was a trend of increasing use of atypical antipsychotics ($p<0.0001$). The likelihood of initiating APS in newly treated dementia patients significantly decreased over time (2013 vs 2010; OR=0.82, 95%CI 0.77, 0.89).

Increased prescribing of APS, particular in males and the older aged population, suggests that clear guidance, particularly in these groups, may be required.

UNCOVERING A NOVEL UPSTREAM REGULATOR OF RECEPTOR TYROSINE KINASE SIGNALLING IN BREAST CANCER

Cruz RGB, Hopkins AM. Department of Surgery, Royal College of Surgeons in Ireland, RCSI Education and Research Centre, Beaumont Hospital.

Introduction: JAM-A is a transmembrane protein which regulates cell-cell adhesion. Its high expression in breast tumour tissue correlates with over-expression of HER2, whilst JAM-A knockdown in breast cancer cells reduces HER2 expression and signalling. HER2 drives tumorigenic signalling through homo- and hetero-dimerisation with other members of the HER family, yet it is currently unknown whether JAM-A might also influence their expression/function.

Methods: A panel of breast cancer cell lines was used to analyse if changes in protein and RNA expression levels of JAM-A influenced those of HER1, HER3 and HER4 and its downstream signalling effector molecules.

Results: JAM-A gene silencing reduced HER3 mRNA and protein expression in estrogen receptor (ER)-positive MCF7 breast cancer cells, and concomitantly reduced the protein expression of its downstream effectors phospho-AKT and -ERK. This translated into a significant reduction in cell viability. Conversely, JAM-A overexpression increased mRNA and protein expression of HER3. Gene silencing of any HER family member did not affect JAM-A protein expression in those cells. In ER-low breast cancer cells which express negligible levels of HER3, JAM-A knockdown instead reduced HER1 and HER4 mRNA and protein expression.

Conclusion: Our results suggest that JAM-A regulates expression of HER receptor tyrosine kinases in a context-specific manner. Ongoing investigations will determine the mechanisms involved and explore the possibility of JAM-A as a future drug target to inhibit HER-dependent breast cancer tumorigenic signalling.

PROTECTIVE EFFECT OF TRPM7 INHIBITION IN A MODEL OF BREAST TUMOUR CALCIFICATION

O'Grady S [1], Morgan MP [1] .
[1] Department of Molecular and Cellular Therapeutics, RCSI, Dublin.

Despite comprising one of the most commonly found mammographic indicators of breast cancer, the formation of mammary microcalcifications remains poorly understood. We have established a human in-vitro model of microcalcification formation utilising the triple-negative cell line MDA-MB-231. Calcified deposits were observed following at least 14 days of incubation with the osteogenesis promoting reagents γ-glycerophosphate, ascorbic acid and dexamethasone.

Altered magnesium homeostasis has been suggested as an important mediator of tissue calcification. We found that a slight increase in magnesium concentration almost completely blocked calcium deposition in our model. Upregulation of the cation channel TRPM7, an important regulator of intracellular magnesium levels was observed in the early stages of calcification (60% increase at day 14, $p<0.01$). Unexpectedly, blockade of this channel by two separate TRPM7 channel inhibitors (2-APB and NS8593) failed to attenuate the protective effect of magnesium in our model. Further studies showed that TRPM7 inhibition blocked calcification in a dose dependent manner even in the absence of additional magnesium.

These results identify TRPM7 as a potential candidate in the initiation of mammary calcification and may provide a possible explanation for the high expression levels of this channel observed in patient samples with associated microcalcifications.
This work was funded by Breast Cancer Now.

NOTES

NOTES

RCSI Royal College of Surgeons in Ireland
Coláiste Ríoga na Máinleá in Éirinn
123 St Stephen's Green, Dublin 2
Tel: +353 1 402 xxxx
Email: xxxxxxxx@rcsi.ie
www.rcsi.ie