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INTERNATIONAL PhD PROGRAMME
RESEARCH PROJECTS

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Summary of available research projects

Research Theme: Cancer

Research project 1: The role of USP11 in ER+ Breast Cancer
Supervisors: Prof Darran O'Connor, Molecular & Cellular Therapeutics (MCT) and Prof Tracy Robson, Molecular & Cellular Therapeutics (MCT).
Research Project Description: Background: De-regulated estrogen receptor (ER) function is a key feature of approximately 70% of breast cancers. Given that the tumourigenic properties of ER primarily lie in its function as a growth-controlling transcription factor, we sought to discover novel modulators of ER transcriptional activity. Using an RNAi loss-of-function screen, we identified the deubiquitinating enzyme USP11 as a key regulator of ER transcriptional activity. Aims: We hypothesize that USP11 influences ER activation through removal of ubiquitin moieties that block acetylation and repress ER transcriptional activity. We propose to:
1) Determine the role of USP11 de-ubiquitinating activity in controlling ER function and the effect modulating USP11 has on the ubiquitination/acetylation balance of ER.
2) Determine the effect of USP11 on the response of ER+ breast cancer cells to anti-endocrine therapy and examine the effect of CRISPR knock-in ER mutations on the ability of USP11 to control ER function.
3) Further validate USP11 as a breast cancer biomarker and evaluate the in vivo effect of USP11 modulation on the growth of ER+ breast cancers and their response to anti-endocrine therapy.
Techniques and Methodology: A combination of immuno-precipitation, mass spectrometry and RNAi will be used to map target lysines and explore the functional relevance of their modification. Impact on response to anti-endocrine drugs will be evaluated using in vitro growth assays and xenografts in nude mice and the impact of ER mutations on these responses assessed. Tissue microarrays will be used to evaluate the clinical relevance of USP11.
Impact on breast cancer research ER remains a rational therapeutic target in both the primary and recurrent setting and the discovery of novel mechanisms controlling ER function offer attractive new therapeutic opportunities.
Host Laboratory: The Molecular Oncology Laboratory at the Dept. of Molecular & Cellular Therapeutics, led by Dr Darran O'Connor (http://www.rcsi.ie/index.jsp?p=256&n=726&a=6338), is a young, vibrant and well-funded research group focused on the identification and mechanistic anchoring of novel cancer biomarkers and therapeutic targets. As part of major national and international research consortia (e.g Breast-Predict Cancer Centre (www.breastpredict.com), RATHER (www.ratherproject.com) and Angiopredict (www.angiopredict.com)) the lab has a network of world-class collaborators in the cancer research field, with ample opportunity for national and international secondment.
Recent outputs:
1. Li B, Ni Chonghaile T, Fan Y, Madden S, Klinger R, O'Connor AE, O'Hurley G, Mallya G, Joseph J, Tarrant F, Conroy E, Gaber A, Chin SF, Bardwell HA, Provenzano E, Dubois T, Linn S, Jirstrom K, Caldas C, O'Connor DP* & Gallagher WM*. Therapeutic rationale to target highly expressed CDK7 conferring poor outcomes in triplenegative breast cancer. Cancer Res 2017 Jul 15;77(14):3834-3845 *Shared Senior Authorship.
2. Mulrane L, Madden SF, Brennan DJ, Gremel G, McGee SF, McNally S, Martin F, Crown JP, Jirström K, Higgins DG, Gallagher WM & O'Connor DP. miR-187 is an independent prognostic factor in breast cancer and confers increased invasive potential in vitro. Clin Cancer Res 2012 Dec 15;18(24):6702-13. 3. Brennan DJ*, O'Connor DP*, Rexhepaj E, Ponten F & Gallagher WM. Antibody-based proteomics: Fast-tracking molecular diagnostics in oncology. Nature Reviews Cancer, 2010 Sep;10(9):605-17. *Equal Contribution
Keywords: Cancer

Research project 2: Breast Cancer Associated Microcalcifications – Investigation of their Relationship with Tumour Molecular Subtype and Potential Consequences for Tumour Progression
Supervisors: Dr Maria Morgan, Molecular & Cellular Therapeutics (MCT) and Prof Leonie Young, Surgery.
Research Project Description: Radiographic mammary microcalcifications constitute one of the most important diagnostic markers of both benign and malignant lesions of the breast. Up to 50% of all nonpalpable breast cancers are detected solely through microcalcifications presenting during a mammogram scan and up to 93% of cases of ductal carcinoma in situ (DCIS) present with microcalcifications. During the past decade, cases of subclinical cancer, that is, breast cancers detected by mammography, have accounted for a progressively increasing percentage of breast cancers. Although the diagnostic value of these microcalcifications in breast cancer is well established, their genesis is not clear. In particular, the question as to whether they are a sign of degeneration or of an active cell process remains a matter of debate. It is generally accepted that the presence of oxalate-type microcalcification [CaC2CO4-2H2O, Type I] appears to be a reliable criterion in favour of the benign nature of the lesion or, at most, of a

lobular carcinoma in situ, whereas calcium hydroxyapatite (HA) [Ca10(PO4)6(OH2), Type II] is generally associated with malignant breast tumours. With the exception of our recent publications, there have been no other reported investigations of the mechanism of calcium deposition or, the potential of differing mammary cell types to generate specific calcium mineral species. No study to date has examined the pro-inflammatory potential of the deposited hydroxyapatite found associated with breast cancer. Furthermore, the potential for mineralisation and the microenvironment regulating it representing a significant feature of selected tumours has not even been considered. This project will test the hypothesis that (i). Mammary cells can generate specific mineral deposits which reflect their molecular subtype and/or state of differentiation; and (ii). mineralisation induced local inflammation may promote an inflammatory niche which contributes to tumour progression. This PhD project will help elucidate the relationship between microcalcifications and the mammary tumour microenvironment, establishing the role for mineralization-associated pro-inflammatory molecules in influencing macrophage function and polarization. The project will further clarify the biological consequence of mineralization-induced epithelial to mesenchymal transition in promoting tumourigenesis through active tissue remodelling. This study will help resolve the unexplained prognostic association of these calcifications with benign and malignant disease of the breast and help to understand the full complexities of one of the most significant markers of pre-invasive breast cancer.
Keywords: Cancer

Research project 3: Modelling tumour – immune system interactions in 3D in vitro model of neuroblastoma using collagen-based scaffolds
Supervisors: Dr Olga Piskareva, Molecular & Cellular Therapeutics (MCT), Dr Caroline Curtin, Anatomy, Prof Fergal O'Brien, Anatomy and Prof. Donal O'Shea, Pharmaceutical & Medicinal Chemistry, RCSI.
Research Project Description: The main challenge in treating neuroblastoma, a paediatric cancer of the sympathetic nervous system, is to combat tumour metastasis and resistance to multiple chemotherapeutic drugs highlighting the unmet need in new more efficient pre-clinical models to study disease pathogenesis, drugs testing and development. Immune therapy holds great promise as a treatment modality for paediatric and adult cancers owing to the specificity of immune effector cells targeted to the tumour, potentially reducing the systemic side effects observed with other forms of treatment. In order to accurately study the interaction between immune and tumour cells, we need to develop an adequate 3D in vitro model system that mimics the native tumour microenvironment at the tissue level.
3D scaffold-based in vitro cell culturing is a recent advancement in cancer research bridging the gap between conventional 2D culture and in vivo tumours. A scaffold is a 3D matrix that provides the necessary support for cells to proliferate, differentiate, deposit extra-cellular matrix and respond to stimuli similar to in vivo biological systems.
To date, the collaborative efforts of Piskareva's and Prof. O'Brien research groups led to the development of a 3D tissue-engineered tumour cell model using collagen-based scaffolds for neuroblastoma). Neuroblastoma cells displayed > 100-fold increased resistance to cisplatin treatment when compared to 2D cultures exhibiting chemosensitivity similar to orthotopic xenograft models. This 3D in vitro model demonstrated a physiological similarity to in vivo models, making evident the potential of this model to serve as a tool to elucidate neuroblastoma pathogenesis and for the development of new drugs. Therefore, by growing cancer cells on the 3D scaffolds and allowing the formation of 'tumour mass', the shortcomings of using 2D cultured cells can be overcome as minimal communication networks and cellular gradients observed within in vivo tumours are re-established.
Here, we suggest advance the current model to study tumour – immune system interactions by characterization of the microenvironment in 3D using a panel of neuroblastoma cell lines with distinct genomic and biological characteristics and incorporating cellular and molecular components of the immune system. Neuroblastoma cell death will be examined by real-time using a patented RCSI Amphiphilic NIR-Fluorescent Probe developed in Prof. O'Shea's lab and optimized to label neuroblastoma cells. This fluorophore permits real-time imaging of cellular uptake, trafficking and efflux without perturbing function. The combination of new generation of fluorophore and 3D in vitro culturing will allow to mimic the tumour immune interactions and to test the response of the platform to anti-GD2 immunotherapy, which entered clinical trials for children with high-risk neuroblastoma.
The major ambitions and aspiration for this project are:

- the development of a new 3D tumour tissue engineered model to study tumour - immune system interactions;
- the reduction and/or replacement of live animals and provide a new platform for pre-clinical testing;

- the acceleration of the paediatric cancer drug development process leading to more effective and tailored therapies.

Keywords: Cancer, Immunology, Tissue-engineering

Research project 4: A key role for FKBPL in the regulation of cancer stem cell signalling and the microenvironment; therapeutic implications for tumour growth and metastasis
Supervisors: Prof Tracy Robson, Molecular & Cellular Therapeutics (MCT) and Dr Darran O'Connor, Molecular & Cellular Therapeutics (MCT).
Research Project Description: Cancer stem cells (CSCs) are a special type of cell found within tumours that are able to undergo unlimited self-renewal and are highly resistant to therapy. Indeed, these cells are left behind and go on to divide rapidly, leading to tumour regrowth. Even more worrying, this population of cells have special features allowing them to move through the body, invading vital organs; a process known as metastasis. We have identified a novel protein, called FKBPL, that occurs naturally in the body and which inhibits tumour blood vessel development, thereby stopping tumour growth. A therapeutic drug derived from the protein and designed to harness its therapeutic effects, has successfully completed phase I cancer clinical trials and was recently granted Orphan Drug status in ovarian cancer by the FDA. However, we have acquired data which suggests that this protein also targets breast and ovarian CSCs by transforming them into a more 'normal' cancer cell, which can be easily killed by chemotherapy. This project will assess the impact of FKBPL on other cells within the ovarian tumour microenvironment that are known to support the growth and survival of CSCs cells in the primary tumour and at distant sites. We will evaluate exactly how FKBPL controls these cells and the implications on the ability of CSCs to become metastatic. Understanding how this protein works will allow us to design future clinical trials that are more likely to demonstrate better response rates in cancer patients.

- Valentine A, O'Rourke M, Yakkundi A, Worthington J, Hookham M, Bicknell R, McCarthy H, McClelland K, McCallum L, Dyer H, McKeen H, Waugh D, Roberts J, McGregor J, Cotton G, James I, Harrison T, Hirst D, Robson T FKBPL and peptide derivatives: novel biological agents that inhibit angiogenesis by a CD44-dependent mechanism. Clin Cancer Res. 2011 Mar 1;17(5):1044-56.
- McClements L, Yakkundi A, Papaspyropoulos A, Harrison H, Ablett MP, Jithesh PV, McKeen HD, Bennett R, Donley C, Kissenpfennig A, McIntosh S, McCarthy HO, O'Neill E, Clarke RB, Robson T. Targeting treatment resistant breast cancer stem cells with FKBPL and its peptide derivative, AD-01, via the CD44 pathway. Clin Cancer Res. 2013 Jul 15;19(14):3881-93.
- Annett S, Robson T. Targeting cancer stem cells in the clinic: Current status and perspectives. Pharmacol Ther. 2018 Jul;187:13-30.

Keywords: Cancer, metastasis, stem cell signalling

Research project 5: Theranostic Combination of Targeted Fluorescence Imaging and Photodynamic Therapy
Supervisors: Prof Donal F O'Shea, Pharmaceutical & Medicinal Chemistry and Dr Brona Murphy, Physiology & Medical Physics.
Research Project Description: Research Project Description: Photodynamic therapy (PDT) is a modern treatment that uses light to locally photosensitize tissues, resulting in the selective killing of targeted cells. Following administration of a photosensitizer, the cancerous lesion is irradiated with low energy light, promoting a set of photochemical reactions to damage the irradiated cell population, resulting in tumor ablation. Although PDT is an effective means of killing cancer cells, photosensitizers generally have little intrinsic selectivity for tumors. Increasing tumor selective accumulation could improve the efficacy of PDT and reduce any risk of side effects caused by photosensitizer accumulation in non-target tissue.
The various types of cells that comprise a tumor mass express many molecular receptors on their surface that distinguish them from normal cells. Nanobodies (smallest active targeting component of an antibody) can selectively recognize and target such surface receptors leading to delivery of a therapeutic payload exclusively at the desired site of disease. This research project will focus on exploring a less harmful approach to cancer therapy through nanobody derivatization to facilitate a controlled and definable conjugation to the highly efficient photosensitizing agents dibromo-BF2-chelatedazadipyrromethenes (ADPMs). The ADPM photosensitizer class has been developed within the O'Shea research team and their covalent combination with nanobody targeting agents would yield a drug-light combination that lends itself to two layers of selectivity. The inherent fluorescence of the ADPM class allows them to be readily visualized in vitro and in vivo thereby allowing for a theranostic approach as imaging of the diseased tissue during treatment. Such an approach will increase treatment efficiency while reducing the undesirable side effects associated with traditional chemotherapies. This truly multidisciplinary project will advance knowledge in the fields of bio-conjugation chemistry, chemical biology and efficacy assessment of targeted PDT.
Keywords: Cancer, chemical biology, synthetic chemistry, live cell fluorescence imaging, in vitro/in vivo testing.

Research project 6: Predicting variability in glioblastoma proliferation and spread using stochastic mathematical models and 3-D-cultures of patient-derived neurospheres.
Supervisors: Dr Brona Murphy, Physiology & Medical Physics and Dr Mark Sturrock, Physiology & Medical Physics.
Research Project Description: Brain tumours are the biggest cancer killer of adults under 40. Brain tumours reduce life expectancy by an average of 20 years, the highest of any cancer. Survival rates have improved little in over 40 years. The most common and aggressive primary brain tumour is glioblastoma (GBM). Despite intense effort to combat GBM with surgery, radiation and temozolomide (TMZ) chemotherapy, 90-95% of patients succumb to the disease within 5 years of diagnosis and nearly all patients experience disease recurrence, usually within 6-8 months of treatment onset (Stupp et al. 2009). New, better, more-personalised treatments are urgently required.
The extensive molecular heterogeneity found between GBM tumours contributes significantly to the limited effectiveness of current therapies and the difficulty in developing new efficacious treatment regimes. The theory that a 'one-size-fits-all' approach to treating this disease is not valid. Instead, as our laboratories and others have published, a more personalized approach is necessary to select the most appropriate drugs for a given patient (Paul et al. 2010; Murphy et al. 2013; Weyhenmeyer et al. 2016). Diagnostic tools that can predict case-specifically if and which treatment (Murphy et al. 2013; Weyhenmeyer et al. 2016) is likely to be beneficial for a specific subset of GBM patients are therefore of high interest, both for innovative clinical trials design (enrolment of patients which are likely to respond) and to optimise/personalise treatment. As we and others have highlighted, for this to work, reliable in silico models of the disease that can accurately predict treatment responsiveness need to be developed (Weyhenmeyer et al. 2016).
Such novel approaches are pursued in the field of mathematical modelling (Sturrock et al. 2015; Neal et al. 2013; Jackson et al. 2015). However, one major weakness of existing studies is that they do not capture variability in rates of glioma growth and spread. Hence, the primary objective of this project is to develop stochastic mathematical models of glioma growth that capture and quantify the variability in glioma growth and spread in vivo. Furthermore, model predictions of tumour growth under current and novel treatment conditions will be validated using spatio-temporal imaging data of 3-D-cultures of patient-derived neurospheres grown under these same treatment conditions. Our project holds tremendous potential to develop clinically relevant tools that can identify patients that will likely benefit from currently approved and novel treatment options. This would be an excellent step-forward for patients as it will spare them toxic treatments that hold no overall benefit to them, as well as helping to preselect patients for clinical trials. The aims of this research address one of the most significant problems in the field of brain cancer, and we are confident that our research can make a significant contribution towards improving GBM treatment and patient survival in the future.
Keywords: Cancer, computational biology

Research project 7: Evaluation of Junctional Adhesion Molecule-A (JAM-A) as a novel potential biomarker and therapeutic target in thyroid
Supervisors: Dr Ann Hopkins, Surgery and Prof Christopher Thompson, Medicine, ERC.
Research Project Description: Thyroid cancer is a growing problem in Ireland and across Western nations in general. Although many cases are surgically treatable, the lack of a national screening programme in Ireland means that some thyroid tumours are diagnosed late and thus patient survival prospects are extremely poor. In conjunction with the fact that genetic mutations are relatively rare in thyroid tumours (compared to, for example, breast or lung cancer); this highlights the importance of seeking or validating new proteins that might contribute to disease progression or open up alternative therapeutic approaches. We propose that the protein Junctional Adhesion Molecule-A (JAM-A) represents a potential biomarker and therapeutic target worthy of investigation in thyroid cancer. The overexpression of JAM-A has already been demonstrated to correlate with disease progression and poor patient prognosis in many solid tumours, but nothing is known about its potential contribution to thyroid cancer. However, the expression of a structurally-similar protein belonging to the same protein superfamily as JAM-A has recently been linked with thyroid cancer severity. This project proposes a joint scientist-/clinician-led approach towards interrogating the role of JAM-A in thyroid cancer. It will span a continuum of translational research: using molecular and cell biology data from patients to inform functional assays and ultimately test the druggability of JAM-A in thyroid cancer settings. Collectively, this will ensure broad-spectrum training for the student and the exciting possibility of defining a novel biomarker and therapeutic target for a cancer which is both intellectually stimulating and socio-economically challenging.
Keywords: Cancer

Research project 8: RET a novel therapeutic target to treat breast cancer brain metastasis
Supervisors: Prof Leonie Young, Surgery and Prof Arnold Hill, School of Medicine.
Research Project Description: Metastatic disease recurrence occurs in up to 30% of breast cancer patients with approximately 20% of these tumours metastasising to the brain. With the advent of better systemic therapies, brain metastases are increasing in incidence and confer poor prognosis, which is compounded by limited treatment options. Breast cancer brain metastases are defined by complex adaptations to both adjuvant treatment regimens and the brain microenvironment. Consequences of these alterations remain poorly understood, as does their potential for clinical targeting. Previous research using experimental models and primary tumour datasets has proposed some mechanisms of disease progression relating to brain metastasis. Mutational analysis on longitudinal breast and metastatic samples by our group and others illustrated acquired mutations affecting HER2 and the PI3k/AKT/mTOR pathway. Although current emphasis for longitudinal profiling of tumours is on mutation-level alterations, these approaches have failed to uncover genomic alterations for site-specific metastasis or the molecular determinants that drive adaptation to treatment. Conversely, transcriptional and epigenetic re-programming develops with higher frequency and has been observed to functionally affect oncogenes and related signalling pathways.
In preliminary studies, we have characterized the brain metastatic-altered
transcriptome across 21 patient-matched primary breast tumours and their associated brain metastases to identify new therapeutic targets. Considerable shifts in breast cancer cell-specific gene expression profiles were observed upon brain colonization, which had a large degree of metastatic selectivity.
Bioinformatic analysis for readily druggable targets revealed recurrent gains in expression of the tyrosine kinase receptors RET and HER2. In preliminary studies, ex vivo patient explants and PDX brain metastatic studies demonstrated significant anti-tumour activity for therapies directed against both RET and HER2.
This project as part of the StAR International Training Programme will address clinically relevant questions arising from these observations.

- What is the molecular profile of the primary tumour of patients at risk of developing brain metastasis?
- How does enhanced tyrosine kinase signalling and in particular RET contribute to brain metastasis?
- What is the efficacy of cabozantinib to inhibit progression to brain metastatic disease?
- Can we use next generation sequencing in multiple models of brain metastasis to define a gene signature to predict response to RET treatment?

A multidisciplinary approach, with input from molecular biologists, bioinformaticians, clinicians and industrial partners, will be taken to address these questions. Retrospective and on-going prospective clinical trials will be used to profile at risk patients. In vitro, ex vivo and in vivo models will be used for mechanistic and functional studies, bioinformatics and biostatistics will be employed for advanced data analysis and computational modelling.
The output of this research will be:
Full evaluation of RET as a new therapeutic target to treat breast cancer patients at risk of and with overt brain metastatic disease ready for commercial partnership.
Publications in high-impact journals
In combination with structured StAR PhD training modules, this research will provide a breath of experience in clinically relevant advanced technologies providing the candidate with a competitive advantage for international post-doctoral and/or industrial placements.
Keywords: Cancer, Breast, Brain

Research project 9: Evaluating the impact of the steroid milieu on cancer cell metabolism in endocrine resistant breast cancer
Supervisors: Dr Marie McIlroy, Surgery and Dr Triona Ni Chonghaile, Physiology and Medical Physics Paediatrics, RCSI
Research Project Description: There is strong epidemiological and historical evidence which suggests that estrogens may not be the sole steroid drivers of breast cancer. Understanding individual inter-tumour steroidogenesis will be critical to evaluating this clinically as it is known that elevated levels of weak androgens are associated with breast cancer risk >2 years prior to cancer detection. We hypothesise that abundant androgenic steroid precursors, such as androstenedione (4AD), promote a metabolically deviant, endocrine resistant breast cancer phenotype. This study therefore sets out to address the impact of the global steroid milieu and its relevance to breast tumour biology, the impact on cell metabolism and response to treatment.
Aims: Using well established models of aromatase inhibitor resistance we will determine the fate of the steroid precursor androstenedione (4AD) in vitro, and pair this with quantification data on the steroidogenic

enzymes present. This will be extrapolated to tumour samples from patients that responded to AI therapy in comparison to those who did not. We will culture primary tumour associated adipocytes in 4AD and then evaluate how it is metabolised. This conditioned medium will be applied to our models of resistance and we can evaluate its impact on cell metabolism, autophagy and stemness.
Techniques and Methodology: Using cell models of AI resistance we can explore the impact of tumour derived steroids on cell metabolism, stemness and autophagy. We can interrogate well annotated clinical specimens to build a comprehensive picture of the steroid milieu in AI resistant and sensitive tumours using highly sensitive LC MS/MS and iTRAQ to evaluate steroidogenic enzymes. Finally, using our models of resistance we can evaluate the potential impact of drugs targeting cell metabolism, such as the novel ATPase inhibitor (HY-111651), either as a solo agent or in combination with Metformin.
Impact on breast cancer research: Steroid levels are known to be altered by diet, exercise and other lifestyle choices; understanding their potential role in breast cancer development could therefore have wide-ranging effects. This research area has the potential to be extrapolated into a large-scale global study that may have a radical impact on our understanding of breast cancer development. The proposed study will re-examine breast tumour biology through a fresh lens and provide information on the impact of the altered steroid environment on cancer cell metabolism.

Research project 10: The spleen as a site of relapse for early T-cell progenitor leukaemia
Supervisors: Dr Triona Ni Chonghaile and Prof Jochen Prehn, Physiology and Medical Physics, RCSI
Research Project Description: Early T-cell progenitor ALL (ETP-ALL) originates from a block at early stages of T-cell development, has a poor prognosis with risk for relapse. We previously identified that ETP-ALL is dependent on the anti-apoptotic protein BCL-2 for survival and is sensitive to the BH3 mimetic, ABT-199 (venetoclax). Patients diagnosed with ETP-ALL present with blast cells in the blood, bone marrow; and often in the spleen and in the lymph nodes. The spleen is an important site for extramedullary haematopoiesis and splenomegaly in patients is often associated with a poor prognosis. Of note, there is very limited research in leukaemia on the splenic microenvironment. In addition, it is not known how ETP-ALL interaction with distinct microenvironments affects the chemosensitivity and anti-apoptotic dependence. We have evidence that the spleen may be a potential site of relapse for ETP-ALL treated with ABT-199. Using cytokine arrays, CRISPR-cas9 technology and BH3 profiling we aim to discern the mechanism. Next, we aim to measure the sites of relapse of ETP-ALL xenograft in vivo to ABT-199 and whether the protective niche in the spleen is driven by cytokine signalling. A recent case report has described the first ETP-ALL patients that have been treated with ABT-199. Our research into sites of relapse following venetoclax treatment could lead to improved therapeutic responses, in this group of patients with high-risk disease.

Secondary Theme(s): Cell Biology

Research project 11: Examining the role of transcriptional and epigenetic alterations regulating microcalcification in ductal carcinoma *in situ* (DCIS)
Supervisors: Dr Maria Morgan and Dr Sudipto Das, Molecular and Cellular Therapeutics, RCSI
Research Project Description: Breast cancer is the most common cancer in women worldwide with incidence rates increasing and survival rates largely varying depending on early detection and treatment. Ductal carcinoma in situ (DCIS) is often regarded as a precursor to invasive breast cancer, which is regulated by several genes which possibly control processes involved in development of common features associated with breast cancer such as microcalcification. Our laboratory has shown extensive
However, despite ongoing research in this specific breast cancer sub-type, the precise cellular/molecular alterations that underpin the DCIS-associated microcalcification remain unknown. This gap in knowledge necessitates an in-depth investigation to identify the genetic/epigenetic alterations that are impacted during the DCIS associated changes such as microcalcification. The primary aim of this project therefore will be to identify the transcriptional and epigenetic alterations, as well as to examine their precise functional role of these alterations in driving DCIS-associated microcalcification in vitro.
To this extent, the project will involve global genomic and DNA methylation analysis using next-generation sequencing based platforms using the well-established pipelines established by our lab of the DCSI cell line models following microcalcification. This will allow identification of gene targets that are altered during the process of microcalcification which will be further examined using *in silico* methods to identify the molecular/cellular pathways that are impacted by these genes. Next, *in vitro* perturbation of these gene targets using knock-in and knock-out methods in the DCIS cell lines followed by functional assays in order to ascertain the phenotypic impact of the genes on DCIS-associated phenotype. Ultimately, for the first time

this project will allow development of a comprehensive profile of genetic/epigenetic induced alterations that largely impact DCIS associated microcalcification. The functional assessment of these gene targets will thereby identification of potential novel diagnostic/therapeutic targets for DCIS patient.

Secondary Theme(s): Tissue Engineering

Research project 12: Modelling metastasis in 3D: from pathway discovery towards targeted drugs

Supervisors: Dr Olga Piskareva, Anatomy and Regenerative Medicine, and Prof Jochen Prehn, Physiology, RCSI

Research Project Description: Paediatric cancer research in general and neuroblastoma, in particular, has very limited preclinical models of metastasis. Keeping in mind that 50% of primary neuroblastomas have already metastasised at the time of diagnosis it is paramount to seek new technological and methodological approaches to understand molecular mechanisms underlying this phenomenon. We aim to model and characterise invasion in 3D tissue-engineered platform that consists of extracellular matrix (ECM) - based hydrogels functionalised to act as 3D structural scaffold supporting neuroblastoma organoids to grow and respond to stimuli in a well-defined environment. We will use PDX-derived organoids to maintain native tumour heterogeneity and invasion phenotypes that can lead to pathway discovery and target validation. Towards this aim, we are taking an integrated approach that combines biological research, molecular and RNA profiling, mathematical modelling and tissue engineering. By the end of the project, we will greatly advance our understanding of mechanisms of invasion and metastasis of neuroblastoma and contribution of its microenvironment to the process which may be further utilised in the development of effective targeted therapies for children with this disease. The project is pioneering a new area of paediatric cancer research both in Ireland and the International neuroblastoma research community.

Research Theme: Infection, Immunity and Inflammation

Research project 13: Investigating novel metabolites on the regulation of microRNAs for the treatment of Multiple Sclerosis

Supervisors: Dr Clare McCoy, Molecular & Cellular Therapeutics (MCT) and Prof Luke O'Neill, Biochemistry & Immunology Dept, Trinity Biomedical Sciences Institute, TCD.

Research Project Description: The study of metabolites has yielded new insights into the mechanisms that mediate immune cell function. Recent advances have highlighted that in addition to regulating energy states in a cell, metabolites can have a major impact on immune cells such as macrophages. For example, succinate, an intermediate metabolite of the Krebs' cycle, has been shown to accumulate and act as a potent signal inducing the inflammatory state of macrophages (1). On the other hand, another metabolite called itaconate can act as an anti-inflammatory molecule, counteracting the actions of succinate, through the inhibition of pro-inflammatory signals and reactive oxygen species (2). Interestingly, succinate has been shown to drive an inflammatory state within the central nervous system of a Multiple Sclerosis (MS) disease model (3), whereas the administration of itaconate can reverse this effect (2). This suggests that the repurposing of metabolites holds huge potential as anti-inflammatory agents in many diseases including MS.

The discovery of microRNAs has led to a very exciting and rapidly growing area of research. microRNAs are extremely small RNA molecules that play a critical role in normal immune cell function. However, for reasons that we don't fully understand, microRNAs are also dysregulated in multiple inflammatory diseases. MicroRNA (miR)-155, a pro-inflammatory microRNA, has been particularly implicated in MS (4). Elevated levels of miR-155 are found in the serum and brain lesions of MS patients (5), while data from Dr McCoy's laboratory has identified that miR-155 is particularly elevated in macrophages that infiltrate and cross the blood brain barrier in a MS disease model. miR-155 activation results in the release of pro-inflammatory cytokines and toxic mediators and contribute to the damage observed in MS brain pathology (4). Significantly, we have shown for the first time that inhibition of miR-155 in macrophages can change their phenotype to an anti-inflammatory state that could potentially promote tissue repair (6, 7). Thus, inhibition of miR-155 in macrophages could offer a novel therapeutic approach for the treatment of MS.

This PhD project will focus on examining a range of metabolites and assessing their impact on miR-155 expression, macrophage metabolism and plasticity, with the aim that a metabolite will be selected for its efficacy in the MS disease model. The PhD candidate will be based in Dr Claire McCoy's laboratory at RCSI who leads a research team investigating the impact of miR-155 on MS disease pathology. She was the

recent recipient of a prestigious President of Ireland Future Research Leader Award. Prof Luke O'Neill will act as a co-supervisor, he is a world leader and expert in Immunometabolism, publishing frequently in Nature, Cell and Science. The successful candidate will have opportunities to engage with Prof O'Neill's lab at Trinity College Dublin, as well as undertake industry secondments associated with his research programme.

References:

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2. Mills EL, & O'Neill LA. Nature. 2018
3. Paraboschi EM. Int J Mol Sci, 2011
4. McCoy CE. Adv Exp Med Biol. 2017
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6. Quinn SR & McCoy CE.J Biol Chem. 2014

Keywords: Multiple Sclerosis, Immunology

Research project 14: Multi-antibiotic resistant Enterobacteriaceae and near-patient environmental decontamination: are current methods adequate in the face of changing epidemiology and enhanced transmission

Supervisors: Prof Deirdre Fitzgerald-Hughes, Clinical Microbiology and Prof Fidelma Fitzpatrick, Clinical Microbiology.

Research Project Description: Patients with infections caused by antibiotic-resistant bacteria are at increased risk of worse clinical outcomes and death. Klebsiella pneumoniae – a common intestinal bacteria that can cause life-threatening infections, is increasingly resistant to a last resort antibiotic, carbapenem, making it a multidrug resistant (MDR) pathogen and an almost untreatable 'superbug'. Carbapenemase-producing (CP) Enterobacteriaceae (CPE) which include mainly K. pneumoniae and Escherichia coli have spread to all regions of the world. CPE are a major cause of hospital-acquired infections such as pneumonia, bloodstream infections, and infections in newborns and intensive-care unit patients. In some countries, because of resistance, carbapenem antibiotics do not work in more than half of people treated for K. pneumoniae infections and hence morbidity rates as high as 50% are reported.

Infections rates involving CPE are rising and outbreaks are increasingly reported globally. To protect patients and prevent transmission in the USA, agencies such as the Centre for Disease Control and Prevention (CDC), Health Protection England and the Irish Health Protection Surveillance Centres provide recommendations for prevention and control of CPE that include contact precautions, patient/staff cohorting, hand-washing, surveillance and antibiotic stewardship. However, guidelines lack emphasis on the environments role in the transmission of CPE. Aggressive decontamination of the environment close to patients colonised with CPE has been pursued in some hospitals but mainly in response to the identification of environmental reservoirs and the finding of ineffective disinfection. Appropriate and effective decontamination of the healthcare environment in relation to CPE requires a more evidence-based understanding of the epidemiology and transmission of CPE in healthcare settings, a goal that this proposal will address.

We showed that CP Klebsiella survive longer than other CPE on surfaces commonly found in patient bed-spaces and are more resistant to disinfectants at twice the recommended concentrations. In this project, we will further investigate the survival of antibiotic-resistant and -susceptible K. pneumoniae and E. coli on surfaces, correlated with the adherence traits of these organisms and bacterial fitness. Bacterial survival on surfaces, decontaminated using current disinfection guidelines, will be investigated under laboratory conditions and the development of disinfectant tolerance will be investigated. In a hospital-based study, we will determine the burden of K. pneumoniae and E. coli in the environment of colonised/infected patients. We will determine the effectiveness of routine surface cleaning in relation to target organisms and simultaneously we will evaluate the cleaning standard achieved on surfaces, based on removal of a fluorescent dye applied to multiple surfaces. The relatedness of environmental isolates and isolates recovered from patients will be investigated by whole genome sequencing to identify routes and reservoirs of transmission. These studies will provide evidence of potential clinical sources of patient acquisition and will determine the relationship between cleaning thoroughness and cleaning effectiveness for recovery of these pathogens. As such, it will inform future infection prevention and control policy with regard to CPE.

Keywords: Public Health, Infection Prevention and Control, antibiotic resistance.

Research project 15: Could cold atmospheric air plasma be a potential novel therapy for treating infected diabetic foot ulcers?

Supervisors: Dr Niall Stevens, Clinical Microbiology and Prof Hilary Humphreys, Clinical Microbiology.

Research Project Description: In Ireland, diabetes mellitus is reported to affect 207,490 people, or 6.5% of the population in the 20-79-year-old age group (www.diabetes.ie). By 2030, this number will rise to 278,850 people; representing an increase of 34.39% in fifteen years. Foot ulcers are common in patients with diabetes and they are the most frequent diabetes-related cause of hospitalisation. The HSE in Ireland estimates that one-in-twenty sufferers will get a foot ulcer in their lifetime and approximately half of these will be infected at presentation. Diabetic foot infections (DFIs) can be associated with significant morbidity and at least one-in-five result in lower extremity amputations. The ultimate treatment goal for a diabetic foot ulcer (DFU) is to achieve wound closure but management is dictated by the severity of the ulcer, presence of neuropathy, vascularity, and importantly, whether there is an infection. The impact of infection and the subsequent amputation of a limb can severely impact the quality of life of patients with diabetes mellitus. Finding new therapies to treat infected ulcers would help improve patient care and prevent the patient losing their leg or foot. Plasma is the “fourth state of matter” and there are different types depending on the gas used. We generate plasma be applying high voltage electricity to a gas. We know that plasma can kill bacteria but we don’t know if it will work on the bacteria that infect the ulcers of patients with diabetes and we also do not know if this treatment is safe to use in patients.
This research proposal aims to investigate the potential of plasma to be a novel and non-invasive therapeutic that could quickly treat infected ulcers, promote healing and prevent the loss of limbs. We believe this would advance care and improve the quality of life of patients with diabetes mellitus.
Keywords: Infection, diabetic foot ulcers, microbiology

Research project 16: Development of small molecule inhibitors of FcεRI
Supervisors: Prof Dermot Cox, Molecular & Cellular Therapeutics (MCT) and Prof Mauro Adamo, Pharmaceutical & Medicinal Chemistry.
Research Project Description: Allergic diseases are one of the most common diseases in the Western world. A report from the American academy of allergy, asthma and immunology indicated that between 40-50% of children are sensitized to at least one allergen. Its most severe presentation is anaphylaxis with an incidence of approximately 20 per 100,000 person-years.
Allergies occur when patients form IgE antibodies to a foreign protein. When subsequently exposed to the protein the IgE-antibody complex binds to the Fc receptor on basophils and mast cells. Binding to FcεRI triggers degranulation of the target cell which specifically releases histamine and other inflammatory cytokines. These agents cause bronchoconstriction and vasodilation leading to difficulties breathing and life-threatening hypotension.
Current treatment of allergic reactions is mainly symptomatic including anti-histamines, steroids, adrenaline and bronchodilators. However, none of these alter the underlying disease, thus, there is a need for more effective agents to treat allergic reactions. One possibility is to target the IgE-FcεRI interaction. This could be achieved by blocking FcεRI with a small molecule. We will use the expertise that we have developed in discovering small molecule inhibitors of FcγRIIa-IgG to develop small molecule inhibitors of the IgE-FcεRI interaction.
There are a number of potential benefits to an orally active small molecule inhibitor of Fcε. Firstly, it will be a lot cheaper than a monoclonal antibody. Secondly, it can be used prophylactically where patients could take a daily tablet during hay fever season to prevent symptoms. Finally, it could also be used in an emergency situation where a tablet could be taken when symptoms develop to prevent further deterioration in the patient.
Keywords: Immunology, pharmacology, drug discovery

Research project 17: Innovative technologies for the development of lead candidates in the treatment of sepsis
Supervisors: Prof Steve Kerrigan, School of Pharmacy and Prof Ger Curley, Anesthesia and Critical Care, Beaumont Hospital.
Research Project Description: Sepsis is the most common condition, and the single biggest cause of mortality, in critically ill patient. Outside of Critical Care Units, sepsis contributes to one-half of all hospital deaths. In addition, sepsis confers a major long-term economic burden on survivors and on society due to functional and cognitive disability. Improved treatment of sepsis could offer meaningful improvements in population health, quality of life and survival. In the developed world, sepsis is dramatically increasing by an annual rate of between 8-13 % over the last decade, and now claims more lives than heart attack, stroke or

colon cancer and breast cancer combined. Sepsis involving multiple organ dysfunction is associated with especially high morbidity and mortality (up to 50%) and consumes a vast amount of healthcare resources. In Ireland, the cost of treating hospitalized sepsis patients was in the region of €250 million in 2013 alone (National Sepsis Steering Committee report, 2015). These figures do not take into account the costs associated with the lifelong follow up that is required post hospital discharge (most survivors experience lifelong complications due to sepsis). This makes sepsis the most expensive condition treated in Irish hospitals. **At present there is no specific anti-sepsis treatment available**, therefore management of sepsis patients relies on therapeutic measures to be initiated as soon as possible after sepsis diagnosis to include administration of appropriate antibiotics, source control measures when necessary and resuscitation with intravenous fluids and vasoactive drugs when needed. Although antibiotics play a key role in fighting the infection treatment success is often poor as downstream events as a result of endothelial dysregulation is not controlled. A January 2018 search of the www.clinicaltrials.gov revealed almost 550 trials of drugs and devices for the treatment of sepsis in various stages of completion, however no therapeutic options have made it to the market yet. It can be conservatively estimated that of upwards of $30billion has already been spent on the objective of developing a higher order therapy for the treatment of sepsis, indicating the market need. Previous approaches have focused on treating or controlling late stage pathophysiological effects (such as inflammation, thrombus formation & coagulation etc), an approach which has resulted in the failure of many compounds in clinical trials as a result of later intervention in the disease progression pathway.

This project will use cutting edge technology to identify lead candidate drugs that target very early in sepsis progression by targeting the human vascular endothelium.

Keywords: Therapeutics, sepsis

Research Theme: Neurological and Psychiatric Disorders

Research project 18: The microbiome as a mediator of focal epilepsy

Supervisors: Prof Gianpiero Cavalleri, Molecular & Cellular Therapeutics (MCT) and Prof Norman Delanty, Molecular & Cellular Therapeutics (MCT).

Research Project Description: Epilepsy is a group of common brain disorders characterized clinically by the occurrence of recurrent unprovoked seizures. There are many different types of epilepsy, both common and rare. Non-lesional focal epilepsy (NLFE) is a common type, whereby the patient has a focal onset of seizures in the brain, without a demonstrable cause visible on good quality MRI brain imaging. Most cases are unexplained and poorly understood. In addition, many patients with NLFE are do not respond to drug therapies, underpinning the pressing clinical need to further explore and understand this type of epilepsy. The gut micobiome is defined as the collection of genes of all the microbes present within the gastrointestinal (GI) tract, and studies of the microbiome examine the interaction of all GI flora within the host. There is increasing interest in the gut microbiome as an important determinant of human health and disease, and emerging results from studies of brain disorders such as multiple sclerosis and neuromyelitis optica, supports the hypothesis that perturbations of the microbiome may be of aetiological significance in neurological conditions. Recent work in a mouse model of epilepsy has illustrated how the ketogenic diet (an effective way to control seizures) appears to act through changes in the gut microbiota. Despite this emerging interest in the microbiome and disease, there has been little if any work in humans on its possible role in the development and treatment of epilepsy.

The project will address three principal questions – 1) are changes in gut microbiota correlated with a diagnosis of epilepsy? We will answer this question by comparing the gut microbiota of people with nonlesional focal epilepsy to that of a healthy control population 2) are distinct gut microbioate profiles associated with response to epilepsy treatment? We will answer this question by comparing the gut microbiota of people with treatment-resistant epilepsy to those who respond to treatment. 3) Does the ketogenic diet impact on gut microbiota in people with epilepsy? We will answer this question by comparing the microbiome of people on the ketogenic diet too those who are not on the diet.

This project will be conducted through the SFI FuturNeuro Centre of Excellence and the Epilepsy Program at Beaumont Hospital,the main tertiary referral centre for complex epilepsy in Ireland, and is the national centre for epilepsy surgery. It will facilitate the PhD student to develop cutting edge skills in the generation and analysis of next-generation sequence data, bioinformatics, statistics as well as exposing them to the clinical neurology at Beaumont Hospital.

Keywords: Epilepsy, microbiome

Research project 19: Molecular mechanisms of blood-brain barrier dysfunction and repair in epilepsy
Supervisors: Dr Cristina Ruedell Reschke, Physiology & Medical Physics and Prof David Henshall, Physiology & Medical Physics.
Research Project Description: Epilepsy is a common, chronic neurological disorder characterized by recurrent, unprovoked seizures. Current treatments fail at least one third of patients and we have no disease-modifying therapies. Thus, there is a critical need for new ideas about patho-mechanisms of epilepsy. The blood-brain barrier (BBB) is a critical structure comprising specialized endothelial cells with tight junctions that physically and chemically separates brain tissue from circulating factors in the blood. Acute injuries to the brain and certain chronic neurological diseases are associated with BBB impairment which allows passage of molecules and cells normally excluded into brain tissue. This is thought to cause inflammation and promote neuronal dysfunction. There is growing evidence that epilepsy is associated with BBB dysfunction. Moreover, when seizures occur they may directly open the BBB and further promote molecular changes that contribute to an enduring state of hyper-excitability.
Several key questions remain unanswered which form the basis for the hypothesis to be tested in this PhD project. What are the molecular changes within the BBB that contribute to barrier breakdown? What is the mechanism initiating and maintaining these changes? What size of molecular pore is required to provoke or maintain epilepsy? How long must barrier breakdown persist in order to be epileptogenic? Can epilepsy be resolved by repairing the BBB? If so, how and is there a time limit on when this is possible?
This PhD research project will involve a multi-disciplinary approach comprising neuroscience, genetics, RNA and protein chemistry, microscopy and neuropharmacology to explore the molecular mechanisms of BBB dysfunction and its repair in epilepsy. The project will feature a strong imaging component, including use of pre-clinical models of epilepsy and two-photon microscopy and magnetic resonance imaging (MRI) to study blood brain barrier function in the living brain. A translational research element will involve opportunities to analyze human brain samples from patients with epilepsy and feature the design and delivery of gene therapy and oligonucleotide-based experimental treatments to restore BBB integrity. The researcher will work with a highly dynamic team of neuroscientists based at the recently launched FutureNeuro Research Centre at RCSI as well as molecular biologists, clinicians and bioinformaticists and our broader network of scientific and clinical collaborators.
In summary, this project will provide a neuroscience-focused researcher with a comprehensive, diverse and cutting-edge training experience that will uncover novel molecular mechanisms and treatments for BBB dysfunction in epilepsy.
Keywords: Neuroscience, epilepsy

Research project 20: Mechanisms of fever-induced epilepsy and related cognitive impairment
Supervisors: Dr Gary Brennan, Physiology & Medical Physics and Prof David Henshall, Physiology & Medical Physics.
Research Project Description: Temporal-lobe epilepsy is a common chronic neurological disorder characterized by spontaneous recurrent seizures. Fever-induced seizures (febrile seizures) are the most common type of seizure in young children and are generally harmless. However prolonged febrile seizures and complex febrile seizures are associated with developmental delay, cognitive impairment and an increased risk of developing epilepsy in later life. Additionally up to one third of patients who develop epilepsy are resistant to current medical treatments and will experience uncontrolled seizures. The disorder is also associated with a higher risk of developing anxiety disorders and depression as well as sudden unexpected death from epilepsy. There are currently no treatments for the prevention of adverse outcomes following a prolonged febrile insult during childhood and a real clinical need remains for the development of novel therapeutics which treat the underlying causes of the disease. The mechanisms by which prolonged febrile seizures cause cognitive impairment and epilepsy however remain poorly understood. The current project will explore the role of a novel class of non-coding RNAs called microRNAs as a potential regulator of febrile seizure-induced cognitive impairment and subsequent epilepsy development. MicroRNAs are a class of small non-coding RNAs which negatively regulate gene expression by binding to target RNAs and blocking their translation. Previous work from our group and others has found that these molecules play an important role in temporal lobe epilepsy. Their role in febrile seizures however is as yet unexplored.
The project will employ a variety of innovative methodologies to explore the role of a novel class of RNAs in specific cellular populations to decipher the role of microRNAs in febrile seizure related epilepsy and cognitive impairment. Specifically we will develop a novel in vivo model of febrile seizures and combine with cutting edge cell specific RNA-sequencing and ChIP-sequencing approaches as well as subsequent intervention strategies.

The inter-disciplinary nature of this project (molecular biology, in vivo pharmacology and bioinformatics approaches) will provide the student with broad training and a unique skillset. The project will be incorporated into the SFI-funded FutureNeuro Research Centre where students will have access to world class research facilities and the chance to work within an integrated team consisting of scientists with diverse backgrounds as well as clinicians and e-health researchers.
Keywords: Febrile seizures, Molecular biology, Neuroscience, microRNAs

Research project 21: KETOGENIC DIET: An in vitro single-cell imaging and molecular analysis approach to determine and therapeutically target the control principles of neuronal bioenergetics for the treatment of epilepsy with ketogenic diet
Supervisors: Prof Jochen Prehn, Physiology & Medical Physics and Dr Susan Byrne, Physiology & Medical Physics.
Research Project Description: Glutamate is the main excitatory neurotransmitter in the central nervous system (CNS)CNS. Glutamate receptor activation imposes a significant work load and energy demand on neurons which requires neurons to increase ATP production. However, excessive activation of glutamate receptors induces neuronal dysfunction and nerve cell death, a process termed 'excitotoxicity'. Interestingly, neuronal excitation and excitotoxic injury is substantially modulated by alterations in energy substrates.
Protective effects of ketone bodies and fatty acids on neuronal excitotoxic injury and in the setting of epilepsy treatment in patients have been described. Their mechanism of action however is poorly understood.
The main aim of this PhD project is to analyse the effects of the metabolic switch imposed by a ketogenic diet on mitochondrial function and neuronal bioenergetics, by using a combined single cell imaging and molecular deep phenotyping approach. First, the candidate will analyse the influence of acetoacetate, beta-hydroxybutyrate and decanoic acid (core components of a ketogenic diet) on basal bioenergetics and neuronal excitability at the single cell level by employing time-lapse confocal microscopy studies. He/she will avail of key a set of established, fluorescent reporters (FRET probes) that were previously characterized by the host laboratory (Connolly et al., J Neurosci, 2014; D'Orsi et al., J Neurosci, 2015). Studies will be done in primary hippocampal neurons under baseline conditions, under conditions of hyperexcitation, and after a prolonged glutamate challenge which is normally toxic to neurons. Next the candidate will perform a systems approach to fully understand and optimise the effects of ketogenic diet on neuronal bioenergetics and excitability. The candidate will perform RNA sequencing studies to analyse the transciptome of neurons exposed to ketogenic diet and to provide insights into the mechanism of action of this novel treatment. Alterations in gene expression in response to ketogenic diet will be validated by PCR and Western blotting, and further interrogated using genetic manipulation of differentially expressed genes that are of relevance for neuronal energy metabolism, excitation and excitotoxic cell death.
Unravelling the mechanisms by which ketogenic diet modulates neuronal bioenergetics, excitability and survival is pivotal for the use and optimization of dietary approaches for the treatment of epilepsy.
Keywords: Neuroscience, epilepsy

Research project 22: Validation of microRNAs as novel diagnostic and therapeutic targets in ischaemic brain injury
Supervisors: Dr Shona Pfeiffer, Physiology & Medical Physics and Prof David Williams, Geriatric and Stroke Medicine.
Research Project Description: Ischaemic stroke is a leading cause of death and most common cause of acquired major disability resulting from death of brain tissue and focal neurological deficits; however, despite decades of research, treatment options remain limited and the lack of therapeutic treatment strategies is a critical clinical problem. To this end, there is a need for biomarkers as clinically useful diagnostic and prognostic indicators for outcome in patients, improving functional recovery through individualised therapeutic strategies. Furthermore, such biomarkers have potential to be developed into neuroprotective agents aimed at rescuing ischaemic neurons from irreversible injury, widening the therapeutic window, improving neurological outcome and facilitating brain recovery.
Endogenous microRNAs (miRNA) are potent regulators of gene function elevated in a wide range of diseases, with crucial roles as regulators of signaling pathways involved in ischaemia-reperfusion injury. The complex nature of the ischaemic cascade has made identification of clinically useful biochemical markers of ischaemia challenging despite statistical associations with stroke and therefore targeting a single pathway may be ineffective. The roles played by miRNAs and their dysregulation in disease, particularly their ability to regulate multiple genes in similar pathways given the heterogeneity of stroke pathophysiology,

combined with remarkable stability in biofluids and easy detection leave them uniquely poised as ideal biomarkers and therapeutic targets in many future clinical trials. Identification of a stable, endogenously expressed biomarker will be of significant clinical value in the development of a rapid blood test based on simple, cost-effective, near-patient technology, thereby contributing valuable and timely information necessary for prompt patient management decisions in the acute setting. Such information would aid in the choice of appropriate therapeutic intervention, treatment and secondary prevention and help with identifying timing of onset. Furthermore, identification of multi-targeting endogenously expressed biomarkers has significant potential for the development of neuroprotective agents, reducing stroke mortality rates. The outcomes of this research will have significant potential for application and translation as effective and feasible interventions, substantially improving patient care and outcome.
Keywords: Neuroscience

Research Theme: Pharmacy, Pharmaceutical Sciences & Chemistry

Research project 23: Bioorthogonal Chemistry and Fluorescent Post-Labeling for Real-Time Tracking of a Platinum-based Anticancer Drug.
Supervisors: Dr Darren Griffith, Pharmaceutical & Medicinal Chemistry and Prof Donal F O'Shea, Pharmaceutical & Medicinal Chemistry.
Research Project Description: Metal-based drugs have a wide range of medicinal applications and are routinely used clinically as therapeutic and diagnostic agents. In particular platinum (Pt) drugs such as cisplatin, carboplatin and oxaliplatin, have played a very important and well documented role in treating cancer and are employed in nearly 50% of anti-cancer regimens.
The cytotoxicity of Pt drugs, which hydrolyse (loss of chlorido or carboxylato ligands) inside cells, has traditionally been primarily attributed to their ability to covalently bind DNA, forming DNA adducts, leading to DNA damage responses and ultimately programmed cell death, apoptosis.
Significantly, it is becoming increasingly clear that the exact biomolecular mechanisms of action of Pt drugs have not been fully elucidated. It has been demonstrated recently for example that oxaliplatin, in contrast to cisplatin and carboplatin, does not kill cells via the DNA-damage response but by inducing ribosome biogenesis stress.
Trackable metal-based drugs which incorporate an organic fluorophore for example offer the prospect of real-time imaging of important biological processes in vitro and providing vital information concerning the biodistribution, cellular transport, subcellular localization, and mechanisms of action and resistance to metallotherapeutics.
The near-infrared (NIR) spectral region (700–900 nm) provides ideal imaging spectral wavelengths, reduced light toxicity and does not interfere with competing endogenous chromophore absorbance. Significantly NIR probes have been successfully employed to image tumours in vitro and in vivo and as sensors for ROS, RNS, thiols, ions, pH and enzyme activities.
Bioorthogonal chemistry describes chemical reactions that can occur in living systems without interfering with native biochemical processes. Bioorthogonal chemical ligation strategies include for example 1,3-dipolar cycloaddition between azides and cyclooctynes, between nitrones and cyclooctynes, oxime/hydrazone formation from aldehydes and ketones, tetrazine ligation and the quadricyclane ligation for example.
This multidisciplinary project, which will incorporate medicinal chemistry, cell biology and imaging, will employ bioorthogonal chemistry to develop Pt anticancer compound surrogates that feature reactive biocompatible handles that can be tagged with a reporter NIR fluorophore in cellulo. Such conjugates will play an important role in the ongoing investigation into the non DNA-binding effects of Pt-based drugs.
Keywords: Cancer, Medicinal Inorganic Chemistry, Imaging

Research project 24: Novel antibiotic candidates against drug-resistant bacteria causing the highest threat to human health
Supervisors: Prof Marc Devocelle, Pharmaceutical & Medicinal Chemistry and Dr Deirdre Fitzgerald-Hughes, Clinical Microbiology.
Research Project Description: ‘A post-antibiotic era—in which common infections and minor injuries can kill—far from being an apocalyptic fantasy, is instead a very real possibility for the 21st century ‘, reported the World Health Organisation (WHO) in 2015. The pipeline for the development of new antibiotics is now

virtually empty, it will take at least 10 years to develop new antibacterial agents and the useful lifespan of an antibiotic (avoiding resistance) can typically be as short as 2 years.
The number of strategies currently identified to potentially delay the emergence of antibiotic resistance is particularly limited. Among them is the therapeutic use of natural molecules which have enabled, for millions of years, the first line of defence against infections in living organisms, the antimicrobial peptides. They have a number of unique features, particularly attractive against antibiotic resistance. Their clinical exploitation is however limited by a number of shortcomings, in particular their potential toxicity.
The host laboratory has developed a unique approach for the generation of PEG-based peptidomimetics of AMPs. Peptidomimetic is a generic term describing a molecule which displays the biological activity of a parent peptide, but which is structurally different. Peptidomimetic conversion is usually apply to address the clinical shortcomings of a parent peptide. Different peptidomimetic candidates of AMPs have been developed worldwide, in particular polymer-based mimetics, where a synthetic polymer backbone replace the peptide's poly-amide backbone. These metabolically stable analogues can be administered at lower doses, increasing thereby the therapeutic window. Some of these candidates have progressed to clinical trials where they have shown significant advantages over classical (approved) antibiotics. However, while polyethylene glycol (PEG) is described as 'the gold standard biocompatible polymer for pharmaceutical and medical applications', it has not been for the generation of polymer-based peptidomimetics to date.
The first PEG-based peptidomimetics were produced by the host lab; they mimicked a Cell Penetrating Peptide, a peptide sequence able to translocate across cell membrane and to act as a delivery agent for a large number of therapeutic molecules. The CPP peptidomimetic was shown to be more efficient than the parent peptide in the delivery of nucleic acids, while being non-toxic. The first peptidomimetics of ultrashort AMPs (tripeptides) were also successfully generated. Despite their small size, they displayed interesting antibacterial and antibiofilm activities, at least equivalent to those of the parent peptides. The approach used to generate these candidates can be adapted to produce larger candidates, approaching the size of the best AMPs know to date.
The aim of this project is to produce this second generation of AMP PEG-based peptidomimetics and to test and optimize their antimicrobial and antibiofilm activities against most bacteria in the WHO list of pathogens causing the most significant threat to human health (Nature, 2017, Vol. 543, p. 15).
Keywords: Pharmaceutical Sciences, Novel antibiotics, Drug-resistant bacteria

Research project 25: Development of an inhalable anti-tubercular therapies to include pre-clinical screening and 3D printing/additive manufacturing.
Supervisors: Prof Sally Ann Cryan, School of Pharmacy and Prof Joseph Keane, School of medicine, Trinity College Dublin, Prof. Andreas Heise, Pharmaceutical & Medicinal Chemistry, RCSI, Ronan MacLoughlin, Science Manager, Aerogen and Honorary Senior Lecturer, RCSI.
Research Project Description: Mycobacterium tuberculosis (TB) is the primary infectious disease killer in the world. In 2016 alone 1.7million people died from TB and 10 million people fell ill [1]. It is the main cause of death related to antimicrobial resistance and the leading killer of patients with HIV. TB is primarily a pulmonary pathogen but current treatment regimens are based on oral and parenteral drug therapy requiring a minimum of 6-9 months for successful treatment. These treatments are lengthy, associated with a high risk of adverse drug reactions and poor patient adherence that is leading to multi-drug resistance (MDR-TB) strains emerging. Therefore, new therapies and treatment modalities are urgently required. By localising new and existing TB therapies to the lungs via aerosol, to target the site of TB infection in the alveolar macrophage (AM), the occurrence of these adverse events can be diminished/eliminated, patient dosing requirements reduced and clinical efficacy enhanced. Recent pharmacokinetic studies have shown that inhaled anti-tubercular drug formulations can achieve not only greater levels of concentration in the lungs than oral formulations but also have longer residence times.
The student will bas based in RCSI'S St Stephens' Green campus but will have an opportunity to spend time in the clinical (St James Hospital) and industrial laboratories (Aerogen, Galway) of their co-supervisors. This project seeks to encapsulate innovative emerging TB therapies into inhalable delivery platforms designed for cell-specific targeting through additive manufacturing. Previous work by our group has investigated the key parameters required for targeting the macrophage using inhalable particle technology [2.3]. Working alongside a clinical research group in St James Hospital Dublin a range of novel host-directed [4] and peptide-based therapeutics that have emerged from research within our teams over the last number of years will first be assessed for their efficacy using a well-established in vitro TB infection model [5]. In the second stage of the project these lead therapeutics will be encapsulated into inhalable polymeric particles using additive manufacturing (3D PRINT) technology being developed within the Drug Delivery and

Advanced Materials team in RCSI. These drug-loaded particles will be characterized pharmaceutically and their cellular targeting and efficacy assessed using well established advanced cellular imaging and infection models respectively [2, 4]. Key to the clinical use of inhalable therapies is integration with an appropriate inhaler device. Working with our industrial collaborators the inhalable therapies will be loaded into devices for aerosol testing including simulation modelling.

For truly advanced treatment modalities to be efficiently translated into the clinical environment a convergence of biomedical sciences and delivery platforms early in a research programme is becoming critical. Overall, this PhD project will integrate screening of novel TB therapies using state-of-the art cellular and biological models combined with pharmaceutical design, advanced additive manufacturing processes and medical device integration to support their clinical translation offering hope to the thousands of patients worldwide who die and fall ill daily due to TB.

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Keywords: Respiratory Medicine, pharmaceutical sciences and bioengineering

Research project 26: Optimisation and Preclinical Evaluation of Targeted Nanoformulations of Anti-TNF-α Therapeutics for the Treatment of Inflammatory Bowel Disease (IBD)

Supervisors: Dr Zeibun Ramtoola, School of Pharmacy and Dr Brian Kirby, Pharmacy.

Research Project Description: Inflammatory Bowel Disease (IBD) is a chronic inflammatory condition that affects the gastrointestinal tract (GIT) of the patient and comprises of Crohn's disease (CD) and ulcerative colitis (UC). In CD inflammation can occur in any part of the GIT, whereas in UC, ulceration is usually limited to the lower part of the GIT, namely small intestine, colon and rectum[1]. IBD patients suffer a wide variety of symptoms including abdominal pain, diarrheoa, rectal bleeding, and anaemia, thus negatively impacting their quality of life[2]. Current treatment comprises the administration of small molecule therapeutics such as 5-•-aminosalicylic acid, corticosteroids and immunosuppressants and more recently, the anti-TNFα monoclonal antibodies (mAbs) including infliximab, adalimumab and certolizumab pegol. While conventional small molecules provide relief from IBD symptoms and maintain symptomatic
remission, disease progression ultimately leads to surgical resection of diseased tissues.

It is now recognised that anti-TNFα mAbs in addition to causing induction and maintenance of remission also promote GI mucosal healing, now recognized as a key goal of IBD treatment, as this reduces the need for surgery[3]. A major disadvantage of the mAb therapy, however, is the resulting severe adverse effects such as leucopenia, serious infection and increased risk of malignancy due to their systemic administration at high doses. In addition, intravenous administration requires skilled personnel and mAb short shelf life after reconstitution, adds to the overall cost of IBD therapy. The development of novel drug delivery strategies of anti-TNFα therapeutics that can enhance their efficacy and reduce their side effects is a priority for treatment of IBD. Biodegradable PLGA nanoparticles of budesonide administered orally in a colitis mouse model, were shown to be preferentially taken up by the inflamed gastrointestinal cells, resulting in enhanced efficacy and reduced side effects[4]. Nanoparticles of biological therapeutics have not been formulated and studied due to their known sensitivity to environmental and processing stressors. We recently studied the effect of various processing stressors and environmental factors on the stability of the anti-TNFα mAb, Infliximab, and designed novel nanoformulations of Infliximab, in non-digestible polymeric envelopes, to provide stability of the mAb[5]. We demonstrated retention of biological activity of Infliximab from these nanoformulations and showed enhanced cell uptake and transport of the nanoformulations in an in-vitro intestinal inflamed epithelial cell model. Treatment with these Infliximab nanoformulations resulted in reduced inflammation and recovery of the epithelial barrier function. The aim of this project is to examine the potential of these novel Infliximab nanoformulations, administered orally, in targeting the inflamed intestinal mucosa of IBD, in a colitis mouse model. As part of this project the nanoformulations will be formulated and optimised for drug loading, drug release and stability. Optimised nanoformulations will be investigated in an in vivo colitis mouse model for their ability to target and interact with the inflamed intestinal tissues, to reduce inflammation and promote healing. Such a targeted strategy may provide increased efficacy and dose reduction, resulting in lower systemic side effects and improve the benefit to risk ratio of anti-TNFα mAbs).

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Keywords: Pharmaceutical Sciences, Anti-inflammatory therapeutics, Inflammatory Bowel Disease

Research project 27: Modification of marketed dosage forms for administration to children
Supervisors: Fiona O'Brien, Pharmacy, RCSI and Prof Matthew Peak, Clinical Research Division, Alder Hey Children's Division
Research Project Description: Can guidance be provided to allow pharmaceutical companies to develop approvable strategies for alternative methods of administration of medicines to children when the marketed dosage form does not provide the required dose and/or is not acceptable to the child or carers?
It may not be viable for a pharmaceutical company to develop and market the many different dosage forms required to satisfy the dose requirements and preferences of children from the preterm neonatal age and immature development through to those of older children and adolescents whose requirements may be closer to those of adults. There is recognition that the ability to modify a marketed dosage form can be an alternative strategy to achieve the required fractional dose or to increase the acceptability of the dosage form and improve adherence to the therapeutic regimen. Modification may involve manipulation of the dosage form at the point of administration, for example splitting a tablet to obtain the required dose or crushing a tablet and administering mixed in food. It may also involve preparation in advance of need, usually by the pharmacist, by preparing extemporaneously an oral suspension from the marketed tablet and a variety of excipients to mask taste, smell or appearance.
Whilst information is included in some medicines Summary of Product Characteristics (SmPCs), little is known or written about what makes an alternative administration strategy that would meet the requirements of medicines regulators. What may be accepted for clinical trials may not be acceptable for the authorised, marketed product. If it is likely that such modifications are needed, there may be financial advantage in investigating alternative administration strategies during the early drug development stage, rather than being required to do this as a condition of authorisation at a later stage.
This project will gather evidence about modifications, alternative strategies and the current experience and opinion of the industry, regulators and clinical practitioners. Working with these interested parties, the project aim is to gather this evidence and to use it to develop a guidance framework including study design templates and risk assessments rather than definitive studies. The framework will allow appropriate dosage form modifications and design of robust studies that can be readily accepted by regulators. The supervisory and supporting team is fully expert in the regulatory context, clinical/pharmaceutical challenges and research methodologies required to deliver a successful PhD.
There are several project phases required to fully investigate this work that will include the following:

- A systematic review of published literature with an equal consideration of the 'grey' literature and experience. Engagement with stakeholders namely the EMA, FDA, pharmaceutical industry, clinical practitioners, carers and children to identify the issues surrounding the development of alternative dosing strategies.
- Examination of the impact of the manipulation of different types of dosage forms where information is lacking, using Pharmacopoeial methods and 'in use' simulations to inform study design.
- Preparation of framework guidance on the conduct of studies to support alternative administration strategies. Critical review of the guidance by stakeholders.

Research project 28: Development and evaluation of a loco-regional drug delivery system for the treatment of pancreatic cancer
Supervisors: Helena Kelly, Cian O'Leary and Seona Rossi, Pharmacy, RCSI
Research Project Description: Pancreatic adenocarcinoma (PDAC) has the highest rate of death per incidence of any cancer and there have been no significant improvement in survival outcomes in the last 40 years. Currently surgery has the best outcomes for patients, however only about 20% of patients are eligible for surgery at time of diagnosis. Up to a further 40% of patients have locally advanced disease, where the tumour has not spread outside the pancreas but is too large for surgical resection.

Two factors that contribute to the poor clinical outcomes associated with PDAC are the tumour stroma and epithelial-mesenchymal transition (EMT) of cells within the tumour. The tumour stroma in PDAC is a dense extracellular matrix (ECM) composed of fibrillar elements, such as collagen and activated fibroblasts with a recent hypotheses being that the ECM could play a role in therapeutic resistance. According to this hypothesis, the stiffness of the ECM impairs blood vessel perfusion, resulting in a poorly vascularised cancer, which ultimately represents a barrier to drug delivery. This results in a need for higher systemic doses of drugs, leading to increased toxicity and patient morbidity, often with limited efficacy. One approach to overcoming the issue of penetration into the dense stroma is direct intratumoural (IT) injection using Endoscopic Ultrasound-Fine Needle Injection (EUS- FNI). A significant constraint of this approach has been the use of aqueous solutions of a single chemotherapeutic agent. The low viscosity of aqueous solutions provide limited retention and duration of action at the tumour site resulting in rapid clearance and transient effect of the drug.

There is also increasing evidence in PDAC suggesting that EMT generates cells with stem cell properties, promoting the Cancer Stem Cell (CSC) phenotype, and enhancing PDAC tumorigenicity and chemoresistance. Recent evidence has suggested that this chemoresistance is associated with development of the EMT phenotype in tumour cells. These factors in combination are considered to contribute significantly to the poor clinical outcomes observed in the treatment of PDAC.

The Kelly lab has developed a drug delivery platform for direct IT injection, which has been shown to be retained at a tumour site for at least 14 days with the ability to offer sustained release of drugs. The platform has also been shown to have inherent chemoablative properties, which may offer synergistic activity with other therapeutic modalities. This project proposes to use this platform to develop a multi-modal loco-regional drug delivery system with a primary aim to reduce tumour burden in PDAC.

Research project 29: Pharmacoengineering of advanced RNA-nanomedicines to treat cystic fibrosis (CF)
Supervisors: Prof Sally-Ann Cryan, Pharmacy, Prof Andreas Heise, Chemistry, and Prof Catherine Greene, Clinical Microbiology, RCSI
Research Project Description: Cystic fibrosis (CF) is an inherited disorder caused by a mutation in a single gene responsible for the production of a protein called the cystic fibrosis transmembrane conductance regulator (CFTR). While potentiator and corrector drugs have improved therapy for some CF patients, there remains an unmet need for advanced therapies to treat the significant number of patients who do not respond to these approaches. MicroRNAs have been found to play an important role in both inflammatory processes and in regulation of CFTR and we and others have determined that exogenous delivery of this microRNA to CF cells can attenuate inflammatory responses and therefore offer a promising new gene therapeutic strategy for CF[1-2].

Respiratory drug delivery is a well-established means of treating respiratory disease and our team is developing technologies for local targeting of RNAi therapies to the CF lungs. CF was one of the first diseases targeted for gene therapy but a number of key challenges have limited translation of inhaled CF gene medicines to-date including: i) inadequate gene delivery efficiency ii) inadequate screening tools for nanomedicines iii) inefficient aerosol delivery. Our team harnesses pharmacoengineering approaches to overcome each of these challenges by integrating advances in our understanding of CF disease with cutting-edge materials science, particle engineering, medical device design and tissue-engineering tools to support the development and clinical translation of RNAi-nanomedicines. We have developed a series of star polypeptide-based gene vectors in RCSI[3,4]. They are built from amino acids and are thus bio-derived, biodegradable and biocompatible by definition. One of the main barriers to gene delivery in the CF patient is the thick barrier mucous that coats the epithelium. We have now tailored these star-polypeptides specifically for delivery to and into respiratory tissues by decorating the surface of the stars with mucous-penetrating polymers.

In the first stages of the PhD project the student will use these novel materials to prepare miRNA-nanomedicines that will be characterized for their mucous-penetrating and pharmaceutical properties. A key roadblock to-date in the development of CF gene therapies has been the lack of valid tools for translation to comprehensively screen new vector systems. To overcome this barrier and to reduce dependence on in vivo models we have developed high content imaging approaches for screening nanomaterials[5] and an innovative 3D collagen-hyaluronic acid bilayered construct that enables culturing of primary human cells in an extracellular matrix (ECM) environment to better recapitulate the human airway[6]. The RNAi-nanomedicines will be screened using these state-of-the-art in vitro models with lead miRNA-nanomedicines being assessed using the 3D model seeded with primary CF cells. Finally, key to the clinical use of inhalable gene therapies is integration with an appropriate inhaler device. The student will work with industrial

collaborators to integrate the miRNA-polypeptide nanomedicines into devices for aerosol testing to create a drug-device combination suitable for clinical translation.
The student will be supported by an experienced, multidisciplinary team on a project focused on the emerging field of pharmacoengineering, at the interface of pharmaceutical sciences and biomedical engineering, and will have an opportunity to work with the team's extensive network of clinical and industrial collaborators.

References:

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Research Theme: Population and Health Systems

Research project 30: Developing, piloting and evaluating a theoretical-based intervention to support endocrine therapy medication taking behaviour in women with stage I-III breast cancer
Supervisors: Dr Kathleen Bennett, Division of Population Health Science and Dr Caitriona Cahir, Division of Population Health Science.
Research Project Description: Almost 3,000 women are diagnosed with breast cancer annually in Ireland. In women with hormone-responsive early breast cancer, 5-10 years of endocrine therapy is recommended to prevent breast cancer recurrence and mortality, with a reduction in cancer recurrence of up to 50%. However, despite the proven clinical efficacy of endocrine therapy, many women (30-70%) do not take their treatment as recommended. To date only a minority of published medication taking behaviour (MTB) interventions have improved MTB or enhanced patient outcomes.
Aims: To develop, pilot and evaluate a theoretical-based behavioural intervention to improve endocrine therapy MTB and health outcomes in women with stage I-III breast cancer.
Methods: The Medical Research Council's framework for the development of complex interventions will be used with the Theoretical Domains Framework (TDF) and taxonomy of Behaviour Change Techniques (BCTs) as the theoretical framework. Three inter-related work-packages are proposed. Work-package 1, building on previous research, will identify and model the demographic, clinical, treatment-related, psychological and health behavioural determinants of endocrine therapy MTB and associated health outcomes (quality of life, side-effects) in women with stage I-III breast cancer using data from the National Cancer Registry Ireland (NCRI) linked to national breast cancer patient questionnaire data (N=1,606, response rate=66%). Work-package 2 will identify the content and implementation options for the intervention by establishing a definitive list of MTB BCTs and their form of delivery to be tested, through Steering Group consensus. The BCTs will be pre-tested in a feasibility study and piloted in a randomized control trial of women with stage I-III breast cancer who have been prescribed endocrine therapy and clinical and support staff, to assess face validity, acceptability, feasibility and any barriers to implementation. Work-package 3 will evaluate the cost-effectiveness of potential interventions to improve endocrine therapy MTB. Conclusion: The results of this research will directly benefit women with breast cancer prescribed endocrine therapy, through improved clinical outcomes and survival and also the healthcare professions involved in their care.
Keywords: Cancer, Population Health, Theoretical-based intervention, endocrine therapy

Research project 31: Patient-Centred Care (PCC) in Sub-Saharan Africa
Supervisors: Prof Ruairi Brugha, Epidemiology and Public Health Medicine and Dr Jakub Gajewski, Division of Population Health Science.
Research Project Description: This PhD studentship, through research undertaken in a sub-Saharan African country (Tanzania or Malawi), will investigate healthcare provider and patient dimensions of patient-centred care (PCC). Little substantive research has been undertaken in Africa on PCC, which is determined by health worker training; the structure and organisation of the health system; and the socio-economic environment in which health workers operate man (1). Given the lack of research from Africa, an exploratory mixed methods study is planned, comprising in-depth interviews of hospital staff and patients, followed by structured surveys of staff and of patients. The successful candidate will have the opportunity to develop

qualitative and quantitative research skills, under the supervision of experienced researchers and working with an international research team from European and African research institutions.
The PhD comprises: 1) a systematic review of the PCC literature, 2) qualitative research to develop an understanding of PCC in an African country context; 3) development and testing of a tool to measure PCC in an African setting, 4) and survey implementation to determine the current state of PCC in the selected country. This will lead to research outputs (scientific articles), led by a candidate who has the drive to become an accomplished researcher; and who may be considering a research career on health in low- and middle-income countries. The platform of the PhD is the 4-year €6 million Horizon 2020 Scaling up Safe Surgery for District and Rural Populations (SURG-Africa) project, 2017-2020 - see www.surgafrica.eu. SURG-Africa is testing a supervision, mentoring and support intervention, in 31 district level hospitals, in Malawi Zambia and Tanzania, filling a major global health research gap.
This project will require fieldwork in Tanzania or Malawi in collaboration with, and supported by, the SURG-Africa research teams. This will provide an excellent opportunity to obtain first-hand experience in conducting studies in resource-limited settings. Structured training will be delivered through the PHS SPHERE programme, with on-the-job training by Brugha and Gajewski, the SURG-Africa research PI and lead, in: (i) qualitative methods; and (ii) quantitative methods. It is expected that findings will lead to the design of a PCC intervention that could form a post-Doc proposal for a candidate interested in a career in global health research.

Reference:

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Keywords: Patient-centred care, health systems, Africa

Research project 32: Solar Water Disinfection Transparent 20L Jerrycan (SODIS-TJC)
Supervisors: Prof Kevin McGuigan, Physiology & Medical Physics and Dr Fidelma Fitzpatrick, Clinical Microbiology.
Research Project Description: According to the WHO and UNICEF, in 20171:

- 2.1 billion people are without access to a safely manage source of water.
- 1.8 billion rely on either unimproved water sources or improved sources that are faecally contaminated.
- 844 million people still lack even a basic drinking water service.
- 159 million people collect drinking water directly from surface water sources.

The 2030 Sustainable Development Agenda agreed by the United Nations (UN) Member States in 2015 calls for universal access to safe drinking water. Therefore, development of sustainable and affordable point-of-use (POU) water treatment technologies to deliver safe drinking water at household or microcommunity level is a priority for achieving Sustainable Development Goal 6 (Clean Water and Sanitation).
Solar water disinfection (SODIS) 2 is a water treatment technique where transparent containers are filled with water and exposed to sunlight for a minimum of 6 h allowing the UV to kill the waterborne pathogens. SODIS is one of the most appropriate household water treatment & storage (HWTS) technologies for treating drinking water in low-income environments because it's:
1. Effective against a wide range of waterborne pathogens
2. Low- or zero-cost in areas where transparent containers are available.
3. Easy to use: very little training is required.

One of the obstacles to SODIS uptake is the workload associated with filling, exposing and managing a sufficient number of standard 2L bottles to provide for the entire household. An objective of the EU WATERSPOUTT project (Grant no 688928 see www.waterspoutt.eu) was to develop a 20L Transparent Jerrycan (TJC) for SODIS purposes. As a result we designed the TJC and demonstrated that the optimum material for its manufacture is polypropylene (PP).
However, in WATERSPOUTT we were unable to source a manufacturer in Europe or Africa who could produce PP TJC prototypes for us. Eventually compromised and the TJCs were made of polyethylene terephthalate (PET) plastic, which is not ideal.
The EU has just awarded Prof McGuigan funding to coordinate a second H2020 Project (PANIWATER Grant no. 820718) which includes funding for the manufacture and evaluation of PP TJCs in India. The successful StAR PhD Researcher will conduct a large scale (~1000 children under 5 yrs) 18 month field trial of the public health impact of the use of 20L PP SODIS TJCs on childhood diarrhea and water quality among disadvantaged communities in Rajasthan in India in cooperation with our local Indian partners. The researcher will be responsible for:

- Conducting baseline survey of incidence of diarrhoeal illness in the study population
- Conducting regular water quality analysis at participating households

- Collection and analysis of pre- & post-implementation data on incidence of diarrhea for the duration of the study.

Almost every household without access to safe water in low-income countries has a jerrycan. If this project is successful it has the potential to replace all of these with PP TJCs which will provide safe drinking water for the most vulnerable communities across the globe.

1. WHO/UNICEF Progress on Drinking Water, Sanitation and Hygiene -2017 Update and SDG Baselines. (https://washdata.org/sites/default/files/documents/reports/2018-01/JMP-2017-report-final.pdf)
2. **McGuigan** et al. Solar Water Disinfection (SODIS): A review from bench-top to roof-top. J. Hazard. Mater. 235–236 (2012) 29–46.

Keywords: Public health

Research Theme: Population Health

Secondary Theme(s): Respiratory Medicine, Psychology

Research project 33: Assessing the application of a novel algorithm for DOSE REduction and Adherence CosT-effectIVEness in adults with respiratory and other chronic conditions (DOSE-REACTIVE)
Supervisors: Dr Frank Doyle, Psychology and Prof Richard Costello, Medicine, RCSI
Research Project Description: Adherence to medications – the process by which patients take their medications as prescribed – is suboptimal, e.g., inhaler adherence is estimated to be as low as 23% in people with chronic obstructive pulmonary disease (COPD). Such non-adherence is associated with poorer health outcomes, but also unnecessary over-prescribing of add-on therapies and resource wastage. Modern technologies could help address these issues. We have developed a potentially ground-breaking novel algorithm that can calculate the optimal drug dose per patient for inhaled (or other) medications, depending on that individual's current adherence rates and any outcome of interest (e.g. exacerbation rates, quality of life, etc.). The algorithm can be applied to any medication where its use can be precisely timed. This suggests that in future, clinicians could prescribe medications and titrate dosages based on individual behaviour patterns, rather than relying on more global clinical guidelines. This PhD will therefore explore the feasibility of including this algorithm in clinical care and devices, integrating with current health data and the potential for use in research and health insurers.

Research project 34: The epidemiology and associated burden of potentially serious alcohol-medication interactions in ageing populations in Ireland and the UK
Supervisors: Dr Gráinne Cousins, Pharmacy and Prof Tom Fahey, General Practice, RCSI, and Dr Steven Bell, Public Health and Primary Care University of Cambridge
Research Project Description: How the body handles alcohol changes as we get older. Ageing lowers the body's tolerance for alcohol; alcohol circulates in an older person's body for a longer time and the effects of drinking last longer. Even at relatively low levels of alcohol consumption, older adults are vulnerable to alcohol-related harms, with exposure to multiple medications exacerbating these harms, due to changes in absorption, distribution and metabolism of alcohol and other medications with age. Alcohol-medication interactions may increase the risk of hypoglycaemia, hypotension, sedation, gastro-intestinal bleeding and liver damage. We recently developed the POSAMINO criteria (POtentially Serious Alcohol-Medication Interactions in Older adults), an explicit set of 38 potentially serious alcohol-medication interactions in older adults. Our preliminary analyses suggest that almost one in five older adults are at risk of potentially serious drug-alcohol interactions, with exposure to POSAMINO criteria involving central nervous system agents, associated with an elevated risk of falls and injurious falls. Given that older adults are often reluctant to reveal a history of excessive alcohol consumption, and healthcare professionals have a lower degree of suspicion when assessing older adults, the question of risk arising from a patient's alcohol consumption may never arise in a consultation. The POSAMINO criteria may be useful in a clinical setting to risk stratify patients at the point of prescribing, allowing for the identification of patients whose alcohol consumption places them at increased risk and who would benefit from a brief intervention. However, prior to informing clinical or public health initiatives, the POSAMINO criteria require further validation, in national and international longitudinal studies, to prospectively quantify the magnitude of risk posed for major health outcomes. The proposed study plans to harness the rich longitudinal information on alcohol consumption and co-occurring medication use among older adults in Ireland and the UK using existing databases such as the Irish Longitudinal Study on Ageing (TILDA), the English Longitudinal Study on Ageing (ELSA) and Whitehall II. This project involves collaboration between the Royal College of Surgeons in Ireland and the

University of Cambridge, and will involve a three-month placement at the University of Cambridge.

Research Theme: Respiratory Medicine

Research project 35: Using induced pluripotent stem cells (iPSC) to evaluate new CFTR-directed and microRNA-based therapies for cystic fibrosis (CF)
Supervisors: Prof Catherine Greene, Clinical Microbiology and Dr Killian Hurley, Consultant Respiratory Physician and Senior Clinical Lecturer, Medicine.
Research Project Description: The recent development of drugs that directly modulate the CFTR protein in patients with CF has delivered a paradigm shift in how CF is treated using personalised medicine. However, a significant number of patients with CF do not have the better understood and more common CFTR mutations and therefore cannot access these lifesaving medications because CFTR modulator medications are only licensed and available to patients with specific mutations (e.g. Ivacaftor for G551D). Several CF preclinical models exist but none are lung-specific and reflect an individual patient's genetic background. Here we propose to engineer a novel in vitro human system to enable the derivation of lung-specific organoids, called bronchospheres, from induced pluripotent stem cells (iPSC) isolated from the peripheral blood of patients with such CFTR mutations. Using forskolin induced swelling of these bronchospheres we will measure lung-specific CFTR function in a high throughput fashion to screen approved and experimental CF therapies. The experimental therapies that will be tested are proprietary inhibitors of microRNA that downregulate CFTR expression. In addition to the high throughput methods we will also assess CFTR function using MQAE and YFP fluorescence-based chloride ion conductance assays in air-liquid interface (ALI) cultures, and immunoprecipitation and western blotting for mature CFTR.
Aims:

- To establish iPSC-derived lung-specific models of CF from patients with rare or unknown CFTR mutations. A placement in Boston University, School of Medicine will be provided to learn the methodology.
- To interrogate these models to study CFTR protein, translation, trafficking and function.
- To test the efficiency of existing and novel CFTR-targetting drugs for patients with rare CFTR mutations.

This PhD project will be co-supervised by a scientist and a clinician-scientist. It is designed to facilitate the acquisition of in-depth cutting-edge and state-of-the-art **cell and molecular biology** skills in a **translational and personalised medicine setting.**
Keywords: Cystic fibrosis and miRNA-based medicines

Research Theme: Vascular Biology

Research project 36: Development of a novel therapeutic for the treatment of sickle cell anaemia
Supervisors: Dr Marian Brennan, Molecular & Cellular Therapeutics (MCT) and Prof Marc Devocelle, Pharmaceutical & Medicinal Chemistry.
Research Project Description: Sickle cell anaemia is a chronic condition that is expensive to treat and requires significant clinical intervention and management. Patients in crisis need acute management that often requires hospitalization. Furthermore, damage to the vasculature leads to joint and organ damage that require frequent monitoring and treatment. Currently patients can be cured by bone marrow ablation followed by a transplant if a match can be found. Unfortunately, this process is very costly, dependent on a donor match and only has a 90% survival rate. This treatment is also only available in specialized treatment centers. Sickle cell anaemia is a disorder resulting from a mutation in the β-chain of haemoglobin. Haemoglobin is the protein responsible for carrying oxygen from the lungs to the tissues where it releases oxygen. In patients with sickle cell disease, oxygen can be successfully carried from the lungs to the tissues, but after the oxygen is released, the haemoglobin protein interacts inappropriately with other haemoglobin proteins. This results in multimers of sickle haemoglobin (HbS) forming long rigid chains which in turn causes erythrocytes to distort into the characteristic sickle shape. The sickle shaped erythrocytes are inflexible and become trapped in the capillaries causing damage to organs. The multimers are more prone to form under certain conditions such as in low oxygen conditions and during infections. The blocking of capillaries leads to severe

pain in patients known as a sickle cell crisis. The crisis can cause permanent damage to organs including the brain, liver, spleen, kidneys and lungs.
In 1998 the anti-cancer treatment hydroxyurea was approved for use in sickle cell disease. Hydroxyurea increases expression of the foetal haemoglobin β-like chain. As the foetal gene is a different gene to the adult one, there is no defect in this protein subunit expressed, and therefore multimer formation is disrupted and thus the red blood cells do not distort.
Although this reduces the number of hospitalizations in patients, approximately one third of patients are refractory to this therapy for a variety of reasons (Yahouédéhou SCMA et al., 2018). Hydroxyurea is not specific and therefore can also have severe side effects including liver and kidney damage as well as increased carcinogenic risk.
Using molecular modelling, we have designed peptides to disrupt haemoglobin multimer formation that can be delivered into the erythrocytes. We plan to test peptides for binding to HbS and assess the disruption of multimer formation in a cell free assay. A range of peptides and peptidomimetics derived from the parent peptide will be assessed to identify the lead peptide. The peptides are designed with a specific peptide tag for delivery into erythrocytes, therefore, we will assess delivery into erythrocytes and perform haemolysis assays in order to determine whether the peptides damage the erythrocyte membrane. We do not expect toxicity from the peptides, however, we will also test for toxicity in endothelial cells, erythrocytes and platelets. We will further test the peptides ex vivo using red blood cells from patients with sickle cell anaemia. Successful development of an alternative therapy for patients with sickle cell disease will provide a much needed treatment that can prevent painful crisis and damage to patient's organs.
Keywords: Vascular biology, Chemoinformatics, Drug Discovery

Research Project 37: How does the macrophage molecular clock modulate thrombogenesis?
Supervisors: Dr Roger Preston and Dr Annie Curtis, Molecular & Cellular Therapeutics (MCT), RCSI
Research Project Description: Cardiovascular disease (CVD) is the leading cause of mortality and morbidity worldwide. A common feature of CVD is vascular inflammation that in turn promotes blood clotting and thrombosis. Notably, innate immune cells such as macrophages and neutrophils have recently been identified to contribute to thrombotic disease development.
Every cell within the body has the ability to track time-of-day, and this system is termed 'the molecular clock'. The molecular clock comprises a network of feedback loops which generate 24 hour rhythms in gene expression. In most tissues, clock driven gene expression peaks or troughs in "rush hours" anticipating dusk and dawn. Importantly, one gene, Bmal1 acts as the 'master clock controller' - Bmal1 deletion prevents cycling of all the other core clock proteins and ablates all circadian gene expression.
Surprisingly, the clinical manifestation of CVD is intrinsically linked to the molecular clock and circadian rhythms – both heart attacks and strokes occur most commonly in the early morning hours and individuals subject to circadian disruption, such as shift-workers, have a significantly increased risk of CVD. Despite this, the biological basis for how circadian disruption drives thrombogenesis and vascular dysfunction remains completely unknown.
In preliminary studies, we have discovered that the macrophage molecular clock may act to modulate several facets of haemostasis to favour thrombosis, however, the mechanism(s) by which the molecular clock mediates these functions is poorly understood. In this project, we propose to fully characterise how different haemostatic processes are regulated by the molecular clock and assess their subsequent impact on blood clot formation and degradation using newly developed tools and techniques. To understand how the molecular clock impacts upon haemostatic gene expression, we will perform comprehensive expression analysis of blood coagulation and fibrinolysis genes using Bmal1+/+ versus Bmal1-/- macrophages to recapitulate macrophages with and without a molecular clock. In addition, we will utilise newly developed techniques to determine how macrophage stimulation of blood coagulation and clot dissolution is regulated by the macrophage molecular clock. Finally, we will consider whether tissue microenvironment modulates the extent to which the macrophage molecular clock contributes to thrombogenesis. **In summary, this project is anticipated to shed new light on the biological basis for circadian control of CVD development and provide a platform for the creation of novel therapeutic interventions to combat CVD in at-risk individuals**.
The project will be performed under the co-supervision of Dr. Roger Preston and Dr. Annie Curtis. The Preston lab (www.prestonlab.com) and the Curtis lab (www.curtisclocklab.com) are both large multi-disciplinary research groups that are currently funded by prestigious awards from Science Foundation Ireland, Bayer Healthcare, Irish Research Council and the National Children's Research Centre. Both have well-established expertise and an international reputation in haemostasis research and circadian

immunology respectively and a strong publication record in this area (e.g. Gleeson et al Blood 2015, Gleeson et al JTH 2017, Sutton et al, Nature Comms 2017 and Early et al. PNAS, 2018).

Secondary Theme(s): Immunology and Systems Biology

Research project 38: Engineering protease-activated receptors to modulate cell signalling output
Supervisors: Roger Preston and Ingmar Schoen, Molecular and Cellular Therapeutics, RCSI
Research Project Description: Protease-activated receptor 1 (PAR1) is a G protein coupled-receptor expressed on the surface of endothelial cells and platelets. It has a critical role in maintaining vascular homeostasis and its dysregulated activation has been proposed to contribute to a range of cardiovascular and inflammatory diseases. Unlike most receptors, PARs become activated not when bound by ligands, but when cleaved by specific proteases. Recent studies indicate PAR1 activation can occur via a number of different proteases, which can promote either pro-inflammatory, prohemostatic signalling activity or anti-inflammatory, cell-protective effects, depending on the activating protease. Analogously, PAR1 activation in platelets can either potentiate platelet aggregation or aid thrombus consolidation. Despite growing recognition of its physiological importance, the molecular basis for how PAR1 signalling 'bias' is achieved by different proteases remains poorly understood. An enhanced understanding of the molecular parameters that control signalling bias is important given the early promise of drugs that tilt PAR1 towards 'cytoprotective' signalling for the treatment of inflammatory vascular disease.
The objective of this study is to define how different proteases confer distinct PAR1 cell signaling outputs. To achieve this, we will utilize state-of-the-art genome engineering approaches combined with advanced microscopy techniques to decipher the structural determinants required to mediate PAR1 signaling bias in different cell types. We will assess whether specific PAR1 molecular regions are differentially altered in response to activation with different proteases, using mutant recombinant versions of PAR1 in new assays of PAR1 signaling that have been developed in our lab. In addition, we will use fluorescence resonance energy transfer (FRET) live cell imaging and super-resolution microscopy to determine PAR1 interactions with other cell surface receptors that we hypothesize contribute to skewing of PAR1 signaling output. Finally, we propose to utilize genome-wide screening approaches to identify and subsequently characterize novel modifiers of PAR1 signaling outcomes.
Collectively, the proposed study is anticipated to reveal novel insights as to how PAR1 structure, activation status and molecular interactions with co-receptors impact upon downstream signaling outcomes. Ultimately, this will enable generation of new pharmacological strategies to facilitate preferential skewing of PAR1 signaling output for therapeutic benefit.
The project will be performed under the supervision of Dr. Roger Preston and project co-supervisor Dr. Ingmar Schoen. The Preston lab (www.prestonlab.com) is a large multi-disciplinary research group that is currently funded by prestigious awards from Science Foundation Ireland, Bayer Healthcare and the National Children's Research Centre. It has well-established expertise and an international reputation in the study of the mechanistic basis of
PAR1 proteolysis and subsequent downstream signaling (Gleeson et al Blood 2015, Gleeson et al JTH 2017). The Schoen lab (http://schoenlab.strikingly.com) is the only dedicated super-resolution microscopy lab in Ireland and has specific expertise in FRET imaging and super-resolution microscopy techniques (Li et al Nature Methods 2018, Früh et al Nature Communications 2015) with a research focus on platelet mechanobiology. Consequently, the PhD student would benefit from a multi-disciplinary approach, with expert tuition provided in molecular biology techniques, recombinant protein generation, CRISPR-Cas9 mediated genome modification, advanced microscopy and cell labeling techniques.

Research Theme: Immunology

Research project 39: Do Novel Post-Translationally modified peptides Exacerbate COPD?
Supervisors: Dr Emer Reeves and Dr Mark Murphy, Medicine, RCSI and Prof Gerry McElvaney, Medicine, Beaumont Hospital
Research Project Description: Chronic obstructive pulmonary disease (COPD), which includes emphysema and chronic bronchitis, is a leading cause of disability and mortality. Globally, COPD has become the third leading cause of death after ischemic heart disease and cerebrovascular disease. Approximately 180,000 people in Ireland have been diagnosed with moderate or severe COPD, and more recently, concerns have been raised over the rate of COPD in Irish women. Cigarette smoking remains the

largest cause of COPD development, but air pollution is a growing contributor, particularly in the developing world. Currently there is no cure and identification of biomarkers and clinical measures that can evaluate respiratory impairment, help prognosticate outcome in patients and identify measures to control symptoms and stabilize health is central to effective and efficient care.

Of major importance, key studies have demonstrated that white blood cells called neutrophils, and neutrophil-derived factors, play a crucial pathological role in the development of COPD. Neutrophils possess both favourable and unfavourable attributes in the airways with advantages involving a role in lung defence against infection, yet disadvantages involving their role in triggering lung tissue damage. Exciting novel data from this laboratory demonstrated that neutrophils express and release an enzyme called Prim2-4. Prim2-4 is the focus of this study and we believe it is a new and significant risk factor for COPD.

The overall aim of this innovative study is to investigate whether Prim2-4 can catalyse posttranslational modifications of essential structural proteins of the delicate lung tissue. Specifically, we aim to investigate whether dysregulated Prim2-4 activity leads to changes in the protein folding of essential extracellular matrix (ECM) proteins of the lung.

We hypothesize that Prim2-4 can cause disassembly of the ECM and that this leads to the generation of ECM peptides that can signal through Toll-like receptors on airway epithelial cells and immune cells. We believe this to be of great importance, resulting in increased levels of proinflammatory cytokines, and mucus secretion, thereby amplifying disease severity. Moreover, in this study detection of specific Prim2-4 altered ECM peptides in plasma will be extrapolated as prognostic biomarkers of disease severity in COPD individuals.

In summary, this study will utilize a well-developed clinical framework of COPD patients for the identification of novel changes to ECM proteins as prognostic biomarkers and as potential therapeutic targets in COPD-associated airway disease.

Secondary Theme(s): Biomaterials and Regenerative Medicine and Cancer

Research project 40: Rhythms under your skin: Enhancing vaccine efficiency using a state-of-the-art dissolvable intradermal microneedle patch and harnessing the skin dendritic cell circadian clock

Supervisors: Annie Curtis, Molecular and Cellular Therapeutics, RCSI and Prof Ryan F. Donnelly, Pharmacy, Queen's University Belfast

Research Project Description: Background: Vaccination is the **greatest success story of modern medicine** with the WHO estimating 10 million deaths were prevented by vaccines between 2010 and 2015. Given that the global vaccine market is expected to exceed $50 billion by 2025, novel vaccine strategies have both human and economic and societal significance. Upon vaccination, dendritic cells (DCs) process antigen into smaller fragments (antigen processing). The DCs then display these antigen fragments on the cell surface (antigen presentation) enabling interaction with T cells, which together with B cells generate the required immune response to afford long lasting immune memory and disease protection. Although much success has been achieved in developing vaccines to induce antibody responses, generating vaccines to induce T cell responses, specifically cytotoxic T cell responses against cancers, has proven problematic. **The inability to induce sufficient T cell responses by vaccination is a major roadblock to translating the ground-breaking developments which we are currently experiencing in the field of cancer immunotherapy.**

24 hour rhythms (termed circadian rhythms) exist in organisms to efficiently align physiology and behaviour with the daily changes of the environment. Circadian rhythms are generated by the cells own molecular clock, generated by a collection of protein encoded feedback loops. We have made the striking discovery that the molecular clock in dendritic cells is a master regulator of antigen processing, presentation and T cell activation. **Therefore we propose to harness the dendritic cell clock to enhance vaccine responses for cancer immunotherapy.**

It is now well-established that a tight semi-contiguous network of dendritic cells reside in the different skin layers, called skin dendritic cells (skinDCs). We have shown that these cells have the capacity to induce robust T-cell responses and their accessibility on the skin make them an ideal target for vaccination. One particularly promising approach **to targeting of these skin DCs is the use of microneedle (MN) arrays**. MN painlessly pierce the epidermis, creating microscopic holes through which drugs, vaccines and other molecules can diffuse. **We will employ a dissolvable microneedle skin patch to deliver antigen to skinDCs with and without a molecular clock and at specific times-of-day to determine the impact of the skinDC molecular clock on T cell responses.** Once this is established we will determine if we can harness this skinDC molecular clock to enhance anti-tumour responses *in vivo*.

Circadian biology and treatment alignment to the optimal time of day is finally being realised as a critical factor for improving drug efficacy, and is being heralded as **Medicine in the Fourth Dimension[1].** Concurrently, the gains made in **Cancer Immunotherapy** are being hindered by our inability to generate vaccines that sufficiently activate T cells. If successful, **this project will allow us to unleash the full potential of the DC through harnessing circadian function to specifically enhance T cell responses for cancer treatment.** This project will combine the research strengths within RCSI along with world-class international collaborations into a compelling project that spans the cutting-edge areas of circadian biology, drug delivery, tissue engineering and cancer immunotherapy.

1. Cederroth et al, Cell Metabolism, 2019. **2.** Kissenpfennig et al, Immunity 2005. **3**. Zaric et al, ACS Nano 2013, **4.** Zaric et al, Journal of Investigative Dermatology, 2015

Secondary Theme(s): Cancer

Research project 41: Development of "FXR-targeted" Nutraceuticals for Treatment of Intestinal Diseases
Supervisors: Prof Stephen Keely, Molecular Medicine and Dr Sudipto Das, Pharmacy and Molecular & Cellular Therapeutics, RCSI
Research Project Description: The incidence of intestinal diseases, such as colorectal cancer (CRC) and inflammatory bowel disease (IBD), is significantly higher in Western societies than elsewhere in the world. Research suggests that the development of such diseases involves many inter-related factors, one of the most important of which is our diet. Indeed, diets high in fat and but low fibre are now strongly linked to the development of both intestinal inflammation and cancer. Also emerging as a critical determinant of disease development is the microbiome. This is the vast community of trillions of bacteria that live in our gut and which is now known to play crucial roles in regulating many aspects of our physiology, including metabolism, immunity, and inflammation. The microbiome communicates with us through producing chemical messengers that interact with receptors expressed on the epithelial cells lining the intestinal wall. Important among these messengers are various nutrients from our diet and bile acids which are produced in the liver. When they enter the colon from the small intestine, the microbiome metabolises nutrients and bile acids, thereby altering their ability to activate specific receptors present on the epithelium. One of these receptors, farnesoid X receptor (FXR), has been shown in preclinical studies to prevent the development of colon cancer and inflammation. With this in mind, FXR has become a hot target for pharmaceutical development. However, development of synthetic FXR modulating drugs is expensive and risky and there are still no FXR-targeted drugs in clinical trials for the treatment of intestinal disease.
Nutraceuticals, or functional foods, provide an alternative approach to treat intestinal disorders. Previous studies in our lab have found that certain chemicals found in common dietary plants (i.e., phytochemicals) can increase levels of FXR expression in intestinal epithelial cells. Given the capacity of FXR to suppress cancer cell growth and dampen inflammation, these findings have important physiological and pathophysiological implications. Thus, diets deficient in FXR-acting phytochemicals could predispose individuals to the development of colon cancer and IBD, while on the other hand, their supplementation to the diet could provide an effective means to treat, or even prevent, the occurrence of these diseases. With this in mind, our laboratory primarily focusses on developing our understanding of how dietary components, the microbiome, and bile acids interact to regulate intestinal physiology and pathophysiology, with a view to exploiting these interactions for disease treatment
This PhD project is based on the hypothesis that, by virtue of their ability to enhance FXR expression in the gut, dietary plant-derived phytochemicals have the capacity to be developed as a new class of "FXR-targeted" nutraceutical. The project consists of three Specific Aims: **i)** To investigate at the molecular pathways by which certain phytochemicals regulate FXR expression in intestinal epithelial cells, **ii)** to investigate the effects of FXR-targeted phytochemicals on cell growth, death, and inflammation in the gut, and iii) to test plant extracts rich in FXR-targeted phytochemicals in a preclinical model of intestinal disease. Overall, we expect this project to further develop FXR-targeted nutraceuticals for the prevention and treatment of intestinal disease.

Research project 42: Novel approaches for the treatment of dry eye disease
Supervisors: Dr Joan Ni Gabhann, MCT and Ophthalmology and Prof Conor Murphy, Ophthalmology, RCSI
Research Project Description: Inflammation is a key unifying factor for a range of ocular surface inflammatory diseases including autoimmune mediated dry eye disease (DED) and complications with corneal transplantation (CT). While each condition has a specific presentation there are elements of overlap

among them, most strikingly (i) immune-mediated inflammation driving disease pathology (ii) use of immune suppression/corticosteroids as a primary treatment option (iii) lack of mechanisms to effectively deliver therapeutics to the ocular surface and (iv) no diagnostic tests that allow identification of patients who will go on to develop further complications. Current therapies for DED, including artificial tears and anti-inflammatory agents, have been proved largely inadequate as they fail to address the underlying inflammatory component. Recently non-coding microRNA species have been shown to regulate inflammation. Synthetic DNA sequences that mimic or antagonise miR function are a new class of drugs which exhibit enhanced stability, target specificity and bioactivity. An ability to effectively modulate miR function and thus ocular inflammation has wide ranging therapeutic and commercial implications. This proposal aims to address these needs by identifying bio-markers and targeting the molecular mechanisms that underpin DED disease pathology, with a strong emphasis on translational application of these findings to develop both **diagnostics** and novel **therapeutics**. We have generated promising preliminary data from epigenetic studies in Sjogrens Syndrome (SS) patients, who present with severe autoimmune mediated DED, where we have identified novel microRNAs (miRs) that contribute to ocular inflammation and the development of DED. We will build upon these findings and the strengths of our multidisciplinary team to progress personalised medicine in ocular surface diseases by developing and optimising an idealised medical device for effective and targeted delivery of anti-inflammatory agents to the ocular surface, which will lead to critical improvements in healthcare in areas of societal and economic burden, generating health and economic impact.

Research project 43: Profiling miRNA expression in acute anterior uveitis
Supervisors: Prof Conor Murphy, Ophthalmology and Dr Joan Ni Gabhann, MCT and Ophthalmology, RCSI
Research Project Description: Uveitis involves acute, recurrent or chronic inflammation of the uveal tract, the middle vascular layer of the eye. Anterior uveitis (AU), which occurs within the anterior chamber of the eye, is a frequent presentation to ophthalmologists. Acute symptoms of pain, redness and photophobia are very debilitating for the young, working-age group of patients it mostly affects. AU is known to be associated with a variety of systemic conditions, in particular spondyloarthritis (SpA), which affects approximately 40% of cases. AU is characterised by the influx of inflammatory cells into the anterior chamber of the eye, which can be appreciated clinically using the slit lamp microscope. Current knowledge of the mechanisms controlling this pathogenic process is limited. Topical corticosteroids are the cornerstone of therapy in AU. Their precise mechanism of action is poorly understood and they can have a wide array of side effects in the eye. A greater understanding of the pathogenesis of AU is required for the development of more effective and targeted therapies. This project proposes a multidisciplinary approach to investigating the contribution of microRNAs to the molecular pathogenesis of AU.

Research Theme: Nursing

Research project 44: The correlation between, subepidermal moisture measurement, epidermal hydration, temperature, pain, ultrasound and the pro-inflammatory cytokines, IL-1α and Total protein in the early detection of pressure ulcers.
Supervisors: Prof Zena Moore and Dr Declan Patton, Nursing & Midwifery, RCSI
Research Project Description: Pressure ulcers (PU), are localised areas of tissue damage arising due to excess pressure and shearing forces, ranging in severity from superficial tissue damage, to full-scale tissue destruction. PUs are common, costly and affect health and social gain. Further, PU's have a particularly negatively on the individual, thus early detection and prevention of these wounds is essential. Sub-epidermal moisture (SEM) is a biophysical marker and is a product of the leak of plasma after the inflammation process increases local vasculature permeability. When tissue damage progresses to a greater number of cells, the inflammation markers increase with the resultant outcome that SEM which started as microscopic oedema grows to a macroscopic scale and becomes detectable on imaging examinations and ultimately with the naked eye. We have undertaken a large number of studies related to SEM and early PU development, and results have shown very exciting results. All individuals who developed a visual PUs always had a preceding abnormal SEM measure. Further, the mean time to PU detection was 6 days earlier (SD: 2 days) using SEM, versus VSA. Currently, although SEM measurement has been shown to have the ability to detect PUs early, this diagnostic modality is still unable to accurately determine the extent of underlying tissue damage.

Further, the clinical relevance of abnormal SEM measures without evidence of a visual PU needs further exploration. This study will investigate the relationship between subepidermal moisture measurement, epidermal hydration, temperature, pain, ultrasound and the pro-inflammatory cytokines, IL-1α and Total protein, in the early detection of pressure ulcers. A model representing these relationships does not exist currently but will be developed by the scholar over the duration of this project.

Research Theme: Respiratory Medicine

Secondary Theme(s): Immunology

Research project 45: Characterizing the immunogenic properties of bacterial extracellular vesicles (EVs) of the Cystic Fibrosis airway

Supervisors: Dr Judith Coppinger, Pharmacy, RCSI and Prof Paul McNally, Paediatrics, RCSI and Children's Health Ireland (CHI), Crumlin

Research Project Description: Recurrent Pseudomonas (P) aeruginosa infection is common in chronic pulmonary diseases including cystic fibrosis (CF), chronic obstructive lung disease (COPD) and a leading cause of hospital acquired pneumonia. Due to the emergence of multiple-drug resistant isolates, it is become increasingly difficult to cure *P. aeruginosa* related lung infections. In individuals with CF, lung disease is characterized by persistent *P. aeruginosa* bacterial infection and neutrophil dominated inflammation that leads to progressive airway destruction and respiratory failure. In addition to established mechanisms of bacterial induced inflammatory cell activation via pattern recognition receptors we hypothesize that bacteria colonizing CF airways may shed EVs composed of immune stimulatory components that could play a role in CF pathogenesis. Outer membrane vesicles isolated from *P. aeruginosa* have been shown to elicit a chemokine response (IL-8) from CF lung cells. Active immunization with bacterial outer membrane vesicles have been shown to stimulate protective immunity against infections in murine models. We propose to characterize bacterial extracellular vesicle (EVs) from the airways of persons with CF and examine if *P. aeruginosa* EVs modulate innate and adaptive immune responses in vitro and can protect mice against pseudomonas lung infection. EVs may have the potential to protect the immunized host against subsequent infection. This PhD project proposes valuable research on EVs from *P. aeruginosa* from CF airways and investigating novel vaccination strategies to diminish pseudomonas infection in individuals with CF. The project objectives include; (1) Characterising *P. aeruginosa* EVs isolated from the sputum of individuals with CF at different stages of disease progression (2) Determine if *P. aeruginosa* EVs cultured from sputum modulate innate and adaptive immune responses in vitro and (3) Examine if mice injected with *P. aeruginosa* EVs can confer protection against infection in murine models. This project builds on research we currently perform at the National Children's Research Centre on extracellular vesicles as mediators of inflammatory signalling in children with CF. The project will be performed at the National Children's Research Centre (adjacent to CHI-Crumlin Children's Hospital) with sections of the project including mouse work to take place at the RCSI and University College Dublin.

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Research Theme: Neuroscience

Research project 46: Targeting mRNA polyadenylation as novel treatment strategy in epilepsy

Supervisors: Dr Tobias Engel, Physiology and Medical Physics, RCSI and Prof José J. Lucas, Molecular Neuroscience, CSIC and CIBERNED

Research Project Description: Epileptogenesis, the process leading to a reduced threshold for seizures after transient brain insults, is associated with large-scale changes in gene expression and processes, such as selective neuronal loss, gliosis and synaptic plasticity which ultimately lead to the formation of seizure-generating neuronal networks, and the development of epilepsy. Targeting single genes has repeatedly failed to alter the development of epilepsy or reduce the percentage of drug-refractory patients, suggesting approaches which target larger signalling networks may be required. First, however, we must precisely

understand which pathological changes contribute to the development of epilepsy and to the maintenance of the epileptic state. Large-scale molecular profiling studies have provided insight into the mechanisms, which may contribute to the formation of aberrant, seizure-generating neuronal circuits, yet we are still far away from a complete picture of the pathological molecular changes occurring during the process of epileptogenesis.
Cytoplasmic polyadenylation is a process by which dormant, translationally inactive mRNA become activated by the elongation of their poly(A) tails. Cytoplasmic polyadenylation element binding proteins (CPEBs1-4) are central factors controlling polyadenylation-induced translation. In the brain, CPEBs mediate numerous cellular processes including long-term potentiation, synaptic plasticity and neurotransmitter receptor expression, processes altered during epileptogenesis. Pilot data produced by the applicant shows, for the first time, that CPEB expression is changed in both experimental models of epilepsy and in drug-refractory epilepsy patient brains suggesting a contribution of CPEBs to seizure-induced pathology. Further, by using mRNA arrays, the applicant has demonstrated mRNA polyadenylation changes affecting up to 20% of the transcriptome during epilepsy. To date, however, neither changes in polyadenylation, nor the contribution of cytoplasmic polyadenylation to disease progression have been studied in the setting of epilepsy.
This highly interdisciplinary PhD project will characterize and decipher an untested layer of gene control contributing to epileptogenesis and provide a new set of therapeutic target genes with a different mechanism of action to better treat patients suffering from epilepsy. By using interdisciplinary approaches (e.g. preclinical mouse models of epilepsy, different transgenic approaches and human-induced stem cells), the student will determine the impact of CPEBs on seizure-induced pathology and the development of epilepsy and identify genes undergoing CPEB-mediated changes in their mRNA polyadenylation status during seizures and epilepsy.

Research project 47: Transfer RNA fragments in the mechanism and treatment of epilepsy
Supervisors: Prof David C. Henshall and Dr Marion C. Hogg, Physiology & Medical Physics, RCSI
Research Project Description: Epilepsy is a neurological disorder caused by hyperexcitable brain networks that give rise to seizures. Many epilepsies have a genetic cause whereas some develop following a brain injury. Epilepsy affects around 50 million people worldwide and approximately 1 in 3 people with epilepsy do not respond to currently available medication. This leaves many people with uncontrollable seizures that can occur at any time with little warning. An improved understanding of the mechanisms of epilepsy is needed to discover new targets for seizure control and disease modification.
Researchers within the FutureNeuro Research Centre at RCSI have made pioneering discoveries about the role of noncoding RNAs in epilepsy (see www.futureneurocentre.ie). Most recently, we identified a completely new class of signalling molecule derived from transfer RNAs (tRNAs) – noncoding RNA molecules that help build the amino acid chains that make up proteins in all cells. We found fragments of tRNAs were elevated in patients with epilepsy in advance of a seizure occurring (Hogg *et al*, J Clin Inves 2019). This suggests it might be possible to predict when a seizure will occur by monitoring levels of these tRNA fragments in the blood. Efforts are now underway to develop a device capable of rapidly measuring these fragments in blood samples to enable people with epilepsy to assess their seizure risk at home. A short video explaining our research can be found at https://youtu.be/rzbKVCzgfCE, and our discovery was reported in the Irish Times here: https://www.irishtimes.com/news/health/irish-breakthrough-in-predicting-epileptic-seizures-may-lead-to-simple-test-1.3920010.
The objective of this StAR PhD project will be to establish when, how and why these tRNA fragments are generated in the epileptic brain and what they are doing. We know that tRNAs are cleaved as part of a highly conserved stress response. Starting with this knowledge, you will model and study seizure-like activity in models of human epilepsy, employing a variety of cell and molecular techniques to identify the link between neuronal activity, stress and tRNA fragment production and release. To understand their function, you will knock-down or over-express individual and combinations of tRNAs using pharmacologic, imaging, genetic and electrophysiological techniques, to study what happens to cell and network behaviour when they are manipulated. The results will advance our understanding of this major new class of molecule linked to epilepsy and new approaches to treatment and prevention.
Your research project will be supervised by experts in tRNA biochemistry and epilepsy and carried out in the world-class laboratories at RCSI including the SFI-funded FutureNeuro Research Centre. This provides access to multi-disciplinary teams of scientists including geneticists, neuroscientists, pharmacologists, biochemists, imaging experts and clinicians. You will gain experience with cellular models of epilepsy including primary cell culture and iPSC-derived neurons, cell and molecular imaging, small RNA purification

and analysis techniques, antisense knockdown, and electrophysiology. When completed, you will have gained a broad range of molecular, cellular and neuroscience techniques along with specialised techniques for epilepsy research, such as electrophysiology and have contributed to understanding new disease mechanisms.

Research Theme: Biomaterials and Regenerative Medicine

Research project 48: Non-viral gene therapy to prevent cartilage degeneration in Osteoarthritis
Supervisors: Dr Caroline Curtain, Dr Oran Kennedy and Prof Fergal O'Brien, Anatomy, RCSI.
Research Project Description: Osteoarthritis (OA) is the most common form of joint disease, affecting one in five people in the EU and results in activity limitations for approximately one in ten. There is no treatment to reverse or prevent the progression of OA, with the two primary options being pain management, or ultimately total joint replacement. For pain management, intra-articular (IA) steroid injections are regularly used to postpone joint replacement. **Crucially steroid injections reduce inflammation, and thus pain, but do not actually target or alter the behaviour of cells or tissues within the joint and have recently been reported to actually increase cartilage loss.** Therefore, there is a major unmet clinical need for novel disease modifying therapeutics proposed in this application. This project will explore the potential for controlled delivery of novel genetic cargos using non-viral vectors to augment and improve the existing practice of IA steroid delivery. **This will allow us to target the cause, as well as the painful symptoms, of the OA disease process.** We have vast experience using non-viral gene delivery methods (with vectors such as polyethyleneimine (PEI) and chitosan) to modulate cell behaviour. More recently, we have optimised a novel GAG-binding enhanced transduction system (peptide (GET system; developed by our collaborator, Dr. James Dixon, University of Nottingham). In this system a multi-domain protein glycosaminoglycan (GAG)-binding capability will be tested in terms of its ability to deliver pDNA, miRNA and siRNA to modulate, and re-balance, anabolic/catabolic activities of chondrocyte cells in OA (using cell culture and explant models). In parallel, human mesenchymal stem cells (MSCs) will also be targeted for transfection with therapeutic pDNA, miRNA and siRNA. These will also be included in OA model systems to determine their ability to modulate OA progression. Therapeutic strategies based on miRNA/siRNA technology are extremely appealing as, unlike protein inhibitors or pDNA delivery (which only targets one protein at a time) - they can intercept entire gene cohorts. This multi-targeting effect on protein expression can modulate several cellular processes, thus makes miRNA/siRNA-based therapeutics particularly valuable and promising. The specific aims of this project are:

(1) To develop therapeutic gene delivery techniques to articular chondrocytes and MSCs using cell culture systems
(2) Functionalise and optimise gene delivery vectors to in situ chondrocytes using explants models of OA (using targeted antibodies and biomaterials)
(3) Test therapeutic efficacy in vivo, using a pre-clinical rodent model of OA

The impact of this research will be to establish optimal gene delivery techniques for modulating the disease process in OA. These will be developed in 2D cell culture systems (chondrocytes, MSCs), 3D explant models and finally tested in a pre-clinical model of OA. The technology proposed here would have the potential to alleviate suffering and enable sustained healthcare benefits for the aging worldwide population. **Keywords:** Osteoarthritis, Tissue Engineering, Regenerative Medicine

Research project 49: Novel polypeptide bioinks for 3D printing of bioactive scaffolds for tissue engineering applications.
Supervisors: Prof Andreas Heise, Pharmaceutical & Medicinal Chemistry and Prof Fergal O'Brien, Anatomy, Prof. Sally Ann Cryan, Pharmacy.
Research Project Description: The development of defined three-dimensional (3D) architecture fabrication for tissue engineering has been a recent emergence within the field. In particular, 3D printing represents a promising rapid prototyping technology for the production of intricate bio-inspired scaffolds/constructs. Highly defined complex structures can be readily developed with computer-aided design (CAD) and deposited with stereolithography, extrusion, or ink-jet based printing. The primary feedstock materials used are natural hydrogels, which encompass the capability to augment native tissue due to their comparative 3D nano-architecture while holding the potential to act as a mimetic of the extracellular environment. Their disadvantage is varying source reproducibility and the limited possibility to modify the materials for example to improve cell compatibility. Hydrogels from synthetic polymers overcome

these drawbacks and have been successfully applied in 3D printing but those often lack the biodegradability and biocompatibility.
Our research is fused on bringing together the best of both worlds by using natural amino acids as building block and apply polymerization technology to convert them into suitable biomaterials. This approach is very successful as the materials are non-toxic, degradable and allow structural manipulation not possible with natural polymers. For example, we found that star polypeptides readily form strong self-supporting and sheer thinning hydrogels – ideal for 3D printing. Here we are seeking a chemist (desirably with polymer experience) for the development of a new class of bioinks based on star polypeptides. These materials could pave the way for the development of tailor-made cell-compatible hydrogel inks which can create bio- functional structures. Notably, the development of printer technology has significantly outpaced the development of new advanced inks and the limited number of suitable bioinks has been identified as the major barrier to progress for the development of tissue engineering applications. The proposed project is timely and fully aligned with national research interests and industry investments: Johnson&Johnson have recently agreed to fully fund a 3D printing laboratory in AMBER (all project (co)supervisors are AMBER PIs). Henkel Ireland also opened a new 3D printing laboratory. There will thus be a high demand for new bioinks which underpins the potential for RCSI to take a leading position in this area.
Keywords: Chemistry, Biomaterials, tissue engineering.

Research Theme: Regenerative Medicine

Research project 50: To develop and model microglia-mediated inflammatory astrogliosis in vivo using a biomimetic 3-dimensional spinal cord model system
Supervisors: Prof Fergal O'Brien and Dr Adrian Dervan, Anatomy and Regenerative Medicine, RCSI
Research Project Description: Spinal cord injury is one of the severest traumatic life-changing events suffered by the human body, frequently incurring devastating physical loss of voluntary control and somatosensation. Due to poor outcomes, research has identified many of the pathophysiological processes that occur following SCI. It is dominated (in part) by the formation of a complex and inhibitory scar at the site of injury. Central to its formation is the development of a complex neuro-immune interaction between sentinel immune cells, microglia and injury-responsive astroglial cells (astrocytes) close to the lesion site. Both cell types contribute to the development and maturation of dense cell layers that regrowing axons find impossible to traverse without trophic support.
In conjunction with industry partners, the tissue engineering research group (TERG) have driven considerable successes in the area of advanced biomaterials for peripheral nerve repair. Recently we have begun a project in partnership with the Irish Rugby Football Union Charitable Trust and the Science Foundation Ireland funded Advanced Materials and BioEngineering Research (AMBER) Centre to design and build a next-generation smart bioscaffold system to treat spinal cord injury. While comprising a multifunctional axon guidance scaffold (AGS) containing extracellular matrix to trophically support axons it will also carry microencapsulated nanoparticle delivery systems containing diverse molecular cargoes (trophic factors, DNA, siRNA) for local targeting of injury responsive cells. Using this combinatorial approach, we aim to provide the optimal engineered conditions required for axons to not only cross the lesion site but also to extend into the distal cord and reform functional connections.
Injured axons are surrounded and damaged by injury responsive 'reactive' astrocytes and microglia, including a scar forming 'A1'-type astrocyte and M1-type microglia subtypes that can arise from injury-induced astroglial-microglial interaction. We have identified these cells as prime targets for therapeutic intervention. Using standard 2-dimensional culturing techniques, in combination with the use of microfluidic isolation methods and a 3D biomimetic spinal cord scaffold system (SCSS) this project will investigate and parse the different signalling pathways that contribute to astrocyte-microglial activation following injury in vitro. Using different physical- and chemical- induced injury methods the spatiotemporal genotypic and phenotypic changes in reactive cells to produce the A1 and M1 phenotypes will be identified. Using pharmacological manipulation, the different astrocyte-microglia and microglial-astrocyte signalling pathways will be investigated. Several poorly understood signalling pathways have been implicated such as the NFκB pathway, inflammatory cytokines and the $P2Y_1$ purinergic signalling. These pathways will be targeted using selective agonists or antagonists via bath application in 2D cultures or through bolus delivery via a fibrin matrix to the SCSS. In addition, by using immunohistochemical analysis to a panel of markers in conjunction with RNA sequencing under specifically controlled injury conditions the spatiotemporal changes in

phenotype in both cell types will be mapped to transcriptomic changes in order to further identify novel pathways.
This novel approach to study isolated and naïve astrocytes and microglia together in 2D and 3D architectures will provide an unsurpassed ability to parse the effects of physical damage and the neuroinflammatory pathways that contribute to glial scar formation. The deliverables from the project will identify key targets and/or pathways and will inform the design of therapeutic DNA- and RNA-containing nanoparticle cargoes for inclusion into the AGS system.

Research Project 51: 3D tissue engineering-based model of craniosynostosis for identifying potential therapeutic targets that might enhance bone repair.
Supervisors: Prof Fergal O'Brien and Dr Arlyng Gonzalez Vazquez, Anatomy and Regenerative Medicine, RCSI. Mr. Dylan Murray, National Paediatric Craniofacial Centre, Children's Health Ireland (CHI)
Research Project Description: Craniosynostosis (CS) is a bone developmental condition that affects 1 in 2000 children worldwide. Children suffering with CS have premature fusion (bone formation) of the skull sutures, which restricts brain growth and may cause severe brain damage. The only currently available treatment is cranial vault remodelling (CVR) which is a highly complicated surgical procedure with significant potential risks involving the remodelling of the skull. However, due to skull re-fusion these children frequently undergo additional surgeries, *highlighting the need for a better understanding of the mechanisms that accelerate bone formation in CS in order to potentially improve current therapeutic strategies*. Non-syndromic CS –primarily associated with micro environmental alterations- is the most common type of CS. However, the lack of appropriate research models for this form of CS, limits the understanding of sutures that behave pathologically.
Working with AMBER, the SFI Research Centre for advanced materials and bioengineering and industry and clinical partners, the RCSI Tissue Engineering Research Group (TERG) have driven considerable successes in the area of advanced biomaterials for bone tissue repair- translating novel biomaterials from the lab to the clinic. Using these biomaterials, and working with Mr. Dylan Murray, Consultant Craniofacial, Plastic & Reconstructive Surgeon at the National Paediatric Craniofacial Centre, Children's Health Ireland at Temple Street, we have started to develop a tissue engineering (TE)-based 3D *in vitro* CS model that emulates the biophysical features of the skull sutures in order to identify the molecular pathways that promote suture fusion in Non-syndromic CS. Building on this research, in this project we will control suture fate in the CS model by utilising activators and/or inhibitors of the specific pathways identified. By using this approach we propose to identify potential targets to control prematurely fusing sutures. Therefore, we propose the use of a TE-based model built with cells from CS patients not only will emulate the native-tissue features better than 2D systems or *in vivo* animal models but will also empower us to improve current therapies by identifying the molecular pathways that control the pathology of the disease which might be used in a broader context for accelerating bone healing in complex bone fractures generally.
In this project, the student will be based in the RCSI TERG, the 2017 Irish Research Lab of the Year, and will work closely with Mr. Murray's team. This will provide a unique opportunity for the student in terms of clinical engagement. The student will receive training in the areas of cell & molecular biology as well as biomaterial fabrication and characterization of the mechanical and biological response of the novel materials. S/he will attend TERG group meetings which take place weekly and will thus have the benefit of support from researchers from a diverse range of backgrounds, including materials science, engineering, pharmacy and the biological sciences in addition to clinical medicine. All necessary equipment and facilities required to complete the project as described are in place in our labs already.

Research Theme: Psychiatry

Research project 52: Towards a new understanding of the biological underpinnings of mental disorder: a multi 'omics' investigation in a longitudinal birth cohort
Supervisors: Professor David Cotter, Psychiatry, Beaumont Hospital & Professor Mary Cannon, Beaumont Hospital
Research Project Description: Mental disorders have traditionally been studied in "silos" as if they are separate entities. However, it is known that there is considerable co-morbidity between disorders and that they share genetic and environmental risks. A new integrative approach is required to advance our understanding of the aetiology of mental disorders.
The study of biological mechanisms underpinning mental disorder has also been guilty of the "silo" approach. Different 'omic' approaches are typically studied separately but there is evidence for functional interdependence between these biological processes and mechanisms. One must also take into account

the concept of development as the vast majority (~75%) of mental disorders begin before age 25.
In this proposal, we will take an integrated approach to examine biological mechanisms underpinning a range of mental disorders in a large longitudinal population-based cohort. We will also examine the mediating effects of biological markers between environmental and genetic risk factors and mental disorder in the context of development.
This is an ambitious, innovative approach incorporating cutting-edge techniques of proteomics and environmental and genetic epidemiology. This project will expand our knowledge of the origins of mental disorders and pave the way for new treatments.
Keywords: longitudinal cohort, psychosis, environmental risk, proteomics