MONASH University

Medicine, Nursing and Health Sciences

Central Clinical School

Honours 2015
Final Oral review seminar program
BMS(Hons) & BSc(Hons)

Honours BMS & BSc Final Oral Seminar Program 2015
Day 1
Date: Monday 26 October
Time: 10am – 3.35pm
Venue: Lecture theatre, Level 5, Alfred Centre

Monday 26 October		
Opening and welcome by Chair: Prof Magdalena Plebanski		10am
Speaker	Miss Suelyn Van Den Helm	10.05am
Project title	***Targeting Apoptotic and Metabolic Pathways in Acute Myeloid Leukaemia***	
Supervisor(s)	Dr Mark Gutheridge, Dr Giovanni Monaco	
Department	ACBD	
Question and answer 10.20am		
Speaker	Miss Jacqueline Boyle	10.25am
Project title	***CRISPR-Cas9 mediated mutagenesis to study the JAK2^{V617F} mutation in Myeloproliferative Neoplasms***	
Supervisor(s)	A/Prof David Curtis, Dr Stefan Sonderegger	
Department	ACBD	
Question and answer 10.40am		
Speaker	Miss Tayla Davidson	10.45 am
Project title	***HDAC1 Dysregulation in Multiple Myeloma***	
Supervisor(s)	Prof Andrew Spencer	
Department	ACBD	
Question and answer 11:00am		
Morning tea 11:05am		
Speaker	Mr William McInnes	11.20am
Project title	***Studying the role of Snai1 over expression and the importance of the SNAG domain in hematopoiesis***	
Supervisor(s)	A/Prof Jody Haigh, Dr Catherine Carmichael	
Department	ACBD	
Question and answer 11.35am		
Speaker	Mr Andrej Terzic	11.40am
Project title	***Investigating Synergy Between a PRMT5 Small Molecule Inhibitor and Other Compounds in Acute Myeloid Leukaemia***	
Supervisor(s)	A/Prof David Curtis, Dr Emma Toulmin	
Department	ACBD	
Question and answer 11:55am		
Lunch 12.00pm		
Speaker	Mr Paul Gill	1:00pm
Project title	***Investigating immune effects of circulating short chain fatty acids in healthy humans***	
Supervisor(s)	Dr Jane Muir, A/Prof Rosemary Ffrench	
Department	Gastroenterology	
Question and answer 1:15pm		
Speaker	Miss Marina Yousef	1:20pm
Project title	***Identification of the cell of origin of Grhl3-deficient skin squamous cell carcinoma***	
Supervisor(s)	Dr Charbel Darido	
Department	Medicine	
Question and answer 1:35pm		

Speaker	Miss Alicia Ware	1.40pm
Project title	***Assessing the effects of adjuvants on immune responses to an EBA175 malaria vaccine***	
Supervisor(s)	Dr Jack Richards, Dr Raffi Gugasyan	
Department	Burnet Institute	
Question and answer 1.55pm		
Speaker	Miss Angela Nguyen	2:00pm
Project title	***Characterisation of Mr1 on B Cells and the effect of Baff On Mait Cells In Mice***	
Supervisor(s)	Prof Fabienne Mackay, Dr Dan Andrews	
Department	Immunology	
Question and answer 2:15pm		
Afternoon tea 2:20pm		
Chair: A/Prof Mark Wright		
Speaker	Miss Rachael Lim	2:35pm
Project title	***Investigation of a Potential Long Non-Coding RNA, AI447881, and Differential Gene Expression Analysis of Follicular and Marginal Zone B cells***	
Supervisor(s)	Prof Fabienne Mackay, Dr William Figgett	
Department	Immunology	
Question and answer 2:50pm		
Speaker	Ms Ellen Mcallister	2.55pm
Project title	***The arginine methyltransferase, Prmt5, is essential for B cell development in vivo***	
Supervisor(s)	Prof Fabienne Mackay, Dr Stefan Sonderegger	
Department	Immunology	
Question and answer 3:10pm		
Speaker	Mr Tian Zhao	3.15pm
Project title	***Acetylcholine Modulation of Cerebellar Function in Spinocerebellar Ataxia Type 1 (SCA1) Mice***	
Supervisor(s)	Prof Elsdon Storey	
Department	Medicine	
Question and answer 3.30pm		
Program Ends 3.35pm		

ABSTRACTS

Speaker	Miss Suelyn Van Den Helm	10.05am
Project title	***Targeting Apoptotic and Metabolic Pathways in Acute Myeloid Leukaemia***	
Supervisor(s)	Dr Mark Gutheridge, Dr Giovanni Monaco	
Department	ACBD	

Acute myeloid leukaemia (AML) is a disease which results from the overproduction of immature white blood cells in the bone marrow. Prognosis remains dismal, with the overall survival of AML patients being <30%. Thus, there is an urgent need for the development of new and effective targeted therapies. Deregulation of metabolic pathways is a classical hallmark of cancer and therapeutic approaches targeting oxidative phosphorylation and/or glycolysis may have clinical benefit in AML patients.

Our laboratory has previously shown that BCL-2 inhibition by ABT-199, down-regulates oxidative phosphorylation leading to impaired ATP production. We examined the possibility that ABT-199 may induce the production of reactive oxygen species (ROS) subsequently triggering cell death. However, ROS scavengers did not impair the ability of ABT-199 to induce apoptosis and we saw no evidence that ABT-199 was able to induce ROS. Furthermore, not only ABT-199, but metformin was also able to down-regulate oxidative phosphorylation in AML cells. Additionally, phosphatidylinositol 3-kinase (PI3K) inhibitor, BEZ235, down-regulated glycolysis in AML cells. Thus, combining metformin or ABT-199 with BEZ235 was examined for their ability to induce a metabolic crisis and apoptosis. Metformin and BEZ235 did not significantly increase AML cell death compared to single agents alone, whereas ABT-199 and BEZ235 synergised to induce cell death in AML cell lines and primary human AML patient samples. Treatment of primary human AML xenograft mice suggested that the combination of ABT-199 and BEZ235 reduced leukaemia burden although statistical significance was not obtained due to limited mouse numbers in the combination arm.

These studies highlight the potential for targeting metabolic vulnerabilities in AML cells. Importantly, combined ABT-199/BEZ235 treatment in mice was not associated with overt toxicity. As agents targeting BCL-2 and PI3K are already in clinical development, such approaches are likely to have favourable toxicity profiles and therapeutic windows and could be fast-tracked toward future clinical trials.

Speaker	Miss Jacqueline Boyle	10.25am
Project title	***CRISPR-Cas9 mediated mutagenesis to study the JAK2^{V617F} mutation in Myeloproliferative Neoplasms***	
Supervisor(s)	A/Prof David Curtis, Stefan Sonderegger	
Department	ACBD	

The JAK2V617F activating mutation in the pseudokinase domain of janus kinase 2 (JAK2) is a characteristic feature of Myeloproliferative Neoplasms (MPNs). The mutation constitutively activates JAK2 signalling pathways, conferring cytokine independence in hematopoietic stem cells (HSCs). Protein arginine methyltransferase 5 (PRMT5) may offer a potential therapeutic window in treating MPNs, owing to its specificity for JAK2V617F. To assess this we proposed the development of an mBa/F3EpoR cell line that endogenously expresses JAK2V617F.

Here, we sought to use CRISPR-Cas9 technology to induce a single base G>T substitution in an erythropoietin (Epo) dependent mBa/F3EpoR cell line to generate the JAK2V617F mutation.
The CRISPR-Cas9 system was validated in our cell line by inducing expression of known sgRNAs targeting mTrp53. Here we demonstrated effective induction of frame shift mutations and depletion of mTrp53 protein expression. Functional validation of our mutant clones through treatment with the PRMT5 inhibitor (CTX034) demonstrated that depletion of mTrp53 correlated to increased survival when challenged with the inhibitor. These findings demonstrated that the CRISPR-Cas9 system was an appropriate tool for the generation of mutations in our cell line.

To generate the desired mJak2V617F mutation, we induced expression of our mJak2 sgRNA in the presence of a homologous donor template harbouring the mutation. Cell lines were deprived of Epo to select for mutants with a survival advantage, namely the mJak2V617F mutation. Proliferation of cytokine independent cells was observed in cell lines expressing the sgRNA irrespective of the presence of a donor template. This may indicate successful generation of the Jak2V617F mutation and other pro survival mutations.

The significance of this research is that by utilising CRISPR, we may generate a cell line that endogenously expresses the JAK2V617F mutation, providing a platform for further research into MPNs.

Ongoing research will determine the mJak2 status in our cell line, and characterise the nature of mutations generated.

Speaker	Miss Tayla Davidson	10.45 am
Project title	***HDAC1 Dysregulation in Multiple Myeloma***	
Supervisor(s)	Prof Andrew Spencer	
Department	ACBD	

Multiple myeloma (MM) is a haematological malignancy affecting plasma cells, which remains incurable despite the introduction of new therapies. Epigenetic modifications, affecting gene expression but not the DNA sequence itself have been associated with adverse outcomes in many cancers where they are often aberrantly expressed. In MM, it has been found that histone deacetylases (HDACS), a group of epigenetic enzymes that remove acetyl groups from histones leading to gene silencing, are overexpressed in human myeloma cell lines (HMCL) and in primary myeloma cells. High levels of HDAC1 expression are associated with poor prognosis in MM patients, with shorter progression free and significantly shorter overall survival compared to patients with low levels of expression. The silencing of HDAC1 is thought to affect the transcription of genes involved in proliferation and apoptosis. Therefore, this project aimed to utilise short interfering RNA (siRNA) to downregulate HDAC1 in HMCL and study its effects on cell phenotype and drug sensitivity in comparison to cells with ongoing HDAC1 dysregulation. Down regulation of HDAC1 in the HMCL OCI decreased cellular proliferation. In HMCL, the pan-HDAC inhibitor LBH589 (1-10nM) reduced cell viability and increased histone acetylation, indicating active gene transcription. HDAC1 silencing increased the sensitivity of OCI to the proteasome inhibitor bortezomib (velcade), LBH589 and the combination of LBH589 and proteasome inhibitor carfilzomib. In conclusion, HDAC1 may play a role in the proliferation of myeloma cells and its downregulation may be implicated in increased drug sensitivity. Further studies will involve the use of a lentiviral Tet-One inducible expression system to knockdown HDAC1 expression, in order to further characterise the role of HDAC1 in MM.

Speaker	Mr William McInnes	11.20am
Project title	***Studying the role of Snai1 over expression and the importance of the SNAG domain in hematopoiesis***	
Supervisor(s)	A/Prof Jody Haigh, Dr Catherine Carmichael	
Department	ACBD	

The EMT modulator Snai1 has not been well characterised in non-epithelial cells to date, however past evidence from the Haigh lab has implicated Snai1 in hematopoiesis using retroviral and transgenic over-expression models. The following phenotypes were observed; increased hematopoietic stem cells and granulocyte/monocyte progenitors, decreased platelets, B and T lymphocytes, and a complete loss of erythrocytes. Results mirroring the combined knock-out phenotypes of two well known hematopoietic genes Gfi-1 and Gfi-1b near exactly. Interestingly the two share a conserved SNAG domain with Snai1, the domain responsible for binding of multiple factors including the epigenetic factor LSD1. Lysine specific demethylase 1 (LSD1) binds through the SNAG domain to give proteins functionality, and is also known to separately play a role in hematopoiesis.

The hypothesis is that over-expressing Snai1 is causing a decrease of LSD1 in the cell, through SNAG domain binding; impairing Gfi-1/1b function. The experiment investigated this by virally over-expressing Snai1 in murine hematopoietic progenitor cells, along with a mutant 'mut5' incapable of binding LSD1 due to a SNAG domain mutation. Results showed that the Snai1 over-expression had increased macrophages and decreased erythroid cells and granulocytes, while mut5 was phenotypically normal. Further to this when LSD1 was inhibited in Snai1 over-expressing cells there was alleviation of the granulocyte and macrophage phenotypes, but not of the erythroid. Meaning there was not complete alleviation of the phenotype, implying either there are other factors at play or when Snai1 recruits LSD1 it stores it and doesn't subsequently activate genes. This however requires further proteomic analysis such as Co-IP or ChIP Seq, to be done in the future. Overall the study indicated that the mut5 mutation alleviated the Snai1 phenotype, and that LSD1 binding was in part responsible the phenotype when Snai1 is over-expressed. Better knowledge of hematopoietic development will help improve current treatments for when this development goes awry.

Speaker	Mr Andrej Terzic	11.40am
Project title	***Investigating Synergy Between a PRMT5 Small Molecule Inhibitor and Other Compounds in Acute Myeloid Leukaemia***	
Supervisor(s)	A/Prof David Curtis, Dr. Emma Toulmin	
Department	ACBD	

Protein Arginine Methyltransferase 5 (PRMT5) is known to be able to methylate histone and non-histone proteins that play a role in many cellular pathways. Overexpression of PRMT5 is thought to be associated with many solid and haematological cancers, including Acute Myeloid Leukaemia (AML). AML is a haematological malignancy that results from the overproduction of immature myeloid cells (or blasts) within the bone marrow. Current treatments for AML have remained fairly consistent for over 30 years and involves treatment with chemotherapeutics. Treatment failure, mortality rates and relapse rates remain remarkably high, stressing the need for novel and more targeted therapies. A small molecule inhibitor of PRMT5 (known as 034) could present as a potential candidate for a novel treatment.

Utilising two leukaemic cell lines (FDM and MLL-AF9 cells), we aimed to assess the anticancer effects of 034 as a single agents, as well as elucidating potential underlying mechanisms in vitro. Furthermore, we also aimed to validate whether 034 is synergistic in combination with other inhibitors.

PRMT5 inhibition (via 034) was found to induce the p53 pathway and cause an increase in the half-life of p53. The potency of 034 was not optimal as a single agent, so therefore its clinical potential in combination trials was assessed. Synergy was not observed when 034 was combined with an MDM2 inhibitor (Nutlin-3a), drugs that disrupt the ribosomal biogenesis pathway (CX-5461 and BEZ235) and standard chemotherapeutics (Daunorubicin and Cytarabine). Synergy was observed when 034 was used in combination treatment with an inhibitor of BCL-2 (ABT-199). The synergy that was observed may pave the way for future treatment options in AML, as the standard therapies currently used in the treatment of AML present with many downfalls.

Speaker	Mr Paul Gill	1:00pm
Project title	***Investigating immune effects of circulating short chain fatty acids in healthy humans***	
Supervisor(s)	Dr Jane Muir, A/Prof Rosemary Ffrench	
Department	Gastroenterology	

Short chain fatty acids (SCFAs) are metabolites produced in the colon during bacterial fermentation of dietary fibre. SCFAs promote gut health and also pass into the circulation, especially acetate. Murine studies have demonstrated that increasing circulating acetate via consumption of a high fibre diet and oral consumption of acetic acid leads to an increase in Foxp3+ T regulatory cells (Treg). These cells promote anti-inflammatory effects in murine models of inflammatory bowel disease and asthma. Therefore, investigation of immune effects of SCFAs in humans is needed to determine if these findings could translate to a clinical setting.

We conducted a single blinded, randomized, controlled cross-over dietary intervention study in a small group of healthy volunteers (n=10). Each participant underwent a vinegar drink dietary period, followed by a high and/or low fibre diet in random order. Each dietary period was 5 days in duration. Blood samples were taken on the 5th day of each dietary period to assess changes to plasma acetate, Tregs and cytokines.

We found that vinegar consumption, low fibre diet (LFD) and high fibre diet (HFD) did not significantly change plasma acetate levels measured by gas chromatography- mass spectroscopy (GCMS). Flow cytometric analysis of Tregs showed that consumption of the LFD was associated with a significant decrease in the proportion of CD4CD25Foxp3+ T regulatory cells. Subsequent analysis of Foxp3 expression on CD4CD25+ lymphocytes showed that HFD was associated with a decline in Foxp3 expression compared to baseline. No significant changes to cytokines levels were observed after consumption of vinegar, HFD or LFD.

Taken together, these results highlight associations between dietary fibre, vinegar and the peripheral immune system that could be investigated further in a larger study. Continuation of these studies will help to develop a study protocol to investigate immune effects of SCFAs in patients with inflammatory conditions.

Speaker	Miss Marina Yousef	1:20pm
Project title	***Identification of the cell of origin of Grhl3-deficient skin squamous cell carcinoma***	
Supervisor(s)	Dr Charbel Darido	
Department	Medicine	

Cancer-initiating cells (CICs) are commonly thought to arise from stem cells (SC) or early progenitor cell populations, rather than differentiated progeny. Our lab has recently identified the Grainyhead-like 3 (GRHL3) transcription factor (TF) as a potent tumour suppressor in squamous cell carcinoma (SCC), although its cell of origin remains unknown. Since Grhl3 is highly expressed in suprabasal cells of the epidermis, we hypothesised that these differentiated cells could expand in the absence of Grhl3, facilitating the accumulation of mutations, leading to neoplastic transformation. To address this hypothesis, we first interrogated markers of self-renewal in SCCs obtained from previous experiments using K14 conditional-knockout (cKO) mice, which has Grhl3 deletion in the whole epidermis. Although proliferation was increased, self-renewal factors were not involved in cKO SCC progression. We then elected to delete Grhl3 in suprabasal cells using a 4-hydroxytamoxifen (4-OHT)-inducible cre-recombinase expressed under the involucrin promoter (IVLCre+). These mice show long-lived cells through maintenance of Grhl3-deletion well-beyond the 4-week turnover point of the epidermis, correlated to loss of the Grhl3 target gene, Pten. Using the DMBA/TPA carcinogenesis protocol (currently at week 27), we found that Grhl3/IVLCre+ mice were more susceptible to SCC-development and, moreover, resultant tumours had Grhl3-deletion, indicating that Grhl3-loss induces CIC to drive tumour development. Furthermore, our data are confirmed in the human skin SCC line, SCC13, by excluding a role for self-renewal factors, and showing activation of the PI3K/mTOR signalling pathway as a result of loss of the Grhl3 target gene, PTEN. Taken together, our results suggest that Grhl3-deficiency increases the longevity of differentiated cells, exposing them to oncogenic mutations, thereby providing insights into SCC origin. Findings are expected to pave the way for developing targeted therapies in Grhl3-deficient skin SCC, potentially translating to other epidermal SCCs, including the head and neck, SCCs associated with poor survival outcomes.

Speaker	Miss Alicia Ware	1.40pm
Project title	***Assessing the effects of adjuvants on immune responses to an EBA175 malaria vaccine***	
Supervisor(s)	Prof Jack Richards, Dr Raffi Gugasyan, Dr Paul Ramsland	
Department	Burnet Institute	

Malaria can be caused by infection with several species of the Plasmodium parasites, but Plasmodium falciparum causes greatest morbidity and mortality globally. Significant advances have been made recently with regards to improved prevention, diagnosis and treatment [1], including the approval of the first ever malaria vaccine, a pre-erythrocytic vaccine called RTS,S [2]. Unfortunately, phase III trials of RTS,S indicate that this vaccine only affords 35-55% protection, and that such responses are short-lived [3].

This study sought to compare vaccine-induced responses to a promising blood-stage vaccine candidate, called erythrocyte-binding antigen 175: Region II (EBA-175 RII). We assessed the vaccine-induced responses to this vaccine when formulated with different four adjuvants currently licensed for human use.

C57BL/6 mice were immunised at four-week intervals with three doses of vaccine containing recombinant EBA-175 RII in combination with the adjuvants Alhydrogel (aluminium hydroxide), Montanide® ISA720, AddaVax™ and glucopyranosyl lipid A-stable emulsion (GLA-SE), or PBS-control. Draining lymph node and splenic tissue samples were collected two weeks after the final immunisation and analysed to determine counts of mature recirculating, germinal centre and plasma B cells, CD4+ and follicular helper T cells, and secretion of IFN-γ, IL-4 and IL-17A. Antibody responses were also assessed by determining titres of total IgG and IgG subclass in serum samples and whether these were capable of inhibiting binding of native EBA-175 to human erythrocytes.
Enhanced IgG production and class-switched subclass responses were observed for all adjuvant groups except Alum; these responses were capable of inhibiting binding of EBA-175 to the erythrocyte. Immunisation with Montanide induced high titres of all IgG subclasses, whilst GLA-SE demonstrated IgG subclass and CD4+ T cell responses highly characteristic of a TH1-polarised responses. Comparisons of other currently available adjuvants and other antigens are required to advance malaria vaccine development as quickly as possible.

1. Cotter C, Sturrock HJW, Hsiang MS, Liu J, Phillips AA, Hwang J, et al. The changing epidemiology of malaria elimination: new strategies for new challenges. Lancet. 2013;382(9895):900-11.
2. First malaria vaccine receives positive scientific opinion from EMA [press release]. London, United Kingdom: European Medicines Agency, 24 July 2015.
3. RTS SCTP. Efficacy and safety of the RTS,S/AS01 malaria vaccine during 18 months after vaccination: a phase 3 randomized, controlled trial in children and young infants at 11 African sites. PLoS Med. 2014;11(7):e1001685.

Speaker	Miss Angela Nguyen	2:00pm
Project title	***Characterisation of Mr1 on B Cells and the effect of Baff On Mait Cells In Mice***	
Supervisor(s)	Prof Fabienne Mackay, Dr Dan Andrews	
Department	Immunology	

Mucosal-associated invariant T (MAIT) cells have recently emerged as a new innate-like subset of T cells responsible for mucosal immunity and defence against bacteria. In recent years, it has been shown that MAIT cells are able to recognise vitamin B metabolites, a microbial by-product of the riboflavin synthesis pathway common to most bacterial species. MAIT cells are able to recognise these antigens in the context of the major histocompatibility class-I related protein (MR1). Here I show that MR1 proteins are expressed intra-cellularly in all cells of the spleen, lymph nodes, blood and peritoneal lavage. To determine whether this ubiquitous intracellular expression also correlated to cell surface expression on B cells, flow cytometric analysis was performed on cells stained with anti-MR1 antibody. It was found that MR1 expression was not significantly detected at the cell surface. Subsequently, we attempted to increase surface MR1 expression through culturing of a newly discovered MR1 ligand, acetyl-6-formylpterin (Ac-6-FP), however no significant upregulation of MR1 from basal levels was detected.

As recent data has suggested a requirement of B cells on MAIT cell development, I sought to determine whether the B cell maturation and survival factor, BAFF, caused an indirect change to MAIT cell frequencies. My data suggested that BAFF-dependent B cells may be dispensable in MAIT cell development as confirmed by flow cytometry and real-time quantitative polymerase chain reaction. Thus, I hypothesise that conventional B cells may be dispensable in MAIT cell development. Flow cytometric analysis of µMT mice showed that MAIT cell frequencies were the same compared to wild-type mice, suggesting that IgA-producing B cells, which still remain in µMT mice, may be sufficient in driving MAIT cell development with conventional B cells being required for later selection and/or expansion of MAIT cells.

Speaker	Miss Rachael Lim	2:35pm
Project title	***Investigation of a Potential Long Non-Coding RNA, AI447881, and Differential Gene Expression Analysis of Follicular and Marginal Zone B cells***	
Supervisor(s)	Prof Fabienne Mackay, Dr William Figgett	
Department	Immunology	

In B cell biology, the exact molecular mechanisms involved in the differentiation of transitional B cells to marginal zone (MZ) or follicular B cells remain unclear. In autoimmunity, MZ B cells have been observed to be contributors to disease pathogenesis. For many years, expressed sequence tag AI447881 has consistently been shown to be the most differentially expressed in MZ B cells relative to Fo B cells. However, nothing is known about it; the sequence of AI447881 has numerous stop codons and does not appear to contain any open reading frames; thus unlikely to be a protein-coding gene but could potentially be a part of a long non-coding RNA (lncRNA). Here, the study presents the finding that AI447881 is not part of a lncRNA but instead part of a protein-coding gene, I830077J02Rik, which encodes for a single-pass transmembrane protein, C1orf162, that has yet to be characterised. The discovery was made from obtaining the full length sequence of AI447881 using the 5' rapid amplification of cDNA end technique and RNA-seq. Additionally, RNA-seq has revealed an enormous number of differentially expressed genes between MZ and Fo B cells. The focus was then shifted to characterising the C1orf162 protein, starting off with determining its possible binding partners. Mass spectrometry of the captured proteins from pull-down assays with recombinant C1orf162 protein that was expressed in E. coli suggested that factor-H (FH) and a factor-H-like (FHL) protein (Protein Gm4788) are the binding partners of C1orf162. Since MZ B cells are exposed to the circulation and that FH and FHL bind to self-markers and C3b to prevent complement activation by the alternative pathway on host cells, C1orf162 could be a self-marker on MZ B cells for FH and Protein Gm4788 recognition for protection from complement activation via the alternative pathway.

Speaker	Ms Ellen Mcallister	2.55pm
Project title	**The arginine methyltransferase, Prmt5, is essential for B cell development in vivo**	
Supervisor(s)	Prof Fabienne Mackay, Dr Stefan Sonderegger	
Department	Immunology	

Arginine methylation is an important post-translational modification that is catalyzed by the protein arginine methyltransferase (Prmt) family of enzymes. Together, these enzymes influence a diverse range of cellular processes through transcriptional and translational regulation. A role for Prmt5 has been proposed in cell differentiation, development, survival and apoptosis. In this project, the importance of Prmt5 in developing B cells was investigated with the aid of a novel B cell-specific Prmt5 conditional knockout mouse model (Prmt5f/f Mb1cre/+). Prmt5 was found to be essential for B cells, with Prmt5f/f Mb1cre/+ mice having a 50 percent reduction in Pro B cells, and a 95 percent reduction in Pre and Immature B cells in the bone marrow. Mature B cells in the periphery were also diminished by more than 98 percent. The absence of mature B cells was confirmed by observing the disrupted architecture of the spleen, absent serum immunoglobulins, and a 2-fold reduction in spleen size and weight. The loss of B cells was not rescued with enforced overexpression of the anti-apoptotic protein, Bcl-2, supporting the hypothesis that Prmt5 is essential for cell survival. Interestingly, we found that Pro B cells in the Prmt5f/f Mb1cre/+ mice increased their expression of cKIT, the receptor for stem cell factor (SCF), indicating a possible function for Prmt5 in the regulation of this receptor.

Speaker	Mr Tian Zhao	3.15pm
Project title	***Acetylcholine Modulation of Cerebellar Function in Spinocerebellar Ataxia Type 1 (SCA1) Mice***	
Supervisor(s)	Prof Elsdon Storey	
Department	Medicine	

Spinocerebellar Ataxias (SCAs) refers to the dominant ataxias that are characterized by progressive cerebellar dysfunctions, in many cases accompanied by degeneration in other brain regions. In Spinocerebellar Ataxia Type 1 (SCA1), degeneration of brain stem will become noticeable after some major symptoms of SCAs appear. There is still no treatment for SCA1, only supportive physical therapy is available for the affected patients. Acetylcholine could potentially contribute to motor function as some parts of cholinergic fibers are arising from the pedunculopotine tegmental nucleus, which is associated with motor movements. The cholinergic input is thought to modulate the speed of supervised learning in Purkinje cells. This project is a part of a much larger study to systematically characterize the changes in the various neurotransmitter pathways affecting Purkinje cells in SCA1 mice. We investigated the expression, transcription, translation and topological distribution of acetylcholine, transporters and its synthesis pathways in the cerebellar cortex, red nucleus, inferior olivary complex and deep cerebellar nuclei. The preliminary data showed that acetylcholinesterase, choline acetyltransferase, muscarinic acetylcholine receptor 2 and nicotinic acetylcholine receptor α-7 transcription were significantly increased in the inferior olivary complex of SCA1 mice in comparison to wild-type mice. Future directions should lean focus on the changes of cholinergic input in the inferior olivary complex.

Honours BMS & BSc Final Oral Seminar Program 2015
Day 2
Date: Tuesday 27 October
Time: 10am – 3.40pm
Venue: Lecture theatre, Level 5, Alfred Centre

Tuesday 27 October		
Opening and welcome by Chair: A/Prof Julie McMullen		10am
Speaker	Miss April Fiedler	10.05am
Project title	***The role of metabolites in epigentic regulation of heart disease***	
Supervisor(s)	Professor David Kaye	
Department	Baker IDI	
Question and answer 10.20am		
Speaker	Mr Jarryd Anthonisz	10.25am
Project title	***Comparison of the effectiveness of nitric oxide and nitroxyl in the diabetic heart and vasculature***	
Supervisor(s)	A/Prof Rebecca Ritchie, Dr Chengxue Helena Qin	
Department	Baker IDI	
Question and answer 10.40am		
Speaker	Miss Georgie Wray-McCann	10.45am
Project title	***Examining the RHOA-YAP/TAZ signalling pathway as a mechanism underlying skeletal muscle adaptation in health and disease***	
Supervisor(s)	Dr Paul Gregorevic, Dr Kevin Watt Dr Robert Bryson-Richardson	
Department	Baker IDI	
Question and answer 11.00am		
Speaker	Miss Nur Rosli	11:05am
Project title	***Deficiency of endogenous Annexin-A1 exaggerates cardiomyopathy in mouse model of type 1 diabetes***.	
Supervisor(s)	A/Prof Rebecca Ritchie, Dr Helena Chengxue Qin	
Department	Baker IDI	
Question and answer 11.20am		
Morning tea 11:25am		
Chair: Prof Franklin Rosenfeldt		
Speaker	Mr Jesse Hansen-Bartel	11.40am
Project title	***Effects of perioperative opioid use as analgesics on survival in patients with glioblastoma multiforme***	
Supervisor(s)	Dr Sashendra Senthi, Dr Jeremy Ruben	
Department	Surgery	
Question and answer 11.55am		
Speaker	Mr Krishen Thayanantha	12.00pm
Project title	***Validation of a Predictive Model for Lung Cancer in Patients with Lung Nodules***	
Supervisor(s)	Dr Jeremy Ruben, Dr Sashendra Senthi	
Department	Surgery	
Question and answer 12.15pm		
Lunch 12.20pm		
Chair: Dr Stuart Lee		
Speaker	Mr Nikolay Kozlov	1:00pm
Project title	***Investigating tDCS in naturalistic second language learning***	

Supervisor(s)	Dr Kate Hoy, Dr Neil Bailey	
Department	MAPRc	
Question and answer 1:15pm		
Speaker	Miss Katerina Lau	1:20pm
Project title	***Neural Correlates of Rapid Commercial Second Language Learning***	
Supervisor(s)	Dr Kate Hoy, Dr Neil Bailey	
Department	MAPRc	
Question and answer 1:35pm		
Chair: A/P Frank Alderuccio		
Speaker	Miss Sasha Seneque	1.40pm
Project title	***Understanding the migration and accumulation of myeloid derived suppressor cells into the ovarian tumour microenvironment***	
Supervisor(s)	Prof Magdalena Plebanski, A/Prof Mark Wright, Dr Mutsa Madondo	
Department	Immunology	
Question and answer 1.55pm		
Speaker	Miss Mahtab Parvaresh	2:00pm
Project title	***Molecular Mechanisms that underpin Dendritic Cell Cross-presentation***	
Supervisor(s)	Dr Mireille Lahoud, Dr Mark Wright	
Department	Burnet Institute	
Question and answer 2:15pm		
Afternoon tea 2:20pm		
Chair: Prof Mark Wright		
Speaker	Mr Raymond Shim	2:35pm
Project title	***Targeting iNKT cells as a novel approach to reduce post-stroke infections***	
Supervisor(s)	Dr Connie Wong	
Department	Immunology, Clayton	
Question and answer 2:50pm		
Speaker	Miss Alyce Nicholls	2:55pm
Project title	***Sympathetic nervous system regulation of innate immune suppression following stroke***	
Supervisor(s)	Dr Connie Wong	
Department	Immunology, Clayton	
Question and answer 3:10pm		
Speaker	Mr Jasper Cornish	3:15pm
Project title	***The function of NFuB1 in follicular B cells***	
Supervisor(s)	Dr Raffi Gugasyan	
Department	Burnet Institute	
Question and answer 3:30pm		
Program ends 3:35pm		

ABSTRACTS – DAY 2

Speaker	Miss April Fiedler	10.05am
Project title	***Investigating the effect of a high fibre diet and acetate on heart failure and fibrosis***	
Supervisor(s)	Professor David Kaye	
Department	Baker IDI	

Fibrosis and inflammation within the heart play a highly pathological role in the development and progression of heart failure (HF), and as such are appealing targets for interventional treatment. Dietary fibre is highly associated with a reduced incidence of a variety of CVDs, including HF. Furthermore, bacterial metabolites produced form fermentation of fibre, notably the short chain fatty acid (SCFA) acetate, are capable of directly influencing immune cell activity and have been used to ameliorate disease in models of asthma, irritable bowel disease and chronic kidney disease. We therefore propose that dietary fibre or acetate supplementation in drinking water may be able to reduce cardiac remodelling and dysfunction. To investigate this hypothesis, these dietary interventions were assessed in the Mst1 transgenic mouse line, which exhibits dilated cardiomyopathy and HF.

Following eight weeks of treatment, cardiac remodeling and function were assessed with echocardiography, hemodynamic analysis and histological assessment of fibrosis. As expected Mst1 mice exhibited extensive cardiac remodeling and features consistent with congestive HF, however dietary intervention with a high fibre diet or acetate in the drinking water was unable to significantly alleviate these symptoms. Despite this there, was a non-significant reduction in collagen mRNA with both treatments, suggesting that the treatments have some potential to alleviate fibrosis in the heart tissue. Assessment of inflammatory markers in the cardiac tissue revealed significantly increased Il6 and Cd8a mRNA in the hearts of Mst1 mice. These findings may indicate that CD8+ T lymphocytes play a role on Mst1 cardiac dysfunction. Finally, while dietary fibre treatment produced no apparent change in disease progression, it did significantly elevate T helper and T regulatory (Treg) cell proportions in the mouse spleen, supporting previous findings of bacterial metabolite influence on Treg induction.

Speaker	Mr Jarryd Anthonisz	10.25am
Project title	***Comparison of the effectiveness of nitric oxide and nitroxyl in the diabetic heart and vasculature***	
Supervisor(s)	A/Prof Rebecca Ritchie, Dr Chengxue Helena Qin	
Department	Baker IDI	

Introduction: Diabetic cardiomyopathy is characterised by impairment of left ventricular (LV) diastolic function, cardiomyocyte hypertrophy, fibrosis and elevated reactive oxygen species (ROS). To date, a specific treatment targeting diabetic cardiomyopathy is not available, and consequently the disease has poor prognosis. The NO• redox sibling nitroxyl (HNO) elicits vasodilatation and enhances left ventricular (LV) contraction and relaxation. The efficacy of HNO has not yet been fully characterised in disease. We tested the hypothesis that the HNO donor isopropylamine NONOate (IPA-NO) offers haemodynamic advantages over the NO• donor diethylamine NONOate (DEA-NO) in the diabetic myocardium. Methods: After 8wks of streptozotocin diabetes (55mg/kg i.v., blood glucose ~30mM and ~20mM) or sham, hearts were isolated from adult male rats, anaesthetised (ketamine-xylazine 100-12mg/kg i.p.) and Langendorff-perfused. Following U46619 preconstriction of the coronary vasculature to 50%, dose-response curves to acute bolus doses of IPA-NO or DEA-NO, both 10pmol-10µmol were performed. A separate cohort of rats followed an identical protocol, but were pre-treated with the soluble guanylyl cyclase (sGC) inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (10µM ODQ), prior to U46619 preconstriction.

Results: HNO vasodilatation was preserved, and the positive inotropic and lusitropic effects were increased in diabetic hearts; in contrast, those of NO• were attenuated. The impaired NO• vasodilatation persisted in hearts from moderately hyperglycaemic rats (6-7U insulin s.c./day, blood glucose ~22mM). The sGC inhibitor ODQ blunted coronary vasodilator, cardiac contraction and relaxation responses to DEA-NO in hearts from non-diabetic rats. However, any remaining response to DEA-NO in moderate and severely hyperglycaemic hearts were resistant to ODQ. In contrast, ODQ significantly blunted the vasodilator, inotropic and lusitropic effects of IPA-NO, irrespective of glycaemic levels.

Conclusion: Haemodynamic responses to HNO are preserved or enhanced in diabetes, whereas those of NO• are attenuated. These may receive contributions from both sGC-dependent and sGC-independent mechanisms.

Speaker	Miss Georgie Wray-McCann	10.45am
Project title	***Examining the RHOA-YAP/TAZ signalling pathway as a mechanism underlying skeletal muscle adaptation in health and disease***	
Supervisor(s)	Dr Paul Gregorevic, Dr Kevin Watt Dr Robert Bryson-Richardson	
Department	Baker IDI	

Yes-associated protein (YAP) and its paralogue, transcriptional co-activator with PDZ-binding domain (TAZ), are the critical transcription factors of the Hippo pathway in mammals, characterised as regulators of cell proliferation and commitment. Stains, a class of cholesterol-lowering drugs can interact with YAP/TAZ in in vitro tumour models in a RHOA-dependent manner. One side-effect of statin administration is myopathy, which forces many patients to discontinue use. Given that a role for the YAP/TAZ in controlling skeletal muscle size and the RHOA-YAP/TAZ interaction in tumorigenesis have recently been identified, we hypothesised that a RHOA-YAP/TAZ pathway exists in skeletal muscle and plays a role in myopathy caused by statin use. In this study, we treated C2C12 myoblasts (mouse skeletal muscle progenitor cells) with 5 μM simvastatin for 24 hrs, which decreased total YAP protein but not YAP transcripts. Lowered YAP levels by simvastatin treatment lowered the C2C12 myoblast proliferation rate by 50%. The reduction in proliferative rate was dependent on YAP but not TAZ. Simvastatin decreases cholesterol production by inhibiting the mevalonate pathway. To promote nuclear YAP, RHOA requires on an intermediate of the mevalonate pathway to localise at the nuclear membrane, geranylgeranyl pyrophosphate (GGPP). A rAAV6:RHOAQ63L vector, which expresses a mutant RHOA constitutively bound to GTP, was injected into the tibialis anterior of eight week old male C57BL/6 mice at 5x109 - 5x108 vector genomes (vg) for two and four weeks. At high doses the vector caused muscle degeneration with variable expression of YAP protein. The lower dose (5x108 vg) did not cause muscle degeneration or affect YAP protein levels, until 5 mg/kg simvastatin was administered in the background, where YAP levels significantly decreased. Contrary to our hypothesis that RHOAQ63L would mitigate the simvastatin-induced YAP reduction, it demonstrated a simvastatin-RHOAQ63L-YAP interaction. Subject to further investigation, RHOA could be therapeutically targeted to combat the myopathic effects of statin treatment due to loss of YAP activity in skeletal muscle.

Speaker	Miss Nur Rosli	11:05am
Project title	***Deficiency of endogenous Annexin-A1 exaggerates cardiomyopathy in mouse model of type 1 diabetes***.	
Supervisor(s)	A/Prof Rebecca Ritchie, Dr Helena Chengxue Qin	
Department	Baker IDI	

Introduction: Diabetes is a chronic inflammatory disease and is associated with an increased risk of heart failure. We have previously shown that deficiency of an anti-inflammatory protein, annexin-A1 (ANX-A1) exaggerates myocardial infarction. However, its impact on other cardiac pathologies has not been investigated.

The aim of this study was to test the hypothesis that deficiency of ANX-A1 (ANX-A1+/+) exaggerates diabetic cardiomyopathy in type 1 diabetic mice. Method: Wild-type ANX-A1+/+ and ANX-A1-/- male mice were allocated to receive either streptozotocin to induce diabetes or vehicle sham (55mg/kg/day, i.p. for 5 days), and were followed for shorter-term (9 weeks) or longer-term (16 weeks) diabetes. Results: Diabetic mice developed marked hyperglycaemia and retarded body weight gain, regardless of genotype and diabetes duration, compared to their non-diabetic sham counterparts. Diabetic mice following shorter-term diabetes, exhibited early trends suggestive of LV diastolic and systolic dysfunction, and cardiomyocyte hypertrophy, compared to their non-diabetic sham counterparts. This was accompanied by increased circulating monocyte levels, and downregulation of the pro-resolving cytokine, LV interleukin-10 (IL-10) expression. Further, ANX-A1-/- diabetic mice exhibited a significant increased in cardiac fibrosis and upregulation of the pro-inflammatory cytokine, LV tumour necrosis factor-α (TNF-α) expression, when compared to ANX-A1+/+ diabetic mice at this time point. After longer-term diabetes, a more marked LV diastolic and systolic dysfunction, cardiomyocyte hypertrophy, and cardiac fibrosis were evident, compared to non-diabetic sham counterparts. Interestingly, ANX-A1-/- diabetic mice exhibited more severe cardiac fibrosis and cardiac macrophage content, compared to ANX-A1+/+ diabetic mice particularly following longer-term diabetes compared to shorter-term duration.

Conclusion: These data suggest that deficiency in ANX-A1 exacerbate diabetes-induced cardiac dysfunction and remodelling. ANX-A1 may thus represent a therapeutic target for the treatment of diabetes.

Speaker	Mr Jesse Hansen-Bartel	11.40am
Project title	***Effects of perioperative opioid use as analgesics on survival in patients with glioblastoma multiforme***	
Supervisor(s)	Dr Sashendra Senthi, Dr Jeremy Ruben	
Department	Surgery	

During the perioperative period, the utilisation of anaesthetics and analgesics are common practice in patients with glioblastoma multiforme (GBM). Pain management is essential for undertaking surgery, and for mitigating discomfort for the duration of a patient's illness. Of interest is a speculative link between opioid use and tumour recurrence, and directly a shorter time to death. Opioids may attenuate immune function in humans, and it has been reported in a number of retrospective studies to decrease survival in various cancer models. Conversely, other retrospective studies have shown no effect on overall survival. This was a single institutional and retrospective study of 69 GBM patients at the William Buckland Radiotherapy Centre. Comprehensive demographics, perioperative data, and survival times, defined as date of diagnosis to date of death (if applicable) were obtained. Univariate Cox regression analysis and multivariate Cox proportional hazard models were conducted to assess the association between perioperative opioid exposure and overall survival. The median amount of perioperative morphine equivalents administered was 2.830 g (range 0.075-8.467) and the median survival of this cohort was 16.32 months (95% CI 13.008 months - 25.392 months). The univariate analysis demonstrated a protective effect for opioid use (HR: 0.84, 95% CI: 0.9997-0.9999, P = 0.043) however the significance of this was lost in the multivariate model after adjustment for important covariants, despite a trend in favour of opioid use on the associated Kaplan-Meier survival curve. Prospective, randomised controlled studies are needed to explore the possible anti-cancer effects of opioid use, and delineate the dose-response relationship between opioid use and overall survival in GBM patients.

Speaker	Mr Krishen Thayanantha	12.00pm
Project title	***Validation of a Predictive Model for Lung Cancer in Patients with Lung Nodules***	
Supervisor(s)	Dr Jeremy Ruben, Dr Sashendra Senthi	
Department	Surgery	

Predictive lung models utilize demographic, clinical and radiographic variables to discriminate benign and malignant tumours in patients with suspicious pulmonary nodules. The TREAT (Thoracic Research and Evaluation Treatment) model is a predictive model developed and validated at the Vanderbilt University Medical Centre (VUMC), to evaluate suspicious lung lesions pre-operatively in patients. This model was retrospectively translated to an Australian population to validate its ability to predict lung malignancy prior to either surgical or radiotherapy treatment. A database consisting of both radiotherapy and surgical patients from the Alfred Hospital, Melbourne was constructed with demographic, clinical and radiographic variables collected. The TREAT model was externally validated at the VUMC and statistical practices of area under the receiver operating curve (AUC) and Brier Score were employed to measure the model's discriminative power. The TREAT model was compared against the Mayo model to assess the model's efficacy and to possibly improve it to better suit the Australian population. The TREAT model was developed in an Australian population of 306 patients with a lung cancer prevalence of 85%. In the Australian population, an AUC of 0.63 and brier score of 0.167 was obtained compared with an AUC of 0.87 and a brier score of 0.12 in the original VUMC population. The TREAT model performed poorly in discriminating between benign and malignant nodules and thus was not validated in the Australian cohort. The Mayo model also performed poorly in the Australian population with an AUC of 0.58 and brier score of 0.23. A new model specific to the Australian population with re-estimated coefficients was developed using the existing variables of the TREAT model and had an AUC of 0.86. This new model had significantly higher diagnostic accuracy prior to treatment compared to the TREAT and Mayo models.

Speaker	Mr Nikolay Kozlov	1:00pm
Project title	***Investigating tDCS in naturalistic second language learning***	
Supervisor(s)	Dr Kate Hoy, Dr Neil Bailey	
Department	MAPRc	

Background: The efficacy of Transcranial Direct Current Stimulation has seldom been investigated in naturalistic settings. Language learning is an example of cognition that is widely used in everyday settings, therefore it is an ideal candidate to assess the efficacy of tDCS in a "real life" condition. It has also been suggested in previous literature that the inter-individual variability of tDCS effect is dependent on one's innate ability for the task. The primary aim of the current study is to investigate whether anodal tDCS to Wernicke's area has an effect on naturalistic language learning. The secondary aim is to determine whether language learning ability can be used as a predictor for tDCS efficacy.

Methods: 24 participants were randomly allocated to active/sham tDCS conditions and undertook a commercial Spanish language course along with tDCS over three days. The participants completed three recall tests during the course and an overall recognition test at the end. A double oddball Spanish phoneme recognition task was performed before and after the course as a more sensitive measure of language learning. Participants were then allocated to a high/low phoneme responder group to determine if learning ability modulated tDCS effects. Results: tDCS had no significant effect on language test outcome or Spanish phoneme recognition. Phoneme recognition accuracy improved after learning compared to baseline, and tDCS had a significant effect on the reaction time for phoneme response. No significant effect of tDCS on language tests was observed in either the low or high phoneme responder groups.

Conclusion: The current findings suggest that tDCS has no significant effect on test scores when combined with a naturalistic language learning program, however a more sensitive measure suggests that while the effect of tDCS may be too subtle to reliably detect with "real life" measures, it does have an effect on reaction time. Predisposition to language learning was not found to have a modulatory effect on tDCS.

Speaker	Miss Katerina Lau	1:20pm
Project title	***Neural Correlates of Rapid Commercial Second Language Learning***	
Supervisor(s)	Dr Kate Hoy, Dr Neil Bailey	
Department	MAPRc	

Background: Commercial rapid language learning programs are rising in popularity and claim to be effective, efficient and accessible. However, there are currently no studies that substantiate these claims. One of the hallmarks of effective language learning is the neural changes that occur. The P300 is a neural marker that changes its amplitude in response to stimuli depending on the degree of recognition. The primary aim of the current study was to investigate whether rapid commercial language learning induced changes in the P300 response and if this change correlated with language learning. The secondary aim of the study was to investigate whether non-invasive brain stimulation during learning was able to enhance the P300 response.

Methods: Twenty-four healthy controls participated in the randomised single-blind between subjects study. Participants underwent a double-oddball phoneme task before and after completing the Elizabeth Smith's Teach Yourself Spanish CD which was conducted over three sessions on consecutive days. Participants were randomly allocated to receive either active or sham stimulation to Wernicke's area during each of the three language learning sessions. Results: The results showed that following language learning in the sham stimulation group ('control group'), participants had a significantly greater P300 maximum amplitude response to the Spanish deviant, which correlated with learning outcomes at a trend level. There was no effect of stimulation on the P300 responses.

Conclusions: The findings suggest commercial rapid language programs are successfully able to induce neural changes associated with second language acquisition. Additionally, tDCS is most likely too broad of an application to be suitable to specifically facilitate such language instruction methods. Further research needs to be undertaken in this area to replicate and extend these findings.

Speaker	Miss Sasha Seneque	1.40pm
Project title	***Understanding the migration and accumulation of myeloid derived suppressor cells into the ovarian tumour microenvironment***	
Supervisor(s)	Prof Magdalena Plebanski, A/Prof Mark Wright, Dr Mutsa Madondo	
Department	Immunology	

Background: Worldwide, over 50% of women diagnosed with ovarian cancer will die as a cause of the disease each year (estimated statistic in 2012). Ovarian cancer is a chronic inflammatory disease and is able to progress due to poor recognition of symptoms and ineffective treatments. The formation of the ascites (fluid in the peritoneal cavity) is a common characteristic for patients with stage III to IV ovarian cancer. Inflammation allows the migration of immune cells into the ascites often leading to a suppressive immune environment.

This study addresses the mechanisms by which myeloid derived suppressor cells (MDSCs) and stimulatory dendritic cells (DCs) migrate into the ascites. Previous data has revealed that tetraspanin CD37 is required for DC migration to lymph nodes. This evidence poses the basis of the study, that CD37 may be involved in the migration of DCs and MDSCs into the ascites.

Method: To investigate this hypothesis, a quantitative research approach comparing wild type (WT) and CD37 knockout mice (CD37-/-) was used. Phenotyping, culturing, and migration assays were performed with blood and bone marrow cells. Flow cytometry was used to acquire the data. Results: DCs expressed more CD37 than MDSCs in the studied tissues, where blood cells expressed the most CD37. As cells migrate within the blood, the results suggested a use for CD37 in migration. However, the migration pattern of cells did not differ between the WT and CD37-/- samples. CD37 may be involved in integrin recycling procedure of migration, as a greater proportion of CD37+ cells engulfed nanoparticles. Conclusion: CD37 was found to be present on DCs and MDSCs when they travel in the blood with a greater expression level.

Future experiments will need to address the cause of this finding, specifically researching the migration of MDSCs to multiple chemokines found within the ascites and reveal whether CD37 co-localises with nanoparticle uptake.

Speaker	Miss Mahtab Parvaresh	2:00pm
Project title	***Molecular Mechanisms that underpin Dendritic Cell Cross-presentation***	
Supervisor(s)	Dr Mireille Lahoud, Dr Mark Wright	
Department	Burnet Institute	

Dendritic cells (DCs) are professional antigen (Ag) presenting cells critical for the stimulation of the adaptive immune system, providing defence against foreign and aberrant cells. DCs are proficient at processing and presenting Ag on major histocompatibility complex (MHC) class I and II, initiating CD8 and CD4 T-cell response, respectively. Although Ag presentation takes place in all Ag presenting cells, cross-presentation is unique to specific DC subsets. Cross-presentation enables exogenous Ags to gain access to the MHC class I presentation pathway, and initiate a cytotoxic T-cell response against foreign invaders. However, the molecular mechanism that underpins Ag cross-presentation is yet to be fully defined. Using microarray comparison of DC subsets and different stages of DC development, a panel of genes selectively expressed by cross-presenting splenic CD8α+ cDC and Flt3L DC subsets upon acquisition of cross-presentation were identified. Four genes were selected for further investigation, these include Formin binding protein 1-like (Fnbp1l), G protein-coupled receptor 33 (Gpr33), Lysosomal-associated protein transmembrane 4-beta (Laptm4β) and GLI pathogenesis-related 2 (Glipr2). These genes were selectively expressed by splenic CD8α+ cDC, and increased in expression upon DC activation, as demonstrated by quantitative RT-PCR. In order to assess the role of these genes in Ag cross-presentation, short hairpin RNA (shRNA) knockdown approach was utilized, and Fnbp1l, Gpr33, Laptm4β and Glipr2 specific shRNA constructs were designed and successfully cloned. A pipeline was developed for analysis of the role and function of Fnbp1l, Gpr33, Laptm4β and Glipr2 in Ag cross presentation. Further studies will focus on using the prepared shRNA constructs for knockdown of these selected genes in a CD8+ DCs model cell line, and investigating changes in Ag cross-presentation, which may reveal novel targets for immunotherapy.

Speaker	Mr Raymond Shim	2:35pm
Project title	***Targeting iNKT cells as a novel approach to reduce post-stroke infections***	
Supervisor(s)	Dr Connie Wong	
Department	Immunology, Clayton	

Stroke causes a large portion of death around the world and is the second largest contributor to mortality in Australia. The burden it creates in the health care system and society is immense and this is predicted to rise due to our ever aging population.

It is now widely accepted that impairment of immunity occurs following stroke, resulting in increased susceptibility to infection. We recently demonstrated that the impaired function of iNKT cells is one of the main mediators of post-stroke immune suppression, and that infection could be prevented by specifically activating iNKT cells with α-GalCer. Therefore, we evaluated the effectiveness of seven α-GalCer analogues on activating iNKT cells to prevent post-stroke immune modulation and infection. Additionally, we investigated the impact of activating iNKT cells with two analogues on other immune cells after stroke. To approach this, we firstly observed the temporal profiles of iNKT cell responses and systemic immunity change induced by each analogue in mice. Secondly, each analogue was administered to mice 1 hr following a mouse model of stroke to determine whether these analogues would improve or exacerbate stroke outcomes at 24 hrs after surgery.

Finally, neutrophil chemotaxis and macrophage phagocytosis assays were performed to examine the mechanism of action of two chosen analogues. While we found that the majority of the analogues tested could activate iNKT cells to alter systemic cytokine production, administration of SKC8-27 reduced infarct size at the expense of exacerbated infection after stroke. In contrast, DB06-9 was superior in reducing infection in all examined tissue without exacerbating brain injury. To explain the decrease in infection rate, mice treated with DB06-9 had enhanced neutrophil and macrophage activity. Ultimately, this study showed that DB06-9 can activate iNKT cells to combat post-stroke immune modifications to offer a novel alternative therapeutic option to improve stroke outcomes.

Speaker	Miss Alyce Nicholls	2:55pm
Project title	***Sympathetic nervous system regulation of innate immune suppression following stroke***	
Supervisor(s)	Dr Connie Wong	
Department	Immunology, Clayton	

Infectious complications are a major contributor to mortality in the post-acute phase of stroke. Clinical observation of stroke patients and recent animal studies have implicated a potential role for sympathetic nervous system (SNS) activation in the development of post-stroke immune suppression. Moreover, the timecourse of infection suggests a role for impairment in the innate arm of the immune system, however the mechanisms that regulate this suppression have not yet been elucidated. Therefore, we aimed to examine the role of noradrenaline (NA) in impairing innate immune cell function and promoting immunosuppression following stroke. Macrophages and neutrophils were isolated from 1) C57BL6 naïve wildtype mice and challenged with increasing concentrations of NA or 2) wildtype mice subjected to a mouse model of stroke, the Mid-cerebral artery occlusion (MCAO), or sham surgery, before being assessed for cell function. NA and MCAO significantly impaired the migration of neutrophils towards the chemokines KC and fMLP, however intraperitoneal administration of the adrenergic receptor blocker propranolol following stroke partially restored migration towards KC only. In addition, there was a shift towards an M2 phenotype in macrophages following treatment with high dose NA or MCAO, however, this was not accompanied by a decreased capacity for phagocytosis. Together, our findings demonstrate that both NA and MCAO can significantly alter the function of cells of the innate immune system, whereby SNS activation may contribute to the regulation of innate immunosuppression following stroke.

Speaker	Mr Jasper Cornish	3:15pm
Project title	***Defining the Critical Roles of the Transcription Factor NFκB1 in Mature B Cells***	
Supervisor(s)	Dr Raffi Gugasyan, Dr Paul Ramsland	
Department	Burnet Institute	

B cells are a vital component of the immune system, with the capacity to secrete highly specific antibodies. While the outcomes of B cell deregulation are well known, the transcriptional regulation of B cell function is still being elucidated. NFκB1 is a transcription factor that is critical for B cell homeostasis and function. Polymorphisms in the human nfκb1 gene are associated with the development of autoimmune disease, and reduced survival outcomes in multiple myeloma patients. In a murine model, deletion of nfκb1 gives rise to a chronic autoimmune disease, characterised by multi-organ immune cell infiltrate and aberrant lymphoproliferation. This pathology is partially mediated by the overproduction of IL-6 by a population of mature NFκB1-deficient follicular B cells. IL-6 is strongly associated with enhanced terminal B cell differentiation, however, the overproduction of IL-6 alone does not explain the full pathology in the nfκb1-/- mice, and disease manifestation remain unclear.

This study utilised immunological techniques to characterise the peripheral B cell compartment in young and ageing nfκb1-/- mice. The expression of ICOS-L, a signalling molecule key to the differentiation of peripheral B cells, was critically assessed, given that ICOS-L deregulation is believed to promote the generation of autoimmunity. ICOS-L expression was markedly increased in the mature and terminally differentiated B cells in the nfκb1-/- mice. Mature follicular B cells from nfκb1-/- mice cultured in vitro also displayed an impaired capacity to downregulate ICOS-L after BCR-crosslinking and TLR-9 stimulation. Ageing nfκb1-/- mice developed a striking overexpansion of plasma cells, assumed to be due to processes triggered by the increased expression of ICOS-L. The findings in this study highlight the importance of understanding the function of NFκB1 in B cells, and how the absence of NFκB1 leads to the deregulation of ICOS-L and the development of multi-organ immune disease.