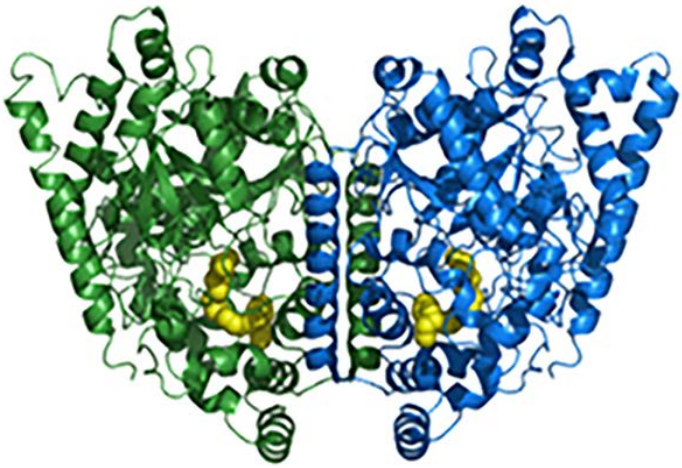
BACHELOR OF BIOMEDICAL SCIENCE (HONOURS)

RESEARCH SYMPOSIUM

Tuesday 23 October 2018
10am to 2:30pm
(Followed by prize giving and nibbles)
Rooms 503-024 and 503-028
FMHS, Ground Floor



THE UNIVERSITY OF AUCKLAND
Te Whare Wānanga o Tāmaki Makaurau
NEW ZEALAND

GENERAL INFORMATION

The Bachelor of Biomedical Science (Honours) Research Symposium will be held at the Faculty of Medical and Health Science, Grafton, in rooms 503-024 and 503-028 on Tuesday 23rd October from 10am to 2:30pm. All Honours students are expected to be present throughout the Symposium.

There will be two parallel streams of presentations (see map for room locations). Each student will have 15 minutes to present a summary of his or her research and then 5 minutes for questions. Each session will have a chairperson and two official markers who will score each presentation according to the three criteria shown below.

The Board of Studies (Biomedical Science) is most grateful to Coherent Scientific and BD Biosciences for providing sponsorship for this event.

Presentation Marking Criteria

Content.....____/30
(Is the context of the work adequately defined)
(Quality, quantity and level of information)

Structure.....____/30
(Does it develop as a clear, logical sequence of ideas and conclusions)
(Is the audience left with a clear idea of the relevance of the work)

Method and delivery.....____/40
(Oral and visual clarity and impact)
(Pacing and audience engagement)
(Did the presentation go significantly under or over time?)
(Response to questions)

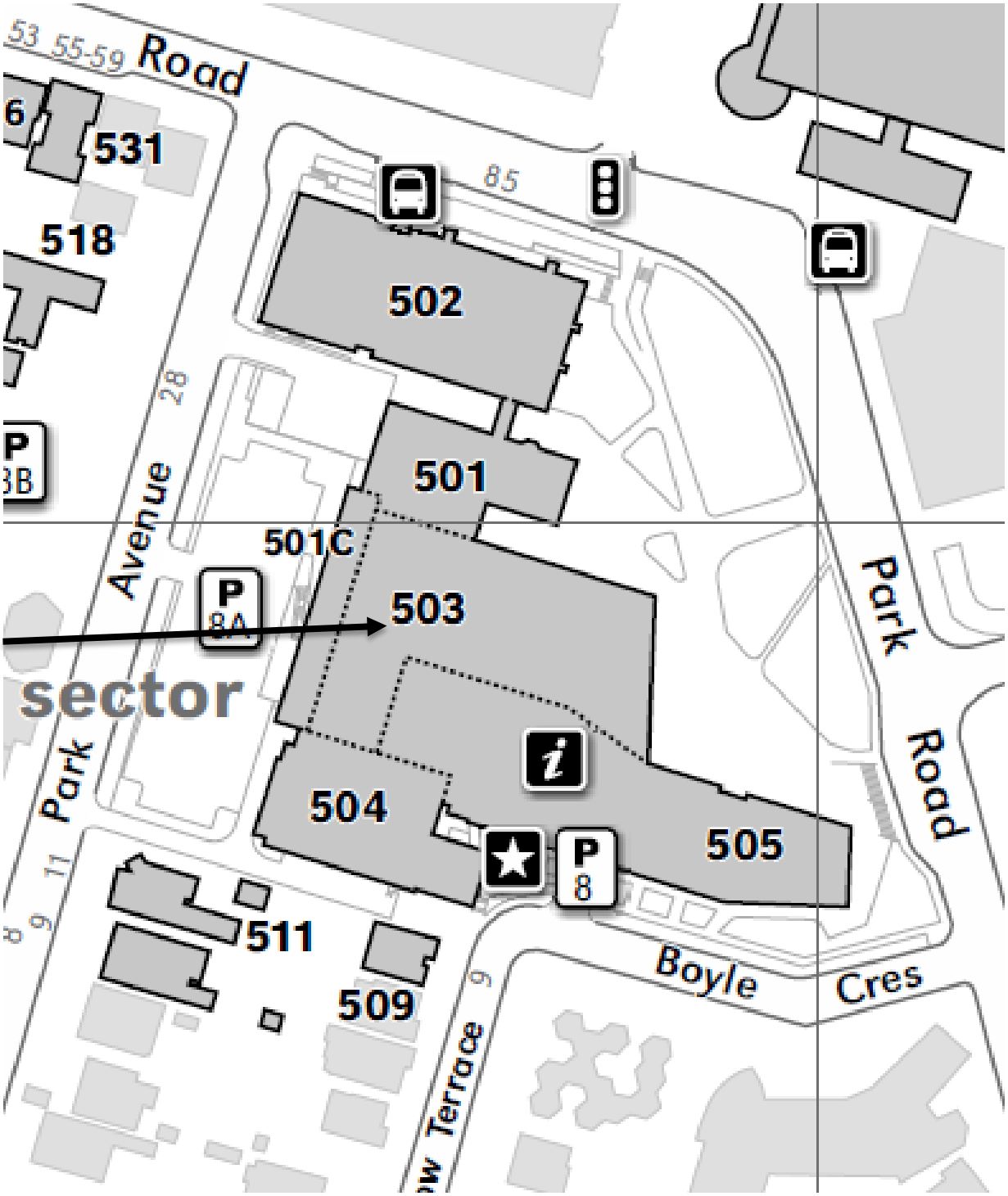
***Total Mark*.....____/100**

Note: Chairperson will warn presenter when 2 minutes and 1 minute remain. Running a little over time is OK but there will be a penalty if the presentation runs significantly over time.
Room numbers for each of the venues and a schematic of their locations are given below.

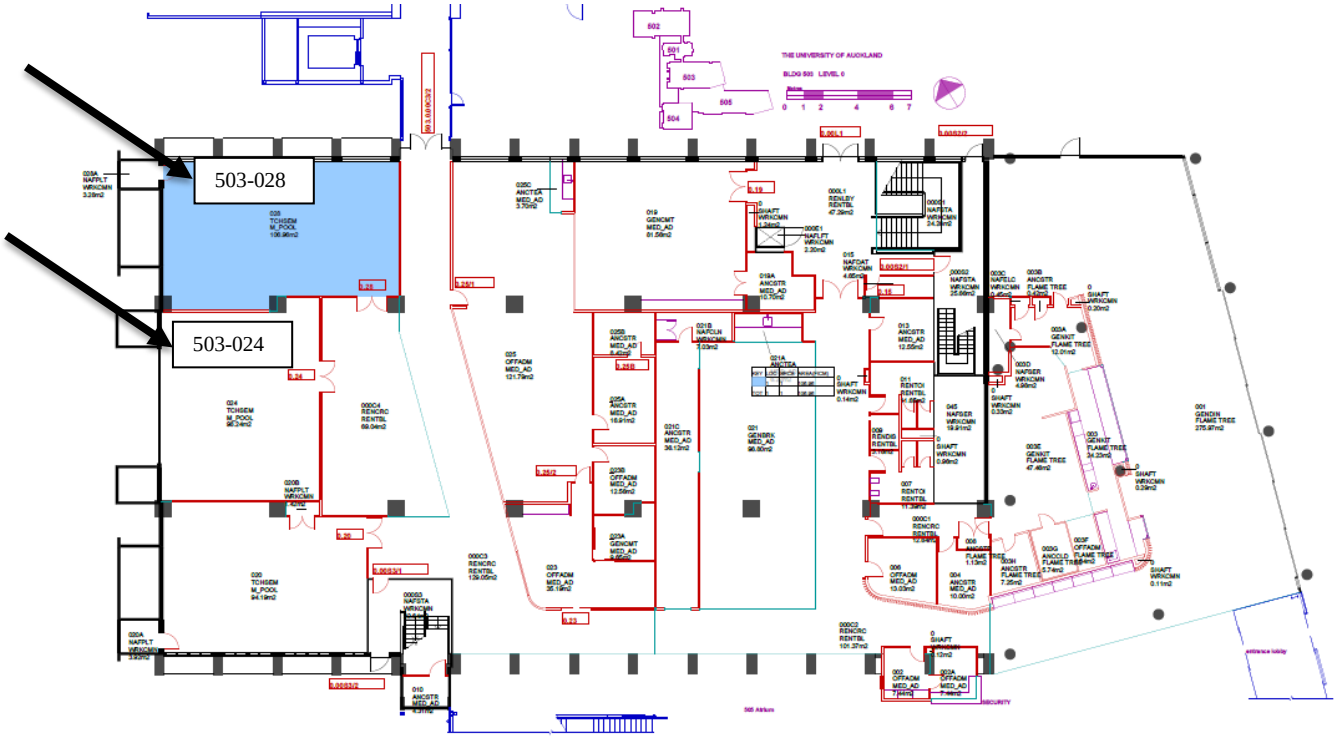
- Venue 1 - Room 503-024
- Venue 2 - Room 503-028

MAP

Location at the Faculty of Medical and Health Sciences



VENUE LOCATION
Venue 1
Room 503-024 and 503-028



VENUE 1: 503 024			
Chairperson: Dr Simon O'Carroll			
10:00am	**Introduction**		
Time	**Name**	**Title**	**Supervisor**
10:10am	Sediqa Amin	Mapping blood brain barrier leakage in Motor Neuron Disease	Emma Scotter
10:30am	Maize Cao	Developing an AAV mediated knockdown of XT-1 and an *in vitro* evaluation of its potential for spinal cord injury	Simon O'Carroll
10:50am	Judith Glasson	Eyes for the Eyes: Hoki fish lens crystallins as ocular therapeutic carriers	Trevor Sherwin Laura Domigan
11:10am	Timothy Ho	Is immune priming a major driver for acute rheumatic fever?	Nikki Moreland
11:30am	Panagiota Kalogirou-Baldwin	How do Melanoma cells cross the blood brain barrier endothelium?	Scott Graham
Lunch 12 noon Manaakitia			
1:00pm	Yu-Jung Lai	Optimisation of a Gene Regulation System for Gene Therapy for Huntington's Disease	Debbie Young
1:20pm	Alexandra McCall	Mesenchymal Stem Cell and Macrophage Crosstalk in Placental Vascular Development	Joanna James
1:40pm	Lisa Mill	Measurable Residual Disease Monitoring in NPM1 Positive Acute Myeloid Leukaemia	Peter Browett Stefan Bohlander Neil Van de Water
Nibbles and Prize giving at 2:30pm (Atrium near entrance of Building 504)			

VENUE 2: 503 028			
Chairperson: Dr Julie Lim			
10:00am	**Introduction**		
Time	**Name**	**Title**	**Supervisor**
10:10am	Shree Senthil Kumar	Do constitutively active melanocortin-4-receptors cause obesity due to impaired intracellular calcium signalling?	Kathy Mountjoy
10:30am	Andrea Soffe	Trigeminal efferent neurons in the hindbrain alar plate in the chicken embryo	Fabiana Kubke
10:50am	Cherry Sun	Are placental macrophages involved in the formation of the first placental blood vessels?	Joanna James
11:10am	Adelie Tan	Characterisation of the neurovascular unit in Huntington's disease using human tissue microarrays	Malvindar Singh-Bains Richard Faull Mike Dragunow
11:30am	Catherine Webb	Insulin receptor expression in the Alzheimer's disease middle temporal gyrus	Maurice Curtis
Lunch 12 noon Manaakitia			
1:00pm	Greta Webb	Human T-cell interactions with melanoma and antigen-presenting cells	Rod Dunbar Daniel Verdon
1:20pm	Petra White	Validation of NODDI-MRI for detection of cortical brain injury following peripheral inflammation in neonatal rats	Justin Dean
1:40pm	Minghan Yong	Expression of Thymidylate Synthase the intracellular target of 5-fluorouracil	Nuala Helsby
Nibbles and Prize giving at 2:30pm (Atrium near entrance of Building 504)			

Venue 503-024

Presented by: Sediqa Amin
Supervisor: Dr. Emma Scotter and co-supervisor Professor Mike Dragunow
Title: Mapping blood brain barrier leakage in Motor Neuron Disease

Abstract
Background: Motor neuron disease is a neurological disorder characterised by motor neuron degeneration in the brain and spinal cord [1]. Disruption of the blood-brain barrier (BBB) causes extravasation of haemoglobin and other blood borne toxins into neuronal tissue, increasing neuronal toxicity and damage [2]. Therefore it is hypothesized that BBB leakage in different regions of brain may play a role in the neurodegeneration and disease pathology.
Objective: To map BBB leakage in different regions of post-mortem ALS brain against pathological TDP-43 spread.
Methods: Immunohistochemistry was performed on 19 brain tissue regions from single control and ALS donors, arrayed as "tissue microarrays", and on motor cortex tissue from 10 ALS and 5 control human post-mortem brains. Markers were chosen to assess neurodegeneration (pTDP-43, NeuN), BBB leakage (haemoglobin), and BBB integrity (PDGFRB, lectin, collagen-IV, claudin-5 and P-gp).
Imaging was performed using a VSlide scanner and Nikon Eclipse microscope. Analysis was performed using MetaMorph software, using custom analysis journals written in-house.
Results and Discussion: We detected reduced vascular coverage by pericytes, reduced tight junction protein claudin-5 and reduced efflux pump P-gp across the brain in ALS, particularly in motor cortex, sensory cortex, cerebellum and upper medulla. We hypothesize that loss of pericyte coverage and claudin 5 may underpin an impairment in BBB integrity causing the extravasation of haemoglobin. In contrast to widespread BBB dysfunction, pathological TDP-43 aggregates were detected almost exclusively in the motor cortex. Compared to the pathological aggregation of TDP-43, BBB dysfunction may be an early feature of ALS.
References
1.Brown, R.H. and A. Al-Chalabi, Amyotrophic Lateral Sclerosis. N Engl J Med, 2017. 377(2): p. 162-172.
2.Garbuzova-Davis, S., et al., Amyotrophic lateral sclerosis: a neurovascular disease. Brain Res, 2011. 1398: p. 113-25.

Presented by: Maize Cao
Supervisor: Dr. Simon O'Carroll and Associate Professor Deborah Young
Title: Developing an AAV mediated knockdown of XT-1 and an *in vitro* evaluation of its potential for spinal cord injury

Abstract
Background: Functional recovery after spinal cord injury (SCI) has limited potential due to complex biochemical processes. Chondroitin sulphate proteoglycans (CSPGs) are molecules upregulated after injury that inhibit axonal growth. By eliminating the glycosaminoglycan (GAG) side chains of CSPGs, the inhibitory effect on axon outgrowth is removed. This project targeted an enzyme involved in GAG synthesis (XT-1) using the AAV vector as a delivery mechanism to see if this could be a viable means of therapy after SCI.
Methods: Artificial miRNA sequences were used for XT-1 knockdown and delivered to rat astrocyte culture using AAV. An *in vitro* model of injury was induced by adding TGF-β1 to the astrocytes, to stimulate CSPG production. Changes in XT-1 expression were assessed using RT-qPCR and changes in GAG biosynthesis were assessed using ELISA. GAGs that were specific to CSPGs (CS-GAG) were quantified using immunocytochemistry.
Results: A significant decrease ($p<0.01$) of XT-1 was observed with XT-1 miRNA compared to control miRNA in basal conditions. However, in TGF-β1 treated cells, XT-1 levels were upregulated and the XT-1 miRNA was found to be less effective, with no difference compared to control miRNA. Changes in GAG or CS-GAG level were unable to be detected with XT-1 miRNA knockdown for both basal and injury conditions.
Discussion: This *in vitro* study did not find correlation of reduced GAG/CS-GAG levels with XT-1 miRNA knockdown. Further investigations should explore the kinetics of the XT-1 enzyme and its regulation factors; as well as assess and fine-tune the *in vitro* environment as a suitable model for SCI.

Presented by: Judith Louise Glasson
Supervisor: Professor Trevor Sherwin and Dr Laura Domigan
Title: Eyes for the Eyes: Hoki fish lens crystallins as ocular therapeutic carriers
Abstract
Background: In the human eye the clarity of the cornea, the front part of the eye, is vital to visual health. The transparent state of the cornea is maintained by specialised stem cells located at the limbus, the region where the cornea meets the whites of the eye. Disease or physical injury can deplete these cells and therapeutic transplantation is required. We have investigated the use of crystallin protein biomaterials as cell carriers for reintroducing cultured stem cells to eye.
Methods: Initial culture experiments assessed the potential toxicity of films to ocular cells over 28 days. Further efficacy as a carrier was investigated using primary corneal epithelial cell lines and limbal tissue explants, combined with LIVE/DEAD™ assays and immunohistochemical cell staining. Cellular gene expression levels were evaluated with droplet digital PCR. The mechanical properties of the films were assessed.
Results: Crystallin biomaterials have proven to be supportive of an adherent and proliferative ocular cell population. The investigated formulations support cell growth, and offer a variety of mechanical properties. Initial testing of larger films show good malleability and texture.
Discussion: The use of Hoki fish eye lens crystallins shows promise as a new therapeutic cell carrier. Biocompatibility and good cell outgrowth, combined with tuneable mechanical properties supports ease of surgical manipulation compared to the current standard carrier of human amniotic membrane. Future work will require additional mechanical testing and investigation of optimal film casting conditions to produce a material fit for purpose.

Presented by: Timothy Ho
Supervisors: Dr Nikki Moreland and Dr Natalie Lorenz
Title: Is immune priming a major driver for acute rheumatic fever?
Abstract
Background: Group A *Streptococcus* (GAS) commonly causes pharyngitis and impetigo, but can also cause serious post-infection syndromes such as acute rheumatic fever (ARF). It has been postulated that repeated GAS infections are required to "prime" the immune system towards autoimmunity, triggering ARF. However, it is unknown if children who develop ARF experience more GAS infections compared to those who do not. The aim of this study is to answer this question by exploiting serum memory and the fact that individuals generate lasting antibody responses against GAS.
Methods: Sera from 12 ARF patients and 12 highly matched healthy controls were obtained from Middlemore Hospital in 2017 as part of the recently completed Rheumatic Fever Risk Factors study (HDEC14/NTA/53/AM02). The serum reactivity profiles were measured in immunoassays against synthetic peptides derived from the hypervariable region (HVR) of the M protein, a major GAS virulence factor. The HVR is encoded by the *emm*-typing region. The synthesised peptides represents 59 GAS *emm-types* and >90% of the circulating GAS strains in New Zealand.
Results: The serum reactivity profiles against the 59-peptide panel were compared between each highly matched case/control pair. Wilcoxon matched pairs test showed a significant increase in the number of HVR reactivities in the ARF cases compared with controls (mean reactivity 12.8 vs 4.9, p=0.001).
Discussion: The significant increase in GAS reactivity observed in ARF sera suggests the number of GAS exposures a child experiences is a risk factor for developing ARF. The has implications for the design of future disease prevention strategies.

Presented by: Panagiota Kalogirou-Baldwin
Supervisor: Dr Scott Graham
Title: How do Melanoma cells cross the blood brain barrier endothelium?
Abstract
Background: New Zealand has one of the highest incidences of melanoma in the world. Most studies estimate that approximately 60-80% of the disease mortality is due to brain metastasis. The goal of this research is to understand the molecular mechanism used by melanoma cells to breach the blood-brain-barrier (BBB) and enter the CNS.
Methods: Electric cell impedance sensing technology was used to determine the effect of New Zealand melanoma cells (NZMs) on the barrier-strength of human microvascular endothelial cells (hCMVECs). Immunocytochemistry was used to visualise key junctional molecules and changes in their localisation and expression after melanoma addition.
Results: The NZMs lines used in this study mediated rapid disruption and opening of the brain endothelial barrier. Melanoma cells rapidly associate to the paracellular junctional space with loss of endothelial expressed CD144, a key adherens junction protein expressed by brain endothelial cells. Antagonism of several melanoma adhesion molecules failed to slow melanoma invasion across brain endothelial cells.
Discussion: NZMs were found to be highly invasive across the BBB endothelial cells. The localisation of the invasive melanoma cells around the junctional space, and rapid changes in barrier integrity following melanoma addition suggests that their invasive nature is heavily dependent on molecular interactions that occur rapidly at the junctional space. The melanoma cells appear to reduce endothelial CD144 expression at the site of invasion. However, the melanoma cells do not abundantly express CD144, which rules out a homomeric interaction facilitating invasiveness. The identity of the adhesion molecules mediating the melanoma invasion are as yet unknown.

Presented by: Amy Lai
Supervisor: Associate Professor Debbie Young
Title: Optimisation of a Gene Regulation System for Gene Therapy for Huntington's Disease
Abstract
Background: Gene therapy is a promising treatment modality but specific targeting and regulation of therapeutic transgene expression in at-risk neurons would improve the safety of gene therapy. In this project, we determine if a weaker human synapsin (hSyn) promoter can drive expression of a regulatory transcription factor in a novel gene regulation system and improve the inducibility of transgene expression in a cell and animal model.
Methods: Transfected HEK293 cells treated with tamoxifen was used as a model of caspase activation, leading to expression of the marker gene, GFP. Immunocytochemistry and immunofluorescence was used to visualise translocation of the regulatory transcription factor. An AAV9 vector containing a calpain-activated cassette was infused into the striatum of transgenic YAC128 Huntington's disease mice and transgene expression was visualised using immunohistochemistry two weeks post-infusion.
Results: In comparison to the strong constitutive CBA promoter, the hSyn promoter drives lower levels of ARF5, and consequently lower levels of GFP in transfected HEK293 cells under basal conditions. Application of tamoxifen at concentrations sufficient to induce caspase-3 activation did not result in any detectable increase in GFP expression. Inducibility of the gene regulation system in GFP expression appears slightly higher in YAC128 mice striatum compared to wild-type mice but ARF5 staining was negligible.
Discussion: The presence of ARF5 is required for transgene expression but whether the cassette is inducible is still unclear. Further studies showing activation of calpain in YAC128 mice is still needed as well as better quantification methods for ARF5 and GFP expression.

Presented by: Alexandra McCall
Supervisor: Dr Joanna James
Title: Mesenchymal Stem Cell and Macrophage Crosstalk in Placental Vascular Development
Abstract
Background: Placentation is fundamental in establishing efficient exchange between mother and fetus. Placental dysfunctional results in pregnancy pathologies such as fetal growth restriction (FGR), where baby is born dangerously small. Placental blood vessel development is essential for optimal exchange, and is impaired in FGR placentae. Both placental-resident macrophages (Hofbauer cells (HBC)) and mesenchymal stem cells (MSCs) have been linked to placental angiogenesis. MSCs reside in close proximity to HBCs during placental development, and may influence HBC function and differentiation via paracrine secretion. However, the mechanisms influencing HBC function in pregnancy are not well understood. This project aims to investigate the influence MSCs have on HBC function in placentation.
Methods: Media conditioned by MSCs (MSCCM) isolated from first trimester, term, and FGR placentae were added to a U937 monocyte cell-line. U937-cells treated with PMA and IL-4 was used as a positive control for M2 macrophage differentiation. Immunohistochemistry was used to identify expression of pan-macrophage (CD68), and M2 macrophage markers (CD163, CD206, CD11b, CD86).
Results: PMA plus IL4 treatment resulted in the expression of CD68, CD86, and CD11b. PMA plus MSCCM resulted in the expression of CD68, CD86, and CD11b. While a proportion of monocytes became activated with CM only, marker expression was not observed in the absence of PMA treatment.
Discussion: These results indicate that MSCCM may not directly activate U937-cells, but may influence differentiation after PMA-activation. Understanding the overall relationship between HBC and MSCs in normal and abnormal placentation will aid the future development of treatments for placental pathologies like FGR.

Presented by: Lisa Mill
Supervisors: Professor Peter Browett, Dr Neil Van de Water and Professor Stefan Bohlander
Title: Measurable Residual Disease Monitoring in NPM1 Positive Acute Myeloid Leukaemia
Abstract
Background: In Acute Myeloid Leukaemia (AML), the level of leukaemic cells remaining in the bone marrow, that is, measurable or Minimal Residual Disease (MRD) is emerging as an important prognostic factor. MRD monitoring post initial induction therapy is important in the assessment of the chemo-sensitivity of leukaemic blasts, and can also be used in detecting impending relapse, allowing for early intervention. NPM1 gene mutations are detected in 30% of all AML cases, and can potentially be used as a leukaemic specific marker for MRD monitoring. The aim of this project was to develop a laboratory based strategy to detect MRD in NPM1 positive AML patients
Methods: NPM1 mutant transcript levels from leukaemic cell lines were analysed by Real Time quantitative PCR (RQ-PCR) assays to compare a mutation specific probe method and a mutation specific primer method. Serial dilutions were performed to assess each method for sensitivity and specificity. Validated assays were piloted in a cohort of NPM1 (type A) positive adult AML patients. MRD data on this cohort of patients was correlated with the NPM1 allelic ratio, and compared to MRD analysis on gDNA using a Next Generation Sequencing (NGS) strategy.
Results: *Sensitivity and Specificity of RQ-PCR assay methods:* The maximal sensitivity reached for the primer specific and probe specific method was 10^{-5} and 10^{-3} respectively. Cross detection of other NPM1 mutations was observed in both primer specific and probe specific assays.
Patient cohort data: The mutation specific primer assay was used to assess x peripheral blood and bone marrow samples from a cohort of y NPM1 mutation positive AML cases. The results of this analysis will be presented including the correlation with clinical features, outcome, NPM1 allele burden and the gDNA MRD analysis.
Discussion: The sensitivity of the primer specific assay makes this method suitable for routine MRD monitoring in the diagnostic settings. The results from this preliminary study will now be validated in a larger, prospective cohort of AML patients.

Venue 503-028

Presented by: Shree Senthil Kumar
Supervisor: Associate Professor Kathy Mountjoy
Title: Do constitutively active melanocortin-4-receptors cause obesity due to impaired intracellular calcium signalling?

Abstract
Background: The melanocortin-4-receptor (MC4R) is critical for energy homeostasis regulation. Human MC4R mutations with impaired signalling cause obesity and paradoxically, cAMP constitutively active (CA) human MC4R mutations also cause obesity. The MC4R signals through multiple signalling pathways, including a less characterised agonist-induced intracellular calcium signalling pathway. The hypothesis is that MC4R calcium signalling is essential to prevent obesity and this calcium mobilisation pathway is impaired in obesity-associated CA mutant MC4Rs. The objective is to develop a quantitative intracellular calcium assay to compare native MC4R and obesity-associated mutant MC4Rs.
Methods: Using Fura-2/AM, a calcium assay was developed and optimised for experimental parameters with agonist stimulation of endogenous muscarinic acetylcholine receptor present in HEK293 cells. The assay was then performed using α-melanocyte stimulating hormone (α-MSH) as an agonist to stimulate transiently transfected MC4Rs in HEK293 cells.
Results: Parameters optimised were time (60minutes - dye uptake; 80minutes **-** ester cleavage), temperature (room temperature **-** dye uptake; 37^0C **-** ester cleavage) and buffer (DMEM **-** dye uptake; high glucose calcium loading buffer **-** ester cleavage). Pluronic acid and probenecid enhanced the cellular uptake and retention of the dye. α-MSH dose-dependently increased calcium for native and CA mutant MC4Rs similarly.
Discussion: A rapid, sensitive and reliable assay to quantitate agonist-induced intracellular calcium in adherent cultured cells was established. Contrary to the hypothesis, obesogenic CA mutant MC4Rs mobilise intracellular calcium similar to native MC4R. Understanding the physiological relevance of the calcium signalling cascade in both native and mutant MC4Rs is important for advancing the understanding of MC4R physiology and obesity.

Presented by: Andrea Soffe
Supervisor: Dr Fabiana Kubke
Title: Trigeminal efferent neurons in the hindbrain alar plate in the chicken embryo

Abstract
Background: In the developing nervous system, it has been historically assumed that all efferent neurons are born in a part of the neural tube called the basal plate. However, previous research has suggested that efferent neurons of the vestibulocochlear (VIIIth cranial) nerve (Kubke, Gilland, Baker, & Carr, 1999) and the trigeminal (Vth cranial) nerve (Kubke, unpublished observations) are present in the neuroepithelium of a different region: the alar plate. The aim of my project was to investigate the presence and location of this group of efferent neurons which may be born in the hindbrain alar plate, in the trigeminal system.
Methods: The trigeminal nerve ganglia of twenty fixed chicken embryos were injected with fluorescent molecule DiI, with the left ganglion subsequently removed as a control. After overnight incubation (to allow dye transport along the neurons), the hindbrain was isolated, cleared in glycerol, and analysed using a fluorescence microscope.
Results: In 90% of embryos, labelled cell bodies (presumed to be efferent trigeminal neurons) were present in the hindbrain alar plate.
Discussion: My findings provide support for the presence of a group of trigeminal efferent neurons in the hindbrain alar plate, which have not previously been reported. In wholemount embryos, it cannot be determined whether these neurons are born in the alar plate, or have migrated there. However, my observations in wholemount embryos, combined with cross-sectional data (Kubke, Gilland, Baker, & Carr, 1999; Kubke, unpublished observations), suggest that some efferent neurons may originate in the hindbrain alar plate.

Presented by: Cherry Sun
Supervisor: Dr Joanna James
Title: Are placental macrophages involved in the formation of the first placental blood vessels?
Abstract
Background: The first fetal blood vessels within the placental villus mesenchyme are derived from local differentiation of mesenchymal stem cells into hemangiogenic progenitor cells. The early appearance of abundant fetal macrophages (Hofbauer cells) during placental vasculogenesis and angiogenesis suggests a possible paracrine involvement of Hofbauer cells in these processes. This study aims to 1) quantify the spatial relationship between vascular structures and macrophages in the first trimester placental villous core and 2) examine the possible paracrine effects of Hofbauer cells on the angiogenic properties of mesenchymal stem cells in the placenta.
Methods: Sections of paraffin-embedded first trimester placenta were immunohistochemically stained for CD68 and CD31 to co-localise macrophages and vascular endothelial cells respectively. U937 monocytes were differentiated to M2-polarised macrophages using PMA and IL-4, and co-cultured with a placental mesenchymal stem cell tube formation assay, which was visualised by time-lapse microscopy to quantify differences in branching points and branch length.
Results: The majority of macrophages in the placental villi are closely associated with vascular structures in the first trimester. The proximity of macrophages to placental blood vessels does not appear to change between 6 and 12 weeks of gestation. U937 cells treated with PMA and IL-4 became plastic-adherent, and upregulated the expression of pan-macrophage (CD68), and M2-macrophage (CD11b, CD163, CD206) markers, indicating their suitability for use in angiogenesis assays. Time-lapse microscopy data are pending.
Discussion: The intimate spatial relationship between Hofbauer cells and vascular structures suggests a paracrine relationship between these cell types in the first trimester placenta.

Presented by: Adelie Yii Shin Tan
Supervisor: Dr Malvindar Singh-Bains, Distinguished Professor Sir Richard Faull and
Professor Mike Dragunow
Title: Characterisation of the neurovascular unit in Huntington's disease using human tissue microarrays
Abstract
Background: Huntington's disease (HD) is a genetic neurodegenerative disease associated with a polyglutamine (CAG repeat) expansion in the *huntingtin* gene. However, the pathological process underpinning disease progression still remains to be elucidated. This study aims to characterise the contribution of non-neuronal cells within the neurovascular unit (NVU) to HD neuropathology using human brain tissue microarrays (TMAs).
Methods: Immunohistochemistry was performed to detect pyramidal cells (SMI-32), mutant *huntingtin* (mHTT) aggregates (1C2,1F8), glial cells (GFAP, Iba-1, HLA-DR), perivascular cells (PDGFRβ, αSMA), and endothelial cells (CD31, Lectin) in two TMAs; a middle temporal gyrus (MTG) TMA (n=28 control vs HD) and a multiregional TMA (n=7 control vs HD). The immunolabeled TMAs were imaged using the automated V-slide scanner and analysed using Metamorph software.
Results: SMI-32+ neuronal density significantly decreased, while IC2+ mHTT aggregate count significantly increased in the MTG compared to controls. Significant relationships were found between 1C2+ mHTT aggregates and age of disease onset, and CAG repeat length. Further investigations into non-neuronal cells revealed subtle changes in the expression patterns and/or number of astrocytes, microglia, and endothelial cells in the MTG. Furthermore, certain astrocytic, microglial, and vascular changes were associated with aggregate number and expression.
Discussion: NVU and neuropathological changes within the MTG TMA implicates the involvement of this brain region in HD. The loss of pyramidal neurons and increased mHTT aggregates in the TMAs recapitulate the changes demonstrated by previous studies utilising copious tissue quantities, reinforcing the TMA platform as a screening platform to study novel molecular changes in HD.

Presented by: Catherine Webb
Supervisor: Associate Professor Maurice Curtis
Title: Insulin receptor expression in the Alzheimer's disease middle temporal gyrus.
Abstract
Background: Insulin resistance is a risk factor and common characteristic of Alzheimer's disease (AD). Tissue homogenate studies show reduced insulin receptor (IR) expression in AD brains, however, such studies lose cellular context. Two splice variants of the IR are differentially expressed; IR-B has been observed in glia, while IR-A is considered the neuronal isoform. We aimed to investigate the *in situ* cell-specific expression of these isoforms in human brain tissue, and how this changes in AD.
Methods: We probed for these splice variants using *in situ* hybridisation on a tissue microarray of normal (n=18) and AD (n=18) middle temporal gyrus cores. This staining was combined with immunohistochemistry to visualise overlap between IR mRNA and the neuronal protein NeuN. We quantified neuron number and total cell number in each core together with the overall and neuron-specific load of IR mRNA.
Results: Overall expression of both isoforms was decreased in AD. However, the mRNA load in each neuron and the proportion of mRNA within neurons was unchanged for both isoforms. While the number of neurons was decreased in AD, total cell number was unchanged.
Discussion: Our findings show reduced IR expression in AD cortex, as literature suggests. However, this can be attributed to a loss of neurons rather than reduced cellular mRNA expression. Therefore, other factors than reduced neuronal IR mRNA expression may cause insulin resistance. Since raising brain insulin levels improves the memory function of early AD patients, understanding insulin resistance in AD is an important foundation for therapeutic development.

Presented by: Greta Webb
Supervisors: Professor Rod Dunbar and Dr Daniel Verdon
Title: Human T-cell interactions with melanoma and antigen-presenting cells
Abstract
Background: Interactions between immune and cancer cells have been of huge scientific interest over the last few decades, due to the potential for the development of cancer immunotherapies from this knowledge. Metastatic melanoma is a cancer that has shown dramatic responses to immunotherapy, but a deeper understanding is needed of the interactions between T-cells and antigen presenting cells in the context of melanoma, and how immune responses to melanoma arise. Numerous immunotherapy trials have shown evidence of antigen spreading, in which effector T-cell responses arise specific to tumour antigens that were not initially targeted by the vaccine or cell therapy. This shows the potential for one T-cell response to effectively lead to another as tumour cells are killed.
Methods: Experiments featured antigen and HLA-mismatched co-culture of melanoma cell lines with T-cell clones and monocyte-derived dendritic cells in order to measure the effects of soluble factors secreted by T-cell clones, T-cell activation in response to antigen presentation, and antigen uptake by APCs. Findings were investigated by flow cytometry.
Results: HLA-DR upregulation by melanoma cells was observed in response to the presence of activated T-cells, which may sensitise these cells to killing by CD4+ T-cells. Uptake and presentation of killed melanoma antigens to T-cell clones by Monocyte-derived dendritic cells was explored, as were the effects of plasmin on uptake and presentation.
Discussion: Relevance to current available immunotherapies will be discussed, as well as the preparation of cancer vaccines and how this ties in to the ability of dendritic cells to take up antigen and prime T-cell responses.

Presented by: Petra White
Supervisor: Dr Justin Dean
Title: Validation of NODDI-MRI for detection of cortical brain injury following peripheral inflammation in neonatal rats

Abstract
Background: Preterm infants have high rates of neurodevelopmental disabilities associated with microstructural MRI changes, however their exact relationship remains unclear. The Neurite Orientation Dispersion and Distribution Index (NODDI) is a novel diffusion MRI technique, proposed to measure neuronal branching. This study aims to validate the relationship between MRI-NODDI parameters and neuronal dendritogenesis during development of the cerebral cortex, and examine cortical changes in a preterm-equivalent rat model of inflammatory brain injury.
Methods: *Experiment 1:* Sprague-Dawley rat pups collected at postnatal day (PND) 1, 14, and 21, and brain tissues impregnated with Golgi solution or fixed for *ex-vivo* MRI-NODDI analysis (9.4T). *Experiment 2:* Rats received daily intraperitoneal lipopolysaccharide (LPS; 0.3mg/kg) on PND1–3, with recovery until collection at PND21. In Golgi tissues, the complexity of pyramidal neurons in the somatosensory and motor cortices were assessed (Neurolucida). For MRI, the changes in fractional anisotropy (FA), orientation dispersion index (ODI), intracellular volume fraction, and isotropic volume fraction were calculated.
Results: LPS was associated with a significant reduction in cortical volume at PND21, without evidence of cell death or loss of neurons. Golgi analysis showed a significant increase in dendritic parameters from PND1 to PND21 with NODDI parameters only reflecting dendritic development to PND14. LPS exposure was associated with a significant reduction in complexity of the motor cortex at PND21. NODDI analysis showed a significant decrease in cortical ODI, and increase in FA, at PND21.
Discussion: Validation of NODDI may provide a novel technique for assessing cortical pathology in preterm-born infants.

Presented by: Minghan Yong
Supervisor: Associate Professor Nuala Helsby
Title: Expression of Thymidylate Synthase the intracellular target of 5-fluorouracil

Abstract
Background: Inhibition of thymidylate synthase (TS) by 5-fluorouracil is key for its action as a cytotoxic drug. Genetic polymorphism alters transcription. Both mRNA expression in the tumour and germline genotype associate with treatment outcomes. In contrast, immunohistochemistry has weak association with outcomes. TS can also auto-regulate its own translation and protein phosphorylation has been reported to affect translation and catalytic activity [1,2]. The aim was to assess the profile of immunoreactive TS in various cells using Western-blotting to characterise variation in TS protein expression and assess whether post-translational modifications can be observed.
Methods: TS protein expression was determined in pooled PBMC, human liver samples (n=19) and tumour cell lines by SDS-PAGE-immunoblotting with anti-TS mAb. Human recombinant protein (rTS) was used as a reference standard. *TYMS* genotype was determined using RFLP-PCR [3].
Results: Variation in immunoreactive TS protein was observed across liver donors. However, multiple immunoreactive bands were detected, with the major band ~3kDa higher than reference rTS. Neoplastic cells expressed a different profile of TS bands. Compared with proliferating cells, differentiated CaCo-2 cells formed an apparent TS dimer, which was resistant to denaturation. Initial studies suggest phosphorylation of rTS retards electrophoretic migration.
Discussion: Different patterns of immunoreactive TS products between different cell types suggest post-translational modifications may complicate correlations between *TYMS* and the protein.
1. Chen et al (2017) Journal of Biological Chemistry, 292: 13449–13458
2. Ludwiczak et al (2016) Molecular BioSystems, 12: 1333–1341
3. Mandola et al (2003) Cancer Research, 63: 2898–2904.