2023

RESEARCH REVIEW

Research For Life

WELLINGTON MEDICAL
RESEARCH FOUNDATION

RESEARCH REVIEW 2023

COVER PHOTO:

Grant recipients: Aaron Stevens, Hannah van der Woude with supervisor Claire Henry, and Afnan Al Abadey

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Research For Life
The Wellington Medical Research Foundation Incorporated

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TRAVEL GRANTS 2023

Editorial

BY PROFESSOR REBECCA GRAINGER

Each year I look forward to reading the reports of science funded by Research for Life grants.

As Chair of the Research Advisory Committee, I have led the rigorous process of evaluating the applications and making funding recommendations to the Board. It's so interesting to read and reflect on the grant reports, including seeing the results to date and next steps. These grants often fund Wellington young scientific talent taking the first steps in a career in medical research. Many of our grant recipients are PhD candidates and the funds support their experimental work. While a PhD is an impressive undertaking and often the final stage in tertiary education, it is really the first stage in a research career; a PhD can be seen as a form of formalised research training. Let me use some of the things I see in the grant reports to explain.

The first thing to notice is that most of the grant reports have a principal investigator, supported by a team. Scientific reports, including journal articles and grant reports, have conventions in the order of authors. The first author is the person who has done most of the work, actually undertaken the design, data collection or generation, data analysis and usually the first draft of the write up. While it depends on the structure and composition of the research team and the career and expertise of the team members, this can be a fairly junior member of the team. This person is learning their craft and has the time and energy to do the hard yards! The final author is usually the laboratory leader or senior team member. This person has the technical skills and detailed knowledge of the topic of research, management skills to organise the team and its work, and hopefully the leadership skills to set the team up for success. The authors in the middle contribute to the team somewhere along the spectrum above, or bring specific expertise or opportunity to the team, with order determined by relative contribution. When the first author is a PhD candidate, the last (often called senior author – in experience, not necessarily age!) author is usually their PhD supervisor. The supervisor is responsible for the PhD candidate's ultimate success in research training. Research for Life is proud to support the research training of PhD candidates and enable the brilliant senior scientists in Wellington to nurture future talent. These teams are necessary to support the medical research happening here on our doorsteps.

The second thing you might notice on reading the grants, is that not all proposed research goes as planned. While that is probably disappointing for our researchers, I am never surprised when I read this. Our experiments can fail when our proposed methods don't work as expected or other barriers prevent completion (I'm looking at you COVID-19). Experiments test hypotheses and results can be different to those expected if our hypothesis is off the mark. Then comes the fun of rethinking the issue and designing more experiments to test revised hypotheses. Like any training, often just as much, or more, can be learnt when things don't go as planned.

The third thing you might notice is the breadth and variety of the research undertaken in Wellington giving me optimism for science in the capital as being an ongoing contributor nationally and internationally. The broad areas addressed range across brain cancer and inflammation, cancer development to treatment, head trauma, heart attacks, infections, and management of preterm birth! We also have this amazing research occurring across so many of our government, education and independent research centres across Wellington including Te Herenga Waka Victoria University of Wellington, University of Otago Wellington, Massey University, Institute of Environment Science and Research (ESR), and the Gillies McIndoe Research Institute. Some of these projects are supported by researchers from the Malaghan Institute of Medical Research. It's wonderful that here in Wellington we have a variety of research active organisations undertaking biomedical science and training the next generation of science talent. Supporting this talent through grants provides them training but also provides a pipeline of talent to keep the organisations at the cutting edge. The research topics are also using science to address a variety of major health needs. As a medical doctor I know there are many wonderful treatments but still so many diseases that do not have cures or enough effective therapies. There is still so much to be done.

So, I hope you enjoy reading the 2023 Research for Life Research Report also. While science is interesting, what it represents is more exciting. Biomedical science is alive and well in Wellington and continues to contribute to finding cures for disease and advancing human health. It's a pleasure and privilege for Research for Life to support this work and thank you for supporting us and biomedical science in Wellington.

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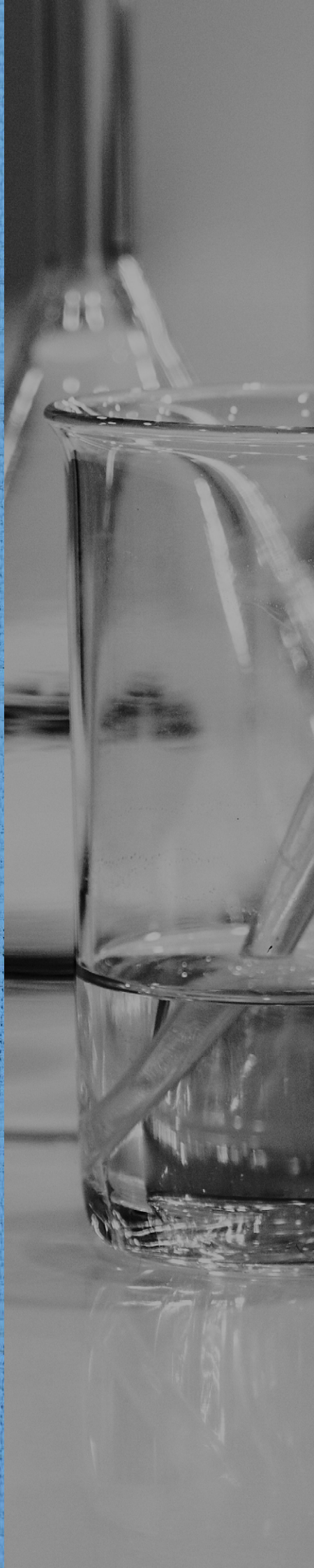


RESEARCH REPORTS

Te Herenga Waka
Victoria University of Wellington

Association of BCL6 with known and novel partners in glioblastoma

A TRIBE, P TEESDALE-SPITTLE, M MCCONNELL

KEY FINDINGS

BCL6 associates with its corepressor NCOR2 in untreated glioblastoma cells and both proteomics experiments and the experiments described below have shown that this association may be lost in response to acute irradiation. This may help to explain the context-dependent activity of BCL6 in glioblastoma cells.

OBJECTIVES

The transcription factor BCL6 is important for the survival and therapy response of glioblastoma cells (Fabre et al., 2020, PLOS One). Previous experiments showed that the role of BCL6 appeared to change when glioblastoma cells were treated with different treatments and this was linked to alterations in which proteins were associated with BCL6 (Tribe et al., 2023, *BioRxiv*). This research aimed to validate the interaction of BCL6 with known BCL6 corepressor NCOR2 in untreated glioblastoma cells and to confirm the loss of this association in response to acute irradiation treatment. Additionally, validation of a novel BCL6 association with metabolic regulator AMPK was investigated.

METHODS

Three glioblastoma cell lines, LN18, NZG0906 and NZG1003, were treated with 10 Gy irradiation or left untreated. After 48 hours, proximity ligation assays were performed to enable high resolution detection of BCL6-NCOR2 and BCL6-AMPK association. Cells were fixed and permeabilised before mouse anti-BCL6 antibody along with either rabbit anti-NCOR2 or rabbit anti-AMPK antibody was added. Oligonucleotide-labelled probes recognising mouse and rabbit antibodies were added. When the two proteins of interest were in very close proximity, the oligonucleotides were able to hybridise and undergo rolling-circle amplification, greatly enhancing the signal. Fluorescent detection oligos then bound to the amplicons and the fluoresence was visualised using confocal microscopy.

RESULTS

As this was the first use of the PLA technique in the McConnell lab, the technique was first validated using abundant target proteins: NFκB subunits p50 and p65 (Figure 1A).

The co-localisation of BCL6 and NCOR2 in the three glioblastoma cell lines was distinct ($p \leq 0.05$) from the non-specific antibody negative controls (Figures 1B-D). This verified the association of BCL6 with NCOR2 in untreated glioblastoma cells. Consistent with previous data, acute irradiation of NZG0906 cells resulted in the loss of the BCL6-NCOR2 association ($p \leq 0.001$) (Figure 1B). However, proximity ligation assays were unable to detect any change in BCL6-NCOR2 association in response to acute irradiation in LN18 or NZG1003 cells (Figure 1C&D). Perhaps explaining this inconsistency, although the difference was statistically significant, the BCL6-NCOR2 interaction signal was only marginally higher than the signal in the non-specific antibody controls. This may indicate that the BCL6-NCOR2 interaction is close to the threshold of detection for this assay.

Similarly, the BCL6-AMPK interaction signal could not be confidently distinguished from the control signal in untreated glioblastoma cells (Figure 1E). Contrastingly, there was a clearer, although not statistically significant ($p \geq 0.05$) trend towards higher signal in the BCL6-AMPK PLAs in irradiated LN18 cells compared to in the controls (Figure 1E). However, the low level of the BCL6-AMPK signal in glioblastoma cells meant that this interaction could not be validated using the PLA method. Future optimisation or the use of another method may allow validation of this novel interaction.

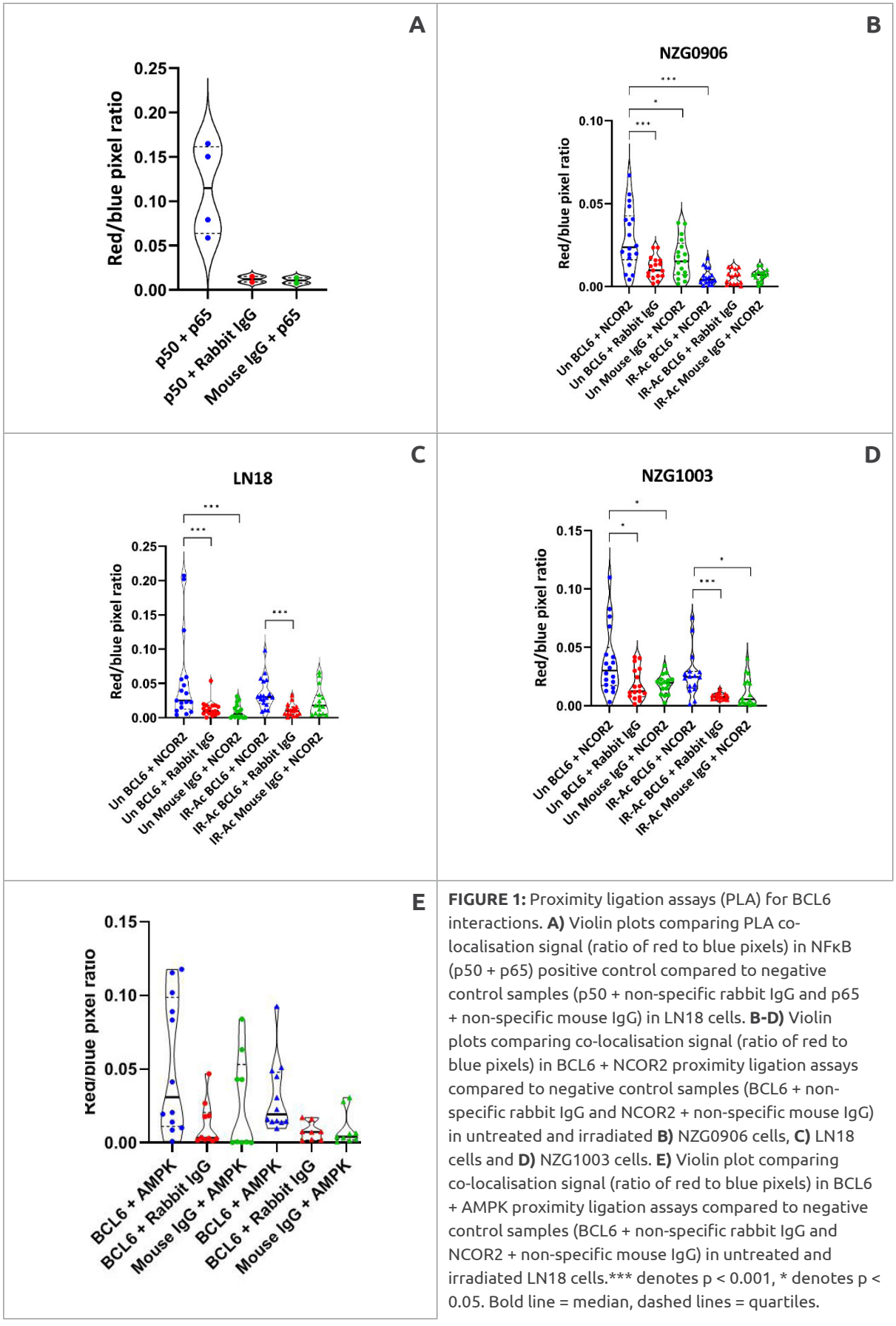


FIGURE 1: Proximity ligation assays (PLA) for BCL6 interactions. **A)** Violin plots comparing PLA co-localisation signal (ratio of red to blue pixels) in NFκB (p50 + p65) positive control compared to negative control samples (p50 + non-specific rabbit IgG and p65 + non-specific mouse IgG) in LN18 cells. **B-D)** Violin plots comparing co-localisation signal (ratio of red to blue pixels) in BCL6 + NCOR2 proximity ligation assays compared to negative control samples (BCL6 + non-specific rabbit IgG and NCOR2 + non-specific mouse IgG) in untreated and irradiated **B)** NZG0906 cells, **C)** LN18 cells and **D)** NZG1003 cells. **E)** Violin plot comparing co-localisation signal (ratio of red to blue pixels) in BCL6 + AMPK proximity ligation assays compared to negative control samples (BCL6 + non-specific rabbit IgG and NCOR2 + non-specific mouse IgG) in untreated and irradiated LN18 cells.*** denotes $p < 0.001$, * denotes $p < 0.05$. Bold line = median, dashed lines = quartiles.

CONCLUSION

This first use of the PLA method in the McConnell lab indicated that it is a very useful technique that can be optimised and utilised in future experiments. Although the technique worked well with the abundant proteins p50 and p65, the low abundance of BCL6 limited detection of its protein-protein interactions. Nonetheless, the BCL6-NCOR2 interaction was validated in untreated glioblastoma cells and its irradiation-induced loss was evident in the NZG0906 cells. However, as the signal was close to the threshold of detection, the novel BCL6-AMPK interaction could not be confidently validated, and neither could the loss of the BCL6-NCOR2 interaction in irradiated LN18 and NZG1003 cells. With further optimisation to improve the detection of low abundance interactions, the PLA technique will be a very useful method for validation of protein-protein interactions detected with proteomics methods in the McConnell lab.

All of these results have been included in the PhD thesis: https://openaccess.wgtn.ac.nz/articles/thesis/BCL6_is_a_context-dependent_mediator_of_the_glioblastoma_therapy_response/21937850.

Some of this work has been included in the following preprint, currently under review at the International Journal of Biological Macromolecules, an Elsevier journal with a JCR Impact Factor of 8.2: https://www.biorxiv.org/content/10.1101/2023.05.29.542686v1.

Te Herenga Waka
Victoria University of Wellington

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

A AL ABADEY, A LA FLAMME

KEY FINDINGS

Specialised brain cells known as microglia were exposed to proinflammatory stimuli and treated with the kappa opioid receptor agonist, nalfurafine. Exposure to nalfurafine led to a switch from proinflammatory responses to functions and markers associated with wound healing and immune resolution including enhanced debris removal, reduced ability to interact with T cells, and reduced production of harmful cytokines. These findings suggest that nalfurafine could aid in resolving inflammation and supporting the brain's repair processes in multiple sclerosis.

OBJECTIVE/S OR AIM

The aim of the research was to determine the effects of kappa opioid receptor (KOR) agonists on microglia in the inflamed central nervous system (CNS). To achieve this, three research aims were proposed:

Research Aim 1: Optimising the isolation and culture microglia from adult mice.

Research Aim 2: Examining in vitro microglial responses to lipopolysaccharide-induced inflammation in the presence and absence of KOR agonists.

Research Aim 3: In vivo characterisation of microglia from KOR agonist-treated healthy and EAE mice.

METHOD/S

I evaluated different methods for isolating microglia whilst considering several factors like purity, yield, viability, cost, sterility, and time efficiency. The successful technique selected was the Mojosort™ Mouse P2RY12+ Selection kit to isolate live microglia from adult mice.

To test whether these isolated microglia responded to proinflammatory stimuli, they were primed with interferon gamma (IFN-γ) and subsequently stimulated with lipopolysaccharide (LPS) for 24 hours. Isolated microglia were treated with various concentrations of nalfurafine and several activation markers were assessed using flow cytometry.

The second stage of the project will involve experiments to verify KOR expression on the isolated microglia using real time quantitative polymerase chain reaction (RT-PCR). I will study how KOR expression changes due to LPS-induced inflammation and subsequent nalfurafine treatment. I addition, I will analyse the production and release of reactive oxygen and nitrogen species by these microglia in response to LPS-induced inflammation and nalfurafine treatment using commercially available assay kits.

To confirm that LPS-induced inflammation mimics in vivo inflammation observed in EAE, we intend to isolate P2RY12+ microglia from EAE mice and a comprehensive comparison between isolated EAE and LPS-induced microglia will be conducted using flow cytometry.

For research aim 3, animals will be subjected to EAE to induce disease progression that mimics MS. After the designated experimental period, brain and spinal cord tissues will be harvested for analysis. Brain and spinal cord tissue will be utilised to assess several parameters including microglial activation markers, reactive oxygen and nitrogen species expression and phagocytosis through flow cytometry. Conversely, the top portion of the spinal cord will undergo immunohistochemistry, enabling the assessment of microglial activation and recruitment. This evaluation will involve observing distinct microglial markers, notably ionized calcium binding adaptor molecule 1 (IBA1). I will also explore how microglial activation coincides with immune cell infiltration and demyelination.

RESULTS OR FINDINGS:

Adult microglia were successfully isolated from the CNS of adult mice and stimulated with IFN-γ and LPS to induce a pro-inflammatory environment. Nalfurafine treatment increased the expression of microglial activation markers associated with the anti-inflammatory M2 microglia phenotype. This suggests that nalfurafine has the potential to facilitate the resolution of inflammation brought about by IFN-γ and LPS.

Furthermore, the phagocytic activity of isolated microglia was enhanced after nalfurafine treatment. This enhancement hints at nalfurafine's ability to support the crucial process of debris clearance, a significant requirement for healthy remyelination within the CNS.

Another significant finding was the reduction in proinflammatory cytokines, such as IL-6, by microglia exposed to nalfurafine. This reduction highlights nalfurafine's potential to dampen harmful inflammatory responses.

CONCLUSION

Significant strides have been made in the project which have yielded exciting results. While there is still further research proposed, I am optimistic about its completion by late 2024.

The findings thus far indicate that nalfurafine holds promise as a candidate for resolving inflammation, supporting debris removal, and mitigating detrimental cytokine production by microglia. These insights contribute to our understanding of potential therapeutic strategies for neurodegenerative diseases such as MS.

Te Herenga Waka
Victoria University of Wellington

Developing a flow cytometry methodology for the detection of neutrophil extracellular traps (*NETs) in human blood

C JONES, A LA FLAMME, P LARSEN, K HALLY

KEY FINDINGS

A novel flow cytometry protocol was developed for the detection of neutrophil extracellular traps (NETs) in human blood samples. This methodology is the most advanced for examining NETs using this technology.

OBJECTIVES

Neutrophil extracellular traps (NETs) are a recently discovered mediator of inflammation. NETs are structures of DNA dotted with neutrophil-derived proteins which protrude from the surface of the neutrophil (a type of white blood cell). NETs were initially appreciated as mediators of bacterial killing, by trapping the bacterial in the web-like structures of DNA, and subsequently neutralising the bacteria with the local concentration of proteins. Additionally, NETs are implicated in sterile inflammation (inflammation in the absence of microorganisms) such as atherosclerosis. NETs are commonly examined under the microscope or by examining cell-free complexes of DNA and neutrophil-derived proteins, however these techniques are limited by subjectivity and challenges in identifying global NET burden, respectively. The overall aim of this project was to overcome these limitations by developing an objective and specific flow-cytometry-based method to identify NETs in human blood samples. The specific objectives were:

Objective 1: Optimize cell-surface staining of key markers of NETs, including 7-AAD (a DNA dye), and neutrophil-derived proteins, including myeloperoxidase (MPO) and neutrophil elastase (NE) for flow cytometry.

Objective 2: Optimise cell surface staining of key markers of neutrophils, including CD66b and CD15.

Objective 3: Translate the flow cytometry panel from isolated neutrophils into whole blood samples from heart attack patients.

METHODS

Flow cytometry is an objective analytical tool that allows for the rapid measurement and characterisation of individual cells. Using this tool, we have developed a seven-colour panel to identify neutrophil-appendant NETs in human blood samples.

To optimise this methodology, we collected blood samples from 10 self-reporting healthy individuals, isolated the neutrophils, and stimulated these cells with the NET-inducing agonist phorbol myristate acetate (PMA) to increase cell-surface expression of NET markers. Two markers (CD66b and CD15) were included in our panel to identify neutrophils, and a viability dye (Zombie NIR) which is expressed by cells undergoing necrosis was incorporated to distinguish NETs from other cell death processes. Cell-surface expression of these 7 markers was examined using the Cytek 3-Laser Aurora Flow Cytometer.

Once this panel was functional in stimulated neutrophils from healthy individuals, we translated the panel into neutrophils purified from whole blood from people admitted to Wellington Regional Hospital after having a heart attack. This allowed us to examine cell surface expression of NET markers in fresh, unstimulated neutrophils from patients experiencing an acute inflammatory response due to a heart attack.

RESULTS

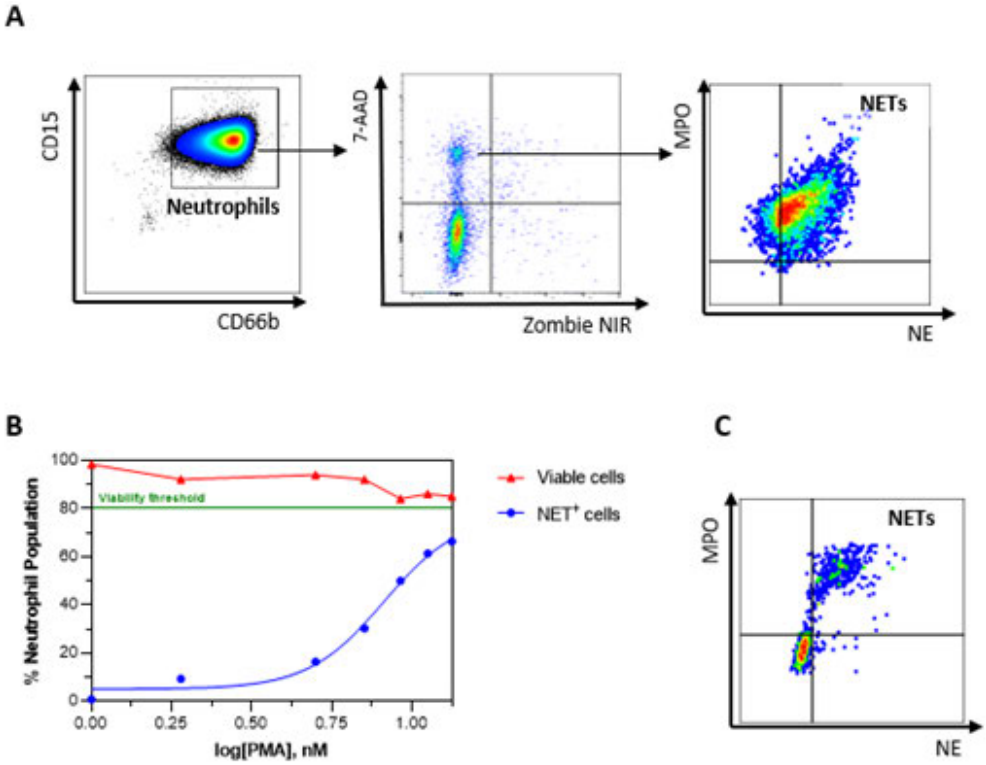


Figure 1. Examining NETs by flow cytometry. *Neutrophils were isolated from blood from healthy volunteers and stimulated with PMA to induce NETosis (A). The proportion of Zombie NIR$^-$7-AAD$^+$MPO$^+$NE$^+$ NETs increased in a dose-dependent manner with PMA stimulation (B). Neutrophils were purified from blood from heart attack patients and stained with our 7-colour panel to examine NETosis in acute inflammation (C).*

Cell-surface staining of surrogate markers of NETosis was successfully optimised in PMA-stimulated neutrophils, and NETs were subsequently identified as Zombie NIR$^-$ DNA$^+$MPO$^+$NE$^+$ neutrophils (**Fig.1A**). These NET$^+$ neutrophils increased in a dose-dependent manner with PMA (**Fig.1B**) and were detectable in fresh blood from people who have had a heart attack (**Fig.1C**).

CONCLUSION

We have optimised a protocol for quantifying NETs in human blood using flow cytometry. Our seven-colour panel is the most comprehensive panel to quantify NETosis by flow cytometry. This protocol can detect NETs in fresh, unstimulated blood from patients experiencing acute inflammation, which is consistent with current literature examining NETs in disease.

Te Herenga Waka
Victoria University of Wellington

The Role of Regulatory T Cells in Remyelination Following Nalfurafine Treatment

M A KIERNAN, A LA FLAMME

KEY FINDINGS

Our research suggests that FoxP3+ regulatory T cells are required for kappa opioid receptor agonist promotion of remyelination. We have demonstrated that selective genetic deletion of the kappa opioid receptor on regulatory T cells significantly impairs nalfurafine-mediated remyelination and recovery from disease compared to wild-type-nalfurafine-treated controls and led to an increased rate of disease relapse.

OBJECTIVE

Multiple sclerosis (MS), a demyelinating disease of the central nervous system, is increasing in prevalence in New Zealand. Currently, treatment options are limited to immunomodulatory agents and generalised symptom management.

Kappa opioid receptor (KOR) agonists, like nalfurafine and U50,488, have been shown to promote remyelination in experimental autoimmune encephalomyelitis (EAE), an animal model of demyelination. This has been correlated with a regression in demyelinating disease. However, the mechanisms governing this remyelination are still largely unknown.

This project aimed to determine whether regulatory T cells are driving this regression in disease and promoting remyelination in the central nervous system.

METHODS

EAE induction and treatments

Male C57BL/6J mice where the kappa-opioid receptor has been selectively knocked out on regulatory T cells (T KOR -/- mice) were induced with EAE. These animals also had a knocked-in yellow fluorescent protein (YFP) under the FoxP3 promoter resulting in fluorescent FoxP3+ Treg.

At 8-12 weeks, mice were immunised subcutaneously (s.c.) in the rear flanks with myelin oligodendrocyte peptide (MOG)35-55 (50ug per mouse; Genescript, Piscataway, NJ USA) suspended in complete Freund's adjuvant (Sigma, St. Louis, MO USA) containing heat inactivated Mycobacterium tuberculosis H37Ra (500ug per mouse; Fort Richard, Auckland, NZ). This was administered on day 0. Animals were then injected intraperitoneally (i.p.) with 200ng of pertussis toxin (List Biochemicals, Campbell, CA USA) on day 0 and 2.

An examiner blinded to treatment allocation would weigh and score mice daily according to the following EAE scoring system: 0, normal; 1, partial tail paralysis; 2, full tail paralysis; 3, paralysis in one hind limb; 4, paralysis in both hind limbs; and 5, moribund. Any animal reaching a score of 4.5 or above (full paralysis in back limbs and weakness in both front limbs) were euthanised and excluded from experimental analysis.

Treatments remained blinded throughout the course of the experiment and were allocated at disease onset (EAE score at least 1) in a consecutive fashion, to ensure the treatment periods remained consistent across groups. Animals were allocated to either nalfurafine (0.01mg/kg), or a vehicle control. Once allocated, treatments were administered daily as an i.p. injection diluted in physiological saline: DMSO: Tween80 in a ratio of 8:1:1.

Tissue processing for histology

Following CO2 asphyxiation, animals were perfused with PBS. Spinal cords were removed from animals and placed in 4% PFA to fix the tissue. Spinal cords were then transferred to 15% sucrose overnight at 4 degrees Celsius, followed by 30% sucrose at 4 degrees Celsius until spinal cords sunk to the bottom of their tube. The cervical region of the spinal cord was removed and embedded in Cryomatrix embedding resin (Thermo Fisher Scientific), frozen in isopentane over dry ice and then stored at -80 degrees Celsius.

Using the Leica CM3050 S cryostat microtome (Leica Biosystems), 5 transverse sections, 20um in thickness, from descending regions (100um apart) of the cervical spinal cord were taken from each animal and mounted on Superfrost Plus slides (Thermo Fisher Scientific) and stored at -80 degrees Celsius until staining.

Black Gold II staining

Slides were first defrosted at room temperature for 20 minutes and embedding resin removed by immersing slides in PBS for 5 minutes. Black Gold II Myelin staining (Sigma-Aldrich) was completed according to the manufacturer's instructions.

Black Gold II quantification

Analysis of Black Gold II-stained slides was performed by a treatment blinded assessor, using ImageJ software. The area of lesions present on sections were manually selected in the white matter and given as a percentage of total white matter for each section.

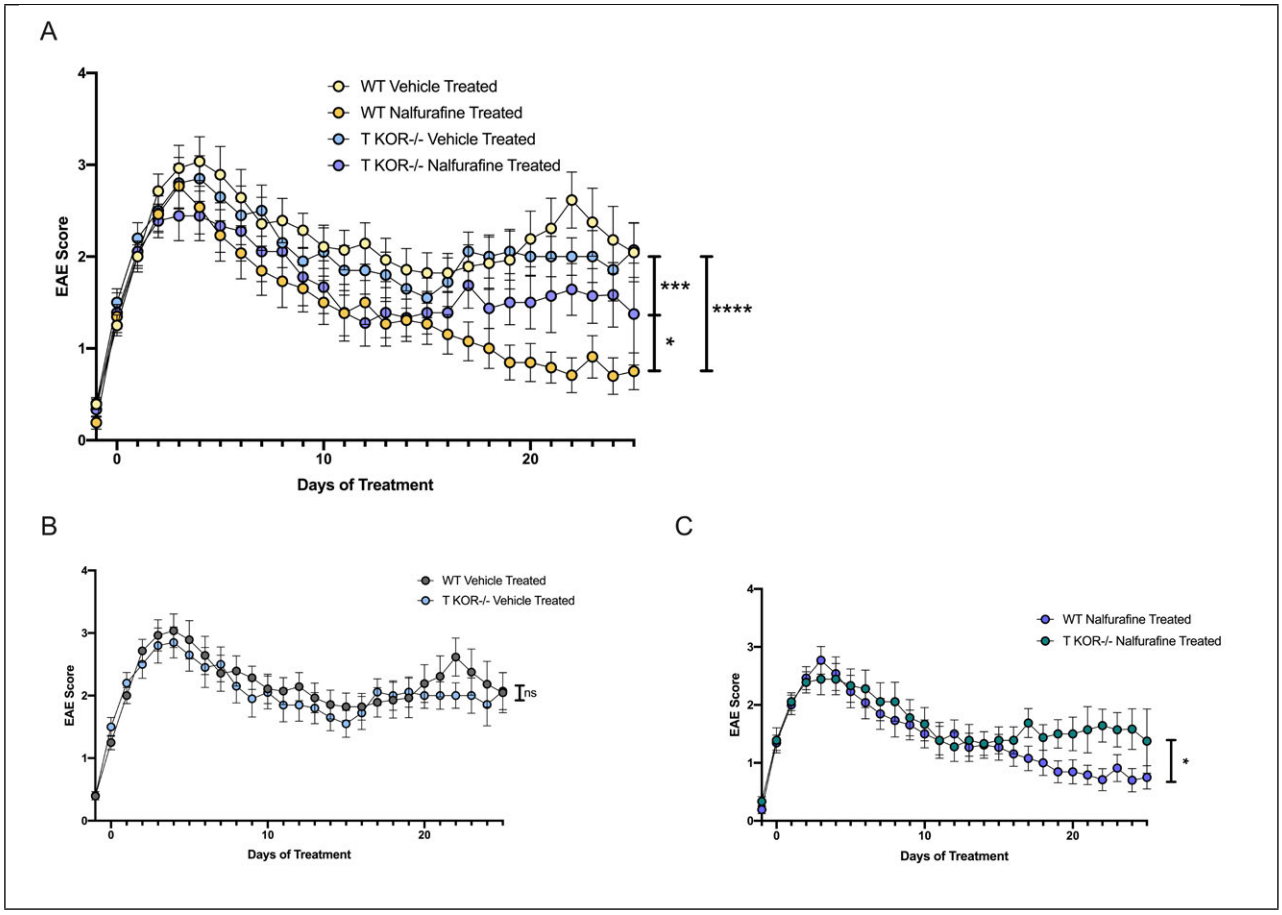
To quantify the myelin present in each section, a region of interest (ROI) was analysed for its percentage of myelin coverage. Images were first converted to an RGM stack, where the green filter was used. The ROI colour was inverted and a threshold determined based on heathy controls. White matter in the ROI was selected and myelin coverage was measured.

These analyses were done on 10 sections per animal (slides in duplicate) and averaged to create a mean value of myelination and lesion percentage for each animal.

Statistical tests

Data is presented as mean +/- standard error of the mean (SEM). For normally distributed data, parametric analyses were used. Specifically, when comparing two groups; an independent samples t- test, and for more than 2 groups; a one-way ANOVA paired with Tukey's multiple comparison test. For non-normally distributed data, nonparametric tests were used. When comparing two groups; Mann- Whitney analysis, and for comparison of more than two groups; Kruskal-Wallis paired with Dunn's multiple comparison.

RESULTS



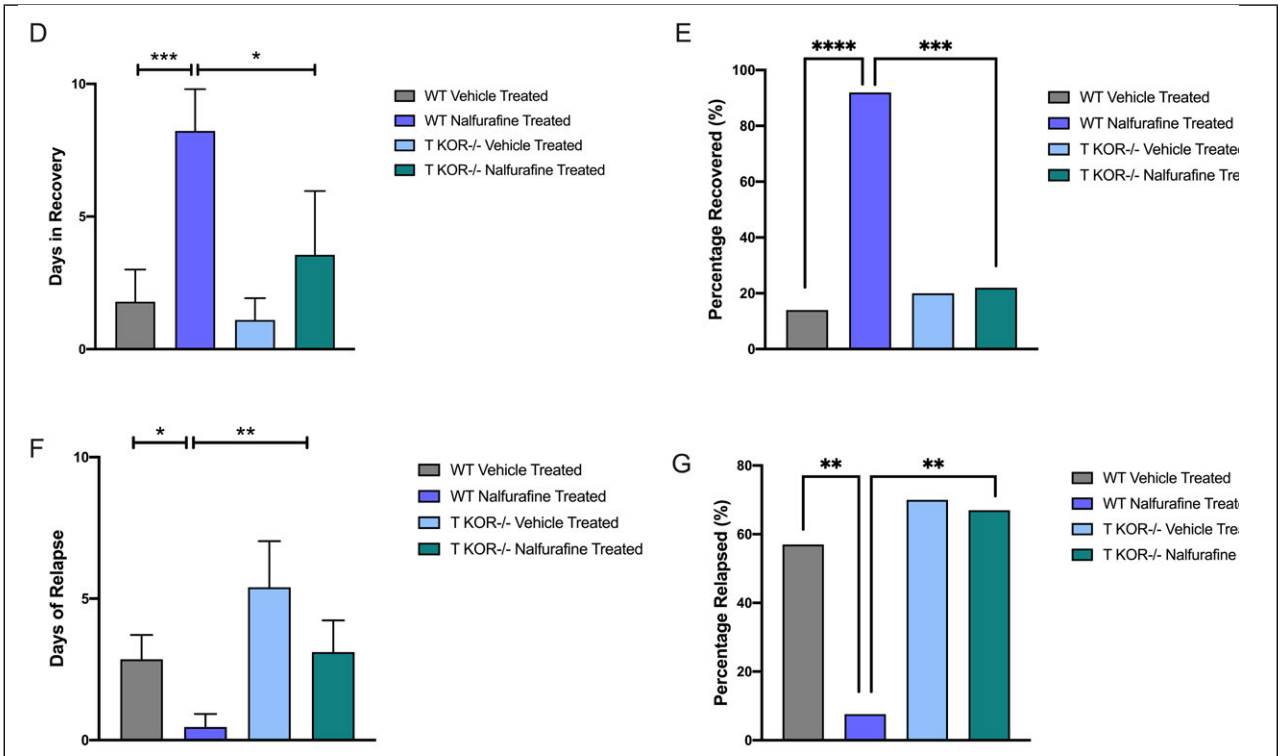


Figure 1: Scores and disease parameters of animals with intact kappa opioid receptor on Treg treated with kappa opioid receptor agonists compared to those with Treg knock-out kappa opioid receptor. (a) Disease scores of animals to day 25 post disease onset (where EAE score >/= 1 is considered onset). Results shown are from 6 independent experiments (n= 9-14) Data sets are mean +/- SEM. *P<0.05, ***P<0.001, ****P<0.0001 by Kruskal-Wallis comparison. (b) Disease scores of vehicle treated animals with or without functional KOR on Treg. (c) Disease scores of nalfurafine treated animals with or without functional KOR on Treg. *P<0.05 by Kruskal-Wallis comparison. (d) Recovery from EAE disease (considered EAE score </= 0.5) expressed as days spent in clinical recovery post disease onset. Presented as mean +/- SEM. *P<0.05, ***P<0.001 by Kruskal-Wallis comparison. (e) Recovery from EAE disease (considered EAE score </= 0.5) expressed as percentage of animals who clinically recovered within 25 days post disease onset. ***P<0.001, ****P<0.0001 by Kruskal-Wallis comparison. (f) Relapse in EAE disease (considered increase of 1 from lowest score post peak disease) expressed as days spent in clinical relapse post disease onset. Expressed as mean +/- SEM. *P<0.05, **P<0.01 by Kruskal-Wallis comparison. (g) Relapse in EAE disease (considered increase of 1 from lowest score post peak disease) expressed as percentage of animals who clinically relapsed within 25 days post disease onset. Presented as mean +/- SEM. **P<0.01 by Kruskal-Wallis comparison.

FIGURE 1 shows an increase in disease severity for T KOR-/- animals treated with nalfurafine, compared to their wild -type control. Nalfurafine mediated recovery from disease is also hindered in these animals, both in the percentage who recovered, and the days spent in clinical recovery throughout the course of disease. Furthermore, a statistically significant increase in the percentage of animals who relapse and the number of days they spent in a period of relapse was observed. This suggests nalfurafine is acting directly on Treg to promote disease recovery and prevent relapse of disease severity.

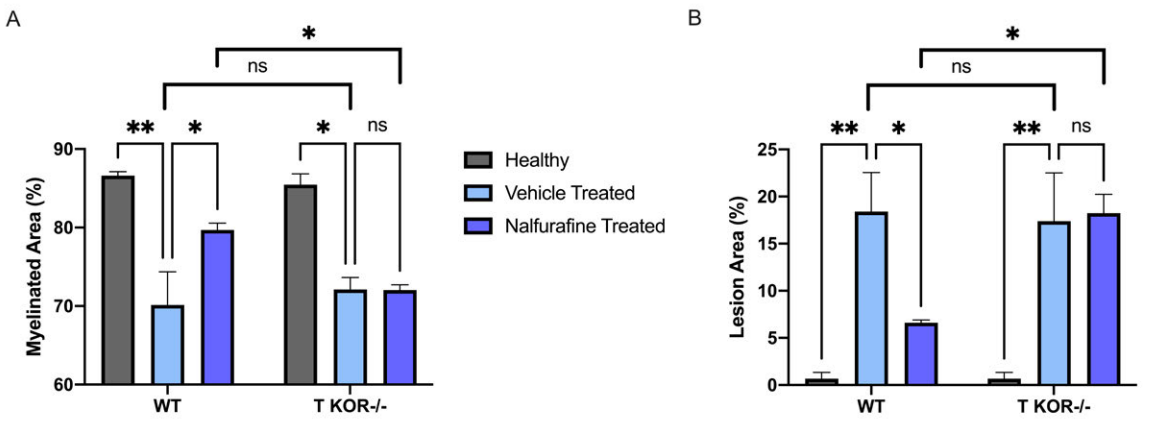


Figure 2: Black Gold II analysis of cervical spinal cord myelination in animals with intact kappa opioid receptor on Treg compared to those with 'knock-out' kappa opioid receptor on Treg (a) Myelination percentage of the cervical spinal cord in animals from each treatment group and healthy age matched controls determined by thresholding. Data sets are mean +/- SEM. *P<0.05, **P<0.01, by Kruskal-Wallis comparison. (b) Percentage of lesion area from the cervical spinal cord in animals from each treatment group and healthy age matched controls determined by blinded quantification. Data sets are mean +/- SEM. *P<0.05, **P<0.01, by Kruskal-Wallis comparison.

FIGURE 2: shows the increased myelination occurring in wild-type animals treated with nalfurafine, compared to vehicle treated controls, is abolished in animals with selective genetic deletion of the kappa opioid receptor on regulatory T cells. Similarly, the resulting lesion area of the white matter in T KOR-/- animals is significantly increased for nalfurafine treated animals compared to their corresponding controls.

CONCLUSION

These findings suggest regulatory T cells are crucial for nalfurafine-mediated remyelination and recovery from EAE, and that this protective effect is mediated through the kappa opioid receptor on regulatory T cells specifically. This research contributes to the understanding and development of therapeutics targeted at promoting remyelination within the central nervous system, which would directly address the cause of disability in MS – demyelination.

Te Herenga Waka
Victoria University of Wellington

Exploring the Immune Modulatory of Kappa Opioid Receptor on Mast Cell Activity

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KEY FINDINGS

Mast cells are known to be highly involved in driving immediate allergies reactions and uncontrolled mast cells activation can result in severe and life-threatening allergic reaction. In this project, we conducted a pilot study to investigate the role of kappa opioid receptor on mast cells activity in vitro. We demonstrated direct effect of kappa opioid receptor agonists in inhibiting mast cells activation and this inhibitory effect have shown to be dose dependent. Together, this data provide insight on potential therapeutic effect of kappa opioid receptor agonists on treating allergies.

OBJECTIVES

Mast cells are important sources of inflammatory mediators and their activation leads to the release of proinflammatory cytokines and chemokines, which can augment inflammation in many pathological conditions such as food allergies. Kappa opioid receptor agonists, such as U50, 488 have been shown to reduce inflammation and modulate mast cells activation. However, whether the anti-inflammatory effect of kappa opioid receptor agonist is dependent on mast cell involvement is not known. Therefore, the overall aim of this project is to explore the effect of kappa opioid receptor agonist on mast cell activity in vitro. The aim of this project will be addressed by three major objectives.

Objective 1: Investigate whether kappa opioid receptor agonist hamper or prevent mast cells activation.

Objective 2: Investigate the dose dependent effect of kappa opioid receptor agonist.

Objective 3: Explore the effect of different type kappa opioid receptor agonist.

METHODS

Mast cells are obtained by inducing differentiation of bone marrow cells under the influence of interleukin-3 (IL-3) growth factor. Bone marrow cells are isolated from the femurs and tibia of C57BL/6 mice, then cultured for 4-8 weeks in CTCM supplemented with 10% FCS, 1% L-glutamine, 1% penicillin/streptomycin, 1% nonessential amino acids, 1% HEPES, 0.1% β-mercaptoethanol, and mouse recombinant IL-3 (final concentration of 10 ng/ml). The differentiation of BMMCs and the purity of the cultures will be assessed every week by visualized using toluidine blue staining and flow cytometry.

To explore the effect of kappa opioid receptor agonists on mast cells activation, mast cells were treated with kappa opioid receptor agonists for 30 minutes prior of stimulation with PMA/Ionomycin for 2 hours. Level of activation of mast cells are measured by the surface expression of CD107a/LAMP-1 on mast cells using Flow Cytometry. Data is then analysed and graphed using Graph Pad Prism 9.

RESULTS

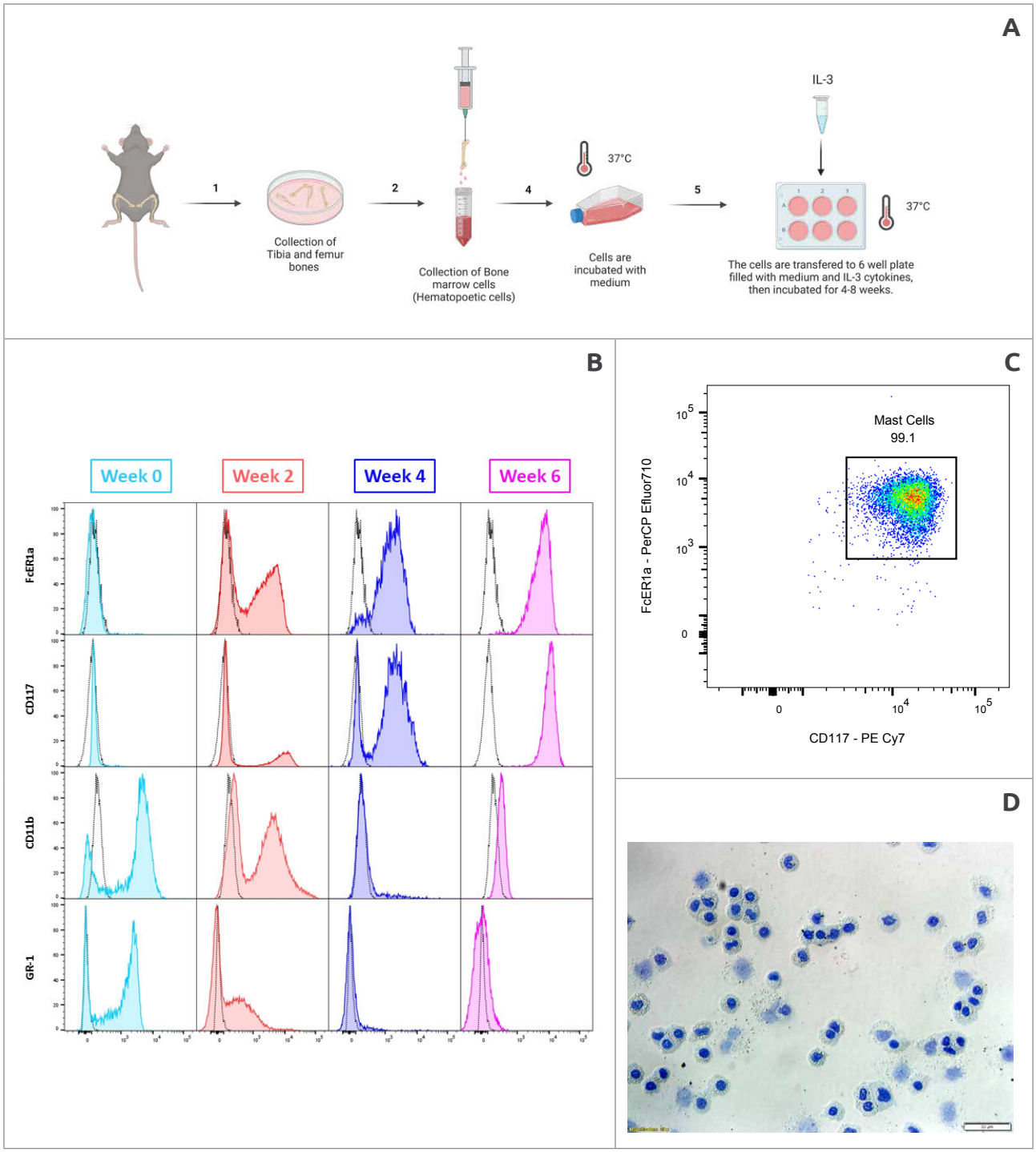

FIGURE 1. Generation of Mast Cells.

A) Schematic diagram of murine mast cells generation from bone marrow cells. (Created using Biorender.com)

B) Changes in cell surface antigen expression during bone marrow cells differentiation.

C) Purity of mast cells determined by double expression of CD117 and FceR1a using Flow Cytometry.

D) Visualisation of mast cells using Toluidine Blue staining.

We have optimised and developed murine mast cells culture model which allowed us to explore the functional properties of mast cells. First, bone marrow cells are isolated from the femur and tibia of the mice, then cultured in the medium containing IL-3 (10 ng/ml) to induce differentiation to mast cells, Figure 1(A). During the differentiation process, bone marrow cells can be observed undergo numerous changes particularly in cell surface antigen expressions. As seen in Figure 1(B), bone marrow cells gain mast cells marker receptors (CD117 and FceR1a) as early as week 2, and completely lost progenitor myeloid expression (CD11b and GR-1) by week 4. These changes are crucial to ensure a successful differentiation of bone marrow cells into mast cells.

CD117/c-kit, a tyrosine kinase growth factor receptor, plays a critical role in regulating cell development, differentiation, and proliferation. CD117 is widely expressed on hematopoietic progenitor cells, however, CD117 expression is lost during differentiation in most hematopoietic system except for mast cells. On the other hand, expression of FceR1a, high affinity of IgE receptor on mast cells are critical for the functionality of mast cells especially interacting with allergen molecules. This interaction leads to cellular activation and release of potent mediators such as histamine, which commonly associated with allergic response. However, FceR1a expression is not distinct to mast cells, in fact, FceR1a is expressed by other immune cells including basophils and monocytes. Therefore, the presence of mast cells is confirmed by double expression CD117 and FceR1a, Figure 1(C). Other than receptor expression, mast cells also can be recognised by its distinct morphology such as oval or irregular shaped cells containing a dense granular cytoplasm, Figure 1 (D). When >90% purity of mature mast cells is achieved, the cells are used for studies.

To induce activation of mast cells, culture are stimulated with PMA (100 ng/ml) and Ionomycin (500ng/ml). Activation of mast cells is determined by the surface expression of lytic granules (CD107a) on mast cells, Figure 2(A). As seen in Figure 2(B), the activation of mast cells can be seen as early as 15 mins and degree of activation increases as the incubation hours increases. The frequency of activated mast cells stimulated for 120 mins was almost 4 fold higher than the mast cells stimulated for 30 mins. We then validated inhibitory function of mast cells using a known antihistamine drug, clemastine fumarate (CLE). We observed a dose dependent effect of CLE, with maximal inhibitory efficacy at 1ug CLE concentration (63.84%), Figure 2(C). Increasing concentration of CLE after 1 ug does not produce a greater magnitude of inhibitory effect.

Since we observed decreased activation of mast cells when treated with CLE, we are interested whether we could observe similar result using kappa opioid receptor agonists such as Nalfurafine and U50-488. Based on Figure 2(D) and Figure 2(E), we observed similar dose dependent effect as CLE when using Nalfurafine and U50, with maximal inhibitory efficacy at 5nM of Nalfurafine (60.46%) and 2uM of U50-488 (69.59%). We also observe difference in potency of kappa opiod receptor agonists on activation of mast cells, Figure 2(D). Nalfurafine shows to be more potent that U50-488 as it require almost a thousand fold less concentration to achieve maximal inhibitory effect compared to U50-488. Although we saw significant reduction of mast cells activation when treated with Nalfurafine, U50-488 and CLE when comparing to untreated group, we did no observe significant difference in reduction of activation between treatment group, Figure 2(F).

CONCLUSION

We have successfully established the development of murine mast cells protocol and direct effect of kappa opioid receptor agonists in decreasing mast cells activity. We also have shown the dose dependent effect of the agonists and potency of different type of agonists on mast cells. This project provides insight on potential therapeutic effect of kappa opioid receptor agonists on mast cells associates disease. This project is still ongoing, and we are currently validating this data in animal model.

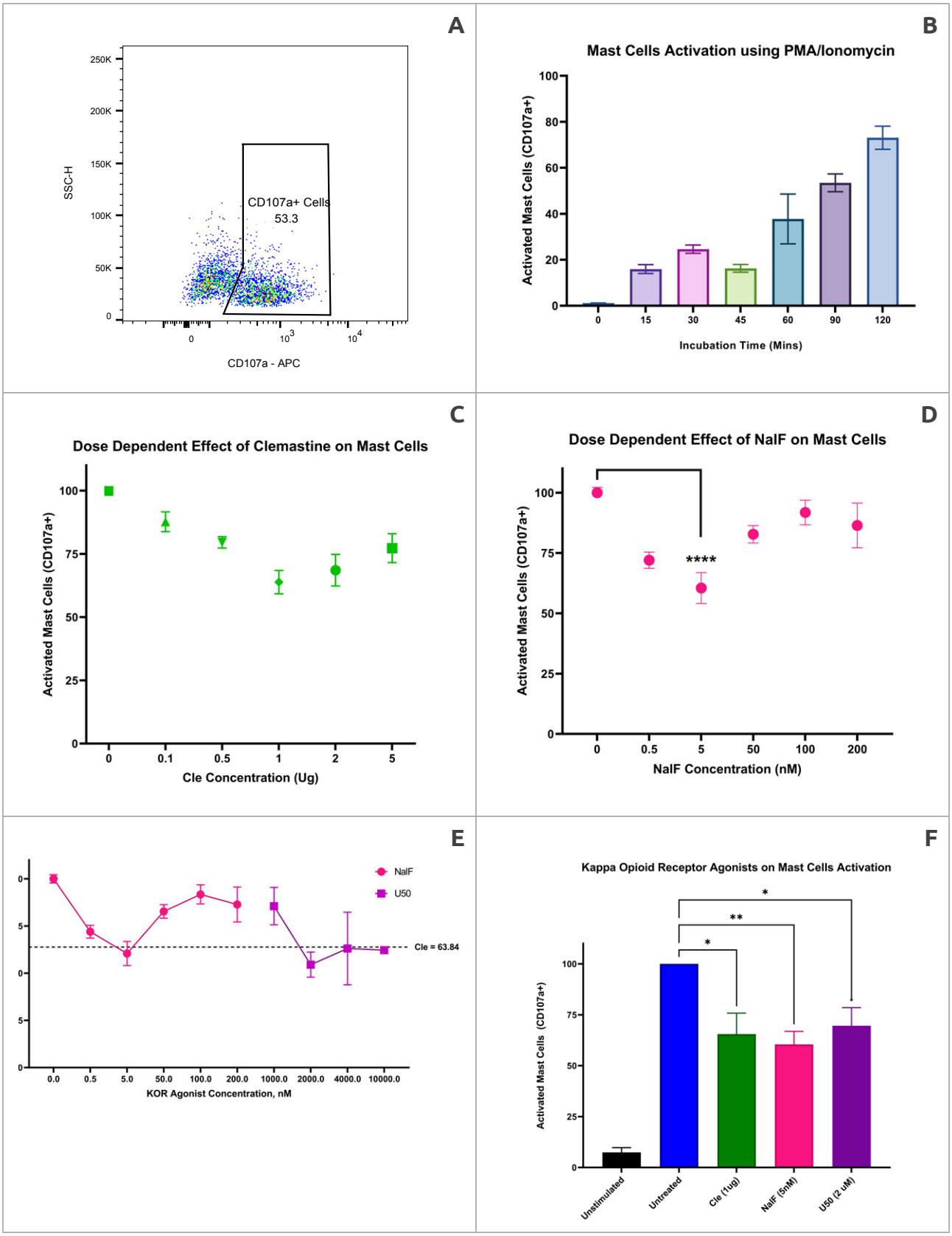

FIGURE 2: Exploring Inhibitory Effect of Kappa Opioid Receptor Agonists on Mast Cells.
A) Activated mast cells population gated by extracellular expression of CD107a.
B) Activation of mast cells are dependent on stimulant incubation time.
C) Dose dependent effect of Clemastine Fumarate (antihistamine) on mast cells activation.
D) Dose Dependent Effect of Nalfurafine on mast cells activation.
E) Difference in potency of Kappa Opioid Receptor Agonists on depleting mast cells activation.
F) Depletion of mast cells activation upon treatment with Clemastine, Nalfurafine, and U50-488.

Te Herenga Waka
Victoria University of Wellington

Engineering self-protection enzymes to enable CAR T-cells to combat solid tumours with precision chemotherapy

POST DOCTORAL FELLOWSHIP INTERIM REPORT

A SHARROCK, R WEINKOVE, M TERCEL, D ACKERLEY

KEY FINDINGS

Through bacterial metagenomic screening and bioinformatics analyses, my research has uncovered two classes of enzymes capable of protecting *Escherichia coli* and human cells against the damage caused by certain DNA alkylating chemotherapy drugs. To enhance the efficacy of these enzymes, I have employed directed evolution techniques and generated three error-prone PCR libraries that will serve as resources to identify improved enzyme variants. Additionally, I have observed that the cellular location influences the activity of these protective enzymes in human cells.

AIMS

Chemotherapy and immune cell therapies are two essential forms of cancer treatment, however they are largely incompatible with one another, as immune cells are inherently sensitive to chemotherapeutic agents. The overarching goal of this research is to identify and evolve novel protective enzymes that can effectively shield engineered immune cells from specific forms of chemotherapy-induced DNA damage.

To achieve this goal, my specific aims are to i) identify candidate DNA repair enzymes from the literature and conduct sequence homology analysis to uncover uncharacterised homologs, ii) use mass screening of the total bacterial DNA extracted from soil to identify novel enzyme candidates capable of protecting *E. coli* against key drugs, iii) conduct in vitro directed evolution experiments to enhance the protective abilities of lead enzyme candidates, and iv) characterize the activity of lead enzymes in cultured human cells, including assessment of how cellular localisation affects activity.

METHODS

A "metagenomic" library of total bacterial DNA extracted from soil was generated using our bespoke methods that promote high-level expression of genes captured by *E. coli* host cells. This provides access to the biochemistry encoded by many thousands of bacterial genomes, and we have shown these libraries can prove a rich source of previously unknown drug resistance genes. The metagenomic library was screened to coverage on medium supplemented with our target chemotherapy drug (identity withheld for IP reasons). This screen resulted in the discovery of four novel enzymes that conferred high level protection to *E. coli.*

To date, the top enzyme from this metagenomic screening and two other enzymes discovered by bioinformatics analyses have been subjected to directed evolution via error-prone PCR. Error-prone PCR involves the use of a polymerase which exhibits a high error rate to randomly introduce mutations throughout a gene and is a powerful approach (pioneered by 2018 Nobel Prize winner Frances Arnold) for improving enzymatic activities that have not previously been selected in nature. The mutated DNA libraries I generated in each error-prone PCR reaction were cloned into a bacterial expression vector and used to transform an *E. coli* screening strain. Colonies from each library transformation were pooled, scraped, and stored as a library glycerol stock. The transformed *E. coli* cells were then plated on media containing my target chemotherapy drug, at levels just above the minimum growth-inhibiting concentration for *E. coli* expressing each non-evolved enzyme. This ensured that only cells that had gained improved resistance elements would be able to survive and replicate to form colonies.

Resistant colonies were harvested, and their plasmids extracted and sequenced to determine the mutations present in the evolved genes and encoded enzymes. To eliminate the possibility of spontaneous plasmid backbone or *E. coli* genomic mutations contributing to the improved resistance, evolved gene variants are being re-cloned and re-tested to ensure the improved drug resistance is maintained.

RESULTS

Metagenomic screening uncovered four novel enzymes that exhibited only low-level sequence similarity to any previously known enzymes. Each of these was found to be capable of protecting E. coli and cultured human (HEK-293) cells against the cytotoxicity of our target chemotherapy drug. One of these four enzymes was selected as a representative and subjected to characterisation using methods such as isothermal titration calorimetry and high-performance liquid chromatography to determine the mechanism of protection however, this remains unclear.

To enhance the desired drug-resistance activity, I have thus far generated error-prone PCR libraries for two enzyme-encoding genes identified by bioinformatics, and one from the metagenomic screening. The sizes of these libraries were ca. 0.5 million, 4 million, and 1.25 million clones, respectively, and error-rates ranged from 1.1-2.7 amino acid mutations per gene. A screening pipeline was developed for each library, which included optimisation of the expression level of each protective enzyme, screening density, and drug concentration. Preliminary library screening was conducted, but thus far has only yielded hits that conferred greater protection to *E. coli* cells through increased expression of the protective enzyme, rather than through increased activity. As our aim is to enhance activity, I am currently adjusting my screening conditions to ensure the selection drives improvement in this direction.

I am also interested to understand whether the protective activities of the enzymes I have discovered can be boosted according to their cellular localisation. For example, DNA repair enzymes might be more active in the nucleus of a drug-exposed cell, whereas drug-detoxifying enzymes might be more active in the cytoplasm, or unaffected by their cellular localisation. I have been modifying my enzymes to direct them to specific cellular locations. I have first tested these proteins in *E. coli* assays – although bacterial cells have no nucleus, this has allowed me to confirm that the modifications I introduced did not negatively affect baseline protective activity. Subsequently, I generated stable human cell lines expressing each modified enzyme. Thus far, I have shown that one of the classes of enzyme I discovered is more protective in the nucleus, while another is more protective in the cytoplasm. This may provide insights to enhance protection of engineered immune cells.

CONCLUSION

This project is ongoing, and our future plans involve the continuation of generating and screening error-prone PCR libraries to optimize the activity of each protective enzyme. Evolved hits will be tested preliminarily in HEK-293 cells, and ultimately in immune cells. This could potentially pave the way for synergistic chemo- and immune cell mediated therapies.

Institute of Environmental Science and Research (ESR), Massey University

Antimicrobial Surveillance for Gonococcal Clinical Samples

X REN, C STRAUB AND C BROMHEAD

KEY FINDINGS

Increasing antimicrobial resistance (AMR) in *Neisseria gonorrhoeae*, the causal agent for gonorrhoea, is becoming a worldwide emergency. We tested a PCR-based, multi-gene panel, high-throughput assay based on the AMR Multilocus Typing Scheme (NG-STAR, 1) for gonococci. We found, with further optimisation, this panel has the potential to expand *Neisseria gonorrhoeae* AMR surveillance in Aotearoa New Zealand.

OBJECTIVES

Objective 1: Determine feasibility of the assays on a variety of clinical samples from different anatomical sites and test formats.

Objective 2: Determine the clinical sensitivity and specificity of assay.

METHODS

DNA Extraction

We tested a variety of DNA extraction methods for the extraction of clinical samples from different anatomical sites and test formats. Methods tested are displayed in Table 1.

SAMPLE FORMAT	EXTRACTION KITS TESTED AND USED (BOLD)
Isolate	**Qiagen Blood and Tissue**, Gentra Bacteria and Yeast
Ames Buffer	**Qiagen Blood and Tissue**
Aptima Urine	Qiagen Blood and Tissue, **QIAamp UCP Pathogen**, Zymo Quick -DNA Urine Kit
Aptima swab	Qiagen Blood and Tissue, **QIAamp UCP Pathogen**
BDQx swab	**Qiagen Blood and Tissue**, QIAamp UCP Pathogen
BDMax Urine	Qiagen Blood and Tissue, QIAamp UCP Pathogen, **Zymo Quick -DNA Urine Kit**
BDMax swab	**Qiagen Blood and Tissue**, QIAamp UCP Pathogen
BD urine	Qiagen Blood and Tissue, QIAamp UCP Pathogen, **Zymo Quick -DNA Urine Kit**

TABLE 1: DNA extraction methods tested.

PCR

Multiplex PCR for short-amplicon and long-amplicon assays were optimised by testing different concentrations and ratios of primer mix, annealing temperatures and Taq enzymes. Due to the limited space, we will not discuss it here. Quantitative PCR (qPCR) was used to estimate the sensitivity of the chosen *Neisseria gonorrhoeae* (NG) assays (2).

Sequencing

Short-amplicon products were sequenced using 2X250bp sequencing format with Illumina chemistry on the MiSeq system. Long-amplicon products were sequenced using rapid and ligation sequencing format on the Oxford Nanopore system.

Bioinformatics

Custom pipelines were built to assemble the PCR products. Antimicrobial resistance alleles were detected by searching the assembled PCR products against the amrfinder (3) database. Validity of the pipeline was tested with known WHO reference isolates (4). Due to the limited space, we will not discuss it here. *Neisseria gonorrhoeae* Multi-Antigen Sequence Typing (NG-MAST, 5) and *N. gonorrhoeae* Sequence Typing for Antimicrobial Resistance (NG-STAR, 1) were not performed due to sequencing fragmentation and quality issues (see results).

RESULTS

Objective 1: Determine feasibility of the assays on a variety of clinical samples from different anatomical sites and test formats.

To accomplish this, we first tested a variety of methods for extracting DNA from clinical samples. We compared the results using DNA concentration and *NG* qPCR value (when possible). We also attempted to compare the qPCR value we obtained to the original diagnostic PCR CT value. However, this comparison was problematic due to differences between screening assays as well as the storage time before performing our DNA extraction. Unfortunately, the COVID-19 pandemic had a major impact on the project: X Ren was an integral member of the response team and there were associated issues with access to laboratory space. This resulted in the majority of the samples being stored for greater than 6-months before extraction. Major difficulties were encountered with Aptima samples where the buffer was not compatible with the lysis buffer in the kits we tried. Eventually we decided to use the QIAamp UCP Pathogen kit for extraction because it has a heating step that reduced some of the compatibility issues.

To investigate how accurate the PCR and bioinformatic pipelines were compared using *in silico* PCR of isolate genomes, isolate PCR result and PCR result obtained from corresponding clinical samples. We were unable to examine NG-MAST and NG-STAR typing because of missing amplicons in short-amplicon PCR set and multiple products for a given amplicon in the long-amplicon PCR set. We were also unable to gather sufficient data points for the comparison of long-amplicon PCR results due to issues with 23S ribosomal RNA amplicon: for 19 of 20 isolates, a 23S PCR product could not be detected. We also found issues with the short *ponA* amplicon, which was due to failure in sequencing the complete fragment. The *ponA* amplicon is large at 494bp and there were not enough high-quality reads in the 2X250bp sequencing format to generate full sequences. We had a number of technical and sample issues but found that the AMR allele detection on isolates was consistent between isolate and its corresponding clinical samples. (Table 2).

SAMPLE TYPE / DILUENT	ALL SHORT-AMPLICON PRODUCT SEQUENCED	ALL LONG-AMPLICON PRODUCT SEQUENCED	AMR ALLELE DETECTION: ISOLATE MATCHES GENOME	AMR ALLELE DETECTION: ISOLATE MATCHES CLINICAL SAMPLE
Isolate	20/20 samples	0/20 samples	6/11	11/12
BDMAX	4/10 samples	0/2 samples	NA	NA
BDUrine	12/12 sample	0/12 samples	NA	NA
BQ	22/28 samples	0/1 samples	NA	NA
Aptima	39/83 samples	NA	NA	NA
AMES	2/3 samples	0/3 samples	NA	NA
negative	0/10 sample	0/10 samples	NA	NA

TABLE 2: Comparison of Sequencing Results

Objective 2: Determine the clinical sensitivity and specificity of assay.

We tested the specificity of the PCR assays on primary clinical samples by examining NG negative samples. These negative samples were obtained from urine, rectum, and throat. All negative samples gave very few sequencing reads for both the short and long amplicon format. The only product generated for the long-amplicon format was 23S RNA, which is not unexpected as 23S is present in all bacteria. These results indicate that the NG AMR amplicon panels are specific for the anatomic sites tested so far.

To investigate the analytical sensitivity of the assay we performed amplicon sequencing on a dilution series of three WHO reference NG isolates and used qPCR as well as a synthetic oligonucleotide (gblock) to estimate the genome copy number. For the short-amplicon format we were able to consistently sequence all 10 amplicons when there are more than 10 copies of the genome for all three isolates. For the long-amplicon format we were able to sequence all 9 amplicons when there are more than 100 copies of the genome for two of the three isolates. Unfortunately, we were only able to do this experiment once.

Due to DNA extraction issues with Aptima samples, we did not assess the limitation of the amplicon assay for these samples. For other clinical samples, where diagnostic qPCR results were available (26 Samples), samples with a CT below 28 cycles consistently yielded all 10 short-amplicon products. We were unable to assess long-amplicon assay due to technical issues with the 23S PCR primer set.

CONCLUSION

Overall, we have shown that the multi amplicon PCR combined with next generation sequencing is a valid approach for determining AMR profile from a variety of clinical samples. However, further optimisation and validation is needed. Two simple modifications to test would be to 1) use 2X300 bp sequencing instead of 2X250bp sequencing because it will ensure full length amplicons are sequenced. 2X300bp format sequencing has improved in quality recently. 2) Increase the concentration of 23S primer in the long-amplicon primer pool to improve PCR efficiency. This work would contribute to antimicrobial stewardship by allowing increased surveillance of the AMR genotypes circulating in NG in Aotearoa New Zealand.

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CENTRE FOR EPIGENETIC AND GENETIC RESEARCH

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Oxidative stress causes epigenetic changes that may promote tumour development

A STEVENS, F RICH, M HAMPTON

KEY FINDINGS

This project aided our understanding of the biological consequences of inflammation associated oxidants on our cells. We demonstrated that oxidants generated by activated white blood cells can change the pattern of methylation at specific regions of human genomic DNA. We anticipate that this interaction may contribute towards the subsequent development or progression of cancer, however it may also be of wider relevance to inflammation associated diseases, such as Alzheimer's.

INTRODUCTION

Oxidative stress is a common feature of inflammation-driven cancers and promotes aggressive tumour developments. This can occur by promoting genomic disruptions, or by modification of key regulatory proteins that direct how genes are turned on and off. Neutrophils are our most abundant white blood cells and accumulate at sites of infection and inflammation where they can produce strong oxidants. At sites of infection and inflammation the heme enzyme myeloperoxidase generates the chemical HOCl, and its primary targets are most likely to be extracellular methionine and amines. Resultant chloramines, such as the glycine chloramine used in this study, will therefore be prominent under physiological conditions. Their lower reactivity provides them the opportunity to pass into neighbouring cells and alter cell function. We hypothesize that exposure to immune derived oxidants can impact the pattern of chemical signatures on genomic DNA. These chemical signatures, such as DNA methylation are important epigenetic 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 cellular and environmental stimuli is a central question for managing health and disease and is a major contemporary challenge in human genetics.

OBJECTIVES

The primary goal of the study was to determine whether sub lethal concentrations of the oxidant glycine chloramine can modify the pattern of DNA methylation. The secondary goal of the study was to perform a bioinformatic prediction of how the impacted gene regions may contribute towards disease development or progression.

RESULTS

This grant aided in optimisation of *in vitro* assays that were designed to interrogate the cellular consequences of oxidant exposure on genomic DNA methylation. Sub lethal glycine chloramine exposure coincided with substantial decreases in DNA methylation, an effect which was conserved over multiple cycles of cell division, without further exposure (Fig 1). The timing of oxidant exposure with relation to cell cycle was crucial. The most substantial effects were observed when exposure preceded DNA replication, which includes active methylation of nascent DNA strands. These findings have direct relevance to situations of prolonged oxidant exposure, as in the instance of chronic inflammation. We also observed that a large proportion of the changes in DNA methylation were situated towards chromosomal ends (Fig. 2), suggesting that these regions are particularly susceptible to modification, and may also impact on the

timing of DNA replication. This may contribute to genomic instability of chromosomal ends when cells are dividing, which may have potential implications for the regulation of telomere length and cellular longevity. DNA methylation changes corresponded with gene activity and analysis of the regulated genes had broader implications than expected. In addition to potential targeting of cell cycle regulation and key tumour developmental pathways we also observed that the results may have relevance to other inflammatory disorders, including Alzheimer's disease and diabetes.

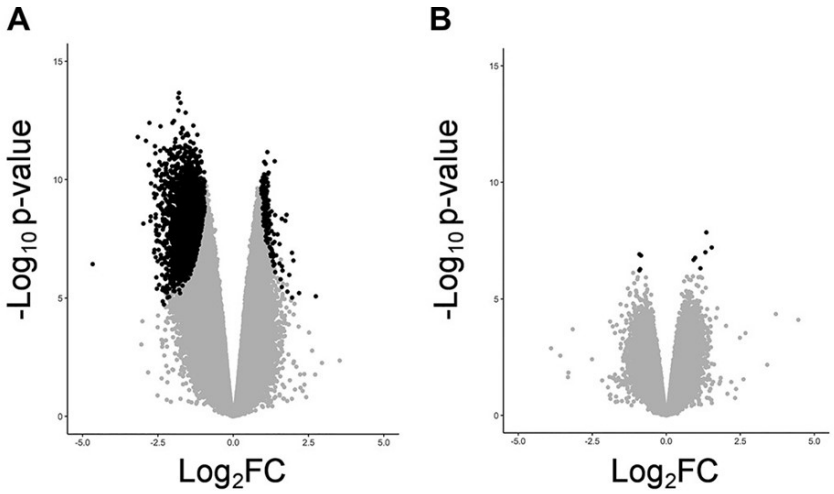


FIGURE 1: Volcano plots displaying log2 fold changes in methylation for all probes on the x-axis versus statistical significance on the y-axis -log10 P-value. Black dots represent significant probes (A) GlyCl exposure at 4 h, (B) GlyCL at 72 h.

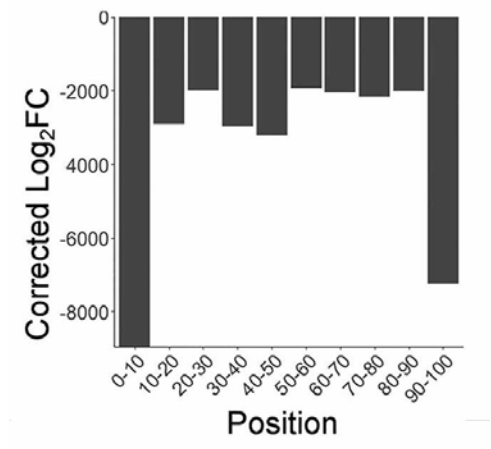


FIGURE 2: Significant DNA methylation changes plotted by chromosolmes position. Data was adjusted for the number of probes that binds per region and total number of probes.

We expanded our research into two interesting new avenues. We have begun validating our preliminary observations in the context of Alzheimer's disease. This has involved optimisation of experimental conditions in a new cell model, which will investigate cellular regulation of the blood brain barrier. Currently the results are validating prior observations and have demonstrated several interesting avenues for future research. We have also expanded this research to investigate the effect of an additional oxidant HOSCN. We have recently established the highest concentration that does not substantially influence viability and growth of Jurkat T-lymphoma tissue cells across a period of 24 hours. Cellular viability was assessed by flow cytometry, and each experiment consists of four independent replicates.

METHODS

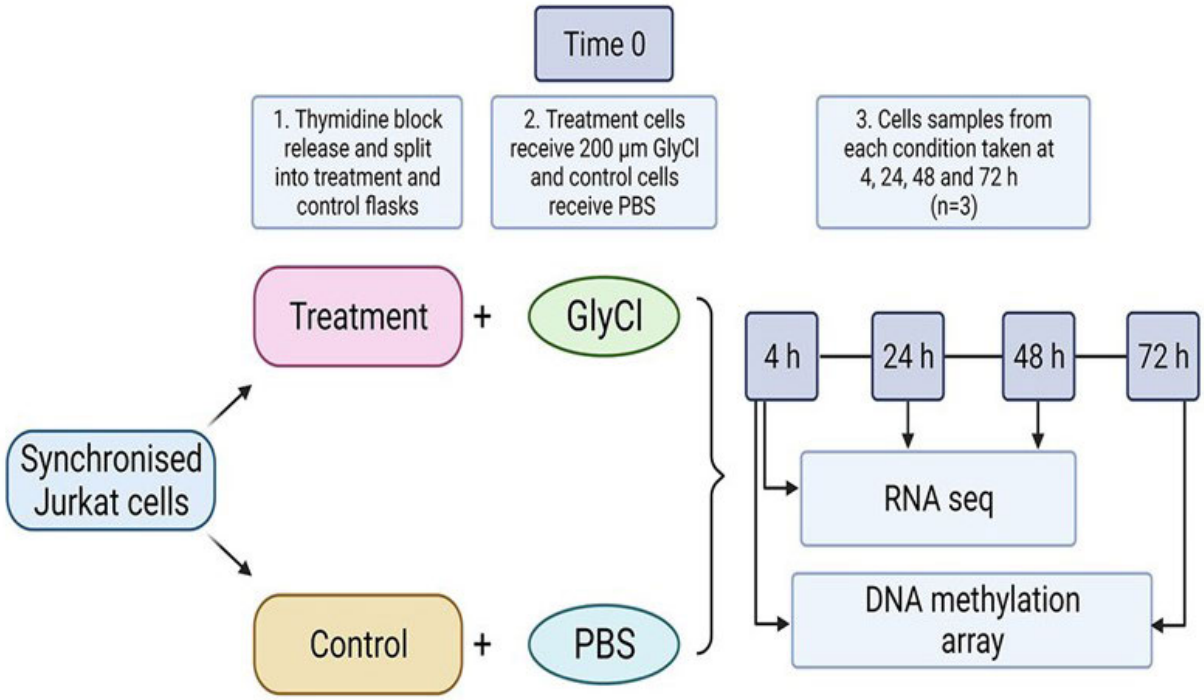


FIGURE 4: Experimental design: Jurkat cells were arrested in S phase of the cell cycle by using a thymidine block. (1) Cells were released from the block and split into treatment or control flasks. (2) The treatment flask immediately received 200µm GlyCl and the control received the volumetric equivalent of PBS (Time O). (3) DNA was extracted at the 4 and 72 h time points, bisulfite converted and DNA methylation was assessed using the illumina methylation EPIC850K array.

CONCLUSION

Our novel findings have demonstrated an interesting avenue for immune directed epigenetic regulation of gene activity. This appears to be a likely mechanism that links chronic inflammation with the subsequent development or progression of cancer and is of potential relevance in additional inflammatory disorders, including Alzheimer's disease. Because inflammation is often a precursor of oncogenic onset, a window of opportunity exists for prevention of malignant progression. For development of preventative or therapeutic options, it is crucial to understand the molecular consequences of inflammation. We are currently building on these findings and plan to expand and replicate the findings in additional disease contexts.

Successful award of funding from the WMRF was crucial for completion and expansion of this research, which has recently been published in the Human Molecular Genetics journal: *"Site-specific decreases in DNA methylation in replicating cells following exposure to oxidative stress"*, (https://doi.org/10.1093/hmg/ddac232).

University of Otago, Wellington

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

S. HUMMEL, K. HALLY, P. LARSEN

KEY FINDINGS

Dual anti-platelet therapy (DAPT) is the current gold standard for treating heart attacks (acute myocardial infarction – AMI) as DAPT prevents cellular components in the blood from aggregating and thus reduces recurrent cardiovascular events. However, heart attacks have a complex pathophysiology, involving a variety of immune cells that can interact with platelets. Neutrophils have gained a new appreciation in the pathophysiology of heart attacks due to their ability to form Neutrophil extracellular traps (NETs), a pro-inflammatory mechanism that has been associated with recurrent adverse cardiovascular events. We found that DAPT seems to prime neutrophils to form NETs.

OBJECTIVES

We aimed to investigate the effects of DAPT on neutrophils and their ability to undergo NETosis.

METHODS

Patient recruitment

We recruited 10 healthy volunteers (five males and five females), aged between 45 to 70, into a prospective interventional study with DAPT (aspirin and ticagrelor) as the intervention. Exclusion criteria for healthy subjects were known cardiovascular or inflammatory disease, disorders associated with platelet dysfunction, acute illness within six weeks prior to recruitment, pregnancy, diabetes mellitus, a history of adverse drug reaction, treatment with cardiovascular and immune-modulating drugs or antiplatelet agents within seven days prior to recruitment, having had a vaccine within the last six weeks or awaiting a COVID-19 test result at any point throughout the study. These healthy volunteers were asked to provide a blood sample before and after treatment with DAPT for isolation of treatment-naïve and DAPT-treated neutrophils.

Administration of DAPT

The healthy subjects received DAPT for two days. DAPT consisted of a combination of aspirin (300mg loading dose on day 1, 100mg maintenance dose on day 2) and ticagrelor (180mg loading dose on day 1, 90mg maintenance dose on day 1 and day 2). This mimics the routine of DAPT administration given to individuals with AMI in Aotearoa New Zealand.

Neutrophil isolation and in vitro NET stimulation

To test the effects of DAPT on the ability of neutrophils to induce NETosis, neutrophils were isolated directly from EDTA-anticoagulated whole blood and resuspended in cell culture media to 2 x 106 neutrophils/mL. Neutrophils were stimulated with various concentrations of PMA (4 nM to 500 nM), or Ionomycin (5 µM to 75 µM), both classical NET agonists, or FSL-1 (0.016 µg/mL to 1 µg/mL), a neutrophil activator, but does not induce NETosis, or left unstimulated, for two hours at 37 □C/ 5% CO2.

Measuring NETosis:

We used Myeloperoxidase activity and SytoxGreen fluorescence as surrogate markers of NETosis. Neutrophils were cultured in the presence of the cell-impermeable

nucleic acid stain SytoxGreen. Briefly, upon NET formation, DNA is expelled from the cells and the cell-impermeable nucleic acid stain is able to bind to the extracellular DNA and the amount of NET formation can be inferred from the fluorescent intensity. Additionally, we utilized the peroxidase activity of MPO, a peroxidase found within NETs. Following neutrophil culture, free-floating MPO was removed by discarding the supernatant prior to vigorous resuspension of the neutrophil monolayer, which contains NET-appendant MPO. Using a colorimetric assay, NET release was inferred proportionally from MPO activity.

Statistics

We calculated signal:noise ratios for all assays using the unstimulated samples as their own control. Samples were then normalized to their pre-DAPT values. We performed an unpaired t-test with Welsh's correction and assumed normal Gaussian distribution (α = 5% significance level). Values are displayed as mean ± SEM.

RESULTS

To assess whether DAPT treatment affects the ability of neutrophils to undergo NETosis, we measured MPO activity and SytoxGreen fluorescence as surrogate markers of NETosis pre- and post-DAPT treatment. Neutrophil MPO activity and SytoxGreen expression was not statistically significant between pre- and post-DAPT treatment across all concentrations of PMA (Figure 1A), Ionomycin (Figure 1B) and FSL-1 (Figure 1C). However, while the differences between pre- and post-DAPT treatment in MPO activity were not statistically significant, a trend of increased MPO activity can be observed in the post-DAPT treatment conditions.

SytoxGreen fluorescence was not statistically significant across all PMA (Figure 1D) and FSL-1 (Figure 1F) concentrations. While SytoxGreen fluorescence was only significantly different between pre-and post-DAPT treatment for neutrophils stimulated with 75 µM Ionomycin ($p=0.049$), a similar trend in increased SytoxGreen fluorescence can be observed in the post-DAPT treatment condition.

CONCLUSION

We assessed MPO activity and SytoxGreen fluorescence as surrogate markers of NETosis and observed a trend of increased MPO activity and SytoxGreen fluorescence in neutrophils stimulated with classical NET agonists, PMA and Ionomycin, post-DAPT treatment. These preliminary results suggests that DAPT treatment may prime neutrophils for NETosis. While the differences between pre- and post-DAPT treatment in MPO activity and SytoxGreen fluorescence were not statistically significant, we cannot exclude a biological significance that exerts effects on the pathophysiology of illnesses such as AMI.

To further investigate the effects of DAPT on neutrophils and their ability to undergo NETosis, we are planning to co-culture treatment-naïve neutrophils, that have not been exposed to DAPT, with platelets that have been treated with DAPT. This will allow us to determine the effects of DAPT on platelet-mediated NET formation and further deepen our understanding of the interplay of these two cells in the context of heart attacks. We aim to complete this study and the associated analyses by the end of the year.

University of Otago, Wellington

Could measuring a protein in the blood help us assess concussion in the emergency department?

A SIK, A ROGAN, D MCQUADE, P LARSEN

KEY FINDINGS

Our study has found that adults diagnosed with a head injury in the Emergency Department have a higher blood level of the protein S100B when compared to the blood levels of healthy individuals. However, experiencing more concussion symptoms, or a higher severity of symptoms, did not seem to relate to having a higher blood S100B level. This could be promising in terms of using S100B to assess head injury patients.

AIMS

Concussion has recently had a lot of attention in sports, medicine, and in the media. It is a common head injury and affects many people in NZ and internationally. The mechanisms of concussion are not well understood yet. What we do know is that it is a form of brain injury that can manifest in different ways in different people and in some cases can affect people's quality of life significantly. We know that it cannot be seen on routine head imaging and can often have normal findings on clinical examination. Because of this, our ability to assess concussion in an emergency department setting is challenging, with a lot of room for improvement.

S100B is a protein that is most abundant in brain cells and other parts of the nervous system. Current evidence suggests that brain cells release S100B in response to injury. Other studies have found that S100B levels in the blood rise when there has been severe brain injury and bleeding in the brain with changes seen on routine imaging.

We aimed to find out whether S100B in the blood could be tested to detect concussion in patients presenting to the emergency department (ED) after having a head injury. We also wanted to learn more about the relationship between S100B blood levels and the number and severity of symptoms patients with head injury experience.

METHODS

For two years (March 2021 to March 2023) we recruited people with a head injury from the Wellington Regional Hospital ED and healthy individuals. All participants were asked to consent to a blood test and complete a survey that scored their symptom number (a total of 22 symptoms) and symptom severity (from no symptoms, 0, to severe, 6, with a maximum total of 132). Headache, nausea or vomiting, dizziness, blurred vision, and difficulty concentrating are some examples of the concussion symptoms in the survey. All emergency department patients were assessed as per normal by ED clinicians and it was recorded if patients were diagnosed with concussion, head injury or another diagnosis. Other data about the participant was collected such as age, sex, ethnicity, and what caused their head injury. All participants had to be 18 or over and able to consent to participate in the study.

Blood samples were then processed and analysed for blood protein levels of S100B. We then compared the protein levels to see if healthy individuals (controls) had a different blood protein level than those diagnosed with concussion, those diagnosed with head injury, or those diagnosed with something else (other). We also looked at how concussion symptom number and severity scores differed between the study groups. Lastly, we compared the S100B levels to the number and severity of concussion symptoms experienced by the participant.

RESULTS

We recruited 83 participants with a median age of 33 years old, with 54% between 18-35, 22% between 35-50, and the remaining 24% over 50. 57% of participants were male, with 47% female. The majority of participants were NZ European (72%) with Māori (12%), Asian (12%), Pacific peoples (2%),

and other ethnicities (1%) making up the rest. The reasons for head injury in participants were sport (21% of head-injured participants), fall from standing (21%), motor vehicle accident (13%), assault (13%), fall from height (5%), and with the rest of participants having other causes of head injury (27%, e.g. work related). Of all the participants, 25% were controls, 37% diagnosed with concussion, 21% diagnosed with head injury, and 17% with another diagnosis.

When comparing the median S100B levels across the four groups (controls, concussion, head injury, and other diagnosis) there was a significant difference between controls and those diagnosed with head injury. Those diagnosed with head injury had a higher median blood S100B level (0.105ug/ml) than healthy controls (0.046ug/ml). There was no significant difference between any other groups and controls, however, in general controls had a lower median S100B blood level compared to those diagnosed with concussion (0.059ug/ml) or with another diagnosis (0.067 ug/ml) (see figure 1 below).

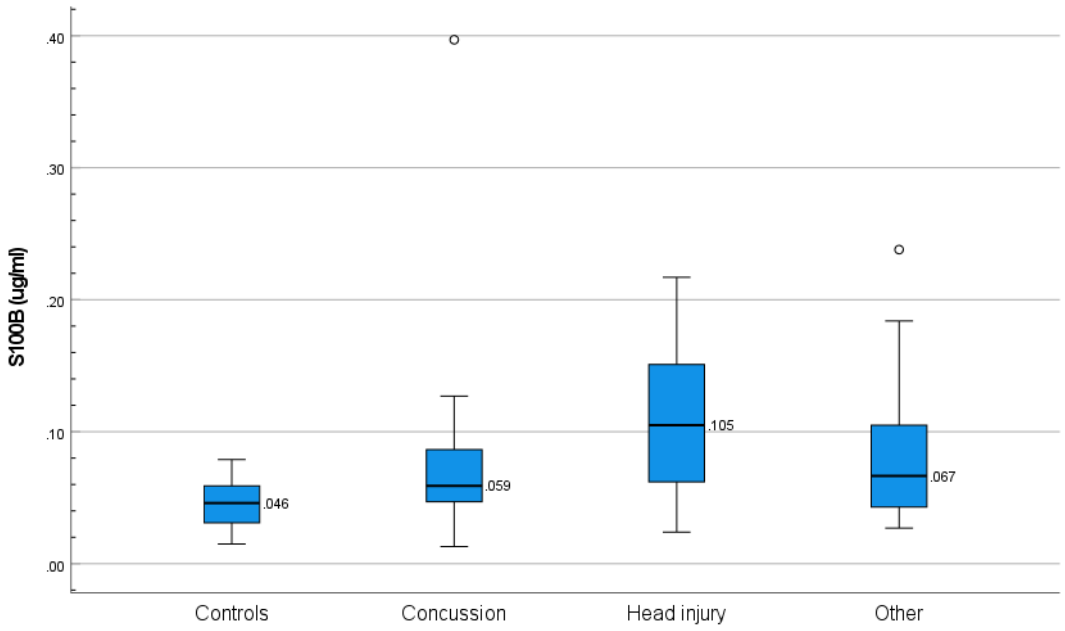


FIGURE 1

In both the number and severity of concussion symptoms across all four groups there was a significant difference in median scores between controls (number 0/22, severity 0/132) and those diagnosed with concussion (number 16/22, severity 40/132), head injury (number 12/22, severity 28/132), and another diagnosis (number 12/22, severity 21/132). There was no significant difference between any of the three groups with a head trauma.

Experiencing a higher number of concussion symptoms was not related to a higher blood S100B level. Similarly, having a higher severity of concussion symptoms was not related to a higher blood S100B level.

CONCLUSION

The difference in blood S100B levels between controls and those diagnosed with head injury was large enough for us to say that we are seeing an actual increase in S100B levels across the two groups. For the other groups, differences in S100B levels are too small to decide whether the difference is simply random chance, or if there is in fact an actual difference. Our study did not have enough participants to detect small differences in S100B levels in the concussion and other diagnosis groups, but it could be that a bigger study with more participants would tell us for sure.

However, even finding the difference between controls and the head injury group is promising as an indication that S100B does rise with head trauma, even with more mild injuries. We plan to do further and more detailed analysis to see if a cut off blood level of S100B would be useful to assess mild head traumas.

Our study did not find a relationship between S100B blood levels and symptom number or severity. It may be that our study was too small to find a difference, or it may be that there is no difference. There are a lot of steps between cell damage and the experience of symptoms, with many other factors influencing a person's experience of an injury.

Gillies McIndoe Research Institute, Wellington

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

(INTERIM REPORT)

B CHANG-MCDONALD[1], C GRAY[1], S HALL[1], JAMES SINCLAIR[2], N WEST[2], K HATTON-JONES[2]

[1] Gillies McIndoe Research Institute, Wellington

[2] Griffith University, Queensland, Australia

KEY FINDINGS

Pending. We are awaiting analysis of the data.

OBJECTIVE

To better understand the spatial heterogeneity of squamous cell carcinoma (SCC) of the tongue at the level of tumour and tumour microenvironment using spatial transcriptomics, a molecular profiling method that allows scientists to measure the gene activity in a tissue sample and map where the activity is occurring.

METHODS

The diagnosis of squamous cell carcinoma of the tongue was confirmed by an Anatomical Pathologist reviewing haematoxylin and eosin-stained histological sections of the tissue (Figure 1). We then sent unstained formalin-fixed paraffin-embedded (FFPE) tissue slides of the cases to Griffith University, Queensland, for analysis by the Nanostring GeoMx® Cancer Transcriptome Atlas (1833-plex RNA panel) which allows comprehensive spatial profiling of tumour biology, the tumour microenvironment, and the immune response. This platform generates spatially resolved gene expression data of over 1,800 genes simultaneously from multiple distinct regions of interest (ROI) which we pre-selected within each single tissue section of a patient's tumour. ROI contained tumour and surrounding stroma that included immune cells and fibroblasts. To ensure pre-selected ROI contained clearly identifiable tumour with surrounding stroma, immunofluorescence staining was done with each slide stained simultaneously with fluorescent markers targeting the pan-cytokeratin marker AE1/AE3 (tumour) combined with the pan-haematopoietic marker CD45, and alpha smooth muscle that stains fibroblasts and smooth muscle cells (Figure 2).

Nanostring Technologies, Seattle, United States of America, had earlier performed spatial transcriptomics on 4 cases of SCC of the tongue. The grant from Research for Life was to go towards performing spatial transcriptomics on a further 4 cases at Griffith University which has the same Nanostring platform. We had been informed that as both centres used the same platform that it would be possible to combine the data from both runs to give a total of 8 cases. However, there were Covid related delays and issues with sourcing and working up identical antibodies as those used in Seattle for the immunofluorescence staining. Given this, we subsequently decided to carry out a single centre study with all 8 cases having spatial transcriptomic analysis performed at Griffith University, Queensland, using the Nanostring platform.

We had a total of 8 cases (4 smokers, 4 non-smokers) all of which were males. The tumours included 2 well differentiated SCCs, 2 well to moderately differentiated SCCs, 3 moderately differentiated SCCs and one moderate to poorly differentiated SCC.

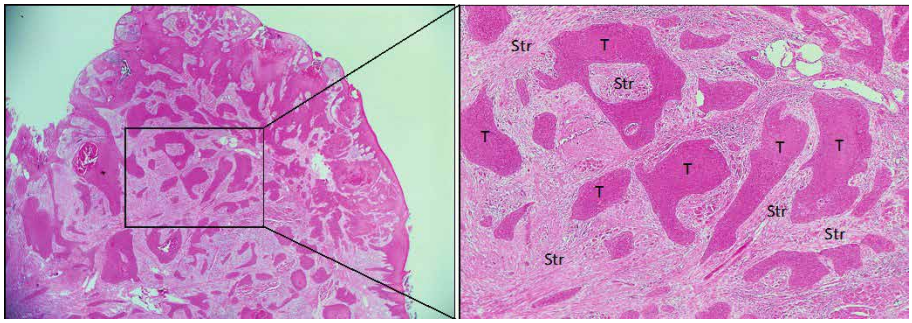


FIGURE 1: Histological section of squamous cell carcinoma of the tongue stained with Haematoxylin & Eosin (T (tumour), Str (stroma)). Original magnification of photograph on left 12.5x, Inset photograph original magnification 40x.

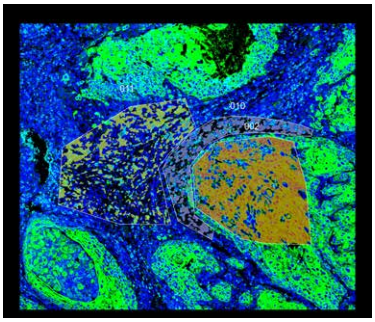


FIGURE 2: Immunofluorescence staining was performed for selection of regions of interest (ROI). The tumour is highlighted in green (cytokeratin AE1/AE3). Original magnification 200x

RESULTS

The analysis of the data obtained from the spatial transcriptomics is pending (Figure 3).

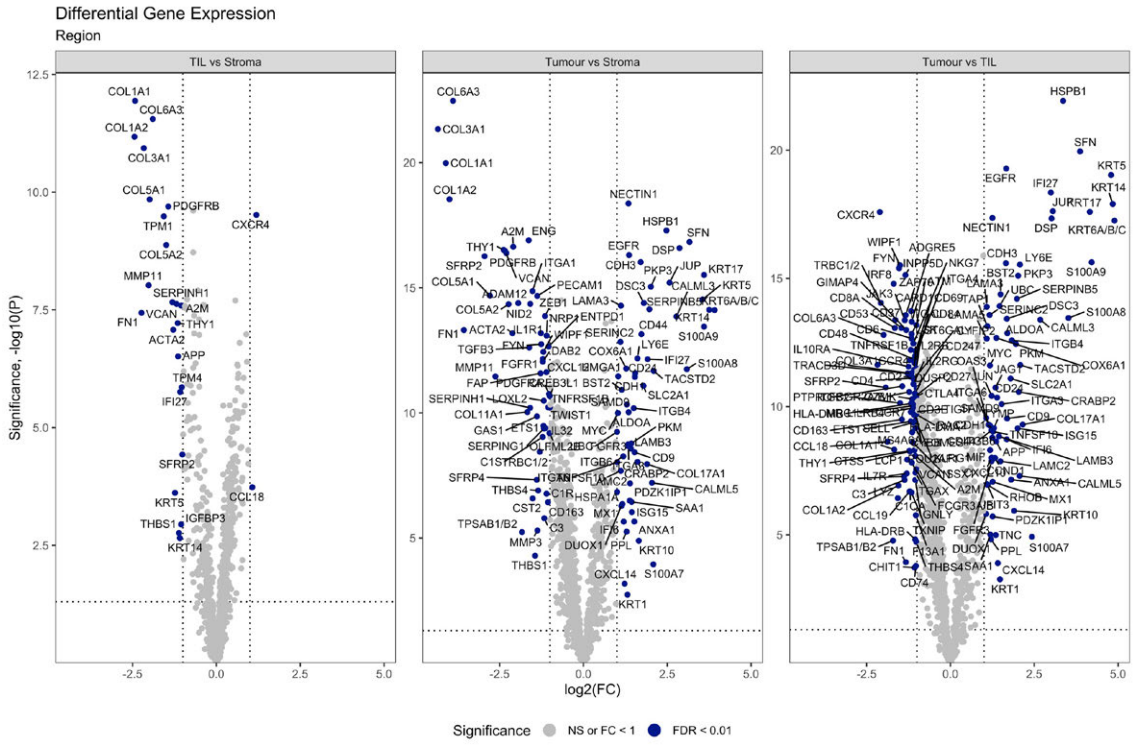


FIGURE 3: Volcano plot from preliminary data. We are awaiting analysis of the data to be completed.

CONCLUSION

Our project was impacted by methodological issues and previous analysis had to be started from scratch as batch effects were observed between Seattle and Griffith University Nanostring centres. All slides had to be re-done and sent to Griffith University to be analysed. Now remedied, we predict that analysis should be completed by November-December 2023 and then preparation of data and manuscript can commence.

Wellington Hospital

C*STEROID trial – Wellington

(INTERIM REPORT)

DR JUDY ORMANDY

Obstetrician and Gynaecologist,
Wellington Hospital

The C*STEROID trial is a randomized controlled trial of antenatal steroids for mothers having a planned caesarean section after 35 weeks' gestation. Compared to babies born vaginally, babies born by planned caesarean section have higher rates of respiratory distress and breathing problems. These breathing difficulties are usually short-term but may require babies to be admitted to the neonatal unit, thereby requiring babies to be separated from their mothers.

The aim of the C*STEROID Trial is to find out whether giving mothers corticosteroid injections before having a planned CS from 35+0 to 39+6 weeks of pregnancy will safely reduce the risk of short-term breathing problems for babies.

We know that administering corticosteroids before birth to mothers giving birth before 35 weeks gestation reduces the rates of respiratory distress, perinatal distress, and adverse perinatal outcomes. What is less clear is if corticosteroid use at gestational ages greater than 35 weeks and specifically prior to a planned caesarean section provides benefit without harm. Corticosteroids before a late preterm birth may be associated with an increase in neonatal hypoglycaemia.

We don't know if corticosteroid use before caesarean section at late preterm and term gestations reduces neonatal respiratory morbidity and/or increases neonatal hypoglycaemia.

The C*STEROID trial will determine whether antenatal administration of corticosteroids prior to planned Caesarean at 35 to 39+6 weeks provides benefits without harm The trial will assess neonatal respiratory morbidity and neonatal hypoglycaemia and aim to assess any impact on later childhood development.

Wellington Hospital is one of seven New Zealand hospitals participating in the C*STEROID trial. The trial started in October 2020 and Wellington joined in October 2021. The trial aims to recruit 2500 women. The COVID pandemic has added challenges to trial recruitment and 581 women have participated so far. Twenty-eight of these women were from Wellington.

This year 15 Australian centres have joined the trial and July 2023 was a record month with 43 participants recruited. Wellington recruited 4 participants in June which was a record for us. It is anticipated that the trial will need to continue for a further 2 -3 years.

We are grateful to the support from Research for Life which means that whanau in the Wellington region have the opportunity to participate in clinical trials.

RESEARCH PROJECT & TRAVEL GRANTS

Research Project Grants 2023

THE FOLLOWING PROJECTS HAVE BEEN APPROVED FOR FUNDING IN THE FINANCIAL YEAR ENDED 30 JUNE 2023 OR ARE LONGER TERM PROJECTS FROM PREVIOUS FUNDING ROUNDS. THESE WILL BE REPORTED ON IN SUBSEQUENT RESEARCH REVIEWS.

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 $6,490 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 received a Research for Life grant of 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.

Evaluating Kappa Opioid Receptor Localisation and Expression in Multiple Sclerosis **MARTIN DALEFIELD**	Martin Dalefield, M.Phil., received a Research for Life grant of 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.

Diagnostic performance of S100B as a rule out test for intracranial pathology in patients presenting to the Emergency Department within 6-hours of head injury that meet NICE CT-head criteria

ALICE ROGAN

Dr Alice Rogan received a grant of up to $13,102 to conduct research investigating the use of blood tests to help diagnose traumatic brain injuries (TBI). TBI are a leading cause of death and disability in New Zealand and common emergency department presentation. TBI incorporates mild (concussion) to severe injuries (brain bleeding or swelling). Doctors must decide who is at risk of more severe injuries and warrant a head CT. This can be difficult, particularly in people with severe concussion or intoxication. This project proposes that Dr Rogan's research will explore whether blood tests can be used as a screening tool to exclude more severe injuries and support doctor's decision-making. This could improve patient care by reducing CT head wait times and enable earlier ED discharge for those without a severe injury. Dr Rogan is an Emergency Medicine Research Fellow within the Department of Surgery at the University of Otago, Wellington and an Emergency Medicine Registrar at Wellington Hospital.

In vitro characterisation of the immunomodulatory pathways in macrophages induced by novel kinase inhibitors

ELYSHA-ROSE GRANT

Elysha-Rose Grant received a grant of up to $12,970 to undertake research to help discover new compounds that alter the activity of macrophages. Macrophages are immune cells that have both pro-inflammatory and anti-inflammatory functions and can cause harm in autoimmune diseases such as multiple sclerosis. Finding new compounds that can alter the function of specific aspects of the immune system, rather than causing widespread suppression, can be potential candidates for treatment of certain diseases. Isolated from marine sponges, Nelliellosides 11B and 13A have shown promising activity alteration in splenocytes and macrophages, with particularly exciting activity induced by (–)-TAN-2483B, a fungal metabolite that increases anti-inflammatory properties of macrophages. Elysha's research will explore the mechanisms by which these compounds generate their unique effects. Elysha-Rose Grant is a PhD candidate under the supervision of Prof. Anne La Flamme at the School of Biological Sciences, Victoria University of Wellington.

Defining the role of immunometabolism in multiple sclerosis

GEORGIA LENIHAN-GEELS

Dr Georgia Lenihan-Geels received a grant of up $21,921 to undertake research to improve our understanding of immune cell metabolism in progressive multiple sclerosis patients. Aotearoa has one of the highest rates of multiple sclerosis worldwide and there is a lack of effective treatment options for those with progressive forms of multiple sclerosis. Recent findings have shown that patients with secondary progressive multiple sclerosis have higher levels of activated immune cells in the blood. Dr Lenihan-Geels' research aims to better understand the influence of these cells on MS disease outcomes, such as fatigue and cognition. Dr Lenihan-Geels is a postdoctoral researcher at Victoria University of Wellington - Te Herenga Waka.

Investigating the Role of T cells in Endometrial Cancer Treatment Response

HANNAH VAN DER WOUDE

Hannah van der Woude, a PhD student at the University of Otago, Wellington, received a grant of up to $10,000 to undertake research investigating the role of immune cells in endometrial (uterine) cancer using novel tissue culture methods. Endometrial cancer incidence is rising in Aotearoa, particularly in our pre-menopausal population, and there is a strong link between endometrial cancer and the obesity epidemic. Despite this, there are limited treatment options available for those who wish to avoid surgery, either to preserve their fertility or because a high BMI carries with it increased surgical risk. Moreover, conservative treatment for endometrial cancer is severely under-researched and response rates are highly variable. Hannah is exploring how conservative endometrial cancer treatment influences immune cell response. This will shed light on how to overcome resistance by effectively harnessing the natural cancer-killing properties of the immune system and provide a foundation for personalising conservative treatment options.

SARS-CoV-2 heterodimer subunit caccines select for broadly neutralising antibodies.

ISABELLE STEWART

Dr Isabelle Stewart received a grant of up to $27,255 to undertake research to understand how COVID-19 vaccines can better protect us against future variants of SARS-CoV-2 (the virus that causes COVID-19). Dr Stewart's research explores how antibodies that can target future SARS-CoV-2 variants develop after immunisation, and how to encourage the development of these cross-protective antibodies. This project involves investigation of new protein-based SARS-CoV-2 vaccines, which have been designed to induce cross-protective antibodies. Dr Stewart is a postdoctoral fellow at Victoria University of Wellington and has worked with Vaccine Alliance Aotearoa New Zealand – Ohu Kaupare Huaketo to help New Zealand make its own vaccine against COVID-19.

Oxidative stress causes epigenetic changes that may promote disease development.

AARON STEVENS

Dr Aaron Stevens, a senior lecturer in the Department of Pathology and Molecular Medicine at the University of Otago, Wellington, received a grant of up to $20,125 to investigate the chemical signatures on genomic DNA. These 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 Stevens' 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 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.

Investigating differences in the peripheral immune signature of females and males with Acute Myocardial Infarction (AMI).

CERYS BLACKSHAW

Cerys Blackshaw received a grant of up to $15,295 to undertake research to help women with cardiovascular disease (CVD). CVD is the leading cause of mortality for women worldwide, but research shows that there are inequalities of care between women and men. International reviews have shown that females have worse health outcomes due to a lack of comparable care between males and females. In a New Zealand context, we have found that females are experiencing less guideline-based interventions but have seen no effect on health outcomes. Cerys' research is investigating if these differences are due to systemic bias in the hospital system, or due to a physical difference in the way the disease impacts women compared to men. Cerys is a PhD student at Otago University, working in the Surgery and Anaesthesia Department at Wellington Regional Hospital.

Investigating the remyelination potential of novel heparan sulfate compound 'HS16-35'

MADELEINE GRIFFITHS

Madeline Griffiths received a grant of up to $14,083 to undertake research to help multiple sclerosis patients. Multiple sclerosis is a chronic neuroinflammatory that may cause vison issues, pain, numbness, spasms, balance problems, sexual dysfunction, and incontinence. With 1 in 1,000 kiwis affected by this disease, there is desperate need for new therapies. Madeline's research is exploring new treatment options to aid repair in the central nervous system following a disease relapse. Madeline Griffiths is a PhD Student in the Cell and Immunobiology research group at Victoria University of Wellington – Te Herenga Waka, supervised by Prof. Anne La Flamme.

A narrative inquiry exploring the experiences of survivors of long-term intensive care and their support people in Aotearoa New Zealand

AMY BEST

Ms Amy Best received a grant of up to $2,000 grant to undertake research to help former critically ill patients and families who have experienced a prolonged stay in the intensive care unit (ICU). Most people admitted to an ICU have a relatively brief length of stay, however, up to ten percent do not recover rapidly, experiencing a prolonged stay (≥8 days). Internationally there is very limited knowledge on prolonged ICU stays as well as the complexities, vulnerabilities, and traumas that a protracted stay creates for survivors and their family. Ms Best's research is creating new knowledge by exploring survivors' and support peoples' lived experience territory across the first year following discharge from hospital in New Zealand. Through the narrative process, health, and healthcare issues as well as the support needs of these people be illuminated, thus, this research has the potential to improve the care and support ICU survivors receive. Ms Best is a PhD candidate at Massey University School of Nursing and a senior staff nurse in the ICU at Wellington Regional Hospital.

Characterising the progenitor to theca cell transition in ovarian follicles

ZARAMASINA CLARK AND SARAH SCZELECKI

Dr Zaramasina Clark and Dr Sarah Sczelecki received a grant of up to $25,008 to undertake research into an underexplored target for treating infertility. The theca cell layer of the ovarian follicle plays a key role during the development and ensuing release of a mature egg (ovulation). Until recently, the origins and identity of theca cell progenitors were unknown. This research will provide some of the first descriptions of key points in this progenitor to theca cell transition enabling the development of novel models to understand ovulation failure, a known cause of infertility and disease. Dr Clark is a Lecturer and Dr Sczelecki is a postdoctoral fellow in the School of Biological Sciences at Te Herenga Waka-Victoria University of Wellington.

Confirmation of a transgene visualization method to characterize and monitor anti-cancer CAR T cell therapies.

ANDREW WILSON

Andrew Wilson received a grant of up to $7,018 to work on a new genetic test for use with cell and gene therapies. Cell and gene therapies, such as chimeric antigen receptor (CAR) T-cells, are becoming a standard of care internationally for treatment of certain blood cancers. New cell and gene therapies are in development worldwide, including at Wellington's Malaghan Institute of Medical Research. Andrew's project will use genetics expertise to develop and optimise a new method to directly see and count the added gene segments inside CAR T-cells. This could help with safety and monitoring of new cell and gene therapies. Andrew has recently completed his PhD studies through the University of Otago Wellington, in collaboration with the Malaghan Institute of Medical Research, and plans to continue a career in the field of genetics and genetic research.

Investigate The Effect of Kappa Opioid Agonism in Food Allergies Model

HASANAH HAMIZAN

Hasanah Hamizan received a grant of up to $18,832 to unpack the potential benefits of the kappa opioid receptor agonists as alternative food allergies treatment. Hasanah's research will explore the little-known mechanisms and immune response (particularly mast cell activity) involved during kappa opioid receptor agonists treatment in animal models. Hasanah is a second year PhD student working under the supervision of Prof Anne La Flamme in the School of Biological Sciences and Centre for Biodiscovery at Victoria University of Wellington.

Modelling the transition between goal-directed to habitual drug-seeking behaviour in the rat

JUAN CANALES

Professor Juan Canales received a grant of up to $16,971 to develop a new model to study the transition from voluntary drug use to habitual, compulsive drug taking. Addiction can be seen as a habit in the sense that it involves a repetitive pattern of behaviour that is extremely hard to break. Drug habits become strongly associated with drug-related cues and contexts, such as drug paraphernalia, that are able to induce uncontrollable urges, contributing to the maintenance of a substance use disorder. Such drug habits often persist in spite of negative consequences. Professor Canales will develop a new animal model of drug habit formation that will provide new insights into the role of learning mechanisms in addiction and pave the way for the design of new medical treatments and interventions for substance use disorders. Professor Canales is Head of School for the School of Psychology at Te Herenga Waka – Victoria University of Wellington.

Structural and functional characterization of the FAM family of neuronal proteins

THOMAS BIRD

Thomas Bird was awarded up to $16,226 to undertake research of FAM171 proteins, an understudied family of neuronal receptors with implications in neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. Thomas' research will provide fundamental knowledge of the structure and function of these proteins that will broaden our understanding of these diseases and may aid in the development of new treatments. Thomas is a PhD student at Victoria University of Wellington.

Generating a single-cell atlas of the murine lung in health and disease

KERRY HILLIGAN AND DAVID SESTER

Dr Kerry Hilligan and Dr David Sester received a grant of up to $10,000 to undertake research into respiratory infectious diseases, such as the flu virus, using a new cutting-edge technology. Spectral cytometry enables the simultaneous analysis of 50+ parameters from each individual sample at a single-cell level, enabling data collection on millions of cells. Dr Hilligan and Dr Sester will use this information to learn about the early immune response following a flu infection to identify potential targets for future vaccines. Dr Hilligan is a Senior Research Fellow and Dr Sester is Head of Cytometry at the Malaghan Institute.

Developing 3D models of breast cancer for therapeutic study

HELENA ABOLINS-THOMPSON

Helena Abolins-Thompson received a grant of up to $24,374 to establish 3D models of different subtypes of breast cancer. She will be comparing integrated spheroid models derived from immortalised cell lines to patient-derived organoid models generated from primary patient samples. This will be done in an exclusively Māori patient cohort. Māori patients are at higher risk of breast, presenting at later and more advanced stages. Confocal microscopy and viability assays will be used to assess the effect of clinically relevant chemotherapies on these models with the wider goal of ensuring Māori are represented in therapeutic testing platforms, which will allow further exploration into more appropriate and equitable treatments for Māori patient cohorts. Helena is a PhD candidate in the Surgical Cancer Research Group at the University of Otago, Wellington.

Investigating differences in the transcriptomic profile of neutrophils in patients with ideal versus adverse cardiac repair after acute myocardial infarction

KATHRYN HALLLY

Dr Kathryn Hally received a grant of up to $16,627 to explore the molecular basis of cardiac healing in patients that have experienced a heart attack. Adverse healing after a heart attack can reduce heart function and conveys an increased risk of experiencing recurrent cardiac events (for example, another heart attack). Dr Hally's research aims to characterise the role of neutrophils in the healing process after a heart attack. Neutrophils are an abundant cell type within the blood that can cause inflammation, and understanding how neutrophils are involved in healing the heart may reveal new targets for therapeutic interventions in the future. Dr Hally will be working with Zoe Moltschaniwskyj, Dr Ana Holley and Associate Professor Peter Larsen to conduct this research. All applicants are a part of the Wellington Cardiovascular Research Group and are based at the University of Otago, Wellington.

Novel flow cytometry techniques to detect extracellular vesicles for diagnosing endometriosis.

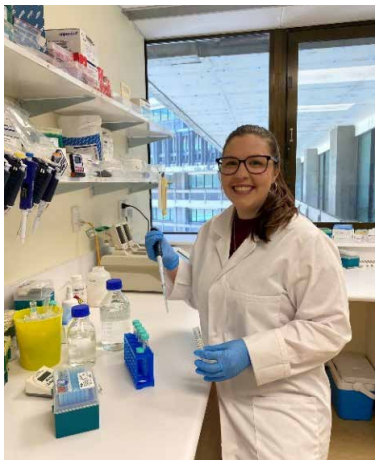
EMILY PATERSON

Emily Paterson (PhD Candidate) received a grant of up to $17,920 for investigating non-invasive biomarkers of endometriosis. Endometriosis is a common, painful disease affecting an estimated one in nine women and gender diverse people. Endometriosis can currently only be diagnosed through laparoscopic surgery, creating a significant diagnostic delay. Emily's research focuses on detecting small particles called extracellular vesicles present within blood using flow cytometry as biomarkers of endometriosis. Her research aims to help reduce the diagnostic delay associated with endometriosis and enable clinicians to triage patients and prioritise those most likely to benefit from surgery.

Travel Grant Reports

EMMA SYMONDS

INTERNATIONAL SOCIETY FOR EXTRACELLULAR VESICLES (ISEV) ANNUAL MEETING IN SEATTLE



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 $1629 to present her research at the International Society for Extracellular Vesicles (ISEV) annual meeting in Seattle, Washington, USA 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.

ISEV is the largest and most prestigious conference in the 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 both 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 NZ 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.

EMILY PATERSON

WORLD CONGRESS ON ENDOMETRIOSIS (WCE) IN EDINBURGH, SCOTLAND

Emily Paterson

With the support of the Wellington Medical Research Foundation, I was able to attend and present my PhD research at the World Congress on Endometriosis (WCE) in Edinburgh, Scotland in May this year. The WCE is the premier international endometriosis conference and is only held once every three years, so it was a real privilege to be able to attend during my PhD.

I presented my research investigating cervicovaginal fluid extracellular vesicles isolated from vaginal swabs and cervical smears for potential diagnostic biomarkers of endometriosis. This was the first international conference I have presented at, and as such was very valuable experience in disseminating my research to other researchers. I was also able to make connections with other scientists who research extracellular vesicles in endometriosis, which will be helpful for my PhD studies and possibly future collaborations.

Many of the keynote presentations discussed the chronic pain, a key symptom of endometriosis. These lectures deepened my understanding of very complex pain pathways and how research into other chronic pain conditions can be useful in the context of endometriosis. This insight has shaped my interest in chronic pain in endometriosis and has sparked an interest in this field for my future research projects.

Myself and my supervisor Dr Claire Henry also did an Instagram takeover of the EndoWarriors Aotearoa Instagram, to share the exciting research being conducted internationally with our endometriosis community in NZ.

Overall, my attendance at the WCE was inspiring, rewarding and extremely valuable for my current PhD and future research.

KERRY HILLIGAN

2023 KEYSTONE SYMPOSIUM ON *INFLAMMATION IN THE LUNG: FRIEND OR FOE IN VIRAL INFECTION* HELD IN UTAH

Kerry Hilligan

Thanks to a Research for Life travel grant, I was able to attend the 2023 Keystone Symposium on *Inflammation in the Lung: Friend or Foe in Viral Infection* held in Utah, United States to present my research investigating "infection interference" in the context of COVID-19. This describes a phenomenon whereby respiratory infections caused by other bugs "interfere" with the ability for the COVID-19 virus, SARS-CoV-2, to infect its host. In this work, my colleagues and I delineated several different mechanisms by which "infection interference" caused by bacterial or parasitic infection can protect against severe COVID-19 in pre-clinical models. This conference was the perfect setting to present our work and I received invaluable feedback from other attendees at both the poster session and following my short talk.

Keystone Symposia are known for their focused programmes as well as providing great networking opportunities - and this conference was no different. I met many interesting people and had many stimulating discussions at the poster sessions and over dinner. It was the first time Keystone Symposia had run a conference with the topic "*Inflammation in the Lung*" and it was an undeniable success, with similar meetings already planned for the future. One particularly successful aspect was that the conference ran simultaneously with another meeting focused on "*Lung Development, Infection, Repair and Aging*" and included shared plenary sessions so that attendees could hear talks on both topics. This encouraged interaction between the immunologists at the "Inflammation" meeting and developmental biologists at the "Lung Development" meeting. I certainly learned a lot from my developmental biologist colleagues that I have brought back home to implement in my future research.

In addition to the cross-discipline benefits of the conference, there were countless outstanding presentations on unpublished research in the "*Inflammation in the Lung*" programme that I was able to share and discuss with my colleagues in New Zealand upon returning. I am extremely grateful to Research for Life for the opportunity to attend such an amazing conference!

KAITLIN BUICK

50TH ANNUAL MEETING OF THE AUSTRALIAN AND NEW ZEALAND SOCIETY FOR IMMUNOLOGY (ASI) 2022 IN MELBOURNE, AUSTRALIA

Kaitlin Buick

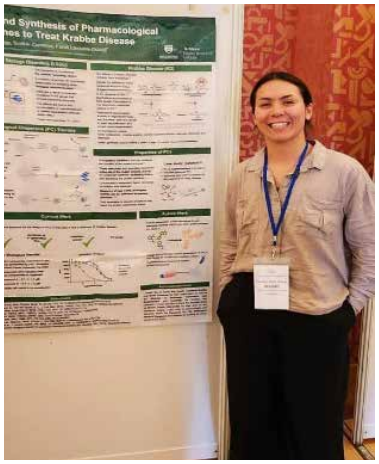
With the generous support from Research For Life, Wellington Medical Research Foundation I was able to attend and present at the 50th annual meeting of the Australian and New Zealand Society for Immunology (ASI) 2022 in Melbourne, Australia. This year the ASI conference had the largest number of attendees, with over 1,000 registrations. This provided many opportunities to interact and engage with a wide range of scientists in different settings across the conference with four days of oral presentations and poster sessions.

The ASI conference gave me my first opportunity to do an oral presentation at an international conference. I was able to present research I conducted as a Research Assistant on investigating the protein-protein interactions occurring between different immune cell populations during the initiation of allergic disease. Through this presentation I was able to present a molecular technique and showcase the value of interdisciplinary collaborations. This was a great opportunity to showcase my work at a large conference and talk to other immunologists about my work.

The ASI conference also coincided with the 25th anniversary of Peter Doherty and Rolf Zinkernagel's Nobel Prize for their discovery around the mechanism whereby T-lymphocytes recognise viral antigen. It was a highlight to have both Nobel Prize winners at the conference and to hear from them both about their research careers and achievements.

LUCY HUGHES

2023 EUROPEAN CARBOHYDRATE (EUROCARB) SYMPOSIUM IN PARIS, FRANCE



Lucy Hughes

I am a final year PhD student at Te Herenga Waka, Victoria University of Wellington. I am doing my postgraduate studies out of the Ferrier Research Institute, the carbohydrate research institute of New Zealand. My PhD is focussed on the synthesis of carbohydrate-based drugs for the treatment of Krabbe disease, a rare, fatal, and currently untreatable neurodegenerative lysosomal storage disorder. My PhD focuses on making pharmacological chaperones* as a means of treating this devastating disease. If we are successful in our approach to developing this treatment, our design approach could serve as the basis for development of pharmacological chaperones for a variety of other, similar, lysosomal storage disorders.

Thanks to the support of the Wellington Medical Research Foundation, I was able to attend the 2023 European Carbohydrate (EuroCarb) Symposium in Paris, France. This conference brought together 600 participants from 36 different countries across the world, including 221 PhD students, industry professionals and university professors. This was an excellent opportunity, bringing together the brightest minds in glycoscience to deliver their recent research results. This was a jam-packed 5-day conference, featuring 11 plenaries, 17 keynote and 144 oral lectures, as well as 224 posture presentations. At this conference, several prestigious carbohydrate science awards were presented also. The awardees gave plenary lectures to highlight the work that resulted in them receiving such awards. It was amazing and extremely motivating to see the research outcomes from these presenters. I was also able to see talks from two of my favourite carbohydrate chemistry researchers, which was a highlight of the whole experience.

I presented a poster on my research project, titled 'Design and Synthesis of Pharmacological Chaperones to Treat Krabbe Disease.' In this poster, I presented the recent biological assay results of the top two drug candidates out of a library of compounds that I have synthesised during my PhD. These compounds have been shown to have very promising biological activity, comparable to that of the gold-standard pharmacological chaperone on the market for another lysosomal storage disorder. My two-hour poster session was packed full of exciting conversation about my topic. My poster was well received by those who read it, and I was approached by researchers who had comments and suggestions on my research topic. This stimulated my thought on areas of my research I had not yet considered, and I absolutely loved being able to talk about my research with other experts in the field.

All of the other talks and posters that I attended and read opened me up to a plethora of research topics in carbohydrate science that I was not aware of yet and also exposed me to new scientific methods that I will be able to implement into my own research at some stage. It was truly amazing to see the breadth and quality of the science being conducted in the carbohydrate research community, and the passion that these researchers have for their topic is motivating. I was able to develop some connections, as well as take down some e-mails of researchers who I would perhaps be interested in contacting for a post-doctoral research fellowship once I finish my PhD. All-in-all, the European carbohydrate symposium was extremely rewarding and an amazing opportunity for me to present my research, learn new science and develop new connections in the field.

MARY BUCHANAN

ROYAL AUSTRALIA AND NEW ZEALAND COLLEGE OF PSYCHIATRISTS (RANZCP) CONFERENCE IN QUEENSTOWN

Mary Buchanan

In my PhD research, we have produced a systematic review of factors that enhance resilience to long term outcomes of cumulative adversity in childhood. The findings of this review suggested that social support and education, as well as internal characteristics such as personality, are key areas for future research to alleviate the burden of cumulative childhood adversity.

Thanks to the travel grant awarded by Research for Life, I was able to present this paper at the Royal Australia and New Zealand College of Psychiatrists (RANZCP) conference in Queenstown in September 2022. Attendees at the conference had a range of backgrounds, and this was reflected in the interesting array of talks.

I presented as a part of a suite of talks on childhood and adolescent mental health. As a result, I was able to connect with other presenters, and audience members who shared an interest in development. This was the first conference presentation of my PhD work, and I benefitted greatly from the range of perspectives in the room. I had many stimulating conversations about my talk and others and made connections with clinicians and researchers.

I was also allowed the opportunity to help moderate a panel of talks from medical students and registrars presenting their research projects. Overall, this was a very valuable experience, and I hope to attend more conferences like this one in the future.

SONJA HUMMEL

THE 71ST ANNUAL SCIENTIFIC MEETING OF THE CARDIAC SOCIETY OF AUSTRALIA AND NEW ZEALAND (CSANZ)

Sonja Hummel

This event took place over four days at the Adelaide Convention Centre in Adelaide, South Australia. In conjunction with the International Society for Hear Research, this conference brought together cardiologists and researchers alike and provided the scientists, doctors, and researchers in the cardiovascular space a meeting place to present and discuss world-class research. As a 3rd year PhD student, I immensely valued the opportunity to attend this meeting.

Having the opportunity to present my research at this renowned meeting allowed me to obtain feedback from experts in the field and hear their view on the strengths and weaknesses of my research, as well as aid in establishing where further work is required. It has been of great benefit to discuss my science with follow researchers as their questions about my experiments, my background, as well as other research aspects, demonstrated that my PhD progress is on a good path. Presenting my poster also benefitted me in improving my presentation and communication skills, both soft skills that are instrumental for a successful career as well as wonderful opportunity to practice for a PhD defence.

As a female in science, I value the opportunities that are actively created to bring women in STEM together. At a 'women in cardiology' afternoon tea, I was able to connect with fellow female colleagues and enjoy encouraging conversations that highlighted the support the female community is providing for one another, as well as inspiration and empowerment to strive for success. This informal afternoon tea was truly motivating, and I am grateful to have attended an event where renowned female scientists took the time to converse with us, to empower us to be role models for other females and to take a stand for woman in science. This was an instrumental experience that further encouraged me to be a confident woman in science.

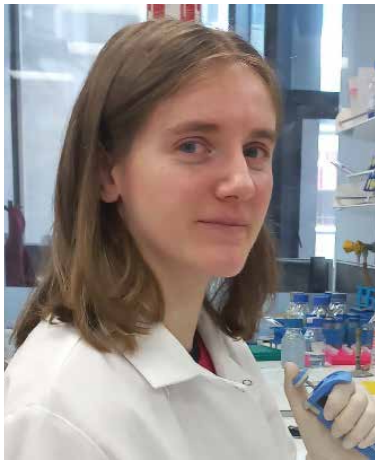
A personal highlight of the conference were presentations from sport cardiologists. Sports cardiology had not been an area of research that I had been aware of previously and listening to professors and clinicians present the fascinating cases and studies of endurance athletes and cardiovascular events has given me insight into the wide range of research areas that exist within the cardiology space.

As a third year PhD student I am actively looking for post-PhD work opportunities. CSANZ provided an immensely expansive repertoire of industry professionals and while their primary presence at the conference was to promote their products and services, I found it highly valuable to engage with these professionals and learn about their individual career pathways. These conversations have given me insight into the varying roles of the industry space as well as connections that I would not have been able to form without attending the conference. Taken together, attending CSANZ has not only allowed me to connect with potential future employers but also allowed me to learn about myself and my research interests and how I want to contribute to this field post PhD.

Overall, I am grateful to have been given the opportunity to attend this conference. I believe, attending this conference has been hugely beneficial for my professional development.

SARAH MESSENGER

2022 APPLIED MOLECULAR MICROBIOLOGY SUMMER SCHOOL REPORT



Sarah Messenger

In September 2022 I attended the ninth John Innes/Rudjer Bošković Summer School in Applied Molecular Microbiology in the wonderful Croatian city of Dubrovnik at the Inter-University Centre. An esteemed faculty of researchers directed the course, and 45 students, from 21 different countries around the world attended and participated. Faculty members presented a series of health-focussed lectures relating to microbes, specialised metabolites, natural product biosynthesis, discovery and isolation, and antibiotic resistance. We participated in small group discussions on various topics to troubleshoot problems or discuss ideas. All students gave a 2-minute flash talk and presented a poster to share their current research. The summer school was a highly immersive experience, with breaks and meals also used to discuss science and connect with students and faculty member alike.

Attending the summer school was an immensely beneficial experience. Topics of lectures were highly relevant to my PhD research, and I was able to gain broader understanding of the field and related lines of research. The most invaluable aspect of the summer school was connecting and networking with my fellow students and the faculty members and it was a privilege to discuss research and ideas with them.

Overall, the 2022 Applied Molecular Microbiology Summer School was an excellent, immersive experience and has been hugely beneficial and inspirational for me, moving forward in my doctoral research. I have established connections with professors, researchers, and fellow PhD students, which I hope can be maintained and lead to collaborations later in my career. I am very grateful for the support and funding I received from Research for Life which enabled me to attend.

MOLLY DORE

BRITISH GYNAECOLOGICAL CANCER SOCIETY (BGCS) CONFERENCE IN ABERDEEN, SCOTLAND

Molly Dore

With the support of Research for life I was able to travel to the UK and present my research at the British Gynaecological Cancer Society (BGCS) conference in Aberdeen, Scotland in June this year. My research is based around conservative management of endometrial cancer and pre cancer (atypical hyperplasia) with the Mirena intrauterine system. Specifically, my PhD is looking into predictive biomarkers that may be able to predict response to the Mirena prior to treatment commencing, alongside identifying molecular features that may be driving treatment resistance in some women.

The theme for the BGCS conference was "Research and Quality Improvement" and presentations focused on clinical and translational research to improve patient care pathways. This was congruent to the work I am carrying out in my PhD. At this conference, I made connections with some leading experts in my field and got insightful feedback on the work that I am currently doing, alongside ideas for future research. It was an incredible and invaluable opportunity to hear about the research that is being carried out around the world and how it compares to the field here in Aotearoa.

Alongside the conference, I was also able to collaborate with Team Womb, an endometrial cancer research group led by Professor Emma Crosbie at the University of Manchester, UK. Prof Crosbie is a pioneer in this space, and also gave a presentation at BGCS. While working with their group, I was involved in clinic work, patient recruitment and assisted in audit writing for a project looking into molecular profiling of endometrial cancer patients in Manchester. Being involved in the clinic work allowed me to observe the treatment pathways and clinician decisions first hand which was an invaluable experience. Both the conference and the collaboration in Manchester were hugely beneficial to the progress of my PhD and personal career development in my final year of study. I am hoping to return to the UK following completion of my PhD to carry out a postdoctoral fellowship with the people I met and worked with in the UK. I am extremely grateful for the support of Research for Life which enabled me to attend this conference.

KATHARINA ROBICHON

50TH ANNUAL SCIENTIFIC MEETING OF AUSTRALIAN AND NZ SOCIETY FOR IMMUNOLOGY

Katharina Robichon

I am a postdoctoral immunologist working at the Victoria University of Wellington and the Wellington Regional Hospital. In 2021, I was awarded a prestigious and highly competitive MBIE Whitinga fellowship, to develop my own independent research in translating findings from bench to bedside. The ASI 2022 in Melbourne provided an excellent platform to promote not only my own research in the Australasian community but also a very good opportunity for me to connect with other scientists in similar fields. This conference had a high focus on autoimmune disease, especially MS with special MS symposiums, and workshops.

I have meet two very inspiring female scientist in the field of MS research and computational science to model the immune system, with whom I have discussed my findings and gain valuable feedback for my own research project developing a personal medicine approach.

Besides being a full-time scientist, I am also the mother of two beautiful daughters, age 4 and 2. Being on a mother and a scientist are both very demanding jobs and sometimes attending a conference is not easy. Even though it was hard to be away from the girls for the first time, being at this critical stage of my research career, the ASI 2022 in Melbourne provided an essential opportunity to establish new networks to share knowledge, technology and expertise. All of which are beneficial when starting my own research group next year. Thank you, Research For Life, for supporting me and made it possible to attend this conference.

Three Minute Thesis (3MT)

Research For Life supports Masters and PhD level research by sponsoring a $500 prize for the University of Otago, Wellington 3MT competition.

This year there were only two applicants and the prize was split between them. Both presentations were of high quality.

SOPHIA NOBLE talked about our 'Unlikely Allies' describing how hookworm therapy might help with our immune system and how parasites being introduced to our bodies might help to treat disease.

ZARA MANSOOR was selected to compete in the Otago 3MT Grand Final in the Doctoral category. Her thesis was based on evaluating an emotion coaching programme for parents of early adolescents with mental health services.

NIWA Science Fair

Research For Life also provides $300 annually for prizes in the Wellington NIWA Science Fair for schools. It rewards projects which look at some aspect of health.

This year's Fair was very successful. There were 271 exhibits, involving 383 students from 28 schools from the Wellington region.

The following exhibits won Research For Life WMRF Special Prizes:

AANYA BADIYANI AND KAAVYA BADIYANI YEAR 7 STUDENTS FROM WHITBY COLLEGIATE

Won $100 with an exhibit called "Vaping - Smoking without Fire".

ZOË ROYLE YEAR 8 STUDENT FROM NORTHLAND SCHOOL

Won $50 with an exhibit called "Eat Like an Athl-Eat: Does healthy eating influence athletic ability?".

ABHITHA BALAKUMAR YEAR 8 STUDENT FROM ST ORAN'S COLLEGE

won $50 with an exhibit called "Lower those levels!".

KOKO JACKSON YEAR 8 STUDENT FROM WADESTOWN SCHOOL

Won $50 with an exhibit called "Heart Hydration".

MARCUS YOUNG A YEAR 9 STUDENT FROM RONGOTAI COLLEGE

Won $50 with an exhibit called "You're All Heart".

Research For Life

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