Research Review 2018

Research For Life

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Editorial

Each evening I saved my thesis onto a floppy disc and hoped that the next day the PhD student in my lab would not need the Macintosh Classic computer we shared. My experimental notes were meticulously handwritten in a hard cover lab book with dot matrix-printed flow cytometry plots stuck in. I can't recall how the plots were then incorporated into my thesis but it is probably blocked from my memory as it was undoubtedly labourious and with suboptimal appearance. To complete the literature review for my work, I had spent hours in the library pouring over the biblically-thick volumes of Index medicus and scouring bibliographies of relevant papers. When I presented my work at a conference, the poster was prepared weeks in advance, as it had to go off to the university printers. Similarly, slides (actually physical slides for a projector) required several weeks for the photography department to prepare. The laboratory research had explored what characteristics were required for immune cells to start and re-start an immune response against foreign bacteria. I made one or two small, novel observations, which still hold true today. That one-year laboratory project was my first foray into medical research. It was 1994.

A decade later I found myself in another lab, again in immunology. Now my focus was on how immune and inflammatory cells orchestrated inflammation in gout. Fortunately the internet was now a thing and most of the scientific literature was available online, vastly improving access to information. Powerpoint and data projectors now meant I could write my talks up to the last minute – not necessarily an advantage. Again, I made a couple of interesting and novel observations. My PhD research was enabled by a generous grant from the then Wellington Medical Research Foundation. This grant was essential to buy the reagents and equipment required for the

laboratory work. My PhD launched an academic career, combining work as a physician with medical research.

Just more than two decades after I was fist in the lab, I am now in the fortunate position to lead teams and collaborate with others in medical research. My focus has moved from the lab to clinical research, but still has the end-goal of improving human health and wellbeing. Information and communication tools for science have changed beyond recognition. A reseacher can access powerful databases of information from anywhere, anytime, via a hand-held computer, or email any international researcher for information or advice. However, the process of science remains the same: find an important topic, ask a question, find out what is already known, construct a hypothesis, test with an experiment, analyse data, and draw conclusions. Then, communicate your results. All this requires time and money and writing applications for grants is part of the cycle of any researcher's year.

One aspect of my work that I thouroughly enjoy is supervising students as they enter medical research. They share the curiosity, excitment and hope that I brought to my first project. We differ in that these students belong to the millennial generation, who grew up during the period of massive technological change and never remember a time without the internet. As a hyperconnected group, they are collaborative and team-oriented, and also not afraid to challenge the status quo. Since they are digital-natives they are adept at managing instant access to information and integrating this into daily life. Millennial researchers also have a heightened need for work to have authentic purpose and real-world outputs. The attributes of millennials are ideally suited for work in medical research, which is about as real-world as science gets. I have noticed that my young millennial students still prepare PowerPoint slides right up to the last moment!

A great privilege in my work now is to foster innovative and exciting medical research by serving on bodies that distribute funding for research. Research for Life (RFL) is the new face for the Wellington Medical Research Foundation, which has been supporting medical Research in Wellington since 1960. After serving on the RFL scientific advisory committee since 2011, I took over from Professor Brett Delahunt as Chair in late 2017. I thank Brett for his 20 plus years of service and leadership and leaving me with a team of dynamic, experienced researchers who bring a broad range of skills, experience and knowledge to the committee.

The research advisory committee advises the Board of RFL regarding priorities for funding grants to support the medical research that is undertaken across the region's universities and institutes. Each grant round the committee are impressed by the extraordinary science happening right here the capital of this tiny island by clever and committed people who would be right at home in esteemed institutions anywhere in the world. The grants provided by RFL often support doctoral students or early-career researchers, many of whom go on to establish careers in medical research. These millennials, with fresh thinking and using current and evolving technology, maintain my optimism for significant developments in medical science that will lead to improvement in human health and reduced suffering from disease.

In the few short decades I have been involved in medical research, the ability to progress medical science has accelerated. There has never been a better time to invest in medical research that can actually lead to better health outcomes. We can all contribute to the investment. Millennials can invest their time in training as medical researchers. Established researchers and their institutes can provide a supportive environment for these young

researchers to learn and flourish. Members of the public can support medical research in Wellington by contributing financially to RFL so more grants can be distributed. Please share my optimism for the future of medical research and the discoveries that will be made. Support this by supporting RFL however you can. I look forward to a future where our current young researchers can look back and tell us how far we have come.

I hope you enjoy reading the fascinating reports in this year's review as much as I have.

Dr Rebecca Grainger

Editor

Research Advisory Committee (2018)

Reports of research work funded by grants prior to the current year

University of Otago, Wellington

Changes in DNA methylation within the HIF3A gene after weight-loss in severely obese individuals either undergoing dietary intervention or gastric bypass

J Krebs, M Benton, J Clapham, A Parry-Strong, R Carroll, R Stubbs, D Macartney-Coxson
University of Otago, Wellington

Aim: Changes in DNA methylation have been correlated with obesity and type 2 diabetes (T2DM). *HIF3A* DNA methylation has been associated with BMI in adults and correlated with infant weight and adiposity. We previously observed significant differential *HIF3A* DNA methylation in subcutaneous adipose before and after gastric bypass. Here we investigate *HIF3A* DNA methylation in blood in severe obesity and subsequent weight-loss achieved by dietary intervention alone or gastric bypass.

Methods: Blood *HIF3A* DNA methylation was analysed by pyrosequencing (EpigenDX) of three specific *HIF3A* CpG loci in two cohorts. One of severely obese individuals at two time points, before and at least 5 years after gastric bypass (n=27); the other of individuals who participated in the Diabetes Excess Weight Loss (DEWL) trial (n=55), sampled at baseline and 12m. Statistical analyses were performed in R.

Results: We observed significant changes in DNA methylation at specific CpG sites within *HIF3A* before and after weight-loss associated with gastric bypass and a controlled diet. The associations between DNA methylation and weight persisted with inclusion of covariates (gender, age) in the model. Furthermore, our data suggest the

possibility that the previously reported relationship between *HIF3A* DNA methylation and BMI is perturbed in T2D and/or severe obesity.

Conclusions: *HIF3A* DNA methylation changes in association with weight-loss and may be perturbed in T2D and/or severe obesity. Increased understanding of the role epigenetic mechanisms play in disease may reveal novel intervention strategies to augment the effects of dietary or pharmaceutical treatments.

Dissemination: This work was presented to the New Zealand Society for the Study of Diabetes (NZSSD) conference 2017.

Demonstrating a Mycobacterial growth inhibition assay models host pathogen interactions

Principal Investigator: A Verrall
Pathology and Molecular Medicine, University of Otago, Wellington

Other investigators: T Blackmore, Victoria University; J Ussher, Department of Microbiology and Immunology (University of Otago, Dunedin)

Background: Each year, *Mycobacterium tuberculosis* kills over a million people and causes nine million cases of tuberculosis disease (TB). A crucial component to better TB control is finding an immune response that is correlated with genuine protection from *M. tuberculosis*, as this could be used to guide the development of vaccines. In field studies we have identified a group of people who are exposed to tuberculosis but remain uninfected. These people are likely to have innate immunity to *M. tuberculosis*.

Mycobacterial growth inhibition assays are experiments in which human immune cells are cultured with mycobacterial species. People who are immune to tuberculosis may also inhibit the growth of mycobacteria better. We sought to develop a mycobacterial growth inhibition assay to validate the associations found in our field studies.

RFL support: RFL supported the purchase of a cell counter for standardisation of cell numbers in the assay. The cell counter is also used by other members of our laboratory when doing cell culture work.

Progress to date: The cell counter has been used when we have performed the growth inhibition assay on whole blood with non-virulent mycobacteria species (*M. bovis* BCG vaccine strain). The growth inhibition assay is sensitive to different inoculation concentrations. We are currently working on two aspects of optimization before the assay can be used in studies. Firstly, the reproducibility of the assay needs to be improved, secondly, we need to prevent BCG stocks degrading in storage.

Continued work on the growth inhibition assay has been the subject of applications for grant funding to the Health Research Council of New Zealand.

Effect of short-term temperature changes on energy expenditure

T O'Donnell, M Fan, J Krebs, S Tzeng
Centre for Translational Physiology and Department of Surgery and Anaesthesia, University of Otago, Wellington

Aim: This PhD study is an investigation into the effect ambient temperature has on the amount of energy expended in humans. In modern society, large proportions of the population spend the vast majority of their time exposed to controlled air-conditioned environments. This is

designed to maximise comfort however there is a risk that this may be limiting daily energy expenditure and predisposing people to obesity.

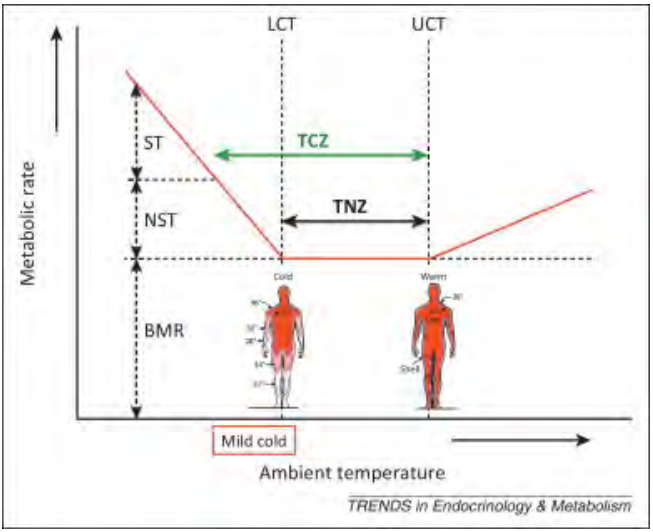


Figure 1: The thermoneutral zone as it is traditionally depicted

Background: The thermoneutral zone is a range of temperature in which resting energy expenditure is not required to increase to warm or cool the body (Figure 1). Previous research suggests that ambient temperatures outside the thermoneutral range results in elevated energy expenditure and presents the possibility of an interventional target for raising daily energy expenditure. In overseas populations this range has varied between 17-26 degrees and we have sought to establish if such a range existed for our unique local population in New Zealand.

Methods: Using the Global Energetics and Environmental Simulation Suite (GENESIS) at the Centre for Translational Physiology, University of Otago, Wellington we are able to alter and control temperature whilst accurately measuring energy expenditure. Participants underwent two separate

protocols which started at 23 degrees for 30 minutes and over 90 minutes the temperature was either decreased down to 11 °C or increased up to 35 °C. Participants were seated in a semi-reclined position wearing standardised scrubs and their expired air continuously collected with a hood calorimeter to quantify energy expenditure.

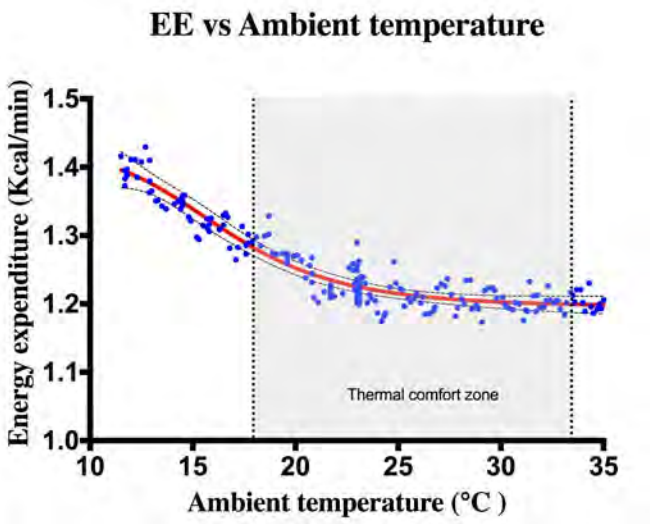


Figure 2: Energy expenditure changes as ambient temperature is modulated

Results: Data collection has now completed on 30 participants (15 female). We did not observe an increase in energy expenditure when ambient temperature was increased up to 35 degrees (Figure 2), however we did observe an increase in energy expenditure as temperature was decreased. Crucially, a portion of this increased energy expenditure occurred within the thermal comfort zone. This implies that there is a temperature range within which interventional temperature decreases could be applied whilst being accepted by participants.

This will be the focus of follow-up studies, particularly looking at the impact on a workplace environment. We are currently preparing the manuscript from this first investigation for submission to a peer-reviewed journal. Portions of this work were also included in an oral presentation by the Chief Investigator at the Recent Advances and Controversies in Measurement of Energy Metabolism (RACMEM) conference in Fribourg, Switzerland, October 2017.

Electron microscopic localisation of upregulated extra cellular matrix influencing gene products in colorectal carcinoma

D Kenwright
Department of Pathology and Molecular Medicine, University of Otago, Wellington

Background: Colon cancer is common affecting 1200 New Zealanders each year. Localising proteins ADAMTS2, Jagged2 and LTBP3 known to correlate with outcome in colorectal cancer, may help identify people with worse outcomes to focus treatment. Localising protein antigen at the ultrastructural level can be unpredictable. Good localisation can be obtained at the light microscope level with poor localisation in the electron microscope.

Aim: Our aim in this study was to assess whether gold immunolabelling using EM could identify the subcellular localization of proteins ADAMTS2, Jagged2 and LTBP3 known to correlate with outcome in colorectal cancer.

Methods: Samples were obtained from 18 people with colon cancer. Tissue was taken from non-necrotic areas of the tumour and from normal appearing colon tissue distant from the tumour. Since one important factor unpredictability of EM localisation of protein antigens is the

effect of the fixative on the antigen, for this reason a range of fixative strengths were used. Glutaraldehyde was used in strengths from 4% down to 0.5% and paraformaldehyde was used alone. As the resin embedding the tissue can also affect antigen binding, two embedding media were used, an epoxy resin and LR White, a methacrylate and less damaging media.

Results: Both ADAMTS-2 and LBP-3 antibodies were used at the light microscope level, with dilutions and incubation times optimised, and good results were obtained. The same dilutions and incubation times were tried for electron microscopy but did not work. The dilutions were reduced from 1/100 progressively down to 1/10 and the incubation times increased from an hour up to overnight. None of these variations gave consistent antibody labelling.

Key finding: Gold immunolabelling is not a suitable method for localising ADAMTS2, Jagged2 and LTBP3 proteins.

Examination of the temporal variance in neutrophil phenotypes across the acute phase of a myocardial infarction [interim report]

A Holley, K Hally, S Harding, P Larsen
University of Otago, Wellington Department of Surgery and Anaesthesia

Background: The intense inflammatory response triggered in an acute myocardial infarction (AMI) allows for the clearance of dead cells and matrix debris from the wound, and for reparative processes such as tissue repair and remodelling to preserve cardiac function to occur. This inflammatory response is extraordinarily complex and tightly regulated, and it is plausible to think that any deviation from ‘normal’ could result in a flawed healing response.

Pre-clinical studies have suggested that a dysregulated inflammatory profile is implicated in maladaptive remodelling (poor cardiac function). The degree of remodelling that occurs following an AMI remains one of the determinants of long-term survival in AMI patients.

However early prediction of pathological remodelling and subsequent risk of adverse clinical outcomes following an AMI remain challenging. Clinical studies identifying patients who experience an excessive or prolonged pro-inflammatory state following an AMI are sparse, in part owing to limitations on what biomarkers to measure, and when to measure them.

Neutrophils are the first immune cells activated and recruited to the infarct site and play an important role as key mediators of the initial inflammatory response. Once the pro-inflammatory response to the acute tissue injury has accomplished the necessary wound clearance, reparative processes – that are poorly understood relatively – are initiated for the suppression of inflammation and the commencement of tissue repair and cardiac remodelling. Currently, very little is known about the neutrophil in this setting, and there is no information on how the neutrophil phenotype changes throughout the acute phase of an AMI, nor is there clear evidence of the implication of prolonged neutrophil activation on the transition from the 'pro-inflammatory' to the 'pro-reparative' phase.

Objective: The objective of this study is to assess the variation that may exist in neutrophil phenotypes at different time points in the acute phase of a myocardial infarction (MI). We aim to examine how the neutrophil phenotype changes between the initial 'pro-inflammatory' phase of an MI to the 'pro-reparative' phase that occurs at a later time point. It is possible that this switch between 'pro-inflammatory' and 'pro-reparative' is delayed in some

patients, and so the proportion and characteristics of patients with a delayed pro-reparative phase will be described as they are of particular interest.

Potential significance: This study will help to understand the temporal variance in neutrophil phenotype patterns over the course of an AMI, and to understand the variance that exists between patients. Understanding what proportion of patients display a prolonged pro-inflammatory phenotype is an important stepping-stone to better understand inflammatory processes implicated in adverse remodelling and allow for the development of targeted interventions to reduce the risk of pathological remodelling in AMI patients.

Project Status: Patient recruitment began in April/May 2018 and is ongoing. We expect to be at our target study population within the next few months.

Blood samples are being processed and stored as recruitment continues.

Final experiments will be conducted in a batch-approach manner at the end of patient recruitment as this is best practice to avoid assay variability.

Optimisation for determining neutrophil phenotype has begun in a small subset of patients in order to confirm that this marker panel is suitable in this population.

Optimisation for cytokine measurement to define the ‘pro-inflammatory’ and ‘pro-reparative’ phases has been conducted in a small subset of patients to ensure that the multiplex technique is suitable in this population.

Intermittent hypoxia in preterm and term infants in the first year of life: effects on growth, and measures of heart rate variability [interim report]

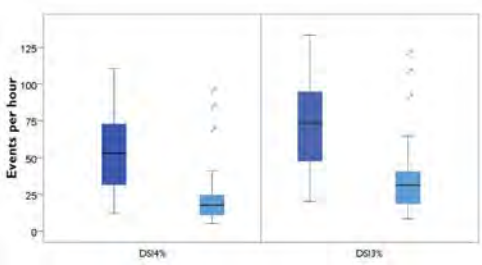
Principal Investigator: D Elder
Other investigators: A Campbell, P Larsen, C Niu (PhD student)
Paediatrics and Child Health, University of Otago, Wellington

Background: New generation oximeters provide much greater detail than previously available about brief drops in oxygen saturation (intermittent hypoxia) in preterm infants. This information appears concerning when first viewed clinically for a preterm infant who is otherwise thought to be well and ready for discharge home from the neonatal unit. This study aims to determine a normal range of values for measures of intermittent hypoxia (IH) for preterm infants in comparison to the same measures in healthy term infants over the first year of life.

Objectives: The research was designed to determine by 12-hour overnight oximetry the range of IH documented on discharge in preterm infants and determine the natural progression of IH in these infants throughout the first year of life in comparison with a group of healthy term infants measured regularly from 42 weeks postmenstrual age (PMA). The particular measures being addressed are the desaturation indices (DSI), specifically DSI3% and DSI4%. These DSI represent the number of events per hour for drops in oxygen saturation that are at least 3% or 4% below baseline.

Results: From Wellington and Lower Hutt hospitals between April and September 2017, 111 infants (55 preterm, 56 term) were recruited to the study. Of the 55 preterm infants, 49 had discharge studies done, followed by 42, 38, and 28 studies done at 42 weeks, 4 months, and

8 months PMA. Of the 56 term infants recruited, 47, 46, and 37 infants had overnight oximetry studies done at 42 weeks, 4 months, and 8 months PMA respectively.



Preterm infants born at 32-35 weeks' gestation showed improvement in IH as seen through the DSI3% and DSI4% between discharge from the neonatal unit and their follow-up at 42 weeks.

At 42 weeks PMA, median DSI4% and DSI3% for term infants (n=46) was 30.8 events/hour (IQR 22.1-35.4) and 50.8 events/hour (IQR 39.6-61.47), higher values than those seen in the preterm infants at 42 weeks PMA.

We have now completed follow-up studies for infants at 4- and 8-months PMA, with preliminary data showing continued improvement in DSI4% and DSI3%. At 4 months PMA, median DSI4% and DSI3% for preterm infants (n=38) was 8.1 and 18.35 events/hour respectively, and for term infants (n=46), 7.95 and 18.1 events/hour respectively. It appears that frequency of IH episodes between term and term infants were comparable at 4 months PMA, though further analysis is needed to account for other factors such as sex and ethnicity. At 8 months PMA, median DSI4% and DSI3% for preterm infants (n=28) was 6.8 and 19.75 events/hour respectively, and for term infants (n=37), 8.3 and 19.8 events/hour respectively. This suggests that preterm infants continue to improve, though it is interesting to see a slight increase in median DSI in term infants. As these data are preliminary, further analysis is needed. Follow-up studies are on-going to 12 months PMA

for recruited infants and are expected to finish in October 2018.

Conclusions: Our data confirm that IH is common in preterm infants ready for discharge from the neonatal unit and rates of IH decrease by 42 weeks PMA (about 3-4 weeks after discharge for some infants). Healthy term infants also exhibit IH in overnight oximetry recordings. When preterm infants at 42 weeks PMA were compared with healthy term infants at 42 weeks PMA the DSI3% and DSI4% levels were higher in the healthy term infants. This means we need to think again about whether the preterm infant rates of IH at neonatal discharge indicate pathology or whether they are developmentally normal for age and level of maturity. Follow-up at 4 months and 8 months shows continued decreases in the DSI3% and DSI4% values but not as marked a change as previously and we still need to determine if these later changes are statistically significant. These data allow us to describe the natural history of IH in these preterm and term infants using modern oximeters to record continuous oxygen saturation overnight in the home environment.

We look forward to sharing our further findings as the study continues and our data are further analysed. The project has expanded to The Children's Hospital of the Fudan University, Shanghai, China where 327 term and preterm infants were recruited by Dr Niu over a 3-month period.

Renal bicarbonate handling, cerebral perfusion and acute mountain sickness [interim report]

M Fan
Department of Surgery and Anaesthesia, University of Otago, Wellington

Background: Due to advanced transportation, millions of people are affected by acute mountain sickness (AMS) worldwide each year. Further, a significant portion of AMS cases can develop into life-threatening high-altitude cerebral oedema and pulmonary oedema. Each year, the number of New Zealanders traveling to high altitude regions (such as the Himalayas) continues to rise, thus placing more New Zealanders at risk of AMS. A better understanding of the role of the kidneys is paramount in the ongoing research in the prevention and treatment of AMS. Findings from this study may lead to early diagnosis and prevention for individuals who are susceptible to developing AMS, which has the potential to reduce incidences of AMS.

Aim: To examine the role of renal bicarbonate excretion on cerebral perfusion and the pathogenesis of acute mountain sickness during high-altitude exposure.

Progress to date: We have recruited 19 of the 30 participants for this study. Of those recruited, we have completed data collection and analysis of data from 13 participants. We expect to complete data collection by the end of October 2018, and complete data analysis by end of February 2019.

We have begun drafting the introduction and methods section of the two manuscripts.

Next steps: We aim to complete this study and submit manuscripts for publication by October 2019, with a report to Research For Life shortly thereafter.

Small non-coding RNAs as early biomarkers for colorectal cancer

K Danielson
Surgical Cancer Research Group, University of Otago, Wellington

Background: Colorectal cancer (CRC) is the most common form of cancer in New Zealand and kills 1200 per year. Early diagnosis of CRC is a critical factor in reducing mortality and the development of novel, non-invasive biomarkers for disease screening is of paramount importance.

Aims: This study examined expression levels of miR-21, miR-29a, and the 5' and 3' fragments of hY1 in the plasma of CRC patients and healthy controls to assess their utility as CRC biomarkers.

Study progress: CRC patients were stratified by stage so that we compared healthy controls (n=17) to early stage patients (Stage I/II; n=32) and those with advanced stage cancer (Stage III/IV; n=29).

Baseline plasma samples were collected from patients with colorectal adenocarcinomas at the time of diagnosis and CRC stage classification was based on histopathological evidence following tumour resection. Control patients were age- and sex- matched and had normal bowel confirmed by colonoscopy. RNA expression levels were measured by RT-qPCR with specific assays against the species of interest. RNA expression levels were quantified using the 2-ΔΔCq method against and exogenous (cel-miR-39) and an endogenous (miR-345) control and are presented as relative expression levels in the graphs below.

miR-29a expression was significantly different across patient groups (p=0.0035, one-way ANOVA) with statistically significant differences observed between

controls vs late stage CRC ($p<0.01$) and early vs late stage CRC ($p<0.5$) on post-hoc analysis (Tukey's multiple comparison). miR-21 expression trended towards statistical significance ($p=0.07$, one-way ANOVA), but no difference was seen between patient groups for the 5' and 3' hY1 fragments (Figure 1).

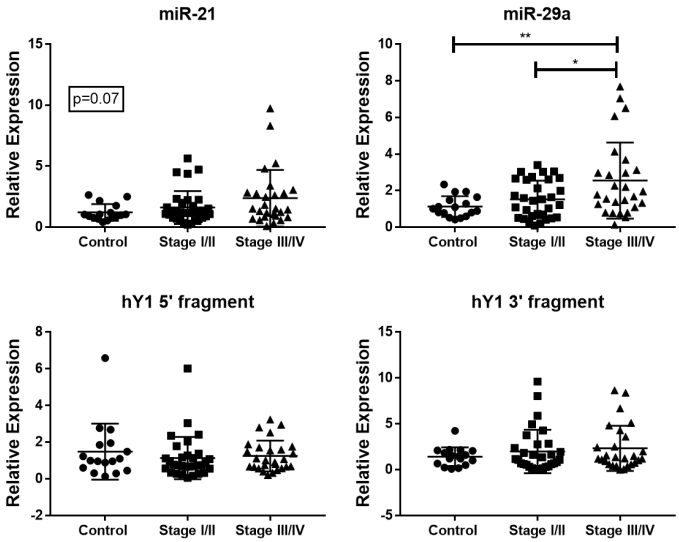


Figure 1: Patient group differences

Potential significance: This work suggests that plasma levels of miR-29a and miR-21 could be useful markers for distinguishing between early and late stage CRC and for discrimination of CRC patients vs controls. Future work will need to extend this analysis to a larger group of patients to validate these findings.

The identification of epigenomic biomarkers predictive of further cardiac events in young patients presenting with myocardial infarction (MI)

M Thunders, A Holley, S Harding, P Larsen
Department of Pathology, University of Otago, Wellington

Background: Globally, ischaemic heart disease is a major contributor to premature morbidity and mortality and is associated with significant economic burden. A significant proportion of new MI patients present as young patients, typically under the age of 55. A recent study by Matsis et al described the clinical characteristics of 1199 patients on the Wellington Acute Coronary Syndrome (ACS) registry. In this cohort 12.8% (154/1199) of the patients presented with MI as young patients. Of this group 36% had none or only one traditional risk factor for MI prior to presenting with their index coronary event and so would have been classified as 'low cardiovascular risk'. 10% of MI patients typically have further events (recurrent myocardial infarction, stroke or death) in the year following the index event. The identification of biomarkers which can assist in the identification and management of young MI patients at increased risk of poor outcomes is therefore needed.

Epigenomic biomarkers are increasingly used in medical diagnostics. Unlike DNA sequences, which are largely the same in every cell, epigenomic changes can occur as a result of dietary, behavioural and other environmental exposures, including physical and psychological stressors. Abnormalities in DNA methylation are associated with many diseases and manifest through inappropriate gene expression. Through comparative analysis of methylome data we can attain a clearer understanding of the molecular mechanisms that underlie pathology in different population subsets. Epigenomic profiling can provide informative biomarkers with potential for epigenomic

reversibility and an attractive and potentially lucrative target for clinical intervention.

Reduced Representation Bisulfite Sequencing (RRBS) combines bisulfite conversion and next generation sequencing (NGS) on an Illumina HiSeq 2000 sequencer to give a cost-effective alternative solution to whole genome methylation sequencing [11]. It covers 2.5% approximately of the human genome but it is highly enriched for promoter specific CG-enriched areas. This produces FASTQ sequence files that can then be analysed using Differential Methylation Analysis Package (DMAP). DMAP is a package developed specifically for the generation of reference methylomes and comparative analysis of RRBS data.

Aim: The aim of our study was to identify epigenomic biomarkers specific to young MI patients identified as 'low risk' who then subsequently experience recurrent events. The identification of such biomarkers would aid in understanding the pathogenesis in this subset of apparently 'low risk' MI patients and could potentially be used as predictors of disease risk and progression to aid in the management of these patients.

Methods: 16 patients with premature MI and who experienced recurrent ischaemic events (stroke or MI) were identified from the Wellington ACS registry. These patients were matched for age, gender, diabetic status and smoking status with 16 controls that had no adverse events within the year of their index event. As part of the ACS registry peripheral blood samples were obtained from all patients upon admission at their index coronary event. The ACS study was reviewed and approved by the Central Regional Ethics Committee (URA/11/05/2016). Patient consent was voluntary and included the use of samples for identification of biomarkers. DNA was extracted from the blood samples, assessed for quantity and purity and used for Reduced Representation Bisulfite Sequencing. The

DMAP bioinformatics tool was used to explore methylation differences in the sequence data between the two clinical groups. Reactome and GOrilla pathway databases were used to explore significant biological pathways common to the identified significantly differentially methylated genes in the patients with recurrent events.

Results: Within the DMAP package diffmeth was employed to analyse differential methylation. Across the group and within case-control pairs' variation were analysed using ANOVA and Fisher's Exact Test respectively. Results from across the group variation analysis identified several candidate genes that are of potential interest to cardiac pathology, for example, the genes DGAT1 and OSBPL5. DGAT1 encodes a protein that functions as a key metabolic enzyme and is potentially associated with obesity and other metabolic diseases. OSBPL5 encodes a protein believed to play a key role in the maintenance of cholesterol balance in the body. Pairwise comparisons across each matched case-control pair resulted in a list of genes that were consistently significantly differentially methylated between all 16 matched pairs. This gene list was input into Reactome and GOrilla pathway databases to see if particular molecular pathways were over represented in the group of patients with recurrent events. Of particular relevance to cardiac pathology the following pathways were identified as over represented in the patients with recurrent events; cell adhesion, platelet degranulation and cardiac electrical conduction, specifically Phases 2 and 3 of the cardiac action potential. The normal sequence of contraction of atria and ventricles of the heart requires activation of groups of cardiac cells controlled by change in action potentials. The action potential has 5 phases (numbered 0-4). Both Phase 2 and 3 sustain muscle contraction. Suppression of these phases could contribute to irregular heartbeat (cardiac arrhythmia) and recurrent ischaemic events. The fact that these biological pathways appear to be operating

differently in the patients with recurrent events points to these genes as key indicators for recurrent ischaemic events and further exploration in a larger dataset is now warranted.

Conclusion: The funds received were used to look at methylation differences between two populations of cardiac patients. Initial results suggest that there are significantly different methylation profiles between the two groups studied. Further analysis will determine whether these epigenomic differences can be used as biomarkers to predict further cardiac events in young cardiac patients presenting with MI.

Victoria University of Wellington

Aptabiotics: aptamer targeted antimicrobials for treating bacterial infections in Cystic Fibrosis [interim report]

D Day and J Soundy
School of Biological Science, Victoria University of Wellington

Background: Cystic fibrosis (CF) is a common genetic disorder that manifests as a multi-system disease that includes chronic pulmonary infections. The genetic defect in CF causes an abnormality in the cystic fibrosis transmembrane conductance regulator protein (CFTR) which results in dehydration of the airway surface, and consequent viscous mucus that is poorly cleared, which facilitates colonisation by pathogens. Infection by *Pseudomonas aeruginosa* is prevalent by the teenage years and is associated with significant morbidity. *P. aeruginosa* is an opportunistic gram-negative pathogen that produces a large number of potent virulence factors that induce inflammation and facilitate establishment of chronic infection. The high level of intrinsic antibiotic resistance,

the formation of biofilms, and differentiation to a mucoid phenotype, make treatment of *P. aeruginosa* infections highly problematic. The presence of mucoid *P. aeruginosa* is the most important risk factor for pulmonary deterioration, such that most CF patients succumb to the microbial infection. While nebulised tobramycin is effective in reducing the bacterial load in the lungs of CF patients to improve outcomes, it is unable to cure infections. Thus, there is an unmet need for effective antimicrobials for treating CF pulmonary infections, particularly for drugs that can circumvent existing antibiotic resistance mechanisms and target bacteria in biofilms.

Study objective and methods: To address this need, we have developed a DNA aptamer that specifically targets P. aeruginosa. Aptamers can be considered as *in vitro* selected chemical antibodies engineered to bind a target molecule or cell with high affinity. We have modified our aptamer by conjugating it with a therapeutic agent such that the aptamer is the delivery agent for a drug conjugate. The therapeutic agent we have attached is a DNA sequence that has been designed to scaffold metallic silver nanoclusters and/or silver ions by virtue of folding into a quadruplex i-motif structure. We call these aptamer drug conjugates aptabiotics. We have validated the antimicrobial action *in vitro* on both planktonic and biofilm grown cells of P. aeruginosa and have shown that the aptabiotics act rapidly to kill cells within minutes of administration and are able to sterilise a *P. aeruginosa* culture (Figure 1, A to C). Using the well-validated *Gallaria mellonella* invertebrate model of microbial infection13, we have demonstrated the efficacy of aptabiotics in prolonging survival in vivo (Figure 1, D).

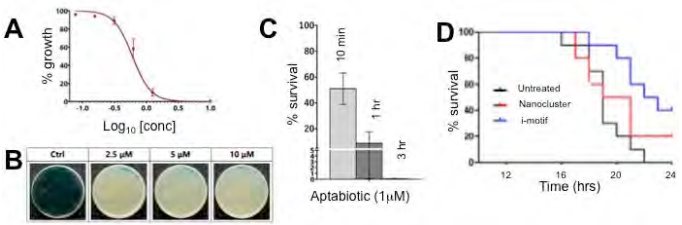


Figure 1. Aptabiotics are potent antimicrobials. (A) Aptabiotic St21Lp17 has an inhibitory concentration of 50% (IC50) = 0.6 μM, and a minimum inhibitory concentration (MIC) of 2.5 μM against *P. aeruginosa* strain PAO1.
(B) Enumeration of colony forming units (CFU) of PAO1 after 18 hr treatment resulted in no detectable colonies for concentrations ≥ 2.5 μM. (C) PAO1 cells are killed rapidly with only 0.09% surviving following 3 hr exposure.
(D) Survival of PAO1-infected *G. mellonella* larvae after treatment with aptamer-silver nanoclusters (red) or aptamer-silver i-motif (blue) compared with the infected, but not drug-treated larvae (black).

The aptabiotics also show potent activity in disrupting mature biofilms such that exposure of a mature biofilm to aptabiotic for 6 hours causes a 90% decrease in biomass relative to a matched untreated control biofilm (Figure 2, A). Importantly, using a checkerboard assay we have also shown our aptabiotics strongly synergise with Tobramycin (Figure 2, B), and unlike many antibiotics, the aptabiotics are not substrates the major efflux pumps in *P. aeruginosa*, thus avoiding a major antibiotic resistance mechanism (Figure 2, C).

Findings to date: Collectively, the present data demonstrate we have developed an antimicrobial that has potent activity against P. aeruginosa, kills rapidly, disrupts biofilms, is not effluxed, is efficacious in prolonging survival in an invertebrate model of infection, and synergises with tobramycin.

Potential significance: We hypothesise that a combination therapy of nebulised tobramycin and aptabiotics will be highly effective in the treatment of *P. aeruginosa* respiratory infections, and that our aptabiotics will prove

invaluable in treating infections in the lungs of cystic fibrosis patients.

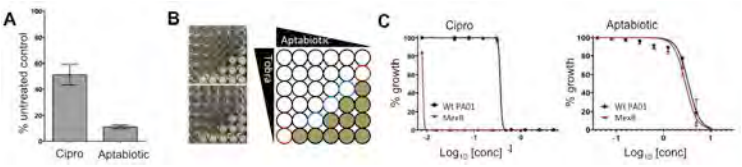


Figure 2. (A) Mature biofilms (44hr) show a 90% reduction in biomass when exposed to aptabiotics at MIC for 6hr, compared with a 50% reduction for ciprofloxacin exposure at MIC. (B) Serial 2-fold dilutions of aptabiotics (left to right) and Tobramycin (top to bottom) show strong synergy (FIC, blue circles). Growth, filled circle; no growth, clear circles; MIC, red circle. (C) *P. aeruginosa* PA01 and an isogenic knock of the efflux pump MexB were compared for susceptibility to ciprofloxacin and aptabiotic St21Lp17. The efflux mutant is more susceptible to cipro but not St21Lp21 indicating St21Lp17 is not an efflux pump substrate.

Assessing early immune cell inflammatory responses to MIS416 in secondary progressive multiple sclerosis [interim report]

C Beyers
Centre for Biodiscovery, Victoria University of Wellington

Background: Our work on MS is particularly important regionally in Wellington as there is a high incidence of multiple sclerosis (MS) in our community. In progressive MS, the peripheral

immune system differs to that of healthy individuals in terms of cell subset composition and number.

Objectives: The project aims to apply flow cytometry to assess cellular function by profiling blood cell responses to treatment on a cohort of patients with secondary progressive multiple sclerosis (SPMS) in our tissue bank. The output measures from this work are cytokine

production, and changes to the expression of regulatory and cellular activation markers on the cell surface.

We have a custom designed antibody panel to show cytokine production and surface expression in peripheral blood mononuclear cell (PBMC) subsets, as well as laboratory protocols optimised for this work.

Findings to date: Our validation work to date has shown proof of concept: profiling cell function in patients with Secondary Progressive MS (SPMS) using cytometry methods is a feasible and appropriate application. We are on track to conclude our experimental validation work early next year.

Project status: The early investment from Research for Life has facilitated our experimental validation and is allowing us to get the best use out of valuable clinical trial samples. We are now poised to apply our methods to acquiring data that we hope will aid in our understanding of SPMS pathology and treatment responses.

We are also excited to report that we have secured access to a new platform for our data collection, with excellent support from the Hugh Green Cytometry Core facility at the Malaghan Institute of Medical Research who have offered us access to their spectral cytometer. Using this platform adds a very novel approach to our methods that we hope will enable new approaches to clinical trial sample analysis in the future.

The project was presented at the 14th International Society of Neuroimmunology Congress in Brisbane in August 2018, with the organisers offering support for an oral presentation.

How do platelets change the immune response to infectious agents?

K Hally, A La Flamme, S Harding and P Larsen
Clinical Research Laboratory, School of Biological Sciences, Victoria University of Wellington

Introduction: Platelets are multi-functional cells that, aside from their role in thrombosis, are increasingly appreciated as mediators of vascular inflammation. Emerging research indicates that platelets have dual roles in activating but also limiting host immune responses. Toll-like receptors (TLRs) are the first-line defence against infectious and injurious 'danger' signals, and we hypothesize that platelets may limit inflammation by regulating aspects of leukocyte function in response to TLR stimulation.

Aim: to determine the extent to which platelets modulate peripheral blood mononuclear cells (PBMC) and granulocyte responses in vitro to TLR4, TLR2/1, and TLR2/6 stimulation in healthy subjects. Specifically, to examine how neutrophil and monocyte activation in response to TLR stimulation changes following platelet co-culture; to examine how granulocyte elastase production in response to TLR stimulation changes following platelet co-culture; and to examine how patterns of PBMC cytokine production change in response to TLR stimulation following platelet-co-culture.

Methods:

Cell isolation and culture. PBMCs and granulocytes from 10 healthy volunteers were either cultured alone or co-cultured in a 1:250 ratio with platelets. Cultures were left unstimulated or stimulated with 1-100 ng/mL of LPS (TLR4 agonist), Pam3CSK4 (TLR2/1 agonist) and FSL-1 (TLR2/6 agonist). Granulocytes ± platelets were cultured for 4 hours and PBMCs ± platelets were cultured for 24 hours at

37°C/5%CO_2. Following stimulation, cell culture supernatant was collected and stored.

Flow cytometry. Neutrophil activation (CD66b expression) and monocyte activation (HLA-DR expression) was assessed by flow cytometry.

Analysis of cell culture supernatant. As a marker of activation, granulocyte elastase production was measured with ELISA. The following cytokines and chemokines were measured in PBMC supernatant by multiplex ELISA: TNF-α, IL-6, IL-10.

Statistical analysis. Data assessing the effect of platelet co-culture are presented as relative change. Differences between measurements were examined using paired t-tests.

Selected results:

Neutrophil activation in response to TLR stimulation is reduced in the presence of platelets. The addition of platelets did not reduce CD66b expression in unstimulated neutrophil cultures, but platelet co-culture differentially reduced neutrophil activation in response to stimulation with all three TLR agonists (Figure 1A). The increase in CD66b expression seen in response to various doses of TLR stimulation was reduced by 11-19% in the presence of platelets.

Platelets reduce monocyte activation in response to low-dose LPS. Following co-culture with platelets, the increase in HLA-DR expression in response to low-dose LPS was reduced by 5% (Figure 1B). This was the only culture condition where the presence of platelets significantly changed monocyte HLA-DR expression.

Platelets reduce granulocyte elastase release in response to low-dose TLR stimulation. Platelets reduced elastase release in response to low doses of each TLR agonist (Figure

2). The increase in elastase production was reduced by 17-21% in response to low-dose TLR stimulation.

Platelets differentially modulate PBMC cytokine and chemokine production in response to LPS and FSL-1, but not Pam3CSK4. The production of IL-6, TNF-α, and IL-10 was assessed for PBMC ± platelet cultures in response to TLR stimulation. In platelet co-cultures and in response to LPS, there was a significant reduction in IL-6 and TNF-α production (14-19%), and increased IL-10 production (19%) by PBMCs (Table 1). In response to FSL-1 stimulation, platelet co-culture increased IL-6 and IL-10 production by 37-62% and 5-14%, respectively, and TNF-α production was reduced by 16-19% in co-culture (Table 1). Platelet co-culture did not alter PBMC cytokine production in response to Pam3CSK4.

Conclusions: In this study, we showed that platelets differentially regulate leukocyte responses to TLR stimulation in a TLR agonist-specific manner. Platelets suppress neutrophil CD66b expression in response to a range of TLR agonists and suppress monocyte HLA-DR expression only in response to low-dose LPS. In modulating certain leukocyte activation patterns, platelets may limit inflammation and, ultimately, limit host damage. Interestingly, the presence of platelets results in a complex modulation of PBMC cytokine and chemokine production. Broadly, these collective results suggest that platelets can push PBMCs away from pro-inflammatory, and towards anti-inflammatory, cytokine production in response to LPS. Combined, these results suggest a dynamic platelet effect on leukocyte activation in response to TLR stimulation.

We are currently pursuing publication of this research, and this research was shared with the scientific community at the Australasian Society for Immunology (ASI) Annual Scientific Meeting in November 2017.

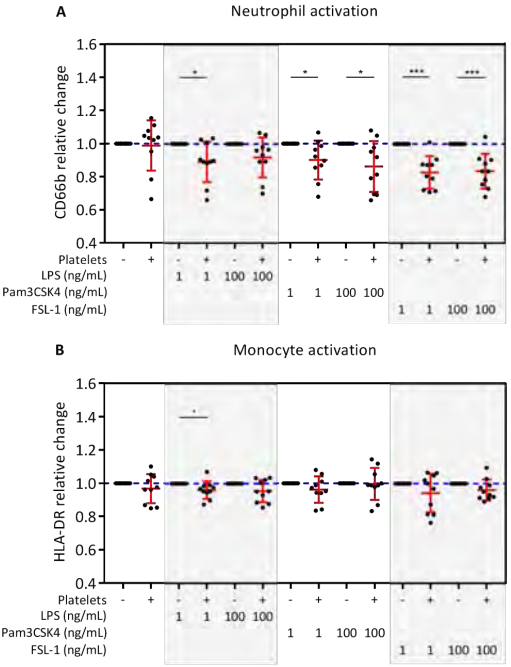


Figure 1: Platelets reduce expression of neutrophil CD66b expression (A) and monocyte HLA-DR expression (B) following TLR stimulation. Expression of these activation markers was measured by flow cytometry in leukocyte only cultures and co-cultures with no stimulation and in response to 1 and 100 ng/mL LPS, Pam3CSK4 and FSL-1. Differences between these relative change measurements were examined using paired t-tests. *p<0.05, ***p<0.001.

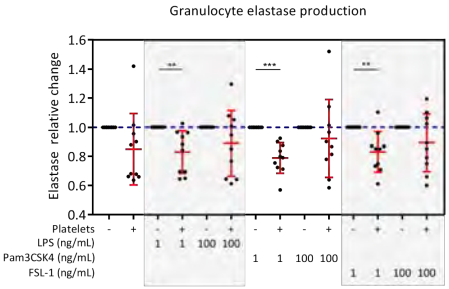


Figure 2. Granulocyte elastase production in response to low-dose TLR agonism decreased with platelet co-culture.

		+ platelets[#]		
Agonist	ng/mL	IL-6	TNF-α	IL-10
Unstimulated	--	0.94 (0.30)	0.95 (0.38)	1.04 (0.26)
LPS	1	0.86 (0.14)**	0.81 (0.09)****	1.02 (0.19)
	100	0.86 (0.16)*	0.85 (0.13)**	1.19 (0.17)**
Pam3CSK4	1	1.25 (0.66)	0.85 (0.26)	1.08 (0.11)
	100	1.24 (0.36)	0.90 (0.15)	1.19 (0.33)
FSL-1	1	1.62 (0.73)*	0.84 (0.09)***	1.05 (0.07)*
	100	1.37 (0.51)*	0.81 (0.16)**	1.14 (0.15)*

Table 1. Relative change in PBMC cytokine production in PBMC-platelet co-culture(+platelets) following TLR stimulation.

[#]all leukocyte only measurements were normalized to 1, and all co-culture (+ platelets) measurements were compared to this normalized response. Differences between these measurements were examined by paired t-tests. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Investigating the therapeutic potential of MP1104 for the treatment of neuropathic cancer pain [interim report]

B Kivell and D Atigari
School of Biological Sciences, Centre for Biodiscovery, Victoria University of Wellington

S Manjumdar
Memorial Sloan-Kettering Cancer Centre, New York

Background: Common chemotherapy drugs such as paclitaxel used to treat breast, ovarian, lung, head and neck cancers can cause serious side-effects that can lead to decreased dose or discontinuation of treatment and reduce effectiveness of therapy. Cancer chemotherapy induced neuropathic pain (CINP) includes pain, numbness, tingling and increased sensitivity to touch and temperature. It is estimated that 30–40% of patients receiving chemotherapy develop CINP. Unfortunately, there are few effective treatments, and CINP can persist for months after chemotherapy treatment.

In our laboratory we have previously shown a novel chemical called MP1104 has high potency and efficacy in acute pain models including thermal and inflammatory pain models. MP1104 is a potent, long-acting analgesic that activates both kappa- and delta-opioid receptors. We hypothesise that mixed opioid drugs like MP1104 may be effective in modulating CINP.

Methods: We utilise a preclinical model of CINP to evaluate pain responses to mechanical force using von Frey filaments. The force that elicits limb withdrawal is recorded over time during CINP (Figure 1a). We also evaluate the time spent responding to a cold stimuli (acetone) (Figure 1b). Utilising this model, the chemotherapy agent paclitaxel elicits long-lasting pain responses after 8 days that is stable after 15 days. This confirmed that the model is similar

enough to human CINP that it can be used to evaluate the effect of MP1104.

Figure 1

The Chemotherapy Drug Paclitaxel Induces Neuropathic Pain

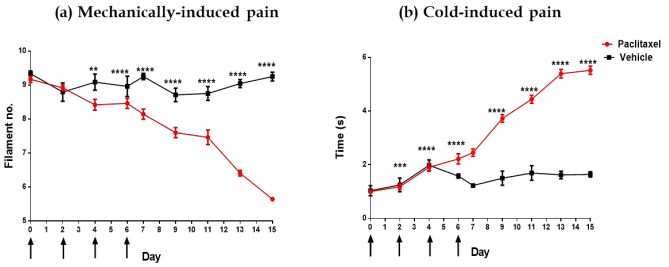
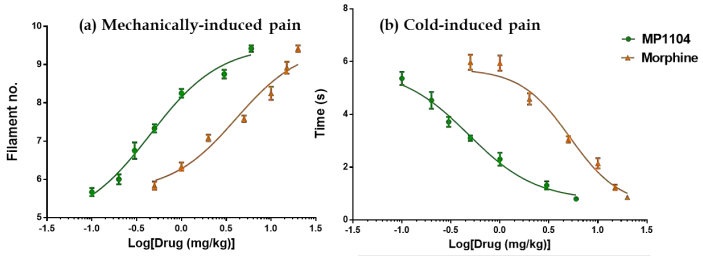


Figure 1: The effect MP1104 agonist on paclitaxel-induced neuropathic pain. (a) Paclitaxel treatment resulted in withdrawal responses to a lower filament number. (b) Paclitaxel treatment increased the time responding to acetone stimulation. Two-way repeated measures ANOVA followed by Bonferroni post-test. Arrows indicate timing of paclitaxel or vehicle injections.**p < 0.01, ***p < 0.001, ****p < 0.0001 for paclitaxel treated animals compared to vehicle-treated.

Findings: In this study we showed that acute administration of MP1104 is more potent than morphine in reducing sensitivity to mechanical and cold stimuli. Our evaluation of dose response effects reveals that MP1104 is 8- to 10- fold more potent than morphine in reducing CINP (Figure 2).

Figure 2

Effects of Acute Administration of MP1104 on CINP



Drug	ED_{50} value (mg/kg)
MP1104	0.45
Morphine	4.07

Drug	ID_{50} value (mg/kg)
MP1104	0.47
Morphine	5.18

Figure 2: Dose-response effects of MP1104 and morphine mechanical and cold allodynia (n = 6 per group). MP1104 displays greater potency than morphine.

Because medications to treat CINP would be likely to need to be administered repeatedly over time to treat this chronic condition, we also evaluated the effectiveness of MP1104 following daily administration following stable induction of CINP (Day 15). Figure 3 shows MP1104 (1.2 mg/kg) has long-lasting effects in reducing mechanical and thermal to control levels. In contrast morphine (10 mg/kg) was only effective for 10-15 days before pain was back to non-treated CINP levels.

Figure 3

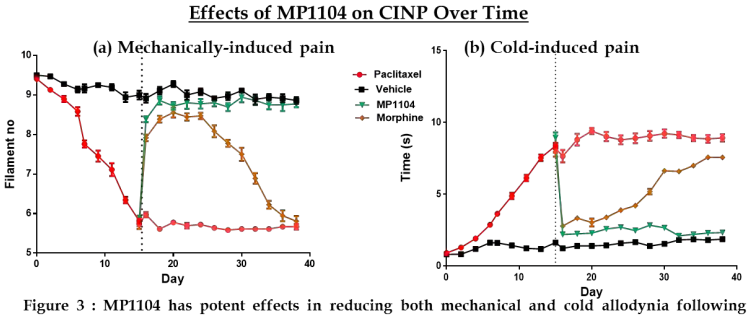


Figure 3 : MP1104 has potent effects in reducing both mechanical and cold allodynia following chronic treatment in mice and unlike morphine did not show significant analgesic tolerance in mice following repeated administration. .****p < 0.0001 compared to paclitaxel control. (n=9 per group).

In summary we have shown MP1104 is also effective in modulating CINP in preclinical models and that MP1104 does not exert tolerance to the analgesic effects.

Research for Life funding covers the cost of reagents and consumables for this project.

Further work: Future experiments will evaluate tissue for cellular changes to begin to elucidate potential mechanisms underlying these therapeutic effects.

The role of the apelinergic system in glioblastoma multiforme

V Venkatesh
Victoria University of Wellington

Aim: Understanding the molecular signalling circuitry that underpins the pathogenesis of glioblastoma is critical to understanding and developing effective treatments for this recalcitrant tumour. The apelinergic system is a signalling pathway primarily consisting of the apelin peptide (APLN) and the apelin receptor (APLNR). The apelinergic system has been implicated in the pathophysiology of several cancers. This project sought to investigate the potential roles of the apelinergic system in glioblastoma.

Findings: Firstly, we have determined that mRNA expression of APLN but not APLNR is upregulated in glioblastoma compared to normal brain tissue. However, APLNR expression is still present in equal amounts to normal brain tissue, which was confirmed in publicly available datasets. In public datasets we also observed APLN and APLNR expression was higher in grade IV glioblastoma than lower grade tumours. These data suggest the apelinergic system may have a role in glioblastoma.

Secondly, we sought to investigate the expression of APLNR in the GL261 mouse model of glioblastoma. Very low expression of APLNR was found in the GL261 tumour tissue contrary to our finding that APLNR mRNA was expressed in human glioblastoma. This lack of expression in GL261 tumours may suggest that model selection is critical for the research of the apelinergic system in glioblastoma and further studies are required to validate this finding.

Finally, an *in vitro* cellular model of the apelinergic system in glioblastoma has been developed and validated.

Capital and Coast District Health Board

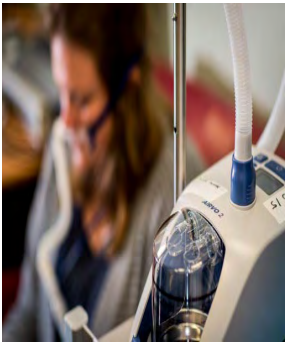
Comparing the effect of nasal high flow (NHF) therapy with non-invasive ventilation (NIV) on PaCO2 in chronic obstructive pulmonary disease patients with chronic respiratory failure

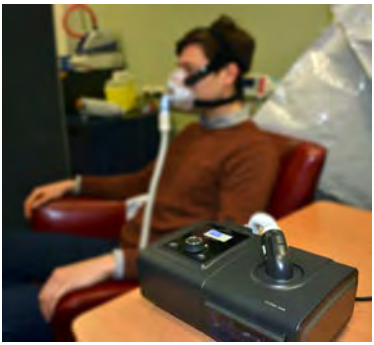
S McKinstry and J Fingleton
Capital and Coast District Health Board

Background: Chronic obstructive pulmonary disease (COPD) is a significant health problem in New Zealand and worldwide. COPD affects 14% of adults aged over 40 in New Zealand and is responsible for over 12,000 hospital admissions every year. Māori and Pacific people carry a higher burden of disease. Acute exacerbations of COPD (AECOPD) requiring hospitalisation are associated with high healthcare costs, reduced quality of life and shorter survival. Around 20% of people presenting with AECOPD develop acute hypercapnic respiratory failure (AHRF), which is a rise in arterial carbon dioxide tension (PaCO2) due to the inability of the patient to expire carbon dioxide. Patients who develop AHRF are more likely to need to go to the intensive care unit (ICU) and are more likely to die. The hospital mortality of patients with AECOPD and ARHF is 12%.

Although there are no New Zealand guidelines on management of AECOPD, British guidelines on the ventilatory management of AHRF recommend that patients with AECOPD and AHRF should have an initial trial of 60 minutes with steroids, titrated oxygen and nebulised bronchodilators. About 20% of patients will improve with this treatment, however, about 80% will require more intensive forms of treatment such as non-invasive ventilation (NIV). NIV is airway and ventilatory support that does not use an endotracheal tube. Appropriate use of NIV reduces the need for subsequent intubation, hospital

length of stay and mortality in patients with AECOPD. However, although less invasive than intubation, NIV requires its own set of equipment, trained staff, close patient supervision, and regular arterial blood gas testing to monitor progress and appropriate adjustments to NIV machine settings. In addition NIV is very poorly tolerated by many patients and there is an unmet need for a well-tolerated, easy to administer treatment to reduce the need for NIV.





Examples of nasal high flow (left) and non-invasive ventilation (right)

Nasal High-flow (NHF) therapy is a gas delivery system that provides heated and humidified air by nasal cannulae. Air administered in this way can be supplemented with oxygen as required. NHF oxygen therapy reduces mortality in comparison to standard oxygen therapy in ICU patients with hypoxic respiratory failure. NHF therapy is also reported to reduce the risk of re-intubation after ICU therapy and after abdominal surgery. To date NHF therapy has not been investigated in a controlled trial in people with COPD with AHRF.

NHF therapy may reduce hypercapnia and the work of breathing and could reduce the need for NIV in AECOPD. Three clinical trials in patients with lung disease show that NHF therapy with oxygen can reduce CO2 levels compared to standard oxygen therapy. Further research at the

Medical Research Institute of New Zealand (MRINZ) shows that NHF therapy is well-tolerated, reduces PaCO2 in patients requiring supplemental oxygen, and in stable COPD causes a modest reduction in PaCO2 and a significant reduction in work of breathing.

Method: With support from the Wellington Medical Research Foundation, we have completed a 24-patient randomised controlled two-way crossover trial in patients with COPD who have chronic hypercapnic respiratory failure. The trial directly compared NHF for 60 minutes, via the myAIRVO 2 device with NIV in the form of bi-level positive airways pressure (BiPAP) support for 60 minutes, in line with current BTS guidelines regarding recommended settings. We used the funding to purchase: 1) a state of the art NIV device (Respironics BiPAP AVAPS-ST 60 Series); 2) NIV consumables (masks and tubing) 3) Sentec (transcutaneous measuring device) consumables (gas cannisters, ear clips, probe membranes, gel).

Results: NIV reduces PtCO2 by more than NHF therapy in hypercapnic COPD, but NHF is better tolerated. NHF should be considered when patients cannot tolerate NIV. The study results suggest a possible role for NHF in patients who do not tolerate NIV. We think this will guide clinicians in the use of NHF in hypercapnic COPD while we await a trial looking at NHF in acute hypercapnic COPD.

Understanding the utilisation of protein in a restricted carbohydrate environment in Type 1 diabetes

J Krebs, J Arahill, P Cresswell, M Weatherall, A Parry-Strong
Capital and Coast District Health Board

Summary: This randomised controlled cross-over study compared post-prandial glucose concentrations and incidence of hypoglycaemia for mealtime bolus insulin calculated for both meal protein and carbohydrate content with ordinary dosing for carbohydrate content alone in adults with type 1 diabetes who usually follow a carbohydrate-restricted diet (less than 100g carbohydrate per day).

Objective: Does addition of bolus insulin adjusted for both protein and carbohydrate meal content reduce post-prandial glucose concentrations and avoid excess hypoglycaemia in individuals with type 1 diabetes habitually consuming a carbohydrate restricted diet.

Methods: In this randomised controlled cross-over trial, 16 participants with type 1 diabetes performed six standardised meal tests measuring serum glucose over 180 minutes. For three of the meal tests they received a bolus insulin dose for carbohydrate content alone and the other three for both carbohydrate and protein. The order of the sets of three treatments was randomised and each participant received both interventions.

All 16 participants completed three test meals under each of the two conditions. Participants were NZ European predominantly; 8 men and 8 women, with an average age of 39 years. The average duration of diabetes was 23 years, and the average daily insulin dose 37 units.

Results: The primary outcome was the time normalised Area Under the Curve (AUC) of glucose measurements. The

mean (SD) AUC glucose concentration for insulin dosing for both protein and carbohydrate was 8.3 (2.1)mmol/L compared with 10.0 (2.2)mmol/L for carbohydrate alone. The difference (95% CI) was -1.76mmol/L (-2.87 to -0.65) P=0.003. The mean (SD) glucose concentration ≥8.0mmol/L was 54.8 (32.4)% for dosing for protein and carbohydrate and 73.7(26.3)% for carbohydrate alone, rate ratio (95% CI) 0.75(0.62 to 0.89), P=0.002. For glucose concentration <4.0mmol/L 5.5 (15.1)% and 2.8 (11.7)%; rate ratio (95% CI): 1.97 (0.90 to 4.27), P=0.087.

Conclusion: In people with type 1 diabetes habitually consuming a carbohydrate restricted diet, the addition of mealtime bolus insulin based on an insulin:protein ratio, to that bolus dose already estimated by the carbohydrate ratio, reduced postprandial hyperglycaemia without a marked risk of hypoglycaemia.

Project Grants 2018

The following projects were approved for funding in May 2018 and will be reported on in subsequent Research Reviews.

Georgina Bird

Victoria University of Wellington

Characterisations of B cells in acute myocardial infarction

The purpose of this study is to characterise what a specific immune cell, the B cell, is doing during a heart attack, with a view to understanding whether B cells play a pathological role in heart attacks. Georgina is a PhD student at Victoria University and is a part of the Wellington Cardiovascular Research Group. Her research focuses on cardiovascular immunology.

Dr Kirsty Danielson

University of Otago, Wellington

Extracellular vesicles as biomarkers of disease

Dr Danielson received a grant towards the purchase of an ultracentrifuge which will be used to isolate and study membrane-enclosed packages called extracellular vesicles that are released by cells in the body. These packages act as a communication system between cells and are being increasingly studied as biomarkers for disease.

Dr Janet Pitman and Ms Sarah Sczelecki

Victoria University of Wellington

Dissecting the genetic regulation in early phenotypes of ovarian cancer

Ovarian cancer has the highest mortality rate of all gynaecological cancers due to the lack of symptoms in patients and screening tests for early detection. This

research will explore genetic changes throughout tumour development using representative animal models, with the aim of identifying potential biomarkers to establish a pre-screening method for early detection. This will facilitate early diagnosis and potentially improve ovarian cancer mortality rate.

Associate Professor Wayne Patrick

Victoria University of Wellington

Resistance is futile: investigating collateral sensitivity to combat antibiotic resistance

This is a war, and one that humankind is losing. Microbes have now evolved resistance to every available class of antibiotic. Rather than focusing on the costly development of new antibiotics, this work will investigate an alternative strategy that manipulates an aspect of bacterial evolution known as collateral sensitivity.

Dr Joanna MacKichan

Victoria University of Wellington

Keeping intruders at bay: investigating a novel mechanism of bacterial inhibition of wound healing

Neisseria meningitidis is a bacterial pathogen that is usually carried asymptomatically in the upper airway tissues, but occasionally can cause severe, invasive meningococcal disease. The early interactions between the bacteria and the host mucosal airway tissues remain poorly understood, even though it can lead to invasive disease or transmission of the pathogen to new hosts. We are studying a bacterial protein that binds host haptoglobin and exploring whether this protein influences the development of invasive disease by compromising host wound repair. We will also investigate whether bacterial binding of haptoglobin can

enhance bacterial resistance to the antimicrobial effects of host haptoglobin.

Dr Katherine Woods

Malaghan Institute of Medical Research

Functionally defining human mucosal associated invariant T (MAIT) cell subsets ex vivo

To undertake studies in a newly described subset of human immune cells, called MAIT cells. MAIT cells are involved in protection from a range of human diseases, including bacterial and viral infections, autoimmune diseases, and cancer. Several different subtypes of MAIT cells have been described in humans, and we don't yet know the role that each of these play in different human illnesses. By comparing how these MAIT cell subtypes are activated and blocked in experimental models, we can figure out which MAIT cells are most important for protection from different diseases.

Lisa Denny

Victoria University of Wellington

Evaluating the mode of action of novel remyelinating compounds in the treatment of multiple sclerosis

MS occurs when the body's immune system recognises the protective myelin sheath of neurons in the central nervous system (CNS) as foreign and launches an attack, leading to severe neurological deficits. Lisa Denny's research is investigating new targets for an effective treatment option that functions to drive the repair of the damaged myelin sheath of the CNS.

Dr Darren Day

Victoria University of Wellington

Enhancing aptamer selection for biomedical applications

Aptamers are a type of drugs that act like antibodies that are finding great use in biomedical applications for diagnostics and as therapeutics. The research will explore new ways of developing aptamers for treating bacterial infections using a cross-disciplinary approach that uses recent advances in computer science and molecular biology.

Travel Grant Reports

The following travel grant reports were received during the year:

D Atigari

National Institute on Drug Abuse (NIDA) International Forum, San Diego, 2018
College on Problems of Drug Dependence (CPDD) Annual Scientific Conference, San Diego, 2018 International Narcotics Research Conference (INRC), San Diego, 2018

At the CPDD and the joint NIDA International Forum/CPDD international research posters' session, I presented my poster titled 'Anti-cocaine and analgesic properties of MP1104, a mixed kappa and delta opioid receptor agonist in rats'. I presented that MP1104 has a longer duration of action compared to the gold-standard analgesic morphine and has anti-cocaine effects with no aversive, pro-depressive nor anxiogenic effects. I also highlighted the new concept of including a delta agonist component to negate the aversive like side-effects of pure kappa-opioid receptor agonists. I presented my exciting research findings

to an international audience specialised in research related to drug addiction and abuse.

At the INRC meeting, I presented a poster titled 'Investigating the anti-addiction and chemotherapy-induced neuropathic pain effects of MP1104, a novel mixed kappa and delta opioid receptor agonist in rodents'. To this specialised audience on opioid research, I presented that MP1104, a mixed opioid agonist, is effective and potent in modulating chemotherapy-induced neuropathic pain in preclinical models compared to morphine. I also showed that chronic administration of MP1104 has reduced mechanical and thermal pain with effects that last longer, moreover MP1104 does not exert tolerance to the analgesic effects compared to morphine.

Attending these conferences put me in touch with some of the major scientists in this area and speaking with them gave some valuable feedback for my research. As a final year PhD student, being at these conferences gave me a chance for networking and looking for post-PhD career opportunities.

Overall, these conferences were packed with a variety of cutting-edge science and data, but it was also a very enlightening and enjoyable experience. My PhD research is likely to be disseminated with two international peer-reviewed publications.

C Beyers

Australasian Society for Immunology (ASI) Conference, Brisbane, 2017

The forum was highly informative, and I had the opportunity to meet and hear from speakers who are leaders in immunological research in the Australasian region and internationally.

I presented a poster of my data where we performed whole blood immune phenotyping on blood samples from patients with progressive multiple sclerosis (MS). Our work in MS is particularly important regionally in Wellington as there is a high incidence of MS in our community. The data I presented detailed how, in progressive MS, the peripheral immune system differs to that of healthy individuals in terms of cell subset composition and number. We showed that in a healthy control cohort there is great variation in the makeup of the peripheral immune system, with inter individual variation being high while intra individual changes over time remain relatively stable. This data builds towards our knowledge base of MS pathology and allows us to develop and test new therapies in patients who suffer from this untreatable form of MS.

The poster session I presented in was very well attended with nearly 600 delegates. I had many researchers show interest in our work and many commented on the high quality of the methods. This allowed me to raise awareness of our laboratory, Victoria University, and Research for Life who all support this area of clinical research.

M Calcott

Natural Products and Synthetic Biology: Parts and Pathways, California, 2018

My research is about synthetic biology approaches to drug discovery. I presented a poster of my work on a metagenomic survey of the natural product diversity of lichen associated bacteria within New Zealand.

Natural products produced by bacteria have been our most important source of antibiotics, however many environments have been exhaustively searched in drug screening programs. Lichens have been largely overlooked

as a source of bacteria in drug screening programs. My results found that lichen associated bacteria are a promising source of new bioactive compounds. My poster was well received, and the conference was exceptionally valuable for networking due to its narrow subject.

J Chandler

Molecular and Cellular Biology of Helminth Parasites: Hydra, 2017 and lab visit at Centre d'Immunologie de Marseille-Luminy, Marseille, France

Molecular and Cellular Biology of Helminth Parasites: Hydra, Greece: This conference was a consortium of parasitologists, immunologists and geneticists. The broad range of disciplines, all centralised around helminth parasites, gave an excellent overview of the current state of the field as a whole. I was given the opportunity to present my research to an audience of world-renowned scientists. This paved the way for constructive discussions around my work in which useful ideas were generated which I am excited to pursue now that I am back in New Zealand. On a personal note presenting at my first international conference was a milestone of my career and significantly boosted my confidence in delivering oral presentations. In addition, I formed a potential collaborative relationship with Professor Andy Fraser from the University of Toronto regarding the metabolic profile of our parasitic worm, *Nippostrongylus brasiliensis*. Furthermore, the networking opportunities gained from this conference were invaluable. I met with many influential scientists, and up and coming peers completing their PhD studies. Forming these relationships is crucial for a successful scientific career and I am very thankful to have had this opportunity in which I can begin to establish such international networks.

Centre d'Immunologie de Marseille-Luminy: Marseille, France: This lab visit allowed me to visit our collaborators Professor Jonathan Ewbank and Professor Nathalie Pujol. Their work focuses on the non-parasitic model animal, *Caenorhabditis elegans* and its immune response to a fungal infection. Both Jonathan and Nathalie visited the Malaghan Insititute on a sabbatical from January-July 2017 and worked with us to adapt their *C.elegans* techniques to our mouse model of human hookworm, *N.brasiliensis*. This collaboration proved extremely successful as we developed a new platform in which we can investigate genetic aspects of *N.brasiliensis*, which we were previously ill-equipped to do so. At the Centre d'Immunologie de Marseille-Luminy they have equipment and tools specialised for molecular analysis of nematodes, including a worm sorter and a microinjector. The worm sorter allows physical sorting of a population of worms based on size and fluorescent tags, similar to our cell sorter at the Malaghan Institute. I was able to see this machine and view what it was capable of. The micro-injector is a specialised mechanical needle attached to a microscope that allows injection of foreign DNA into a worm, that then gets incorporated into its genome, generating a transgenic worm. This machine would allow us to delete or insert certain genes of interest into our parasitic worms, thus providing an cohesive technique to investigate genes of interest of *N.brasiliensis*, improving our understanding of how parasitic worms interact with their host. In addition, I presented my research to Professor Jonathan Ewbank's lab group and got their feedback on this work which was beneficial.

L Connor

46th Annual Scientific Meeting of The Australasian Society for Immunology, Brisbane, 2017

I was fortunate to present my research on the induction of Th2 immune responses by Dendritic cells at the poster session. I had insightful and helpful discussions with prominent immunologists also working on Dendritic cells. I had a fantastic discussion with Dr Shalin Naik, a highly successful newcomer, who has been developing novel techniques to track Dendritic cell fate using barcoding technologies. I was also selected to chair a session in the infection and immunity workshop, this provided a great opportunity for public exposure.

The keynote talks were outstanding, I particularly enjoyed Professor Federica Sallusto from University of Lugano, Switzerland. She presented some beautiful work examining the function of human T cells. Her laboratory has extensively characterised T cell fate and function in human disease. Professor Sallusto is a pioneer in her field, identifying critical markers expressed by T cells that can be used as reliable surrogates of human T cell function. Professor Shane Crotty from La Jolla Institute, USA presented a very interesting talk on neutralising antibodies against HIV. The development of antibody-based vaccines for the protection against HIV has been hampered due to the ability of HIV to escape immune recognition. Professor Crotty and his team show that the immune system can detect HIV neutralizing epitopes, but they are incredibly rare. To overcome this issue, vaccine strategies must compensate by utilizing high affinity immunogens that specifically target the immune cells recognizing HIV neutralizing epitopes, without activating immune cells that respond to HIV but produce unhelpful antibodies that compete with the ‘good’ (broadly neutralizing) antibodies.

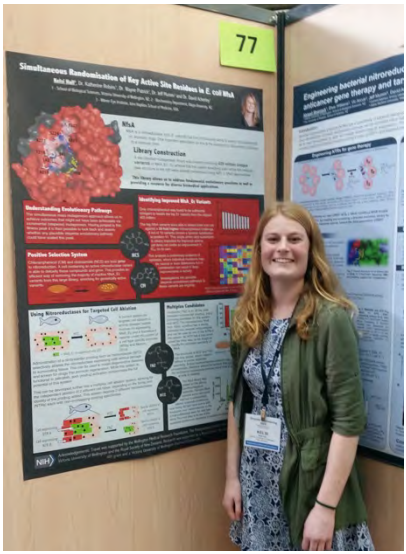
Attendance to the 46th ASI Annual Meeting was highly beneficial, in addition to the outstanding presentations and networking with the Australasian immunology community, I also had the chance to visit many of the vendors present at the meeting, who were able to offer excellent lab start-up packages, saving me time and money to set up my laboratory.

K Hall

24th Enzyme Engineering Conference, Toulouse, 2017

The conference began with the plenary lecture from Frances Arnold, a pioneer of directed evolution, one of the main themes of my doctoral research. It was great listening to her speak about all her successes and she really set the stage for the rest of the week's presentations.

The rest of the conference was split up into 7 different sessions with each session focussing on a different theme of enzyme engineering. These sessions included a wide range of topics including: synthetic biology, computational design, functional based discovery and biocatalysis. There was a wide range of speakers from both academia and the enzyme engineering industry providing a lot of variety in the presentations.



The conference allowed for many networking opportunities as evening meals were arranged at several nearby hotels as part of the conference programme. This allowed us to engage with other participants in a more social setting.

The highlight of the conference was having the opportunity to present some of my work in one of the poster sessions. This was the first time I was presenting work from my PhD. It was great being able to talk to like-minded people about my research and listen to their advice for future experiments.

Images: Capitole de Toulouse, Town Hall – conference venue; Kelsi Hall with her poster presentation.

K Hally

Australasian Society for Immunology (ASI) Annual Scientific Meeting, Brisbane, 2017

My research has focussed on platelet immunology in patients with acute myocardial infarction (heart attacks). In particular, I am interested in determining how platelets may be alternatively activated by inflammation during a heart attack, and how this may relate to patient outcome. The work I presented at the ASI meeting focussed on how platelets participate in inflammation by their interaction

with white blood cells in response to an inflammatory stimulus.

Summary of findings presented: We can conclude that platelets regulate inflammation in a leukocyte- and agonist-specific manner, and platelets have anti-inflammatory responses that are currently underappreciated in the literature. We believe this research is important translational science from a cardiology point-of-view. Firstly, we have demonstrated a novel anti-inflammatory platelet-specific pathway that may contribute to limitation of inflammation during and following myocardial infarction. Secondly, anti-platelet medication is guideline-recommended for patients with myocardial infarction to inhibit platelet activation, but this treatment strategy may also be inhibiting more subtle anti-inflammatory effects needed for reparative processes after myocardial infarction. The results of this project have opened up several avenues of research, including fully characterizing these anti-inflammatory platelet responses in myocardial infarction and investigating the effects of anti-platelet therapy on these platelet-leukocyte interactions. These avenues are the subject of ongoing research by our laboratory.

This study was conducted as a part of my PhD at Victoria University of Wellington under the supervision of Professor Anne La Flamme, Dr Scott Harding and Associate Professor Peter Larsen.

R Martin

12th International Society for Physical & Rehabilitation Medicine (ISPRM) Conference, Paris, 2018 and Cochrane Rehabilitation Workshop

The Research for Life travel grant allowed me to travel to the International Society of Physical and Rehabilitation Medicine International Congress in Paris, July 2018, to present two posters outlining findings from my PhD research evaluating the effectiveness of therapeutic horse riding in positively influencing health outcomes for children experiencing disability.

As a lecturer of health professionals who work in rehabilitation services across New Zealand, it was beneficial for me to see both the range and focus of current rehabilitation research, innovative technologies and clinical practice. I also attended a number of short workshops within the conference which explored ways to improve health outcomes for people who experience disability, particularly those with long-term health conditions.

Before this conference, I attended a two-day workshop in which a small group of international researchers discussed innovative thinking around synthesising rehabilitation research evidence and making it more accessible for consumers, health service providers and funders. I found this workshop to be particularly productive in that I was able to continue to develop papers for publication with other international researchers and to explore opportunities for future international research collaborations.

Titles of poster presentations: 1. *Therapeutic horse riding: a context facilitating learning and flourishing for children experiencing disability*. 2. *What works for which riders, and to what extent? Evaluating the effectiveness of a NZ therapeutic riding intervention using a single-case*

experimental design (Abstract published in Annals of Physical and Rehabilitation Medicine, Volume 61, Supplement, July 2018, Pages e522-e523)

J Mayer

Australasian Society for Immunology Conference, Brisbane, 2017

The conference focused on a broad range of immunological questions ranging from clinical perspectives on cancer, autoimmune disease and vaccinations to basic scientific questions of immune regulation, innate and adaptive immunity and mucosal immunology. Many international speakers were invited, and I was especially inspired by the keynote lectures from Professor Federica Sallusto and Professor Judith Allen which focused on novel scientific approaches and cell types that were related to my own research.

I was selected to present my own research at the 'DC and Immune Regulation' workshop and with a poster during the conference poster session. Around 70 researchers attended the workshop and many interesting discussions arose afterwards and during my poster presentation. I was also able to cement two future collaborations with laboratories in Australia, one of which is located in Brisbane itself, and I took the opportunity to visit the laboratory after the conference and give a seminar in front of the institute.

M Rich

Gordon Research Conference on Enzymes, Coenzymes and Metabolic Pathways at New Hampshire, USA

At the Gordon Research Conference on Enzymes, Coenzymes and Metabolic Pathways at New Hampshire, USA earlier this year, I presented a poster describing the

discovery of novel nitroreductase enzymes from the DNA of bacterial species living in NZ soil and lichen, and won a prestigious poster award from the American Chemical Society Division of Biological Chemistry.

My research, conducted in Prof David Ackerley's group at Victoria University of Wellington, focuses on the application of nitroreductase enzymes that are able to activate potent anti-cancer prodrugs, in the targeted gene therapy strategy known as gene-directed enzyme prodrug therapy (GDEPT).

In GDEPT, nitroreductases are selectively delivered to cancerous tissues and can subsequently activate systemically-administered inert prodrugs to their cytotoxic forms, allowing for selective killing of the tumour cells. Nitroreductases can also activate radioisotope-labelled PET imaging probes to temporarily cell-entrapped forms, which allows a clinician to rapidly confirm localisation of the nitroreductase solely to cancerous cells before prodrug administration. The ability to rapidly discover and evaluate previously unexplored enzymes from nature has enhanced Prof Ackerley's group's ability to find nitroreductases with the broad activity profiles that they are seeking.

The generosity of Research For Life allowed me to attend the Gordon Research Seminar (GRS) and subsequent Conference (GRC) on Enzymes, Coenzymes and Metabolic Pathways, which is part of the prestigious Gordon Research Conference series.

These international Gordon Research Conferences comprise over 200 focused topic meetings, attracting established and emerging leaders in their fields and encouraging presentation and discussion of new and yet-unpublished research. Selection of conference attendees is

extremely competitive, with each individual applicant heavily scrutinised to determine the prestige of their research group as well as the fit of their research for that particular GRC. GRCs are typically held at isolated conference locations, to nurture an informal community atmosphere, with attendees sharing accommodation and meals to encourage networking.

At this GRC, over five discussion sessions took place over the course of the day, from 9 am to 9 pm, with poster sessions and optional workshops occurring in the afternoon. Attendance at this GRC allowed me the opportunity to meet with and discuss ideas with established and emerging leaders in the enzymology field. I was able to learn from the cutting-edge research presented by enthusiastic leading experts over the intensive six-day conference. I also attended a workshop on newly developed web-tools from the Enzyme Function Initiative, which have been designed to facilitate the discovery of enzymatic and metabolic functions of unknown enzymes. Through the use of sequence similarity networks and genome neighbourhood networks, researchers can predict the function of enzymes in prokaryotes that may be essential for growth or pathogenicity or involved in the production of novel anti-cancer compounds. These networks are rapidly becoming commonplace and used by researchers worldwide, and it was extremely valuable to be able to learn these skills first-hand. I also learned about new techniques developed for enzymology and metabolic pathway engineering, which will be useful in my own research as well as conveying to other researchers in New Zealand.

My attendance at this GRC, as well as promoting New Zealand research at a prominent international forum, allowed me to receive helpful and positive feedback for the

research I presented on, which we intend to submit for publication early in 2019.

K Robins

Enzyme Engineering XXIV, 24-28 September, Toulouse, 2017

The “Enzyme Engineering XXIV” in Toulouse, France, which was held 24-28 September 2017 is a leading conference in the field of enzyme engineering and is held every two years attracting some of the biggest names in the field. I particularly enjoyed the plenary lecture from one of the pioneers in the field, Frances Arnold. I was also interested to hear from an American biotech company that was working on a project that was similar to my research.

I presented my poster entitled “Development of a selection to recover improved DNA ligase enzymes during directed evolution”. My work aims to evolve improved DNA ligase enzymes which are better able to anneal two blunt-ended DNA substrates. This has the potential to expand the applications of next generation DNA sequencing technologies, a technique with many medical applications both in research and in the clinic. I spoke with many of the attendees during my poster session, and most were very interested in the upcoming publication of this work.

As well as all the excellent talks and posters, there were plenty of networking opportunities throughout the conference. Sit-down lunches and dinners were provided most days and we took these opportunities to sit with different groups of people at each meal. I thoroughly enjoyed this conference and learnt a lot about the exciting work being done in the field.

A Sharrock

Johns Hopkins School of Medicine, Baltimore

Earlier this year, I was lucky enough to be invited to travel to the Wilmer Eye Institute at Johns Hopkins School of Medicine, Baltimore, to conduct research in the Mumm lab for two months. Thanks in part to this grant, I was able to take up this fantastic opportunity. From March until May I worked in the Mumm Lab at the Wilmer Eye Institute where I learnt all sorts of new skills to do with zebrafish husbandry and the creation of disease models.

During this time, I was able to gather a large amount of exciting data for my thesis, some of which will hopefully contribute to an upcoming publication. A highlight achievement during my time in the Mumm Lab was finding that one of my engineered nitroreductase enzymes appeared significantly more active with our target drug than the Mumm lab's current 'top' nitroreductase.

Another highlight was attending a seminar given by Jennifer Doudna, a world-renowned biochemist best known for leading the discovery of CRISPR-Cas9 gene editing, a technology that has the potential to eradicate previously incurable diseases.

As well as providing me with an invaluable experience, my research trip to the Wilmer Eye Institute also strengthened the collaboration between our lab here in Wellington, and the Mumm Lab in Baltimore. I believe that collaboration is a key aspect of science, and to really push forward progress

and innovation in a field, researchers from across the scientific spectrum must join forces.

Image: The Wilmer Eye Institute at Johns Hopkins School of Medicine, Baltimore

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R van de Wetering

International Drug Abuse Research Society (IDARS) conference in Dubrovnik, 2017

The 6th biennial meeting of the International Drug Abuse Research Society (IDARS) was held September 4-8 2017 in Dubrovnik, Croatia. IDARS is an international scientific organisation that promotes and fosters the research and collaboration of scientists around the world with focus on the molecular, cellular, and behavioural mechanisms of drug addiction.

The conference presentations began with a session on the neurobiology of opioid addiction. George Koob gave an enlightening talk about opioid addiction and hyperkatefiea, his newly coined term describing a hypersensitivity to emotional distress in the context of opioid abuse. This was followed by a session on the role of BDNF in alcohol and drug addiction, and a session on alcohol and tissue injury. The final session for the day was a 3-hour poster session where I was able to discuss my PhD research with some of the leading researchers within the field from across the world.

The third day commenced with a session discussing cocaine abuse. A highlight of this session was Jean Zwiller's talk on cocaine abuse and epigenetics. This was followed by a session about methamphetamine, MDMA, and bath salts where my supervisor, Susan Schenk, presented some of the other work within our lab detailing the pharmacology of the subjective effects of MDMA and MDMA self-

administration. A session on HIV-infections and drug abuse closed the presentations for the third day.

On the final day, there were two sessions discussing Marijuana/cannabinoids, followed by a session on emerging targets for medication development for substance use disorders. This session was of particular interest for me as this is the area of my PhD research. The final session focused on dopamine, NMDA receptors and ketamine abuse.

The IDARS conference gave me the opportunity to meet a large number of drug addiction researchers from around the world and to put faces to the names I had read about. I received some great feedback on my work (some positive and some less so) that will help me to continue my PhD research programme. The conference also gave me exposure to a large number of different procedures, methods, and modern ideas about drug addiction, which was invaluable.

Notes

Research For Life

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