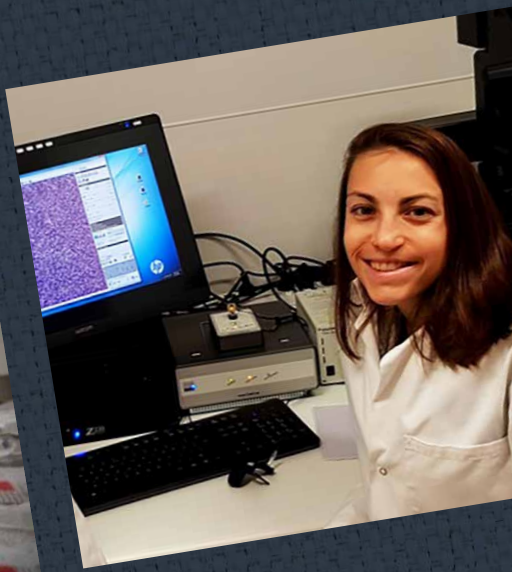
2022

RESEARCH REVIEW



Research For Life

WELLINGTON MEDICAL
RESEARCH FOUNDATION

RESEARCH REVIEW 2022

COVER PHOTO:
Grant recipients Anna Tribe,
Nur Hasanah Hamizan and Sarah Sczelecki.

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Research For Life
The Wellington Medical
Research Foundation
Incorporated

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RESEARCH PROJECT GRANTS 2022

TRAVEL GRANT REPORTS

Editorial

BY PROFESSOR REBECCA GRAINGER

What a difference a year makes. When writing this report in 2021 our borders were shut to visitors, returning New Zealanders had to secure a place in MIQ, and we were still under the traffic light COVID-19 restrictions.

As we emerged from the Delta lockdown the city streets remained quiet and a trip to the South Island found tourist hot spots desserted making for an amazing but slightly strange holiday. Over this unexpected long weekend in September 2022 (to mark the passing of Queen Elizabeth II) Wellington is buzzing with visitors, few masks are seen, and the strange life that we lived under COVID-19 seems to be fading in my memory.

While memories fade, the COVID-19 pandemic will be a historic event of some significance. While looking back many observations will be made and new conclusions come to but the COVID-19 pandemic has confirmed the critical importance of scientific research in protecting human health and well being. This highlights the importance of Research For Life in the Wellington scientific community as a key body in supporting the development of talented health and medical scientists here in the capital—those scientists that will address the many human health challenges in the future.

But before we look to the future, what are my reflections on the role of science during the COVID-19 pandemic? First the development of effective COVID-19 vaccines in record time is one of the miracles of modern science. The first dose of the Pfizer vaccine was administered on 8th December 2020. Since then over 12.7 billion doses of COVID-19 vaccines have been administered to about 68% of the world's population. As the COVID-19 virus evolves new strains, the vaccines still seem to protect against severe disease and death but not infection, and boosters are required as immunity wanes. Efforts continue internationally in vaccine development with a pan-coronavirus vaccine or vaccines with longer lasting efficacy key targets. We are so fortunate to have talented scientists working on this right here in New Zealand, Wellington: The Malaghan Institute of Medical Research is a key member of the Vaccine Vaccine Alliance Aotearoa New Zealand – Ohu Kaupare Huaketo, in collaboration with the University of Otago, Victoria University of Wellington – Te Herenga Waka, the Medical Research Institute of New Zealand and many other institutes across the country. We are likely to be heading into a significant and exciting period of improvements in vaccine science, which has such direct and immediate positive benefits for human health.

While biomedical science has played a critical role in the diagnosis, management and prevention of COVID-19, we must also acknowledge other contributors to the COVID-19 response. There were many scientists outside the traditional non-biomedical sciences who had key roles. Epidemiologists, informaticians, and mathematical modellers all played critical roles in providing scientific advice to our Government that contributed to the COVID-19 response. Science is a team sport and we need team players who bring a variety of skills and expertise. The pandemic has also highlighted the importance of understanding what motivates humans to behave in ways that help each other, and the importance of good science communication. We can't just do the science, we have to communicate the messages to others in a way that supports them to act in positive ways.

In this 2022 issue of the Research For Life research report we provide summaries of the science supported through our grants to Wellington's talented emerging scientists. The 10 reports cover biomedical and clinical studies across a wide range of areas: infection to vaccination, cancer detection to cancer treatment, and neurodevelopment to neurological injury. While the focus of many is on very fundamental aspects of disease or disease management, this painstaking and detailed work forms the foundation of future innovations that can impact directly on human health. Many of our grants support doctoral students, post-doctoral fellows or early career scientists. These people will be the leading scientists of tomorrow. I am also delighted that RFL has been able to award its first Postdoctoral Fellowship, which you will hear more about at the Annual General Meeting and in the Annual Report. This will provide vital funding to continue career development for Wellington's finest, and provide a bridge from learning science to leading science.

I would like to thank the members of the Research Advisory Committee who do an outstanding job of reviewing the grant applications to provide advice to the Board on funding recommendations. I also extend my sincere thanks to Kate Ward our secretary who has provided the most excellent administrative support one could wish for. These people make the work of Research For Life a pleasure, as it is already a privilege.

I hope you enjoy reading the reports this year and continue to support Research For Life in its important work of supporting the development of outstanding medical science, and scientists in Wellington.

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RESEARCH REPORTS

Te Herenga Waka Victoria University of Wellington

A sticky situation: investigating meningococcal adherence during carriage

BM OTTO, JK MACKICHAN

KEY FINDINGS

Our studies showed that the meningococcal protein HpuA does not bind human extracellular matrix proteins, but does mediate autoaggregation, or clumping, of bacteria. We also found that a conserved Cys-Cys motif in HpuA was dispensable for binding to epithelial cells or for autoaggregation.

OBJECTIVES

The goal of this project was to characterise a protein from *Neisseria meningitidis* that we recently showed binds to human epithelial cells. This protein, HpuA, has long been known to play a role in bacterial acquisition of iron from the host. We previously showed that HpuA also mediates adherence to host cells and inhibits epithelial cell wound repair and migration. We hypothesise that epithelial cell wounds or disruptions could provide a portal of entry for meningococci to migrate from the nasopharynx to the bloodstream, with the inhibition of wound repair enhancing the risk of invasive disease.

Our overall aim was to better understand the role of HpuA in enabling meningococci to persist asymptomatically in the host nasopharynx. Our two specific aims were to (1) test HpuA-mediated binding to candidate human proteins, and (2) carry out site-directed mutagenesis of HpuA to identify motifs that play a role in adherence. The site-directed mutagenesis altered a highly conserved Cys-Cys motif in the protein that does not play a role in host iron acquisition. We further tested whether HpuA has the additional capacity to mediate autoaggregation, or clumping, of the bacteria.

METHODS

All assays were carried out with an *E. coli* protein expression strain with a plasmid encoding an inducible copy of the *N. meningitidis* gene *hpuA*. Prior to each experiment, two cultures were grown, one with HpuA expression induced by IPTG, and one repressed by the addition of glucose.

To assess HpuA-mediated binding to purified extracellular matrix (ECM) proteins, purified human collagen types I, III, and V, and fibronectin (Merck) were coated onto glass coverslips using carbonate-bicarbonate coating buffer. Coverslips were washed, blocked, and a suspension of the *E. coli* expressing HpuA was added, along with 1% mannose to block non-specific binding. After incubation, the coverslips were gently washed, and adherent bacteria were detected with Syto-9 green fluorescent nucleic acid stain (Thermo Scientific). Coverslips were imaged using a fluorescent photomicroscope (Olympus B63) with a FITC filter. Images were analysed using ImageJ.

A site-directed mutant of HpuA was designed, replacing the Cys-Cys motif, at positions 106 and 107, with two serines. This motif was chosen because it is highly conserved across HpuA homologues from different species, but is dispensable for haem acquisition, suggesting it is important for another function. The mutant gene was synthesised by Twist Biosciences and cloned into the pET28a *E. coli* expression vector. A549 lung epithelial-like cells were routinely cultured in DMEM supplemented with 10% foetal calf serum. *E. coli* strains expressing either wild type or the Cys-Cys mutant were treated with either IPTG or glucose to induce or repress expression of the HpuA variants. Expression of the heterologous proteins was confirmed by an SDS-PAGE gel of whole cell pellets. The bacteria were added to A549 epithelial cells at an estimated multiplicity of infection of 10. The precise number of bacteria used for the infection was quantified by plating out dilutions and counting colonies. Bacteria and host cells were co-incubated for 30 minutes at 37°C. To quantify host cell-associated bacteria, the cells were

gently washed with PBS to remove non-adherent bacteria and were lysed with 1% saponin. The lysates were plated out and viable colony-forming units were enumerated. Since *E. coli* can damage A549 cells, additional replicates were washed with PBS to remove the bacteria, then fixed in ice-cold methanol for 5 minutes and examined under a phase contrast microscope.

Bacterial autoaggregation was assayed using a sedimentation assay. The *E. coli* strains with wild type and mutant HpuA induced or repressed were suspended in LB at an OD600 of 1.0. The bacteria were incubated at 37°C, without shaking, for 24 hours. At periodic intervals (hourly for the first six hours, and at 24 hours) 50 µl was carefully removed from the surface of the culture and used to measure the optical density.

RESULTS

Our first aim was to test candidate host proteins to see if they bound to HpuA. Our first candidates were extracellular matrix (ECM) proteins. Many bacterial pathogens bind to these, but they were of interest because of their role in cell migration during wound repair. Four ECM proteins were chosen because *N. meningitidis* had previously been shown to bind to them. *E. coli* strains expressing meningococcal HpuA were incubated on coverslips coated with collagen types I, III, and V, and fibronectin. However, no binding was detected between the *E. coli* strain expressing HpuA and any of these ECM proteins.

We also measured the ability of *E. coli* expressing a site-directed Cys-Cys motif mutant of HpuA to bind to human epithelial cells. The mutant variant was cloned in *E. coli* and we confirmed that it was expressed in the presence of IPTG. We assessed the ability of *E. coli*, expressing either wild type or mutant HpuA, to adhere to A549 lung epithelial cells. Low levels of adherence were seen, but no significant differences were noted between the HpuA wild type and the Cys-Cys mutant. Microscopic examination of the A549 monolayer suggested that the cells were not damaged by the *E. coli* strains.

Previous microscopy revealed that HpuA-expressing *N. meningitidis* strains formed clusters of microcolonies on the surface of epithelial cells, in contrast with little binding seen for an HpuA deletion strain. While the microcolonies could be due to clustering of the HpuA host receptor, we asked whether HpuA could be mediating autoaggregation, or clumping, of the bacteria. Bacterial autoaggregation can promote bacterial survival in the host by clustering of microcolonies, maximising the effects of toxin delivery, or evading phagocytosis. To detect HpuA-mediated autoaggregation, we carried out a sedimentation assay with *E. coli* strains with induced or repressed HpuA expression, both wild type and mutant. These assays surprisingly revealed that wild type HpuA confers autoaggregative properties on the *E. coli* strain expressing it, a novel phenotype for HpuA. However, the autoaggregative phenotype did not require the conserved Cys-Cys motif.

CONCLUSION

Many bacterial pathogens bind to the extracellular matrix proteins, e.g., collagens, that form scaffolding for tissue. ECM proteins also play a key role in wound repair, providing an anchor for re-epithelialisation. Although *N. meningitidis* has previously been shown to bind to ECM proteins, we did not detect ECM binding mediated by HpuA. The human receptor for HpuA remains unknown, but work is ongoing to identify it. Similarly, the conserved Cys-Cys motif in HpuA was found to be dispensable for both bacterial autoaggregation and binding to epithelial cells. Future studies will express truncated versions of HpuA to identify the part of the protein that is important for host cell binding.

Te Herenga Waka Victoria University of Wellington

Can a positive environment reverse the effects of a maternal viral infection on the brain of the children?

BA ELLENBROEK, L PENG

KEY FINDINGS

We found that rats reared in an enriched environment showed significant changes in protein expression within the medial prefrontal cortex, especially to proteins involved in the outgrowth of neurons. Together these data suggest that a more diverse environment substantially changes the communication between brain cells of the prefrontal cortex, an area of crucial importance in multiple mental disorders, such as schizophrenia, major depressive disorders, and autism spectrum disorders.

OBJECTIVE

The original objective of our project was to (1) identify how the brain (in particular the frontal cortex) is altered by prenatal exposure to a drug that mimics a viral infection and (2) whether rearing animals in an enriched environment would counteract some of these changes. Unfortunately, the SARS-Cov2 pandemic substantially impacted our research, and as a result, we had to limit our analysis. Our previous behavioural results showed that the effects of enrichment were stronger and more pervasive than the effects of the prenatal viral-mimetic drug, and we therefore focused on whether an enriched environment altered the protein composition of the frontal cortex in rats.

METHODS

Brain samples from the medial prefrontal cortex were collected from 28 male Sprague Dawley rats raised from infancy to adulthood in either a nourishing environmental enrichment condition or a more restricted standard housing condition. The environmental enrichment (EE) condition, compared to standard housing (SH), featured a larger enclosure with more rats and additional novel objects, such as toys and running wheels.

The protein composition was holistically assessed by quantitative proteomics analysis using liquid chromatography-mass spectrometry (LC-MS) coupled with isobaric labelling by tandem mass tagging (TMT). The proteins from each brain sample were extracted and further processed into peptides that were then individually labelled with a unique TMT tag. The labelled peptides were pooled into their respective batches and further fractionated into eight fractions to increase coverage of the brain proteome. The fractions were submitted to the LC-MS (Figure 1), and raw LC-MS data were collected from the runs.

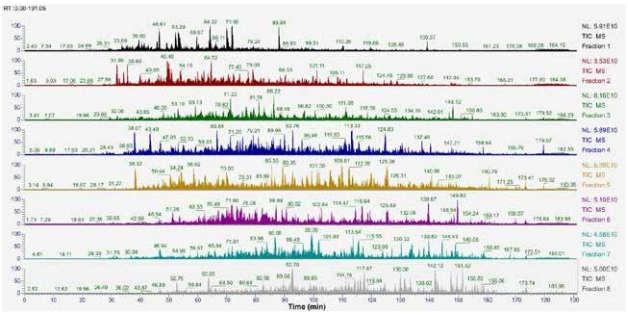


FIGURE 1: Representative LC-MS chromatograms of fractions from prefrontal cortical brain tissue.

The raw LC-MS data was processed in Proteome Discoverer 2.4 to determine the peptide spectral matches and reporter ion intensities. These data were further processed in R using the MSstatsTMT package to determine protein identities and abundances from the individual samples. Finally, data were grouped according to housing condition and compared for relative differences in protein expression using a linear mixed-effects model.

RESULTS

Because of constraints related to the behavioural experiments performed prior to the brain collection for proteomics, the animals (in both the enriched and standard housing) belonged to two different age cohorts: Cohort I: 82 – 86 days and Cohort II: 104 – 113 days old. Although the age difference is not particularly large, we first investigated age difference in proteins abundance. We did not detect any difference in protein levels between younger and older rats that were kept in standard housing. Intriguingly, we did find several proteins that were significantly different between younger and older animals that were reared in an enriched environment. More importantly, we found a number of proteins that were differentially expressed between rats in an enriched environment versus those that were housed in standard cages. The most important proteins of significant differential expression are summarized in Table 1. While most of these proteins were downregulated in enriched animals compared to controls, OX-2 membrane glycoprotein, neuritin and the tubulin beta-2B chain protein were expressed more in enriched animals. Interestingly, the tubulin beta-2B chain protein is also expressed more in younger compared to older animals reared in an enriched environment.

Protein	EE – Young vs Old	EE vs SH
OX-2 membrane glycoprotein		↑
Neuritin		↑
Tubulin beta-2B chain	↑	↑
M7GpppX diphosphatase	↓	↓
2,4-dienoyl-CoA reductase (mitochondrial)		↓
Glycine amidinotransferase (mitochondrial)		↓
Dihydropyrimidinase-related protein 5		↓
Guanylate cyclase soluble subunit alpha-1		↓
Hyaluronan & proteoglycan link protein 1	↓	↓
Cytosol aminopeptidase		↓

TABLE 1: Major proteins that are differentially expressed either as a function of age or of housing. See text for more details.

CONCLUSIONS

Although it is still too early to draw firm conclusions, the findings so far clearly show that rearing animals in an enriched environment significantly alters the prefrontal cortex. It is noteworthy that the two proteins most strongly upregulated in enriched animals are neuritin and the tubulin beta-2B chain protein. Neuritin plays an important role in neuroplasticity (the ability to adapt to external stimuli) and recent findings have suggested a role for this protein in both major depressive disorders and schizophrenia. Tubulin 2β is a protein also involved in structural alterations in the brain, and is thought to be particularly involved in development, differentiation, and maintenance of cortical neurons.

In addition to these proteins that are more abundant in animals reared in an enriched environment; several other proteins involved in neurodevelopment are significantly less abundant after rearing in enrichment. These include dihydropyrimidinase-related protein 5, a protein involved in neuronal migration, axonal guidance, and synapse formation. Likewise, hyaluronan & proteoglycan link protein 1 is a protein involved in neuronal plasticity. In line with our overall hypothesis, it was recently shown that a genetic deletion of hyaluronan & proteoglycan link protein 1 leads to increased axonal sprouting. Furthermore, the downregulations of 2,4-dienoyl-CoA reductase (mitochondrial), glycine amidinotransferase (mitochondrial), and cytosol aminopeptidase indicate lower energy production and hence lower cellular oxidative stress in animals following enrichment.

Taken together, our data confirm our hypothesis that rearing in an enriched environment increases the ability of the rat's brain to form new connections (plasticity), which could offer opportunities for treatment of mental disorders that would not involve drug treatment.

Te Herenga Waka Victoria University of Wellington

Characterizing B cells within epicardial adipose tissue of patients with cardiovascular disease

G BIRD, K HALLY, S HARDING, A LA FLAMME, S GALVIN, P LARSEN

(in association with authors from the Wellington Cardiovascular Research Group, University of Otago, Wellington, and Wellington Regional Hospital)

KEY FINDINGS

We determined that heart fat routinely removed during heart surgery could not be utilized for expanding our understanding of B cells in heart attack patients. We conducted an initial study to investigate two *in vitro* activation models for human-derived B cells. From this work, we demonstrated exciting preliminary results for future human *in vitro* studies into B cells responses in heart attack patients.

OBJECTIVE

This study was designed to investigate B cells resident within adipose tissues (fat) surrounding the human heart. To further develop our understanding of their role in coronary artery disease (CAD) and acute myocardial infarction (AMI, heart attack). Beyond knowing that B cells are present within the epicardial adipose tissue (EAT), limited human clinical evidence exists surrounding the immunological characteristics of B cells. CAD is a chronic sterile inflammatory disease; therefore, this study was designed to investigate the phenotype and function of B cells resident within EAT across patients with differing CAD profiles. Our original aims for this research were:

1. Development of a clinical protocol for isolating B cells from EAT deposits derived from the Right Atrial Appendage (RAA) of the human heart.
2. Development of *in vitro* assays to probe the functional characteristics of EAT-resident B cells.
3. To phenotype EAT-resident B cell subsets and activation status using flow cytometry.
4. To investigate how the cellular numbers, phenotype, and functional characteristics of EAT-resident B cells differed from B cell populations within the patient's peripheral blood. These same parameters would then be investigated in between patients with either no CAD or CAD.

METHODS

Patient recruitment: Potential patients were eligible to be recruited into the study if they had been scheduled for a coronary artery bypass graft (CABG) procedure, with or without prior AMI. Healthy participants were required to have no known diagnosis of cardiovascular disease. This study was reviewed and approved by the HDEC (17/CEN/212, 19/CEN/129), and all participants provided voluntary written and informed consent.

Adipose tissue and blood collection: Adipose tissue samples were collected during CABG surgery without altering the clinical procedure to collect EAT deposits (RAA derived) and the aortic fat pad (AFP, which represents the perivascular Adipose Tissue (PVAT)). Whole blood samples were collected from the patients prior to surgery (5-10 mL) and were collected into EDTA-anticoagulated blood tubes.

Processing of the stromal vascular fraction (SVF) and peripheral blood mononuclear cells (PBMCs): The adipose sample was weighed and digested to isolate the SVF from the adipose tissue, which was stained and analysed on the same day. PBMCs were isolated and cryopreserved for later use.

Overall, we attempted three different digestion protocols to determine which would give the greatest B cell yield from EAT and AFP tissue.

Immunofluorescent staining: Briefly, all samples were stained using the appropriate antibody cocktails for experiment outcomes, incubated for 30 minutes at 4°C, fixed with 1% PFA, washed, and resuspended in staining buffer for analysis on the Cytek Aurora. Single-stained reference controls were established for unmixing ahead of these experiments. Subsequent data analysis was performed on FCSExpress and FlowJo.

In vitro B cell functional assays: B Cells from patient PBMCs were isolated by immunomagnetic negative selection (EasySep B cell isolation kit, Stemcell Technologies) and quantified. Isolated B cells were then stimulated through either a T cell-independent (TI) stimulation using CpG ODN2006 (1 µg/mL; Toll-like receptor 9 agonist; Novus Biologicals) or T cell-dependent (TD) stimulation using recombinant human CD40L (5 µg/mL, R&D Systems) + IL-21 (12.5 ng/mL, R&D Systems). B cells were incubated ± stimulants in complete media for 7 days. Activation profiling markers (CD21, C69 and CD86) were measured at baseline and on day two post-stimulation. All samples were run in duplicate with controls. Supernatant from each sample well, including plate controls was taken off at day two post-stimulation to measure cytokine production of IL-6 and IL-10 using BD Human Inflammatory Cytometric Bead Array kit (CBA; BD Biosciences, Franklin Lakes, NJ, USA). At day seven, post-stimulation supernatant from each well (including plate controls) was aspirated to measure IgM and IgG (total) antibody production using BD CBA Flex Sets for Human IgM and Human IgG total (BD Biosciences, Franklin Lakes, NJ, USA).

RESULTS

Patients: 19 patients (66 (± 8) years, 15 Male, 2 Females) were recruited into this prospective, observational cohort study, from Wellington Regional Hospital between May 2021 and October 2021. Two patients were withdrawn from the study following enrolment. PBMC samples were collected with permission from nine patients. Four healthy age and sex-matched participants were recruited into the study for *in vitro* experiments.

Phenotyping Panel: We optimized a 17-color antibody panel within human PBMCs. This panel identifies 11 human B cell subsets, including the putative B1 cell which has limited clinical assessment in humans. This panel is also capable of probing activation through CD21, CD24, CD69, and CD86 expression. We only applied the original 8-color antibody panel during adipose optimization. *In vitro* research used an adapted panel of 13 antibodies tailored to the experiment.

Adipose Tissue Results: Our EAT samples were consistently very small in size (average 0.11g; Interquartile range 0.045 g – 0.19 g). Initially, attempts were made to optimise the number of B cells collected from this small amount of adipose tissue. However, the maximum number of B cells isolated from EAT was 70, with a median of 33 (interquartile range 3.75 - 57.25). This number was far too low to conduct any meaningful investigations. Due to B cells remaining low and after discussion with the cardiothoracic surgical team, we additionally collected the AFP samples in this study to determine whether B cell yield could be enriched. The AFP was collected from eight patients and yielded only slightly higher amounts of tissue (median 0.3g, interquartile rang 0.055 g – 0.387g). In addition, there was also a modest increase in B cells; the maximum was only 342 (median 113.8, interquartile range 33.5 – 159). It was therefore determined B cell numbers were too low to pursue for the primary aim and any *in vitro* functional studies and collections were halted.

Cell Culture Pilot Results: Two *in vitro* stimulation conditions were investigated to explore the feasibility of using these conditions for future cell culture. B cell-produced antibodies and cytokines shape both the adaptive and innate immunity in sterile inflammation. For this reason, we wanted to investigate if the chosen stimulation conditions would result in IgM, IgG, IL-6, and IL-10 production, which have key roles in ACS. In this study, TD was observed to result in increased IgG and IgM production, whereas TI resulted in more modest IgM production. In particular, IgG production was very small. Moving forward, targeting TI for IgM production only would be recommended. IL-6 production was increased under both conditions; however, TI drove greater amounts. IL-10 production was limited under both conditions with minimal amounts produced, which will be interesting to investigate in a larger cohort. Part of investigating the stimulation conditions was using activation markers to assess the B cell response. Our chosen markers were measurable on day two and successful at denoting activation status within the analysed B cell populations.

CONCLUSION

This research determined it was not feasible at this time to pursue B cells from the EAT (RAA derived) or AFP for our proposed aims. However, we have developed an expansive B cell phenotyping panel for future use in studies in CAD and AMI patients. Pilot results from our *in vitro* stimulation conditions using TD and TI activation of B cells demonstrated exciting preliminary results for a human *in vitro* study of B cell function, designed for further pursuit in AMI.

Te Herenga Waka Victoria University of Wellington

Harnessing innate-like T cells as mucosal adjuvants for novel influenza vaccine development

T PANKHURST, K BUICK, K BUTTON, L CONNOR

KEY FINDINGS

In this project we show that intranasal immunisation of innate-like T cell agonists co-administered with haemagglutinin (HA) protein from influenza A virus induced potent antibody responses which protected mice against influenza infection. We show that both innate-like T cell populations, natural killer T (NKT) cells, and mucosal-associated invariant T (MAIT) cells can be targeted in this manner by intranasal delivery of agonists αGC and 5-A-RU/MG respectively, co-administered with HA protein. Together, the data generated in this project show that innate-like T cells are promising vaccine targets that can be utilised as cellular adjuvants in mucosal vaccines.

OBJECTIVES

Protective immune responses against respiratory viruses are initiated by the mucosal immune system, however most licensed vaccines are administered parenterally and are largely ineffective at inducing mucosal immunity. The development of safe and effective mucosal vaccines has been largely hampered by the lack of a suitable mucosal adjuvant. In this study we explore a novel class of adjuvant that harness innate-like T cells and aim to determine whether they can stimulate antibody responses against HA protein (a glycoprotein expressed on the surface of influenza A virus responsible for viral adhesion). Thus, antibodies raised against HA protein have potential to prevent viral infection and protect the vaccinated individual.

Objective 1: To determine whether innate-like T cell-based mucosal adjuvants can induce antibodies against co-delivered HA protein derived from X31 influenza A virus.

Objective 2: To determine whether innate-like T cell-based mucosal adjuvants co-delivered with HA protein can protect mice against X31 influenza A viral infection.

METHODS

C57BL/6J mice were immunised intranasally with either HA protein alone, in combination with NKT cell agonist αGC, or in combination with MAIT cell agonist 5-A-RU/MG. As a positive control, an additional group of mice received HA protein in combination with licensed adjuvant AddaVax delivered subcutaneously. Mice received 3 total homologous immunisations spaced 2 weeks apart. Blood was collected to measure antibodies one week later, and then all mice were infected with X31 influenza A virus two weeks later and were monitored daily for a two-week period (Fig 1a).

Production of HA-specific antibodies were measured by ELISA. 96-well ELISA plates were incubated with 20μg/mL HA protein overnight. 10% BSA was applied to prevent non-specific binding, followed by application of serially diluted serum samples taken from mice. IgG-HRP, TMB and stop solutions were applied sequentially.

RESULTS

To determine whether MAIT and NKT cells could be targeted as mucosal adjuvants in an influenza vaccine, mice were immunised intranasally with compounds that activate MAIT and NKT cells, namely αGC and 5-A-RU/MG respectively. Mice received either agonist intranasally mixed with HA protein derived from X31 influenza A virus three times with two-week intervals between each dose. One week after the final third dose, blood was collected to analyse the production of HA-specific antibodies by ELISA (Fig 1a).

As seen in figure 1b, both innate-like T cell-based adjuvants were able to induce significant titers of HA-specific IgG

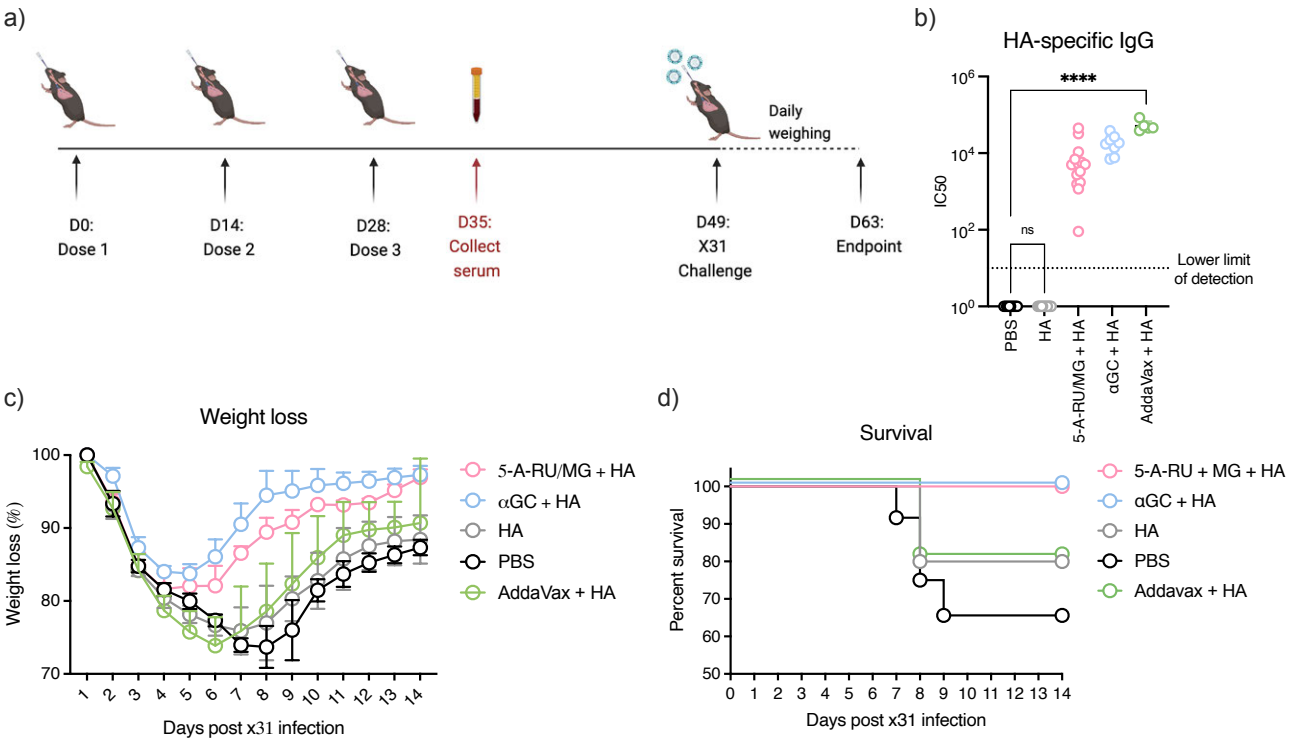


FIGURE 1:
a) Schematic of intranasal vaccination schedule and influenza challenge in mice. Created using biorender.com.
b) IC50 values denoting HA-specific IgG titers measured in the serum of mice by ELISA.
c) Weight loss as a percentage of initial body weight of immunised mice following X31 influenza challenge.
d) Survival rates of immunised mice following X31 influenza challenge.

antibodies compared to PBS treated mice and HA treated mice that did not receive any adjuvant. Mucosal vaccination of innate-like T cell compounds αGC and 5-A-RU/MG generated comparable titers of antibodies to mice that were treated subcutaneously with licensed adjuvant AddaVax. Immunised mice were then assessed for vaccine-induced protection against live influenza virus infection. Three weeks after the final third vaccine dose, mice were challenged intranasally with X31 influenza A virus and monitored daily for weight loss (Fig 1a). Animals exceeding 30% loss of their initial weight were culled. All groups of mice experienced a drop in weight loss within the first three days following infection (Fig 1c). However, only mice that were vaccinated prior with either αGC or 5-A-RU/MG combined with HA protein demonstrated less weight loss overall, and an ability to regain their weight back to normal levels compared to unadjuvanted controls.

Furthermore, 100% of αGC and 5-A-RU/MG treated animals survived infection, whereas 20% and 35% of HA- and PBS-treated animals respectively did not survive the infection (Fig 1d). Surprisingly, mice immunised subcutaneously with HA protein and AddaVax lost weight more rapidly than all other treatment groups (Fig 1c) and almost 20% of animals did not survive infection (Fig 1d), suggesting that the mucosal route of immunisation and targeting NKT and MAIT cell-based adjuvants can provide enhanced protection against live influenza infection.

CONCLUSION

With support from Research For Life we have successfully established a mucosal influenza vaccination regimen harnessing innate-like T cells as cellular adjuvants. We have shown using these methods that robust levels of influenza HA-specific antibodies can be produced which positively correlated with immunised mice being protected from a lethal dose of live influenza.

Te Herenga Waka Victoria University of Wellington

"I get by with a little help from my friends: How do mesenchymal stromal cells enhance survival of cancer cells after therapy?"

HELENA ABOLINS-THOMPSON AND MELANIE J. MCCONNELL

KEY FINDINGS

Breast cancer cells treated with a range of therapies often used in clinical practise – DNA damaging doxorubicin (adriamycin) chemotherapy, microtubule targeting paclitaxel, and a kinase inhibitor dactolisib - gain a survival advantage from being around the non-cancerous 'stromal' cells that are a normal component of a tumour. This survival is mediated largely by cancer-to-stromal cell contact and is associated with increased communication from the cancer cells. This communication could be a target to block that survival advantage, and increase the efficacy of these therapies.

AIMS

1] to determine the range of clinically relevant injuries that can be mitigated by mesenchymal stromal cells (MStC) in the tumour microenvironment

2] to determine how MStC co-culture alters the gene expression of a stressed cancer cell.

METHODS

The 4T1 breast cancer cell line was stably transfected with the red fluorophore TdTomato, as it had more stable fluorescent signal than the eGFP that was originally proposed. Mesenchymal stromal cells were isolated from the leg bones of wild-type mice, and cultured with the red 4T1 cancer cells. The co-cultures were treated with several therapies and the eventual fate of the red 4T1 cells determined – were they killed by therapy, or survived? The red cells were then removed from the cultures and the effect of treatment +/- MStC was determined, by using microarray to analyse the gene expression signatures.

RESULTS

Aim 1: Our preliminary data indicated that a cell injured by DNA damaging chemotherapy, recovered faster when the damaged cell was in culture with another cell type, than on its own. DNA damaging chemotherapy increased the rate of cancer cell: stromal cell contact. Survival after therapy was increased substantially if the treated cell made physical contact with a stromal, non-cancerous cell. In this project we extended these data. First, we established the dose at which the microtubule targeting agent paclitaxel, and the kinase inhibitor dactolisib damaged 4T1 breast cancer cells. These doses were also tested against the non-cancerous MStC cells derived from bone, and shown to have little effect on viability. Then, we exposed a co-culture of both 4T1 cancer cells and MStC to each drug, and to the DNA damaging drug doxorubicin as used previously. A red fluorescent marker, TdTomato, was built into the cancer cells. Using this fluorescence, we specifically followed the fate of those cancer cells. As seen in the preliminary data, exposure to therapy led to more contact being made between the cancer cells and the stromal cells. Also as seen previously, cancer cells that physically contacted the stromal cells had a much higher rate of survival than those that did not, and both survived better than cancer cells treated with the same dose on their own. This demonstrated that effect was not dependent on DNA damage, but was a conserved phenomenon seen in response to multiple stressors.

Notably, this happened even when the stromal cells were also exposed to the therapy. This was an improvement on our preliminary data and better modelled the situation that would occur in a tumour, where all the components of a tumour are exposed to therapy - the cancer cells and the

non-cancerous components such as fibroblasts, immune cells, blood vessels, etc.

Aim 2: The 4T1 cancer cells had been modified to express a fluorescent marker, the red protein TdTomato. This was utilised to separate the cancer cells from the stromal cells before and after treatment, to measure gene expression. Flow cytometry was used to select the red fluorescent 4T1 cancer cells out of the co-culture, and the RNA was extracted, in triplicate experiments. In parallel, RNA was also extracted from 'mono' cultured 4T1 cells, to examine how the response to each chemotherapy was altered by co-culture. These were used to query the Clariom microarray platform, which allowed the quantitation of expression of 20,000 genes in each sample. A series of comparisons were made, first to identify the effect of each therapy on the 4T1 cells alone, and then to identify the effect of therapy on 4T1 cells in co-culture. Each effect was defined as the set of genes that were up- and down-regulated in response to therapy. Next, the gene set of treatment on the mono-culture was compared to the effect of treatment on the co-culture.

Across the three treatment types, there were a series of commonly induced pro-apoptosis genes, that were lost when the 4T1 cancer cells were co-cultured and then treated. This was consistent with the increased survival, and clearly demonstrated that the biological effect could be observed in the gene expression data. The data was then examined to identify potential signals from the treated cancer cells that could have led to enhanced interactions with the stromal cells. While the analysis has been complex, there were several potential genes and pathways identified, including cytokine production, suggesting that more communication occurred in co-cultured cells in the face of therapeutic stress. These pathways are under further investigation.

CONCLUSION

A wide range of stressors have now been shown to lead to more contact between cancer cells and their normal cellular friends and neighbours. This contact has been shown to reduce the effect of chemotherapy and increase the survival of the cancer cell. There is evidence of increased communication between the normal and cancer cells. Work is ongoing to identify the signals and messengers involved.

Dissemination of the data, and progression of the research findings:

This work fulfilled the requirements for a Masters in Biomedical Science thesis by Helena Abolins-Thompson, for which she was awarded an A grade.

Helena has presented the data as a poster at a national cancer research conference, the QMB Cancer meeting in August 2022 – rescheduled from August 2021. The support of Research for Life was gratefully acknowledged in this presentation.

The gene expression analysis has been complex, but the manuscript describing this work is in preparation and will be out for review by the end of 2022.

The data generated here also formed the basis for a funding application to the Health Research Council in 2021. While the EoI was selected for progression at into the second round, the application was not successful at the final round. Additional collaborations are under development to progress this further.

The red fluorescent 4T1 cell line generated for this project has formed the basis for a collaborative HRC-funded project for another Masters student, Danielle Lewthwaite, and this work is on-going.

Te Herenga Waka Victoria University of Wellington

Real-time brain health measures in clinically relevant ovine stroke models

DG THOMAS, FG HARRISON, A LITTLE, A KING, AJ SORBY-ADAMS, YC TZENG, R TURNER, S OBRUCHKOV

(in association with authors from the University of Adelaide and University of Otago, Wellington)

KEY FINDINGS

We tested the feasibility of a prototype device for detecting clinically relevant biomarkers for tissue damage in the early stage of ischemic stroke using single-sided Magnetic Resonance (MR).

OBJECTIVES

Changes visible with MRI such as ADC and PWI are known to provide excellent sensitivity to the early stages of ischemia. However, access to this imaging modality is limited by availability and cost, particularly for acute diagnosis. Our prototype portable magnetic resonance device has been designed to measure these changes in real time. While conventional MRI systems produce images of the whole head, they require large, expensive and complicated hardware. Our system measures the same physical phenomenon, but only in a single spot, similar to a stethoscope, enabling the device to be portable (30 kg) and inexpensive. This will increase the accessibility of MR for this application, potentially allowing faster diagnosis in rural or remote settings.

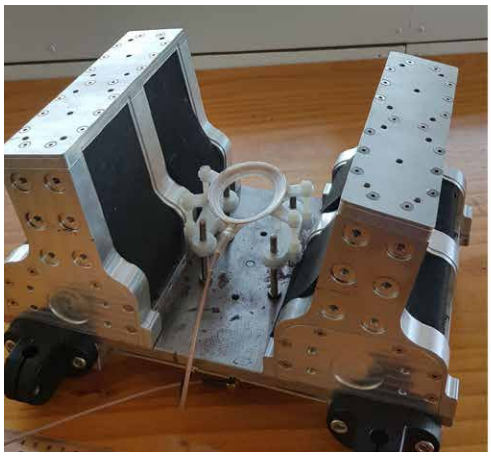
Our major objective in this project was to test the sensitivity of the prototype device to changes in brain Apparent Diffusion Coefficient (ADC). Following the restriction of blood flow to brain tissue, ADC is known to decrease within minutes of the injury, providing a positive indicator for ischemia in the brain. This is due to the dysregulation of fluid inside neurons. The ADC signal can only be detected using MRI, which is uniquely sensitive to changes in tissue microstructure due to its underlying physics.

METHODS

Working with biomedical researchers at the University of Adelaide and the South Australian Health and Medical Research Institute, we tested the feasibility of measuring the brain in real time using our prototype device. The team in Adelaide has developed a protocol for inducing both permanent and temporary ischemic strokes in the middle cerebral artery. The protocol allows us to measure the changes in the brain within an hour of the stroke occurring, which is the most important time for diagnosis. It also allows us to monitor changes that occur following reperfusion, which simulates a successful treatment through thrombolysis or thrombectomy. The facility in Adelaide also hosts a 3T MRI system, allowing images to be taken for comparison to our device readout. The protocol for this study was approved by the Animal Ethics Committee of the University of Adelaide.

For the permanent stroke animals (n=6), the system made a control measurement of the brain before surgery and monitored the brain for up to 4 hours following surgery. The animal was then imaged at the MRI suite. The temporary stroke animals (n=5) had a control measurement, up to 2 hours of monitoring while occluded, and 2 hours of measurement following reperfusion. The prototype was configured to measure ADC and T_2 during the monitoring stages. The coronavirus pandemic meant that we were unable to travel to complete these experiments in person.

One of the main challenges was understanding the position of the device's sensitive region, the spot that it takes measurements from. While an MRI system obtains an image of the whole head, our system is limited to the sensitive region shown in Figure 1. To limit the uncertainty from the head position, we constructed a guide plate using 3D printing, which supported the head in the correct position.



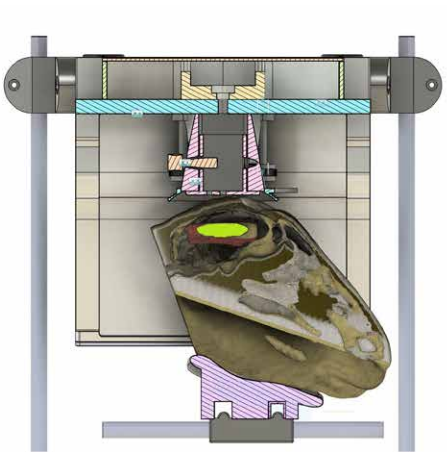


FIGURE 1: The prototype device, showing the magnet with a 30 cm ruler for scale. The right image shows a CAD rendering of the device and its sensitive region (in green) which targets the region of the brain where the stroke occurs. The 3D printed guide piece is shaded in lavender.

RESULTS

Generally, our results showed the expected changes in ADC and T_2 for the hyperacute stage of stroke. Due to the small number of animals, statistical analysis is not possible. Figure 2 shows monitoring results from one of the permanent stroke animals that show the general trends we observed, as well as ADC (above) and T_2 weighted (below) MRI images.

The two blue points at the start of the monitoring are the control measurements, done before surgery. The device was removed, and the surgery to occlude the MCA was completed (indicated by the blue shading). Once the surgery was complete and head closed, the device was placed onto the head again, and monitoring was started. We see a decrease in ADC following the occlusion, which continues to decrease over the monitoring period. This is in line with the ADC MRI image, which shows an ADC of 710±90 μm2/s for the control hemisphere, and 450±80 μm2/s for the stroke-affected hemisphere. In addition to the ADC change, there is also a slow increasing trend in T_2 during the monitoring, which has also been seen in other MRI stroke studies. Unfortunately, a quantitative T_2 mapping sequence was not available for the scanner, although the qualitative T_2 weighted image shows some variation between the stroke and control hemispheres.

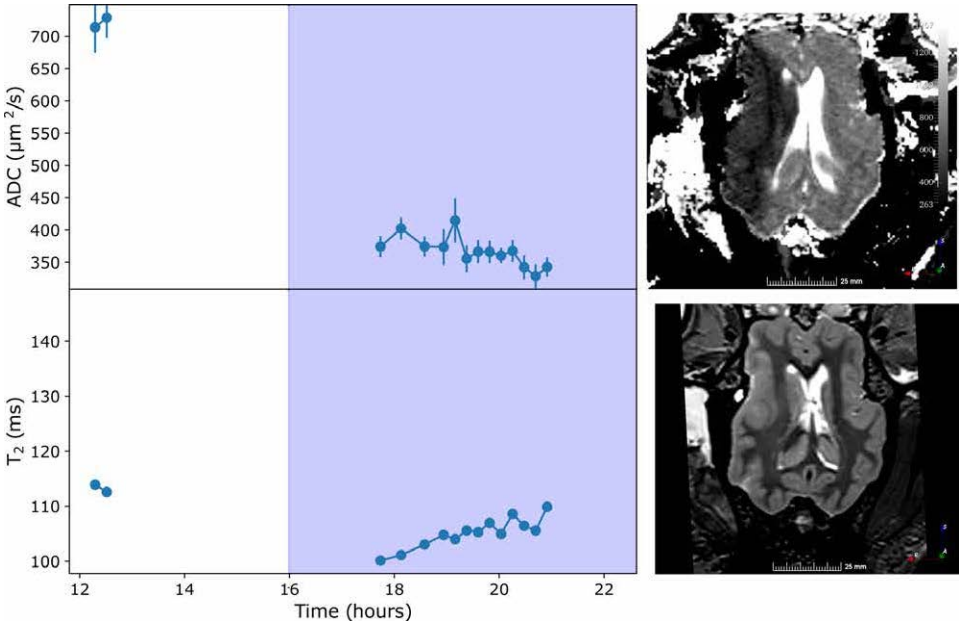


FIGURE 2: Device monitoring outputs, showing quantitative ADC and T_2 over time on the left. ADC (upper) and T_2 -weighted (lower) MRI images on the right. The device detects decreased ADC following the stroke, indicated by the light blue shading. The image also shows decreased ADC in the stroke-affected hemisphere.

CONCLUSION

This project has tested the feasibility of monitoring the health of brain tissue in the hyperacute stage of ischemic stroke. While this pilot study is relatively small, only 11 animals, there are some promising results that suggest that, with further development, our system could be useful for the rapid diagnosis and monitoring of stroke.

Te Herenga Waka Victoria University of Wellington

Synthetic analogues as potential new therapeutics

J MILLER, O ZUBKOVA, R FURNEAUX, P TYLER, B KIVELL

(in association with authors from the Ferrier Research Institute)

KEY FINDINGS

Synthetic bioactive compounds, generally based on natural products or analogues of such, are an unlimited source of potential new therapeutics for the treatment of disease. The potency and selectivity of these compounds for their targets and their bioavailability in the body can be modulated by structural alterations to the lead compounds. At Victoria University of Wellington and the Ferrier Research Institute, we have been screening new compounds in three different areas: 1) heparanase inhibition by heparan sulfate mimetics in cancer, specifically breast cancer and colorectal cancer, 2) DNA methyltransferase inhibitors in leukemia, and 3) new opioidergic compounds for pain control that are non-addictive and show little drug tolerance. We also reviewed βIII-tubulin overexpression in cancer.

OBJECTIVES

The main aim of this project was to screen novel synthetic compounds and their derivatives for bioactivity against human cancer cell lines with the intention where possible of further development of lead compounds in preclinical animal models. We also investigated a new opioid compound for control of chronic pain in a rodent model and reviewed the regulation of βIII- tubulin expression in cancer cells and its effects on drug resistance and metastasis.

METHODS

Cultured cancer cell lines were treated with novel compounds synthesised by organic chemists at the Ferrier Institute to determine if the compounds could prevent cell proliferation or were cytotoxic to the cells. A standard cell proliferation assay, the MTT assay, was used to screen the pure compounds for bioactivity, and attempts to identify the mechanism of action for further action were made in selected cases. New compounds for opioid pain control were supplied by an overseas collaborator.

RESULTS

Over the last two years, we have been working on heparan sulfate (HS) mimetics that inhibit the enzyme heparinase, a project led by Dr Olga Zubkova's group at the Ferrier Research institute. Heparanase stimulates tumour growth by releasing growth factors from the extracellular matrix and stimulates metastasis by breaking down the matrix, allowing cells to escape the tumour and enter the blood stream. Two types of HS mimetics were being tested – tetramers (Tet-29) and trimers (Trimer-6J). Tetramers have 4 sulfated-arms extending out from a dendrimer core (Figure 1).

Trimers have one of the arms removed to allow, in future experiments, a fluorescent tag to be attached as a label to follow the compound's distribution in tissues. These lead compounds were tested in a mouse model using human colorectal cancer cells by collaborators at Otago University in Professor Sarah Young's group (Figure 2).

The heparan sulfate tetramer (Tet-29) inhibited colorectal tumour growth by 40-50% in mice and improved overall survival; however, the trimer (6j) showed little change from the saline control (DPBS). Some preliminary experiments were also carried out with Tet-29 in human metastatic breast cancer cell growth in mice with mixed results. Further experiments are in progress with the HS mimetics. A paper was published in 2022 in the journal *Chemistry-an Asian Journal*.

Professor Richard Furneaux and Peter Tyler's groups at the Ferrier Institute were screening DNA methyltransferase (DMT) inhibitors as potential drugs to treat blood cancers (leukemias). The target enzyme DMT attaches methyl groups onto DNA which affects the cancer cells' growth and differentiation by epigenetic regulation of gene expression. Decitabine, effective in some leukemias, was used as a

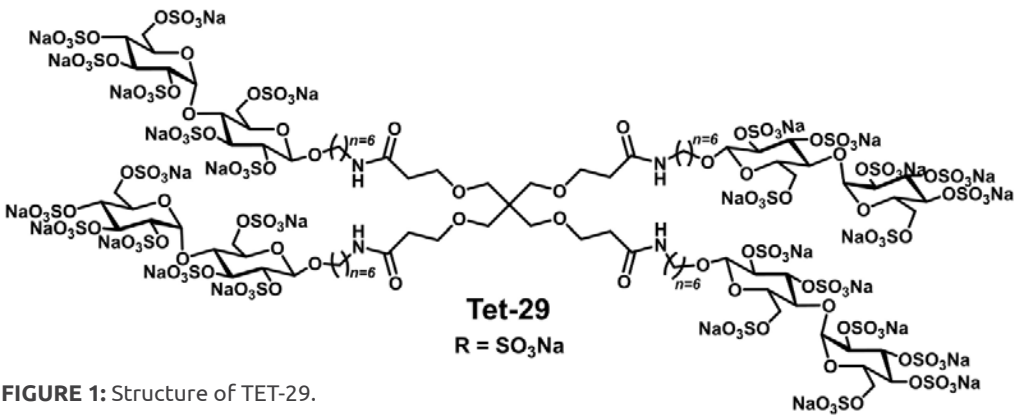


FIGURE 1: Structure of TET-29.

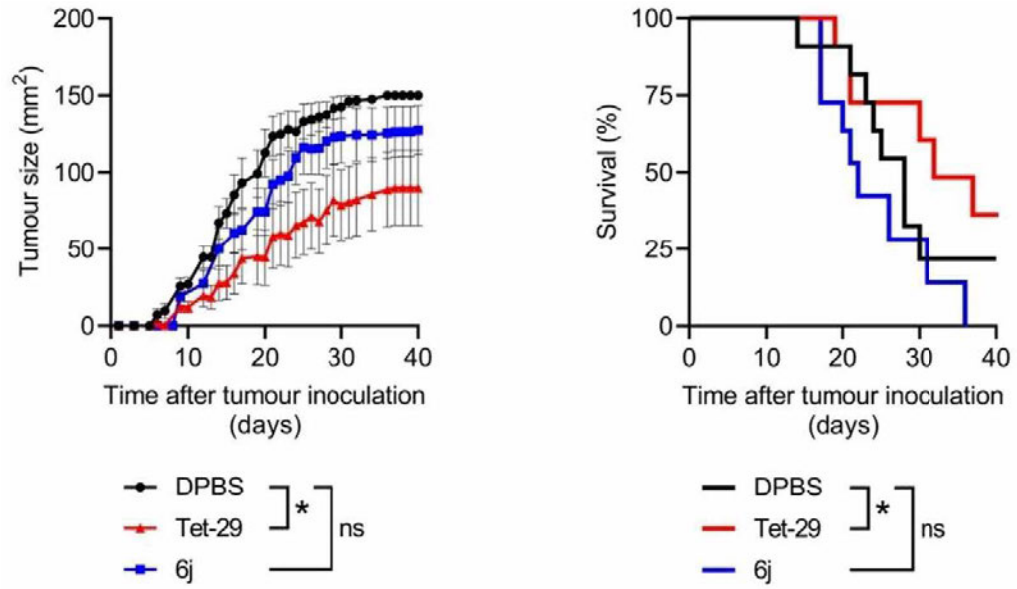


FIGURE 2: Colorectal Cancer Cells in Mice.

positive control. The four novel DMT inhibitors tested showed inconclusive results and further studies on the compounds were discontinued.

In Associate Professor Bronwyn Kivell's lab, different opioid compounds were investigated for pain relief in rodent models. A novel mixed kappa/delta opioid agonist MP1104 was compared to morphine, a mu opioid, and the mixed agonist was shown to be highly effective against chronic pain but much less addictive than morphine and also less susceptible to tolerance. Tolerance to a drug is measured as a decrease in potency with time after long-term use requiring increasing concentrations of the drug over time to maintain a constant level of pain relief. A paper on this work was published in 2021 in the journal *Neuropharmacology*.

We also carried out a review on the role of increased βIII-tubulin expression in cancer and its effects on cancer cell resistance to drugs and the metastasis of the primary tumour cells to other tissues in the body. Potential new therapeutic treatments of βIII-tubulin-expressing cancers were also discussed. This review was published in 2021 by the journal *BBA – Reviews on Cancer*.

CONCLUSIONS

The search for new compounds that are effective against cancer and chronic pain will continue to be pursued. The rewards for developing an effective anticancer drug with good potency and few side effects make the search all worthwhile. Chronic, non-addictive pain control has also long been an area needing further investigation.

Institute of Environmental Science and Research (ESR) and, University of Otago, Wellington

Investigating the mRNA expression of genes differentially methylated in the livers of guinea pigs born prematurely, or at full-term

DONIA MACARTNEY-COXSON AND MAX BERRY

KEY FINDING

We have observed significant gene expression differences for *CRY1* in the livers of adult male guinea pigs born pre-term vs at-term.

OBJECTIVE

To investigate the mRNA expression level of candidate genes for which we observed significant differential DNA methylation in the livers of adult guinea pigs born either at full-term, or prematurely.

METHOD

Samples: We previously examined DNA extracted from the livers of 12 animals. Here we extracted RNA from the same samples. Approximately 10mg of snap frozen sample was processed using a Qiagen RNeasy kit. We obtained good quality RNA (Agilent Bioanalyser RNA integrity number ≥ 7.5).

RT-qPCR: 500ng total RNA was reverse transcribed and RT-qPCR performed on 2µl 1/5 dilution cDNA. *β2 microglobulin* (B2M), *Beta-actin* (ACTB), and *GAPDH* were assayed as endogenous controls. The combined mean expression (Ct, cycle threshold) for these controls was used to normalise target mRNA expression using the ΔCt method (ΔCt = $Ct_{targetmRNA}$ – mean$Ct_{controls}$). Each sample was run in duplicate initially, for duplicates with a standard deviation of > 0.5 Ct the sample was repeated in triplicate.

Gene selection for RT-qPCR analysis: We focused our mRNA expression analysis on genes: a) in which we had observed significant differential DNA methylation (FDR < 0.05, ≥ 10% absolute DNA methylation difference across a number of CpG sites within the gene b) where this differential methylation occurred within or near the transcription start site and/or c) where the literature revealed a potential role in metabolism. We also checked for known expression of the gene in human liver (GTeX portal The Genotype-Tissue Expression (GTEx) project, https://gtexportal.org/home/). Primer/probe design used BioSearch RealTimeDesign Software (https://rtd.biosearchtech.com/design/inputSequences.aspx) and the current guinea pig reference genome which is incomplete. A total of nine candidate genes selected using the above criteria were assayed: *CRY1*, *ERG, GPX4, KDM2B, PCCB, PIK3CD, PPARA, UBXN6*, and *VPS13D*. Candidate gene primer/probe combinations were ordered from dnature diagnostics and research ltd (www.dnature.co.nz). TaqMan assays were used for endogenous controls (*B2M*: Cp04183281_m1, ACTB: Cp03755212_g1, GAPDH: Cp03755742_g1), with 1µl used per reaction.

RESULTS AND DISCUSSION

We previously observed significant differences in DNA methylation between liver samples from adult (9m) male guinea pigs born at full-term (n=6) vs prematurely (n=6).

Hyper-DNA methylation within gene promoter regions is classically associated with reduced gene expression. We therefore sought to investigate mRNA expression of a number of genes for which we observed significant DNA methylation difference within or close to the transcription start site, selecting nine genes for this analysis: *CRY1*, *ERG, GPX4, KDM2B, PCCB, PIK3CD, PPARA, UBXN6,* and *VPS13D*. This was performed in the same samples on which we performed DNA methylation analyses.

Optimising RT-qPCR: First we optimised primer and probe concentrations for each candidate gene assay, resulting in the use of 600nM primer and 200nM probe for each candidate gene.

RT-qPCR: Results can be seen in Figure 1. We observed a significant difference in mRNA expression for *CRY1* (adjusted P Value [Benjamini-Hochberg] = 0.009). A greater level of *CRY1* expression was observed in the liver of guinea pigs born at term, compared to pre-term. We previously observed a differentially methylated region (335bp, containing 10CpG loci) with significantly more methylation in livers from the pre-term (mean 47%) vs term (mean 27%) guinea pigs. Thus, our mRNA expression data is consistent with the traditional DNA methylation dogma that with increasing levels of DNA methylation one observes less gene expression.

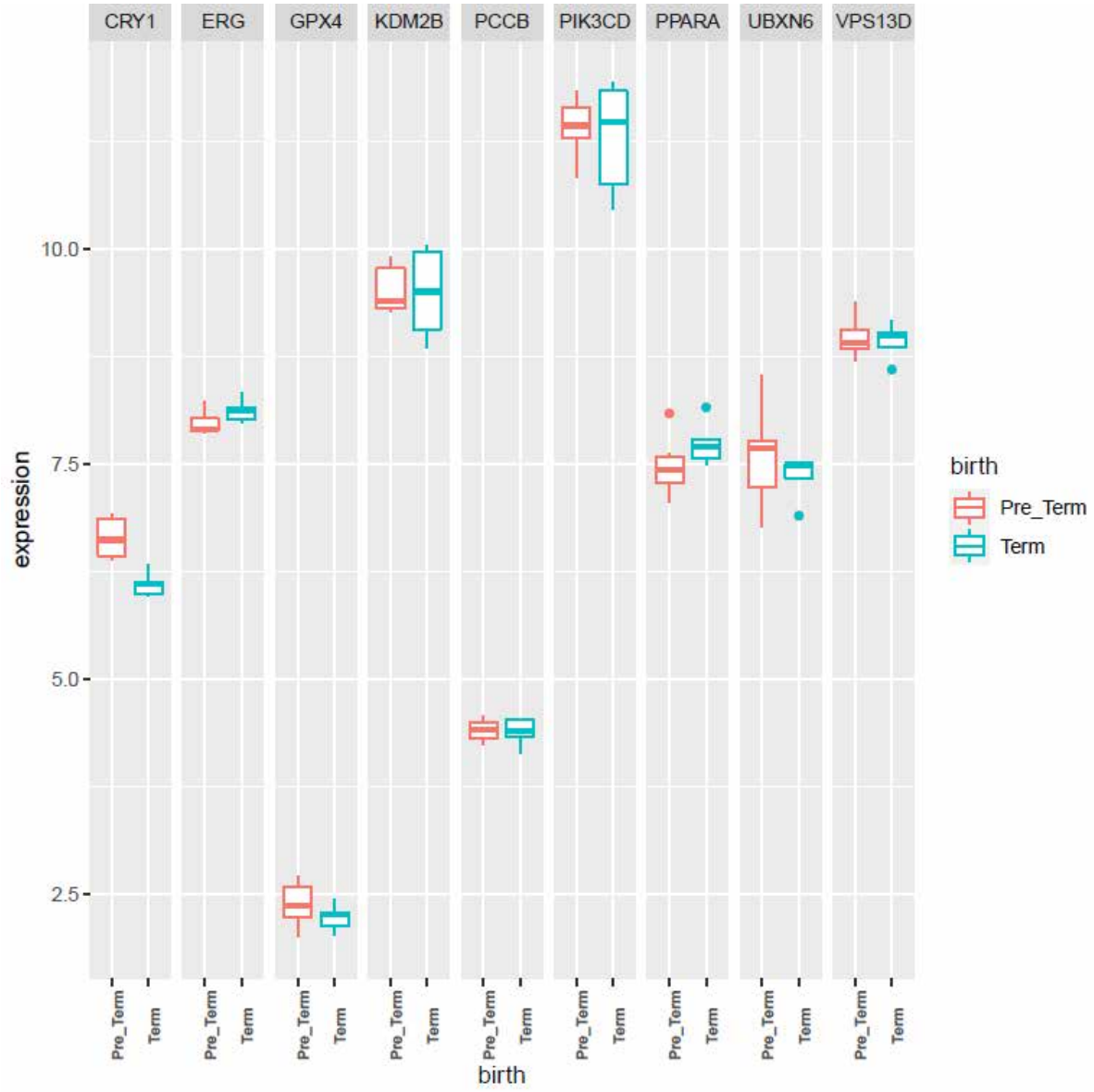


FIGURE 1: Box plots illustrating mRNA expression for candidate genes. Expression levels for *CRY1, ERG, GPX4, KDM2B, PCCB, PIK3CD, PPARA, UBXN6,* and *VPS13D* in liver samples from adult male guinea pigs born either pre-term (red, n=6)) or at term (green, n=6) are shown. Expression is normalised using the ΔCt method i.e. $Ct_{targetmRNA}$ – $meanCt_{controls}$

Premature birth is associated with an increased risk of metabolic complications in later life. Epigenetic mechanisms, which include DNA methylation, present prime candidates for mediating changes in genomic functions which affect disease risk as a result of the premature environmental 'assault' of preterm birth. An emerging literature reporting that epigenetic changes are associated with preterm birth in humans supports this, however these studies are necessarily restricted to blood. Here we utilised our first in world small animal model of preterm birth in guinea pigs to explore this further, concentrating our initial analysis on the liver which plays a critical role in metabolism.

We have observed significant differences in the DNA methylation and mRNA expression of *CRY1*. This gene encodes a transcriptional repressor involved in the regulation of circadian rhythm. Although poorly understood, there is a close relationship between the circadian clock and metabolism. Disruption of circadian rhythm biology in mammals is associated with a number of conditions including obesity, insulin resistance and metabolic syndrome. This is an exciting observation and provides strong support for our continued exploration of the role epigenetic perturbations may play in later life metabolic complications associated with premature birth.

Our current analysis focussed on males, we will seek to expand our analyses to female guinea pigs and extend our analyses of gene expression to other loci at which we have observed DNA methylation changes.

CONCLUSION

We have observed significant DNA methylation and gene expression differences for *CRY1* in the livers of adult male guinea pigs born pre-term vs at-term. This supports the hypothesis that epigenetic mechanisms prime candidates for mediating changes in genomic functions which affect disease risk as a result of the premature environmental 'assault' of preterm birth.

University of Otago, Wellington

Novel biomarkers for endometrial cancer

CLAIRE HENRY

KEY FINDINGS

We created endometrial cancer cells that are resistant to the Mirena, as a model to investigate non-response to this treatment. We investigated release of cell information packages (called vesicles) in Mirena resistant cells. We identified potential types of vesicle RNA that could tell us whether a cancer is responsive or not. We were able to measure these types of RNA in blood samples from women treated with the mirena.

OBJECTIVES

Endometrial cancer (EC) is the most common gynaecological cancer in Aotearoa New Zealand, and incidence rates are rising, particularly in younger women. Surgical resection (hysterectomy) is the standard of care for women with endometrial cancer. However, it is becoming more common that other treatment options are needed for women with significant co morbidities (high body mass index being the top risk factor for endometrial cancer) and/or who have not completed their families.

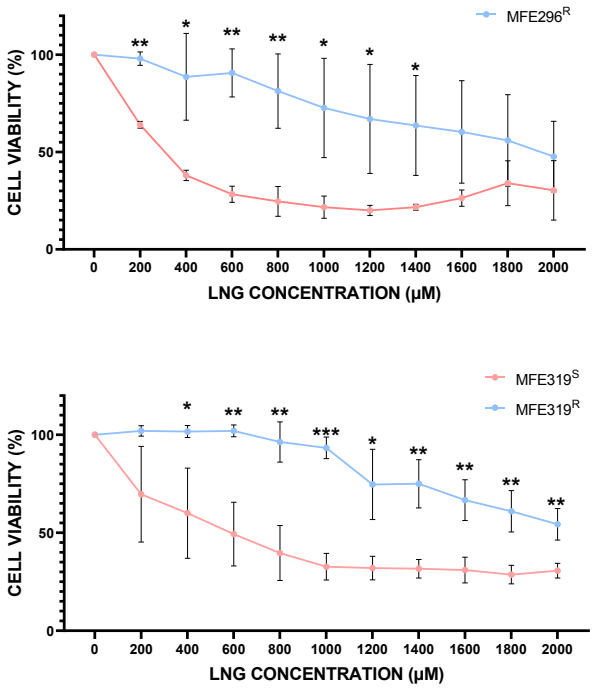
Traditionally used as a contraceptive, the Levornogestrel Intra Uterine System (LNG-IUS), also known as Mirena, may fill this role. It delivers progestogen directly to the uterus to inhibit oestrogen stimulated proliferation. Preliminary evidence shows that treatment of early-stage endometrial cancer or complex hyperplasia (pre-cancer) with the Mirena is only successful in approximately 60-70% of women. There are no predictors of response to LNG-IUS treatment; these are needed to determine who will benefit or who will still need surgery as a first line of care.

Our overall goal is to improve the treatment and detection of EC. Specifically for this project we will investigate molecular and cellular predictors of response to Mirena treatment for complex hyperplasia and early-stage EC. Our objectives were to investigate molecular changes in LNG resistant cells in the aim to inform biomarker discovery for endometrial cancer, specifically:

1. Create LNG resistant cell lines
2. Characterise the EV profile of Mirena resistant cell lines
3. Determine clinical efficacy of measuring mRNA from EVs in patient plasma samples

RESULTS

Resistant cell lines were created in MFE296, MFE319 (Figures 1 & 2) and 3x primary patient derived cells (Table 1).



FIGURES 1 & 2: Cell line resistance in increasing concentrations of LNG. Resistant clone (blue) and sensitive (red). A) MFE296 B) MFE319

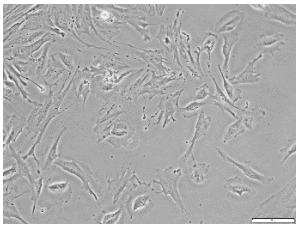
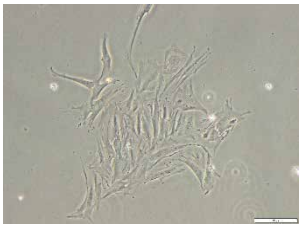
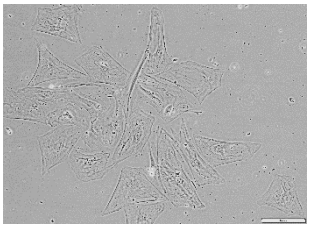
	GB #13	GB #16	GB #23
FIGO Stage/grade	Stage: IIIB Grade: 2	Stage: IB Grade: 3	Stage: IB Grade: 1
Histology	Endometrioid adenocarcinoma	Clear cell	Endometrioid
Tumour Size	39 x 25 x 17 mm	36 x 33 x 20 mm	50 x 30 x 16 mm
Nodal involvement	No	No	Staging incomplete – nodes not removed due to technical challenges of surgery; high BMI
Depth of myometrial invasion	23.5mm of 25mm	10mm of 17mm	2mm of 10mm
Molecular staining	Loss of MLH1/PMS2	P53/P16 overexpression, wt1 (-), napsin A (-), ER (-), HBM E-1 (-) vimentin (+)	MSI stable
Representative cell images	 200µm	 200µm	 200µm

TABLE 1: Patient derived cell line characteristics

EVs were characterised according to MISEV2018 guidelines, including particle size analysis via qNano (Figures 3-4), Western blotting (Figure 5), and electron microscopy (Figure 6).

The EV cargo were analysed via RNA seq to identify RNA markers of LNG resistance.

We investigated expression of mRNA and miRNA. mRNA was unable to be detected in EVs (qRT-PCR CT values >35). Therefore, we continued with miRNA analysis.

We conducted small RNA seq (perkin elmer NEXTFLEX small RNA library prep) in collaboration with genomics Otago. We are also collaborating with Sunali Meta (Otago) for downstream RNAseq analysis/ miRNA differential expression analysis. We have identified miRNAs that are upregulated in EVs from resistant cells (Figure 7). We validated these through qRT-PCR (Figure 8).

We measured candidate markers in plasma collected from women treated with the Mirena, and those who were not. miRNAs of interest were able to be identified in plasma. miR-31 and miR-182 were overexpressed and indicative of non-response to LNG. It is important to note that over the last year, with the impact of COVID, patient recruitment has been low. RNA seq analysis, validation and collaboration is ongoing.

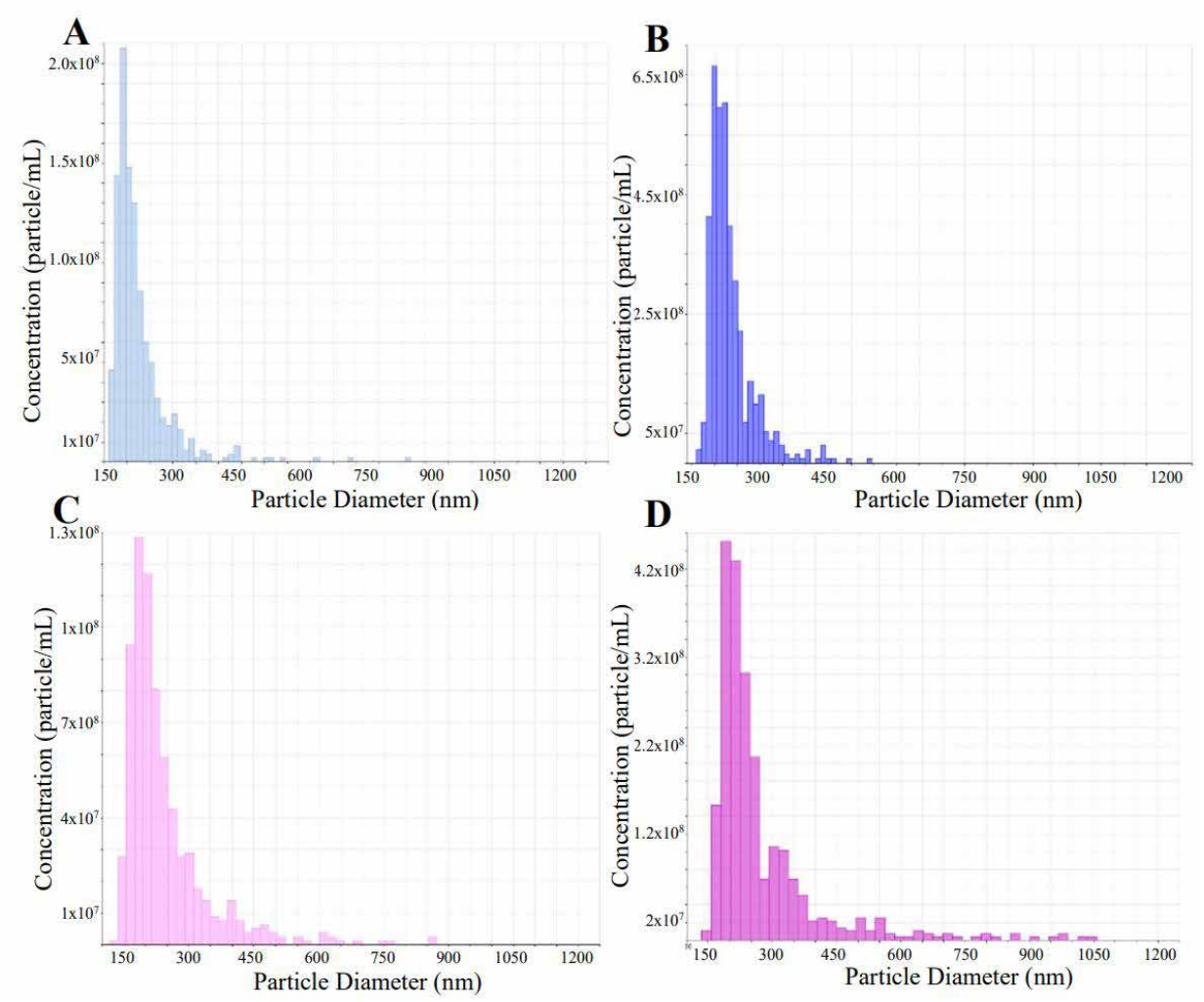


FIGURE 3: Representative size distribution histogram of EVs as measured by TRPS. Concentration of EVs (particles/mL) is plotted against particle diameter (nm). A) MFE296S. B) MFE296R.

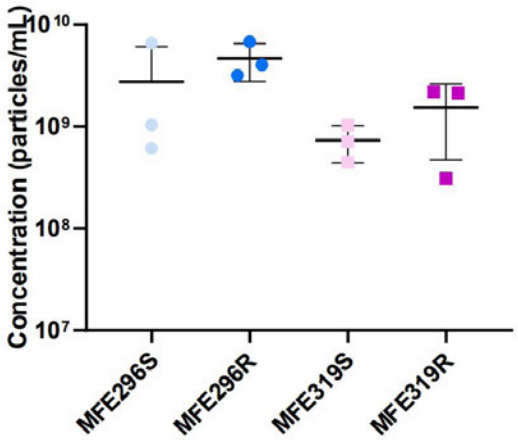


FIGURE 4: Concentration of EVs from cell culture media as measured by TRPS. Data is presented as the mean ± SD (n=3) with individual values plotted.

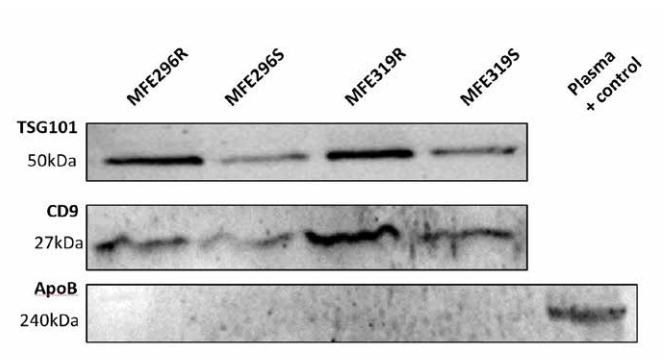


FIGURE 5: Detection of EV specific protein markers TSG101 and CD9, and contaminant marker ApoB in EV lysates from cell culture media. Plasma was included as a positive control for ApoB.

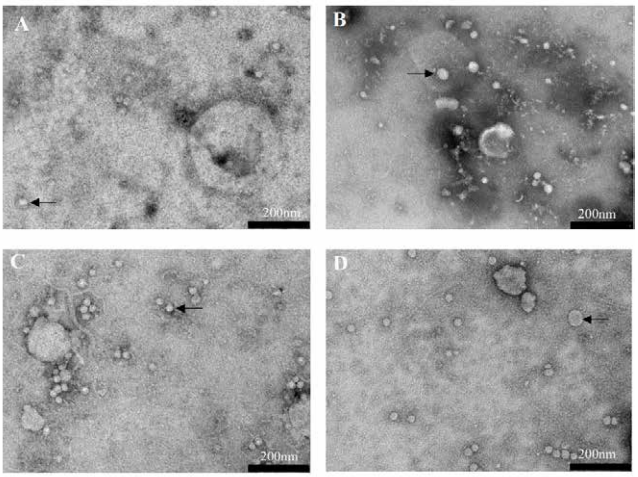


FIGURE 6: Negative stain electron microscopy images of EVs isolated from cell culture media. EVs were isolated from fresh cell culture media and all fractions pooled and concentrated for imaging. Arrows indicate example intact vesicles. A) MFE296R. B) MFE296S. C) MFE319R. D) MFE319S.

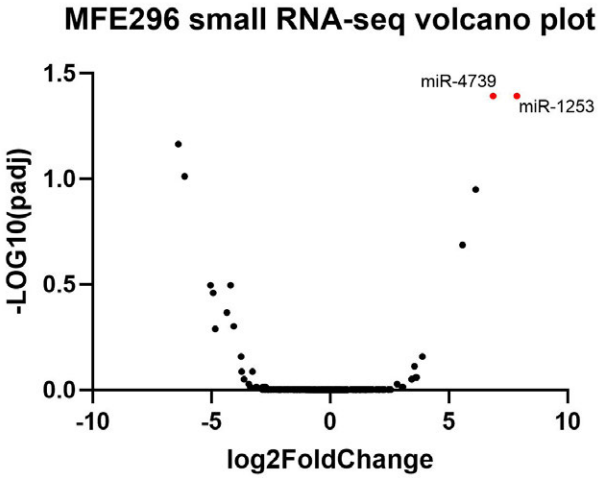


FIGURE 7: Volcano plot of RNA seq data highlighting potential biomarkers of resistance (red).

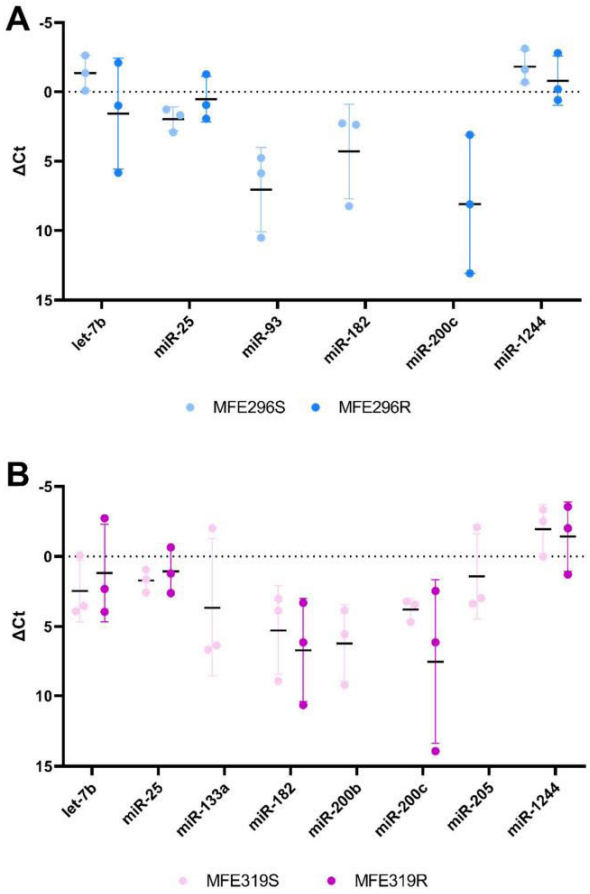


FIGURE 8: Validation miRNA expression in endometrial cancer cell line EVs.

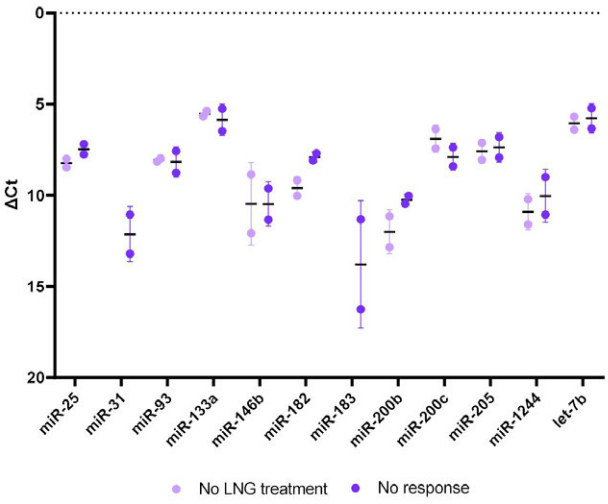


Figure 9. miRNA expression in patient plasma samples.

CONCLUSION

The support for this project has been invaluable for myself (PI Henry) as an early career researcher in Wellington. I have been able to create collaborations with other Otago staff, for example Dr Richard Easingwood in Dunedin for electron microscopy and solidify working relationships with Wellington staff such as Kirsty Danielson. The support for this project has lead to:

1. A successful HRC emerging researcher grant to investigate the function of EV cargo in treatment resistance and to further work towards creating a blood based biomarker test,

2. The establishment of a multi-centre study of the Mirena (LNG-IUS) where we recruit women being treated from CCDHB, Hutt Valley DHB and Mid Central DHB and collect blood and tissue samples,

3. Growth of my research group: retained Molly Dore as a PhD student, and Emily Paterson as an Hons (2021) and now PhD student (2022), both who worked on aspects of this project in 2021.

Molly Dore has gone on to investigate stem cells in endometrial cancer LNG-IUS resistance, in which she received a RFL grant for, and Emily Paterson has started her PhD in endometriosis and EVs, drawing upon the skills she has developed from this project.

We work closely with the gynaecological oncology team at wellington hospital who allow this work to be closely aligned with translational outcomes. I am part of the NZ Gynae Cancer Group, and am involved in the development of new guidelines to aid the use of the LNG-IUS for treatment of endometrial cancer. I am also part of the Early-Mid Career Research Committee where we support researchers to career development, mentorship, and grant application.

We are still completing some of the final RNA seq analysis, with collaborators in Dunedin with Dr Sunali Mehta and Post Doc Debina Sarkar. At completion of this data we will prepare our manuscript for publication.

We have published the data on the development of the cell lines, and behavioural characteristics of LNG resistance: Dore M, Filoche S, Danielson K, Henry C. Characterisation of Levonorgestrel-Resistant Endometrial Cancer Cells. Cancer Manag Res. 2021 Oct 14;13:7871-7884. doi: 10.2147/CMAR.S327381. PMID: 34703309; PMCID: PMC8523362.

From this work we have completed a review of endometrial cancer EVs and recommendations for working with miRNA biomarkers, currently under review in Translational Oncology.

University of Otago, Wellington

The immunomodulatory role of tumour-derived extracellular vesicles in colorectal cancer

O BUCHANAN, L CONNOR, K HALLY, K DANIELSON

KEY FINDINGS

In this study, we identified and characterised monocyte populations from the peripheral blood of healthy volunteers. We have also isolated and characterised extracellular vesicles from colorectal cancer (CRC) cell lines and are in the process of analysing the influence of extracellular vesicles (EVs) on monocyte phenotypes and function. This will enhance our understanding of how colorectal cancer cells communicate with host immune cells.

OBJECTIVES

The aim of this study was to investigate the immunomodulatory role of tumour-derived extracellular vesicles (tEVs) in CRC. Specifically, this project involved investigating monocyte subsets and function in response to CRC-derived tEV application. This included developing a cell-culture model to study PBMC-derived monocytes over time and optimising a flow-cytometry panel. Ongoing results from this study are currently being analysed and completion is expected by October 2022. Further results from this study may provide insight into the mechanisms by which CRC cells influence the immune and inflammatory response.

METHODS

Healthy volunteers were recruited from the Wellington region. Written and informed consent was obtained prior to blood collection (HDEC 19/CEN/192).

tEVs were isolated from two CRC immortalised cell lines: DLD1 (Stage III colorectal adenocarcinoma) and SW837 (Grade IV rectal adenocarcinoma). Cells were cultured under standard conditions for 48 hours and tEVs were isolated from culture media using size exclusion chromatography (SEC). tEV size and counts were assessed by tunable resistive pulse sensing using the qNano gold nanoparticle counter (Izon).

Peripheral blood mononuclear cells (PBMCs) were isolated from the peripheral blood of 3 healthy volunteers and cells were cultured with tEVs or dummy EVs (as below) for 6 hours. The same healthy volunteers were used for each experiment and PBMCs were pooled prior to antibody staining.

tEVs were applied at 2 different doses to reflect a ‘high’ and ‘low’ exposure. The cell-surface expression of markers for identifying and assessing monocyte phenotype were examined on a Cytek Aurora Spectral Flow Cytometer using an eight-marker panel.

RESULTS

We demonstrated identification of classical, intermediate and non-classical monocytes with the application of tEVs over 6 hours of culture. Preliminary results indicate no shift in cell count across monocyte subsets (Figure 1). The percentage of total monocytes was counted for classical, intermediate and non-classical monocytes and there were no significant differences between the three subsets across tEV treatments.

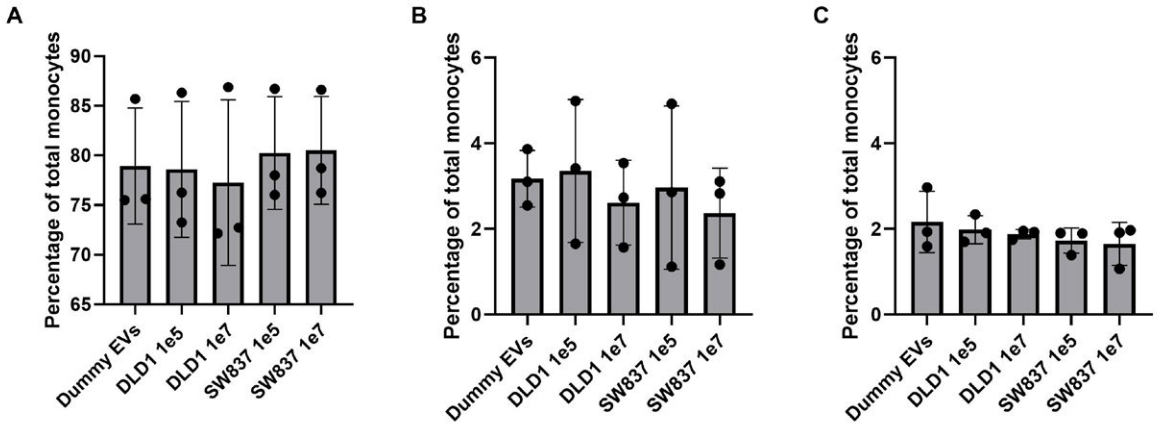


FIGURE 1: Cell counts of (A) classical monocytes, (B) intermediate monocytes (C) non-classical monocytes. Counts are shown as percentage of total monocytes within each experimental run (n=3). Data shown as mean +/- standard deviation and analysed with a one-way ANOVA with paired multiple comparisons.

CONCLUSION

We found no significant shift in the number of cells across monocyte subsets in response to tEV application. We are in the process of measuring the expression of the eight markers in monocytes, in response to tEV treatment. This will involve an in-depth analysis to assess any significant shifts in expression of phenotypic and functional markers across all three monocyte subsets. This information can be applied to future studies which may investigate the effects of tEVs on other immune cell phenotypes and functions.

University of Otago, Wellington

The single-cell transcriptome of rectal cancers before chemoradiation therapy

A GREENWOOD, K DANIELSON, N TUCKER

(in association with the Masonic Medical Research Institute (MMRI) in Utica, New York)

KEY FINDINGS

The genetic landscape of a rectal cancer sample, resistant to chemoradiation, has been profiled, at the single-cell level, to reveal major intra-tumour heterogeneity and a low immune cell component. Analysis of multiple cancer samples, from responders and non-responders, is underway to confirm this and determine exact genetic drivers of treatment-resistance.

AIM

The aim of this study was to recruit and collect pre-treatment and post-treatment biopsies from six rectal cancer patients at Wellington regional hospital with a varied response to chemoradiation therapy. We then aimed to profile the genetic landscape, at a single-cell resolution, using single-cell sequencing (sc-seq) and compare responders and non-responders to treatment. This information is key to understanding the contribution of individual tumour cells, with divergent biological behaviours, to treatment-resistance.

METHOD

In response to COVID-related disruptions to clinical collections, we were able to recruit for this study between the period of August 2020 to October 2021. This study was approved by the Health and Disabilities Ethics Committee (20/NTB/19). Patients at Wellington Regional Hospital with a clinical diagnosis of advanced rectal cancer, who were expected to undergo long course chemoradiation, were eligible for inclusion.

All biopsies were shipped to the MMRI, Utica for sc-seq. A microfluidics technology was used to reduce samples into single-nuclei suspensions. Libraries for individual nuclei (up to 200,000 nuclei from each sample) were prepared using the 10X Genomic platform for Illumina sequencing. Pre-processing of data and quality control metrics have been completed for two samples and the remaining samples are currently being analysed.

RESULTS

Of ten eligible patients, eight provided written informed consent. Biopsies were successfully collected from five participants where the rigid-sigmoidoscopy procedure could be performed, and cancerous tissue was large enough to be identified and biopsied by a colorectal surgeon. All five participants completed chemoradiation over six weeks. After surgical resection of the tumour, three patients were classified as ‘responders’ (complete or near-complete response) and two as ‘non-responders’ (partial or no response).

Bioinformatic analysis of a single sample (from a non-responder) has been completed to demonstrate, at an initial low resolution, cellular diversity within the tumour microenvironment. Figure 1 presents cell clustering where cells are aligned in space based on their genetic similarities. Most strikingly, three large, distinct malignant cell clusters are present and make up the majority of cells within the

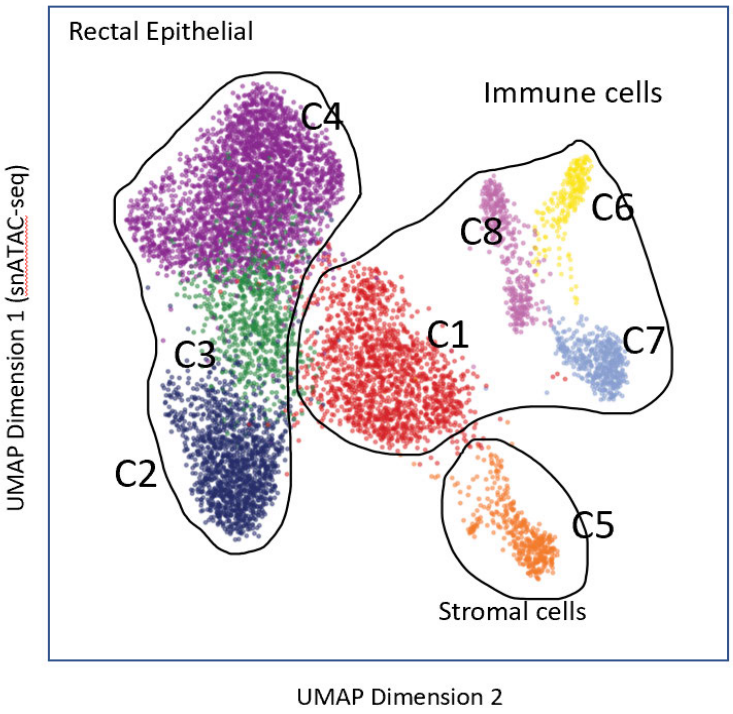


FIGURE 1: Cell diversity from single-cell sequencing. Data is from a post-treatment rectal cancer biopsy resistant to chemoradiation. A total of eight genetically distinct cell clusters were detected (C1-C8) representing malignant epithelial cells, immune cells and stromal cells.

tumour. This demonstrates the level of the intra-tumour heterogeneity present, which likely contributes to treatment resistance. Smaller clusters of immune cell populations are present which may suggest low immunogenicity (tumour-induced immune response) and could be involved in resistance.

More in-depth analysis to characterise specific cell subtypes is currently underway for this sample and will be used as a template for analysis of all samples in this study. This will allow for between-response comparisons to highlight genetic and cellular drivers of treatment-resistance.

CONCLUSION

Sc-seq has been performed on rectal cancer biopsies collected from participants with a varied response to chemoradiation. Initial bioinformatic analysis on a single sample has found several distinct malignant tumour clusters demonstrating a large amount of within-sample heterogeneity and low immune cell component, which are known contributing factors to treatment-resistance. In-depth analysis is currently underway for all samples to make between-response comparisons to compare cellular diversity and find genetic drivers of treatment-resistance.

University of Otago, Wellington

Assessing genomic variability between multiple instances of the Jurkat clone E6-1 cell line

A WILSON, N DASYAM, R WEINKOVE, M THUNDERS

(in association with the Malaghan Institute of Medical Research, Wellington)

KEY FINDINGS

Jurkat clone E6-1 cell lines obtained from various laboratories around Aotearoa New Zealand show diverse genomic landscapes that may have unfavourable implications for translational research. Genomic diversity between cell lines was assessed using various cytogenetic and molecular techniques, and mutational profiles deviated when compared to newly obtained Jurkat clone E6-1 cells from the supplier.

OBJECTIVES

Initially, the aim of this research was to characterise various cell lines using cytogenetic methods and compare the observed chromosomal abnormalities to reported karyotypes from the supplier. Due to the temporary closure of the University of Otago Wellington campus shortly after obtaining funding from Research for Life, the aims of the project were adjusted to reflect this change in circumstances. Therefore, we aimed to obtain a fully comprehensive genome-wide survey on multiple instances of one cell line to investigate genomic diversity between laboratories. The Jurkat clone E6-1 cell line, derived from a paediatric case of T-lineage acute lymphoblastic leukaemia, is widely used in translational cancer research in New Zealand. Cells were obtained from three different laboratories around Aotearoa New Zealand. Four samples of Jurkat clone E6-1 cells (three from New Zealand laboratories, and one newly obtained from the ATCC) were used in genomic analyses to investigate heterogeneity between cell lines that are reportedly identical.

METHODS

Jurkat clone E6-1 cells were split into two separate aliquots and cultured for different downstream applications. Using the first aliquot, cells were cultured for cytogenetic analysis according to clinical guidelines. Once harvested, cells were delivered to the Wellington Regional Genetics Laboratory for karyotyping. 20 cells in metaphase were analysed for each case of the Jurkat clone E6-1 cell line. To confirm karyotypic findings and to detect structural variants below the limit of detection for karyotyping, DNA was extracted from the second aliquot of each Jurkat clone E6-1 sample for microarray analysis at the Wellington Regional Genetics Laboratory. Using the same DNA sample extracted for microarray studies for each Jurkat clone E6-1 cell line, whole exome sequencing was outsourced to Custom Science, Auckland, to provide an insight on the genomic landscape of each cell sample at a single nucleotide level. Gene panels were constructed to filter down variants: one panel focussed on genes associated with maintaining genomic stability (452 genes), and a second panel including genes associated with T cell signalling and proliferation pathways (169 genes).

RESULTS

Prior to the karyotyping of in-use Jurkat clone E6-1 cells, it was essential that the karyotype for newly obtained Jurkat clone E6-1 cells was obtained to establish a cytogenetic baseline. The karyotype for newly obtained Jurkat clone E6-1 cells was **46,XY,del(2)(p23),del(18)(p11.3)[20]**, which can be inferred as a male diploid karyotype with a deletion on chromosome 2p and a deletion on chromosome 18p identified in 20 metaphase cells (example karyotype in Figure 1A). After establishing the initial karyotype for Jurkat clone E6-1 cells, it was then possible to analyse the three in-use Jurkat clone E6-1 cells obtained from three research facilities around Aotearoa New Zealand (hereafter referred to as laboratory 1, 2, or 3).

Karyotype results for Jurkat clone E6-1 cells from laboratory 1:

81~89,XXYY,del(2)(p23)x2,add(5)(p15)x2,del(18)(p11.2)x2[cp20] –male hypotetraploid line with deletions on chromosomes 2p and 18p, and additional material on chromosome 5p, formed from a composite karyotype of 20 metaphase cells where every cell analysed was different (example karyotype in Figure 1B).

Karyotype results for Jurkat clone E6-1 cells from laboratory 2:

45,X,-Y,del(2)(p23)[20] – male diploid karyotype with a loss of chromosome Y and a deletion on chromosome 2p identified in 20 metaphase cells (example karyotype in Figure 1C).

Karyotype results for Jurkat clone E6-1 cells from laboratory 3:

46,XY,del(2)(p23)[9]/47,idem,+20[11] – male diploid karyotype with a deletion on chromosome 2p detected in 9 metaphase cells. An additional subclone was detected with trisomy 20, alongside the deletion on chromosome 2p, in 11 metaphase cells (example karyotype in Figure 1D).

All karyotype results were confirmed by microarray studies (not shown), the results of which also revealed subtle structural variants that were below the limit of detection for conventional cytogenetics.

Whole exome sequencing highlighted variability in the mutational profiles at a single nucleotide resolution of each Jurkat clone E6-1 cell line analysed in this research. Firstly, newly obtained Jurkat clone E6-1 cells were analysed to establish a baseline mutational profile, then the mutational profiles of each in-use cell line were directly compared to newly obtained cells. Jurkat clone E6-1 cells from laboratory 1 featured pathogenic mutations in *LCK* and

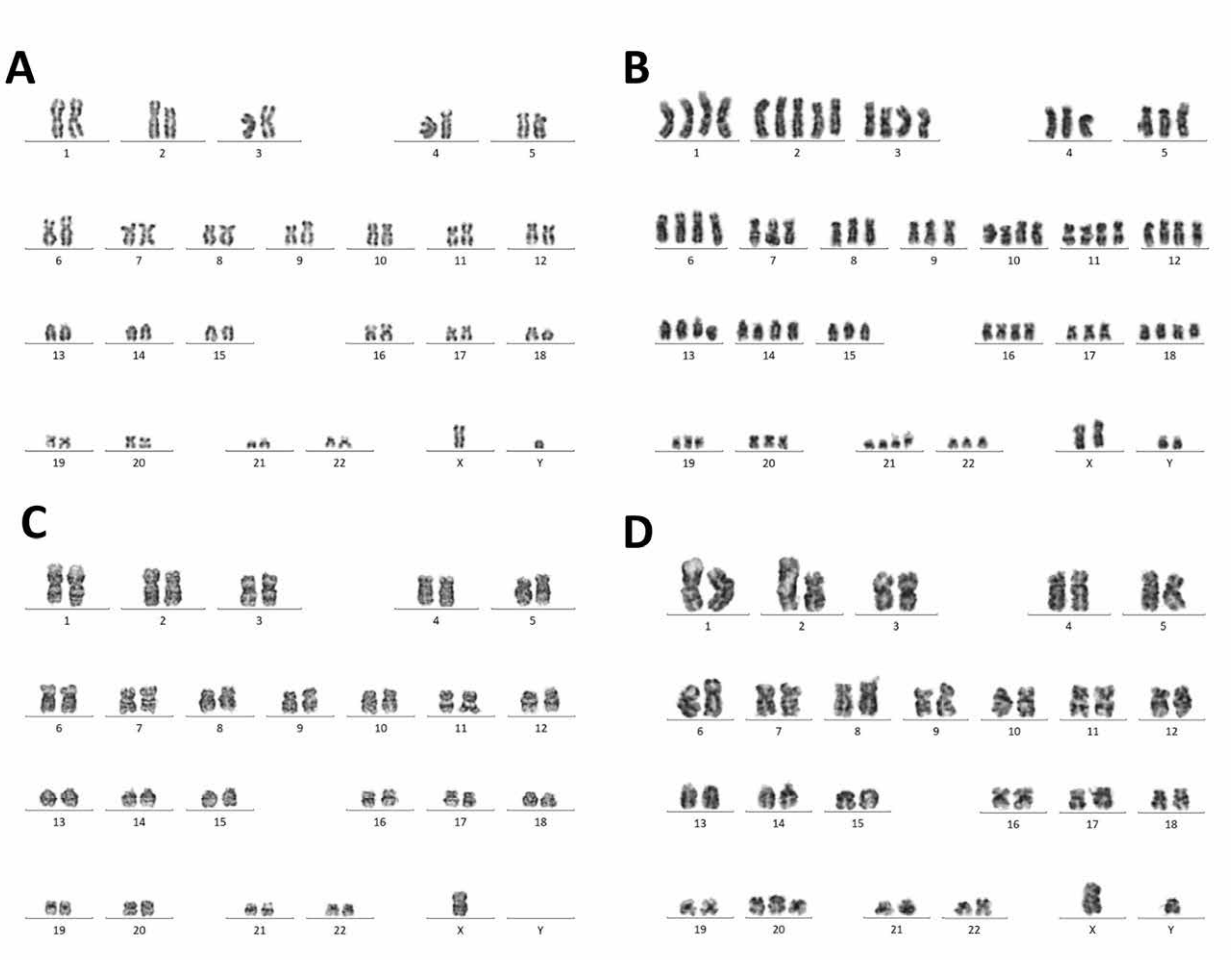


FIGURE 1: Example karyotypes for Jurkat clone E6-1 cell lines. A: newly obtained, B: laboratory 1, C: laboratory 2, D: laboratory 3.

IL2RA; both of which are critical genes in T cell development and proliferation pathways. Jurkat clone E6-1 cells from laboratory 2 featured a damaging frameshift mutation in *CD3G*, a critical gene involved with the formation of the T cell receptor CD3 complex, and resulted in a premature termination at codon 71. Jurkat clone E6-1 cells from laboratory 3 feature a damaging frameshift mutation affecting *BAX*; a gene involved in the maintenance of genomic stability. In addition to these damaging mutations, whole exome sequencing data provided insight into the number of different structural variants present in each sample. Table 1 provides information on the frequency of each type of structural variant called.

CONCLUSION

Our data highlights genomic diversity, at both chromosomal and DNA levels, between each Jurkat clone E6-1 cell line analysed in this research. Overall, we would recommend that researchers be mindful of the genomic landscape of their cell lines and would encourage genomic analyses of cells before use in key experiments. We wish to acknowledge each laboratory who provided Jurkat clone E6-1 cells for genomic interrogation, and the Wellington Regional Genetics Laboratory for their aid and technical guidance throughout this project.

	Newly obtained	Laboratory 1	Laboratory 2	Laboratory 3
Deletions	28	36	33	33
Insertions	30	39	39	45
Tandem duplications	12	14	13	14
Interchromosomal translocations	12	50	26	32
Intrachromosomal translocations	24	26	20	30
Total	**106**	**165**	**131**	**154**

TABLE 1: A comparison of structural variants detected by whole exome sequencing for all instances of the Jurkat clone E6-1 cell line analysed in this study.

University of Otago, Wellington

Describing the circulating monocyte response in patients with a heart attack

K HALLY, A HOLLEY, L FERRER-FONT, S HARDING, A SASSE, P LARSEN

(in association with Victoria University of Wellington, the Malaghan Institute of Medical Research and Wellington Regional Hospital)

KEY FINDINGS

Monocytes are a key type of inflammatory cell in blood that are involved in driving inflammation during a heart attack. Here, we show that the function of monocytes is dynamic within the first three days following a heart attack in humans. Their inflammatory activity peaks at between 12- and 36-hours after individuals start experiencing chest pain, and these monocytes become exhausted by day 3 (approximately 60-72 hours) post-onset of chest pain (Figure 1).

AIMS

Heart attacks are a leading cause of death and disability in New Zealand and, for those that survive, life after a heart attack is far from free of health complications. The heart starts to repair soon after a heart attack but, in some patients, the quality of this repair process is compromised. This can result in growth or expansion of the region of the heart which is damaged during a heart attack (known as the 'infarct'). Currently, we do not know how to identify, and therefore treat, patients whose heart does not repair optimally.

We think that monitoring inflammation may be an effective way of identifying these high-risk individuals. But first, we must understand the timing of inflammation after a heart attack: when does inflammation peak in most individuals following the onset of chest pain, and when does this inflammation resolve? This type of information will assist us in determining the optimal sampling time for biomarker discovery in this context: sample too early, and inflammation

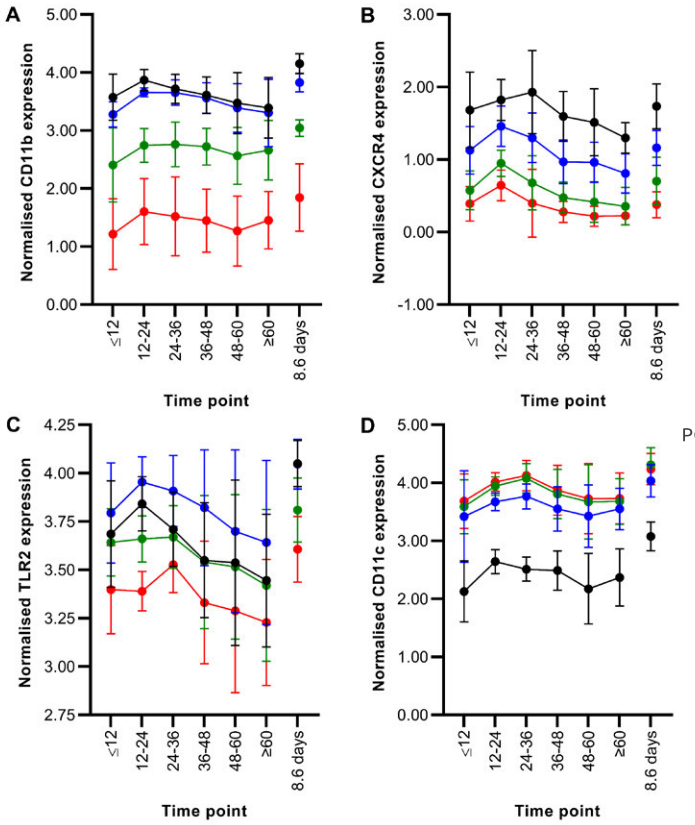


FIGURE 1: The trajectory of circulating monocyte activation in patients with a heart attack. Monocytes were analysed by flow cytometry from a peripheral blood sample from 17 patients at multiple timepoints during hospitalisation for a heart attack. Activation was monitored by a panel of markers including CD11b (A), CXCR4 (B), TLR2 (C) and CD11c (D). Four monocyte subsets were identified as follows: classical (black), intermediate (blue), SLAN(-) non-classical (red) and SLAN(+) non-classical (green). Mean ± standard deviation is shown for ≥5 patients per time point.

may not have reached its peak yet; sample too late and inflammation may be resolving.

More specifically, we are interested in monitoring the activation status of monocytes, which are important blood immune cells that participate in inflammation during a heart attack. Moreover, we can monitor these monocytes within peripheral blood, which is a relatively non-invasive way of collecting this information (a 5 mL venous blood draw).

METHODS

We have recruited 17 patients admitted to Wellington Regional Hospital with a heart attack. These patients have not had a heart attack before and have been treated invasively with percutaneous coronary intervention (stenting) of the culprit coronary artery.

We have drawn blood from these individuals each day from enrolment to discharge (1-3 blood draws per patient). We have also taken blood from these individuals at between 7 and 11 days after their onset of chest pain. This is a remote time point after hospital discharge.

In these blood samples, we have measured monocyte immune status using full spectrum flow cytometry, which is a technique used to measure the physical characteristics of a cell type of interest.

RESULTS

Description of the patients: the patients that we have recruited into this study were mostly male (88% of our patient cohort), were 63 years old on average and had a high rate of hypertension (47%), dyslipidaemia (71%) and diabetes (29%). Most patients presented with ST-segment myocardial infarction (STEMI; 82%), which is a condition where the coronary artery is completely blocked and is associated with more severe outcomes than non-ST segment myocardial infarction (NSTEMI; 18% of our patient cohort).

Blood sampling: for most patients (92% of the cohort), two or more blood samples were analysed during their hospital stay. For 12 patients (71%), we also sampled blood at an average of 8.6 days after their symptom onset. All blood samples were processed within four hours of drawing.

Mapping the trajectory of monocyte activation following a heart attack: by flow cytometry, we have identified four subsets of circulating monocytes which, based on the literature, differ in their immune function. For each of these subsets, we tracked the expression of proteins on the surface of these cells that are associated with particular monocyte functions. For example, Figure 1 illustrates the trajectory of markers associated with cellular adhesion (CD11b, CD11c) and sensing the environment for pro-inflammatory molecules (CXCR4) and damage- or pathogen-associated molecules (TLR2). Each monocyte subset has a distinct expression pattern for these markers. For example, classical monocytes (represented by the black line in Figure 1) express the highest levels of CD11b and CXCR4 but the lowest levels of CD11c compared to all other monocyte subsets (represented by the blue, green, and red lines). Although there is significant inter-individual variation (as shown by the error bars indicating standard deviation in Figure 1), we can see that there is a trajectory of monocyte activation that peaks at between 12- and 36-hours after the onset of chest pain, and (in most cases) drops towards the later time points recorded (Figure 1). At the remote time point (approximately 8.6 days post symptom onset), we see that expression of these markers is higher than during the in-hospital time points. This may indicate a priming of monocytes following a heart attack, although a longer follow-up (perhaps at 6 weeks and 6 months post-heart attack) will give us greater insight into how monocytes behave once a patient has stabilised.

CONCLUSION

We have demonstrated that monocyte activation is dynamic during a heart attack, with a peak at between 12- and 36-hours after the onset of symptoms. We also demonstrate that this dynamicity is not confined to the in-hospital period, with monocyte activation high at a time point remote to hospitalisation. Mapping this trajectory is important for understanding the role of monocytes in response to a heart attack and indicates that sampling at a time point that is personalised for each patient (based on their timing of symptom onset) is an important measure for biomarker discovery in this clinical context.

CENTRE FOR ENDOCRINE, DIABETES, AND OBESITY RESEARCH (CEDOR)

Wellington Regional Hospital

Whole exome sequencing in primary hyperparathyroidism: prevalence of predisposing germline

[INTERIM REPORT]

RW CARROLL, M BENTON, D MACARTNEY-COXSON, E WALSH, S HARPER, V BOYLE, M ELSTON

KEY FINDINGS

This study is investigating how frequently underlying predisposing genetic mutations are present in people with primary hyperparathyroidism. This is the first study of its kind in Aotearoa New Zealand.

OBJECTIVES

This is a multicenter study to assess the prevalence of inherited genetic mutations predisposing to primary hyperparathyroidism and related endocrine disorders by whole exome sequencing, in a cohort of New Zealanders with primary hyperparathyroidism. Ethics approval, Māori research advisory group endorsement, and locality approval were received in 2019-20, and recruitment began in late 2020.

METHODS

A cohort of individuals with primary hyperparathyroidism suspected to have an increased likelihood of predisposing germline variants has been recruited. Selection was both retrospective and prospective, and included patients with confirmed primary hyperparathyroidism who are <50 years of age, have multigland disease, have additional endocrine issues suggesting multiple endocrine neoplasia, and/or individuals with a family history suggesting an increased risk of primary hyperparathyroidism or associated endocrine disorders. Participants have been recruited through the endocrine departments in Wellington and Hamilton.

Participants have provided either a 4ml blood sample (collected EDTA vacutainers and stored at -80°C) or a saliva sample using DNA collection kits (Oragene). Samples have then been transported to ESR (Porirua, Wellington) where they will be stored at -80°C until DNA extraction is performed using a Qiagen Blood DNA extraction kit. Whole-Exome sequencing will be performed on an Illumina NextSeq 550 using the Illumina Nextera DNA exome kit. The sequencing will focus on several key genes either to be involved in parathyroid function or where mutations have previously been confirmed as pathogenic. The presence of any known mutations or variations will be reported.

PROGRESS

This is an interim report as enrolment for the study is continuing but nearly complete. Approximately 60 participants have now provided consent and the required blood or saliva samples for the study. Samples are currently being processed at ESR, Wellington and we are hopeful that results will be available within the next few weeks. Ethics approval has been received for enrolment of further cohorts including patients with multiple endocrine neoplasia and familial paraganglioma/phaeochromocytoma syndromes.

The COVID pandemic has unfortunately delayed completion of this study for several reasons. Firstly, enrolment numbers fell around lockdowns, and secondly, the laboratory processing the samples have been involved in COVID exome sequencing as part of the national response. Furthermore, it became clear early in the project that the requirement

for a blood sample was an obstacle to recruitment for some participants, either because of fear of venepuncture, or because of the requirement to attend a central centre for blood collection so samples could be immediately stored at -80°C. We therefore obtained ethics approval in late 2021 to use saliva samples in place of blood samples using a kit designed for this purpose. Saliva samples can be stored at room temperature until processing, and participants can therefore provide samples at the point of consent, often obtained in clinic. This has substantially improved recruitment and will inform our use of whole exome sequencing in the clinic setting going forward.

CONCLUSION

We aim to complete recruitment of cohorts over the next 12 months with results published and disseminated in 2023.

RESEARCH PROJECT & TRAVEL GRANTS

Research Project Grants 2022

THE FOLLOWING PROJECTS HAVE BEEN APPROVED FOR FUNDING IN THE FINANCIAL YEAR ENDED 30 JUNE 2022 AND WILL BE REPORTED ON IN SUBSEQUENT RESEARCH REVIEWS.

## Oxidative stress causes epigenetic changes that may promote tumour development **DR AARON STEVENS**	Dr Aaron Stevens, a senior lecturer in the Department of Pathology and Molecular Medicine at University of Otago, Wellington, was awarded up to $10,000 towards the study of the chemical signatures on genomic DNA which are important regulators of gene activity and act at the interface between the environment and gene regulation. The methylation of cytosine alters DNA structure and gene expression and impacts all aspects of cell function. Understanding how DNA methylation patterns are regulated by environmental stimuli is a central question for managing health and disease and is a major contemporary challenge in human genetics. Dr Steven's team has recently demonstrated that oxidants generated by activated white blood cells can change the pattern of methylation at specific regions of human genomic DNA. They hypothesize that this is a key driver in the development and progression of inflammation-associated cancer. In this project they aim to understand the molecular consequences of this interaction, which will aid in the development of biomarkers for early diagnosis of cancer risk.

## Antimicrobial Surveillance for Gonococcal Clinical Samples **DR XIAOYUN REN AND DR COLLETTE BROMHEAD**	Dr Xiaoyun Ren and Dr Collette Bromhead were awarded up to $25,000 to establish an assay to allow the detection of multiple antimicrobial resistance mutations directly from gonorrhoea clinical samples. Increasing antimicrobial resistance in Neisseria gonorrhoeae, the causal agent for gonorrhoea, is becoming a worldwide emergency. Surveillance of antimicrobial resistance phenotype and mutations that confer resistance are an essential part of combating resistance in Aotearoa New Zealand. However, only a small percentage of gonococci undergo phenotypic resistance testing, creating a big gap in antimicrobial resistance surveillance. This assay, when validated, will greatly increase our understanding of the resistance landscape of gonococci in Aotearoa New Zealand. Dr Xiaoyun Ren is a senior scientist at the Health and Environment group, ESR. Dr Collette Bromhead is a senior lecturer at Molecular Microbiology, School of Health Sciences, Massey University.

Uncovering heterogeneity in cellular and molecular landscapes in human oral tongue squamous cell carcinoma using digital spatial transcriptomics

DR BRIDGET CHANG-MCDONALD

Dr Bridget Chang-McDonald, an Anatomical Pathologist and Research Fellow at the Gillies McIndoe Research Institute in Wellington, was awarded up to $15,000 to better understand how the spatial organization of the microenvironment of tumours affecting the oral cavity shapes further metastatic spread. Head and neck cancer accounts for approximately 3-5% of all cancers and are more common in men and in people over age 50. The overall 5-year survival rate of patients with head and neck cancer is around 63%; however, patients with more advanced disease with metastatic burden have a much lower survival rate. Dr Chang-McDonald's research aims to better understand how the spatial organization of the microenvironment of tumours affecting the oral cavity shapes further metastatic spread.

SPLIT ENZ: Survivorship of Patients post Long Intensive care stay, Exploration/Experience in a New Zealand cohort

DR LYNSEY SUTTON-SMITH

Dr Lynsey Sutton-Smith was awarded a grant of up to $6490 to undertake research on the survival journey post Critical Illness and the Post Intensive Care Syndrome (PICS). This is the first research undertaken in New Zealand which shows patients who have been critically ill have a challenging recovery. It is estimated that a third of ICU survivors have issues with cognitive dysfunction, mental health disturbances, and physical function leading to disability and reduced health related quality of life. This research is key to understanding the recovery journey that ICU survivors undertake and the level of disability they endure in the year post critical illness. Lynsey is a Clinical Nurse Specialist in the ICU at Wellington Hospital and obtained her PHD with the University of Otago, department of Psychological Medicine.

Investigating endometrial cancer stem cells as drivers of Mirena® treatment resistance

MOLLY DORE

University of Otago, Wellington PhD student, Molly Dore was awarded up to $31,855 to undertake research to improve treatment options for endometrial cancer in Aotearoa. Aotearoa New Zealand has one of the highest rates of endometrial cancer (EC), and rapidly rising in younger women. The Mirena® is a long-acting reversible contraceptive device that has gained traction as a possible alternative treatment option to surgery for women with EC. However, early evidence shows that for 1 of 3 women the Mirena® does not work. This research will investigate the role of cancer stem cells in the mechanism of resistance in women that do not respond to the Mirena® and hopefully develop a clinically available test to determine which women will respond and which won't prior to treatment.

The effects of dual anti-platelet therapy (DAPT) on the platelet-neutrophil axis and the formation of neutrophil extracellular traps (NETs)

SONJA HUMMEL

PhD student Sonja Hummel was awarded up to $19,099 to undertake research to help people suffering from heart attacks. Heart attacks are a leading cause of death and disability worldwide and are responsible for around 30% of deaths in the western world. Sonja's research is investigating how current treatment options for heart attacks are affecting the immune system to better understand how the immune system contributes to the recovery and risks associated with heart attacks. Her research aims to improve the pathway of care for patients who are living with the consequences of heart attacks. Sonja is a PhD student in the Department of Surgery and Anaesthesia at the University of Otago in Wellington.

Association of BCL6 with known and novel partners in glioblastoma

ANNA TRIBE AND DR MELANIE MCCONNELL

Anna Tribe and Melanie McConnell was awarded up to $12,000 to undertake research to explore the role of a protein called BCL6 in the therapy resistance of the brain cancer glioblastoma. Glioblastoma is an extremely aggressive cancer with no cure. The treatments we have available are not very effective and research has shown that BCL6 may be involved in helping glioblastoma cells to survive therapy. Anna's research investigates what BCL6 does in glioblastoma before and after treatment. Understanding how this protein helps glioblastoma cells to resist therapy may enable the development of new treatments to target BCL6 and help cancer treatments to be more effective against glioblastoma. Anna Tribe is a PhD student at Victoria University of Wellington.

Investigating how kappa opioid receptor agonists impact microglia in the inflamed central nervous system

AFNAN AL ABADEY

Afnan Al Abadey was awarded up to $13,382 to investigate treatments for multiple sclerosis. The prevalence of multiple sclerosis in New Zealand is approximately 1 per 1000 individuals and results in a range of symptoms including but not limited to fatigue, bladder and bowel dysfunction, visual impairments, and chronic pain. Afnan Al Abadey's research will focus on understanding how these potential treatments may impact an important cell type in the brain, known as microglia. Afnan Al Abadey is a PhD student at Victoria University of Wellington.

Evaluating Kappa Opioid Receptor Localisation and Expression in Multiple Sclerosis

MARTIN DALEFIELD

Martin Dalefield, M.Phil., was awarded up to $18,000 to conduct research into multiple sclerosis, an autoimmune disorder that is the most common cause of non-traumatic neurologic disability in young people. Several studies have found that drugs that target the kappa opioid receptor are beneficial in promoting recovery in preclinical models of multiple sclerosis, but it remains unclear whether these preclinical results will translate into benefits for human patients. By working with the post-mortem brains of patients with multiple sclerosis, Mr. Dalefield will work to clarify the specific location of the kappa opioid receptor and how this changes in MS. This will allow us to better understand how drugs targeting this receptor can used to benefit patients with multiple sclerosis. Mr. Dalefield is a PhD student in the School of Biological Sciences at Victoria University of Wellington supervised by Associate Professor Bronwyn Kivell.

The following travel grant awarded before 30 July 2022 will be reported on in 2023.

SARAH MESSENGER	Sarah Messenger, a PhD student at Victoria University of Wellington, was awarded a travel grant of up to $3,000 to attend the 2022 Applied Molecular Microbiology Summer School in Dubrovnik, Croatia, to learn about natural products and present her research. Sarah's research interests lie in the engineering of natural product gene clusters to produce medically significant compounds that could be used as anti-cancer or antibiotic drugs. Her intention is to revolutionise current methods of natural product engineering to generate novel and exciting drug leads to combat the rising level of antimicrobial resistance and provide new avenues for treatment of cancer.

Travel Grant Report

EMMA SYMONDS

INTERNATIONAL SOCIETY FOR EXTRACELLULAR VESICLES (ISEV) ANNUAL MEETING, LYON, FRANCE

Emma Symonds

Emma Symonds, a PhD student in the Department of Surgery and Anaesthesia at the University of Otago, Wellington, received a travel grant of up to $2660 to present her research at the International Society for Extracellular Vesicles (ISEV) annual meeting in Lyon, France in May this year. Emma's research lies in improving autologous fat grafting as a breast reconstruction option for women that have had a mastectomy as part of their breast cancer treatment. Her intention is to investigate the interaction between cell types in both the donor and breast tissue to improve variable graft retention rates currently associated with autologous fat grafting.

Emma reported that ISEV is the largest and most prestigious conference in her field, bringing together the most esteemed researchers and academics from all over the world. The program consisted of a four-day conference as well as an educational day. Attendance at this conference allowed Emma to develop her scientific communication skills and network with scientists and clinicians in her field.

Emma's presentation was extremely well received and there was a great deal of interest in the very novel work she is doing in the field of post-mastectomy breast reconstruction. The numerous poster sessions allowed Emma to discuss and develop ideas about her work with both cancer researchers and academics involved in tissue reconstruction, something that has been greatly beneficial to her PhD and the progress and development of the project as a whole.

All in all, this conference was of great value to both Emma's work, PhD, and personal development. She gained skills and knowledge outside of the scope of what is available in New Zealand and was able to share her research with the EV and cancer communities. Emma has developed connections that will prove to be vital for her future career and ambitions.

ANNA TRIBE

NEW ZEALAND SOCIETY OF ONCOLOGY (NZSO) CONFERENCE 2021, ROTORUA

Anna Tribe

I was fortunate enough to be awarded a Research for Life travel grant to attend the New Zealand Society of Oncology (NZSO) Conference 2021 in Rotorua. As my PhD project focuses on the brain cancer glioblastoma, this was a valuable opportunity for me to learn about the cancer research occurring around New Zealand and to share my own research. Due to Covid-19, the conference was postponed to January 2022 and then changed to an online conference a few days prior to starting. Unfortunately, this meant that I was not able to present my poster in person or to meet and network with other cancer researchers as I had hoped. However, the conference was still extremely valuable to me in several ways.

It was great to see so many interesting talks from both NZ and overseas cancer researchers. I was particularly inspired by the extensiveness and thoroughness of the research described by one of the international delegates, Professor Karin de Visser. It was also valuable to hear talks from medical oncologists and public health researchers, to gain perspectives on oncology which I do not usually get as a biomedical researcher. It was especially eye-opening to hear several talks examining the inequities in cancer outcomes for Māori. This context is very important for me to keep in mind and to act on where possible in my future research.

Most valuable to me was the opportunity to connect with a fellow PhD student with experience of the little-known technique I am using in my project. Without attending this conference, I would not have known that anyone else in New Zealand was using this technique. While it would have been ideal to meet in person, I have benefitted from discussion of the technique and from his insights into data processing and follow-up.

Attending NZSO 2021 has broadened my knowledge of cancer research and of the wider cancer field, as well as enabling me to make connections benefitting my own research.

[As the Rotorua conference went online, Anna was granted the remaining funds towards her attendance at the British Society for Proteome Research. Anna's report follows.]

In July 2022, I attended the British Society for Proteome Research (BSPR) conference held in Oxford, UK. This was a meeting of international experts in the field of protein research working on cutting edge applications of proteomics technology. The invaluable opportunity to attend this conference was made possible by funding from Research for Life. Thanks to this support, I was able to present my research at an in-person conference for the first time during my PhD. I am passionate about proteomics and this conference enabled me to learn from international experts in the field and to receive their feedback and insights into my work.

The conference began with a full-day workshop on protein-protein interactions, which was of great relevance to my research. This was an excellent opportunity to learn from proteomics experts from Harvard and EMBL-EBI and to meet other PhD students working in similar research areas to me. The following three days consisted of talks on a wide range of research using proteomics. I was fortunate enough to hear talks from Nobel Prize winner Peter Ratcliffe, Oxford Nanopore Technologies founder Hagan Bayley, and well-respected scientists in the field, such as Anne-Claude Gingras. As proteomics is still under-utilised, this was the first time I have had the chance to hear about so many different applications of proteomics techniques and technologies. This inspired me to think of new ideas for future research projects and I brought back what I had learned to my lab group at Victoria University of Wellington. I hope that these ideas and techniques will be utilised to enrich future research in New Zealand.

The conference also provided many opportunities for young researchers to present their research and network with experts. I presented a poster on my research and gave a one-minute pitch of my research before the poster session, along with other young researchers. I was able to engage with many experienced scientists during the poster sessions. Their feedback on my work was very valuable and I made connections that will be useful for my future career.

I am extremely grateful for the support of Research For Life which enabled me to attend this conference. The experience has enriched my knowledge of the proteomics field and has greatly benefited my PhD project.

DÉANNA SHEA

ANNUAL SYMPOSIUM FOR EXTRACELLULAR VESICLES, AUCKLAND AND 16TH CONGRESS OF THE FEDERATION OF ASIAN AND OCEANIAN BIOCHEMISTS AND MOLECULAR BIOLOGISTS

Déanna Shea

With the support of Research For Life I was able to present findings of my PhD work discussing features of extracellular vesicles and how their study can contribute into diagnosis of diseases. Specifically, in developing new therapies for diseases associated with lungs. I presented my work at the Annual Symposium for Extracellular Vesicles held in Auckland and at the 16th Congress of the Federation of Asian and Oceanian Biochemists and Molecular Biologists, both via Zoom. In my presentation I discussed characterization of breath proteome of extracellular vesicles from exhaled breath condensate that I have conducted through ethical study with 59 participants. My study suggests that breath exosomes can be used in future studies contributing into understanding lung disfunction including cancer.

CHAOLAN ZHENG

2022 NEW ZEALAND MELANOMA SUMMIT (VIRTUAL)

Despite the struggles brought by COVID-19, the Melanoma Network of New Zealand Incorporated (MelNet) did an amazing job of creating a jam-packed two day multi-disciplinary conference covering up-to-date research for melanoma prevention, diagnosis, treatment and care. I was honoured to present research on the rare side effect of a commonly used immunotherapy for metastatic melanoma, Pembrolizumab. Though initially planned to be held in Auckland, social gathering limitations meant going virtual was the best option for everyone. MelNet were extremely resourceful and asked us to present our research through a pre-recorded video that could be posted alongside our poster presentation online. This allowed a similar experience to real-life, and questions could be asked through the conference website or email correspondence.

New Zealand's melanoma incidence rate is the world's highest. It is the third most common cancer in New Zealand and approximately 350 kiwis die from melanoma each year. Immune checkpoint inhibitors are a relatively new therapy that have changed the treatment landscape for various malignancies. Pembrolizumab is a monoclonal antibody that activates the immune system against cancer cells. This can lead to an 'overactive' immune system that interferes with a person's neuromuscular pathways, leading to conditions like immune-related myasthenia gravis (irMG). I presented a case report and literature review conducted by myself, Dr Dugena, Dr Taylor and Dr Wong, on the characteristics, management and prognosis of ocular irMG caused by Pembrolizumab. Despite irMG being a rare side effect, the increasingly common use of immunotherapy means that awareness is necessary among clinicians to allow timely diagnosis and management. We found that irMG was incredibly debilitating and potentially fatal, with high rates of mortality (30%) and treatment resistance. Poor prognosis was associated with multiple organ system involvement and other immune-related manifestations. Further research into irMG is important given no clear medication regimen was correlated with resolution of symptoms. Pembrolizumab was almost always withdrawn which can be devastating for those experiencing cancer regression. Though minimal data exists on the possibility of rechallenging patients, case reports of successfully mitigating irMG relapse with steroids are promising. The sphere of unknown that surrounds immunotherapy is slowly being uncovered, and I am honoured to play a part in it.

The Melanoma Summit was a fantastic opportunity to contribute to the growing research around melanoma treatment. I am extremely grateful for Research for Life's support in attend this sharing of knowledge and discussion of future priorities. It was an invaluable experience to collaborate with other clinicians and learn from peoples' years of experience in the field of skin cancer.

SARAH SCZELECKI

SOCIETY FOR THE STUDY OF REPRODUCTION (SSR) JULY 2022

Sarah Sczelecki

The quantity and quality of the research presented at the Society for the Study of Reproduction (SSR) Conference was remarkable. This was the first full, in-person, SSR conference hosted since 2019, which was reflected in the detail of the research presented.

The overarching theme of the conference was inequality in reproduction research. Firstly, Kelle Moley, discussed inequalities in women's health and research innovation. The key messages were that women's health is still being overlooked in the healthcare field and that most patents are still being led by male. Furthermore, there's evidence that female inventors are more likely to patent technologies that benefit women directly and leading to a new wave of "Femme Tech" providing better health outcomes for women. Additionally, Yvonne Maddox spoke about the inequalities of reproductive health outcomes between races in the USA, most starkly highlighting maternal mortality rates during childbirth. This sparked discussion about how research needs to include different ethnic cohorts, as most research still focusses on studies of Caucasian males. This limitation of research is echoed in Aotearoa and as scientists we must endeavour to do better to ensure equal outcomes of our research for all.

The key take-home message directly related to my work was the ubiquity of RNA sequencing and multi-omics approaches to answering research questions. The exposure to international research programmes throughout the conference provided validation that my transcriptomics-based PhD project is at the forefront of the field. Moreover, it demonstrated that the skills I have developed as a PhD are in demand across all reproductive research fields and therefore will be beneficial for potential future career ambitions. I was surprised that there was limited focus on microRNA/small RNA sequencing, however, this proves that my research will be advancing the field in a topic still in its infancy.

TESSA PECK

AUSTRALASIAN WINTER CONFERENCE FOR BRAIN RESEARCH 2022

Tessa Peck

After multiple postponements we were finally able to gather in Queenstown, at the end of August, for the Australasian Winter Conference for Brain Research (AWCBR). This 3-day meeting was part of the larger Queenstown Research week and brought together many scientists from various areas of research under the broad category of neuroscience.

Thanks to the support of the Wellington Medical Research Foundation, I was able to present my work investigating the mechanism of a heparan-sulfate (HS) -mimicking compound for the treatment of multiple sclerosis (MS). This mimetic was developed at the Ferrier Institute for treatment of metastasising cancers, and it was a pleasure to present my PhD research looking into its applicability in the neuroinflammatory disease MS. My poster focused on HS-mimetic's potential for inhibiting inflammatory trafficking into the central nervous system (CNS) by reversing disease-induced blood brain barrier permeability. This attracted interest from many students from the fields like Parkinson's and Alzheimer's disease research as both diseases are known to have an initial neuroinflammatory trigger.

The opportunity to discuss my work, and the work of others, with young scientists from different fields was invaluable. I gained several insights into methods used by other groups to visualise and quantify disease-associated changes at the blood brain barrier, and I was able to bring some new ideas back to my own lab group.

Overall, my attendance at AWCBR was very rewarding. I left Queenstown with many new insights and a new level of motivation to try out new techniques in my own research.

HANNAH VAN DER WOUDE

QUEENSTOWN RESEARCH WEEK CONFERENCE

Hannah van der Woude

I am a second year PhD student in the Department of Obstetrics, Gynaecology and Women's Health at the University of Otago Wellington. My PhD is focussed on endometrial cancer research. Specifically, I have been working on developing a new method of studying endometrial cancer which is more biologically relevant compared to current research methods and therefore better reflects the biology of a patient. Establishing this methodology for endometrial cancer will allow novel therapeutics to be tested quickly, and be more likely to succeed in clinical trial.

I received $1,639 from Research For Life to attend the Queenstown Research Week conference in August/ September 2022 (postponed from 2021). This was my first introduction to a scientific conference and was an exciting opportunity to meet other researchers and learn about the work that is being conducted throughout New Zealand. This was invaluable as, in my early career stage, I am interested in institutes that produce good quality research where I could apply for postdoctoral roles in the future. Additionally, the conference gave me the opportunity to present my research to a room of fellow cancer scientists, building my confidence in science communication and networking.

Queenstown Research Week is one of the largest biology conferences in New Zealand, bringing together researchers from all over the country. The grant money received went towards paying for my conference registration, return flights from Wellington to Queenstown, accommodation, and transport to and from Queenstown Airport. I attended both the Cancer Satellite and the Queenstown Molecular Biology (QMB) meetings which spanned over four days.

As a cancer biologist, I was fascinated in the various methods and techniques used to research cancer throughout the country. My presentation and poster were well received and gave me the opportunity to discuss my research with others who are interested in implementing my model into their own work. It also allowed me to brainstorm future projects and work through problems with people I wouldn't normally interact with.

Overall, attendance at Queenstown Research Week was hugely beneficial to my current research and future career.

THREE MINUTE THESIS (3MT) WINNER

UNIVERSITY OF OTAGO, WELLINGTON HEATS

In late June 2022, Research For Life supported Masters and PhD level research by sponsoring a $500 prize for the University of Otago Wellington 3MT winner.

There were presentations in research areas ranging from treatment for substance abuse to novel diagnostic opportunities for endometriosis.

The event was won by Yasmin Nouri, from the Department of Pathology, who talked about her research into a new type of cancer treatment, CAR T-cell therapy, where the patient's own immune cells, their T-cells, are genetically modified with a new receptor that recognises cancer.

She competed against Henry Fane De Salis, from the Departments of Medicine and Psychological Medicine, who presented his research on the 'Love you back to life' residential treatment programme for substance abuse; Emily Paterson, from the Department of Obstetrics, Gynaecology and Women's Health, who talked about novel diagnostic opportunities for endometriosis; and Julie Artus from the Department of Psychological Medicine, who discussed inpatient psychiatric services for children and adolescents in New Zealand.

Their presentations were judged by Dean and Head of Campus Professor William Levack, Dr Claire Henry, a Research Fellow in the Department of Obstetrics, Gynaecology and Women's Health on the Wellington campus, and Carolyn Jenkins, the Graduate Wellbeing Coach at the Graduate Research School in Dunedin. The event was held in the Horne Lecture Theatre at Wellington Hospital.

From left to right: Yasmin Nouri, Emily Paterson, Julie Artus, Henry Fane De Salis.

Yasmin Nouri also won the University's Grand Final. Yasmin is in the last year of her PhD and is supervised by Dr Michelle Thunders.

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